

Measles

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10 Measles

- Measles Aerosol Vaccine Trial
- Molecular Surveillance of Measles in India
- Isolation and Genotyping fo Measles viruses from specimens referred by WHO - SEARO, measles laboratories
- Molecular Epidemiology of Measles in North-East India
- Measles M gene sequencing based diagnosis of subacute Sclerosing PanEncephalitis (SSPE)

Measles Aerosol Vaccine Trial

Collaborators : King Edward Memorial (KEM) Hospital and National AIDS Research Institute (NARI), Pune
NS Wairagkar, NJ Shaikh, LV Hungund, NS Kumbhar, RS Tomar

Measles group is involved in Phase I Measles aerosol vaccine trial since 2006 and the project continued in this year. Group 1 (18-35 years) was followed up to their 12th visit of the subjects in June 2007. Total of 19 subjects completed the follow-up for one year. The data was sent to WHO and preparation continued for screening and recruitment of Group 2 (5-17 years).

Data Safety monitoring Board DSMB gave approval to go ahead with Group 2 (5-17 years) screening. Accordingly, 119 subjects of this group were screened for clinical, hematological and biochemical criteria and 20 subjects were chosen for inclusion in the study. These were vaccinated with aerosol route and were followed up with regular visits on day 1,3,7,10,14, 28 and 90. Acute toxicity and safety data was assessed on 14 and 28 day. Data was submitted to DSMB, which permitted us to go ahead with Group 3 (1-4 years) in the trial. WHO Product Development Group (PDG) meeting was organized in Pune, which reviewed the trial findings and initiated preparations for next phase of the trial.

DSMB gave approval to go ahead with Group 3 (1-4 years) screening. Accordingly, 64 subjects of this group were screened for clinical, hematological and biochemical criteria. 15 subjects were found eligible on all the criteria and were vaccinated with measles vaccine by aerosol route. The subjects were closely monitored till day 28 as post vaccination follow-up. The group will be monitored for the safety aspects for one year till visit 12.

PRNT support was extended to all the three sites Pune, Kolkata and Chennai. Over 400 specimens from Screening, day 28 and day 90 post vaccination were screened with PRNT for measles antibodies.

GCLP external audit

GCP and GCLP compliance is the basic requirement for participation in International Vaccine trials. Since NIV measles laboratory is a part of Measles Aerosol Vaccine trial, attempts were made to improve the compliance. Various steps taken for GCLP compliance include, Implementation of EQAS program with Health Protection Agency; establishment of Internal Quality Assurance Unit at NIV and implementing IQA SOPs including, internal panel creation and testing, SOP based, protocol based and facility based internal audit; Development of departmental manual with study plan and SOPs required for the vaccine trial; preparation of Quality Manual for PRNT; designing and implementation of Equipment Calibration Program in the department. All these programs were undertaken in the reporting year, staffs were trained internally and at two external GLP workshops. This was the major work done initiating the whole process of GCLP compliance in NIV labs.

External QMS Professionals judged GCP and GCLP compliance this year. They conducted 2 external audits of the Measles lab. After the first audit, Corrective Action Plan (CAP) was prepared for the Scientific Audit Report and preparation for compliance to GLP continued. In the final scrutiny, Audit observed a huge improvement in the lab as compared to first audit and all the critical and major issues were addressed with due care and adherence to the ICH guidelines. **NIV's Measles lab is now declared as GCLP compliant lab for vaccine trial** leading to honor of being the first **GCLP compliant ICMR laboratory**.

Molecular Surveillance of Measles in India

Collaborators : *MeaslesNetIndia*

NS Wairagkar, RS Tomar, LV Hungund, NJ Shaikh

Measles group has completed measles virus genotyping project, funded by WHO-IVR. Measles genotypes circulating in 17 states of India were sequenced. The Measles Sequence Database is created at NIV and has over 200 sequences. This database is growing day by day. *MeaslesNetIndia* (NIV-ICMR research network for Measles and SSPE) established for the project is expanded to 27 centers after addition of 10 centers from North-East region India.

