

Molecular Epidemiology of Measles in India, 2005–2010

Niteen Wairagkar,¹ Deepika Chowdhury,¹ Sunil Vaidya,¹ Sarika Sikchi,¹ Naseem Shaikh,¹ Laxman Hungund,¹ R. S. Tomar,¹ D. Biswas,² K. Yadav,² J. Mahanta,² V. N. R. Das,³ Prasanna Yergolkar,⁴ P. Gunasekaran,⁵ D. Raja,⁵ R. Jadi,¹ Nalini Ramamurthy,⁶ A. C. Mishra,¹ and MeaslesNetIndia collaborators^a

¹WHO Regional Measles Reference Laboratory, National Institute of Virology, Pune; ²Regional Medical Research Center, Dibrugarh; ³Rajendra Memorial Research Institute of Medical Sciences, Patna; ⁴WHO National Measles Laboratory, Bangalore Field Unit of National Institute of Virology, Pune; ⁵WHO National Reference Laboratory for Measles and Rubella, King Institute of Preventive Medicine, Chennai; and ⁶World Health Organization-South East Asia Region Office, New Delhi, India

Measles is a childhood disease that causes great morbidity and mortality in India and worldwide. Because measles surveillance in India is in its infancy, there is a paucity of countrywide data on circulating *Measles virus* genotypes. This study was conducted in 21 of 28 States and 2 of 7 Union Territories of India by MeaslesNetIndia, a national network of 27 centers and sentinel practitioners. MeaslesNetIndia investigated 52 measles outbreaks in geographically representative areas from 2005 through June 2010. All outbreaks were serologically confirmed by detection of antimeasles virus immunoglobulin M (IgM) antibodies in serum or oral fluid samples. Molecular studies, using World Health Organization (WHO)–recommended protocols obtained 203 N-gene, 40 H-gene, and 4 M-gene sequences during this period. Measles genotypes D4, D7, and D8 were found to be circulating in various parts of India during the study period. Further phylogenetic analysis revealed 4 lineages of Indian D8 genotypes: D8a, D8b, D8c, and D8d.

This study generated a large, countrywide sequence database that can form the baseline for future molecular studies on measles virus transmission pathways in India. This study has created support and capabilities for countrywide measles molecular surveillance that must be carried forward.

Measles is a childhood disease that causes great morbidity and mortality throughout the world. Worldwide, the number of reported measles cases recently declined by 67%, from 852,937 in 2000 to 278,358 in 2008, and deaths attributed to measles declined by 78% [1]. However, risk for illness and death from measles still exists in countries with variable routine vaccination

coverage, as in India, where measles is a significant public health problem. Though various studies have described the measles epidemiology in India, the country lacks knowledge generated from systemic countrywide measles surveillance. In the absence of countrywide case-based surveillance in other countries, outbreak surveillance coupled with molecular epidemiology studies have provided important data for mapping transmission routes, documenting the elimination of endemic virus strains, and indicating risk groups [2, 3]. Taking a similar approach, the National Institute of Virology (NIV) in Pune, India, was instrumental in initiating outbreak surveillance and molecular studies that detected the presence of and described the circulation of indigenous genotypes D4, D7, and D8 [4, 5].

Measles virus (MeV), an enveloped virus with a single-stranded, negative-sense RNA genome, is a member of the genus *Morbillivirus* within the family Paramyxoviridae. MeV is highly contagious and causes a disease characterized by high fever, cough, coryza, conjunctivitis, and

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^aPlease see the Appendix for a list of the MeaslesNetIndia collaborators.

Correspondence: Niteen Wairagkar, MD, Regional Reference Measles Laboratory, National Institute of Virology, 20 A Ambedkar Rd., Pune, India, 411001 (niteenv@yahoo.com).

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appearance of a maculopapular rash. MeV is a monotypic virus serologically, but genetic variability exists among wild-type strains [6]. The protocols and nomenclature for genetic characterization of wild-type MeVs have been standardized by the World Health Organization (WHO), which helped in detecting circulation of 23 genotypes of MeV [7]. In June 2010, investigators detected a new genotype of measles provisionally known as d11 (awaiting final designation by the WHO) [8]. Molecular studies have been instrumental in many countries for tracking national and international transmission pathways of MeV strains [3, 9–13]. During 2005 and 2006, virological surveillance activities expanded throughout Southeast Asia, and D4, D7, D8, D9, D5, and G3 genotypes have since been detected in the countries of the region [14].

Only limited genotype data from Maharashtra and Tamil Nadu states was available till 2005 [4], and the study was extended to other geographic areas of India subsequently [15]. The countrywide network of collaborating centers was established, outbreaks of measles were investigated, and the baseline sequence database of measles genotypes circulating in all the geographic areas of India was created. This study

describes the molecular epidemiology of measles from 2005 to 2010.

