FROM DIRECTOR'S DESK

The year has been quite hectic for the National Institute of Virology (NIV), Pune due to the threat of Ebola as 'the sword of Damocles'. Entire NIV was engaged in countering the Ebola scare for preparedness in virus diagnosis, logistic support, conducting workshops, trainings and lectures to create awareness and emergency handling among healthcare workers in India and abroad. NIV provided timely diagnosis to 37 Ebola suspected samples referred from different parts of India and WHO-SEARO countries during July 2014-March 2015 using ***Filoviridae*** specific RT-PCR, Ebola and Marburg specific RT-PCR and Ebola Zaire specific real time RT-PCR. Crimean Congo Hemorrhagic fever (CCHF) and Kyasanur Forest Disease (KFD) dominated among the other important issues for NIV as both the viruses were active during the year and showed geographic expansion. CCHF cases in Jodhpur, Rajasthan and KFD cases in the tribal areas of Malappuram district, Kerala with case fatalities have been major areas of activity and NIV has been successful in providing timely diagnosis and case management. Case management of acute encephalitic syndrome (AES) in Gorakhpur and Muzaffarpur had remained as challenging as before. Attempts to diagnose the putative Gorakhpur agent from the non JEV component of AES cases using the Next Generation Sequencing (SOLiD technology) resulted in the detection of human Parvovirus 4. Studies are needed to confirm its role in AES.

As part of the routine diagnostic services to the nation, NIV has been active in providing diagnosis of dengue and influenza in epidemic proportions. Pune reported one of the largest outbreaks of dengue with the circulation of multiple serotypes. NIV has been instrumental in the management of dengue in collaboration with NVBDCP and Pune Municipal Corporation. The country also witnessed a high number of influenza associated deaths during the year and NIV coordinated the management through multicentric activity. Circulation of A(H1N1)pdm09 was found to be the dominant type during the year. Though there was a scare of introduction of MERS CoV in India, it was ruled out by NIV using appropriate tests.

One of the major contributions of NIV during the year was the active role it has taken in Translational Research. An independent cell has been created at MCC, Pashan campus with the induction of senior scientists under the active participation of the Director. NIV has produced two products (anti-Chandipura virus IgM antibody detection assay and a monoclonal antibody based antigen capture ELISA for detection of Japanese encephalitis virus from mosquitoes) which are in the process of translating to private companies. NIV has also filed two patents both nationally and internationally.

FROM DIRECTOR'S DESK

Despite acute financial crunch, NIV has contributed tremendously in the human resource development and academics. The Diagnostic Virology Group, a facility established at NIV to train staff of the newly constituted virology network of DHR, has imparted hands on training to 80 staff members and academicians in the areas of cell culture, serology, molecular diagnosis, biosafety practices, etc. In academics, 05 Ph Ds was awarded by the Savithribhai Phule Pune University in different disciplines to scholars who worked under the guidance of NIV scientists. Excellent results were produced by the M. Sc. Virology course conducted by NIV with majority of students getting placements in reputed institutions for higher education both in India and abroad.

The major achievement of NIV during the year was the highest number of research publications (n=83) in peer reviewed national and international journals with an average impact factor of 3. It is a matter of great pride to me and the institute as three of our scientists received prestigious awards from Indian Council of Medical Research and Pune Municipal Corporation for their outstanding contribution to science.

As contributions to the Nation, NIV has given timely diagnostic support to more than 20 viruses of public health importance during outbreaks by testing several thousand samples. NIV was also active in investigations for several viral diseases in addition to its involvement during outbreaks to diagnose the etiologic agents. NIV is the major supplier of diagnostic kits for diagnosis of dengue, chikungunya and Japanese encephalitis in India to NVBDCP, research institutions and health care systems (Hospitals, PHCs, etc). During the calendar year, approx. 5000 kits were supplied in India and to WHO for SEAR countries. NIV has also developed diagnostic kits for West Nile virus, CCHF virus, KFD virus and hepatitis viruses for both serological and molecular diagnosis which are in the process of standardization.

Due importance is given for basic research in multidisciplinary areas of virology. Significant progress has been made in understanding the pathogenesis and replication of different viruses, their genetic susceptibility, etc. NIV has initiated a proactive research for hunting novel viruses harbored by bats and during the current year 72 bats belonging to Pteropus species from West Bengal and Assam were screened for viruses. Nipah virus RNA and Nipah virus antibodies were detected in three bats, suggestive of active circulation of the virus in the area. A new paramyxovirus, the Tioman virus has been isolated from bats for the first time in India. The data warrants an urgent need for active surveillance in the country for these highly pathogenic viruses. NIV has a bioinformatics and data management group with highly talented scientists who have expertise in analysis of epidemiological and serological data, bioinformatic sequence and structure analysis, mathematical modeling, etc. The scientists are responsible for comparison of genomic sequences and advanced phylogenetic and molecular clock analysis to study the epidemiology of arboviruses.

FROM DIRECTOR'S DESK

NIV collaborates with other scientific institutions both in India and abroad to conduct advanced studies leading to break-through research. The major collaborators include DBT, DST and CDC (Atlanta, USA). WHO has already recognized NIV as the collaborating center for arboviruses and reference center for hemorrhagic fever viruses. Additionally, WHO has recognized NIV as a reference center for other viruses, ***viz.,*** measles and avian influenza.

NIV also provides core facilities ***i.e.,*** electron microscopy, animal house, micro-array and library to scientists and students of national and international institutions to conduct research. Units of NIV at Gorakhpur, Bangalore and Kerala have made significant progress in terms of laboratory development and providing diagnostic support to the respective State governments. The Bangalore unit has become totally functional at its new location.

It is indeed my pleasure to thank the scientists of NIV for the achievements by their dedication and hard work. I also thank Dr R Lakshminarayanan, Senior Administrative officer and his staff for logistic support in procuring equipments and chemicals/reagents in time. The continued support of Indian Council of Medical Research and Department of Health Research is also greatly appreciated. I extend my sincere gratitude to Dr VM Katoch, DG, ICMR and secretary DHR for his continued support and encouragement extended to us during his tenure.

Director

INSTITUTIONAL COMMITTEES

SCIENTIFIC ADVISORY COMMITTEE

Members	Role
Dr. S.P. Thyagarajan Professor of Eminence & Dean (Research), Sri Ramachandra University,No.1 Ramachandra Nagar, Porur, Chennai - 600116.	Chairman
Dr. A.P. Dash Former Consultant, World Health Organization, Regional Office for South East Asia (SEARO), Indraprasth Estate, Mahatma Gandhi Marg, New Delhi-110002 Regional Adviser, Vector borne and Neglected Tropical Diseases Control, WHO.	Expert, Vector Biology
Dr. D.C.S. Reddy, WHO Consultant 204, Radium Apartment, Dr.Baijnath Road, Near Post Office, New Hyderabad Road, Lucknow-226007.	Expert, Epidemiology
Dr. Randeep Guleria Professor, Department of Medicine, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110 029	Expert, Influenza
Dr. Sridharan Gopalan, Former Professor, CMC Vellore. No.114, Govinda Nivas, Indira Nagar, Perumugai, Vellore-632009.	Expert, Virology
Dr. Sarala Subbarao Former Director, National Institute of Malaria Research Block 13, Apt. 704, East End Apartments, Mayur Vihar-Phase I Extension, New Delhi-110096.	Expert, Vector Biology
Dr. Shubhada Chiplunkar Head, Department of Immunology, Tata Memorial Centre, Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Sector 22, Kharghar, Navi Mumbai-410208.	Expert, Immunology and Diagnostic Vaccine
Dr. Shekhar Mande Director, National Centre for Cell Science, NCCS Complex,SP Pune University Campus, Ganeshkhind, Pune- 411007.	Expert, Molecular Biology
Dr. D.A.Gadkari, ICMR Consultant & Former Director, NIV Shilpayatan Apartments, 2/13 Erandwane, Pune-411004.	Expert, Virology

INSTITUTIONAL COMMITTEES

Dr. Soumitra Das Department of Microbiology and Cell Biology, Room No. 252, Indian Institute of Science, Bangalore-560012.	Expert, Hepatitis
Dr. M.D.Gupte, ICMR Chair (Epidemiology) K-502, West End Village, Bhusari Colony, Paud Road, Kothrud, Pune-411038.	Expert, Epidemiology
Dr. (Mrs). Nilima Kshirsagar, ICMR Chair (Pharmacology), Dean, Prof and Head, Department of Clinical Pharmacology, New MS Building, 1st Floor, Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.	Expert, Clinical studies
Dr. Sharma Vinod Prakash, NASI-ICMR Chair, Distinguished Professor (Public Health Research), Centre of Rural Development & Technology, Indian Institute of Technology, Hauz Khas, New Delhi-110016	Expert, Public Health
Dr. K. Kolandaswamy, Director, Public Health, Directorate of Public Health and Preventive Medicine, Govt. of Tamil Nadu, Chennai-600006.	Expert, Public Health
Dr. A. C. Dhariwal, Director, National Vector Borne Disease Control Program, Directorate General of Health Services, Ministry of Health and Family Welfare, 22, Sham Nath Marg, New Delhi-110054.	Expert, Vector Borne Diseases
Special Invitee	
Dr. S.Mehendale Director, National Institute of Epidemiology, R-127, 3rd Avenue, Tamil Nadu Housing Board, Ayapakkam, Chennai-600077.	Epidemiology
Dr. Shekhar Chakrabarti Former Director, National Institute of Cholera and Enteric Diseases (NICED), P-33, CIT Road, Scheme XM, Beliaghata, P.O.Box 177, Kolkata-700010	Molecular Biology
Dr. Jagdish Deshpande Former Director, Enterovirus Research Centre, Haffkine Institute Compound, Acharya Donde Marg, Parel, Mumbai-400012	Virology
Dr. J. Mahanta Former Director, Regional Medical Research Centre, North East Region, Post Box No.105, Dibrugarh-786001	Epidemiology

INSTITUTIONAL COMMITTEES

INSTITUTIONAL HUMAN ETHICS COMMITTEE (IHEC)

Name & Designation	Role
Dr. Dileep Kadam Professor & Head, Department of Medicine, B J Medical College, Pune-411001	Chairperson
Dr. Devendra T. Mourya Director, National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune-411001	Member Secretary
Dr. Babasaheb V. Tandale Scientist 'E', National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune-411001	Member Coordinator & Basic Medical Scientist
Dr. Sheila Bhave Consultant in Pediatric Research, Department of Pediatrics, KEM Hospital Research Centre, Pune-411011	Clinician
Dr. Arun Risbud Scientist 'F', National AIDS Research Institute, 73, 'G' Block, MIDC, Bhosari, Pune-411026	Basic Medical Scientist
Dr. Vikram Padbidri, Consultant, (Microbiology & Infection Control), Department of Microbiology & Infection Control, Jehangir Hospital, 32 Sassoon Road, Pune-411001	Basic Medical Scientist
Dr. (Mrs.) Seema Sahay Scientist 'E', National AIDS Research Institute 73, 'G' Block, MIDC, Bhosari, Pune-411026	Social Scientist
Adv. Milind Heblikar, Advocate, Office No. E-202, Business Court S.No.707, Mukundnagar, Pune-411037	Advocate
Mr. Shrikrishna Deshmukh, A/P Makhai, Tal-Shirur, Dist. Pune-412208	Person from Community

INSTITUTIONAL COMMITTEES

INSTITUTIONAL ANIMALS ETHICS COMMITTEE (IAEC)

Name & Designation	Role
Dr. Geeta Vanage Scientist F, National Institute for Research in Reproductive Health (NIRRH), J.M. Street, Parel, Mumbai-400012	Main Nominee
Dr. Vijay Subhashrao Jagdale "Darshan Nagari" Building, B-5, Flat No.7, Keshav Nagar, Chinchwad, Pune-411033	Link Nominee
Dr. Pandit Virbhadra Nandedkar C/o. Adv. Devanand Nandedkar, Plot No.29, Flat No.6, Malti Complex, Roshan Housing Society, Vishar Nagar, Aurangabad	Scientist from outside the Institute
Shri Nikunj Sharma C-38, Brij Vihar, Single Storey, PO Chander Nagar, Near Bal Bharti School, Ghaziabad-201011, Uttar Pradesh	Socially Aware Nominee
Dr. Jayati Mullick Scientist E & Group Leader, Avian Influenza Group, National Institute of Virology, Microbial Containment Complex, 130/1, Sus Road, Pashan, Pune-411021	Chairperson & Biological Scientist
Dr. Paresh Sumatilal Shah Scientist D, Dengue GroupNational Institute of Virology, 20-A, Dr Ambedkar Road, Post Box 11, Pune-411001	Scientist from different biological discipline
Dr. Kalichamy Alagarasu, Scientist C, Dengue Group National Institute of Virology, 20-A, Dr Ambedkar Road, Post Box 11, Pune-411001	Scientist from different biological discipline
Dr. Manohar Lal Choudhary, Scientist C, Influenza Group, National Institute of Virology, 20-A, Dr Ambedkar Road, Post Box 11, Pune-411001	Scientist from different biological discipline
Dr. Daya Vishal Pavitrakar Technical Assistant, Encephalitis Group, National Institute of Virology, Microbial Containment Complex, 130/1, Sus Road, Pashan, Pune - 411 021	Veterinarian
Dr. Dilip Rewa Patil Scientist C & Group Leader, Animal House Group, National Institute of Virology, 20-A, Dr Ambedkar Road, Post Box 11, Pune-411001	Scientist In charge of Animal House facility

INSTITUTIONAL COMMITTEES

INSTITUTIONAL BIOSAFETY COMMITTEE (IBSC)

Name & Designation	Role
Dr. D. T. Mourya, Director, National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune-411001	Chairman
Dr. Kavita Lole, Scientist E, Group Leader, Hepatitis Division, National Institute of Virology,130/1 Sus Road, Pashan, Pune-411021.	Member Secretary
Dr. Arvind Sahu, Scientist G, National Center for Cell Science, SP Pune University Campus,Ganeshkhind, Pune-411007.	[DBT Nominee]
Dr. V. Ghole, Ex-Professor and Head, Department of Environmental Sciences, SP Pune University, Ganeshkhind, Pune-411007	[External Expert]
Dr. C. G. Raut, Scientist E, In-Charge, National Institute of Virology Bengaluru Unit, Rajiv Gandhi Institute of Chest Disease Premises, Near NIMHANS, Someshwarnagara 1st Main, Dharma Ram College, Bangalore - 560 029	Veterinary Faculty expert, Member
Dr. Yogesh Gurav, Scientist D, Epidemiology Group,National Institute of Virology, 130/1 Sus Road, Pashan, Pune-411021.	Medical Faculty expert, Member
Dr. Tejaswini Deshmukh, Scientist B, Hepatitis Group,National Institute of Virology, 130/1 Sus Road, Pashan, Pune-411021	Molecular Biology expert, Member

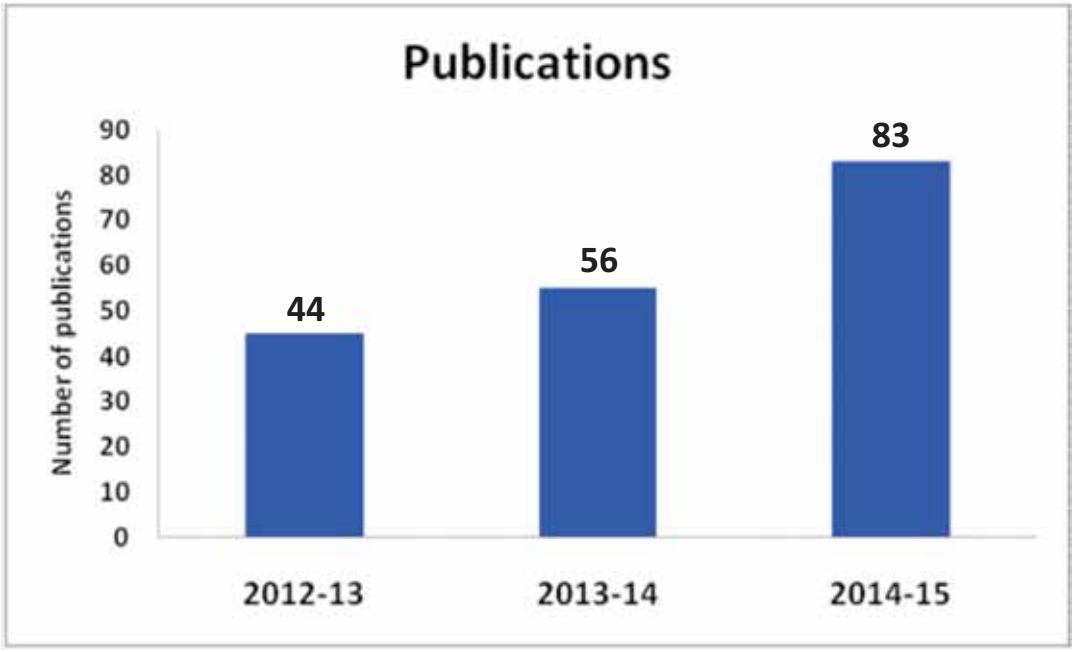
PERFORMANCE INDICATOR

- SCIENTIFIC PUBLICATIONS
- SERVICE TO THE NATION
- HUMAN RESOURCE INDICATORS
- ACADEMICS
- FINANCIAL INDICATORS

PERFORMANCE INDICATORS

SCIENTIFIC PUBLICATIONS (1st April, 2014 – 31st March, 2015)

Publications over the years:



Summary of scientific publications 2014-15:

Total	:	83
Peer reviewed journals	:	80
Book chapters	:	2
Technical article	:	1
Average Impact Factor	:	2.97 (of 75 publications)

PERFORMANCE INDICATORS

SERVICE TO THE NATION

A. PUBLIC HEALTH CONTRIBUTIONS

Highlights:

1.	Total number of outbreaks investigated in 2014-15	:	5
2.	Total number of samples tested	:	39,886
3.	Total number of Diagnostic kits supplied to various hospitals/centres across India	:	4,767
4.	Products/Technology developed	:	02
5.	Response to National calamity	:	01

PERFORMANCE INDICATORS

Details of Outbreaks investigated

Date	Area of outbreak	Etiological agent
10/4/2014	Satara, Maharashtra	Hepatitis E
18/5/2014	Mallappuram, Kerala	Kyasanur Forest Disease
14/6/2014	Muzzaffarpur, Bihar	Acute Encephalitis Syndrome
23/7/2014	Siliguri, West Bengal	Encephalitis
10/12/2014	Wai, Satara, Maharashtra	Hepatitis E

Products/Technology developed

Multiplex real time RT-PCR for simultaneous detection of DENV/CHIKV

PCT application titled "RNAi agent for inhibition of chikungunya virus" has been filed on 2nd July, 2014.PCT application no. is PCT/IN2014/000441.

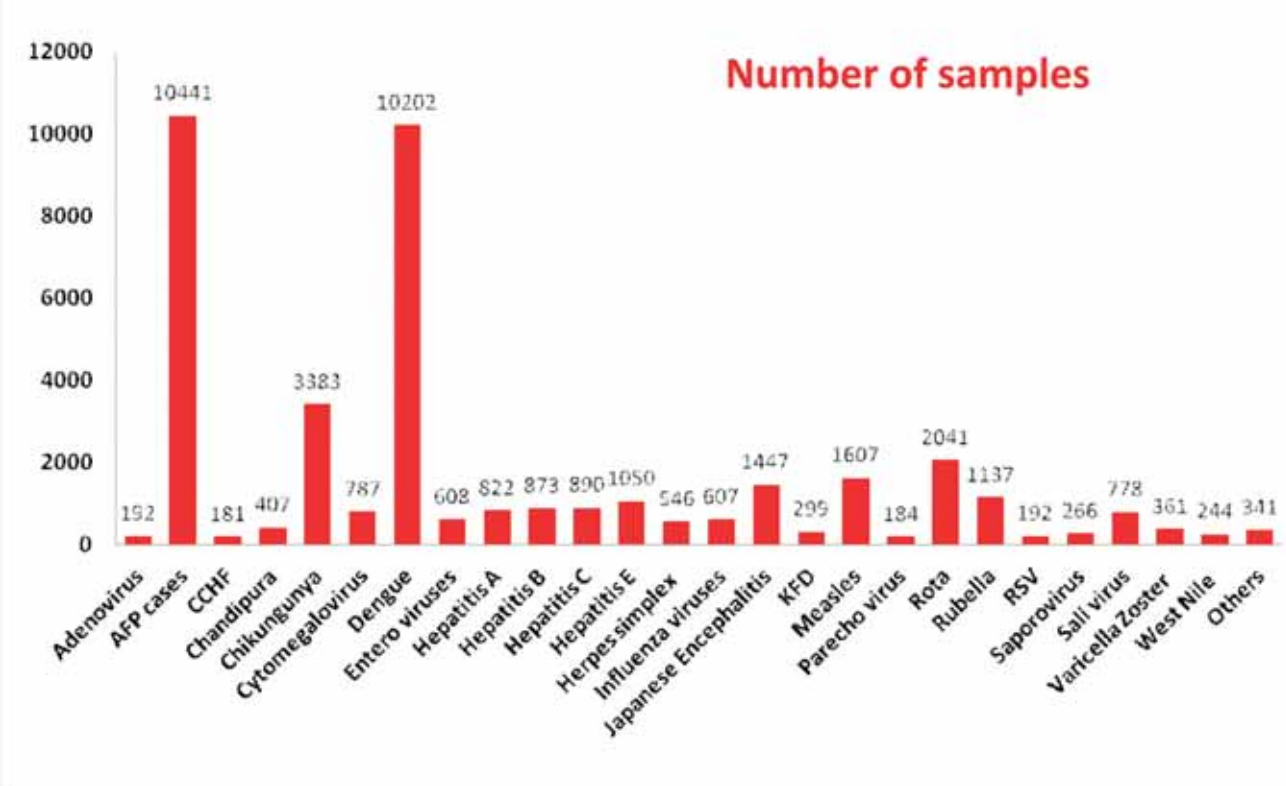
Response to National calamity:

Deployment of Public Health Team to flood affected areas in Jammu and Kashmir, September-October 2014.

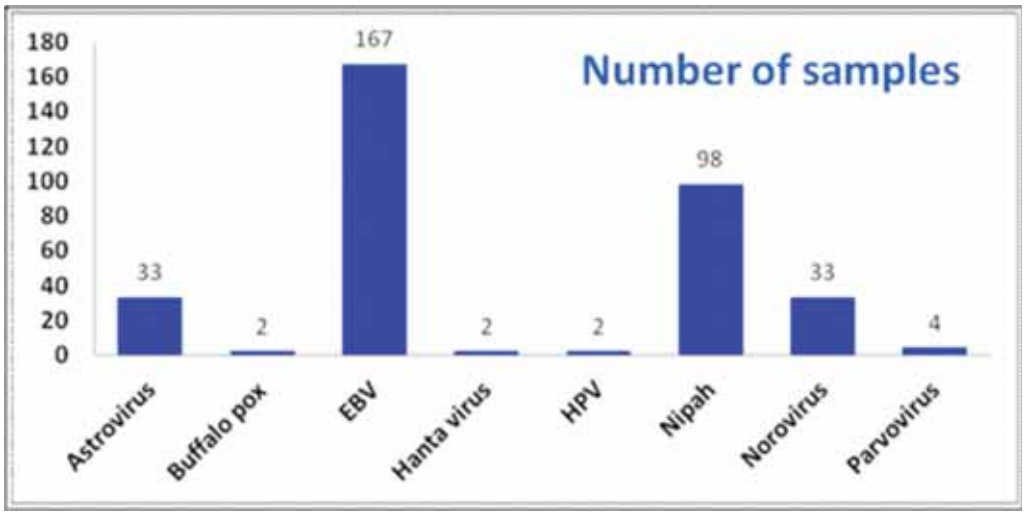
In the backdrop of the flood in Jammu & Kashmir in September 2014, Public Health teams were deployed by the Emergency Medical Relief, Ministry of Health and Family Welfare, Government of India to the flood affected districts of Kashmir region. A team comprising of Dr. YK Gurav, Dr. M Gokhale and Dr V Gopalkrishna from the National Institute of Virology, Pune worked cohesively with the authorities of health services in Kashmir for the surveillance of infectious diseases, entomological surveillance, implementation of public health measures and outbreak investigation of viral diseases and response.

PERFORMANCE INDICATORS

B. REFERRED AND OUTBREAK SAMPLES TESTED

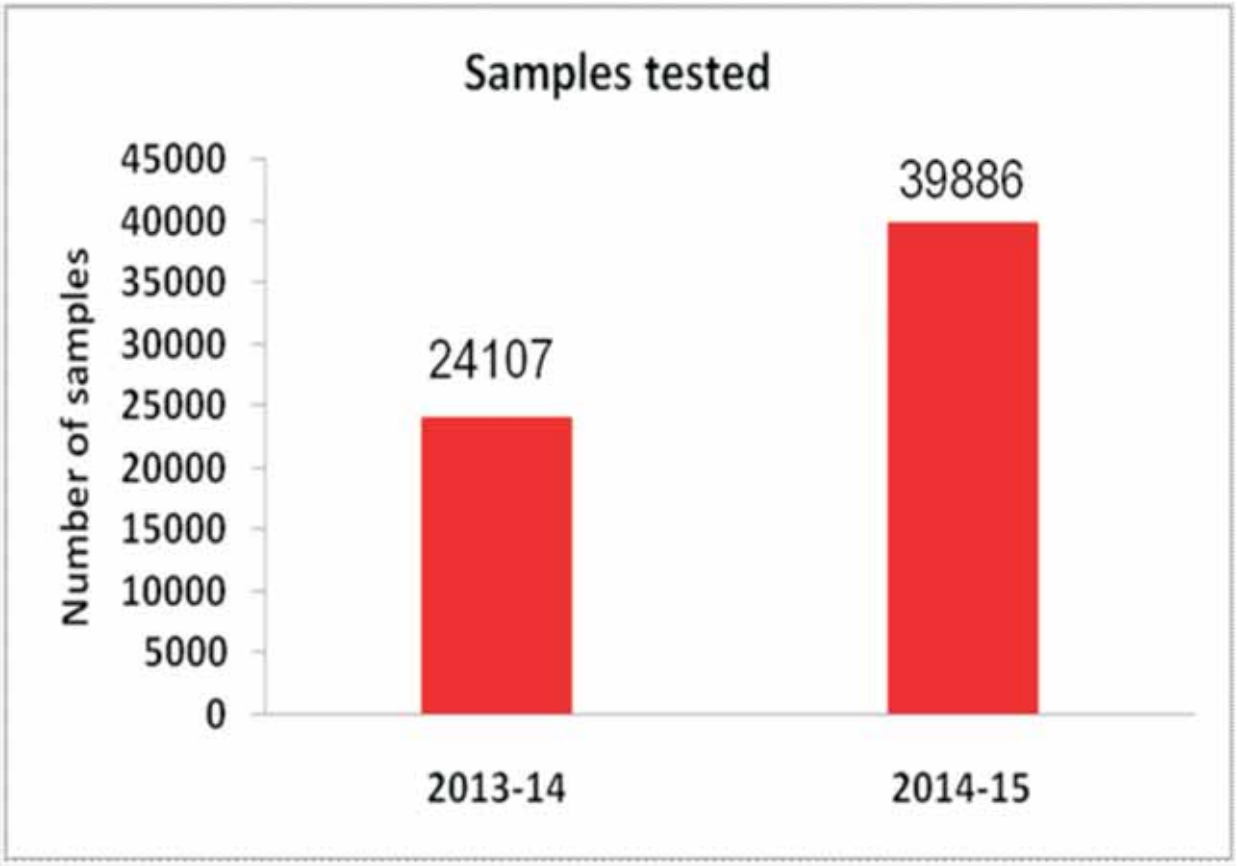


Others include



PERFORMANCE INDICATORS

Sample testing over the years



PERFORMANCE INDICATORS

ACADEMICS

Academic Programs

NIV offers PhD in various disciplines including Biotechnology, Microbiology, Biochemistry, Health Sciences and Basic Medical Sciences (interdisciplinary biomedical research in Virology involving Bioinformatics/Biophysics/Computational techniques) under the affiliation of Savitribai Phule Pune University (University of Pune).

Since 2005, NIV has been conducting Two year full-time M.Sc Virology course in collaboration with the Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University. The Institute provides in-house facilities for teaching, training laboratories and hostel facilities for M.Sc students at the Microbial Containment Complex, Pashan Campus.

PhD

Number of recognized guides	16
No. of students pursuing PhD	29
No. of scholars awarded PhD degree in 2014-15	05

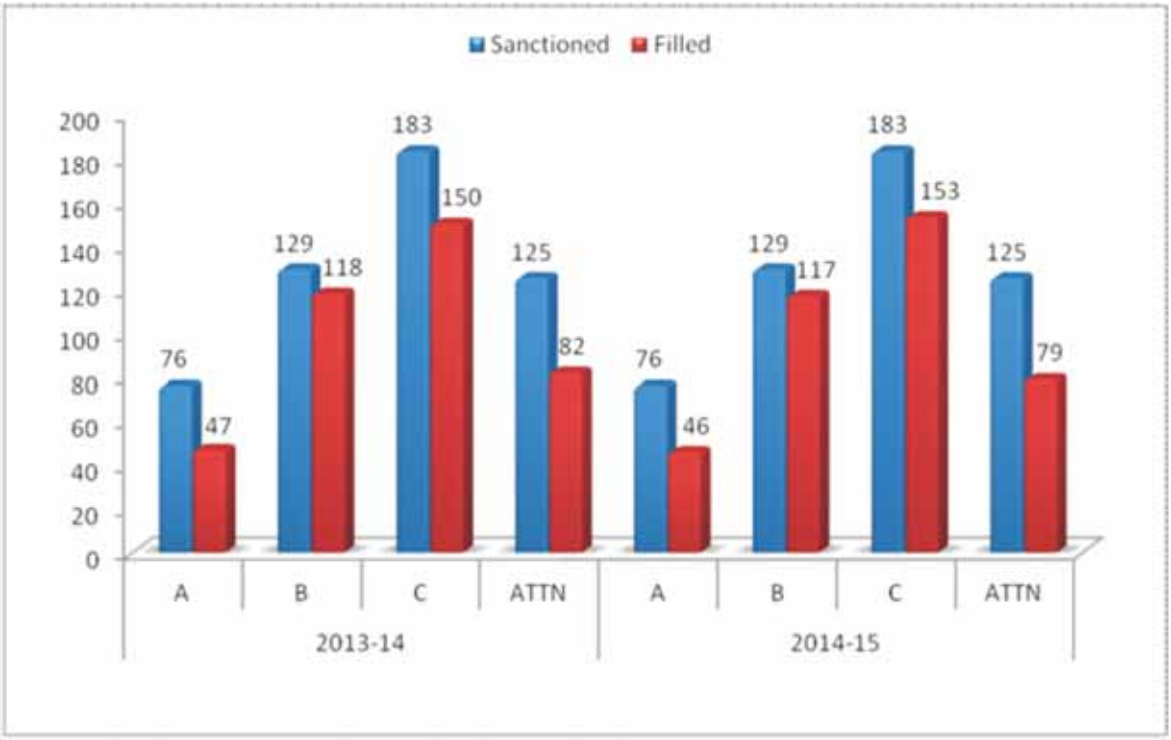
M.Sc Virology

Total no of seats	25
Present strength:	
First Year	21
Second Year	22

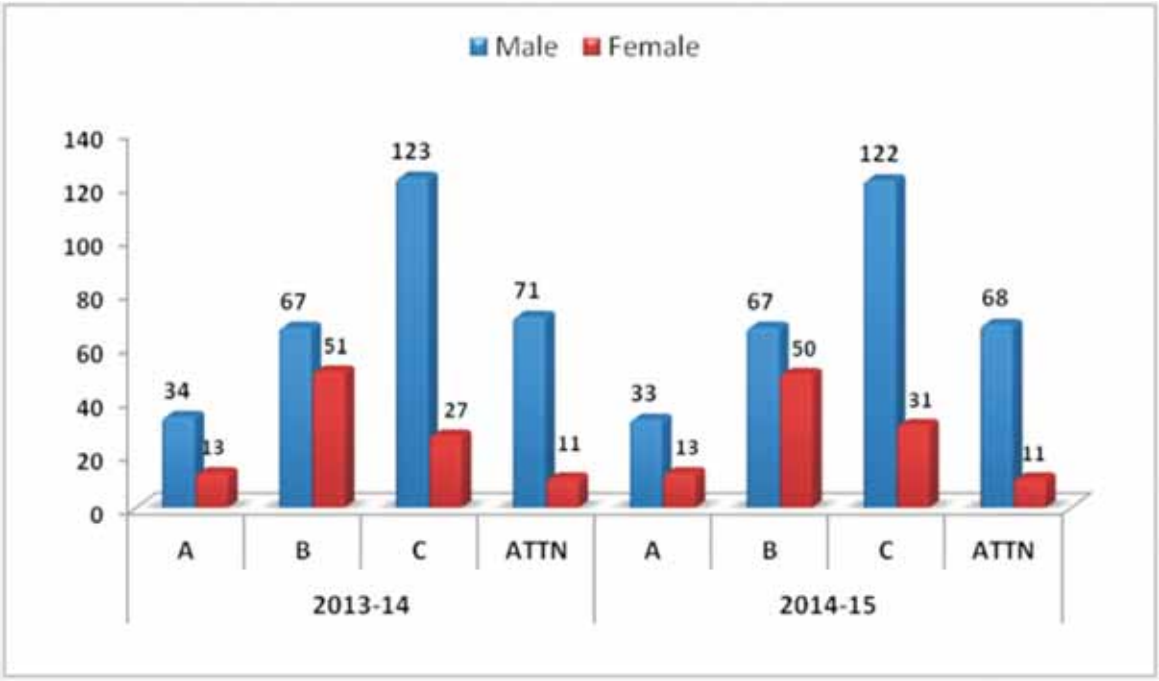
PERFORMANCE INDICATORS

HUMAN RESOURCE INDICATORS

Group wise Staff Strength



Group wise gender distribution



PERFORMANCE INDICATORS

C. WORKSHOPS, TRAININGS & CONFERENCES ORGANIZED BY NIV.

NIV, Pune provided training for human resource development in SEAR countries for improving laboratory bio-safety and bio-security, pathogen detection and diagnosis through WHO-SEARO

Date	Workshop organized for preparedness on emerging infections
26th-30th May, 2014	WHO Regional Laboratory Workshop on 'Influenza and novel viruses' for participants from 9 South East Asian Countries
19th-20th August, 2014	WHO-NIV Regional Laboratory Workshop on 'Biosafety in Laboratories' for participants from SEAR countries.
10th November, 2014	WHO-SEARO-sponsored Workshop on International Air Transport Association (IATA) Awareness, Cold Chain Management (CCM) and Dangerous goods declaration.
10th-14th November 2014	Workshop on the "Preparedness for laboratory diagnosis of Ebola virus"
26th November, 2014	WHO-SEARO-sponsored Workshop on International Air Transport Association (IATA) Awareness, Cold Chain Management (CCM) and Dangerous goods declaration.
26th-30th November, 2014	Workshop on the "Preparedness for laboratory diagnosis of Ebola virus."
25th-28th March, 2015	Workshop on 'Biosafety and preparedness to handle infectious materials in laboratory setting,' for participants from Medical Research Institute, Sri Lanka.

NIV Pune provided diagnostic reagents for surveillance, detection, diagnosis of infectious diseases like MERS CoV, H7N9, CCHF and KFD to SEAR countries through 'WHO-SEARO'.

Diagnostic molecular diagnosis reagents provided to WHO-SEARO for SEAR countries

Period	MARS CoV	Other RI viruses	Influenza A & B	Ebola
Jan 2014-June 2015	11	4	11	2

PERFORMANCE INDICATORS

NIV, Pune conducted workshops and trainings to enhance awareness on biosafety and biosecurity and public health diseases within the country.