Measles outbreaks in Jammu and Kashmir, Agartala, Orissa, West Bengal and Madhya Pradesh were investigated in the current year of reporting. RT-PCR and sequencing was done and genotypes of measles circulating in these states were detected (**Fig. 1**).

The project period was over in August 2007. During the total period of two years, we have investigated 32 outbreaks, detected around 172 strains with genotypes belonging to D4, D7 and D8. Genotype D7 is detected for the first time in India. The interim project report is submitted to WHO-IVR for review. The comments on the report are awaited.

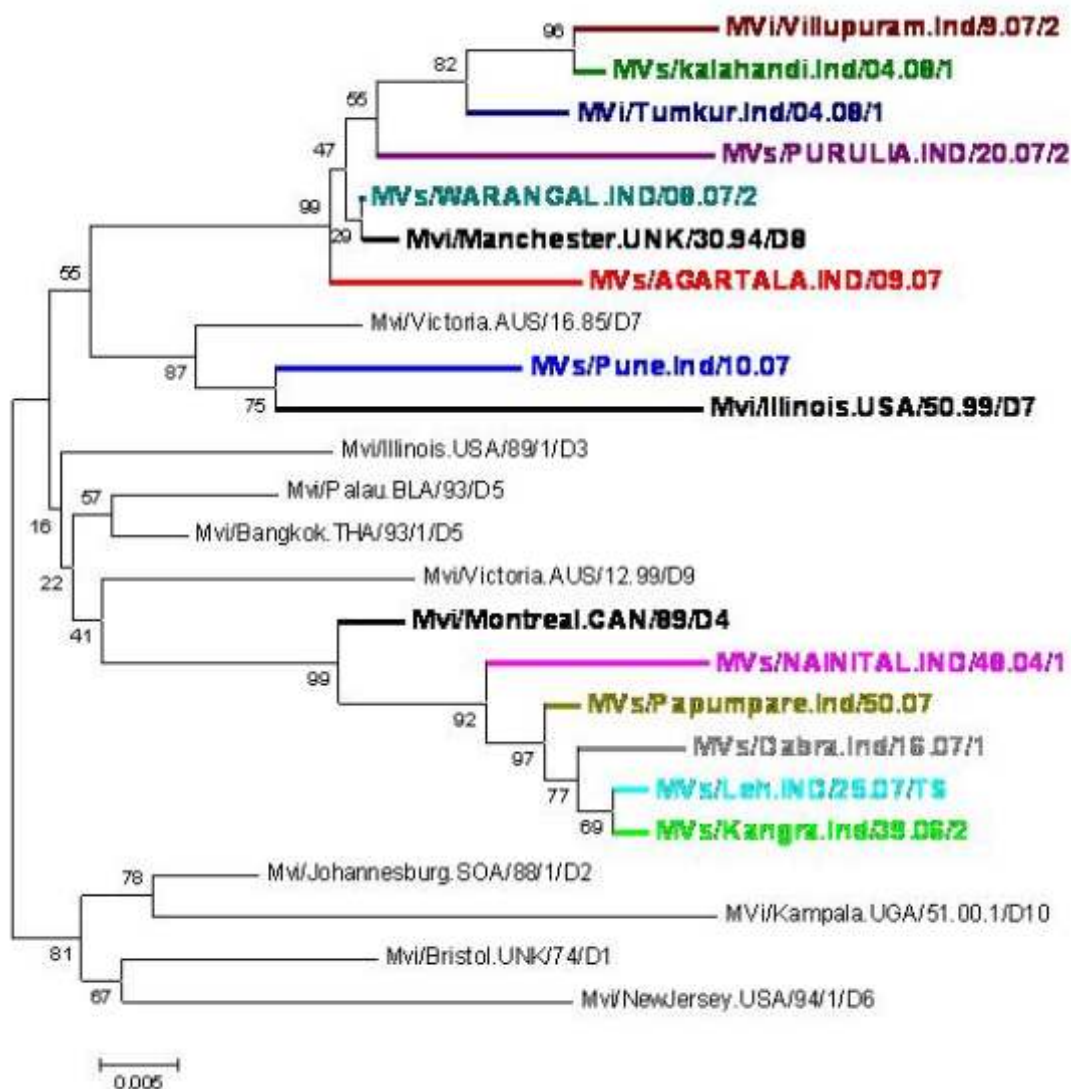


Fig. 1: Phylogenetic Analysis Of N Gene Sequences

Jammu & Kashmir State

Collaborators : Dr. SM Kadri, Directorate of Health Services, Kashmir, Dr. S Slathia, SMGS Hospital, Govt. Medical College, Jammu