MATERIALS AND METHODS

Establishment of MeaslesNetIndia Network

To accomplish the objectives of this study, a voluntary participatory network of 17 centers in India was started in August 2005 and named MeaslesNetIndia [15]. In 2006, MeaslesNetIndia enrolled private pediatric and general practitioners from various states, in the first large-scale public–private partnership for measles-related work. Subsequently, in 2008, network expanded to include 10 centers from the northeastern region of India. Network centers included Indian Council of Medical Research (ICMR) centers, medical colleges, and other research centers, giving geographical representation throughout the country (Figure 1). The participating sentinel-site collaborators were trained at 3 workshops on various aspects of this study, including outbreak investigation strategies, specimen collection, laboratory diagnostics, and the role of each collaborator. These centers performed outbreak investigations using local public

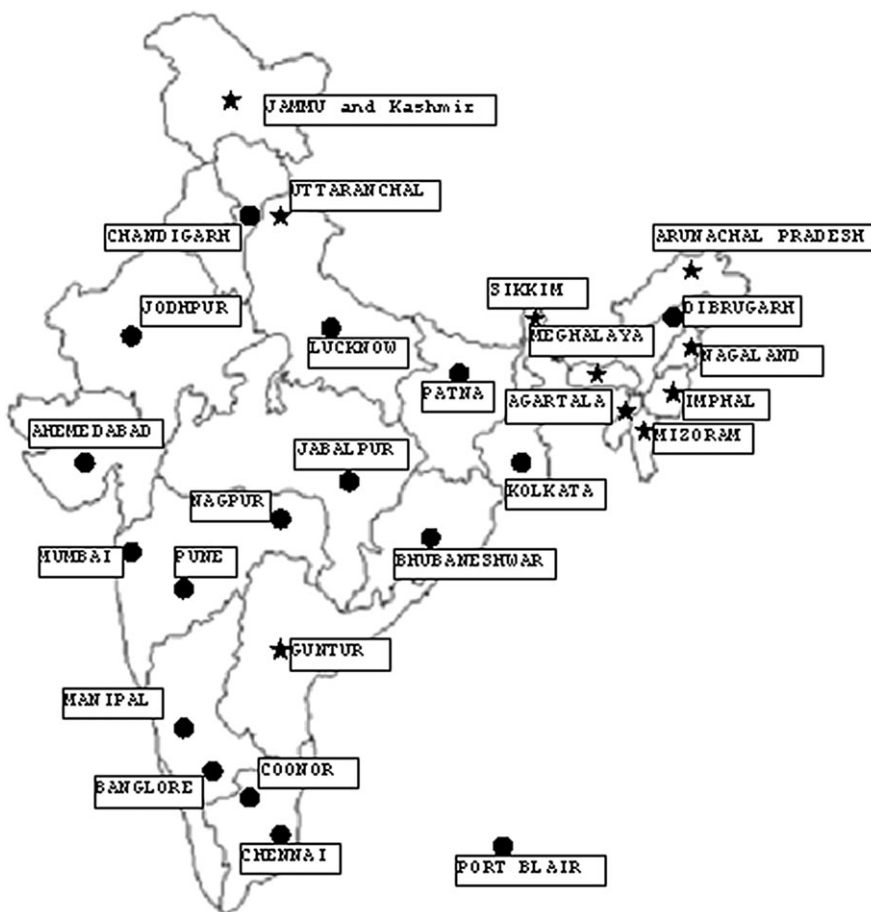


Figure 1. MeaslesNetIndia: geographic representation.

health resources. Financial assistance was made available through the WHO for the training of participants, logistics, and testing at NIV, Pune.

WHO-SEAR Measles and Rubella Laboratory Network

The World Health Organization Regional Office for South-East Asia (WHO-SEAR) measles laboratory network was established in 2003 and has expanded in a phased manner to 19 laboratories in 2009, with 1 regional reference laboratory and 18 national laboratories, 5 of which are in India. The laboratories were recruited through consultation with the national governments. In India, WHO has designated NIV at Pune as Regional Reference Laboratory for Measles and the King Institute of Preventive Medicine (KIPM), Chennai, as National Reference Laboratory for Measles and Rubella. All laboratories in the network have been accredited, and all contributed isolates and clinical specimens for virus characterization.

Outbreak Investigations by MeaslesNetIndia

During 2005–2010, the MeaslesNetIndia network investigated 52 suspected measles outbreaks from 23 states. Investigators used the WHO definition of measles case, which is any person with fever of 38°C or greater, and maculopapular rash (ie, nonvesicular), and any 1 of the following: cough, coryza, or conjunctivitis; any person in whom the health professional suspected measles was included in the investigations [16]. Outbreak was defined as the occurrence of 5 clinical measles cases in a 15-day period from a population of approximately 5000. Appropriate specimens for serologic confirmation and virus isolation were collected and transported in appropriate cold-chain conditions to NIV for laboratory studies. Sporadic cases were investigated from Imphal (Manipur state), Dimapur (Nagaland), Dabra (Madhya Pradesh), and Valsad (Gujarat), areas where no outbreaks were reported. Informed consent was obtained as appropriate using standard forms translated into local languages by the collaborators.

Specimen Collection

Investigators collected throat swabs and urine specimens for virus isolation and blood samples for serology from 5–10 representative acute measles cases in each outbreak. Oral fluid proved to be the most convenient sample for collection and transport to the laboratory in Indian climatic conditions wherever serum could not be obtained [17]. Collected clinical samples (throat swabs, urine, oral fluid, and blood) were processed and stored appropriately until transportation to NIV at Pune. For molecular studies on 1 case of fulminant subacute sclerosing panencephalitis (SSPE) and 1 case of measles inclusion body encephalitis (MIBE), brain and serum/cerebrospinal fluid samples were referred to NIV from Human Brain Tissue Repository of the Department of Neuropathology, National Institute of Mental Health and Neuro Sciences, Bangalore. A total of 753

sera, 283 throat swabs, 80 oral fluids, and 302 urine samples from 845 measles cases were received at NIV during the study period.

Serological Studies

We serologically confirmed all outbreaks as measles by immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) with WHO-recommended kits (Dade Behring, Siemens, or Microimmune) using manufacturers' instructions. Oral fluid samples were also tested by Microimmune kits for detection of IgM antibodies to measles. The samples that tested negative for measles IgM were tested for rubella IgM using Microimmune kits.