Date	Workshop organized for preparedness on infectious diseases
26th-30th May, 2014	Training on "Culture Based Viral Diagnosis".
23rd-27th June, 2014	Viral diagnostic laboratory (VDL) workshop: Faculty to training workshops conducted by Viral diagnostic laboratory (VDL)Module 3: Viral Diagnostic PCR
23rd-29th June, 2014	Training on 'Influenza and RSV virus detection by Multiplex RT-PCR' for representative participants from 12 Regional VDL Laboratories.
15th July, 2014	Onsite training on 'Detection of 18 respiratory viruses by Duplex Real Time PCR' for Bengaluru field unit staff.
16th July, 2014	One day Training program on Basic Biosafety 'Biosafety Measures in BSL-2 laboratories' for staff of the Enteric Virus group, NIV.
18th-19th September, 2014	Workshop for 'Awareness of Engineering Staff on the Crucial Aspects in Maintenance of BSL-2 and BSL-3 Laboratories'.
10th November-9th December, 2014	Training on various aspects of virology like lab set up, biosafety, biosecurity, research, diagnostic, public health and related techniques for MD (Microbiology) students from AIMS, BG Nagara, Karnataka.
17th-20th November, 2014	"XVth Three days training for 'Working in High Containment laboratory-Training program for BSL3 practices and work" for NIV staff and students.
5th December, 2014	2nd Joint (ICMR-ICAR) meeting on Avian Influenza for 'Nationwide avian influenza surveillance plan.'
16th December, 2014	Training on 'Detection of Influenza Viruses by using Real Time RT-PCR' for participants from VDL Regional Laboratories.
5th January, 2015	Workshop on 'Laboratory Technicians Roles in Field Outbreak Investigations.'
21st-22nd January, 2015	RCVDL Training program on "Training in Biosafety & Biosecurity."
10th-16th February, 2015.	Training on 'Detection of Influenza Viruses by using Real Time RT-PCR' for participants from Gorakhpur Unit.
23rd-26th February, 2015.	RCVDL training program on 'Sequence Analysis and Interpretation.'
1st-31st March, 2015.	Training on various aspects of virology like lab set up, biosafety, biosecurity, research, diagnostic, public health and related techniques for MD (Microbiology) students from Bangalore Medical College & Research Institute, Bengaluru
2nd-5th March, 2015	Training on 'Detection of Influenza Viruses by using Real Time RT-PCR' was provided to 4 participants
12th March, 2015	Workshop on "Preparedness of H1N1 influenza outbreak"

PERFORMANCE INDICATORS

NIV, Pune conducted workshops/training to create/enhance awareness on biosafety and biosecurity and diagnosis within the country for emerging highly infectious agents

Date	Conference / Workshop/ Seminar / Meeting
7th-11th July, 2014	Training on 'Molecular and Serological Diagnosis for KFD virus.'
10th July, 2014	Training on 'Use of anti-CCHF Bovine IgG ELISA and anti-CCHF Sheep/Goat IgG ELISA kit.'
26th August, 2014	NIV-AFMC Workshop on 'Ebola Preparedness.'
1st 5th December, 2014	Training on 'ELISA and PCR for KFD diagnosis.'
2nd February, 2015.	Organised training workshop on "Hospital Preparedness and handling Ebola Virus Disease" jointly by RGICD, & NIMHANS and delivered a lecture on "Laboratory diagnosis of EVD"

NIV, Pune provided diagnostic ELISA kits to WHO-SEARO for surveillance and detection, diagnosis and mitigation of infectious viral diseases in other SEAR countries.

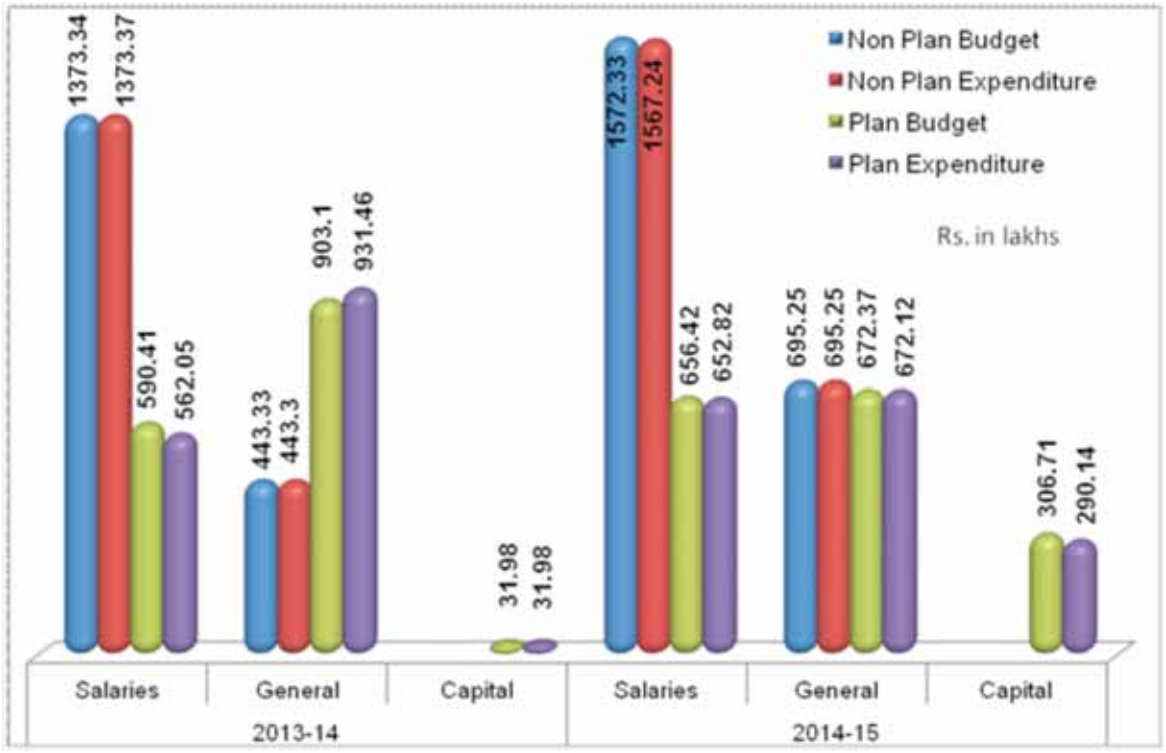
Diagnostic ELISA kits provided for SEAR countries

Period	JE	DEN	CHIK	CCHF	KFD	Ebola
Jan 2014 - Jun 2015	88	25	20	4	4	2

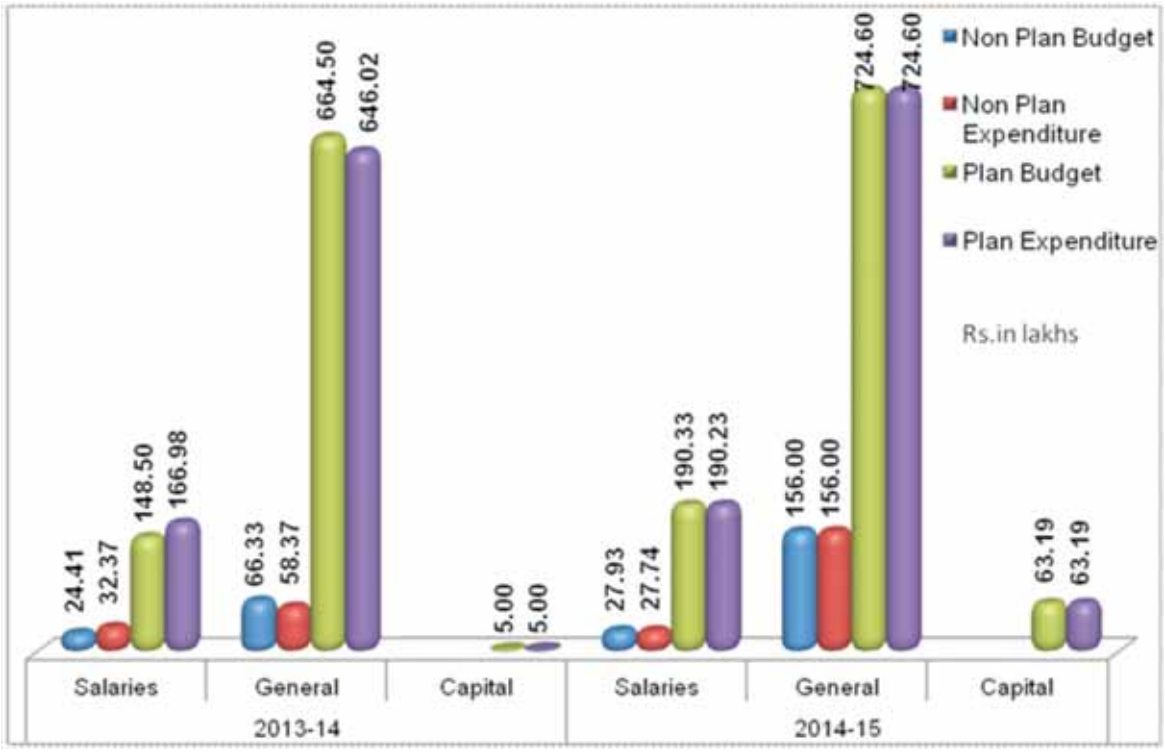
PERFORMANCE INDICATORS

FINANCIAL INDICATORS

Budget NIV, Pune

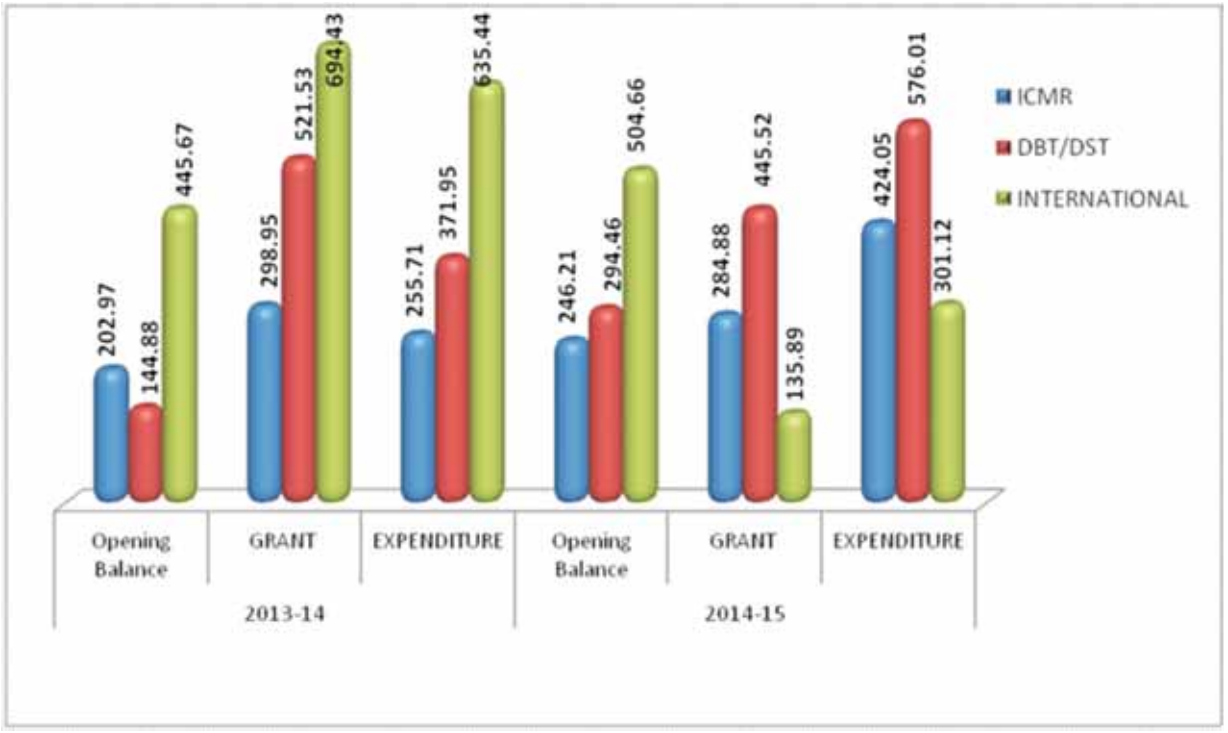


Budget MCC, Pune

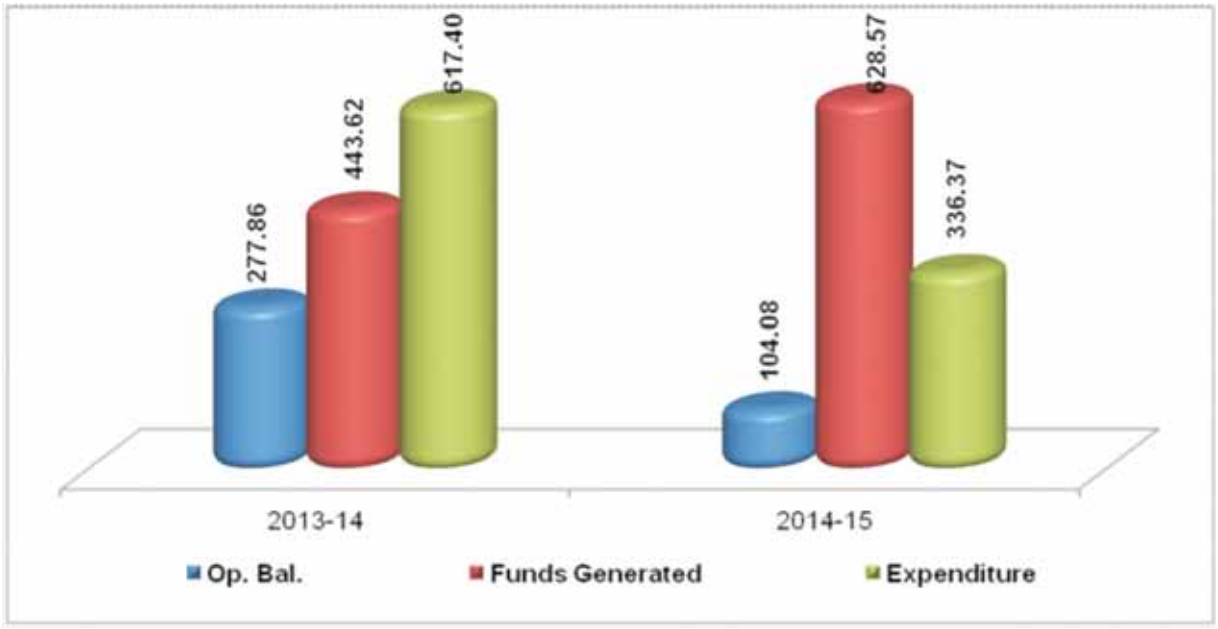


PERFORMANCE INDICATORS

Budget Extramural Projects

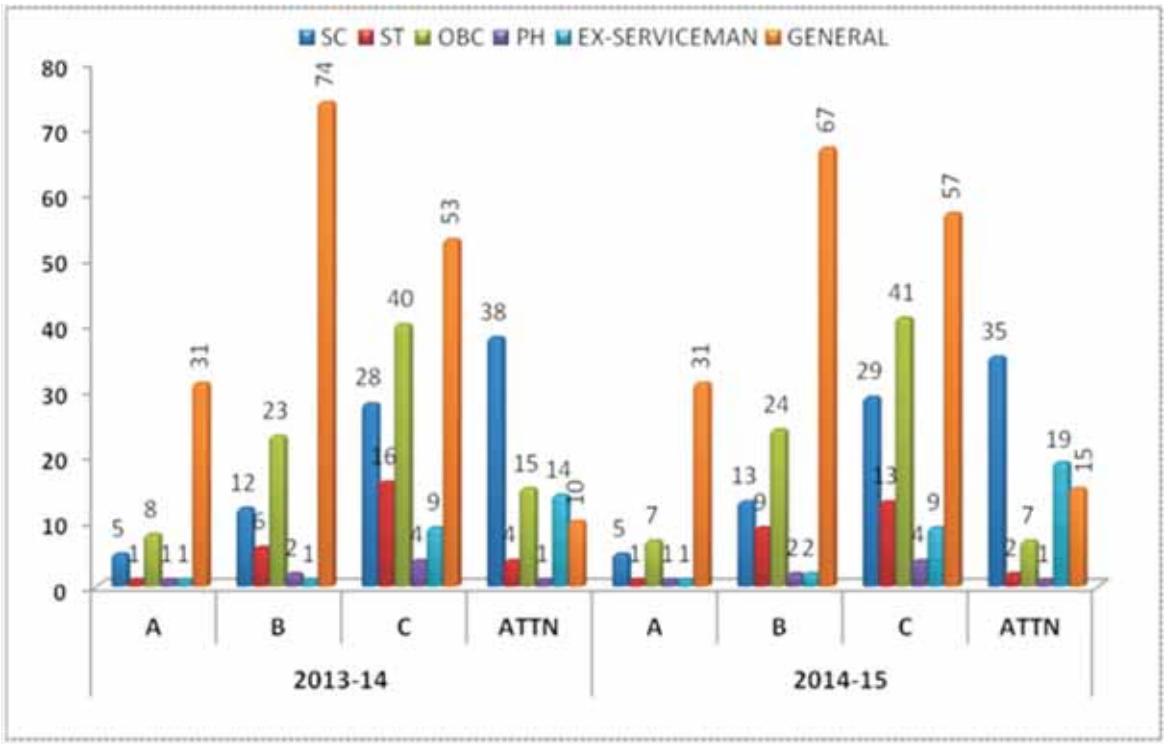


Budget Generated Funds

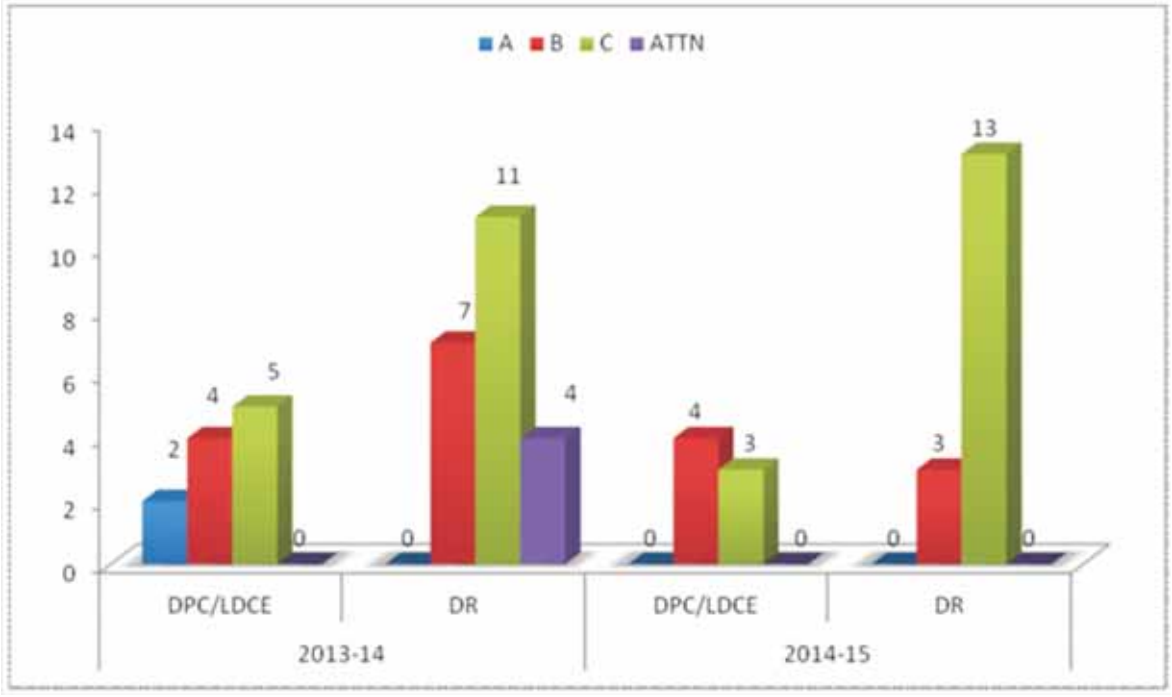


PERFORMANCE INDICATORS

Group wise category distribution



Group wise vacancies filled



PERFORMANCE INDICATORS

Apprentices engaged

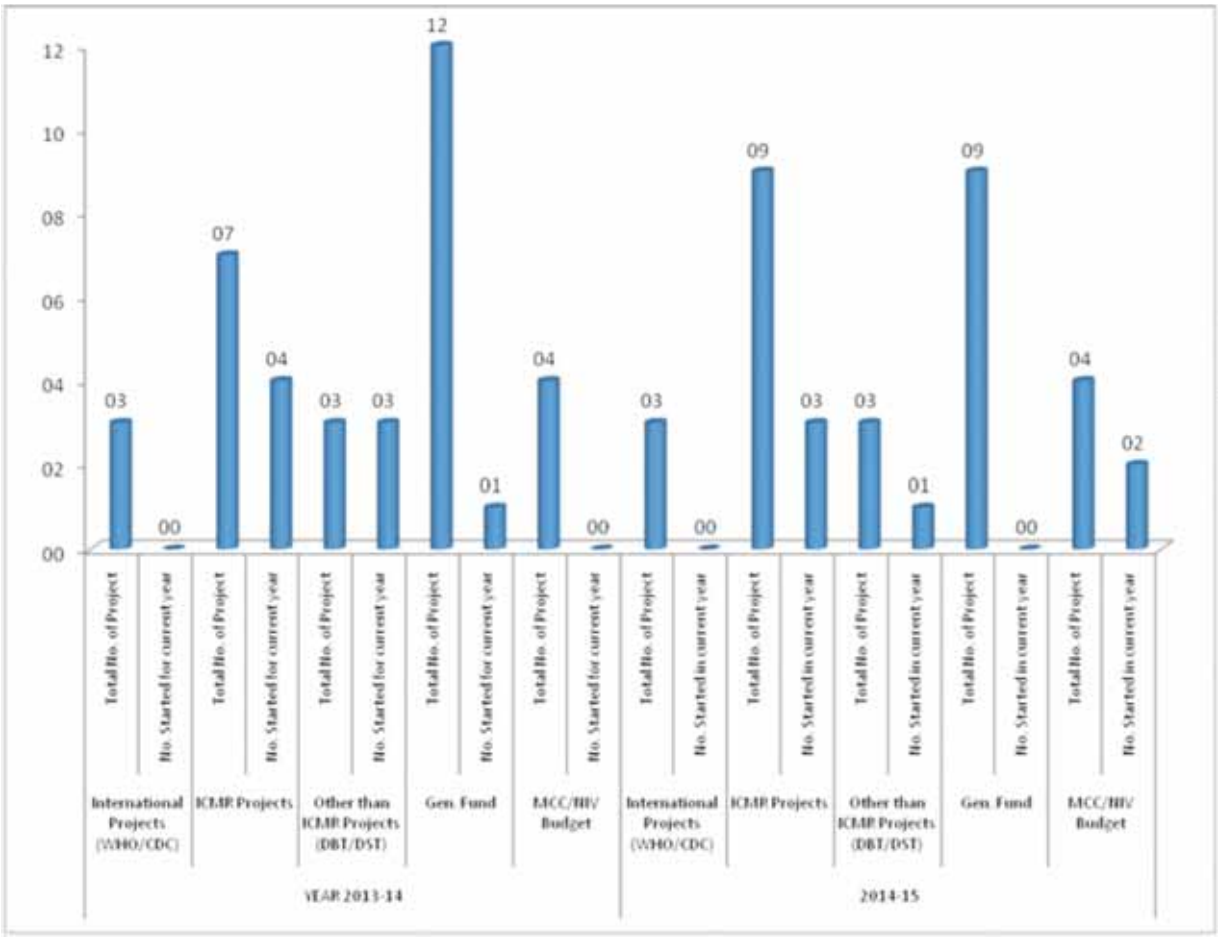
Legend: Blue, Red and Green bars represent Engineering, Administration and Library, respectively.

Training programmes attended by Administrative Staff Members at ISTM, New Delhi during 2014 - 15

S.No.	Name & Designation	Course Title and Code	Duration	From	To
1	Mr. B.K.Wadke, Private Secretary	Establishment Rules (RT)	5 days	26.05.14	30.05.14
2	Mr. Anil Gaikwad, Section Officer	Reservation in services for SC / ST / OBC (RIS)	3 days	07.10.14	09.10.14
3	Mrs. V.V.Shendye, Section Officer	Reservation in services for SC / ST / OBC (RIS)			
4	Mr. H.S.Pasalkar, Assistant	Administrative Vigilance Disciplinary Procedures – 3 (AV3)	12 days	09.03.15	20.03.15

PERFORMANCE INDICATORS

Staff employed in funded projects



MAXIMUM CONTAINMENT LABORATORY

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Mr. Aacharya M	Technician-'A'
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MAXIMUM CONTAINMENT LABORATORY

MCL1301: Diagnostic support/diagnosis for referred samples of viral hemorrhagic fever of unknown etiology and outbreak investigation

PD Yadav, G Chaubal, A Shete, DY Patil, P Kokate & DT Mourya

Funding agency: Intramural Project duration: 2013-2015

1. Diagnostic service for suspected Ebola samples

The 2014 Ebola epidemic is the largest reported in the history, affecting multiple countries in West Africa. Looking at the dreadful scenario, passengers coming from affected countries were screened at all International airports and samples from suspected individuals were sent to NIV for further testing. The Maximum Containment Laboratory (MCL) at NIV provided timely diagnosis for all the referred Ebola samples received from different parts of India and WHO-SEARO countries. Thirty seven human samples (32 blood samples and 5 semen samples) were screened using Filoviridae family specific RT-PCR, Ebola and Marburg specific RT-PCR and Ebola Zaire specific real time RT-PCR during July 2014-March 2015. All the infectious work on the suspected samples was carried out in Biosafety level-4 laboratory of NIV only.

All the serum samples (n=32) tested negative for Ebola virus both by RT-PCR and Real time RT-PCR assays. However, semen samples (5 specimens of the same recovered person at different time intervals) referred by NCDC, Delhi tested positive for the virus by Real time RT-PCR and IgG ELISA. Sequencing of the PCR product targeting the L gene, Nucleoprotein gene and Glycoprotein gene revealed its identity with the 2014 Ebola Zaire strain (Zaire Ebola virus isolate /MLI/14/Manoka-Mali-DPR4). Isolation of the virus from semen sample using Vero-E6 and Vero-CCL81 cells was unsuccessful. Sequential samples showed presence of Ebola viral RNA upto 120 days in semen.

2. Investigation of a nosocomial outbreak of Crimean Congo hemorrhagic fever (CCHF) in Goyal hospital, Jodhpur, Rajasthan State [2015]

PD Yadav, A Shete, DY Patil, P Kokate & DT Mourya

CCHFV is enzootic in southeastern Europe (Bulgaria, Albania, Kosovo, and Greece), southern Russia, and several countries in Africa, Asia and the Middle East. After the first outbreak of CCHF in India during 2011, many episodes of sporadic cases were recorded in Gujarat and Rajasthan. Outbreaks are usually attributable to handling infected animals or humans. On 16th January 2015, a 30 yr old male from Jodhpur was hospitalized in Goyal Hospital, Jodhpur, Rajasthan. The patient developed thrombocytopenia and hemorrhagic manifestations and died in the hospital on 18th January, 2015. Three nursing staff from the same hospital also developed similar symptoms and one succumbed to the infection. Blood and serum samples of the index case and the nurses were tested at NIV, Pune using CCHF virus specific Real time RT-PCR, RT-PCR and anti-CCHF humanIgM ELISA. All the samples tested positive for CCHF by all the three tests. After this episode, a three member medical team from the state ministry visited Goyal hospital and

on their recommendations, samples from 5 nursing staff and 37 contact cases from Pokhran area (village of the suspected index case) were referred to NIV, Pune for diagnosis of CCHF. The nursing staff tested negative for CCHF, while one of the contacts tested positive for CCHF viral RNA and CCHF IgM antibodies. All the five CCHF positive samples were amplified for CCHF virus partial nucleocapsid (N) protein (the S segment) sequence and were found to be closely related to Afghanistan strain with 99.0% nucleotide identity. Data on the genetic analysis suggested the CCHF viral strain responsible for the outbreak to be new which could be a recent introduction from the neighboring countries. The earlier CCHF outbreaks in Gujarat (2011) were caused by Tajikistan strain.

3. Investigation of post outbreak samples of suspected Kyasanur Forest disease (KFD) cases referred from Karnataka State, India.

PD Yadav, A Shete & DT Mourya

Kyasanur Forest disease (KFD) is a tick borne viral hemorrhagic fever, caused by KFD virus (KFDV, family ***Flaviviridae***). The disease was first reported from Kyasanur forest area of Karnataka in 1957 and remained confined to three talukas (Sagar, Soraband and Shikaripur) of Shimoga district until 1972. The virus is transmitted to humans and monkeys by the bite of infected ticks mainly by the nymphs. Though ***Haemaphysalis spinigera*** is the principal vector, several other species of ticks also transmit the virus. Geographic expansion of KFDV activity was observed in the recent years as the virus activity was detected in Uttar Kannada, Dakshina Kannada, Chikmagalore, Udupi and Chamrajanagar districts of Karnataka as well as Nilgiri District in Tamil Nadu and Wayanad and Malappuram districts in Kerala. A total of 97 human blood samples collected from patients admitted to various primary health centers of Chikmagalur, Kodagu, Mangalore, Shimoga, Udupi and Uttara Kannada districts were referred to NIV for diagnosis after the outbreak of KFD in Shimoga district (January to March, 2014). Five tick pools collected from Honnavara taluka, Uttaa Kannada were also referred for virus diagnosis. Human samples were tested for anti-KFDV IgM antibodies by IgM ELISA and KFDV-RNA was tested by real time RT-PCR. Tick pools were homogenized using Geno-grinder®, centrifuged and the supernatants were subjected to real time RT-PCR. All the infectious work was carried out in BSL-4 laboratory. Of the 97 suspected human samples, 35 samples tested positive for KFDV by real time RT-PCR, while 17 samples tested positive for anti-KFDV IgM antibodies. Both IgM antibodies and viral RNA was observed in 7 samples. However, all the tick pools tested negative for KFDV.

4. Investigation of a case of KFDV infection in a tribal population of Malappuram district in Kerala in 2014

PD Yadav, BV Tandale, B Anukumar, & DT Mourya

On 12th May, 2014, one human serum sample of a tribal woman hailing from Nilambur, Malappuram district of Kerala, bordering the Nilgiri district of Tamil Nadu (Figure 1) was received for diagnosis of KFD. The patient, an anti-natal of one month amenorrhea had developed fever, myalgia, and hematemesis and recovered from disease. The Tribal Medical Officer and the District

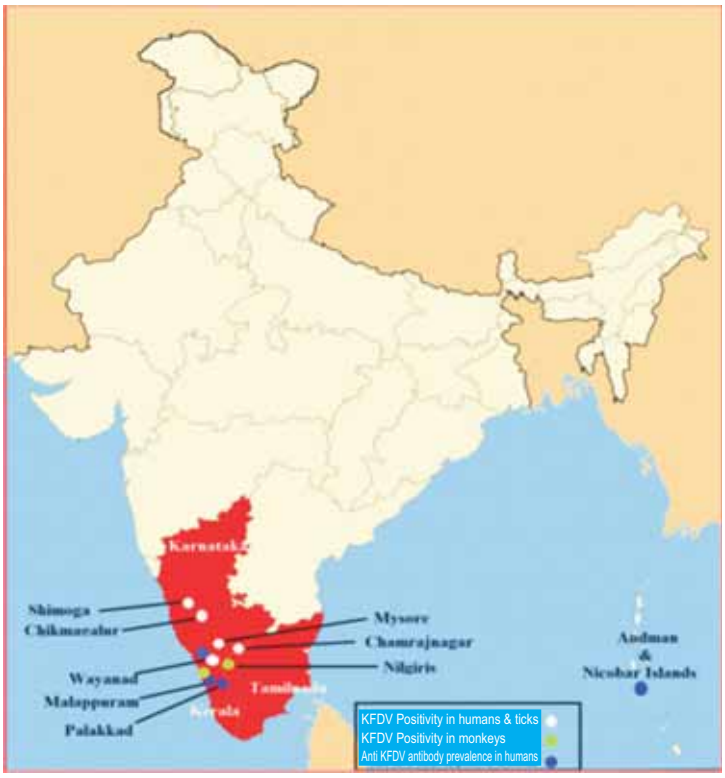

Figure 1: Presence of KFDV in India

Surveillance officer of Malappuram district reported the death of two people with similar symptoms in the area in April, 2014. NIV team visited the affected areas and collected 26 blood samples from the tribals with febrile illness and tested for KFDV by Real Time RT-PCR and anti-KFD human IgM antibody ELISA. A few samples (n=7) were also tested for dengue virus. One hundred and seven tick pools collected from Wayanad district, Kerala were also tested for the presence of KFDV by real time RT-PCR.

The index case tested positive for anti-KFDV IgM antibodies, but KFD viral RNA could not be detected. Of the 26 samples collected by the NIV team, three samples including one from the family where death was reported were found to be positive for anti-KFDV IgM antibodies. None of the tick pools tested positive for KFDV. All the seven samples tested for dengue tested negative for anti-dengue IgM and dengue RNA.

5. Serological investigation of human serum samples collected from Maharashtra State and Andaman and Nicobar Islands for CCHF and KFD IgG antibodies.

PD Yadav, A Shete & DT Mourya

Retrospective analysis of human blood samples collected from Deonar (Mumbai), Radhanagari (Kolhapur) and Andaman and Nicobar Islands for hepatitis investigation were screened to determine the presence of anti-CCHF IgG and anti-KFD IgG antibodies using Vector Best (CCHF) and indigenous ELISA (KFD) to demonstrate the possible circulation of the viruses previously.

Anti-CCHF IgG antibodies were detected in1/76 samples collected from Deonar and 1/66 samples collected from Radhanagari. Anti-KFD IgG antibodies were detected in 3/164 samples collected from Andaman and Nicobar Islands.

MAXIMUM CONTAINMENT LABORATORY

MCL1308: Nationwide Serosurvey of Crimean Congo hemorrhagic fever virus in domestic animals

DT Mourya, PD Yadav, PS Sathe, A Shete, B Pattnaik (ICAR) & G Sharma (ICAR)

Funding: Intramural [accepted by DHR for funding] Project duration: 2013-2015

Objective: Cross-sectional serosurvey of Anti-CCHF virus IgG antibody in domestic animals in India.

In the background of confirmed CCHF activity in Gujarat, Rajasthan, Himachal Pradesh and Uttar Pradesh both by human infections and seroprevalence in animals (Gujarat), a countrywide cross-sectional sero-survey was conducted among domestic animals to determine CCHFV activity in collaboration with Indian Council of Agricultural Research (ICAR). A total of 5636 (bovine 4781; sheep and goats 855) serum samples collected from 22 states and one Union Territory were screened for CCHFV specific IgG antibodies using two indigenously developed ELISA kits (Bovine anti-CCHF IgG ELISA kit and Sheep/goat anti-CCHF IgG ELISA kit). The study showed 5.43% bovine samples (260/4781) and 10.99% sheep/goat samples (94/855) positive for anti-CCHF IgG antibodies (Figure 2). Sero positivity was found to be higher in goats and sheep than in bovines. State wise maximum seropositivity was detected in Orissa for bovines (31.3%) and Himachal Pradesh for goats and sheep (53·1%).

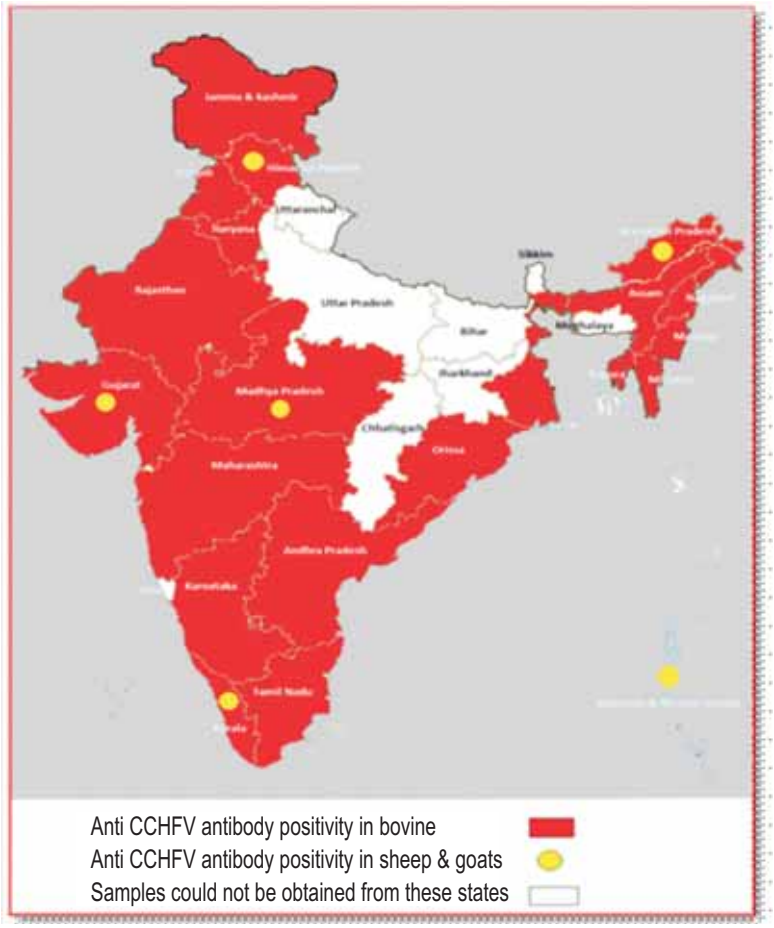

Figure 2: Anti-CCHF IgG positivity in domestic animals in 22 states and one Union Territory of India

MAXIMUM CONTAINMENT LABORATORY

MCL1403: Survey of KFDV in ticks and KFDV antibodies in rodents in potential risk areas in the neighbouring states of Karnataka (Kerala and Tamil Nadu)

NIV team: DT Mourya, PD Yadav, MD Gokhale, & AB Sudeep

VCRC team: P Jambulingam & C Sadanandane

Funding: Intramural Project duration: 2014-2016

In view of the estimated annual incidence of 400-500 cases of KFD in different districts of Karnataka and the recent observation of geographic expansion to the neighbouring states, a study was carried out to detect the presence of KFDV in tick populations in adjoining ecological settings in Tamil Nadu and Kerala. A total of 168 tick pools comprising of different species collected from Nilgiri (54) and Satyamangalam (43) districts of Tamil Nadu and Wayanad (48) and Malappuram (23) districts of Kerala were screened by Real time RT-PCR. All the samples tested negative for KFD viral RNA.