Three outbreaks of measles were investigated in J & K in the district of Leh, Kargil and Jammu in 2007. Age group ranged from 6 months to 28 years. Below 12 months only female children were affected. From 13 months to 156 months more number of males were affected than females.

Leh Outbreak: A total of 355 cases of measles were detected in Leh district. No complications or casualty was found. The immunization coverage in J & K is around 85%. March second and third week was the peak of outbreak. Three serum samples were sent to NIV. All cases were from adolescent age (all 13 yrs). Serologically the outbreak was confirmed to be measles. T.S & / or urine were not available for RT-PCR.

Kargil Outbreak: Measles outbreak occurred in Kargil district (population of 1,07,138) from 1.05.07 to 25.06.07. A total of 38 cases (6mths to 28 yrs) were detected. Representative samples were collected from four cases. First case in 6 months old female of whom T.S was positive for measles by RT-PCR. Second case (1 yr old, Female) has received measles vaccine was also positive by RT-PCR. Third case (2 yrs male) was vaccinated for measles and showed symptoms only fever & rash. IgM ELISA was positive. Fourth case was 28 yrs pregnant female in her last trimester. She presented with fever, rash, cough & cold. Measles IgM antibodies were detected. She delivered a normal baby with no complications. Measles genotype D4 detected in all these cases.

Cluster from Jammu: Sporadic cases of measles were admitted in Government Medical College, Jammu from Nov. 2006 to Feb. 2007. All 5 cases were admitted with history of fever, cough, rash and lymphadenopathy. IgM ELISA was positive in 1/5 sera collected. Throat swabs of these cases were PCR positive and D4 genotype was detected from all four cases.

Tripura State

Collaborators : Dr.S Debbarma, Agartala Govt. Medical College, Tripura

Agartala Outbreak: MeaslesNetIndia collaborator from Agartala, Tripura, investigated small cluster of 6 measles like cases in the month of March and April 2007. Four cases were from Agartala and two cases were from a locality 15-18 kms from Agartala. No complications were found. Age group ranged from 8 months to 17 years. Male to female ratio was 4:2. Most of the cases (4/6) were unvaccinated. Maximum cases occurred in the age group of 3-6 years. A total of 24 samples were sent, out of which 6 were serum, 6 urine, 6-throat swab (TS) and 5 oral swab (OS) samples. Serological study showed IgM antibodies to measles in only one serum sample and also RT-PCR positive result from throat swab and oral fluid sample of the same case. Rest of the serum samples, TS and oral fluid samples were negative. This measles confirmed case was 17 years old and not vaccinated for measles. The genotype detected was D8.

West Bengal State

Collaborators : Dr. AK Biswas, Deputy Chief Medical Officer of Health, Purulia, Dr. Bishnupada Bag, Assistant Chief Medical Officer of Health, Murshidabad and Dr. Shobhan De , FETP Scholar, Purulia

Purulia Outbreak : An outbreak of measles occurred in Kantadin, Jhalda villages of Purulia WB in February 2007. Age group of case ranged from 3 and half years to 7 years. M:F ratio is 3:4. Only one case was vaccinated. 7 serum samples, 7 T.S and 7-urine samples were received. IgM ELISA was positive for 6/7 serum samples. RT-PCR is under process.

Neturia outbreak : block was investigated in May 2007, where serum, urine and TS were collected from five cases each. Age ranged from 3.5-10 yrs of age (M: F ratio 3:2). None of the cases were vaccinated. Five serum samples were tested for IgM ELISA and all five were positive. 3/5 TS were positive for RT-PCR. The genotype detected is D8 in all the 3 cases.