Virological Studies

We isolated the virus and identified measles strains according to the standardized methods at NIV, Pune, NIV Bangalore, and KIPM, Chennai, using B95a cells or Vero-SLAM cell line (from Dr. Yanagi via Centers for Disease Control and Prevention [CDC]-WHO for Network Laboratories) according to the standard CDC - WHO protocol. [18, 19] In brief, we inoculated processed throat swabs and urine samples onto the cell lines and observed them for cytopathic effect (CPE). We blind-passaged inoculated cells up to 3 times before discarding them as negative for virus isolation. We harvested cells when the CPE was maximum (3+ to 4+) in the third-passage culture flasks. All isolates were identified as measles by immuno-fluorescence assay or RT-PCR and sequencing.

Molecular Studies

We performed reverse-transcription polymerase chain reaction (RT-PCR) and sequencing studies on the clinical samples from suspected measles cases and MeV isolates obtained or sent by the collaborators. We extracted viral RNA from infected cell lysates or clinical specimens using TRIzol LS reagent according to the manufacturer's instructions. RNA pellets were dried and resuspended in 50 µl of nuclease free water and stored at -70°C until amplification by RT-PCR. We performed sequencing studies by standardized protocols using WHO-recommended primers. We used primers MV63 (5'CCT CGG CCT CTC GCA CCT AGT 3') and MV60 (5'GCT ATG CCA TGG GAG TAG GAG TGG 3') to sequence the 456 bp fragment of the N-gene COOH terminal according to WHO recommendations. We used primers H2 (GAT CAT CCA CAA TGT CAC CAC), H4R (AGA TGA CAC ATT GTA ACC TCG), H4 (CGA GGT TAC AAT GTG TCA TCT), H6R (CGT ATG AAG GAA TCC TGT TAT), H7 (CCG AGA TTC AAG GTT AGT CCC), and H10 (GTA TGC CTG ATG TCT GGG TGA) to amplify the 1854 bp complete-coding region of the H gene [3, 4, 7].

Sequence Analysis

We derived the sequences of the measles N-gene RT-PCR products by automated sequencing with primers MV60 and

MV63 and the BigDye terminator 3.1v chemistry using reaction conditions that were recommended by the manufacturer (ABI 373, ABI 3100, Perkin Elmer-Applied Biosystems). We proof-read and edited sequences with Sequencer™ (Gene Codes Corporation). Measles N- and H-gene sequences obtained in this study were confirmed with both strands and aligned with WHO standard reference N- and H-gene sequences [14] using software ClustalX version 1.83. We conducted a phylogenetic analysis using MEGA version 4 [20]. We assessed the robustness of the groupings using bootstrap resampling of 1000 replicates, and we visualized the trees with MEGA programs. We used National Center for Biotechnology Information Basic Logical Alignment Search Tool (NCBI BLAST) analysis to find percent nucleotide identity (PNI) with the related strains.

Submission to Public Genetic Databases

We prepared sequence cards for all the sequences, including the available clinico-epidemiological information on each strain. We deposited 243 representative nucleotide sequences (N and H gene) in GenBank (under accession numbers EU812245-EU812316, FJ387130-FJ387157, FJ765064-FJ765087, FJ979795-FJ979797, FJ968742, FJ719484-FJ719489, FJ223133-FJ223168, FJ19763, GU306169-GU306174, GU827659, GU953671-GU953672, GQ420695-GQ420699, HM567314-HM567315, HM452160-HM452161, HM358867-HM358877, AY873971-AY873981, DQ987230-DQ987235, DQ335129-DQ335131, DQ345392-DQ345393, AY953414-AY953418, AY957557-AY957558, DQ923615, AY841168, HQ148303-HQ148309). All the sequences were deposited into the WHO Sequence Database and the MeaNS database of the WHO Measles Laboratory Network.

Additionally, the Indian Measles Sequence Database is maintained at NIV for future use as part of the measles elimination program. We performed detailed analyses to find the intragenotypic diversity of measles strains circulating in India.

RESULTS AND DISCUSSION

This paper provides an overview of measles-outbreak investigations and molecular studies conducted during 2005 till June 2010, giving comprehensive baseline data for India. Of the total 28 states and 7 union territories in India, 23 were covered during this investigation. A total of 52 outbreaks and several sporadic cases (where outbreaks were not reported) were investigated. Table 1 describes the key data elements (geographic location, number of cases, age range, serological confirmation, and genotypes detected) from the outbreaks investigated by MeaslesNetIndia. The details of individual outbreak investigations have been published elsewhere [21–24]. Because the aim of the study was to create countrywide baseline molecular data on circulating genotypes of MeV, we investigated only representative outbreaks from the study period.

Table 2 describes the age distribution among 845 clinically suspected measles cases from whom samples were collected and confirmed as measles by IgM ELISA. This study population neither reflects the extent of the outbreaks nor does it include all cases. Convenient sampling could not be ruled out. The proportion of each age group for cases investigated, samples tested, and positive measles results did not differ significantly from each other, indicating representativeness. The overall measles IgM-positivity rate was 62.8%, and was highest among persons aged 1–3 years, as Table 2 indicates. We found no significant variation of measles seropositivity across the age groups. Rubella cases were detected sporadically in our study. Though a study in southern India detected rubella cases in field investigations of measles outbreak [25], we didn't detect extensive presence of *Rubella virus* in the study period. We found persons aged 1–6 years (51%), and a notable proportion of cases (30.3%) was recorded among persons aged 6–15 years, as shown in Table 3. Small proportions of cases were <1 year of age. Measles in infants, especially among those in orphanages, have been documented in India in previous studies in Pune and Chennai [5, 22]. Table 1 gives reported age ranges from each outbreak in the study period, taken from records maintained by the public health authorities of the respective area. The range of age groups affected by outbreaks in different geographic areas indicates the diversity of measles transmission patterns in India. This finding calls for standardized investigation of outbreaks, in which all cases are considered and the extent of the outbreak and the age group of the study population are not limited. Studies in Kashmir and Tamil Nadu documented cases among persons >5 years of age [21, 23].