MCL1302: Teaching and training program on "General biosafety issues and working in BSL-3 laboratory"

DT Mourya, PD Yadav, J Mullick & SD Pawar

Funding: Intramural Project duration: 2013-2015

1. Workshop on "Preparedness for laboratory diagnosis of Ebola virus" for the Medical Research/diagnostic laboratories in India

Figure 3 A: Trainees in complete PPE (Tyvek suit and PAPR)

Figure 3B: Trainees and NIV staff during the Workshop on "Preparedness for laboratory diagnosis of Ebola virus."

In view of the dreadful global scenario of Ebola virus epidemic, a workshop on the "Preparedness for laboratory diagnosis of Ebola virus" was organized at NIV, Pune (Figure 3B). As part of preparedness to handle the Ebola outbreak situation as per the directive of the Ministry of

Health and Family Welfare, Government of India. Staff from 16 laboratories (BSL-3 and BSL-2 level) of India was trained in two phases (1st: 10th-14th November, 2014, 2nd: 26th-30th November, 2014).

Participants were guided with standard operating procedures of sample handling, use of powered air purifying respirator (PAPR), molecular diagnosis, waste management and International Air Transport Association (IATA) awareness, Cold Chain Management (CCM) and dangerous goods declaration. Adequate information and materials were provided explaining emerging and re-emerging diseases, current scenario of Ebola outbreak and vectors involved in the transmission of the disease, good laboratory practices, biosafety measures required during the sample collection from patients/contact person and hospital management. The setup of a BSL-3 facility; why specified areas should be allocated for changing into Tyvek suits, donning and doffing of personal protective equipments (PPE), working in the facility were also explained (Figure 3A). Tyvek suits (part of PPE) and Ebola virus diagnostic reagents were provided to all the centers as a measure of preparedness.The feedback received from the participants, showed that out of the 16 laboratories, 13 laboratories are capable of diagnosis of Ebola virus.

2. Workshop on 'Biosafety preparedness to handle infectious materials in laboratory settings' for staff of Medical Research Institute, Sri Lanka

DT Mourya & PD Yadav

A workshop on "Biosafety preparedness to handle infectious materials in laboratory settings" was organized by NIV, Pune at the Microbial Containment Complex, during 25th-28th March, 2015 on request of Sri Lanka Regional WHO and SEAR-WHO office (Figure 4).

Figure 4: MRI team, Sri Lanka during workshop on 'Biosafety preparedness to handle infectious materials in laboratory settings'

Five staff members of the Medical Research Institute (MRI), Sri Lanka were trained for BSL-3 laboratory including design, biosafety aspects, workflow, equipments and laboratory management. Both lectures and hands on simulated procedures for handling of suspected infectious samples and diagnosis by Molecular and Serological tests (CCHF specific Real Time RT-PCR and CCHF IgM and IgG ELISA), use of PPE, PAPR and biological waste management was included. The importance of high containment laboratory in providing diagnosis, good laboratory practices, biorisk assessment and shipment of infectious samples as per the WHO guidelines as well as hands on laboratory exercises on emergency plans in biosafety laboratories, working in the facility and donning and doffing of PPE were also explained.

3. Joint NIV-AFMC workshop on Ebola preparedness

DT Mourya, PD Yadav, SD Pawar, BV Tandale & YK Gurav

Figure 5A: Demonstration of Ebola preparedness by NIV scientists at AFMC, Pune.

A workshop on 'Ebola preparedness' was organized jointly by the NIV Pune and the Armed Forces Medical College (AFMC), Pune at the Dhanwantari auditorium (AFMC) on 26th August, 2014. The drill was carried out in the presence of doctors of AFMC, KEM hospital, Pune and Sassoon General Hospital alongwith officials of the Pune Municipal Corporation (PMC) and State Health Officials. Emphasis was given to rapid response to the patient in quarantine, disinfection strategies, awareness and field training of paramedical or medical

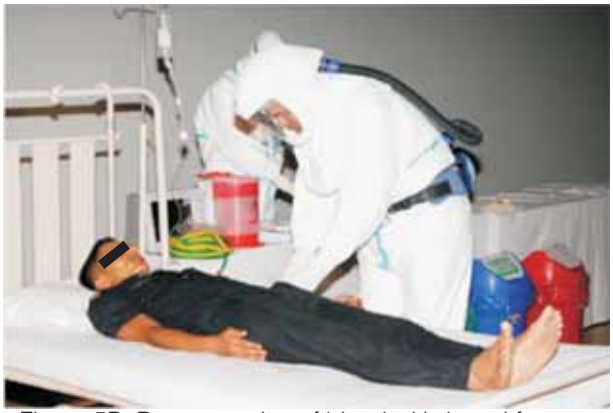

Figure 5B: Demonstration of blood withdrawal from an Ebola suspected patient.

Figure 5C: Dr. DT Mourya, Director, NIV, Pune receiving a token of appreciation from the Head of AFMC, Pune

Figure 5D: Demonstration of effective disinfection of ambulance which carried the Ebola suspected patient

Figure 5E: Demonstration of proper packaging of sample collected from an Ebola suspected patient

personnel on Ebola case management, proper data and sample collection procedures, preventive measures to be taken by nursing and primary healthcare responders and a demonstration of donning and doffing of PPE by NIV scientists (Figures 5A-E).

MCL1304: Preparation of reagents for highly infectious diseases

1. Development of anti-CCHF Sheep and Goat IgG ELISA assay

DT Mourya, PD Yadav, PS Sathe, & A Shete

Funding: Intramural Project duration: 2013-2015

Indian economy and rural development is dependent on agriculture, livestock farming and dairy industry. CCHF virus belongs to Bio-safety level-4 and is highly pathogenic to both humans and animals. The virus has been found to be endemic in Gujarat since 2011. However, the disease burden especially in the animals is not estimated due to the lack of commercially available diagnostic ELISA kits for the detection of anti-CCHF IgG antibodies in sheep and goat. Therefore, an effort was made to develop a kit for the diagnosis of CCHF IgG to determine the presence of anti-CCHF IgG antibodies in sheep and goats across the country.

An anti-CCHF sheep/goat IgG ELISA was developed and standardized at NIV, Pune with 84.62% and 82.61% sensitivity and specificity respectively which is at par with the CDC reagents. This is the first indigenously developed anti-CCHF IgG detection kit for sheep/goat samples. The kit is cost effective, safe to work with in a BSL-2 laboratory and can be used for screening a large number of animal samples. The kit was validated internally in three laboratories at NIV, Pune (Avian influenza, VDL and Hepatitis) and by three external laboratories, ***viz.***, NIHSAD (formerly HSADL), Bhopal, MRC, Jabalpur and BJ Medical College, Gujarat.

2. Development of anti-CCHF Bovine IgG ELISA

DT Mourya, PD Yadav, PS Sathe, & A Shete

People involved with livestock management are at high risk of contracting CCHF as they live in proximity to animals and get the infection through the bite of infected ticks. Veterinary personnel as well as people who are involved in meat trade are also at risk of contacting the disease either by tick bites or through contact of the infected body fluids of animals. Early diagnosis is therefore important to check the spread of the virus. PCR based assays cannot be used for testing of animal samples due to low viremia in the animals. Therefore, antibody detection is the only criteria for early warning signs. Only one kit is available commercially for screening of anti-CCHF IgG antibodies in cattle serum samples which is highly expensive. Therefore, an attempt was made to develop an ELISA in-house.

During the study, a new ELISA has been developed and standardized at NIV to detect anti-CCHF IgG antibodies in cattle serum samples. The new test has a diagnostic sensitivity and

specificity of 80.56% and 96.05% respectively, which is at par with the CDC provided reagents. Due to the inactivated nature of the antigen, the kit can be used in any laboratory setting across the country. This is the first indigenously developed anti-CCHF IgG detection Kit which is cost effective, sensitive, rapid, user friendly as a large number of samples can be tested at any level of clinical settings (public health centers and hospitals etc). The kit was validated internally in three laboratories at NIV, Pune (Avian influenza, VDL and Hepatitis) and by three external laboratories, *viz.,* NIHSAD (formerly HSADL) Bhopal, MRC, Jabalpur and BJ Medical College, Gujarat.

3. Optimization of anti-KFD IgM human ELISA

PD Yadav, A Shete, & DT Mourya

Geographic expansion of KFDV to newer areas in Karnataka and to adjoining states viz., Tamil Nadu and Kerala has been detected recently. This has necessitated an urgent need for diagnostic reagents and kits for early diagnosis of the disease in non-endemic areas of Karnataka and the adjoining States. Currently, no diagnostic ELISA kit is available for specific antibody (IgM and IgG) detection of KFDV. NIV has developed and standardized a new ELISA for the detection of anti KFD IgM in human serum samples. This newly developed indigenous ELISA kit is intended for the qualitative detection of IgM antibodies in the serum of patients presenting clinical signs and symptoms consistent with febrile illness and hemorrhages suspected of KFD. The assay is designed for providing the presumptive diagnosis of KFDV in sporadic cases and outbreak investigations. It can be used at any level of clinical settings, public health centres and hospitals due to the inactivated antigen. The kit has been validated internally in three laboratories at NIV, Pune (Avian influenza, VDL and Encephalitis group) and externally at three reputed laboratories viz., NIHSAD (formerly HSADL), Bhopal, MRC, Jabalpur and KGMC, Lucknow and found to be working satisfactorily.

4. Development of anti KFD human IgG antibody ELISA

PD Yadav, A Shete, & DT Mourya

Despite routine vaccination and control measures, KFD cases continued to be reported in Karnataka State since1999. Recent reports have shown the geographic expansion of the virus to the neighbouring states like Tamil Nadu and Kerala. China has also reported the prevalence of KFD like virus in 2009. The emergence of KFD outside of Karnataka warrants the development of a rapid, sensitive and specific screening assay against the virus for early diagnosis. NIV has developed an anti-KFD IgG ELISA kit for the detection of IgG antibodies in human serum. The assay has been checked for specificity using a panel of IgG antibody negative and positive human serum samples against Japanese encephalitis (JE), West Nile (WN), dengue and Leptospira. Cross reactivity was also tested with rheumatoid (RH) positive and negative human serum samples. None of them showed any cross reactivity except dengue virus which showed 20% cross reactivity that is common across the flaviviruses. The assay can be used at any level of clinical set up, public health centres and hospitals with a BSL-2 level laboratory due to the inactive nature of the antigen. Validation of the kit was carried out internally by three laboratories of NIV

(Avian influenza, VDL and Hepatitis) and externally by three national laboratories (NIHSAD (formerly HSADL), Bhopal, MRC, Jabalpur and BJ Medical College Gujarat.

5. Development of anti-CCHF human IgG antibody ELISA

PD Yadav, A Shete, & DT Mourya

CCHF is a severe acute febrile illness caused by CCHF virus with an overall case fatality rate of 5–50%. Person-to-person transmission of CCHF through direct exposure of blood or other secretions and nosocomial transmission are well-documented. Serological screening allows CCHF affected areas to be identified, as antibody prevalence in human is a good indicator of virus circulation.

The newly developed anti-CCHF human IgG ELISA has been found useful for the detection of anti-CCHF IgG antibodies in human serum. This is the first indigenously developed anti-CCHF IgG detection Kit. The kit is cost effective, safe to work in BSL-2 laboratory and can be used for CCHF surveillance in human serum samples.

The indigenously developed assay has been compared with anti-CCHF IgG ELISA VectoCrimean IgG kit (Gold standard kit, Vector BEST Company, Russia) and the specificity and sensitivity of the kit was found to be 99.0% and 80.0% respectively, which is at par with the gold standard. All these results suggest that the newly developed test can be used for screening CCHF IgG antibodies in human serum samples. Tests to determine cross reactivity with rheumatoid (RH) positive and negative samples, Hantaan, Rift Valley Fever (RVF), chikungunya, dengue, JE and WN positive samples was also carried out. None of them showed any cross reactivity with the anti CCHF IgG detection assay.

6. Development of anti-CCHF Human IgM ELISA

PD Yadav, A Shete, & DT Mourya

Gujarat and parts of Rajasthan continued to report cases of CCHF since 2011. In addition to the enhanced surveillance, access to early, sensitive and specific laboratory diagnosis is a key factor in increasing the preparedness against the disease. Currently, only one commercial diagnostic ELISA kit (Vector BEST) as available for detection of anti-CCHF IgM antibodies in humans, but, is very expensive. Therefore, a new CCHF IgM Capture ELISA kit has been developed at NIV for qualitative detection of IgM antibodies in serum/CSF of patients presenting clinical signs and symptoms of CCHFV. Cross reactivity was tested with RH positive and negative samples, Hantaan, RVF, chikungunya, dengue, JE and WN positive samples. The assay was also compared with anti-CCHF IgM ELISA from VectoCrimean IgM kit (Gold standard kit, Vector BEST, Russia) and found to be 100% concordant in specificity and sensitivity. Three laboratories of NIV, Pune have performed the internal validation of the kit and validation by external agencies is in progress. The preliminary results are suggestive of the efficacy of the newly developed assay for screening CCHF IgM antibodies in human serum samples.

7. Standardization of RT-PCR for detection of Malsoor virus

PD Yadav, T Nikam, A Shete & DT Mourya

Malsoor virus (MV), a novel Phlebovirus isolated from bats *(Rousettus leschenaulti)* from Malsoor village of Mahabaleshwar, Maharashtra State was characterized at the molecular level. Initial studies have shown that MV is genetically related to the Severe Fever with Thrombocytopenia Syndrome (SFTS) and Heartland viruses (HRTV), both causing severe human diseases. SFTSV is a newly identified pathogenic member of Phlebovirus, which causes fever with thrombocytopenia syndrome in humans and has a geographic distribution in China, Japan and USA. Looking at the disease causing potential of SFTSV and HRTV in humans, sero-positivity against SFTSV in domestic animals and its close relatedness to the newly isolated MV, it becomes imperative to study this novel Phlebovirus for assessment of infection in humans. Nucleocapsid (S) gene based nested RT-PCR has been developed which could detect up to 100 copies of viral RNA of MV. The specificity of the assay was assessed using a panel of viruses along with a positive and negative control and was found to be highly specific for MV. The assay can be used to screen both bat and human samples. Retrospective analysis of bat samples using the optimized RT PCR could detect MV positivity of 19/51 samples collected from Mahabaleshwar, Maharashtra.

8. Standardization of Real Time-PCR for detection of Nipah virus.

PD Yadav, GY Chaubal & DT Mourya

Nipah virus (NiV) is a highly pathogenic paramyxovirus that causes acute encephalitis with high morbidity and mortality. NiV has been classified as a Biosafety level-4 pathogen due to high mortality rate and lack of drugs or vaccines. In India, presence of NiV was detected during a focal outbreak in Siliguri (2001) followed by Nadia (2007) in West Bengal. Since the existing real time PCR assay was found to be less sensitive than the nested RT-PCR assay, it was difficult to quantify the virus and establish a relationship between the virus titre and disease severity. An attempt was therefore made to develop and standardize a new Real time RT-PCR using sequences specific for the Nadia case (GenBank accession number – FJ513078.1)

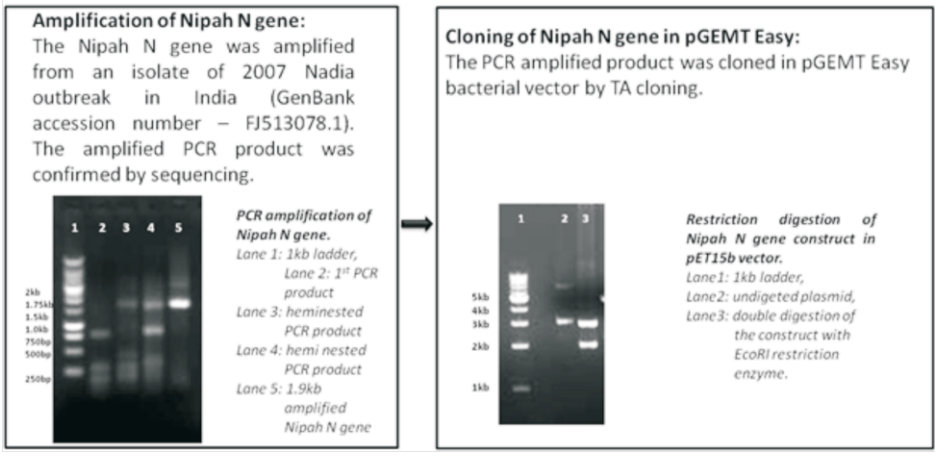

Figure 6: PCR amplification of Nipah N gene
(A) and its subsequent cloning in pGEMT Easy vector
(B). This clone has been developed as an in-house positive control for Nipah realtime RT PCR and Nipah RT-PCR systems.

for virus detection as well as for quantitating the in-house developed positive controls. To develop the assay, N gene was amplified, cloned in the pGEMT Easy vector system (Figure 6) and used to check the sensitivity of the currently used Real time primers, newly designed primers and to generate *in-vitro* transcript. An in-house positive control was also designed. However, the newly designed primers were found to be less sensitive than the existing primers as the detection level was upto 1000 copies and 100 copies respectively. A fresh batch of primers has been developed and will be tested for sensitivity and specificity for NiV. The developed assay can find direct use in diagnosis of the suspected samples as well as to re-check the earlier negative samples.

9. Detection of KFDV specific antibody using recombinant NS1 protein based ELISA

PD Yadav, GY Chaubal, S Jena, & DT Mourya

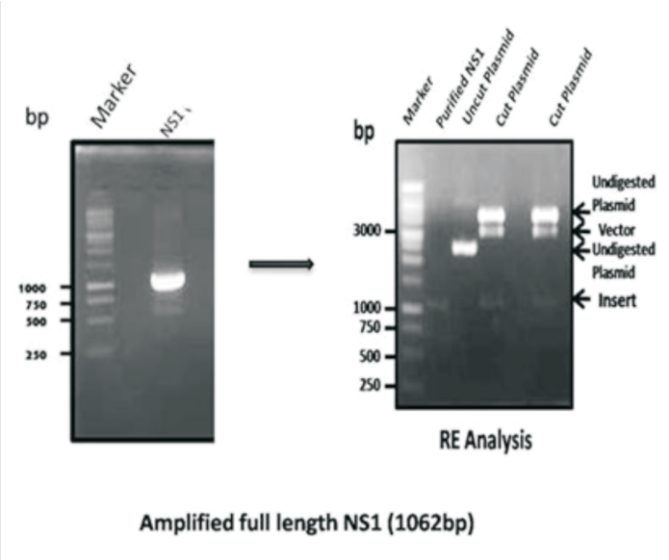

Figure 7A: The figure depicts 1062 base pair PCR amplified NS1 gene. The gene has been cloned in pET28a vector using BamHI and NotI restriction enzymes. The figure shows the release of insert upon double digestion of the constructed pET28a NS1 clone.

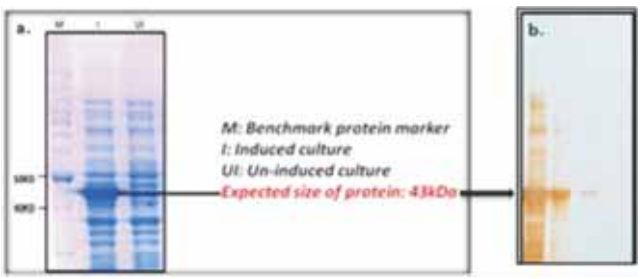

Figure 7B: SDS PAGE image of the NS1 expressed protein stained with Coomassie staining depicting the expressed KFD NS1 protein. Western blot using anti-KFD antibodies raised in mice

Rapid diagnostic methods for KFD, based on nested RT-PCR, real-time RT-PCR and IgM capture ELISA have been developed recently. The current project was proposed on the probability of using the non-structural protein (NS1) of KFDV as a potential biomarker for early diagnosis of the disease as seen in the case of dengue virus. Full length KFDV NS1 protein was expressed in prokaryotic expression system and was used subsequently in an indirect ELISA. In brief, KFD NS1 gene was cloned in pET28a bacterial expression system and confirmed by restriction digestion and sequencing and expressed in BL21 expression cells using IPTG (1mM) at 37°C for 4 hr for induction of protein (Figure 7A). The expressed protein was purified under native conditions using nickel chromatography column. Figure 7B shows the SDS PAGE image and KFD specific Western blot for the expressed NS1 protein. The purified protein was shown to be specifically reacting with KFD positive serum in the Western blot. No reactivity was obtained when the purified recombinant NS1 protein was checked with KFD negative serum. This is the first report of development of KFDV recombinant NS1 protein based assay for detection of infection specific antibody.

As our aim is to develop an antigen capture ELISA using recombinant NS1 protein, the purified protein was used for immunizing mice to develop anti-NS1 antibodies. Eleven 6 to 8 weeks old Balb/c mice were immunized with 5µg purified recombinant NS1 protein (in sterile PBS) with Freund's adjuvant. Four control mice were immunized with sterile PBS in Freund's adjuvant. Anti-NS1 immune serum was also raised in rabbits and currently we are developing the NS1 capture ELISA using anti-NS1 mouse serum for coating and purified anti-NS1 antibodies raised in rabbit for capture of NS1.

10. Detection of Kyasanur Forest Disease (KFD) virus in tick population from Wayanad district of Kerala State

R Balasubramanian & PD Yadav

Since KFDV activity in Kerala State was reported in 2014 after deaths of monkey and humans in the forested areas of Nilambur, Malapuram, a study was initiated to survey the tick populations for KFDV in the forest sites in Wayanad and Malapuram districts. The staff of NIV Kerala Unit collected 27 tick pools from the potential risk areas of Wayanad district and sent to NIV Pune for virus detection. All the tick pools were homogenized using homogenizer (Geno-grinder); viral RNA was extracted from the supernatant and screened for KFDV using Real time RT-PCR. Out of the 27 pools, one pool of ticks collected from 73 GM colony, Wayanad tested positive for KFD viral RNA.

MCL1404: Multi-site epidemiological and virological survey of Nipah virus: Special emphasis on North East Region of India

DT Mourya, J Mahanta, D Biswas, MS Chadha, PD Yadav, M Gokhale & SA Khan

Funding: ICMR (Extramural) Project duration: 2015-2017

In India, among the bat species, P. giganteus is suspected to harbor NiV and has been attributed for initiation of the Siliguri and Nadia outbreaks in 2001 and 2007, respectively. The current scenario of huge population of pigs and bats in close contact with human beings is suggestive of the potential risk of spread of the virus. Since the virus has been reported repeatedly from Bangladesh, it was proposed to determine the prevalence of NiV in bats in the North-east region of India which share boundary with Bangladesh.

With the necessary approvals and permission from forest authorities of the concerned states, a total of 62 *P. giganteus* bats were captured from Cooch behar (n=39) and Jalpaiguri (n=6) districts of West Bengal and Dhubri district (n=17) of Assam in March 2015. *R. leschenaulti* bats from Mynaguri area of Jalpaiguri district (n=12) were also captured. Necropsy of the bats was performed in the field following biosafety protocols using PPE and PAPR. Serum, brain, liver, spleen, lung, intestine and kidney samples, throat swab, rectal swab and urine samples were collected. All the organs were transported to NIV, Pune in liquid nitrogen while serum samples were transported under refrigerated condition.

Liver/spleen and kidney samples of 74 bats (62 *P. giganteus* and 12 *R. lescchenaulti*) were screened using Nipah specific real time RT-PCR. Seven bats were found to be positive for NiV viral RNA in either kidney or liver/spleen samples. A total of 35 serum samples (29 *P. giganteus* and 6 *R. leschenaulti)* were tested for anti-Nipah IgG ELISA and two samples *(P. giganteus)* tested positive.Attempts to isolate NiV from liver/spleen and kidney samples using Vero-E6 and Vero-CCL81 cells are under process.

MCL1303: Testing and rectification of different components of the BSL-4 facility and preparation of basic documents:

DT Mourya & PD Yadav

Funding: Intramural Project duration: 2013-2015

For smooth and uninterrupted functioning of the BSL-4 facility various documents related to engineering facility for breathing air system, process air system and inter locking biosafety doors, document of GMP facility, etc have been completed. All the deep freezers (-80°C and -20°C) installed in the facility have been defrosted at regular intervals to increase the durability of the compressors. Proper protocol and schedule for autoclaving of infectious waste have been designed in order to save the resources. Awareness has been created among the staff for saving electricity which helped in reducing the energy consumption in the facility. Preventive and breakdown maintenance was carried out on many laboratory and engineering related instruments.

MCL1405: Novel Virus like particle based vaccine for Kyasanur Forest Disease

DT Mourya, KS Lole, PD Yadav, G Chaubal

Funding: Intramural Project Duration: 2014-2017

Formalin inactivated tissue culture based (Chick embryo cell culture) KFD vaccine is currently used to immunize people in the endemic areas. The vaccine has been reported to have serious drawbacks with respect to its production and protection as it failed to confer adequate protection during the 2011–2012 outbreaks in Karnataka. It has a short-lived immunity which necessitates having periodic boosters. This warrants an urgent need to circumvent these issues and improve upon the existing vaccine technologies. We plan to develop a protein-based (virus like particle based) vaccine that overcomes the problems and cost associated with the conventional KFD vaccine and avoids the issues associated with the requirement of Maximum Containment facility to produce the vaccine. For that, prM–E encoding region of the virus genome was cloned as a single fragment or independently in a baculovirus transfer vector, pFastBac and pFastBac Dual promoter vector, respectively. Cloned constructs were confirmed by restriction digestion and sequence analysis. Sf9 cells (DH10MaxBAC cells) were transformed with the recombinant transfer vector plasmids to get the recombinant baculovirus in the form of closed circular DNA (bacmid). Sf9 cells were transfected with recombinant bacmids using cationic lipid

Cellfectin. The resultant baculovirus isolates were screened for the gene inserts and for protein expression in Sf9 cells. Monolayers of Sf9 cells (about 9 x 10^5 cells/well in 6 well plates) were infected and harvested after different time intervals (48-144 hr). Cell pellets as well as supernatants were analyzed on 12.5% SDS-PAGE. Gels were either stained directly or blotted onto nitrocellulose membrane. Further expression of prM/E by the recombinant baculovirus expressing envelope from the p10 promoter and PrM from the pH promoter are in progress.

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INF1001: Diagnostic Services/Outbreak Investigation:

MS Chadha, VA Potdar & ML Choudhary

Funding: Intramural Project Duration: Ongoing

Clinical samples (n=673) referred by the Maharashtra State health authorities for Influenza diagnosis assayed by real time RT PCR for influenza viruses showed a total positivity of 20.80% (140/673); which included 30 A(H1N1)pdm09, 84 A(H3N2) and 26 Type B. Of these, isolation was attempted for 62 samples in MDCK cell line which yielded 38 isolates [11A (H1N1) pdm09, 15 A(H3N2) and 12Type B Yamagata].

Outbreak of Influenza A(H1N1)pdm09 in 2015

A resurgence of influenza in 2015 with substantial morbidity and mortality was observed in India. Influenza virus was detected in 831 samples out of the 2476 [812 A(H1N1)pdm09, 16 Type B and 3 A (H3N2)] clinical samples referred from Pune and other parts of Maharashtra (Figure 1). Thirty two of the 59 clinical samples inoculated in MDCK cell line yielded virus isolations [28 A(H1N1)pdm09, 2 A(H3N2) and 2 Type B Yamagata].

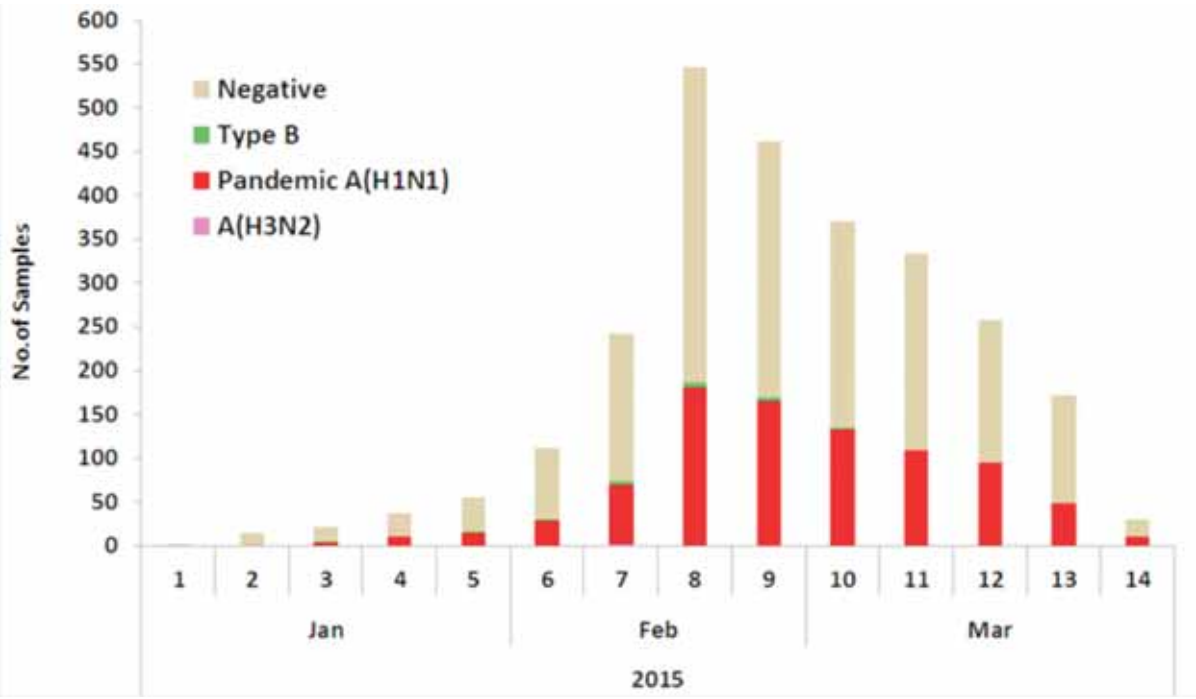

Figure 1: Outbreak of Influenza A(H1N1)pdm09 in 2015

A total of 114 samples were referred from Surat (13), Jaipur (71), Gorakhpur (2) and Nagpur (19) for quality assessment, drug susceptibility testing and molecular characterization. Ninety seven samples were confirmed as A (H1N1) pdm09 by real time RT PCR.

Eight cases of suspected MERS CoV were referred to NIV for confirmation but all tested negative for the virus.

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Genetic Analysis: Phylogenetic analysis of 35 HA gene (including 19 isolates from 2014 and 16 isolates from 2015 and whole genome analyses of 8 A(H1N1)pdm09 isolates was carried out; isolates were found to be close to the A/California/07/2009, 2009-2015 vaccine component (Figure 2) and no pathogenic markers were observed.

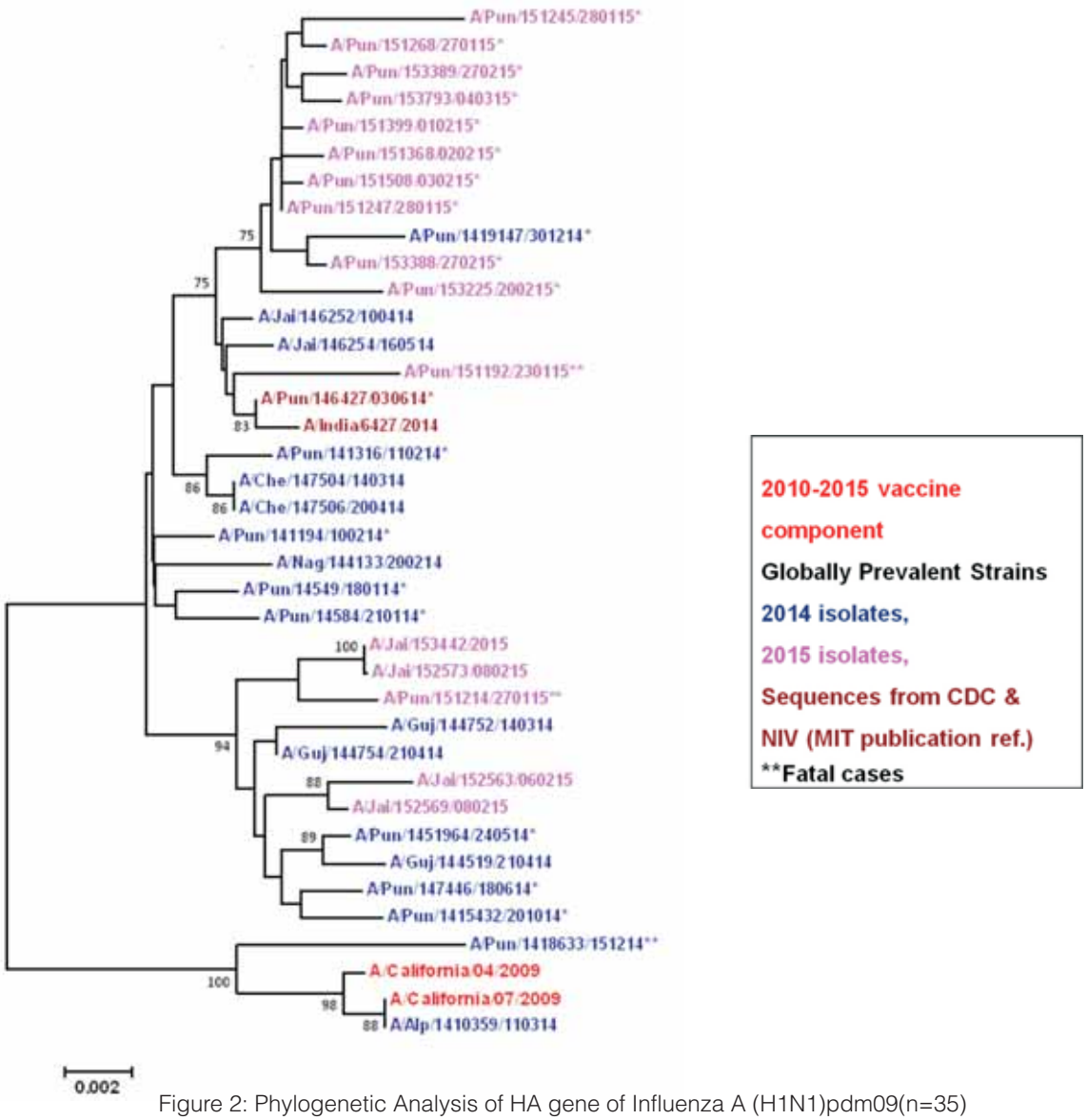

Figure 2: Phylogenetic Analysis of HA gene of Influenza A (H1N1)pdm09(n=35)

INF1003: Multisite Epidemiological and Virological monitoring of human Influenza Surveillance network in India Phase-II.

MS Chadha & VA Potdar

Funding: ICMR-DHHSCDC Project Duration: 15th September 2009-14th September 2014

Surveillance in Pune: Respiratory specimens (n=385) collected from influenza like illness (ILI) patients attending various dispensary/hospital outpatient departments in Pune were screened

and 57 samples were found positive for influenza (37 A(H1N1)pdm09, 15 A(H3N2) and 5 type B). Of the 57 positives, 29 PCR positive samples were processed for virus isolation in MDCK, which yielded 6 A(H1N1)pdm09, 9 A(H3N2) and 3 type B virus isolates.