Murshidabad outbreak : Measles outbreak occurred in Dihigram village, Suti-II block of Murshidabad in the month of May 2007. 11 cases were reported from this village with a population of 1750 and less than 10 years population of 350. Serum and urine samples were received from 11 cases. Age group ranged from 8 months-7 yrs. All were unvaccinated. 9/11 serum samples were positive for measles IgM ELISA. All urine samples were negative by RT-PCR.

Madhya Pradesh State

Collaborators : Dr.Ketkar, Ketkar Hospital, Gwalior

A small cluster of five measles cases was investigated at Dabra in Madhya Pradesh in the month of March 2007. Age group ranged from 6 months to 6 years. More number of males (4/5) were affected than females. Only one case was vaccinated for measles. All 5 sera were positive by IgM ELISA. Three oral fluids were positive by RT-PCR and all were D4 genotype. (Annexure- phylogenetic tree from N gene sequences)

Orissa State

Collaborators : Dr. Tapas Kumar Patra, Chief District Medical Officer, Kalahandi

Cluster of 8 measles cases in Kalahandi, tribal district of Orissa was investigated with the help of public health officials. Age group of cases ranged from 4 to 8 years. M:F ratio is 5:3. History of measles vaccination was unknown in all the cases. Eight serum samples and 6-urine samples were received from eight patients. Measles IgM ELISA was positive for 7/7 serum samples. RT-PCR for measles N gene was positive for 2/6 urine samples. Both the sequences belong to measles D8 genotype. Kalahandi is tribal district in Orissa state where measles related malnutrition is a major public health problem.

Isolation and Genotyping of Measles viruses from specimens referred by WHO - SEARO, measles laboratories

NS Wairagkar, NS Kumbhar, Deepika Khedekar

In this year, **WHO SEARO recognized Measles lab of NIV, Pune as the Reference Measles Laboratory** with the assigned task of virus isolation and measles genotyping for WHO Measles Laboratory Network. As a part of this assignment, 13 isolates were referred from National Measles Laboratory, Chennai and 4 isolates were referred from National Measles Laboratory, Bangalore. All the isolates were confirmed as measles at NIV and were sequenced for N and H gene. Measles genotypes D8 (12 isolates), D4 (1 isolate) were detected in Chennai and D8 (four isolates) in Bangalore. Sequences were deposited in WHO's Global Measles Sequence database. This database will be used to track the transmission pathways of measles strains.

Molecular Epidemiology of Measles in North-East India

Collaborators : Regional Medical Research Center, Dibrugarh

NS Wairagkar, RS Tomar, LV Hungund.

In collaboration with RMRC, Dibrugarh, a new project on Molecular Epidemiology of Measles in North-east India was initiated this year with funding from the ICMR's NE Initiative. The objective of this project is to create, develop and strengthen ICMR's Measles Network in Northeastern states. The strategy would be to investigate the measles outbreaks, confirm serologically and try isolations of measles strains circulating in the region and conduct molecular studies including sequencing for understanding the molecular epidemiology of measles in NE region. The WHO-India-funded workshop was organized at Dibrugarh to train the collaborators from ten centers from NE Region on methodology for measles outbreak investigations, collection of specimens, serological studies etc. 23 collaborators attended the workshop from 10 centers initiating the MeasleNetIndia-NE network. The staff of project based at RMRC Dibrugarh was trained in outbreak investigations, collection of specimens, serological and virus isolation techniques. Attempt will be made to strengthen the virology lab at RMRC for measles diagnostics.

Measles M gene sequencing based diagnosis of subacute Sclerosing PanEncephalitis (SSPE)

Collaborators : Dr. SK Shankar, NIMHANS, Bangalore

NS Wairagkar, Deepika Khedekar

Subacute sclerosing panencephalitis (SSPE), a post-measles progressive neurological disorder is still common in India because of indifferent vaccination compliance. However, the acute fulminant form of SSPE is extremely rare. An unusual case of fulminant SSPE in an 18 year old man from south India with an ultra-short course of 19 days presenting with hemiparesis in absence of myoclonus and progressive cognitive decline, is reported. At autopsy, unlike classical SSPE, oligodendroglia containing measles viral antigen was sparse despite florid necrotizing leukoencephalitis with acute demyelination. Since histopathology alone could not finalize the diagnosis in this case, molecular methods were used.