Suspected cases among persons >15 years of age were encountered in outbreaks investigated in post-tsunami camps in Andaman, East Siang in Arunachal Pradesh, Supoul in West Bengal, Kangra in Himachal Pradesh, Kargil in Jammu, and Kashmir states. Serological confirmations were done by IgM ELISA on serum or oral fluid specimens. Of 64 suspected measles cases among persons aged >15 years, 33 were confirmed as measles by IgM ELISA, including 3 pregnant women. Adolescent outbreaks in Jammu and Kashmir [21], an outbreak in Arakkonum, Vellore district of Tamil Nadu [26], and an outbreak among medical students in Manipal, Karnataka, (Arun Kumar, personal communication) were also confirmed as measles by laboratory studies. These reports suggest the presence of a susceptible adolescent population in some areas, probably where they neither had an opportunity for immunization nor were naturally exposed to measles. The susceptibility of this age group must be investigated and documented further in nationwide studies.

Table 4 describes the seasonality of laboratory-confirmed measles cases. Data indicated measles transmission throughout the year in India, with pattern variations between geographic areas. The seasonal patterns would clearly be detected by countrywide laboratory surveillance. Seasonality in this table is

Table 1. Measles Outbreaks Investigated by MeaslesNetIndia.

State	Period	Number of cases	Age range	Serology confirmation	Measles genotypes
Andaman	Feb 2006–Mar 2006	51	8 mo–33 y	Yes	D8
Andhra Pradesh	Nov 2006–Apr 2007	22	1–13 y	Yes	D4, D8
Arunachal Pradesh	Dec 2007–Nov 2009	21	1.4–22 y	Yes	D4, D8
Assam	Mar 2008–May 2009	25	6 mo–17 y	Yes	D8
Bihar	Dec 2005–Apr 06	310 (3 deaths)	8 mo–26 y	Yes	D4, D8
Chandigarh	Feb 2006–July 2006	25	5 mo–5 y	Yes (1 Rubella IgM+)	D8
Chhatisgarh	Nov 2006	60	6 mo–7.10 y	Yes	NA
Gujarat	Nov 2008–Apr 2010	84 (6 deaths)	3 mo–17 y (>5 y: 47%)	Yes	D4, D8
Himachal Pradesh	Sep 2006	30	4–12 y	Yes	D4
Jammu & Kashmir	Mar 2007–May 2007	401	8 mo–15 y (>15 y: 126 cases)	Yes	D4
Karnataka	Mar 2004–Jun 2004 (Retrospectively investigated)	12	Adult medical students 20–27 y	Yes (2 IgM+)	D8-like (partial sequence)
Karnataka	Feb 2003	1	Fulminant SSPE case	NA	D7
Karnataka	Jun 2005	924	1–15 y	Yes	D8, D4
Kerala	2010	130	<1–15 y	Yes	D8
Madhya Pradesh	Mar 2007	5	6 mo–6 y	Yes	D4
Maharashtra	Jan 2006, Mar 2006 Apr 2010	330 (1 encephalitis case)	6 mo–16 y	Yes	D4, D7, D8
Manipur	Nov 2009	1	11 y	No (IgM NEG)	D8
Nagaland	Apr 2008–Nov 2009	18	4 mo–12 y	Yes	D8
Orissa	Aug 2006–Jan 08	33	7 mo–11 y	Yes	D8
Sikkim	May 2008–Jul 2008	22	2 mo–27 y	Yes	D4, D8
Tamil Nadu	Jan 2005–Jan 2007	129 (13 virus isolates)	<1–15 y	Yes	D4, D7, D8
Tripura	Mar 2007–Apr 2007	6	8 mo–17 y	Yes (17 y old IgM +)	D8
Uttar Pradesh	Jan 2006–Feb 2006	10	9 mo–11 y	Yes	D4, D8
Uttar Pradesh	Oct 2006	12	3–8 y	Yes	NA
Uttaranchal	Oct 2005, Jan 2007	96	6 mo–14 y, one 23-year-old pregnant woman	Yes (Pregnant, IgM measles +)	D8
West Bengal	Feb 2006–May 2007	96	3 mo–16 y	Yes	D8

NOTE. NA, not applicable; IgM, immunoglobulin M.

an indication of circulation of measles strains that could be transmitted to other areas.

Measles Genotype Circulation in India

Table 1 described the indigenous measles genotypes circulating in India in the states covered in this study. During the study period, D4, D8, and D7 genotypes were circulating in various parts of India. Though circulation of D4 and D8 have been documented since 1996 [4] and continued in the study period, the D7 genotype was found to have limited circulation in 3 cities only [5]. Genotype A had not been detected in this period. In this study, we detected no additional D7 genotypes, which indicates either limited circulation of D7 or insufficient molecular

surveillance in the country. During the study period, investigators documented measles genotypes D4, D7, and D8 only.

Intragenotype Differentiation

On further phylogenetic analysis of all the D8 N-gene sequences (Figure 2), we found that the Indian measles D8 genotype could be differentiated into 4 distinct lineages, labeled D8a, D8b, D8c, and D8d.