Activity of Referral Centre: Reconfirmation of Isolates: A total of 102 (15 A(H1N1)pdm09, 49 A(H3N2) & 38 type B) isolates and 288 clinical samples were received from regional centers for reconfirmation. Tests were carried out and the results are given in Table1.

Table 1: Samples (clinical/isolates) received for reconfirmation

Center (clinical/ isolates)	Subtype	Samples received (Clinical/ Isolates)	Real Time PCR		Virus Isolation	
			Concordant (Clinical/ Isolates)	Discordant (Clinical/ Isolates)	Concordant	Could not grow
Delhi (67/16)	A(H1N1) pdm09	1/0	1/0	0/0	0	0
	A(H3N2)	6/3	6/3	0/0	3	0
	Type B (Yamagata)	10/13	10/13	0/0	13	0
	Negative	50/0	50/0	0	0	0
Chennai (17/5)	A(H1N1) pdm09	1/3	1/3	0/0	3	0
	A(H3N2)	3/0	3/0	0/0	0	0
	Type B (Yamagata)	1/2	1/2	0/0	2	0
	Negative	12/0	11/0	1/0	0	0
Dibrugarh (20/20)	A(H3N2)	0/16	0/16	0/0	7	0
	Type B (Yamagata)	0/4	0/3	0/1	3	0
	Negative	20/0	20/0	0/0	0	0
Kerala (17/17)	A(H1N1) pdm09	1/1	1/1	0/0	1	0
	A(H3N2)	15/15	15/15	0/0	3	1
	Type B	1/1	1/1	0/0	1	0
Kolkata (0/22)	A(H1N1) pdm09	0/8	0/8	0/0	0	0
	A(H3N2)	0/8	0/8	0/0	0	0
	Type B	0/6	0/6	0/0	0	0
Lucknow (85/12)	A(H3N2)	5/7	3/7	2/0	0	5
	Type B (Yamagata)	2/3	2/3	0/0	1	2
	Type B (Victoria)	2/2	2/2	0/0	1	1
	Negative	76/0	75/0	1/0	0	0
Nagpur (25/10)	A(H1N1) pdm09	3/3	3/3	0/0	2	0
	Type B (Yamagata)	7/7	7/7	0/0	2	1
	Negative	15/0	13/0	0/0	0	0
Srinagar (57/0)	A(H3N2)	19/0	19/0	0/0	6	0
	Type B	38/0	38/0	0/0	5	0
Total		288/102	284/101	4/1	53	10

Genetic Analysis: HA1 gene of isolates from the referred samples were sequenced and phylogenetically characterized; 19 A(H3N2) isolates were found to be similar to A/Victoria/361/2011; 2012-14 vaccine components (Figure 3). Twenty one type B isolates phylogenetically showed that both the lineages were in circulation with majority isolates (n=19) clustered with Yamagata lineage 2014-25 vaccine component B/Massachusetts/2/2012-like virus (Figure 4). The remaining 2 isolates clustered with Brisbane/60/2008 Victoria lineage component.

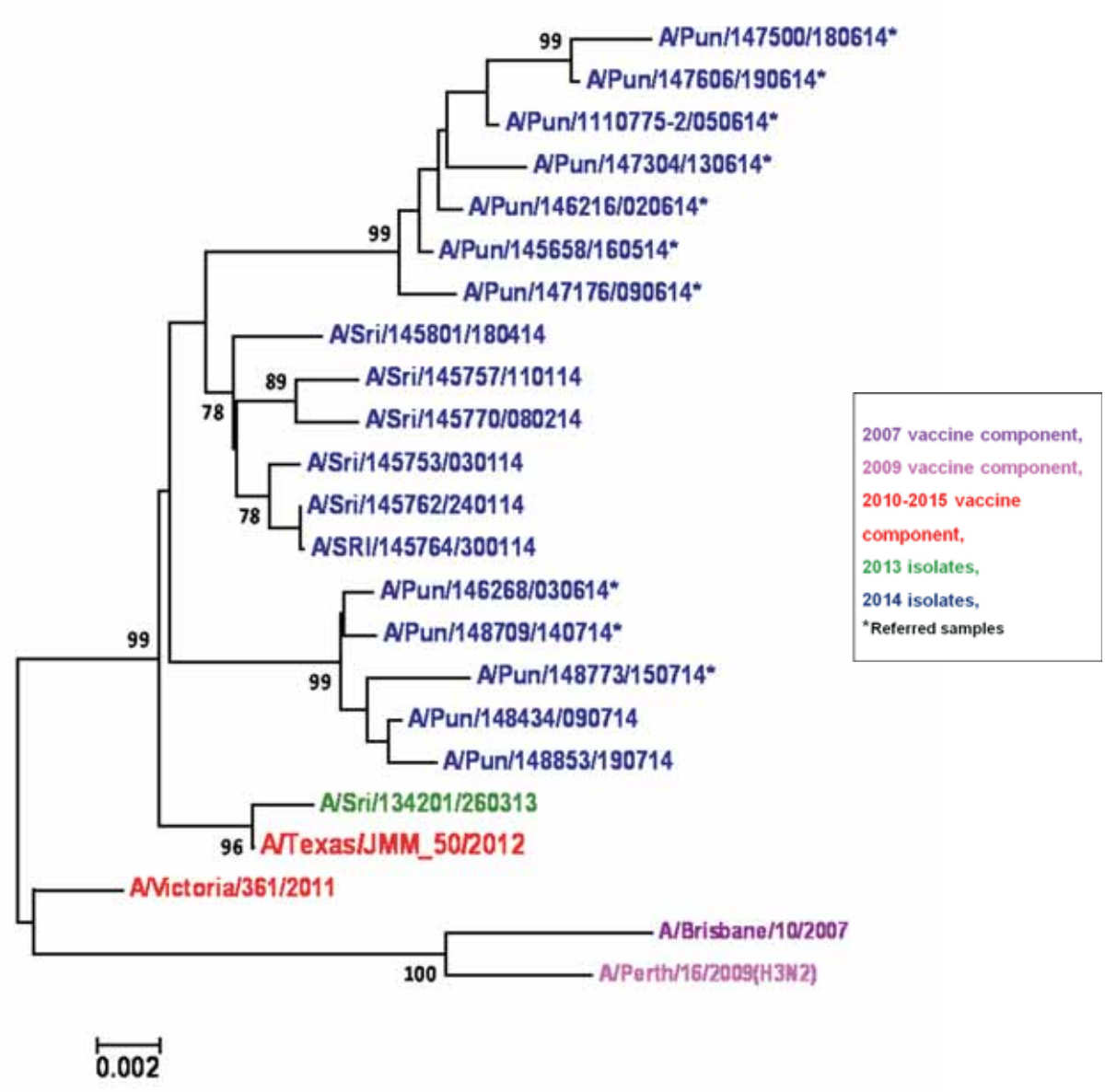

Figure 3: Phylogenetic Analysis of HA gene of Influenza A/H3N2 (n=18)

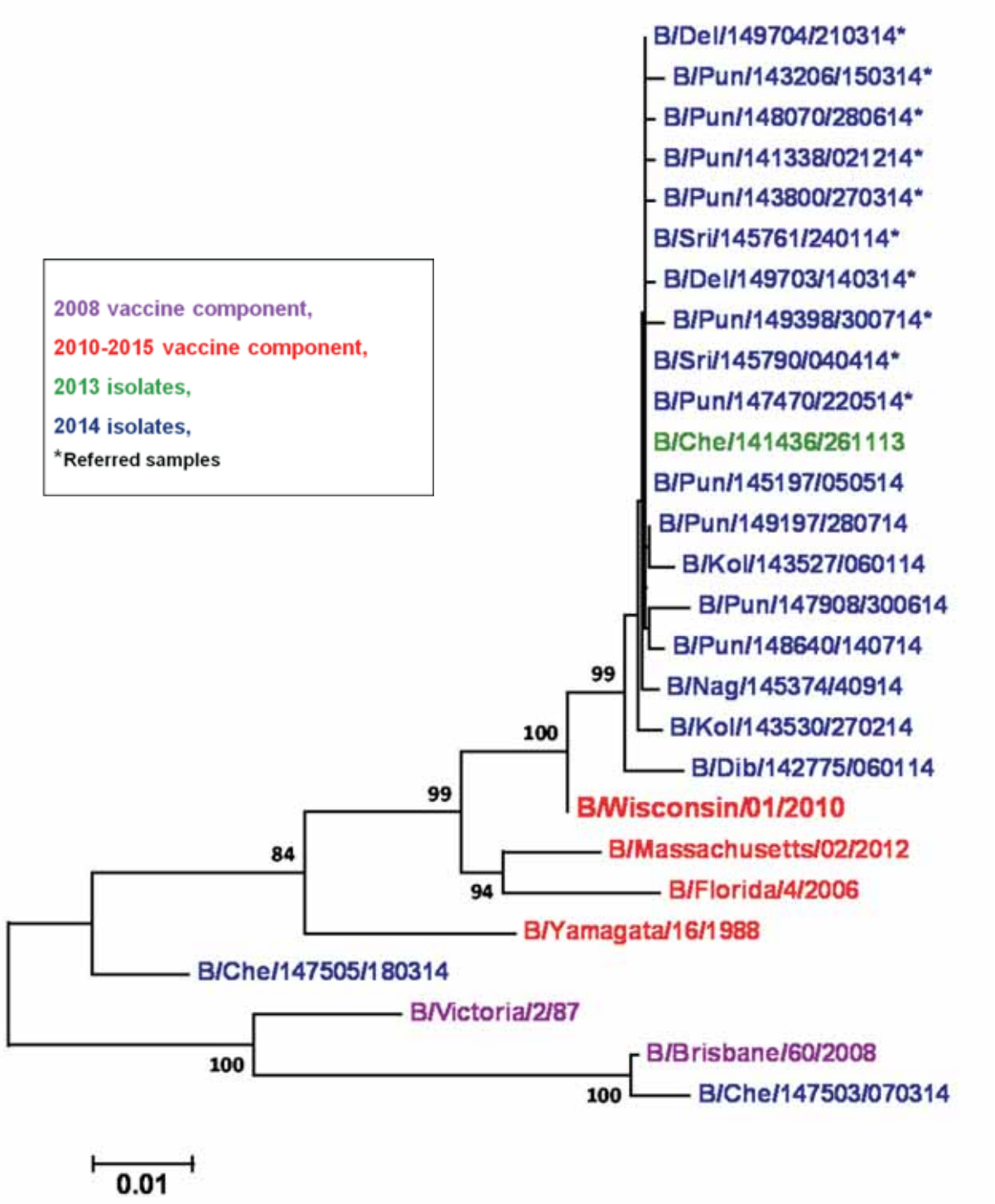

Figure 4: Phylogenetic Analysis of HA gene of Type B (n=21)

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Contribution to Global Influenza Network: Virological data for 20541 samples including 4698 A(H1N1)pdm09, 547 A(H3N2) and 284 type B from the network was submitted to the Global Influenza Surveillance and Response System (Flu net) and 33 isolates were submitted to the collaborating centre at CDC.

Quality Control for regional Labs & participation of referral lab in external QA/QC:

WHO External Quality Assessment Programme (EQAP): Panel 13 of 10 vials comprising of dried Triton X- 100 inactivated virus was received by the NIV referral laboratory in April 2014. All the samples were tested for Influenza A(H3), Influenza A(H5), Influenza A(H1)pdm09, Influenza B and other less commonly recognized Influenza A viruses. All the results were found to be concordant.

For Regional Labs, the QA/QC panel for isolation and Real time PCR were sent and both the results were satisfactory.

INF1203: Assessment of Neuraminidase Inhibitor (NAI) susceptibility in influenza viruses.

VA Potdar & MS Chadha

Funding: Intramural Project Duration: 2013-2016

- M2 ion channel inhibitor is effective for Influenza A viruses and single-nucleotide polymorphism (SNP) between residues 26-34 in M2 protein is responsible for resistance. Eighteen influenza A (H3N2) & 35 A(H1N1) pdm09 viruses were confirmed to have developed resistance to M2 inhibitor by S31N mutation.

- Neuraminidase inhibitor (NAI) is effective against Type A & Type B influenza viruses. Substitution at amino acid H274Y in N1 subtype is known to occur clinically and cause clinical resistance. However, depending on the NA subtype {N1:D199N, I223R, N295S, N2: E119V, R292K, N294S ,Type B: R150K, D197E/N, I 221T, N294S, G407S}amino acid substitutions are known to occur leading to reduced susceptibility *in vitro* but their clinical impact is unknown. During the reporting period, 939 influenza A(H1N1)pdm09 positive clinical samples and 39 isolates from A(H1N1)pdm09 outbreak were assessed by allelic discrimination for H275Y. Two isolates with corresponding clinical samples showed amplification with 275Y allele which is the marker for resistance. Further phenotypic assay and NA gene sequencing confirmed the reduced susceptibility of the two isolates by high IC50 values (444.3, 840.7nm, Figure 5) and H275Y mutation in the NA gene.

- Ninety seven of 114 referred clinical samples from other states and 37/385 A(H1N1) pdm09 positive clinical samples collected through active surveillance were tested by allelic discrimination for H275Y and found to be sensitive.

- Nineteen influenza A (H3N2) and 18 type B isolates obtained through ICMR–MSM network were tested for Oseltamivir susceptibility by functional assay and were found sensitive to NAIs.

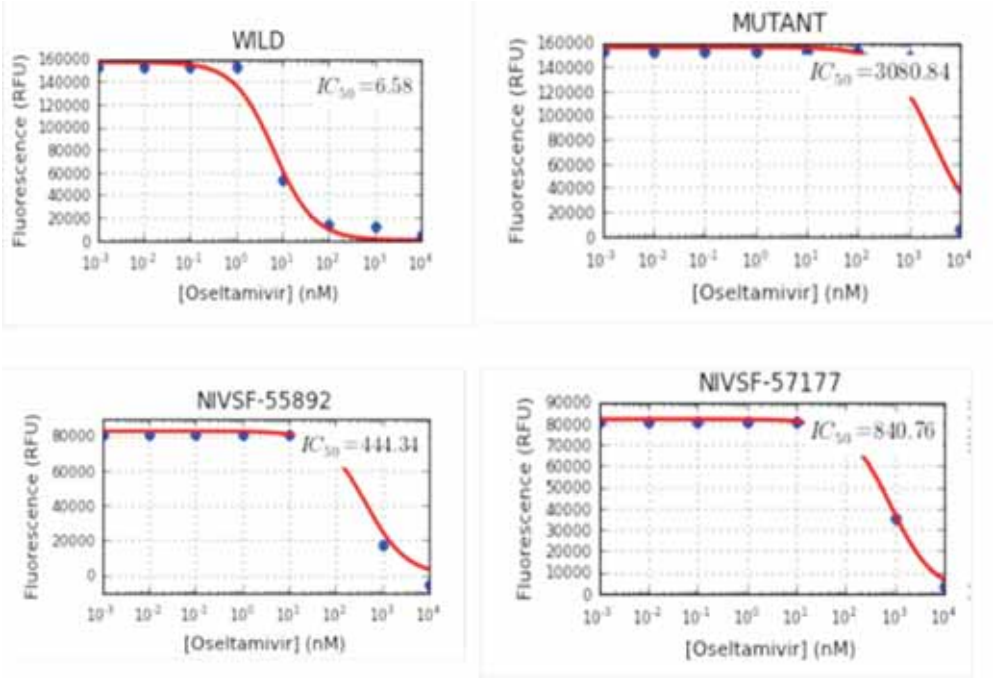

Figure 5: Neuraminidase Inhibition assay

INF1201: Association of respiratory viruses with pneumonia among hospitalized children below 5 years of age

MS Chadha, VA Potdar & ML Choudhary

Funding: Intramural Project Duration: 2014-2017

Pneumonia, an acute respiratory infection, is the single largest cause of deaths among children worldwide. Respiratory viral infections are implicated in approximately 50% of community-acquired pneumonia in young children. The Integrated Management of Childhood Illness case definition for pneumonia was used to screen the hospitalized children. All the samples were tested by real time RT-PCR for the following viruses: A(H1N1)pdm09, A/H3N2, Influenza B, Respiratory Syncytial virus (RSV), Metapneumovirus, Parainfluenza virus 1-4, Rhinovirus and Adenoviruses. A total of 173 samples (75 (2014); 98 (2015) were collected from pneumonia patients which included 101 males and 72 females. Respiratory viruses were detected in 87/173 (50%) samples which included 25 RSV A & B, 22 Rhinovirus, 17 A(H1N1)pdm09, 12 Adenovirus, 6 Parainfluenza and 5 Human metapneumovirus. Co-infections were found in 4/87 samples (RSV-A & Rhinovirus: 1; RSV-B & Rhinovirus: 1; HMPV & Rhinovirus: 1; PIV-3 & Adenovirus:1). The details of the patient age groups and positivity are shown in Table 1.

Table 1: Patients age group with positivity

Age Group in Year	Positive	Negative	Total	Percentage
Upto 1 Year	51	47	98	52.0%
>1 to d"2 Year	18	16	34	52.9%
>2 to d"3 Year	8	11	19	42.1%
>3 to d"4 Year	8	7	15	53.3%
>4 to d"5 Year	2	5	7	28.6%
Total	87	86	173	50.3%

INF1202: Immunogenetics of severe pandemic H1N1pdm09 infections

ML Choudhary, K Alagarasu & MS Chadha

Funding: Intramural Project Duration: 2014-2017

Susceptibility to severe A(H1N1)pdm09 is multi-factorial involving pathogen, host and environmental factors. There are inter-individual variations in the outcome of A(H1N1)pdm09 infections with high rates of morbidity and mortality among younger patients. To detect the single nucleotide polymorphisms (SNP) in the genes coding for pattern recognition receptors, antiviral response genes, cytokine and chemokine genes are associated with A(H1N1)pdm09 virus severity. As per WHO Severe acute reparatory illness (SARI) definition, patients with ILI and shortness of breath or difficulty in breathing and requiring hospital admission are considered as SARI patients. Patients who were hospitalized for severe disease due to A(H1N1)pdm09 infection and laboratory confirmed (positive with real time RT-PCR) were considered for the study. Ambulatory ILI patients with laboratory confirmed A(H1N1)pdm09 infection were considered as mild cases. Blood was collected from 67 severe and 60 mild patients. Of the 67 severe patients, 46 were in ICU/ventilator which included 22 death cases. Genomic DNA was isolated from whole blood using commercial kits and studies to determine the SNPs for different genes i.e. TNFA, TGFB, IL10, IFNG, MBL, VDR, CCL2 and CD209 is under progress by duplex PCR or Real Time PCR. Revised version of the project has been submitted to DBT for funding.

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AVI1301: Survey of avian influenza in wild birds during the winter migratory season, 2013 to 2016 in Maharashtra State

SD Pawar, S Pande (Ela Foundation), AV Jamgaonkar, AL Thormothe & J Mullick

Funding: Intramural Project Duration: 2013-2016

The emergence of highly pathogenic avian influenza (HPAI) H5N1 virus in wild birds and H7N9 virus in China necessitated monitoring for influenza viruses in animals and humans in India. Avian Influenza (AI) surveillance work involved trapping (Figure 1), ringing and collection of cloacal, tracheal, environmental and serum samples from wild and migratory birds.

Processing of 1071 samples from 20 avian species from Pune district revealed the absence of AI viruses in wild birds during the winter migratory bird season 2014-15.

Figure 1: Ring recovery of black-headed ibis, which was tagged during AI surveillance jointly conducted by Ela Foundation and the National Institute of Virology, Pune in March 2014.

1. Lack of evidence of H5 & H9 AI virus seroprevalence during 1954 to 1981 in India

SD Pawar, AV Jamgaonkar, U Umrani & SS Kode

India reported outbreaks of the highly pathogenic AI (HPAI) H5N1 virus in poultry, resident, wild and migratory birds in 13 states from 2006 to 2014. A retrospective seroprevalence study for AI viruses was conducted using avian sera samples collected during 1954 to 1981 for arboviral investigations. A total of 557 serum samples from 41 species comprising of 15 avian families were tested for the presence of antibodies against H5N1, H9N2 and New Castle Disease (NDV) viruses by Hemagglutination Inhibition (HI) and Microneutralization (MN) assays. All the samples were negative for the presence of antibodies against AI H5N1 and H9N2 viruses while two Jungle Crow (Corvus macrorhynchos) samples and one wild duck sample were tested positive for antibodies against NDV.

2. Susceptibility of AI H9N2 viruses to Oseltamivir carboxylate (OC) using embryonated chicken eggs as a model

DS Tare & SD Pawar

In continuation of the work on use of eggs to study susceptibility of H9N2 viruses to Oseltamivir, a total of 73 H9N2 virus isolates from Maharashtra and West Bengal were tested using fluorescence-based Neuraminidase Inhibitor (NAI) assay. All the isolates were found susceptible to Oseltamivir.

3. Detection of AI viruses from environmental samples using novel virus concentration/precipitation technology

SD Pawar, SS Keng, AL Thormothe, NM Godbole, J Mullick & DT Mourya

High & low pathogenic AI viruses namely H5N1, H9N2, H11N1 and H4N6 were tested using a novel water precipitation method, developed at NIV, Pune. This was performed using tap, dam and sea water samples. Various doses of viruses [10-1000 infectious particles (IP)] were spiked and the virus was detected using Real-Time RT-PCR. Ten IP of H5N1, 50 IP of H9N2, H11N1 and 100 IP of H4N6 viruses could be detected. Interestingly, this methodology did not affect survivability of the tested AI viruses. In conclusion, this could be useful for testing water/environmental samples for detection of AI viruses during AI surveillance & outbreaks. Further work on this aspect is in progress.

AVI1302: Operation and maintenance of High Containment Laboratory (SERVICE PROJECT).

J Mullick, SD Pawar & AB Khare

Funding: Intramural Project Duration: Ongoing

High Containment Laboratory also known as Biosafety Level 3 (BSL-3) laboratory provides a safe environment for the personnel working with HPAI H5N1 virus and other highly infectious agents that require containment. Thus, we are constantly working for the smooth operation and maintenance of the BSL-3 laboratory. During this period, the BSL-3 laboratory has supported the experiments of several investigators working with HPAI H5N1 and pandemic influenza 2009 (H1N1pdm2009) as well as experiments in primates with Parvovirus 4 (from Gorakhpur by members of the Hepatitis department). Additionally, we have provided several trainings on General Biosafety and specialized BSL-3 training for staff and students of NIV. A WHO-NIV Biosafety training for participants from SEAR countries was also conducted (Figure 2).

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Figure 2: Glimpses of Biosafety trainings conducted by the AI department during 2014-2015

AVI 1101: Studies on host-virus interactions of low-pathogenic and high-pathogenic avian influenza viruses.

J Mullick, MM Thube, SD Pawar & P Shil

Funding: Intramural Project Duration: 2012-2017

Influenza A viruses circulate globally and continue to be a potential threat for both humans and animals due to their potential to cause epidemics and pandemics. They infect a broad range of hosts among which aquatic birds are known to be the natural swarm representing a global reservoir of influenza genes. Influenza viruses infecting birds are termed as avian influenza (AI) viruses and are broadly classified as low-pathogenic (LPAI) and high-pathogenic (HPAI) viruses primarily on the basis of pathogenesis. Both the forms are known to circulate in poultry and migratory birds. Though the primary virulence characteristics that separate the HPAI from the LPAI lies in the ability of the virus to get cleaved by host proteases, the role of viral genes such as polymerase (PA), non-structural protein (NS1) and many more cannot be ruled out. In this study, we are attempting to understand the molecular basis of differences of the LPAI and HPAI in modulating the host's innate immune response. Earlier we have seen that two strains of LPAI H9N2 are phylogenetically distinct from each other with respect to the NS1 gene. Homology modeling of the respective NS1 proteins also supports the data. Comparison of their replication kinetics in MDCK cells and A549 cells

suggest that Strain 2 replicates rapidly than the Strain 1 virus (Figure 3). Studies by gene expression analysis suggest that Strain 1 induces higher innate immune response than Strain 2 until 24hr which coincides with the interferon inhibiting ability of the respective strains. Further studies on gene expression and molecular modeling are ongoing.

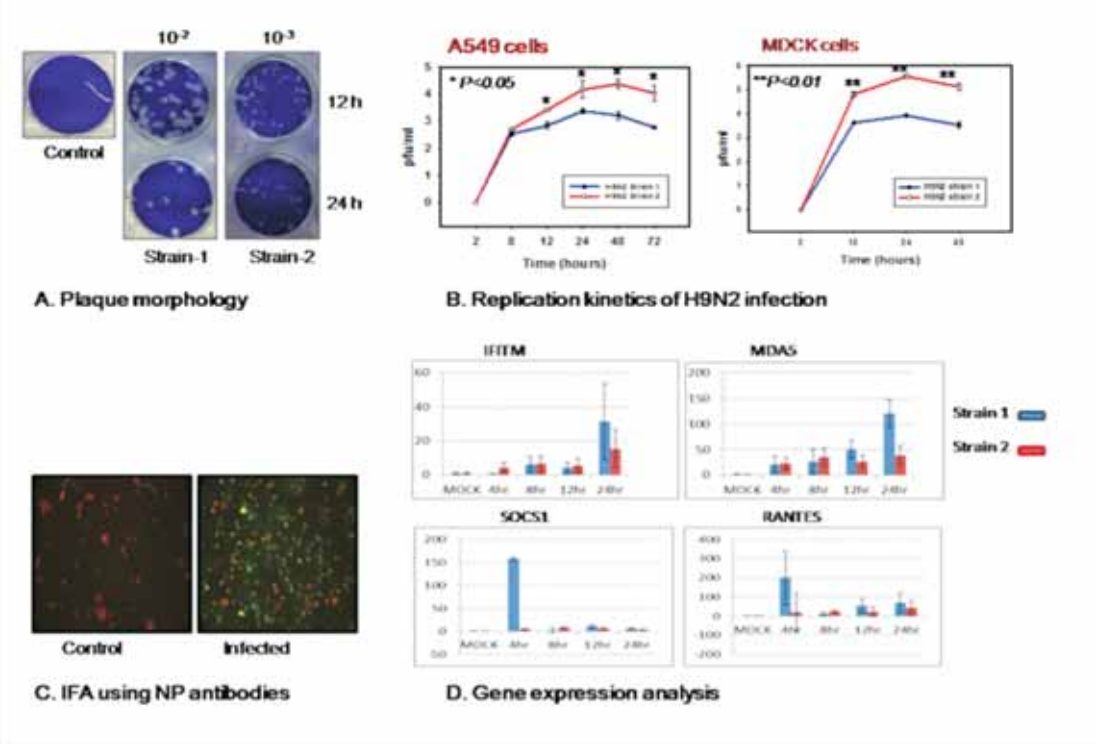

Figure 3: Replication kinetics and gene expression analysis of AI H9N2 virus infection.

AVI1008: Studies on the inflammatory mediators in osteoclast differentiation.

J Mullick, R Kasbe, MR Wani (NCCS) & AB Sudeep

Funding: Intramural

Project Duration: 2011-2016

Viral infections are known to trigger inflammatory mediators such as interleukins, interferons and complement anaphylatoxins. Involvement of complement anaphylatoxins C3a and C5a in dengue virus and Ross River virus (RRV) infections with the latter affecting the bones and resulting into severe monocytic inflammation of the bone, joints and skeletal muscle tissues has been reported. In addition to the primary role of complement in innate immunity, it is also known to play a major role in differentiation. In the present study, an attempt was made to determine whether complement imparts any role in Chikungunya virus (CHIKV), also an arthritogenic Alphavirus, leading to osteoclast differentiation and progression of virus induced polyarthritis, similar to that observed in RRV infection. Our earlier results have shown the involvement of complement pathways in osteoclast differentiation. During this period we have initiated experiments in complement knockout mice infected with CHIKV. In addition, bacterially expressed and purified

mouse C5a and mouse C5a des-arg have been identified by MALDI-TOF. Further work on gene expression during osteoclast differentiation and CHIKV pathogenesis in mice is ongoing.

AVI 1303: Role of mitochondria in vitamin D metabolism & innate immune response during influenza infection. (ICMR-SRF Project, submitted to DBT)

NM Godbole, J Mullick & S D Pawar

Funding: Intramural Project Duration: 2014-2016

Toxicity of vitamin D in human lung epithelial A549 cell line was assessed using MTT assay. Differential susceptibility of A549 cells to high & low pathogenic AI viruses was also studied. Further work on the association of virus infection & Vitamin D in A549 cell line is in progress.

COLLABORATIVE PROJECTS

An International Collaborative study with The CONsortium for the Standardisation of Influenza SeroEpidemiology (CONSISE) Working Group

SD Pawar & SS Kode

CONSISE is a global partnership to develop influenza investigation protocols & standardize seroepidemiology to inform health policy. Following infection with novel influenza virus most people develop specific antibodies that persist for months, which are quantified using MN and/or HI assays. An international within laboratory (lab) comparison of the 2-day ELISA (WHO) & the newly developed 3-day HA-MN protocol using influenza viruses was performed. Eleven labs participated in the study. We established and performed 2-day and 3-day assays using AI H5N1 virus. In conclusion, the ratio of titres between the 2-day and 3-day assay was similar. Overall, in most labs, there was good correlation between the results obtained using the two day assay protocols. Further work on standardization of serological assays is in progress.

CONSISE collaborative study to assess the variability of ELLA in measuring influenza virus neuraminidase inhibiting antibody titers

SD Pawar & SS Kode

Neuraminidase-inhibition (NI) antibody titers can be used to evaluate the immunogenicity of influenza vaccines. Reproducibility of newly developed Enzyme-Linked Lectin Assay (ELLA) from different labs is not well studied. Using CONSISE network, a study was conducted to evaluate the reproducibility of ELLA. Twenty laboratories that had no previous experience with ELLA were provided materials and guidance to establish the assay. We established ELLA in our laboratory and determined NI antibody titers using a set of reference standards in 3 independent assays and the data was submitted for statistical analysis. The results demonstrated that the ELLA provides reproducible results within each laboratory. As a result of this study, the various technical parameters of ELLA were assessed and those that were critical to the success of the assay were compiled with a view to develop a consensus ELLA protocol.

AVIAN INFLUENZA GROUP

CDC-NIV Collaborative Project: Surveillance for Avian Influenza H7N9 virus in humans

SD Pawar, BV Tandale, RS Mali, VA Potdar, SS Kode & MS Chadha

Funding: Intramural & Supplemental Funding from CDC, Atlanta, USA (under Cooperative Agreement No 5U51IP000333CDC) Project Duration: 2013-2014

The emergence of a novel AI H7N9 virus in eastern China has caused an alarming situation. Human infections of H7N9 virus have been associated with poultry exposure. The present study was conducted to determine H7N9 virus infections among poultry workers (as a high-risk group) in Maharashtra and North-Eastern India during 2014. A total of 540 poultry workers from Eastern Maharashtra and North-Eastern states (West Bengal, Assam) were enrolled in the study during January to November 2014. None of the workers were found positive for either influenza A virus infection or for the presence of antibodies against H7N9 virus.

AVI1006: Role of complement during Influenza infection (NCCS Collaborative Project).

A Sahu (NCCS), A Anujrattan, SD Pawar & J Mullick

Funding: Intramural Project Duration: 2010-2015

Complement system is a key component of the innate immunity evolved to protect host from a wide repertoire of pathogens including viruses. The emergence of pandemic influenza A(H1N1)pdm09 virus and the highly pathogenic H5N1 influenza virus in the last two decades has created considerable public health concern and rekindled global interest in understanding factors influencing influenza virus pathogenesis. Earlier studies implicated the role of classical pathway (CP) in controlling seasonal influenza infection, but its role in coping with A(H1N1)pdm09 infection was not studied. Further, no efforts were made to understand the role of individual complement pathways during influenza infection. Our ***in vitro*** studies showed that the virus is efficiently neutralized by CP, but is completely resistant to the alternative pathway (AP) unless coated by antibodies indicating that the viral surface is not amenable for C3 attachment. ***In vivo*** infection studies in C3-/- mice revealed severe weight loss and 100% mortality as early as 9th day post-infection. The mice also showed prolonged viral infection and increased degree of pulmonary consolidation, strongly establishing the role of complement in controlling the A(H1N1)pdm09 pathogenesis. Infection studies in C4-/- (deficient in CP/LP) and factor B-/- (deficient in AP) mice showed significant weight loss with 30%-40% mortality suggesting co-operativity among the complement pathways (Figure. 4). Further our data show that CP-mediated neutralization of the virus is enhanced by AP owing to activation of the AP-loop, and that blocking the signaling mediated by C3a and C5a, which are generated during complement activation and mediate protective immune response, resulting in severe weight loss and mortality in infected wild-type mice. Together our data indicate that complement activation pathway co-operativity during influenza infection influence the outcome by efficient neutralization of the virus as well as enhancing protective immune response against the virus.

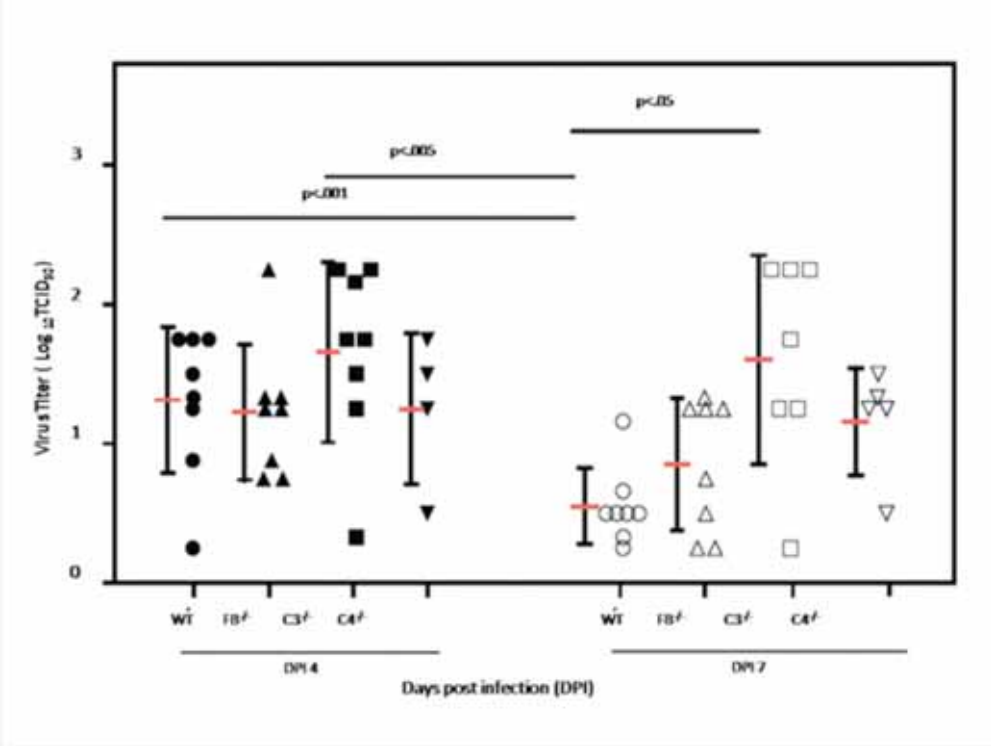

Figure 4: Virus titer from lungs of wild type (WT), C3 knockout (C3-/-), C4 knockout (C4-/-) and factor B knockout fB-/-) mice infected with Influenza A(H1N1)pdm09 virus.

NABL Accreditation of BSL-4, Human and Avian Influenza Laboratories as per ISO/IEC 17025:2005

The documentation for ISO/IEC 17025:2005 NABL Accreditation has been completed. Quality system teams were formed and Quality Manual, Procedure Manual, Standard Operating Procedures (SOPs) and other required documents were prepared. We also completed Internal Auditors training course conducted by the Standardization Testing and Quality Certification Directorate, Department of Electronics and Information Technology, Government of India at Pune and executed internal audits of these departments. Quality system was established and applied to NABL office, New Delhi for acreditation.

Dr. SD Pawar, Mrs. SS Kode and Mr. SS Keng worked as Quality Manager, Deputy Quality Manager and Technical Manager, respectively to establish the Quality system as per ISO/IEC 17025:2005 guidelines.

HEPATITIS GROUP

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HEPATITIS GROUP

HEP1310: Development of HCV genotype 3 replicons and infectious molecular clones; identification of adaptive mutations and understanding their biological mechanisms.