Measles M gene sequencing is important to confirm the diagnosis of SSPE. PCR and Sequencing M gene protocols were standardized on RNA extracted from brain tissue sample. Sequence of 1008 bp was obtained from position 3438nt to 4445nt of measles genome. Bang.Ind-SSPE strain showed maximum similarity (92%) to

measles virus isolate MVs/Dundee.UNK/82/SSPE (DQ190374) (**Fig -2**).

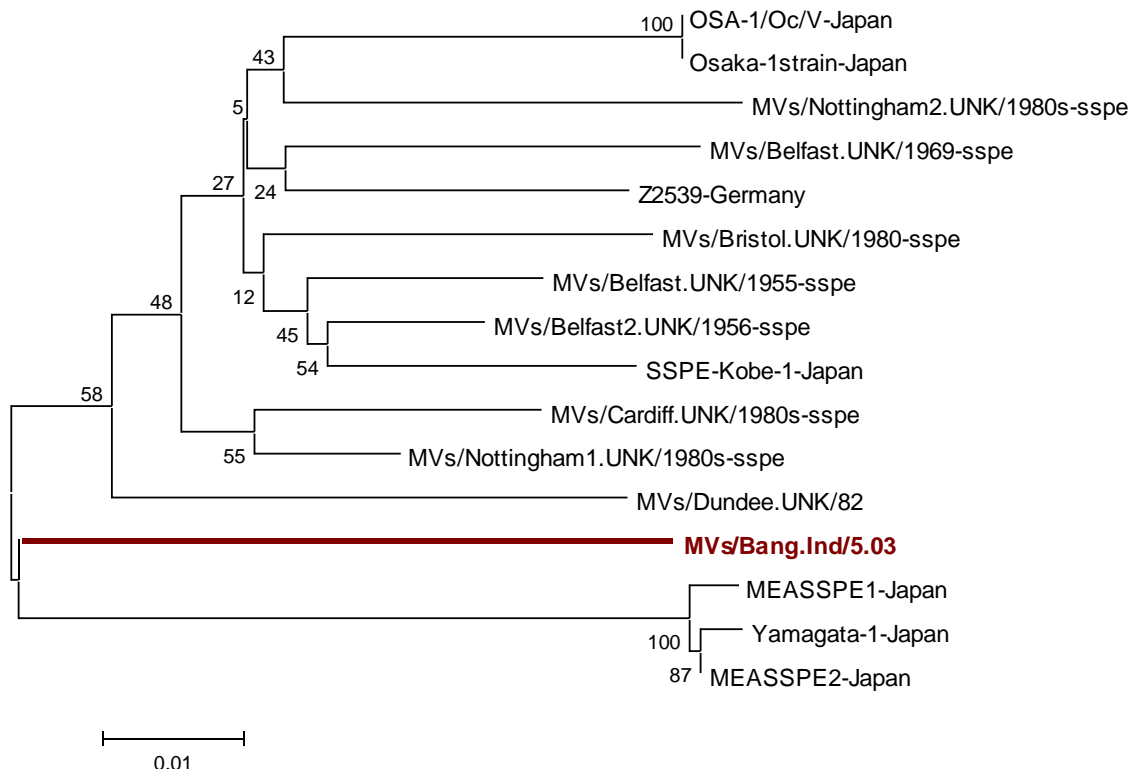


Fig. 2 : Phylogenetic analysis of Measles Matrix gene sequences

Biased Hypermutations in the matrix gene (mainly U to C) have been known as characteristic of SSPE. We compared M gene nucleotide sequence of Bang.Ind-SSPE strain to that of D7 measles virus isolate from Boston, MA 1983 strain, (Bo83/US/83 Genbank accession No. U01985), and M gene sequence of Edmonston strain (Genbank accession No.K01711). A considerable amount of mutations were found along the entire length of M gene. Compared with Boston strain there were mutations at 91 sites along the M gene-coding region, majority (80.2%) of the mutations were U-to-C transitions. Similarly compared to Edmonston strain, 80.9% were changes from U to C.