We used MVi/Tumkur.Ind/04.08 (Genbank accession number FJ719484) as the reference strain for the D8a genotype. D8a was the most diverse genotype circulating from 2005 to 2010, in the geographically diverse states Maharashtra, Karnataka, Andhra Pradesh, Orissa, Gujarat, Sikkim, Kerala, and Manipur.

Table 2. Age-Group Distribution of Investigated Cases and IgM Positivity

Age	IgM + for measles Number of positive results/ number of samples tested (%)	IgM + for rubella Number of positive results
0–6 mo	11/22 (50%)	
6 mo–1 y	37/57 (64.9%)	
1–3 y	86/128 (67.2%)	
3–6 y	155/240 (64.6%)	1
6–12 y	130/205 (63.4%)	3
12–15 y	14/23 (60.9%)	
>15 y	31/62 (50%)	5
Unrecorded	9/16 (56.2%)	
TOTAL	473/753 (62.8%)	9

NOTE. IgM, immunoglobulin M.

Circulation of D8a was detected mostly in central, western, and southern India. We designated MVi/Gulberga.Ind/14.07 (Genbank Accession number FJ979796) the reference strain for the D8b genotype that was circulating from 2005 through 2009 in the states of Assam, West Bengal, Uttar Pradesh,

Table 3. Age-Group Distribution of Investigated Cases and IgM Positivity

Age	Cases investigated N (%)	Samples tested n (%)	IgM + for measles n (%)	IgM + for rubella n
0–6 mo	22 (2.6)	22 (2.9)	11 (2.3)	
6 mo–1 y	63 (7.5)	57 (7.6)	37 (7.8)	
1–3 y	146 (17.3)	128 (17)	86 (18.8)	
3–6 y	278 (33)	240 (31.8)	155 (32.8)	1
6–12 y	231 (27.3)	205 (27.2)	130 (27.5)	3
12–15 y	25 (3)	23 (3)	14 (3)	
>15 y	64 (7.6)	62 (8.2)	31 (6.6)	5
Unrecorded	16 (1.9)	16 (2.1)	9 (1.9)	
TOTAL	845 (100)	753 (100)	473 (100)	9

NOTE. IgM, immunoglobulin M.

Himachal Pradesh, Arunachal Pradesh, Nagaland, and Andaman and Nicobar islands. We designated MVs/Papumpare.Ind/51.07/1 (Genbank Accession number EU812248) the reference sequence for the D8c genotype. No isolate as yet has been made from this genotype. Circulation of this genotype was seemingly