KS Lole & VA Arankalle

Funding: DBT (Extramural) Project Duration: August 2013-July 2016

In the absence of an efficient cell culture system and small animal model, HCV replicon has been extensively used as a tool for understanding the mechanisms of HCV replication, proliferation and antiviral testing. Due to extensive genetic heterogeneity in HCV, it is desirable to develop replicating system for each genotype separately. Genotype 3 being the most prevalent in India, we developed full length cDNA clone for genotype 3a. It was decided to develop two subgenomic replicons (with Neo and RLuc reporters). We chose to generate subgenomic replicons of HCV using Gibson assembly method. This new strategy helps in reducing cloning steps by which we can minimize accumulation of nucleotide changes in the cloned sequences which normally occur during assembling of the overlapping fragments to generate complete clone. Both the clones were completely sequenced to confirm successful generation of subgenomic clones. Common adaptive mutations such as S2210I which was reported to increase the replication efficiency of genotype 1a (H77), 2a (JFH 1), 2a (JFH 2) clones as well as mutations reported for HCV 3a, S52 or S310 clones, were also introduced into the subgenomic replicons using site directed mutagenesis (Table 1). Overall, eight subgenomic clones were developed.

Table 1: Changes introduced in the clones

Clone	Amino acid Change	Region
S52	V→A	NS3
	T→P	NS3
	P→S	NS3
	D→H	NS3
	P→L	NS3
	M→V	NS3
	T→A	NS3
	T→I	NS3
S310	R→G/K	NS5B
	S→I	NS5A

During HCV replication, the negative sense RNA strand of the genome is generated as replication intermediate. Presence of negative sense RNA indicates active replication of the HCV genome in the cell culture system. During virus replication, both positive sense genomic RNA (high copy number) and negative sense RNA (low copy number) exist simultaneously in an infected cell. Accurate detection of the negative sense is hampered by the presence of high copy number of positive sense RNA. For optimization of specific RNA detection, we synthesized both negative sense and positive sense RNAs by *in-vitro* transcription and mixed these molecules in various proportions and used them as templates for standardization of the negative strand detection by RT-PCR. When positive sense RNA was processed using negative strand specific tagged primer,

there was a false positivity up to 10^6 copies per reaction. However, at 10^5 copies of positive sense RNA, reaction showed complete negative result indicating that the negative sense RNA detection can be carried out in presence of 10^5 copies of genomic RNA without noise. We are currently testing replication competence of subgenomic clones harbouring Neo gene in human hepatoma cells, *Huh7.5*, by detecting resistance to G418.

HEP 1019: Attempts to identify putative Gorakhpur agent using SOLiD technology

VA Arankalle, KS Lole, KP Kushwaha

Funding: Extramural (ICMR) Project Duration: May 2011 till June 2014

Massive seasonal outbreaks of non-JE-AES with high mortality (13.9%-21.3%) continue to occur in Gorakhpur district and the adjoining areas of Uttar Pradesh since 2006. Next Generation Sequencing (NGS) platform, Ion Torrent Personal Genome Machine (PGM, Life technologies, USA) was used to analyze the presence of etiologic agent in the clinical specimens. We investigated 144 pediatric cases (119 in 2011 and 25 in 2012) including 10 fatal cases. These included 83 (57.6%) children below the age of five, 27.1% children (39/144) in 5-10 year age group and 15.3% children (22/144) in 11-15 year age group. Of these, 22 acute-phase specimens were used for NGS experiments. Analysis of CSF/serum pooled samples identified 12 contigs of Human Parvovirus 4 (HPARV4). These were closely related to the sequences reported from CSF of two AES patients previously reported from South India. On screening of additional samples using nested PCR, 15/51 (29.4%) CSF and 6/31 (19.3%) serum samples showed presence of PARV4 DNA (Figure 1). Full genome analysis of the representative samples classified these sequences as genotype 2. Within genotype 2, the Indian sequences from Gorakhpur (this study) and South India formed distinct clusters, the percent nucleotide identity being 98.1 ± 0.005%.

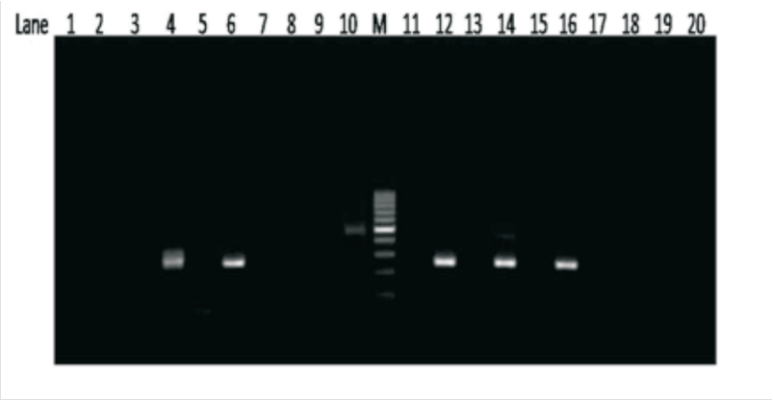

Figure 1: Ethidium bromide stained gel picture showing amplification of a 256bp nested PCR product. Lanes: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 indicate test samples while lanes 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 show negative controls processed simultaneously; M=100bp molecular weight marker

Full genome sequencing and phylogenetic analysis:

One CSF and two serum samples were positive for PARV4 DNA after the first PCR indicating high viral load and were used for full genome sequencing. Sequences of 4936, 4937 and repeat 4936 nucleotides were generated. Phylogenetic analysis (Figure 2) classified these sequences as genotype 2. Within genotype 2, the Indian sequences from Gorakhpur (this study) and South India formed distinct clusters, the percent nucleotide identity being 98.1 + 0.005%. Genotype 2 was further subdivided into two groups with a percent nucleotide difference of 3.8 + 0.005%. The *intra-genotypic* difference was 7.4 – 8.9%.

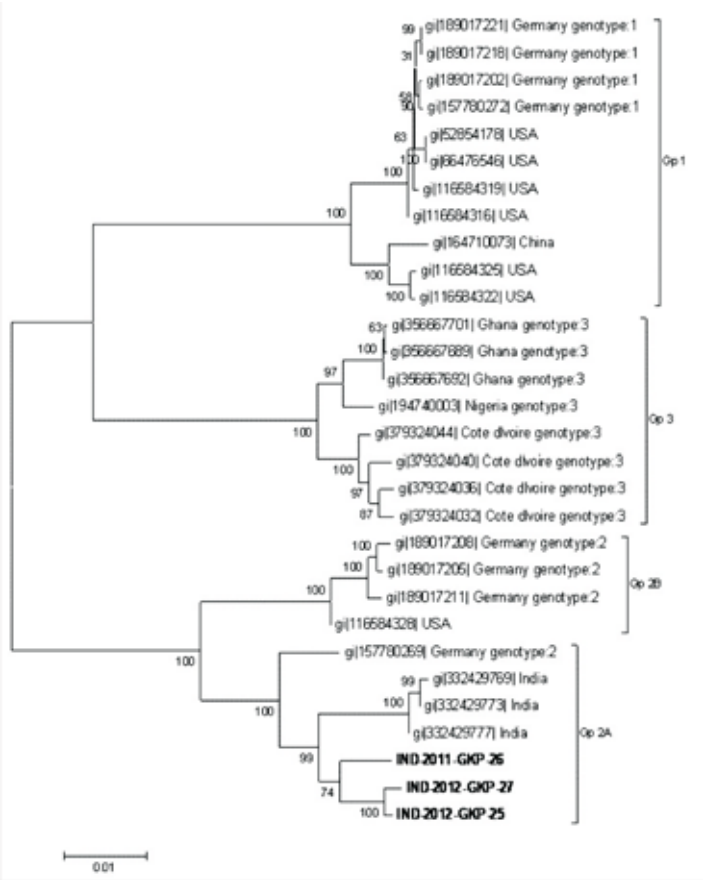

Figure 2: Phylogenetic analysis of almost full genomes sequenced during this study, shown in bold (Accession numbers KJ541119, KJ541120 and KJ541121). Full genome sequences available in the Genbank database are denoted by the respective accession numbers. Percent bootstrap support is indicated at each node. Genotypes are designated as brackets.

Partial NS1 sequences from 24 additional samples showed identical results. An ELISA for the detection of IgM-anti-PARV4 antibodies, employing recombinant VP2 protein, showed 72.9% IgM-anti-PARV4 antibody positivity. None of the samples from apparently healthy children, 109 and 25 from Pune and Gorakhpur, respectively, were positive for IgM-anti-PARV4 antibodies.

HEPATITIS GROUP

Serological and molecular data provide strong evidence for the association of PARV4 with the seasonal outbreaks of AES. Though epidemiology of HPARV4 in India is not understood, high positivity rates in CSF and serum do suggest strong association of this virus with AES in Gorakhpur. Studies are required to assess if HPARV4 is just an indicator of another encephalitis-causing agent or the agent itself.

HEP1317: Development of virus like particles (VLPs) of hepatitis A virus; development of diagnostic assay and application/evaluation as an immunogen in mice

KS Lole, SL Hundekar

Funding: Intramural Project Duration: 2014-2017

Hepatitis A virus (HAV) infections occur in early childhood, mainly as sub-clinical infections leading to a life-long immunity. Improvements in sanitation and hygienic conditions have contributed to a shift from high to intermediate endemicity resulting in increase in the number of susceptible adolescents/ young adults, especially in urban and semi-urban areas in India. With increasing age, clinical manifestations of the disease become more severe. HAV outbreaks in the recent past have indicated need of routine hepatitis A vaccination. Replication of HAV in culture is slow and has poor yields, thus the production of inactivated vaccines is difficult and expensive. We will be attempting to develop HAV VLPs using baculovirus/mammalian expression system and evaluate them as coating antigen in diagnostic ELISA and as an immunogen in mice.

HAV structural genes (P1-2A) and 3C protease were cloned either separately or at tandem (each gene with independent polyhedrin promoter) in baculovirus transfer vector, pFastBac1. Recombinant baculoviruses were developed (Bac P1-2A, Bac 3C and Bac P1-2A/3C). Insect cells (Sf9) were infected and both cell pellets and supernatants were monitored for protein expression by ELISA. Assumption was that the co-expression of P1-2A and 3C in a single cell will lead to P1-2A processing by 3C (to yield VP1, VP2, VP3) and probably result in secretion of these proteins. To check this, cells were either co-infected with the Bac P1-2A and Bac 3C recombinants or with the Bac P1-2A/3C recombinant and cell pellets and supernatants were harvested after different time intervals. Cell lysates and supernatants were tested for protein expression in an ELISA using anti-HAV antibody positive human serum samples (Table 2).

Table 2: Testing of infected Sf9 cell pellets for HAV proteins in ELISA

Recombinants	Positive control	Negative control
Bac P1-2A	1.11	0.08
Bac 3C	0.91	0.09
Bac P1-2A+ Bac 3C	1.0	0.10
Bac P1-2A/3C	0.45	0.14

Infections with BacP1-2A or Bac 3C recombinants alone showed significant levels of protein expression, however, these proteins remained cell associated. There was no significant increase in

the reactivity due to co-expression of P1-2A and 3C. This indicated that there is no processing of P1-2A by 3C in Sf9 cells. This was also confirmed by analyzing cell lysates in a Western blot. None of the infected cell supernatants were reactive in ELISA indicating absence of protein/VLP secretion.

HEP1316: Investigating the role of papain like cysteine protease in hepatitis E virus replication

KS Lole, S Desai

Funding: Submitted to DST (Feb 2014) Project Duration: 2014-2017

This study focuses on the viral papain-like cysteine protease (PCP) from a human strain (genotype 1, GenBank: DQ459342.1) and a swine strain (genotype 4, GenBank: AY723745.1) of hepatitis E viruses (HEV). A putative papain-like protease domain is encoded by the 440–610 amino acid residues in ORF1 polyprotein of HEV. The protease domain is flanked by the Y-domain and proline hinge region. Conservation of the X-domain, which has been exclusively found in association with viral papain-like proteases, suggests that HEV encoded protease bears similarity to proteases observed in other positive strand RNA viruses like alphaviruses and rubella virus. The postulated role of PCP is polyprotein processing of ORF1 polyprotein. It is not entirely clear whether the ORF1 polyprotein is processed into biochemically distinct units by the viral protease. Papain like cysteine protease has a putative catalytic diad having Cys and His at its active site. To confirm the active site we have mutated Histidine residues at the 458 and 497 positions, in genotype 1, and substituted it with neutral amino acids. Amino acid residue at the 497 position, in genotype 4 was substituted with histidine. The protocol for the expression and purification of PCP was optimized. Confirmation of the protein expression was done by Western blot analysis. Additional construct of PCP was developed, which included *proline-rich* region and macro domain region. These constructs will be analysed to see whether these neighboring regions have any influence on the enzymatic activity of the protease.

HEP1318: Development of ORF2 protein based ready-to-use ELISA kit for diagnosis of hepatitis E

TM Deshmukh & KS Lole

Funding: Intramural Project Duration: 2014-2015

HEV continues to be a significant health problem in India. Several water-borne epidemics of varying magnitudes were documented in India since the discovery of HEV. The disease occurs in sporadic and epidemic forms and is the predominant cause of acute hepatitis and acute liver failure in India. Though assays are available commercially for hepatitis E diagnosis they canot be used for routine diagnosis due to high cost. (MP Diagnostics, formerly Genelabs, Singapore, (₹ 20,000/- for 96 tests by ELISA and ₹ 8,000/- for 20 tests rapid immuno-chromatographic-assay),

Very often we need to investigate acute hepatitis outbreaks of varying magnitudes. We reported the utility of an ELISA based on ORF2 protein expressed in insect cells for detection of

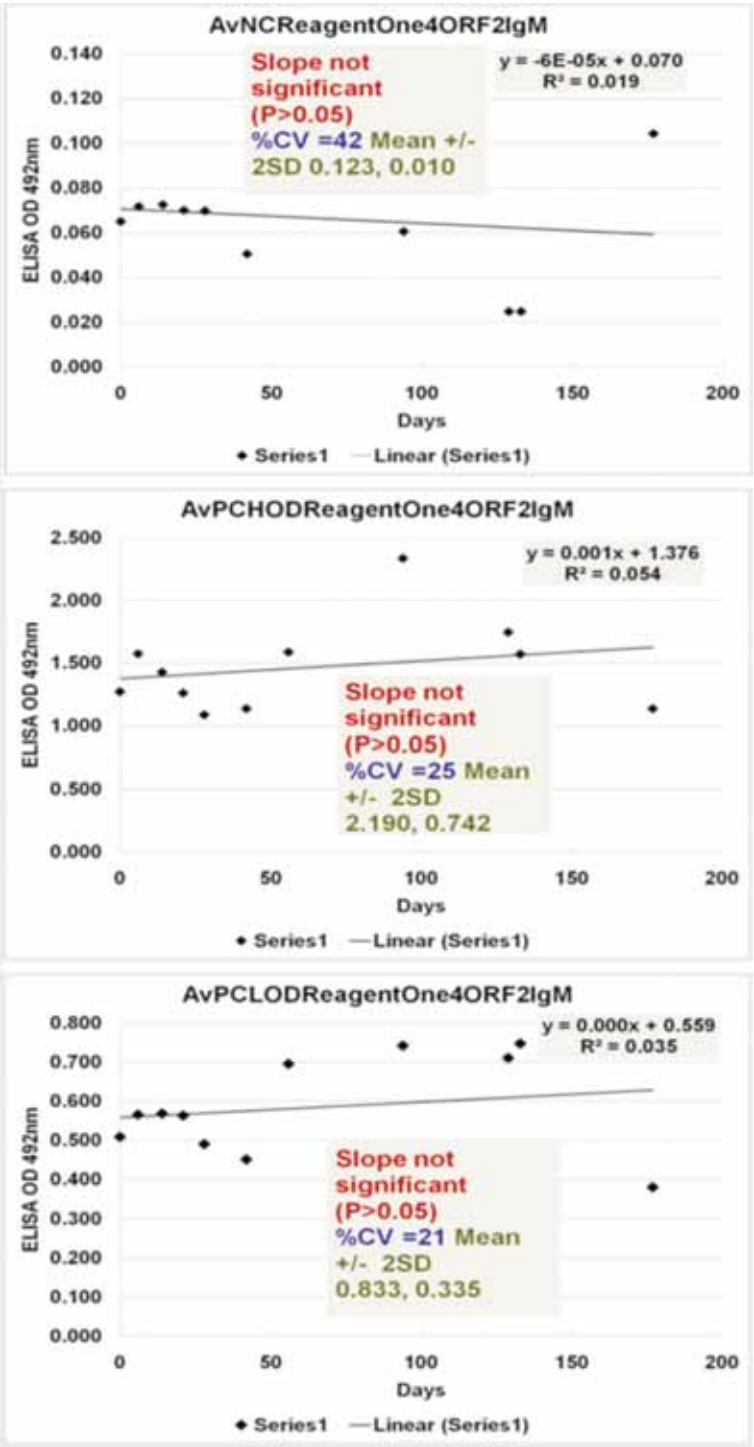

Figure 3: Scatter plot of NC, PC HOD, and PC LOD. OD values obtained over the testing period under 4°C assay condition in ORF2 IgM ELISA using Reagent 1.

HEV infection and was found more sensitive as compared to the Genelabs assay. We use the in-house developed ELISA for routine diagnosis of HEV infection during outbreaks. However, the assay components have limited stability and need to be prepared fresh before every test, making it technically demanding and resource consuming. Adaptation of the ELISA to ready-to-use kit by improving the stability of the assay components would facilitate hepatitis E diagnosis simple and cost-effective.

Performance of anti-HEV antibody detection ELISAs (IgM and IgG) based on ORF2 and T1NE (bacterially expressed neutralizing epitope region protein present within ORF2 protein) proteins were tested over a period of 6 months. Stabilizing reagents used were coating stabilizers (Reagent 1 and Reagent 2) and conjugate diluent (Reagent 3). Following controls were tested in quadruplicate; negative control (NC), positive control with high OD (PC HOD) and positive control with low OD (PC LOD). Performance was tested under 6 assay conditions; Routine Fresh, Routine 4°C, Routine 37°C, Reagent 1, 4 and 37°C, Reagent 2, 4 and 37°C, Reagent 3.

Representative results of OD value (NC, PC HOD, PC LOD) distribution over the testing for ORF2 protein based anti-HEV IgM antibody detection ELISA are given in Figure 3.

Note: Irrespective of the ELISA assessed and the stabilizing reagents used, change in OD values of all the 3 types of controls was insignificant over the testing period under all 6 assay conditions. Use of stabilizers improved the performance of all ELISAs assessed.

HEP1304: Study of T regulatory cells in Hepatitis E infection

AS Tripathy & SB Rathod

Funding: Intramural Project Duration: 2013- 2016

Reports from the Indian subcontinent indicating high mortality in patients with hepatitis E infection has resulted in attempts to understand HEV pathogenesis. Understanding the immune correlates that contribute to the host immune response leading to recovery may help in designing an efficacious vaccine/immune based treatment strategy. Reports have indicated that immune response to HEV infection underlies the pathogenesis of the disease. To gain insight on how HEV infection influences the overall expression profiles on the PBMCs, we analyzed and compared the alterations in unstimulated and HEV rORF2p stimulated immunophenotypic expressions (by flow cytometry), and gene expression patterns (by Taqman Low Density Array, TLDA) of activatory, inhibitory, homing, integrin, ectonucleotidase machinery, co-stimulatory, inflammatory markers and Treg associated cytokines on HEV rORF2p stimulated and unstimulated PBMCs of 43 acute HEV patients, 30 recovered individuals and 43 controls. The phenotypic expressions of key molecules CTLA-4, GITR, CD103, CD25, CD69, IL10 and TGF-β, in the acute patients, TGF-β, in the recovered individuals were significantly elevated on both unstimulated and stimulated PBMCs. Gene expression array data revealed upregulations of CD25, PD1, CD103, CCR4, IL10 and TGF-β, on both unstimulated and HEV rORF2p stimulated PBMCs of acute patients. The observed upregulations of inhibitory, integrin, activatory and Treg associated cytokine genes (Figure 4) on the PBMCs of acute HEV patients complemented by their frequency data suggest them as the major players in the fine tuning of immune response in self-limiting Hepatitis E infection.

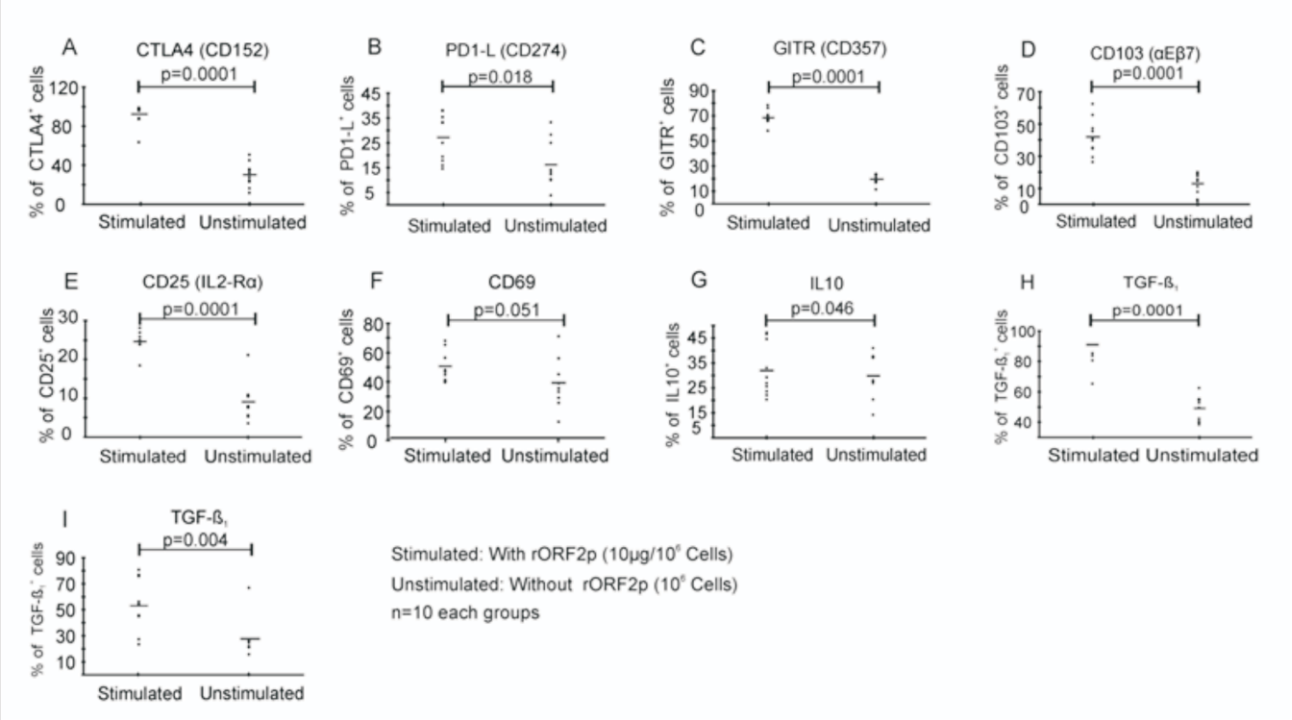

Figure 4: HEV rORF2p stimulated peripheral expression profiles in hepatitis E patients by flow cytometry HEV rORF2p stimulated expression of inhibitory (CTLA-4, PD1L, GITR), activation (CD25, CD69), integrin (CD103), and Treg associated cytokines (IL10, TGF-β.) on PBMCs of AVH-E (A-H) and recovered (I), n= numbers of patients. Each dot represents an individual data point and the horizontal lines represent the mean. The ANOVA with Bonferroni post-hoc corrections was used to compare differences among groups. Data are representative of mean ± SD.

HEP1302: Immunological memory in Hepatitis E infection

AS Tripathy, VA Arankalle, S Kulkarni

Funding: Intramural Project Duration: 2013-2014

Whether HEV infection confers long lasting immunity against re-infection or not is debatable. Long term maintenance of memory responses is the basis of most vaccine development studies. At NIV, we have developed a candidate vaccine against HEV. Balb/c mice were immunized (5µg rNEp + liposome-based adjuvant) with the candidate vaccine to determine longevity of vaccine induced immune response. Unimmunized mice and mice inoculated with adjuvants were used as controls. Anti-HEV IgG titers were detected high till 54 wks post immunization (PI). Spleens were harvested at different time points PI and staining was done for immunological memory markers. Memory B cells were high at 16 wks PI. Functionality of memory B cells was assessed by ELISPOT at 24 wk PI. Spots were seen in splenocytes from immunized mice, while no spots were seen in controls. This study shows that the vaccine candidate is highly immunogenic in mice.

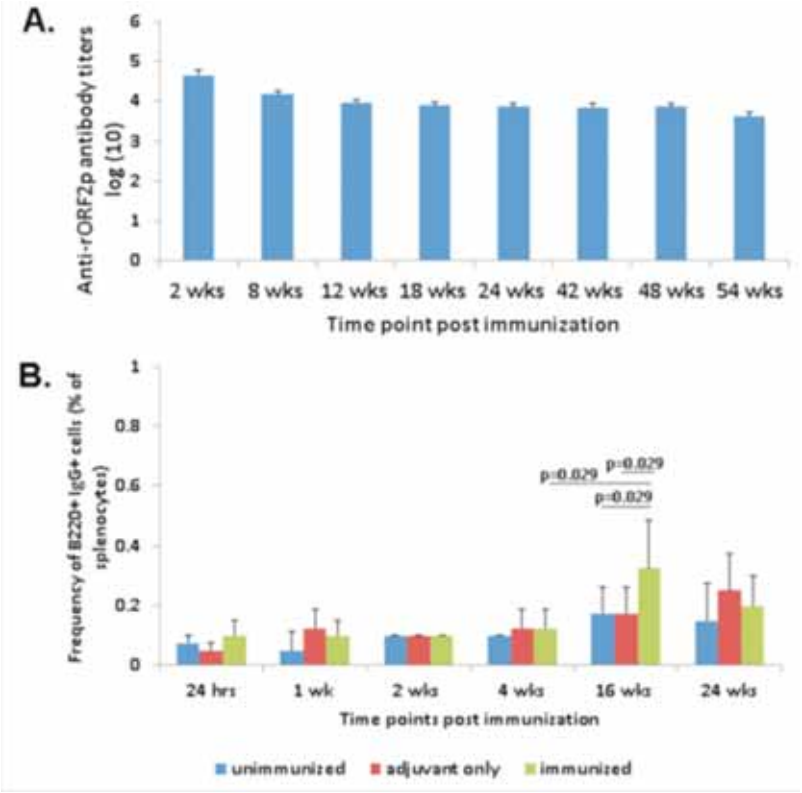

Figure 5: (A) Antibody titres in immunized mice at different time points (B) Frequency of memory B cells in unimmunized, adjuvant only and immunized groups

Similarly, B and T cell memory were assessed in patients with HEV infection. Thirty one acute HEV patients, 10 convalescent individuals and 16 healthy controls from Vilaspur and Wai areas of Maharashtra during HEV outbreaks were assessed for percentage of various immunological memory markers by staining and flow cytometry. Memory B and Th cells were high in controls as compared to acute and convalescent groups. HEV specific memory responses assessed by stimulating patients' PBMCs with affinity purified HEV-rORF2p followed by staining yielded absence of memory response in acute patients (Figure 5).

HEP1009: Study of the involvement of host factors in Chikungunya infection

AS Tripathy, MA Ganu & P Jaywant

Funding: Approved by ICMR

Project Duration: 2014-2017

The re-emergence of Chikungunya in an explosive epidemic form in India during 2005 warranted the need to understand the role of host and host genetic factors in chikungunya pathogenesis. Patients attended by Dr. Ganu at the Sanjeevan Hospital, Latur, Maharashtra were selected as the study population. Three camps were organized for collection of chikungunya samples in 2014. During the 1st camp (April 2014), 73 samples were collected and screening of the samples detected the presence of anti-CHIK IgM and anti-CHIK IgG in 4 and 67 patients, respectively. In the 2nd camp (August 2014), 23 samples were collected and screened for antibodies. Out of these, 20 were positive for anti-CHIK IgM and 22 were positive for anti-CHIK IgG. In the 3rd camp (December 2014), 54 samples were collected, out of which, 27 were positive for

anti-CHIK IgM and 47 samples were IgG positive. Cytokine assay was carried out for these samples by stimulating the PBMCs with purified CHIKV antigen. For assessment of cytokine polymorphism, DNA was extracted from 116 samples of chronic chikungunya infection; 93 of acute chikungunya patients, 13 of recovered individuals and 58 healthy controls by QIAamp DNA Blood Mini kit. Cytokine polymorphism was assessed for 24 samples by PCR-Sequence specific primer methodology (One lambda Kit, Biogenuix). Polymorphism of cytokines TNF a-308, TGF β1-10, TGF β1-25, IL10-1082, IL10-819, IL10-592, IL6-174, IFNβ+874 were assessed for 24 patients'samples. Expression of killer immunoglobulin receptor (KIR) has also been assessed phenotypically on natural killer cell (NK) in anti-CHIK IgG negative controls (n=38) by performing surface marker staining with CD3, CD56, CD158a (KIR2DL1) and CD158b1/b2 (KIR2DL2/DL3). Expression of KIR markers will be assessed on chikungunya patient's samples.

HEP1011: Studies on the involvement of natural killer cells and cytokine signalling inhibitors in chikungunya virus infection

AS Tripathy & S Thanapati

Funding: Intramural

Project Duration: 2012-2016

NK and NKT cells are the important sentinels of innate immune responses that play a major role in the control of viral pathogenesis. With this background, we have carried out a study to characterize NK (CD56+CD3–) and NKT-like cell (CD56+CD3+) responses early after chikungunya infection. Expression profile and functional analysis of T/NK/NKT-like cells were carried out in 56 acute, 31 convalescent chikungunya patients and 56 control individuals. Percentage of NK cells was high in patient groups, whereas percentage of NKT-like cells was high only in convalescent group. Percentages of NKp30+CD3-CD56+, NKp30+CD3+CD56+, CD244+CD3-CD56+, CD244+CD3+CD56+ were high, while NKG2D+CD3-CD56+, NKG2D+CD3+CD56+ were low in the patient groups. Percentages of NKp44+CD3-CD56+ cells were high in the patient groups, while NKp44+CD3+CD56+ cells were low in the acute cases compared to controls. NKp46+CD3-CD56+ cell percentages were high in both the patient groups. Higher percentages of perforin+CD3-CD56+ and perforin+CD3+CD56+ cells were observed in acute and convalescent patients, respectively. Cytotoxic activity was observed in acute patients compared to controls. IFN-ã expressions on NK cells of the convalescent patients and on NKT-like cells of both the patient groups were indicative of the regulatory role of NK and NKT-like cells. Current data collectively (Figure 6) showed that higher expression of activating receptors on NK/NKT-like cells and perforin+ NK cells in acute patients could be responsible for increased cytotoxicity. Classical switch of expression of perforin+ NK cells in the acute phase to perforin+ NKT-like cells in the subsequent convalescent stage allows us to establish unequivocally that NK/NKT-like cells mount an early and efficient response to CHIKV. Study assessing the molecular mechanisms leading to limit viral dissemination/establishment of chronic disease will be helpful towards the understanding of NK/NKT-like cell mediated control in chikungunya infection.

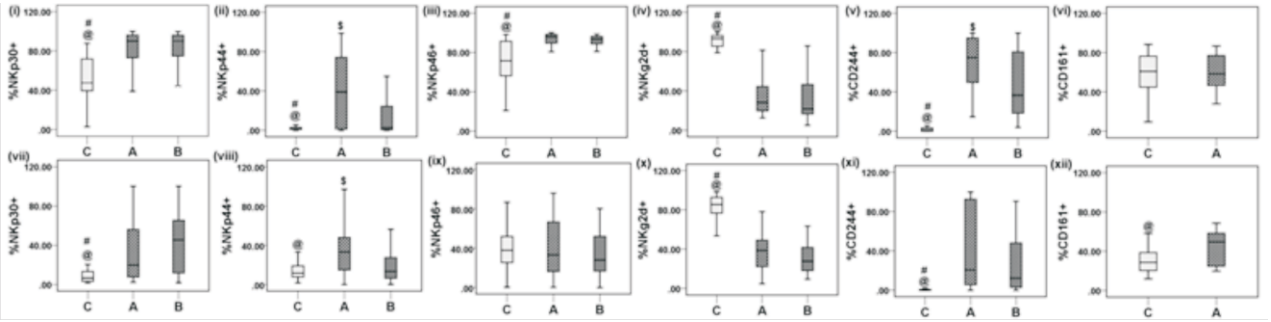

Figure 6: Patterns of NK receptors (NKRs) on NK and NKT-like cells. Frequencies of NKRs were enumerated from the whole blood of 30 control subjects, 30 acute and 31 convalescent patients. Male/female ratios are 0.87, 0.6 and 0.29 in control, acute and convalescent, respectively. Box plots (i-vi) show percentages of NKRs+ NK (CD3-CD56+) cells as (i) NKp30, (ii)NKp44, (iii) NKp46, (iv) NKG2D, (v) CD244, (vi) CD161. Box plots (vii-xii) shows FACS analysis of percentages of NKRs+ NKT-like (CD3+CD56+) cells as (vii) NKp30, (viii) NKp44, (ix) NKp46, (x) NKG2D, (xi) CD244, (xii) CD161. CD161 percentage was enumerated in acute patients and controls. Kolmogorov-Smirnov-test was used for intergroup comparison. p value <0.05 is considered significant

HEP1301: Outbreak investigation and providing diagnosis

KS Lole & AS Tripathy

Funding: Intramural

Project Duration: Ongoing

Table: Samples processed for hepatitis viruses during the year 2014-15

		Molecular/Serological testing						
Sr.No.	Particulars	HAV IgM	HEV IgM	HBsAg	HCV RNA	HBV DNA Quan-titative	HEV RNA	HAV RNA
1	From Drug Controller of India	–	–	773	773	–	–	
2	Chronic Hepatitis B and C patients	–	–	–	1	87	–	
3	Sporadic acute viral Hepatitis patients	364	331	109	–	–	–	
4	From outbreaks of viral Hepatitis	396	634	–	–	–	–	
5	Water samples	–	–	–	–	–	54	
6	Stool samples	–	–	–	–	–	–	22

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ENC1301: Investigations of human clinical specimens collected during encephalitis outbreaks and diagnostic services to suspected Japanese encephalitis and Chandipura encephalitis patients from India.

VP Bondre, RG Damle, SA Mahamuni, DV Pavitrakar, VK Jadhav, RS Gunjikar, VM Ayachit, V Sankararaman & DK Butte.

Funding: Intramural Project Duration: Ongoing

In the capacity of WHO Collaborating Center for arbovirus research, Encephalitis group is involved in investigation of referred as well as clinical specimens collected during viral encephalitis outbreaks occurring in different parts of India. Results are communicated to the concerned health authorities. As a part of routine investigations, virus isolation from clinical specimens and their genetic characterization is carried out to generate baseline data on different viruses circulating in India. Simultaneously, strengthening of the existing diagnostic assays through optimization of reagents and assay controls to increase the sensitivity and reproducibility of the diagnostic test is also carried out.

1. Diagnosis of human clinical specimens:

A total of 515 human clinical specimens comprising of 302 sera and 199 CSF were processed for detection of IgM antibodies against Japanese encephalitis (JE) and Chandipura (CHP) infection by MAC ELISA and antigen detection by JE, CHPV and flavi RT-PCR. Few specimens received from Gujarat processed for virus isolation in tissue culture and in infant mice yielded an isolate each of CHPV (148974) and dengue 1 (148933). A total of 90 clinical specimens (64 Sera, 26 CSF) and 42 CSF specimens were processed for virus isolation in tissue culture and in infant mice, respectively. In addition, diagnostic support was also extended for suspected encephalitic cases from other countries.

2. Development of Real Time-PCR assay for detection of Chandipura virus:

Efforts were made to develop in-house real time RT-PCR assay for CHPV infection from human clinical samples. Primers were designed to amplify the region of CHPV genome spanning 750 nucleotides of the G Protein. Amplification of the target region was carried out using RNA from CHPV (Human strain 1210269). The amplicon was cloned in Topo cloning vector using TOPO-TA cloning kit as per manufacturer's instructions. The cloning of insert in vector was verified by PCR and sequencing. In order to develop internal standards for real time PCR, in-vitro transcription of the linearized DNA and preparation of quantitative standards is in progress.

3. Validation of JE MAC ELISA, anti-CCHF Human IgG ELISA, NIV KFDV IgG ELISA and Anti-HEV IgM ELISA, NIV CCHF virus IgM ELISA:

Validation of six different batches of JE MAC-ELISA kit was carried out and reports were communicated to the diagnostic reagent facility of NIV. Additionally, inter-laboratory validation of Anti HEV IgM Detection Kit, NIV KFDV IgG ELISA Kit, NIV anti CCHFV Human IgG ELISA Kit and NIV CCHFV Human IgM Capture ELISA Kit was carried out.