Our strain was compared with 110 M gene nucleotide sequences (SSPE and classical measles) available in GenBank and NCBI database. Five unique mutations in our SSPE strain were noted as Adenine substituting Guanine or Cytosine (at position 170/1008 M gene) and Cytosine substituting Thymine (at positions 600/1008, 614/1008, 780/1008, and 981/1008). Comparison from deduced amino acid sequences showed three unique mutations All these findings confirmed our diagnosis of SSPE and have pointed out some unique mutations in our SSPE strain, which were not reported before. Standardisation of M gene sequencing will pave way for more studies on SSPE in India.

Publications

- Vaidya SR, Wairagkar NS, Raja D, Khedekar DD, Gunasekaran P, Shankar S, Mahadevan A, Ramamurty N.. First Detection of Measles genotype D7 from India. **Virus Genes**, 2008 Feb; 36(1): 31-4.
- Narasimha Rao S, Wairagkar NS, Murali Mohan V, Khetan M, Somarathi S. Brain Stem Encephalitis Associated with Chandipura in Andhra Pradesh Outbreak. **J Trop Pediatr**. 2008 Feb; 54(1): 25-30.

- Cohen BJ, Audet S, Andrews N, Beeler J; WHO working group on measles plaque reduction neutralization test. (E. Arias Toledo, S. Audet, J. Beeler, W. Bellini, J. Bennett, M. Bentley, D.W.G. Brown, D. Doblas, D. Featherstone, A. Heath, A.M. Henao Restrepo, R. Parry, J. Ruiz Gomez, N. Shaikh, N. Wairagkar). Plaque reduction neutralization test for measles antibodies: Description of a standardised laboratory method for use in immunogenicity studies of aerosol vaccination. **Vaccine**, 2007; 26/1:59-66.
- Sapkal GN, Wairagkar NS, Ayachit VM, Bondre VP, Gore MM. Detection of measles virus in aerosol vaccine. **Am J Trop. Med Hyg.** 2007 Dec;77(6):1139-45

Workshops / Conferences / Seminar / Meetings attended

NS Wairagkar

- Residential conference of Measles Laboratory Coordinators, Udaipur, 3 - 5 March 2008.
- WHO meeting of 9th Product Development Group of Measles Aerosol Vaccine Project, Geneva, 28-31 January 2008.
- WHO meeting of 8th Product Development Group of Measles Aerosol Vaccine Project, Pune, 31st October-2nd November 2007.
- WHO fifth Global Measles and Rubella Laboratory network meeting, Geneva, 26-28 September 2007.
- WHO-Ministry of Health - India Technical Advisory Group on Measles Control, New Delhi, September 2007.
- Asia Pacific Dengue Prevention Board Meeting, Colombo, Sri Lanka, 20-24 June 2007.
- WHO-NIV workshop on MeaslesNetIndia- molecular surveillance network for measles in NE India, at RMRC, Dibrugarh, 23-24 July 2007.
- Eight Advanced Vaccinology Course, Fondation Marieux, Annecy, France, 14-28 May 2007.
- WHO Meeting on Measles Aerosol Project Review, Mumbai, 10th May 2007.

LV Hungund

- WHO-NIV workshop on MeaslesNetIndia- molecular surveillance network for measles in NE India, at RMRC, Dibrugarh, 23-24 July 2007.
- IBSC workshop on Biomedical Waste Management, Pune 29 Oct, 2007.

NJ Shaikh

- UNDP/WHO/World Bank workshop on Good Laboratory Practice. Bangalore, 29-31st Oct 2007.
- WHO Meeting on Measles Aerosol Project review, Mumbai, 10 May 2007.

VS Bhide

- UNDP/WHO/World Bank workshop on Good Laboratory Practice, Bangalore 29-31 Oct, 2007.

RS Tomar

- IBSC workshop on Biomedical Waste Management, Pune, 29 Oct, 2007,.

Trainings/Workshops/Seminars organized

WHO-NIV workshop on MeaslesNetIndia-molecular surveillance network for measles in NE India, at RMRC, Dibrugarh, 23-24th July 2007.