Table 4. Seasonality Pattern of Measles Transmission



NOTE. ■ Measles transmission
□ Transmission not detected

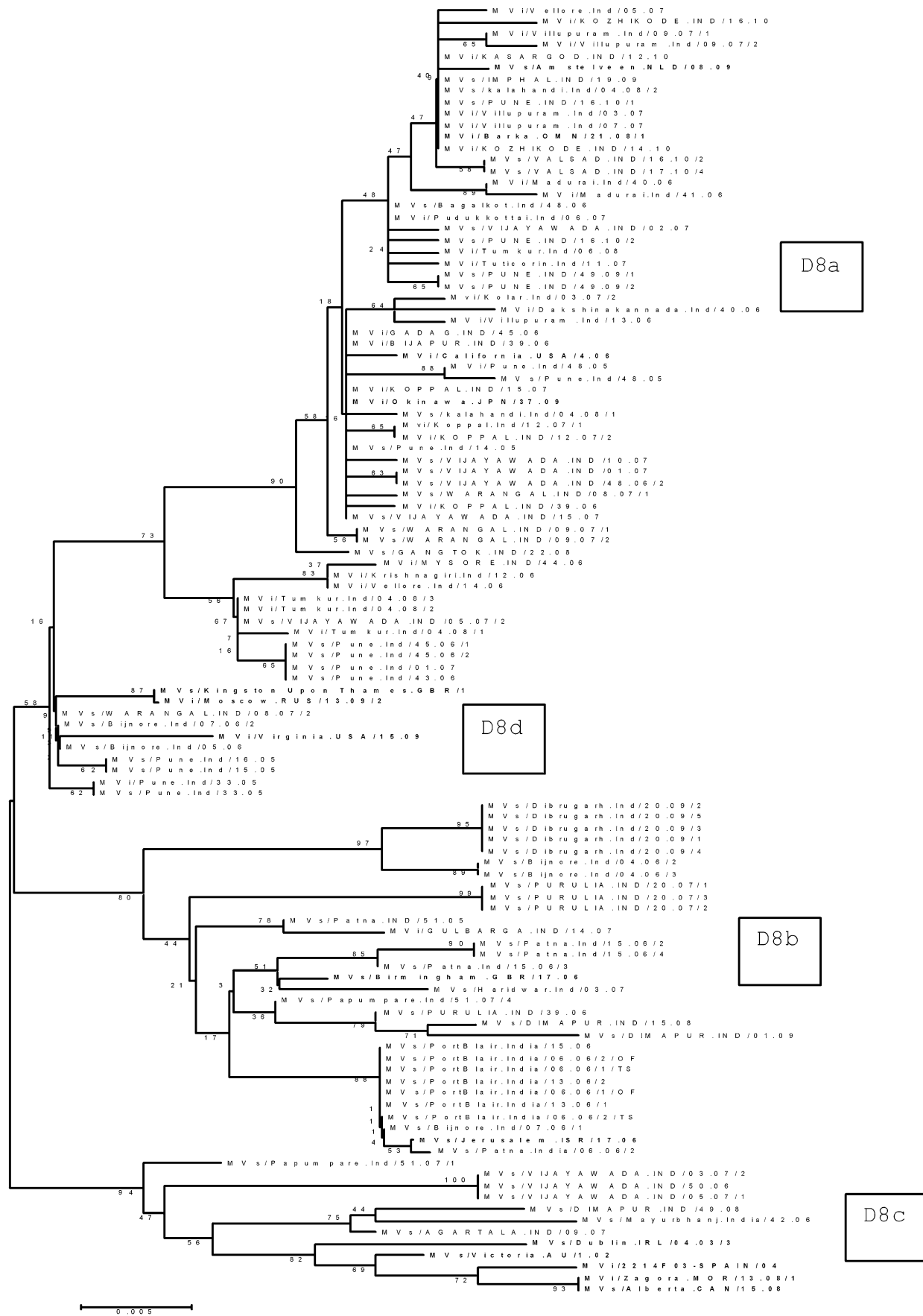


Figure 2. Indian D8 lineages (N gene).

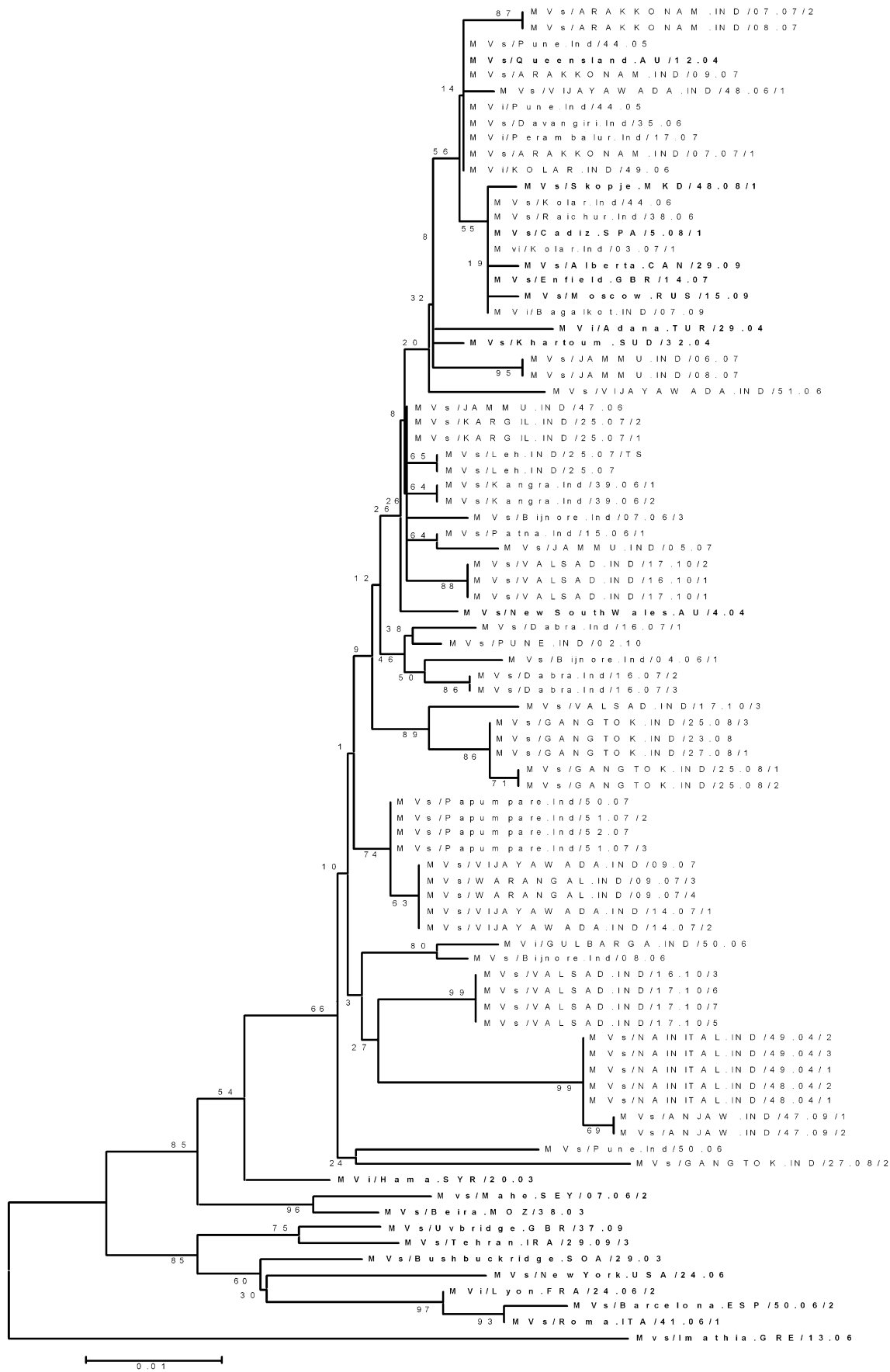


Figure 3. Indian D4 genotype (N gene).