4. Potency testing of Recombinant buffalo interferon tau (rBuIFN-T) corresponding to activity of reference IFN

Interferon tau (IFN-T) is known to utilize interferon alpha receptors sensitive to interferon alpha and thus is expected to have anti-viral properties. As it is less toxic compared to other interferons, it is being increasingly investigated as a potential therapeutic compound. Recombinant buffalo IFN-T (BuIFN-T) expressed and purified at NDRI Karnal was tested for anti-viral activity against CHPV infection using standard bovine interferon Tau B as test control. The interferon assay for detection of activity of rBuIFN-T as against the standard interferon tau was carried out. The assay concluded that, the test sample rBuIFN-T exhibits IFN activity corresponding to 10IU/ml of standard IFN at 10^{-7} dilution after reconstitution of the given lyophilized protein in 300µl of sterile PBS (Figure 1).

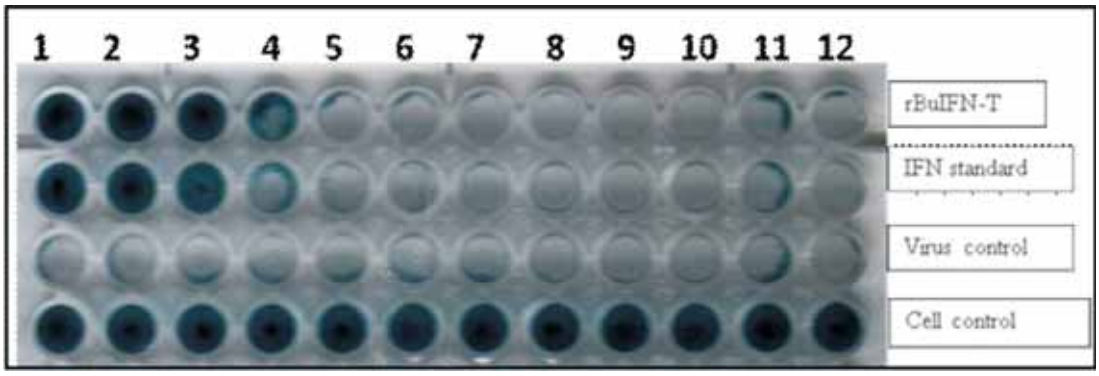

Figure 1: Potency testing of the Recombinant buffalo interferon tau (rBuIFN-T) in MDBK cells with CHPV challenge system. The assay was performed with a broad range of test IFN dilutions starting from 10^{-4} to 10^{-15} dilution (well 1-12) and showed the effective IFN response with end point at 10^{-7} dilution showing 50% reduction in CHPV infection corresponding to 10 IU/ml of standard IFN dilution, which starts from 1000IU with 10 fold dilutions in the subsequent wells. (well 1-12) and showed the effective IFN response with end point at 10^{-7} dilution showing 50% reduction in CHPV infection corresponding to 10 IU/ml of standard IFN dilution, which starts from 1000IU with 10 fold dilutions in the subsequent wells.

ENC1503: A rapid test for the detection of Chandipura virus infection

RG Damle, VP Bondre, SA Mahamuni, VS Bhide & DK Butte

Funding: Intramural Project Duration: 2014-2015

An antigen capture ELISA was developed for detection of CHPV using mouse anti CHPV antibody and rabbit biotinylated antibody as capture and detector antibody, respectively. The test detected 60 picograms of CHPV from the purified preparation. In a comparative study by plaque assay, which detected 7 PFU/0.1 ml, the ELISA detected CHPV up to 10^{-9}. Further studies to improve the ELISA are in progress.

ENC1303: Development of an infectious cDNA clone of a neuropathogenic West Nile virus lineage 1 strain. (Project completed)

VP Bondre, DV Pavitrakar & VM Ayachit

Funding: Intramural Project Duration: 2013-2014

Our previous studies on West Nile virus (WNV) strains isolated from human cases in India suggest substantial variation at the genetic level reflecting variable pathogenesis. This study

describes the development of a reverse genetics system for a neurovirulent WNV isolate 68856 and its characterization. Full length viral cDNA was amplified and cloned into the bacterial artificial chromosome (BAC) under the transcription control of T7 promoter. The RNA transcripts obtained by ***in vitro*** transcription were infectious in mammalian cells upon transfection. Cytopathic effect caused by synthetic RNA transcripts in the mammalian cells (Figure 2a), detection of cell associated viral protein by immunofluorescence assay, and recovery of genetic markers in the progeny virus genome marked the successful development of the reverse genetics system for WNV (Figure 2b). Replication potential (Figure 2c) and plaque morphology (Figure 2d) of the newly expressed virus in mammalian cells along with its antigenic cross reactivity with the parental virus suggests synthesis of biologically identical, replicative virus. Neuropathogenesis studies in murine model in comparison with the parental virus clearly indicate that the three genetic changes occurred in the recombinant virus during ***in vitro*** transcription has no impact on viral pathogenesis. The stable infectious cDNA clone generated from the neurovirulent Indian WNV strain will serve as a valuable experimental tool to study the viral factors contributing towards pathogenesis, host–virus interaction and immune evasion.

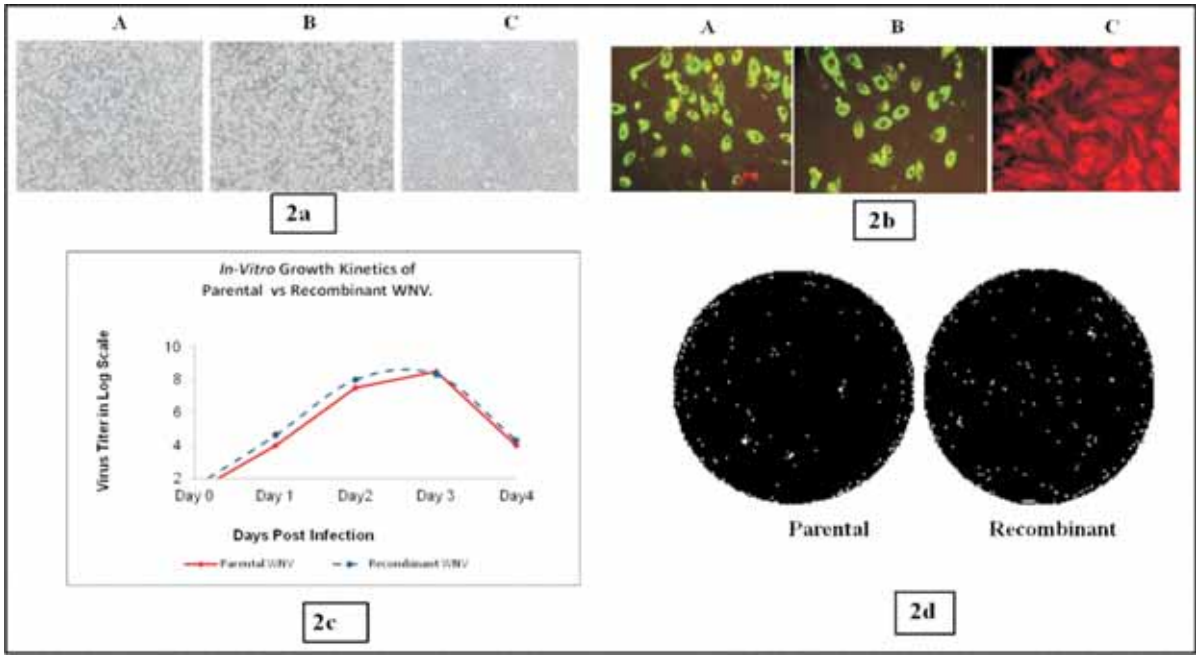

Figure 2: Characterization of infectious cDNA clone of WNV 68856; 2a: BHK-21 cells (A) infected with 68856; (B) transfected with the ***in vitro*** transcribed full length viral RNA transcripts generated from the full length cDNA clone showing CPE at 48hr post transfection. Degenerative changes were not observed in (C) vector control. 2b: Immunofluorescence assay performed on BHK-21 cells. (A) Infected with WNV68856p and (B) Transfected with full length RNA transcribed from full length cDNA detects expression of WNV protein in BHK-21 cells at 48hr post transfection. (C) BHK-21 cells transfected with RNA transcribed from cloning vector (MpBAC) as vector control at 48 hrs post transfection. 2c: In-vitro growth kinetics of parental and recombinant 68856 in BHK-21 cells. BHK-21 cells were infected at 1MOI, supernatant was collected at the indicated time points (0, 24, 48, 72 and 96 hr PI) and virus titer was determined by plaque assay in BHK-21 cells. 2d: Comparative plaque morphology of parental WNV (68856p) and recombinant WNV (68856r).

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ENC1304: Mechanism of apoptosis in the pathogenesis of West Nile virus infection.

VP Bondre & S Mundhra

Funding: Intramural Project Duration: 2013-2015.

The *in vitro* growth kinetics of highly virulent Indian WNV lineage 1 isolate 68856 and a low virulent lineage 5 isolate 804994 was carried out in human neuroblastoma cells (SK-N-MC). Experiment confirmed that the L1 strain has higher replication potential over the L5 strain (Figure 3a) as observed by higher death of neuronal cells as ascertained by MTT assay. Percentage cell survival was reduced to 26% in case of WNV68856 infected SK-N-MC cells while it was 90% in WNV804994 infected cells after 72hr post infection (Figure 3b).

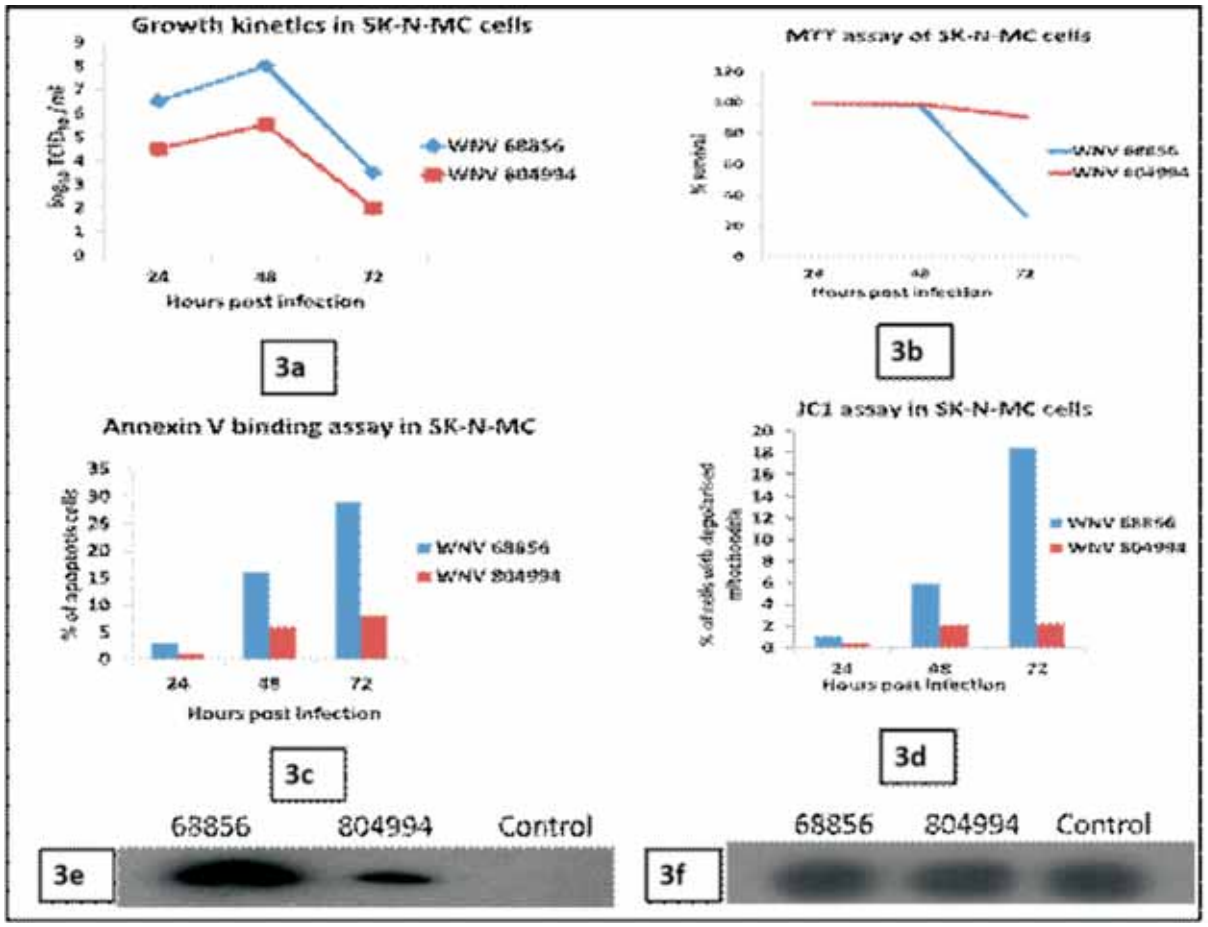

Figure 3: Induction of apoptosis in SK-N-MC cells after infection with WNV strains; 3a: Titration of culture supernatant of SK-N-MC cells infected with 68856 and 804994 strains of WNV on BHK-21 cells at 24, 48 and 72 hour post infection (hpi). 3b: MTT assay of SK-N-MC cells infected with 68856 and 804994 at 24, 48 and 72 hpi. 3c: Annexin V binding assay of SK-N-MC cells infected with 68856 and 804994 at 24, 48 and 72 hpi. Values represent percentage of apoptotic infected cells minus those of control cells. 3d: JC-1 staining assay of SK-N-MC cells infected with 68856 and 804994 at 24, 48 and 72 hpi. Values represent percentage of infected cells with depolarised mitochondria minus those of control cells. 3e: Western blot of cleaved PARP-1 24kDa fragment in SK-N-MC cells infected with 68856 and 804994 at 72 hpi. 3f: Western blot of â-actin as loading control in SK-N-MC cells infected with 68856 and 804994 at 72 hpi.

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To assess the WNV induced apoptosis, different apoptosis detection assays were carried out. In DNA fragmentation assay, 68856 infected cells (SK-N-MC) showed a higher intensity of bands at regular intervals of 200bp, characteristic of apoptosis, as compared to that in 804994 infected cells. The loss of plasma membrane integrity due to apoptosis was analyzed on flow cytometer by running SK-N-MC cells infected with both the strains after labelling with FITC labelled Annexin-V and Propidium iodide. At the end of 24, 48 and 72 hours the number of 68856 infected cells undergoing apoptosis was always higher as compared to 804994 infected cells (Figure 3c). The shift of fluorescence from red to green of the JC-1 stain in damaged mitochondria on infection with 68856 and 804994 was quantified on flow cytometer and found to be higher for the former (Figure 3d). Cleavage of Poly (ADP-ribose) polymerase (PARP-1) by active caspases in apoptotic cells was studied by immunoblotting using antibodies against the cleaved 24kDa fragment. Higher cleavage of PARP-1 was observed in 68856 infected cells than in 804994 infected cells after 72 hr of infection (Figures 3e & 3f). The data indicates higher degree of apoptosis induction in neuronal cells by WNV68856.

ENC1401: Role of Japanese encephalitis virus envelope protein Histidine residues in membrane fusion.

VP Bondre & DN Mali

Funding: Intramural Project Duration: 2014-2017

Membrane fusion is an essential step in flavivirus replication. Low pH mediated conformational changes in viral envelope protein triggers fusion of the virus envelope with cellular membrane. Post membrane fusion release of viral nucleocapsid into the cell cytoplasm ensures initiation of virus replication and thus successful infection. Membrane fusion is the conserved phenomenon for entry of enveloped viruses including the flavivirus into the cell. Conserved Histidine (His) residues located on the distal surface of the viral envelope protein are mainly considered as putative pH sensors during membrane fusion process in different viruses. Among the flaviviruses, His residues are conserved in the envelope protein of all the members of Flaviviridae. However, the exact mechanism of membrane fusion and the role played by different conserved E protein His residues in membrane fusion process of JEV is not yet fully explored. This study is proposed to determine the role of conserved His residues in the membrane fusion of JEV. In order to study the mechanism of endosomal membrane fusion process of wild type JEV 0945054, labelling of virus with fluorescent lipophilic tracers i.e. DiOC or DiD Carbocyanines is under progress. In order to synthesize JEV sub viral particles 'C-PrM-E' region of 2088nt from JEV 0945054 was amplified spanning region from 390-2478nt in complete genome. Cloning of this fragment in pcDNA 3.1 vector is in progress.

ENC1402: Development of DNA Microarrays for detection of flavivirus infections associated with central nervous system (CNS) infection.

VP Bondre, A Chakraborty, RG Damle, V Sankararaman & M Tupekar.

Funding: Intramural Project Duration: 2014-2015 (Completed)

To develop a molecular diagnostic tool for detection of medically important flaviviruses in a single assay, DNA probe array seems promising. Complete genome sequences of flaviviruses

associated with CNS infections were archived from GenBank and aligned using Mega 6.0 software. Based on the analysis of alignment file total no. of 3 family specific and 6 virus specific probes were designed. These were further analysed for their specificity by BLAST analysis with human and other micro-organism sequences. The selected probes will be used for testing their sensitivity by using reference strains available in the institute. These probes will be used for development of microarray based diagnostics for detection of flavivirus infection.

ENC1402: Development of microneutralization assay followed by ELISA for detection of neutralizing antibodies to Chandipura virus

RG Damle, AA Patil & VP Bondre

Funding: Intramural Project Duration: 2014-2015 (Completed)

Microneutralization test followed by ELISA was developed which gives end point result in the form of ELISA OD values. Additionally, time required for this test is 20 hr in comparison to 48 hr required for the test by CPE method. Eighty clinical samples were tested in both systems concurrently. 50 samples belonged to post CHPV outbreak 2009 from Panchamahal, Gujarat and 30 randomly selected samples referred to NIV from different parts of India with wide range of age group. New test was evaluated using conventional NT by CPE method and found 46 specimens positive and 34 specimens negative for anti-CHPV antibodies by both the tests. Sensitivity, specificity, PPV and NPV of the new test was 100%.

RD/Tech/01/03/2009: Development of monoclonal antibodies (MAbs) to Chikungunya virus (Project completed)

RG Damle, V Neethi, S Gosawi & PS Sathe

Funding: Intramural Project Duration: 2009-2014

Considering the limited stock of anti-E MAb which is presently used as a detector antibody in NIV MAC ELISA kit, new anti CHIKV MAbs were developed to replace the existing MAb. Thus, MAbs to CHIKV capsid protein were developed and screened for their suitability as detector antibody in NIV MAC ELISA. The new C MAb test was evaluated where NIV MAC ELISA was used as a 'gold standard'. Samples (N=293) were tested parallely in both the tests which included 85 CHIKV positive, 181 negative and 27 equivocal. The new C MAb ELISA successfully detected recent CHIKV infection up to 5 months post onset similar to NIV MAC ELISA.

The new test has specificity 90%, 82% sensitivity and 82% agreement. Samples were positive if P/N value was above 2.0; samples were negative if P/N was below 1.5 and samples were equivocal if P/N was between 1.5-2.0. In inter-day and intra-day precision testing, the mean SD value was less than 1.0 and co-efficient of variance was less than 10% in all cases and the values were within the acceptable limits. 34 specimens with known POD of infection were tested in both the tests. Except for one equivocal specimen collected within 15 days POD in C MAb ELISA, rest of the specimens (9 specimens collected between 16-30 days POD and 7 collected between 1-5 months POD of infection) were positive by both the tests indicating that the C MAb ELISA detected the recent CHIKV infection (Figure 4).

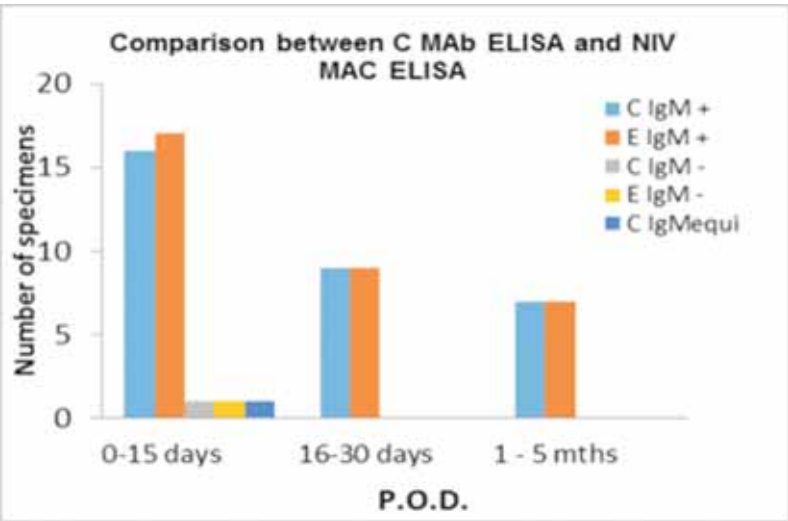

Figure 4: Comparison between C MAb ELISA and NIV MAC ELISA.

DENGUE & CHIKUNGUNYA GROUP

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DENGUE & CHIKUNGUNYA GROUP

DEN1301: Diagnostic support during outbreak investigations (Service project)

A Singh, MB Kakade, A Salunke & PS Shah

Funding: Intramural Project Duration: Ongoing

During this period, three outbreaks of dengue (Ahmednagar in June, Lonand in August and Katra in Gondya) were investigated.

In Ahmednagar, investigations were carried out in Nagar, Telkudgaon, Renuka-nagar, Tajnapur, Shahpur and Gunwadi villages during 2nd-3rd June, 2014 and 25 blood samples were collected. Fever, headache and body pain were the major symptoms reported by the patients. Upon screening of the samples, 18 (72%) tested positive for dengue specific IgM antibodies. Dengue viral RNA was detected in early post onset day (POD) of illness samples by multiplex RT-PCR. DENV-2 and DENV-3 were found responsible for the outbreak.

An outbreak of dengue like illness was reported in Lonand (Satara district) characterized by fever and chills in August 2014. The NIV team visited Lonand and collected 16 blood samples of which 7 (43%) tested positive for dengue specific IgM antibodies. One early POD sample was subjected to multiplex PCR and the serotype was determined as DENV-4.

Thirty five blood samples were referred by District Medical Officer, Katra (Gondiya district) during an outbreak of dengue like illness characterized by fever, headache and vomiting/nausea. Twelve samples tested positive for dengue specific IgM antibodies. Of the seven early POD samples, three tested positive by multiplex PCR as DENV-3 (2) and DENV-2 (1) serotypes.

CHK1301: Chikungunya fever outbreak in rural Pune, Western India

AR Deoshatwar, D Parashar, MD Gokhale & A More

Forty-three serum samples received by NIV from Talegaon, Pune during October 2014 tested positive for anti CHIKV IgM antibodies (48.8%). The NIV team visited the affected areas in Talegaon, collected 67 serum samples and found 40 patients (59.7%) positive for CHIKV IgM antibodies and 11 for viral RNA either by nested RT-PCR or real time RT-PCR. One sample was positive for both IgM and viral RNA. CHIKV infection was confirmed in 76% of the cases.

DEN1302: Dengue Diagnostic services (Service project).

A Singh, SK Pandey, M Bote, T Raut, R Bachal, M Seervi, N Sarthi, PS Shah & Cecilia D

Funding: Intramural Project Duration: Ongoing

Pune city witnessed a massive outbreak of dengue in the year 2014. A total number of 3383 referred serum samples were screened for DENV specific IgM antibodies out of which 2002 (59.2%) samples tested positive. In addition, 3512 samples received from PHCs from Pune and other districts of Maharashtra were also screened for DENV specific IgM and 784 samples tested positive. Results were communicated within 24-48 hr and weekly updates were posted on the NIV

website. Results were also communicated to the Joint Directorate of Health Services and to NVBDCP on a monthly basis. Early POD samples were processed for serotyping and the samples positive for DENV RNA were stored at –80°C in aliquots for isolation studies. DENV-4 which normally occurs sporadically in India was the dominant serotype observed in July-August, 2014 in Pune while DENV-2 dominated in the later part of the year. However, in the rest of Maharashtra, the pattern was different and DENV-2 dominated with 50 cases followed by DENV-3 and DENV-1 with 26 and 7 cases, respectively. DENV-4 is believed to cause milder disease in primary infections but moderate to severe disease in secondary infections. Therefore the rise in number of DENV-4 cases is a serious concern as it may lead to greater severity of dengue in future.

Quality Control for NS1 tests

During the period, we carried out quality control (QC) for various NS1 tests for Pune Municipal Corporation hospitals for 195 samples, of which 143 were found to be concordant. The group is also involved in dengue management in collaboration with Maharashtra Health authorities and media, emphasizing the need for vector control at the community level and more circumspect diagnosis and treatment.

DEN1304: Evolution of dengue viruses based on full genome analysis

J Patil, B Anukumar, MB Kakade, K Alagarasu & D Cecilia

Funding: Intramural Project Duration: 2013-2015

Monitoring of DENV serotypes and genotypes has proved to be important in understanding the evolution, introduction and dissemination of the viruses. During 2014, we reported the emergence of genotype I (GI) of DENV-1 for the first time in Kerala in 2013. During the current year we analysed samples obtained from Tirunelveli outbreak of 2012 and found presence of DENV-1 GI in the samples. The genotypes of DENV-2, 3 and 4 circulating in Tirunelveli (2012) and Kerala (2013) were IV, III and I respectively, the same as reported earlier by our group. Sequencing of the envelope (E) gene revealed that the DENV-1 viruses from Tirunelveli and Kerala were closest to Sri Lankan 2009/10/12 in GI cluster as shown in the ML tree (Figure1). DENV-1 viruses from other regions of India reported earlier clustered in GIV (AM/AF) which has been collapsed in the figure-1. Comparison of amino acid sequences revealed substitutions in the Tirunelveli and Kerala E gene which were not shared with the Sri Lankan viruses.

Full genome sequence of the Kerala isolate was determined and was compared with 32 other isolates of genotype I of DENV-1. The similarity between Kerala isolate and Sri Lankan viruses was the highest, 98.5% nucleotide similarity and 97.8% amino acid similarity as against 95.6% nucleotide and 96.3% amino acid similarity with the consensus sequence of other genotype I viruses. Comparison of amino acid sequences for each viral protein revealed largest number of changes in the E, NS2A, NS3 and NS5 proteins. The core, prM and NS1 were the most conserved. Larger number of changes in the non-structural genes suggested a possible effect on the efficiency of virus replication, which needs to be investigated.

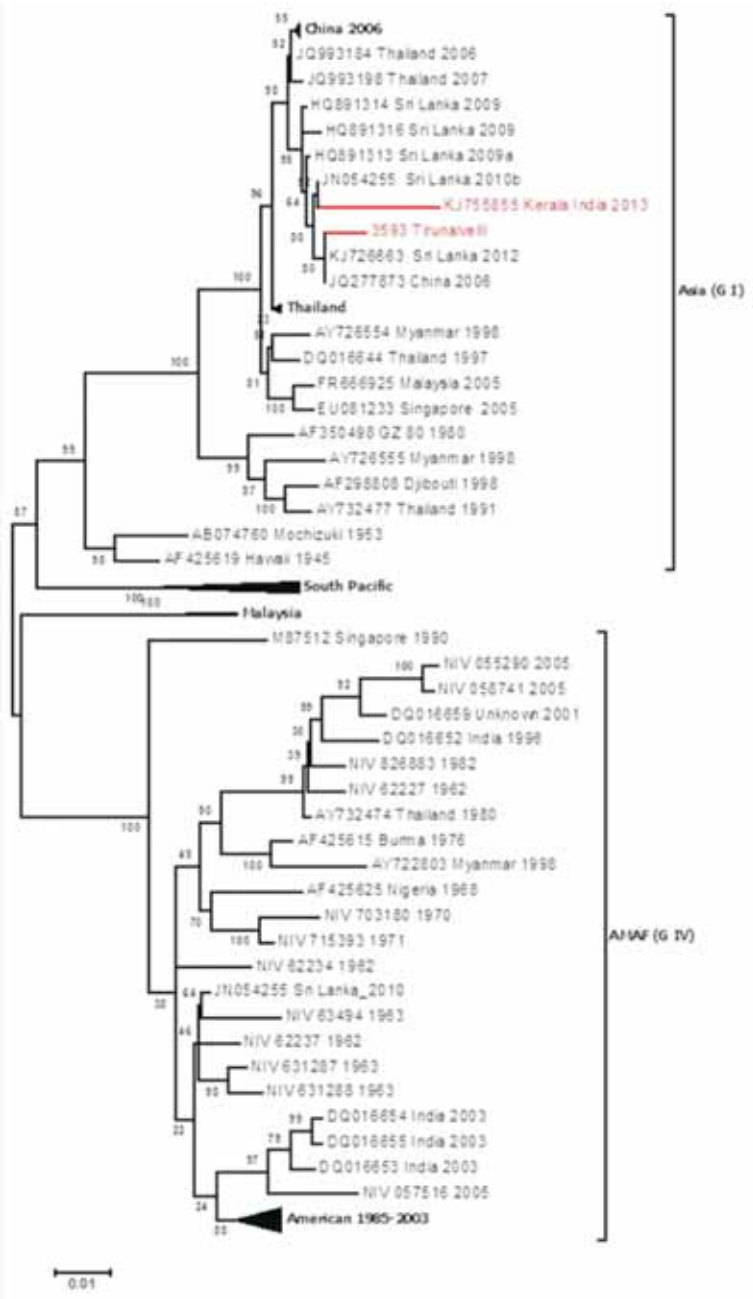

Figure 1: Maximum Likelihood (MEGA 5) tree of DENV-1 viruses

DEN1305: Disease burden of hospitalized dengue in Vadu area in rural western India

P Shah, A Deoshatwar, D Parashar, A Singh, M Seervi & D Cecilia

Funding: Extramural (ICMR, awaiting funds) Project Duration: Two years

Previous studies by NIV have shown that exposure to DENV occur by the age of 15 in Vadu area. A study was therefore planned to determine the population of Vadu in the age group of 5-15 years for sero-prevalence to DENV. Vadu DSS database was used as a sampling frame. Sample size was determined to be ~ 800. Considering 10% drop-outs/year [or loss to follow up for various

reasons] the sample size was proposed to be 1000 from 22 villages. A total of 1000 blood samples were collected after obtaining consent from parents (520 males and 480 females). Sero-prevalence was determined by detection of dengue virus-specific IgG using PanBio-IgG ELISA kit and 180 (18%) were tested positive. When data was analysed according to the age groups, it was found that there was very little exposure till the age of 5 (Figure 2). The samples were also tested for CHIKV specific IgG using an indigenous ELISA and 44 (4.4%) tested positive for CHIKV IgG. Antibody positivity for both DENV and CHIK IgG were detected in 22 samples.

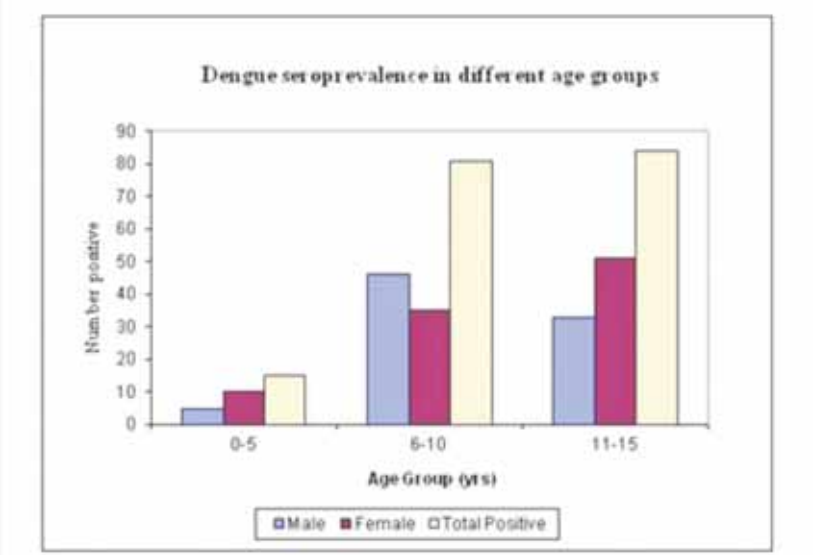

Figure.2: Samples for determining dengue seroprevalence were tested for DENV specific IgG using PanBio-IgG indirect ELISA kit. Samples with Panbio units greater than 11 was considered positive

DEN1306: Characterization of the T regulatory cell response in Dengue

H Tillu, A Tripathy & Cecilia D

Funding: Intramural Project Duration: 2012-2015

Pathogenesis of dengue is immune mediated. $CD4^+$ $CD25^+$ $Foxp3^+$ Regulatory T cells (Tregs) suppress immune response and may contribute to protection. Earlier studies have shown higher Treg (conventional and effector) frequencies correlated with milder disease outcome and higher levels of pro-inflammatory cytokines indirectly contribute to severity by exerting an inhibitory influence on Tregs.

During this year we assessed gene expression of Treg associated genes; $Foxp3^+$, IL-10 and TGF-beta in dengue patients and controls. The patients were categorized into mild and moderate using WHO classification (2009). Primers for real time RT-PCR were designed for IL-10 and published sequences were used for the other genes. Real time assays were standardized using RNA isolated from PHA-stimulated PBMCs from apparently healthy individuals. The efficiency of all three assays were >90%. Lower mRNA levels were observed for all three genes in dengue

patients compared to controls. The decrease was significant for IL-10 and TGF-beta. This difference was seen even when sub-groups of mild and moderate cases were compared to controls. However, comparison of mild and moderate cases revealed higher mRNA levels of all three genes in mild cases (Figure 3). This indicated a dichotomy in the mRNA and protein expression levels for IL-10 and TGF-beta in dengue.

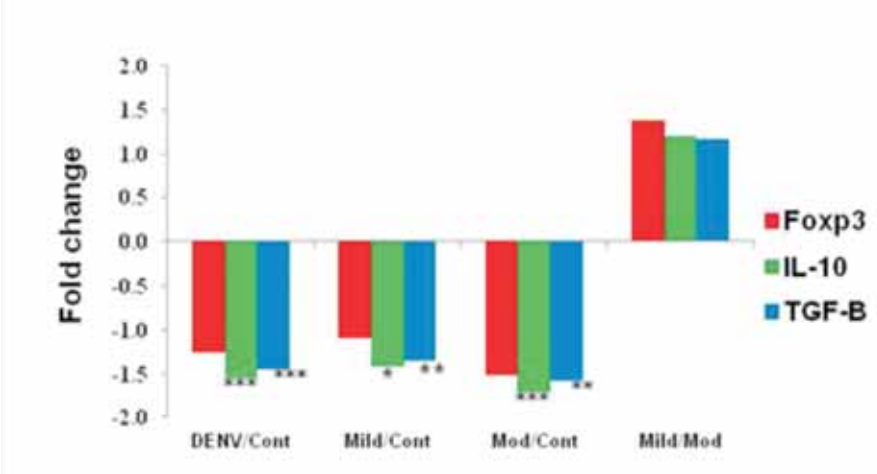

Figure 3: Relative PBMC gene expression pattern of Treg associated gene. The expression of Foxp3, IL-10 & TGF-â was normalized to 18S rRNA and compared between different patient categories and controls. Students t-test used to calculate statistical significance *pd"0.05, **pd"0.01 & ***pd"0.001.

DEN1311: Role of Dengue virus Core protein in viral replication

A Tiwary & Cecilia D

Funding: Intramural Project Duration: 2012-2015

DENV core (DENV-C) protein is a highly basic protein which binds to the viral genome and forms the nucleocapsid. DENV-C has been shown to localize in the nucleus of infected cells and has been reported to bind to hnRNP k, DAXX, histones and several other proteins resident in the nucleus. The functional significance of these associations is still not understood. We have earlier shown that the core protein of DENV localizes in the nucleus in infected cells and this pattern depended on the cell line.We further analysed the nature of the association in the context of its dynamics.

Location of the core in the nucleus was suggestive of its interaction with the nucleolus. To confirm this, co-localization studies were carried out with cells transfected with DENV-C-GFP labelled with anti-fibrillarin antibodies-TRITC. Confocal images showed 100% merging of red and green fluorescence, confirming the targeting of C protein to the nucleolus. Further, we explored the kinetics by studying the fluorescent recovery after photo bleaching (FRAP) of DENV-C-GFP transfected cells. FRAP analysis (Figure 4) suggests that at any given time, 33% of DENV C protein localized in the nucleolus is immobile ***i.e.***, stably bound to the nucleolar components, whereas 67% of the protein is mobile and available for exchange between nucleus and cytoplasm. The dynamics suggested a possible irreversible interaction of DENV-C with a nucleolar component/s and that the interacting component shuttles between nucleolus and nucleoplasm.