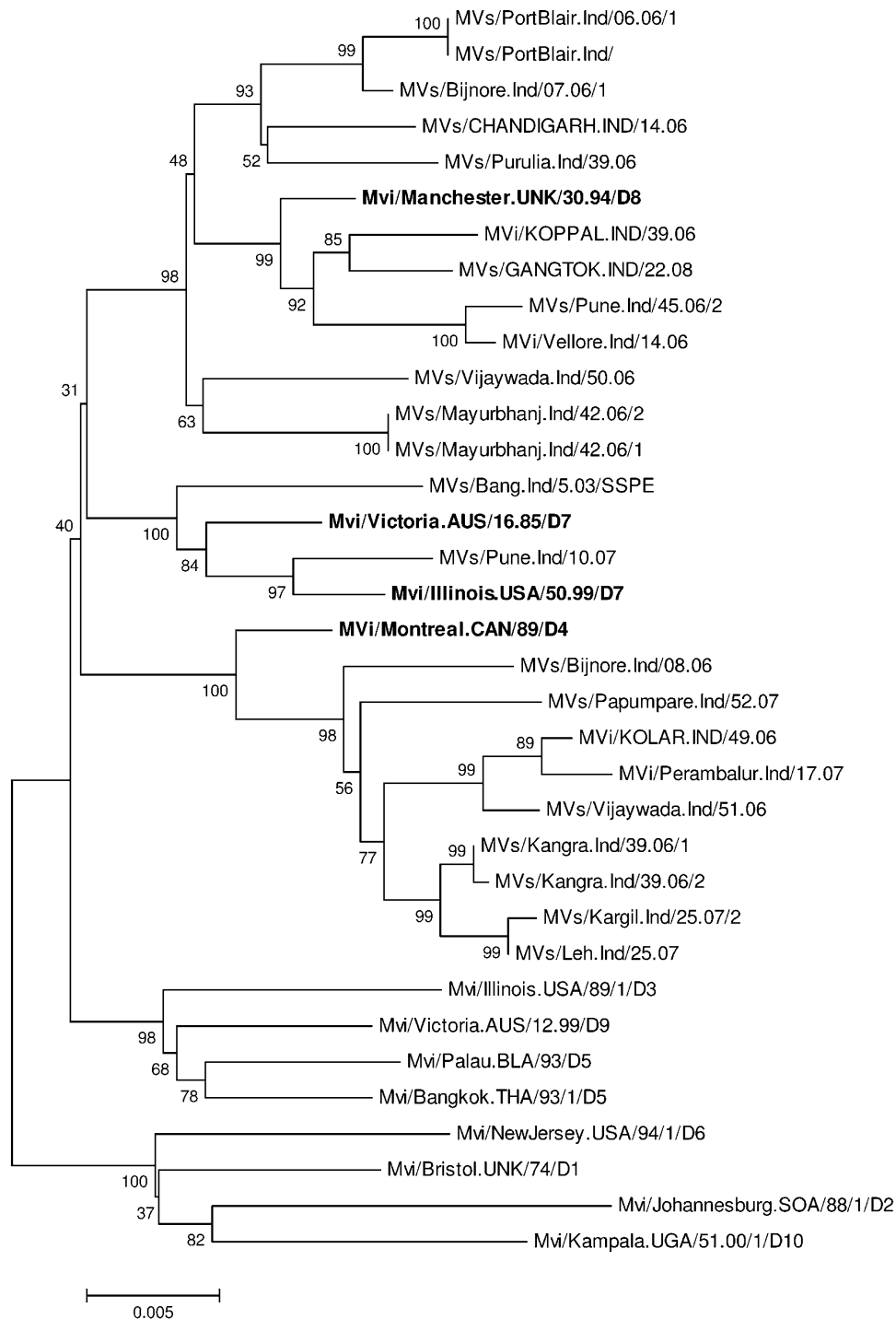


Figure 4. Phylogenetic analysis of H-gene sequences from India.

limited to Andhra Pradesh, Orissa, and northeastern states of Nagaland and Tripura during 2006–2008. We designated MVi/Pune.Ind/33.05 (Genbank Accession number FJ223159) the reference strain for the D8d genotype. Circulation of this genotype was limited between 2005 and 2007 to Maharashtra, Andhra Pradesh, and Uttar Pradesh. Our study did not document further detection of this genotype.

Previous studies have documented of H1, D4, and D6 genotypes in China [26], the Russian Federation [10], Indian Ocean islands [27], and Kenya [28]. Multiple lineages of genotype B3 have been detected in association with endemic circulation of measles in Africa [29]. It would be interesting to see how the circulation of these Indian D8 subclusters would evolve in time, especially under pressure of vaccination campaigns in some

areas. Genetic heterogeneity of dominant genotype D8 in India appears to be due to the presence of multiple, cocirculating lineages of this virus, indicating unrestricted transmission across geographic areas, somewhat similar to circulation of the dominant H1 genotype in China [26].

A Kenyan study [28] found subclustering of D4 into 4 different groups: Montreal-like, India-like, Johannesburg-like, and Ethiopia-like subclusters. Phylogenetic analysis could not differentiate the Indian D4 genotype (Figure 3) into distinct lineages. With multiple chains of transmission, the genotype could emerge with a distinct pattern in the future, which should be studied by countrywide surveillance.

To date, the D7 genotype has been found in only 3 cases from India, but analysis of the H-gene sequences reveals genetic diversity that suggests 3 different lineages of D7 circulating in India, each of which evolved over time [5]. Studies associated MeV genotype D7 with measles cases in Europe during the late 1990s and early 2000s [30], but the genotype has not been detected in Europe since 2005 [31]. The link between Indian and European D7 genotypes is not now clear. Subsequent data from both continents and molecular evolution studies might explain a link.

For all the genotypes detected, N-gene data correlated well with H-gene data (Figure 4).

We documented cocirculation of D4 and D8 genotypes in multiple areas in our study. Cocirculation of D8b and D8c has been noted in 1 outbreak from Arunachal Pradesh. Other countries have also documented cocirculation of genotypes, probably due to multiple chains of transmission during an outbreak [32, 33].