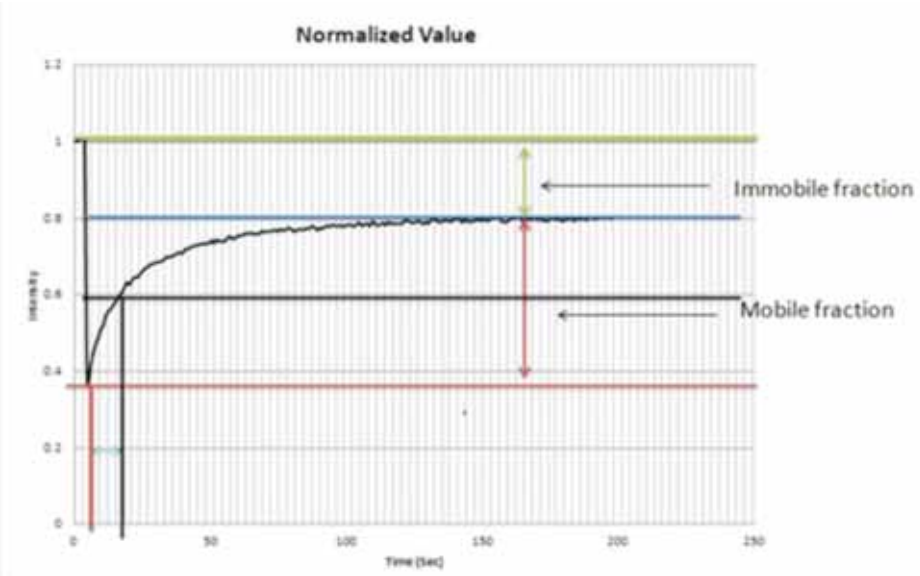

Figure 4: FRAP analysis of GFP-Core transfected BHK-21 cell. Normalized FRAP curve showing mobile and immobile fractions using data collected from 10 cells.

DEN1403: Development of reference serum bank for dengue diagnostics

A Singh, M Seervi & P Shah

Funding: Intramural Project Duration: 2014-2015

Presently, no single diagnostic test is available to confirm dengue infection with 100% sensitivity and specificity. Therefore, there is a need for developing serum bank to evaluate commercially available dengue diagnostic tests, both serological and molecular. A panel of sera from dengue cases positive for DENV specific IgM, IgG, NS1 and DENV specific RNA is planned to be developed. Hospitals run by Pune Municipal Corporation were visited and 186 samples were collected out of which 86 were found to be positive for DENV specific IgM antibodies. A few of the early POD samples were subjected to DENV NS1 specific ELISA test and nine tested positive. When tested by multiplex RT-PCR, all the nine samples were found positive for viral RNA (DENV-1=2; DENV-2 =3; DENV-4=4). All the positive sera will be lyophilized and retested after reconstitution for the respective tests. The panel of sera will be tested for qRT-PCR, NS1 antigen and DENV-specific IgG and appropriate samples will be processed for virus isolation.

DEN1008: Immune Response (non-MHC) gene polymorphisms in Dengue Disease Pathogenesis

K Alagarasu, RV Bachal, H Tillu, AP Mulay, MB Kakade, PS Shah & D Cecilia

Funding: Intramural Project Duration: 2011-2014

Cytokines play an important role in dengue disease pathogenesis. Single nucleotide polymorphisms in the cytokine genes affect cytokine levels. In the present study, the association of

single nucleotide polymorphisms (SNPs) in the cytokine genes, TNF, IFNG, IL1B, IL8, IL0, IL17A and IL17F with dengue disease severity was investigated using polymerase chain reaction based methods in 87 dengue fever (DF), 45 dengue hemorrhagic fever (DHF) cases and 108 apparently healthy controls (HC). Frequency of T/C genotype of IL17F rs763780 was found significantly lower in dengue cases (includes both DF and DHF) (8.3%) as compared to HC (17.6%) [P = 0.033, OR with 95% CI 0.43 (0.19-0.95)]. Frequency of IL8 rs4973 A/T genotype was lower in DHF cases (33.3%) compared to HC (50.0%). Under overdominant genetic model (A/T vs. A/A +T/T), IL8 rs4973 A/T genotype was negatively associated with DHF compared to HCs [p =0.029, OR with 95% CI 0.43 (0.20-0.93)]. Similarly, frequency of IL10 rs1800871 A/G genotype was lower in DHF cases (20.0%) compared to DF cases (39.5%). Under overdominant genetic model, A/G genotype of IL10 rs1800871 was significantly negatively associated with DHF compared to DF cases [p =0.014, OR with 95% CI 0.35 (0.15-0.84). Significantly higher frequency of the combined genotype IL10 A/A-IFNG A/T and lower frequency of the combined genotypes IL10 A/G-IL1B A/A, IL10 A/G-IL8 A/T and IL10 A/G-IL17F T/T were observed in DHF cases compared to DF (Table 1). The results suggest that heterozygous genotypes of IL8 rs4973 and IL10 rs1800871 are associated with reduced risk of DHF. Combinations of IL10 rs1800871 and pro-inflammatory cytokine genotypes influence the risk of DHF.

Table 1 Frequencies of combinations of IL10 rs1800871 genotypes with different pro-inflammatory cytokine genotypes in dengue patient

IL10	IFNG	IL1B	IL8	Percentage combined genotype frequency				Controls vs. DHF		DF vs. DHF	
				IL17F	DHF	DF	HC	Odds ratio with 95% confidence intervals	P value	Odds ratio with 95% confidence intervals	P value
A/A	A/T	-	-	—	31.8	15.1	29.9	1.09 (0.47-2.47)	NS	2.62 (1.00-6.82)	0.046
A/G	A/T	-	-	-	6.8	22.1	9.3	0.71 (0.12-2.96)	NS	0.26 (0.05-0.97)	0.051
A/G	-	A/A	-	-	2.2	16.3	14.9	0.13 (0.00-0.89)	0.046	0.10 (0.00-0.69)	0.017
A/G	-	-	A/T	-	2.2	17.6	18.4	0.10 (0.00-0.68)	0.016	0.11 (0.00-0.75)	0.023
A/G	-	-	-	-	6.7	21.1	16.8	0.35 (0.06-1.32)	NS	0.27 (0.05-1.00)	NS
A/G	-	-	-	T/T	17.8	38.4	28	0.55 (0.20-1.40)	NS	0.35 (0.13-0.89)	0.027

BDM1401: Structure-based design and evaluation of lead compounds targeting CHIKV nsP binding sites (Collaborative Project)

D Parashar, SS Cherian, P Patil, K Inamdar & G Subramanian

Funding: Intramural

Project duration: 2014-2017

Despite numerous efforts, an effective antiviral agent against the CHIKV remained elusive. In this context, we studied the antiviral effects of some known/identified protease inhibitors against CHIKV in Vero cells. Five compounds ***viz.,*** benzamidine, leupeptine and three synthesized compounds (NIV-1, 2, 3) were tested for cytotoxicity and anti-viral effect. Of the five compounds,

four caused toxicity at <1mM concentrations and therefore further antiviral studies with these compounds were not pursued. In contrast, Benzamidine was non-toxic upto concentrations of 2.5mM. This compound was further evaluated for antiviral activity. Cells were infected with CHIKV at 1 MOI and treated with different concentrations of Benzamidine at 48hr and 72hr PI. MTT assay was used to determine cell viability. The most effective concentration of Benzamidine that protected cells against CHIKV was 300µM added to cells at 48hr PI.In silico studies indicated the possible role of Benzamidine in targeting the nsP2 cysteine protease activity of CHIKV. Future plans are to synthesize derivates of Benzamidine and test for their antiviral activity and also test it in the primary human fibroblast cells.

Evaluation of kits and other tests:

Routine validation of kits and tests are carried out in the laboratory. The number of kits and tests validated during the year is given in Table 2.

Table 2: Number of samples tested/kits validated for DRF

Sr.No.	Type of kits	Samples tested (nos)	Validated	Evaluated for Government of Maharashtra and commercial companies
1	DENV IgM by ELISA:	7000	–	–
2	DENV serotyping by multiplex RT-PCR	277	–	–
3	CHIKV IgM by ELISA:	644	–	–
4	DENV IgM ELISA	–	30	–
5	CHIKV IgM ELISA	–	09	–
6	DENV NS1	–	–	10 kits

ENTERIC VIRUSES GROUP

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ENV1301: Investigation of outbreaks and diagnostic services

Funding: Intramural Project Duration: 2013-2015

1. Investigation of diarrheal cases for viral etiology

V Gopalkrishna, NS Chothe, SS Ranshing & PR Patil

Cases of acute gastroenteritis were reported from Miraj and rural areas of Sangli district (Maharashtra) during November 2014 and 33 faecal specimens were referred to NIV, Pune. Three samples (9%) tested positive for rotavirus by ELISA. Positive samples were subjected to multiplex PCR for VP7 (G) and VP4 (P) typing that revealed presence of G1P(8) genotype. The specimens were also tested for other enteric viruses by RT-PCR/PCR that showed positivity for Enterovirus(6%), Astrovirus(3.2%) and none of them were positive for Adenovirus.

2. Detection and characterization of Adenoviruses associated with Kerato-conjunctivitis

V Gopalkrishna, NS Chothe, PR Patil & SS Tikute

Cases of conjunctivitis were reported from Pune (Maharashtra) India, during November 2013-November 2014 and were clinically diagnosed as kerato conjunctivitis. A total of 23 eye swabs were collected from conjunctivitis patients from the National Institute of Ophthalmology and H. V. Desai Eye Hospitals, Pune were characterized during the year at molecular level. Fourteen (60.8%) specimens tested positive for Adenovirus (AdV) by PCR using Hexon region. All the AdV positive strains were further amplified using penton region. Sequencing of the PCR products revealed the presence of AdV8 (n=11), AdV3 (n=1), AdV4 (n=1) and AdV37 (n=1). AdV 8 strains showed 99.80-100% nucleotide identity with AdV8 Japanese strain (AB746853);AdV 37 with 100% identity with USA strain (KF268203); AdV 4, 100% identity with Chinese strains (KF006344);AdV 3, 100% identity with USA (KF268123) strains respectively. One specimen showed co-infection with Adeno and enterovirus while the rest (n=22) tested negative for enteroviruses.

3. Detection, and characterization of enteroviruses associated with Hand, Foot and Mouth disease

V Gopalkrishna, PR Patil, NS Chothe & SS Tikute

Cases of Hand, Foot and Mouth disease (HFMD) were reported from Pune (Maharashtra) India, during October 2013 to November 2014 and 158 clinical samples were collected from 70 HFMD cases from five different local hospitals. Ninety-six of the 158 (60.8%) samples were tested positive for enterovirus by RT-PCR using 5'NCR. Eighty-six (89.6%) of the EV positive strains were subjected for VP1 gene amplification followed by cycle sequencing indicating the presence of CVA16 (n=53), CVA6 (n=29), Echo-12 (n=3) and CVA4 (n=1). The study revealed, CVA-16, CVA-6 as major and other EV types as minor etiological agents associated with HFMD. Phylogenetic analysis on the basis of full VP1 gene of CVA-16 strains showed clustering of the Indian strains with B1c subgenotype which has been reported rarely worldwide.

ENV1302: Assessment of diversity in group A rotavirus

SD Chitambar, VS Tatte, SS Ranshing & G Pradhan

Funding: Intramural Project Duration: 2013-2015

Different lineages/genotypes have been identified within the 11 genes of group A rotaviruses. This diversity remains a challenge to the efficacy of the currently available group A rotavirus (GARV)

vaccines. Hence, genomic and antigenic analysis of rotavirus strains circulating in a particular geographic region is essential. The objective of the study was to isolate common and unusual group A rotavirus strains in cell culture and to determine their genogroups. Earlier, two unusual G9P [4] rotavirus strains were isolated one each in 2009 and 2011. Phylogenetic analysis of the VP7 and VP4 gene sequences showed that the isolates belonged to sublineages c of the lineage III (Figure 1) and P[4]-V lineage (Figure 2). Divergence of 0.4-0.8% / 0.0-1.5% nucleotide / amino acid was noted within the VP7 and VP4 genes of these strains. Isolation of another set of three rotavirus strains with unusual G-P combinations [G1P [4] (2010), G9P [4] (2010) and G12P [6] (2013)] was attempted. Only one of the rotavirus strains with G9P [4] specificity showed adaptation (2+CPE) in MA-104 cell line at passage 3 level. Cell culture supernatant in rotavirus antigen capture ELISA showed positivity. Isolation of three rotavirus strains with common G-P combination (G9P [8]) is in progress. A total of 60 stool samples positive for group A rotavirus by ELISA during 2011-2013 were selected for the study. Multiplex PCR was carried out for genotyping of VP7 (G) and VP4 (P) gene. Rotavirus strains with genotype combinations of G1P[8] (n=17), G2P[4] (n=14), G9P[4] (n=12), G9P[8] (n=6), G12P[6] (n=5), G12P[8] (n=3), G2P[6] (n=2) and G1P[6] (n=1) were subjected to full gene characterization of VP7, VP4, VP6 and NSP4 genes. Phylogenetic analysis of all G1P [8], G9P [8], G12P [8], G12P [6], G2P [6] and G1P [6] showed E1-C1 constellation, while G2P [4] and G9P [4] strains showed E2-C2 constellation for NSP4 and VP6 genes, respectively. Further analysis of nucleotide/deduced amino acid substitutions of the study strains as compared with prototype strains are in progress.

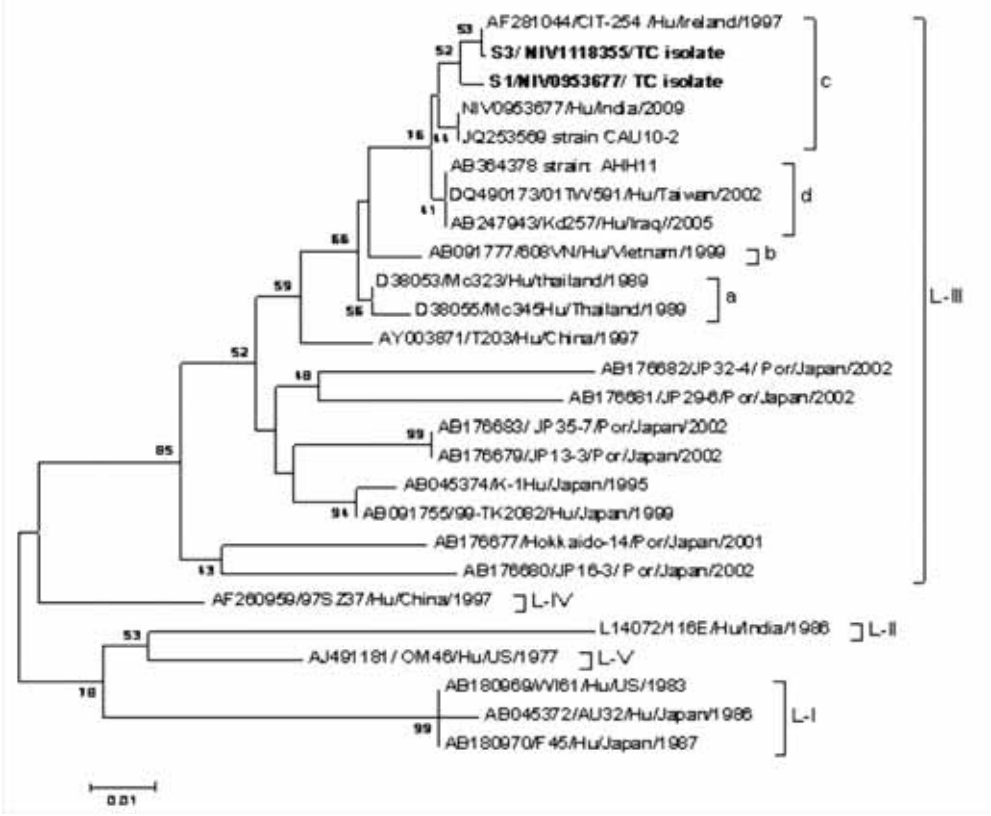

Figure 1: Phylogenetic tree based on the nucleotide sequences of VP7 gene (785-917nt) of G9P[4] rotavirus isolates grown in MA-104 cell line . The strains of the present study are highlighted in bold

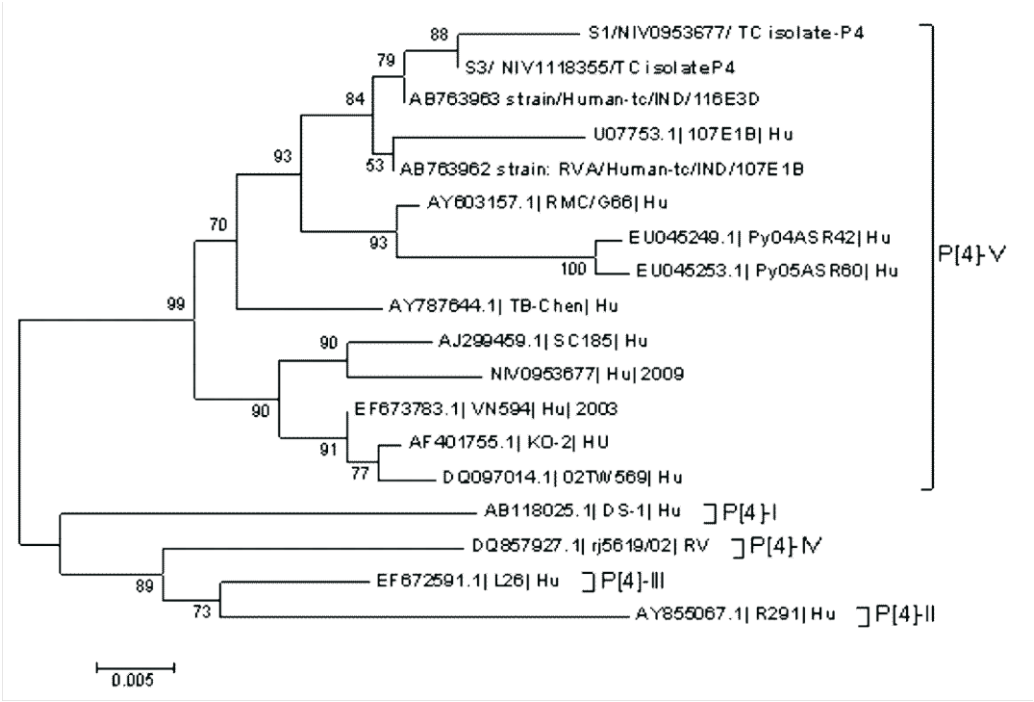

Figure 2: Phylogenetic tree based on the nucleotide sequences of VP4 gene (67-846 nt) of cell culture isolates of G9P [4] rotavirus strains. The strains of the present study are highlighted in bold.

ENV1304: Development of Real time PCR for group A Rotavirus

M S Joshi, SS Ranshing, SD Chitambar

Funding: Intramural Project Duration: 2013-2015

Group A rotaviruses (GARVs) are the most important causative agent of severe acute diarrhoea in infants and young children world-wide. Development of rapid and sensitive diagnostic assays is essential for diagnosis of rotavirus infection. Real time PCR (qRT-PCR) offers several advantages over the traditional RT-PCR such as increased sensitivity, higher throughput and faster turnaround time and quantification of viral loads. In a study carried out earlier, different genomic regions of GARV were evaluated for development of real Time PCR and use of VP6 gene derived primers and probes were found to be suitable. Complete VP6 gene of the human rotavirus 3 (HRV 3) was amplified by RT-PCR and cloned into pGEM-T Easy vector. Further, the presence of the insert in the plasmid was confirmed by restriction digestion followed by PCR. Run off transcription was done by RiboMax™ Large scale RNA production system with T7 polymerase (Promega, Madison, USA) and RNA was purified using TRIZOL LS reagent (Invitrogen, USA) according to the manufacturer's instructions. Serial 10-fold dilutions of the quantified RNA (1.2 x 10^{12} molecules/microlitre) were used as standard RNA. A total of 362 stool samples including known ELISA positive (n=151) and negative (n=211) were screened by the newly developed quantitative real time PCR assay. Analysis of the results is in progress.

ENTERIC VIRUSES GROUP

ENV1305: Development of immunoassays for detection of Norovirus infection using recombinant norovirus GII.4 capsid proteins

R Kulkarni, KS Lole & SD Chitambar

Funding: Intramural Project Duration: 2013–2016

Norovirus (NoV) is the second most common cause of non-bacterial acute gastroenteritis after rotavirus in western India. NoVGenogroup II genotype 4 (GII.4) has been identified as the predominant genotype in human infections in India. The objective of the study is to develop immunoassays for detection of NoV infection using the recombinant NoV capsid proteins. Recombinant virus-like particles (rVLPs) representing the predominant NoV GII.4 genotype (GII.4-DenHaag strain) have been developed in our laboratory by cloning and expression of the viral capsid protein (VP1) in baculovirus system (Annual report 2013-14). Hyper immune serum was generated against the rVLPs by immunization of New Zealand white rabbit and albino guinea pig. IgG was purified from the hyperimmune serum using Protein G column. IgG-HRP conjugate was prepared by HRP labeling of the purified IgG. In an ELISA using the purified IgG as the capture antibody and the IgG-HRP conjugate as the detector antibody, detection of NoV antigen in human stool specimens (known to be positive for homologous NoV type by RT-PCR) was achieved. Further improvement of the NoV antigen capture ELISA is in progress.

ENV1306: Diversity of non-rota enteric viruses in patients with acute gastroenteritis

Funding: Intramural Project Duration: 2013–2016

Though rotavirus is the leading viral etiological agent identified in acute gastroenteritis, association of non-rota enteric viruses in acute gastroenteritis has been reported among infants and young children. Recently, Parecho, Sali, Klasse, Cosa, Sapo and Human Boca viruses have been found associated in patients with acute gastroenteritis. However, not much information is available from western India. The present study has been undertaken to find out the diversity in such non-rota enteric viruses circulating in western India.

1. Detection and genetic characterization of Sapovirus (SaV) strains in children with acute gastroenteritis

N Lasure & V Gopalkrishna

Sapoviruses (SaVs) are members of the family Caliciviridae, associated with both sporadic and epidemics of acute gastroenteritis. Of the five SaV Genogroups, GI, GII, GIV and GV are known to infect humans. GI and GII genogroups are predominantly associated with acute gastroenteritis worldwide while GIV and GV are rarely reported. The objective of the study is to detect and characterize the circulating strains of SaVs to understand their genetic diversity in sporadic cases of acute gastroenteritis. In continuation to the ongoing study, faecal samples (n=266) collected from children < 5 years, hospitalized for acute gastroenteritis from January 2007 to December 2008 were analysed for the presence of SaV. Detection was carried out by PCR amplification of RdRp-Capsid junction region (424bp). SaVs were detected at a frequency of 3.5% (9/266). Co-infection with Rota, Adeno and Astrovirus was observed in 44.4% (4/9) cases. Infections occurred in children<2 years of age and were reported throughout the year with peak activity observed in

summer (44%). Severity assessment of SaV infected cases revealed severe infection in 44% and very severe in33% of cases. Phylogenetic analysis revealed circulation of GGI (22%), GGII (56%) and GGIV (22 %) and shared nucleotide identities between 92.8-98.5%, 95.5-96.3% and 94.8-95.2%, respectively with their respective prototype strains.

2. Detection and molecular characterization of Sali/Klassevirus strains from children with acute gastroenteritis

N Lasure & V Gopalkrishna

Sali/Klassevirus (SKV) is a member of the genus Salivirus A of the family Picornaviridae. Recently, detection of these emerging viruses in faecal samples of children with acute gastroenteritis suggested their etiological role. However, not much information is available on the virus in western India. The objective of the study is to determine the prevalence of SKV and to delineate the genetic diversity. Faecal samples (n=778) collected from children hospitalized for acute gastroenteritis from January 2007 to December 2011 were screened for SKV by PCR amplification of 2C (354bp) region, typing on the basis of 3D (688bp) and capsid region (852bp). Twenty out of 778 (2.6%) samples tested positive for SKV. Co-infections with Rota, Noro, Adeno, Astro and enterovirus were observed in 45% (9/20) of the cases. Infections observed in children <3 years, majority occurring between 0-24 month age group. Though infections occurred throughout the year, peak activity was reported in monsoon. Severity assessment of SKV infected cases revealed mild to very severe infections. Genotyping of the strains revealed, nucleotide identity between the strains was higher in 3D region (95.6-100%) as compared to the capsid region (91.2-100%). Positive strains shared 88.5-90.7% nucleotide identity with Salivirus A (prototype strain) in the capsid region and 95.9-100% in the 3D region. Phylogenetic analysis of the strains revealed maximum identity with strains reported from South Korea, 92.6-99.5% (3D region) and Japan, 94.9-98.9% (capsid region).

3. Genetic diversity of enteroviruses (EVs) and human Parechoviruses (HPeV) in patients with acute gastroenteritis

PR Patil, N Chothe & V Gopalkrishna

Enteroviruses (EV) and human parechoviruses (HPeVs) have been reported to be associated in children with acute gastroenteritis worldwide. However, no data on these viruses are available from India. The present study was conducted to determine the prevalence, circulation pattern, and to understand genetic diversity of the virus strains in children hospitalized for acute gastroenteritis. Faecal specimens (n=184) collected from children hospitalized for acute gastroenteritis during January to December 2011 were tested for EV and HPeV-RNA by RT-PCR using 5'UTR specific primers followed by genotyping of the positive strains by VP1gene amplification. Thirty two (17.4%) and 26 (12.5%) faecal specimens tested positive for EV and HPeV, respectively. Majority of EV and HPeV infections were noted in children <1 year, reported as severe disease. Twenty four (75%) EV strains were genotyped and phylogenetic analysis of the strains revealed the presence of fifteen genotypes belonging to three different species (EV-A:2 [8.3%], EV-B:9 [37.5%] and EV-C:4 [16.7%]) and E14 was the predominant EV type observed. Eighteen (69.2%) HPeV strains were

genotyped and a total of 6 different genotypes (HPeV1, HPeV2, HPeV5, HPeV7, HPeV8 and HPeV14) were identified and HPeV-1 (61.1%) was found to be the predominant type. The study documents circulation of HPeV-2 as a rarely identified genotype.

ENV1307: Susceptibility of different strains of mice to Coxsackie virus A-16 (CV-A16) and Enterovirus–71 (EV-71) isolated from HFMD patients

SS Tikute & V Gopalkrishna

Funding: Intramural Project Duration: 2013–2016

HFMD is a childhood viral infection and cases were reported during 2009-2010 from southern and eastern parts of India. CVA-16 and EV-71 strains have been isolated in our laboratory in RD cell culture. The present study has been undertaken to determine the susceptibility of mice to the CV-A16 and EV-71strains. CV-A16 and EV-71 (10^5 $TC1D_{50}$/ml) were administered in ICR, infant mice by oral and intra peritoneal routes. Four groups of mice were used for the experimental purpose. Age and strain matched mice were mock inoculated with normal tissue culture fluid (TCF) as control group. All the experimental and control group mice were observed daily for clinical changes. Mice were sacrificed on post inoculation day (PID 11) for virus detection. Mice inoculated with CV-A16 strains by the IP route have shown sickness on day 3 and by the oral route on day 5. Subsequently, mice were found to develop hind limb pyrolysis followed by death. A few mice recovered after 11^{th} day PI. The organs viz., brain, heart, liver, lung, kidney, spleen, intestine and skeletal muscles were collected for histopathological study. Sections of 3-5µm thickness were stained with haematoxylin-eosin and observed under microscope. Histopathological study has shown major changes in brain, heart and skeletal muscle in IP inoculated mice. Minimal neuronal degeneration and dilated ventricle was observed in brain and heart, respectively. Minimal focal vacuolations in cardiomyocytes with infiltration of inflammatory cells was observed. In skeletal muscle, mild focal degeneration on day 3 and severe degeneration of myocytes on day 7 was observed.

ENV1308: Molecular characterization of non-polio enterovirus strains from patients with acute flaccid paralysis

LV Rongala, CG Raut & SD Chitambar

Funding: Intramural Project Duration: 2013-2016

Coxsackievirus-B3 (CV-B3) of the enterovirus-B (EV-B) species is known to cause varied spectrum of infections (from asymptomatic to fatal) in humans. Comparative genomic analysis of CV-B3 strains, detected in 10 paralytic and 5 asymptomatic individuals from south-western India during 2009-2010 was carried out. Phylogenetic analyses of complete and partial 5'NCR, structural (P1) and non-structural (P2-P3) genomic regions revealed that all of the strains carried inter-genotypic mosaic recombination, which was further confirmed by Simplot analyses. Only P1 region of the study strains was similar to that of the CV-B3 while other genomic regions (P2-P3 and 5'NCR) were close to other EV-B types (CV-A9, CV-B4, E7, E20, E21, E24, EV-B74, and EV-B86). The segregation of the study strains into groups A and B was noted on the basis of phylogenetic trees, recombination patterns and predicted structures of domain-II of 5'NCRs. The groups showed mean genetic distance of 14.4% representing different meteorological zones, however, not portrayed different phenotypes. These data support the concept of independent evolution of structural and

non-structural genome regions and indicate differential genetic composition of CV-B3 strains circulating in different meteorological zones. The study suggests the need of a concurrent analysis of the viral and host variables and their interaction, to unveil the feasibility of prevention of the disease.

ENV1309 & ENV1310: National Hospital Based Rotavirus Surveillance Network (Referral & Peripheral Site)

SD Chitambar, V Gopalkrishana, PJain & R Ghuge

Funding: ICMR (Extramural) Project Duration: 2013-2016

The study involves a national hospital based surveillance of rotavirus disease and strains among children <5 yr for determination of the age and seasonal distribution and prevalent G-P types at multiple-centres in India. The NIV, Pune is one of the referral centres and represents the west zone comprising of four peripheral sites; Pune (KEM Hospital, Bharati Hospital and Shaishav Clinic), Mumbai (LTMGH & MC), Ahmedabad (BJMC) and Surat (SMIMER) and a Regional centre; RMRC Belgaum with two clinical recruitment sites (CRSs), JNMC, Belgaum and KIMS Karad. A total of 1618 stool specimens were collected during 2014–2015 from Pune (n=262), Mumbai (n=293), Ahmedabad (n=206), Surat (n=332), Karad (n=277) and Belgaum (n=248) sites from children <5 yr of age, admitted for acute diarrhoea. Rotavirus positivity was detected in 542 specimens in the range of 18.9%-53.1% from all sites with a mean value of 33.5%. The highest rotavirus positivity (40.4%- 46.9%) was noted in 7-12 month's age group during the post monsoon season i.e. October to December (45.7%). However, seasonal positivity to rotavirus was found to vary between different CRSs. Genotyping of VP7 (G) and VP4 (P) genes was carried out for every third ELISA positive stool specimens and hence a total of 163 of 542 rotavirus strains were attempted for typing. 151 (92.6%) of the specimens containing rotavirus were typed for both VP7 and VP4 genes. Two strains were nontypable for VP4 gene, while other two though tested positive in VP6 RT- PCR, was found nontypeable for both VP7 (G) and VP4 (P) genes and eight strains (4.9%) contained mixed genotypes. The data showed predominance (57.1%) of G1P[8] strains in circulation. G2P[4], G9P[8], G12P[8], G9P[4], G1P[6], G9P[6], G12P[6] and G12P[11] strains were detected at low levels (0.6%- 9.2%).

ENV1311: Development of an oral HEY (hen egg yolk) antibody formulation for prophylaxis and therapy against rotavirus diarrhea

SD Chitambar & GR Ghalsasi (Venky's (India) Ltd, Pune)

Funding: VIL, Pune Project Duration: January 2013-December 2017

Earlier work for preparation of anti-rotavirus IgY was continued further by upscaling, purification and characterization of human rotaviruses (HRV-1, HRV-9), immunization of SPF birds with purified HRVs, monitoring serum antibody generation by ELISA and collection of eggs from immunized birds. Extraction and purification procedures to recover better yield of anti-HRV IgYs were standardized. Purity of the products is being checked.

ENTERIC VIRUSES GROUP

EPD1002: Community-based surveillance of viral disease/Syndrome in JanataVasahat in Pune city, Maharashtra

YK Gurav, SS Ranshing & V Goplakrishna

Funding: Intramural Project Duration: 2013-2015

Acute diarrheal disease (ADD) surveillance was undertaken by community health workers among children <6yr age in Janata Vasahat in Pune (Maharashtra), India. Twenty four stool specimens were collected from children with ADD and six specimens (25%) tested positive for group A rotavirus (Gr.A RV) by ELISA.

ENV1401: Generation of polyclonal antibodies against simian (SA-11) rotavirus strain for detection of rotavirus in ELISA

SS Ranshing, V Gopalkrishna & SD Chitambar

Funding: Intramural Project Duration: Feb 2014- Jan 2016

Rotavirus is a major cause of acute diarrhoea in infants and young children worldwide. In India, rotavirus diarrhoea accounts for 22% of deaths, 30% of the hospitalizations, 8.3% outpatient visits annually. Rapid diagnosis is very important for management of patients with diarrhea as well as preventing antibiotic administration. The present study is being carried out to improve the indigenous antigen capture ELISA developed earlier for rotavirus detection. Study was continued further by immunization of rabbits and guinea pigs with purified rotavirus (SA-11), purification of anti-rotavirus IgGs by affinity chromatography and conjugation of purified IgGs with horse-raddish peroxidise/biotin. Rotavirus confirmed for inactivation was included as a positive control in the ELISA test. The precision and cut off value of the test was determined after optimizing the concentration of ELISA reagents. Cross reactivity of the reagents with non-rota enteric viruses was determined by ELISA. The performance of the test was compared with that of the commercially available kit. Stability of the reagents was checked and found to be satisfactory.

ENV1402: Development of a biosensor based assay using chicken egg yolk antibodies for rapid diagnosis of rotavirus infection

SD Chitambar, MS Joshi & V Gopalkrishna

(In collaboration with Dr. A. Michael, PSG college of Arts and Science, Coimbatore and Dr. (Mrs) S. Berchmans, CECRI, Karaikudi, Tamilnadu)

Funding: ICMR (Extramural) Project Duration: Sept 2013-April 2015

The aim of the study is to develop a rapid, indigenous ELISA and a novel biosensor based kit using recombinant VP6 chicken egg yolk antibodies for diagnosis of group A rotavirus. Simian 11 (SA11) strain of group A rotavirus was selected for cloning and expression of VP6 gene. Complete VP6 gene was amplified using RT-PCR and full length nucleotide sequence was obtained. Analysis of full length sequence indicated presence of genotype I-2 of VP6 gene. Complete VP6 protein was expressed in the bacterial expression ***E.Coli*** system. Standardization of expression with IPTG

concentration and incubation time was carried out. Expression of the VP6 protein was confirmed using Rotaclone ELISA kit. Further purification and analysis of the expressed protein is being done by affinity chromatography, SDS-PAGE and Western Blot assays.

New Projects Initiated:

ENV1501: Identification and molecular characterization of group C rotaviruses in human and animals with acute gastroenteritis from western India: A retrospective study

MS Joshi & V Gopalkrishna

Funding: Intramural Project Duration-2015-2018

Rotavirus is the most important viral pathogen associated with gastroenteritis, and is classified into eight groups (A–H), of which groups A, B, C and H have been detected in both humans and animals. Group A rotavirus is the leading cause of acute gastroenteritis in children while Group B rotavirus infection is rare. Molecular and epidemiological studies of Group C rotavirus (GCR) indicated 2.3-23.7%, 19.5-46% and 5.5-5.7% positivity in humans, pigs and bovines, respectively. It has been suggested that GCR might be an emerging zoonotic infection in humans due to its low prevalence in humans and a high prevalence in pigs. GCR is globally distributed in sporadic and epidemic cases of gastroenteritis patients affecting all age groups. However, role of GCR in causing gastroenteritis in both humans and animals is unknown and never reported from India. The aim of the present study is to detect and characterize GCR in humans, bovines and pigs with acute gastroenteritis, to elucidate evolutionary relationship and time scale stasis or dynamics existing in GCR. The complete nucleotide sequences of all the 11 genes of GCR strains available in GenBank (n=879) were aligned using Clustal X version 1.83. The nucleotide sequences suitable for designing of diagnostic primers were detected in VP6 gene of human, porcine and bovine GCR strains. RT-PCR assay for detection of GCR has been standardized using newly designed primers. Screening of GCR in retrospective stool specimens collected from acute gastroenteritis patients is under progress.