Though measles surveillance in India is in its infancy, during the preceding 15 years only Clade D genotypes (D4, D7, D8) have been detected in India, whereas surrounding countries have detected D4, D7, D8, D9, D5, H1, d11, and G3 genotypes [8, 14]. These external genotypes were not imported into India between 1995 and 2010. The possibilities of missing genotypes or of importation of other genotypes must be further studied and documented with continuous countrywide molecular surveillance in India.

In the study period, sequencing was performed on autopsied specimens from a case of SSPE [34] and a case of MIBE (Shankar et al unpublished data). There is a paucity of studies documenting genotypes identified from SSPE cases [35, 36], but such cases could be a good resource in India to find missing links in past measles transmission.

Though we investigated only representative measles outbreaks in the laboratory, the baseline sequence data generated from our study will be useful in the future, to track transmission pathways of Indian strains worldwide and to document importation of globally circulating genotypes into India. NIV at Pune is an accredited WHO reference laboratory for measles in India that will continue the MeaslesNetIndia work and molecular surveillance to monitor the circulation of measles lineages in the

Indian states. NIV efforts would be complementary to WHO-SEAR efforts. The molecular database would expand with the expansion of laboratory-based measles surveillance in India. Increased international collaborations, real-time outbreak investigations supported by molecular studies, and a global sequence database would help document the pathways of measles transmission to, from, and within India. The current study has demonstrated the effective use of molecular methods to monitor the measles scenario and the changing epidemiology of measles in modern India.

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MeaslesNetIndia Collaborators:

National Institute of Virology, Pune; National Institute of Epidemiology, Chennai; National Institute of Cholera and Enteric Diseases, Kolkata; Regional Medical Research Center, Dibrugarh; Rajendra Memorial Research Institute of Medical Sciences, Patna; Desert Medicine Research Centre, Jodhpur; Regional Medical Research Centre (ICMR), Andaman & Nicobar Islands; Regional Medical Research Centre for Tribals, Jabalpur; Regional Medical Research Centre, Bhubaneswar; NIV, Bangalore Field Unit, Bangalore; Dept. of Virology, Manipal Medical College, Manipal; Pasteur Institute, Coonoor; Dept. of Preventive Social Medicine, Government Medical College, Nagpur; Dept. of Microbiology, BJMC, Ahmedabad; Dept. of Virology, PGI, Chandigarh; Sanjay Gandhi Post Graduate Institute, Lucknow; Assam Medical College Dibrugarh, Guwahati Medical College, Assam; State Epidemiologist, Naharlagun, Arunachal Pradesh; Regional Institute of Medical Sciences, Manipur; North Eastern Indira Gandhi Regional Institute of Health & Medical Sciences, Shillong, Meghalaya; Pediatricians, Dimapur, Nagaland; Government Medical College, Agartala, Tripura; Aizwal Civil Hospital, Mizoram; Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim; World Health Organization National Reference Laboratory for Measles and Rubella, King Institute of Preventive Medicine, Chennai.

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APPENDIX MeaslesNetIndia Collaborators

Arun Kumar,⁷ Biswas AK,⁸ Bag B,⁸ Debberma S,⁹ Dhole TN,¹⁰ De Shobhan,⁸ Das PK,⁸ Dwibedi B,¹¹ Gupta SN,¹² Illankumar M,¹³ Jampa L,¹⁴ Ketkar A,¹⁵ Kumar Navin,³ Kumar Rajeev,¹⁶ Khobragade P,¹⁷ Kadri SM,¹⁸ Mahadevan A,¹⁹ Murhekar M,²⁰ Gupte MD,²⁰ Martolia H,²¹ Mazi Dipankar,⁷ Ratho RK,²² Shankar SK,¹⁹ Slathia S,²³ Sugunan AP,²⁴ Vittal CSN,²⁵ Vijaykumar G,²⁶ Vashishtha VM.²⁷

³Rajendra Memorial Research Institute of Medical Sciences, Patna; ⁷Manipal Centre for Virus Research, Manipal University, Manipal, Karnataka; ⁸Directorate of Health Services, West Bengal; ⁹Government Medical College, Agartala, Tripura; ¹⁰Sanjay Gandhi Post Graduate Institute, Lucknow, Uttar Pradesh; ¹¹Regional Medical Research Center, Bhubaneswar, Orissa; ¹²Regional Health and Family Welfare Training Centre, Kangra, Himachal Pradesh; ¹³Station Health Organization (Navy), Mumbai, Maharashtra; ¹⁴Directorate of Health Services, Naharlagun, Arunachal Pradesh; ¹⁵General practitioner, Gwalior, Madhya Pradesh; ¹⁶Happy Family Hospital, Roorkee, Uttarakhand; ¹⁷United Nations Children's Fund (UNICEF); Raipur, Chhatisgarh; ¹⁸Regional Institute of Health and Family Welfare, Srinagar, Kashmir; ¹⁹Human Brain Tissue Repository, Department of Neuropathology, National Institute of Mental Health and Neuro Sciences, Bangalore; ²⁰National Institute of Epidemiology, Chennai, Tamil Nadu; ²¹Directorate of Health Services, Dehradun, Uttarakhand; ²²Post Graduate Institute of Medical Education and Research, Chandigarh; ²³General hospital, Jammu; ²⁴Regional Medical Research Center, Portblair; ²⁵NRI Medical College, Chinakakani, Guntur; ²⁶Kakatiya Medical College, Warangal, Andhra Pradesh, and ²⁷Mangla Hospital & Research Center Bijnore, Uttar Pradesh, India.