ENV1502: Development of a multiplex PCR assay for detection of viral pathogens associated with diarrheal diseases

VS Tatte & V Gopalkrishna

Funding: Intramural Project Duration: 2015-2018

Diarrhoea due to viral and bacterial pathogens is a major public health problem. Among the viral pathogens, rotavirus followed by NoV, adenoviruses, Astro and Sapo viruses contribute in causing the disease. In recent years, several novel enteric viruses i.e., Aichi virus, enteroviruses, parechoviruses, Sali/klassie and human Boca viruses have been found associated with acute gastroenteritis. Conventional diagnostic methods for routine detection of enteric pathogens are either laborious or with limited sensitivity and specificity. Development of a simple, rapid and cost effective multiplex PCR for simultaneous detection of majority of the enteric pathogens in diarrhoea cases will be very much useful for conducting epidemiological studies or outbreak investigations where processing of large number of samples are involved. The objective of the study is to develop a multiplex PCR assay system for detection of enteric viruses i.e., Rota, Noro, Sapo, Enteric adeno,

ENTERIC VIRUSES GROUP

Astro, Entero, Parecho, Aichi and Boca viruses. Faecal specimens initially tested positive for different enteric viruses were selected for the standardization of the multiplex PCR. Thirty percent faecal suspension of all the specimens was prepared using 1X PBS, pH 7.4 and RNA was extracted using the Mag Max™ 96 Viral RNA Isolation kit (Life Technologies). Detection of enteric viruses using one step RT-PCR protocol was standardized for the study using the Qiagen one Step RT-PCR kit. The specimens showing positivity for single enteric virus were selected for further standardization of the multiplex PCR.

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DIAGNOSTIC REAGENT FACILITY

WORK DONE:

The department has the following priorities:

- Production and supply of MAC ELISA diagnostic kits.
- Standardization and validation of new test (kits) for viral diseases.
- Conduct training (hands on and class room) for DMOs & DHOs.

PRIORITY 1 : Production of MAC ELISA diagnostic kits

Production and supply of MAC ELISA diagnostic kits to sentinel centers and 15 Apex laboratories engaged in laboratory diagnosis of JEV, Dengue and CHIKV continued as per their need. During the year, 4767 MAC ELISA kits were supplied to SSH and Apex labs under the National Program including that sent to the WHO SEAR for onward transmission to the neighboring countries (Table 1).

Table 1: Supply of MAC ELISA kits in 2014-15

	JE	DEN	CHIK	TOTAL
National Program	386	3343	945	4674
WHO-SEAR, New Delhi	68	15	10	93
Total	454	3358	955	4767

Three hundred fifty sentinel centers have been identified across the country including Arunachal Pradesh, Mizoram, Sikkim, Kashmir (Srinagar), Andaman & Nicobar (Port Blair) and Lakshadweep islands. The kits were supplied to the centers based on their requirements. Supply of kits continued throughout the year as focal outbreaks continued in and around the sentinel centers (Figure 1).

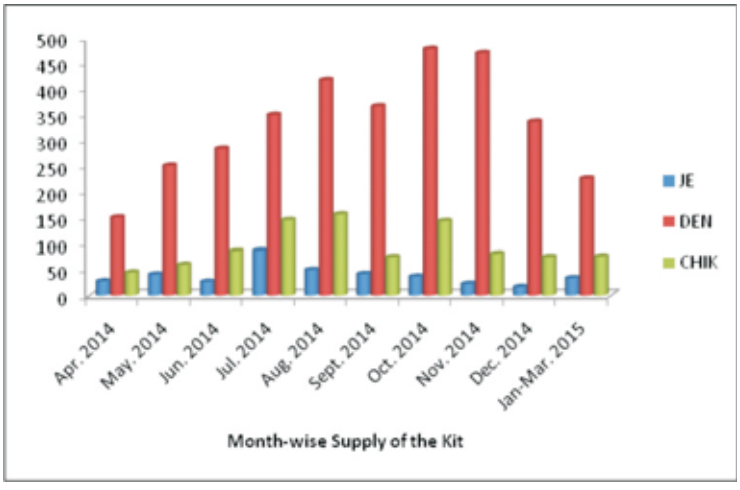

Figure 1: Month-wise supply of MAC ELISA kits under the National Program

DIAGNOSTIC REAGENT FACILITY

PRIORITY 2: Standardization and validation of new test (kits) for viral diseases

The following kits were validated during this period:

- **CCHF Cattle IgG ELISA and CCHF Goat/Sheep IgG ELISA**

  The above kits have been standardized and validated for all the parameters. Details of reagent, and protocols (SOPs) for production and validation of these kits were transferred to the BSL-4 lab as instructed by the Director, NIV. The BSL-4 staff was also given hands on training for up-scaling the production and validation of these kits including quality approval of the raw material. The BSL-4 laboratory was also provided support for testing of the bovine, goat and sheep samples to detect the presence of anti-CCHF IgG (paper published).

- **JE/WN Combo IgM ELISA**

  JEV and WNV are placed in the family ***Flaviviridae*** and belong to a common serogroup. Both the viruses cause encephalitis in humans and are transmitted by bite of infected mosquitoes. During a natural infection, antibody response against these viruses is generated against the envelope protein that has a similar peptide sequence. It is therefore difficult to identify the etiological agent responsible for Encephalitis based on the antibody specificity. The antiviral antibodies cross react in all the serological tests except in the neutralization test or reactivity at a higher dilution.

  After the WNV outbreak in USA, the CDC, Fort Collins developed an ELISA that would differentiate WNV antibodies from SLE antibodies based on the higher reactivity of the antibody to the causative agent in the serology test. Similarly, a JE/WN combo ELISA was standardized.The test could identify JE specific antibody based on the higher P/N ratio for JEV than P/N ratio for WN. The test however, could not confirm the WN outbreak based on the hypothesis, due to the non availability of WN positive samples.

- **JE Pig IgM ELISA and JE Pig IgG ELISA**

  Standardization has been initiated using pig samples collected from JE endemic area. The samples that were positive for HI antibodies and showed reduced titer after β mercaptoethanol treatment indicated IgM antibodies. JE HI positive pig samples were tested for IgG and IgM by the new ELISA.

- ➢ One sample that was IgM positive showed reduced HI after β mercaptoethanol treatment was positive by MAC ELISA indicating presence of IgM.
- ➢ Thirteen samples that were positive for HI were found to be negative for IgM antibody but were positive for IgG antibodies by the new ELISA.
- ➢ Three out of 33 HI negative samples were found to be positive for JE IgG by the new ELISA.

DIAGNOSTIC REAGENT FACILITY

- **JE Antigen Capture ELISA (pig)**

  JE antigen detection ELISA was standardized using the cell culture grown JE antigen. The test could detect JE genotype I (0944400) and genotype III (08977 & 0714922) and did not show cross reactivity with the WNV (821566 & KLU 181), Dengue and CHIKV antigen grown in cell culture.

PRIORITY 3 : Conduct training (hands on and class room)

Staff of the sentinel centers and apex laboratories were provided routine training (hands-on laboratory tests and class room), to conduct effective diagnosis of the virus infection during outbreaks.

MEDICAL ENTOMOLOGY & ZOOLOGY

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MEDICAL ENTOMOLOGY & ZOOLOGY

ENT1401: Entomological studies during virus disease outbreaks.

Gokhale MD & Sudeep AB

Funding: Intramural Project Duration: 2014-2016

Entomological studies were continued during the year to understand the arbovirus vector ecology in the context of arbovirus disease outbreaks. The objectives of the study were to determine vector bionomic in the disease affected areas and to isolate etiological agents from the field collected vectors (mosquitoes, sand flies and ticks). One hundred and twenty eight Aedes *aegypti* mosquitoes and 125 sandfly specimens belonging to Phlebotomus and Sergentomyia genera were processed for Dengue virus and Chandipura virus isolation. Three mosquitoes were tested positive for dengue while no virus could be detected in sand flies.

ENT1302: Studies on the bionomics of Phlebotomine sand flies in Nagpur division of Maharashtra and Andhra Pradesh and to determine their role in transmission of Chandipura virus.

Sudeep AB, Ghodke YS & Gokhale MD

Funding: Intramural (submitted to ICMR; project sanctioned) Project Duration: 2014-2016

Chandipura virus (CHPV) has become endemic to Vidarbha region and Warangal district of Maharashtra and Andhra Pradesh, respectively as sporadic cases continue to report every year. During an outbreak of CHPV in Vidarbha region during July 2012, the virus was isolated from *Sergentomyia* spp. of sand flies for the first time (Reported in last year's AR). Therefore, it was proposed to study the bionomics of Phlebotomine sand flies from the above areas to study the transmission dynamics of CHPV. The objectives of the study are determination of species composition, density and distribution of sand flies; isolation and characterization of CHPV and other sand fly borne viruses from sand flies and human serum as well as the transmission dynamics of CHPV disease/encephalitis. In the previous year, molecular tools for species authentication using mitochondrial Cytochrome c oxidase subunit I (COI) gene and ribosomal 18S rRNA have been standardized. During the reporting year, sequences from *Phlebotomus papatasi, Ph argentipus, Sergentomyia babu, Ser bailey, and Ser punjabensis* was amplified and compared with voucher specimens deposited in the NCBI database. BLAST analysis showed 98-99% identity confirming authentication of species. A cyclic colony of *Phlebotomus papatasi*, the major vector of CHPV, has been successfully established in the laboratory.

ENT1303: Studies on chikungunya virus attenuation during serial passaging *in vitro.*

Sudeep AB, Parashar D & Gokhale MD

Funding: Intramural Project Duration: 2013-2016

CHIKV has been a major public health problem in India since 2005 and affected more than five million people in 21 states/UTs. Despite the high infection rate and morbidity, no licensed vaccine or anti-viral compounds are available. Recent studies have shown the potential of live attenuated vaccines. It is therefore proposed to attenuate CHIKV strains by serial passaging *in vitro* and study the mechanism involved at the genetic level. Serial passaging of three strains of CHIKV

(Asian, ECSA A226 & ECSA A226V) has been completed (58 passages) ***in Vero E6*** cell line. Despite serial passaging for more than 50 passages complete attenuation could not be achieved. Loss of virulence could be observed due to delayed commencement of CPE. Whole genome sequence analysis at different passage levels (at every 10th passage) is being carried out to determine the changes in the genome.

ENT1305: Development of cell lines from certain medically important species of hard ticks using conventional and modern tools.

Sudeep AB

Funding: Intramural Project Duration: 2013-2016

Incidence of tick borne virus infections has shown an increase in the recent years globally. In India, Crimean Congo hemorrhagic fever has caused outbreaks in Gujarat since 2011 while Kyasanur Forest disease virus has shown geographic expansion to neighboring states from its epicenter in Shimoga district in Karnataka. Virus isolation, being the goldstandard in diagnosis and for the development of diagnostics and vaccines, availability of cell lines from the vectors would be advantageous. Primary cultures were set up with embryonic tissues from different species of ticks *viz. Hemaphysalis intermedia, H. bispinosa, Hyalomma marginatum* and *Boophilus microplus.* Though tissue attachment is seen, proliferation of cells could not be observed. Attempts to develop cell cultures are in progress.

ENT1202 Studies on local/migratory birds and aviphillic mosquitoes of Alappuzha district, Kerala state in relation to West Nile virus (In continuation to last year's AR).

Sudeep AB & Gokhale MD

Funding: Intramural Project Duration: 2011-2014

Susceptibility and replication potential of *Culex gelidus* mosquito to six viruses of public health importance in India was studied. The mosquito replicated JEV, CHIKV, Chandipura virus (CHPV), Ingwavuma virus (INGV), Chittoor virus (CHITV) and Umbre virus (UMBV). JEV, CHPV, CHIKV and CHITV yielded >5log$_{10}$TCID$_{50}$/ml virus while UMBV and INGV yielded approx 4log virus. JEV, CHIKV and CHITV could be detected in the saliva of the infected mosquitoes showing the competence of the mosquito to transmit these viruses while CHPV, INGV and UMBV could not be detected in the saliva of the infected mosquitoes suggesting its incompetency to transmit the viruses (Figure 1).

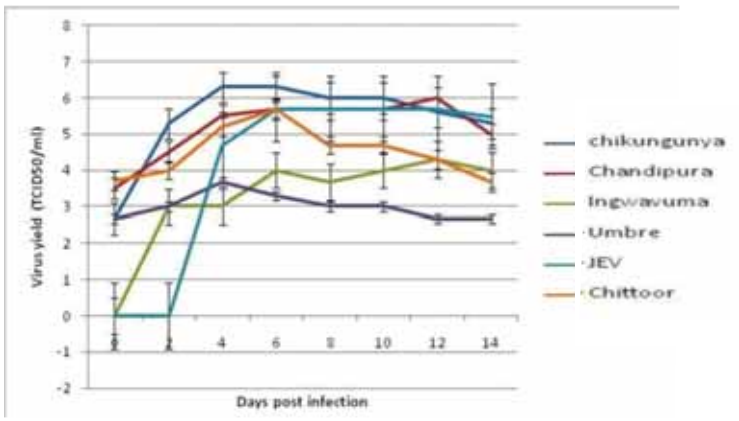

Figure 1: Growth kinetics of certain virus of public health importance in *Cx gelidus* mosquitoes.

MEDICAL ENTOMOLOGY & ZOOLOGY

ENT1306: Studies on the dynamics of JE virus multiplication in different vectors to understand the differential susceptibility.

Gokhale MD

Funding: Intramural Project Duration: 2013-2016

Morphometric measurements with reference to the six taxonomic characters on the 4th stage larvae of *Culex quinquefasciatus, Culex tritaeniorhynchus* and *Culex gelidus* have been completed. Susceptibility of this species to G1 and G3 strains of the Japanese encephalitis virus isolates from Gorakhpur was attempted. It was observed that the mosquito species is susceptible to both the virus strains by both inoculation and membrane feeding routes.

Miscellaneous studies:

Ingwavuma virus studies: *Culex tritaeniorhynchus* mosquitoes were infected by the membrane feeding route with Ingwavuma virus (INGV). The virus was detected in the head squashes by Immuno-fluorescence test. Transmission of the virus by feeding on infant suckling mice was attempted. However, horizontal transmission of the INGV could not be demonstrated.

Susceptibility of *Aedes aegypti* to Dengue-1 virus (Hawaii and Kerala strains) was studied. It was observed that the Kerala strain of Dengue-1 virus is more infective than the Hawaii strain by inoculation and oral feeding routes. The virus from Kerala isolate was detected by 4th post infection day in the head squashes of mosquito by the Inoculation method.

Number of samples tested (virus-wise details from each Group):

Mosquitoes: *Aedes aegypti* specimens (n=128) were received from Gondia (12 localities), Nagpur (3), Wardha (1), Chandrapur (8) and Bhandara (1) districts of Maharashtra as referred samples for dengue virus detection. Dengue virus was detected in 03 Aedes aegypti female individuals collected from Dhanla and Seloti areas in Nagpur district and Pombhurna in Chandrapur district.

Sandflies: One hundred and twenty five sandfly specimens belonging to genus *Phlebotomus* and *Sergentomyia* were received from 6 districts of Gujarat viz., Dahod, Vadodara, Narmada, Kheda, Panchmahal and Nadiad. All the sandflies were processed in tissue culture but no virus could be isolated.

Ecological survey: Survey of vectors of arbovirus diseases was conducted in the flood affected areas of Kashmir during the month of September 2014. During the period of survey, eleven localities in three districts (Bandipora, Baramulla and Srinagar) were visited. Presence of *Culex bitaeniorhynchus, Culex quinquefasciatus* was observed in Bandipora. Water logging/flooding areas in and around other localities, as well as the residential potable and non-potable containers were negative for the presence of container breeding mosquitoes.

MEASLES GROUP

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MEASLES GROUP

MMR1101: Establishment of rapid and reliable assay for detection of measles, mumps and rubella neutralizing antibody

Vaidya SR

Funding: Intramural Project Duration: 2012-2015

Measles, mumps and rubella are vaccine-preventable diseases; however, limited epidemiological data are available from low-income or developing countries. Thus, it is important to investigate the transmission of these viruses in different geographical regions. In this context, a cell culture-based rapid and reliable immuno-colorimetric assay (ICA) was established and its utility was studied using 23 measles, 06 mumps and 06 rubella virus isolates and three vaccine strains. Detection by ICA was also compared with plaque and RT-PCR assays. In addition, ICA was used to detect viruses in throat swabs (n = 24) collected from patients with suspected measles or mumps. Similarly, ICA was used in a focus reduction neutralization test (FRNT) and the results were compared with those obtained by a commercial IgG enzyme immuno assay. For this purpose, a panel of archived serum samples was used; 98 sera (from 60 male and 38 female subjects aged 1 month-27 years) for measles; 94 sera (from 55 male and 38 female subjects aged 1 month–27 years) for mumps and 85 sera (from 50 male and 35 female subjects aged 1 month–27 years) for rubella. For the challenge experiments, wild-type measles (genotype D8), mumps (genotype C) and rubella (genotype 2B) were used in the neutralization tests. Measles and mumps virus were detected 2 days post-infection in Vero or Vero-human signaling lymphocytic activation molecule cells, whereas rubella virus was detected 3 days post-infection in Vero cells. The blue stained viral foci were visible by the naked eye or through a magnifying glass. In conclusion, ICA was successfully used on 35 virus isolates, three vaccine strains and clinical specimens collected from suspected cases of measles and mumps. Furthermore, an application of ICA in a neutralization test (i.e., FRNT) was documented and this may be useful for sero-epidemiological, cross-neutralization and pre/post-vaccine studies.

MMR1202: Genetic and antigenic characterization of measles, mumps and rubella virus isolates

Vaidya SR

Funding: Intramural Project Duration: 2013-2015

Five mumps virus (MuV) isolates from Maharashtra and two MuV isolates from Tamil Nadu and Uttar Pradesh were subjected to complete genome sequencing. Initially, mumps isolates were genotyped based on the standard phylogenetic analysis of small hydrophobic (SH) gene. All structural and non-structural genes and their amino acids were compared with the MuV full genome sequences available in the GenBank. The genetic differences in the antigenic sites were also compared between the Indian isolates and the six vaccine strains. The SH gene analysis revealed that five MuV isolates belong to genotype C and two belong to genotype G strains. The genotype C viruses had 57, 55, 55 and 59 amino acid substitutions in Pune 2008, Chennai 2012, Osmanabad 2012 and Kushinagar 2013 MuV isolates, respectively when compared to the

reference sequence. The genotype G strains had 39 and 26 amino acid substitutions in Pune 1986 and Pune 2012 isolates, respectively when compared to the reference sequence. In conclusion, the complete genome sequencing of the Indian mumps strains obtained in years 1986, 2008, 2012 and 2013 may be useful for understanding the epidemiology of mumps in India as well as globally. The study indicates a need to strengthen surveillance of mumps virus in India.

Reports from the mumps-immunized countries suggest differences in mumps virus neutralizing antibody titers when tested with the vaccine and wild type viruses. Such reports are unavailable from countries like India where mumps vaccine is not included in the routine immunization program. Therefore, cross neutralization studies was performed using a panel of serum samples that was challenged with two wild types (genotypes C and G) and Leningrad-Zagreb vaccine strain (genotype N). Result shows that all the serum samples effectively neutralized mumps wild types and a vaccine strain. However, significantly lower level of FRNT titers was noted to wild types than to vaccine strain ($P<0.05$, t test). Full genome sequencing of measles and rubella virus isolates is in progress.

MMR1201: Routine Laboratory Activities: Outbreak Investigations/Diagnostic services provided to Local/State health authorities/WHO-Reference laboratories

Vaidya SR

Funding: WHO & Intramural Project Duration: Ongoing

Measles Group has been providing diagnostic services to the local hospitals, medical practitioners, and State health authorities. During the year 2014-15, 214 serum samples (Male-118; Female-96) were referred for measles diagnosis from fifteen hospitals and institutions (Table 1). Majority of the suspected measles cases belonged to 0-15 years (189, 88.3%) except 25 adult cases (age ranged between 17-34 years). Of the 214 suspected measles cases, 11 had history of measles vaccination (by documentation or parent's recall) during childhood (Routine National Immunization Policy is one dose of measles after 9 months of age; revised in 2010 to two doses before completion of 24 months). Of the 214 serum samples, 151 (70.56%) were found positive, 57 were negative and 6 were equivocal for Measles IgM antibody. Out of the 63 measles negative and equivocal serum samples, seven were found to be positive, 53 negative and 3 equivocal for rubella IgM antibody. Measles was confirmed in 8-suspected cases (age range 1-7 yrs; 3 male & 5 female) that were vaccinated during their childhood that indicated primary vaccine failure. Measles infection was confirmed in 15/25 adult cases. Laboratory reports were submitted to the concerned authority to undertake control measures and case management. Similarly, weekly/monthly reports were provided to the World Health Organization (WHO).

MEASLES GROUP

Table 1: Serum samples referred from hospitals/institutions

Hospitals/Institutions	Number of Serum samples
Armed Forces Medical College, Pune	13
Aundh Civil Hospital, Pune	18
B J Medical College, Pune	3
Bharati Hospital and Research Centre, Pune	11
Government Medical College, Surat	10
Gujar Children's Hospital, Pune	5
KEM Hospital, Pune	1
Nair Hospital, Mumbai	1
Poona Hospital and Research Centre, Pune	1
Smt Kashibai Navale Medical College and General Hospital Pune	3
Sub-District Hospital, Manchar	6
PHC Pimpalgaon Budruk	4
Unique Children Hospital, Pune	1
Dr Naidu Infectious Hospital, Pune	64
Sasoon General Hospital, Pune	10
Yashwantrao Chavan Memorial Hospital, Pune	63
Total	214

During this year, we received 100 representative throat swabs collected from different outbreaks or sporadic cases from the States of Maharashtra, Haryana, Uttar Pradesh, Jharkhand, Rajasthan, Gujarat, Delhi, Madhya Pradesh, Chhattisgarh and Punjab. Sixteen wild type measles strains were isolated using Vero hSLAM cells (Table 2).

Table 2: Details of the measles virus isolates (wild types) obtained during 2014-15

Sr No	Age (yr)/ Sex	Place	Specimen EIA	MeV IgM RT-PCR	MeV	Genotype
1	6/M	Pune (Maharashtra)	TS	Positive	Positive	D8
2	11/M	Pune (Maharashtra)	TS	Positive	NA	NA
3	5/M	Faridabad (Haryana)	TS	NA	Positive	D8
4	11/F	Pune (Maharashtra)	TS	Positive	NA	NA
5	9/M	Bhilwada (Rajasthan)	TS	NA	Positive	D8
6	2.11/F	Sagar (Madhya Pradesh)	TS	NA	Positive	D8
7	9/M	Raipur (Chattisgarh)	TS	NA	Positive	D8
8	4F	Panna (Madhya Pradesh)	TS	NA	Positive	D8
9	1.2/F	Valsad (Gujarat)	TS	NA	NA	NA
10	2/M	Pune (Maharashtra)	TS	Positive	NA	NA
11	3/F	Rajkot (Gujarat)	TS	NA	Positive	D8
12	2.6/F	Rajkot (Gujarat)	TS	NA	Positive	D8
13	2/F	Jabalpur (Madhya Pradesh)	TS	NA	Positive	D8
14	3/F	Sagar (Madhya Pradesh)	TS	NA	Positive	D8
15	2.6/F	Delhi	TS	NA	Positive	D8
16	9.11/M	Sagar (Madhya Pradesh)	TS	NA	Positive	D8

NA = not available

MEASLES GROUP

MMR1201: Outbreak based measles surveillance in the State of Maharashtra

Vaidya SR

Funding: WHO/Intramural Project Duration: Ongoing

The state of Maharashtra has launched outbreak based measles surveillance since October 2012 and NIV started receiving 3-5 representative serum samples from each suspected outbreaks since January 2013. Between April 2014 and March 2015, the State health agencies had investigated 113 suspected measles outbreaks in 25 districts. Altogether, 553 serum samples (Male-273; Female-275 & unknown-5) were referred for laboratory diagnosis of measles/ rubella to NIV. Majority of the suspected measles cases belonged to 0-15 years (532, 96.2%) except 21 adult cases (age ranged between 15½ -30 yrs). Of the 553 suspected measles cases, 117 had history of measles vaccination (by documentation or parent's recall) during childhood. Results are summarized in Table 3. Overall, 371 (67.08%) clinically suspected measles cases were serologically confirmed at NIV. Similarly, rubella IgM positivity was noted in 36 of 182 measles IgM negative and equivocal samples (Table 4). Measles confirmed in 76-suspected cases that were vaccinated during childhood may indicate primary vaccine failure. Amongst the 21 suspected adult cases, 13 were laboratory confirmed measles cases.

Table 3: Laboratory diagnosis performed on referred serum samples

District	Referred sera (n)	MeV IgM Positive	MeV IgM Negative	MeV IgM Equivocal	RuV IgM Positive	RuV IgM Negative	RuV IgM Equivocal
Ahmednagar	42	35	7	0	4	3	0
Akola	7	6	1	0	0	1	0
Amravati	5	5	0	0	0	0	
Aurangabad	5	5	0	0	0	0	0
Bhandara	5	5	0	0	0	0	0
Chandrapur	33	18	13	2	6	9	0
Gadchiroli	20	13	7	0	1	6	0
Gondia	31	24	6	1	4	3	0
Gr. Mumbai	121	80	39	2	2	39	0
Jalgaon	10	3	7	0	4	3	0
Latur	15	13	2	0	0	2	0
Nagpur	51	33	15	3	0	18	0
Nandurbar	5	5	0	0	0	0	0
Nashik	29	11	18	0	4	14	0
Osmanabad	5	5	0	0	0	0	0
Palghar	10	7	2	1	0	3	0
Parbhani	10	6	4	0	2	2	0
Pune	34	20	13	1	1	13	0
Raigad	5	1	3	1	0	4	0
Sangli	5	5	0	0	0	0	0
Satara	10	10	0	0	0	0	0
Solapur	20	6	12	2	7	7	0
Thane	55	41	12	2	1	13	0
Wardha	15	9	6	0	0	6	0
Yavatmal	5	5	0	0	0	0	0
Total	553	371	167	15	36	146	0

MEASLES GROUP

Of the 113 suspected measles outbreaks, 93 were confirmed as measles outbreak, 8 were confirmed as rubella outbreak, 9 were mixed outbreaks of measles and rubella and three outbreaks (i.e. two from Gr Mumbai and one from Thane) could not be confirmed (Table 4). Altogether, 36-suspected measles outbreaks were reported from the Greater Mumbai and Thane Districts that included 30-confirmed measles and 3-mixed outbreaks of measles and rubella. Altogether, 34-suspected outbreaks were reported from the Vidarbha region that included 30 confirmed measles cases; three rubella and one mixed outbreak of measles and rubella. A limited number of suspected measles outbreaks (n=7) were reported from the Marathwada region that includes six confirmed measles and one mixed (measles and rubella) outbreak. This indicates the need to strengthen the measles surveillance activity in all the districts of Maharashtra.

Table 4: Region-wise split up of the suspected measles outbreak reported in Maharashtra

Region and number of Suspected outbreaks	Number of confirmed measles outbreak	Number of confirmed rubella outbreak	Mixed outbreak (measles and rubella	Non- measles and non-rubella outbreak
Vidarbha (34)	30	3	1	-
Marathwada (7)	6	-	1	-
Gr. Mumbai and Thane (36)	30	-	3	3
Kokan (3)	3	-	-	-
North Maharashtra (9)	5	2	2	-
Western Maharashtra (24)	19	3	2	-
Total (113)	93	8	9	3

1. Measles in the States of Jharkhand, Haryana, Rajasthan, Delhi, Madhya Pradesh, Gujarat, Punjab & Chattisgarh

Altogether, 114 throat swabs or urine specimens collected from the suspected measles cases were referred to NIV for virus detection and genotyping. The specimens received from the states or union territories i.e. Jharkhand, Haryana, Rajasthan, Delhi, Madhya Pradesh, Gujarat, Punjab and Chattisgarh were processed. Circulation of measles genotypes D4 (n=23) and D8 (n=63) strains were detected in these states. The measles virus sequences have been deposited in the WHO global measles sequence database i.e. MeaNS/Genbank (Table 5).

MEASLES GROUP

Table 5: Measles virus genotypes detected from different States/Union Territories

State	No.of cases referred	Specimens processed	Measles genotype	GenBank ID
Jharkhand	1	1	D4	KM034772
Haryana	3	3	3 D8	KM04377, 7 KM043778, KP221890
Rajasthan	8	8	5 D8, 2 D4	KM034773, KM034774, KM034775, KP221905, KP221906, KP221915, KP221916
Delhi	13	13	9 D8	KM364585, KM364592, KM364589, KM364590, KM364591, KM364586, KM364593, KM364587, KM364588
Madhya Pradesh	15	15	12 D8	KP221903, KP221904, KP221907, KP221908, KP221913, KP221914, KP221917, KP221918, KP221919, KP221920, KP221921, KP221922
Gujarat	33	33	22 D8, 2 D4	KP221909, KP221910, KP221911, KP221891, KP221892, KP221894, KP221898, KP221895, KP221896, KP221897, KP221899, KP221900, KP221901. 11-sequences to be submitted
Chattisgarh	1	1	D8	KP221912
Punjab	2	2	D8	KP221889
Daman & Diu	2	2	NA	-
Maharashtra	36	36	10 D8, 18 D4	KM034761 - KM034772. 16 sequences to be submitted

Altogether 77 measles N gene PCR products were referred to NIV from four national measles laboratories i.e. NIV Unit, Bangalore (n=38), SGPGIMS, Lucknow (n=14), KIPM & R, Chennai (n=18) and Government Medical College, Guwahati (n=7) for virus sequencing and genotyping. Circulation of measles genotype B3 (n=1), D4 (n=6) and D8 (n=70) has been evidenced. For the

first time, circulation of measles genotype B3 strain has been reported from Mathura, Uttar Pradesh.

2. Mumps outbreak investigation support

An outbreak of fever with parotitis was reported in a boarding school or Ashram-Shala (Vyara, Kanjan village, Tapi District, Gujarat) during the 2nd week of January, 2015). Altogether, 157 students aged between 5 to 13 years were affected. The local epidemiologists collected serum and throat swab specimens and referred to NIV for laboratory investigation. Eight out of 9 suspected mumps cases were serologically confirmed (5-female & 3-male). However, mumps virus could be neither isolated nor detected by RT-PCR.

Between the 4th and 6th week of the year 2015, an outbreak of fever with uni- or bi- lateral parotitis was reported from Lakhangaon village (Primary Health Centre, Dhamani, Ambegaon) in Pune district of Maharashtra. For the laboratory confirmation, blood, throat swabs, oral swabs and urine specimens were collected from the suspected patients. Serum samples from the suspected patients (n=35; 21-males and 14-females) were tested for mumps specific IgM antibodies by the commercial EIA. Presence of mumps RNA in throat swabs, oral swabs and urine specimens was confirmed by the SH gene RT-PCR. Virus isolation was attempted using Vero cells. Mumps specific IgM antibody was found in 23 of 35 serum samples (65.7%). Laboratory confirmed mumps patients belonged to 0-15 years. Neither Mumps RNA could be detected in throat swabs, oral swabs or urine specimens nor could mumps virus be isolated.

For serological confirmation of the mumps outbreak, the local epidemiologist referred five serum samples (age range 4-16 years, 1 male & 4 female) from Kangra at Dharamshala, Himachal Pradesh. Mumps infection was confirmed in three cases. Another two samples were referred from the local hospitals, Pune which were serologically confirmed for mumps, out of which one case (6 years male) was a mumps encephalitis patient.

Mumps IgM antibody reports were submitted to the concerned health authorities. Mumps virus genotype could not be detected from the sporadic or outbreak cases.

EPIDEMIOLOGY GROUP

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EPIDEMIOLOGY GROUP

EPD1001: Investigation of outbreaks/clusters/cases for viral etiology

BV Tandale, YK Gurav, AR Deoshatwar & DT Mourya

Funding: Intramural Project Duration: 2010-2015

1. Kyasanur Forest Disease outbreak in Malappuram district, Kerala

Following detection of anti KFDV IgM antibody in referred serum, field investigation was undertaken during 18th–22nd May, 2014. The recovered index case was a tribal woman with febrile illness for two weeks and gum bleeding. Two suspected deaths (husband and wife, aged 25-30 years) were reported (by verbal autopsy) approximately 3 months back of the incident. A monkey death was also reported 3 months back in the same area. The NIV team collected 26 sera and tested for anti KFDV IgM and KFDV RNA as well as anti-Dengue IgM and Dengue RNA. Acute and convalescent sera of the recovered case and one of her family members tested positive for the anti KFDV IgM while none tested positive for anti-Dengue IgM and Dengue RNA. Serum collected from another febrile patient of the diseased family also tested positive for anti KFDV IgM and KFDV RNA. The recovered index case was confirmed as the case of KFD based on the positivity for anti KFDV IgM antibody in both acute and convalescent sera. The PCR positivity of blood sample from an additional case from the same area and anti KFDV IgM antibody positivity in a family member of the recovered case and a febrile patient from the nearby area substantiates the recent KFDV activity in the area.

The tribal health authorities undertook enhanced surveillance of fever cases among the tribals in the area along with health education measures. Vaccination of the tribal population was recommended with procurement of vaccine from the Karnataka Government.

2. Acute Encephalitis Syndrome among children in Muzaffarpur, Bihar

BV Tandale, AR Deoshatwar, VP Bondre, PD Yadav & DT Mourya

In the first week of June 2014, AES cases increased considerably in Muzaffarpur, Bihar. Majority (64%) of the patients were between 2-5 years of age followed by 5-12 yrs (23%) and <2 yrs of age (9.8%). Male-to-female ratio among the hospitalized cases was 1:0.9. Mushahari block reported the highest number of cases in the highly affected Muzaffarpur district. Samples were collected from 8 death cases, 9 discharged patients and 100 contacts of AES cases. Secondary data on weather, litchi maturation, harvesting, pests and pesticides was collected from the National Research Center on Litchi [NRCL], IARI, Pusa, Muzaffarpur.

Children presented with sudden onset of generalized seizures and rapid progression to unconsciousness. In many cases, the progression to death was rapid i.e., within 24-36 hr of onset of seizures. Secondary data from the hospitals indicated that blood glucose levels were low at the time of admission in most cases.

The team collected blood samples from AES patients. First lot of 20 samples [8 cases and 10 contacts] was negative for the known encephalitis causing viruses like JEV, CHPV, WNV and NiV. In the second lot, 46 cases were sampled (40 sera, 4 CSF, 6 urine, 1 stool and 1 throat swab) and investigations revealed no known encephalitis causing viruses to be associated with the outbreak. The clinical and epidemiological features were not suggestive of infectious cause.

EPIDEMIOLOGY GROUP

3. Acute encephalitis syndrome in North Bengal Districts, West Bengal

BV Tandale, YK Gurav, VP Bondre, R Damle, PD Yadav & DT Mourya

An unusual rise in AES cases was reported from the North Bengal Medical College and Hospital (NBMCH), Siliguri, West Bengal, India in July 2014. Investigation was carried out during 22nd-29th July, 2014 to detect the etiology and study the clinico-epidemiological profile. Investigations were also carried out as a member of the Central team (BVT) constituted by the Government of India.

A total of 398 AES cases mostly of adults (70.8%, 282/398) were line listed. Majority of the cases were from Jalpaiguri (158) followed by Cooch Behar (83) and Darjeeling (70). Adolescents (10-19 years) were 49 and children (< 9 years) were 67. The overall case fatality ratio among the AES cases was 31.6% (115/398).

Clinical features among the AES cases included acute fever (121), altered sensorium/behaviour (104), headache (53), seizures (26), vomiting (36), diarrhoea (3) and breathlessness (2). Mild to moderate CSF pleocytosis with a lymphocytic predominance and elevated CSF protein was observed. Laboratory investigations for JEV were carried out on 271 (68%) cases. Clinical specimens included both sera and CSF from 151 AES cases; only sera from 120 cases and urine specimen from 45 cases. JEV was confirmed in 134 (49.4%) cases and among these, 79.1% (106/134) were adults. Death among the JE cases were 36 (CFR for JE=26.9%). All urine samples tested negative for NiV.

Increasing trend of case load of JE was observed among adults in North Bengal districts in West Bengal state.

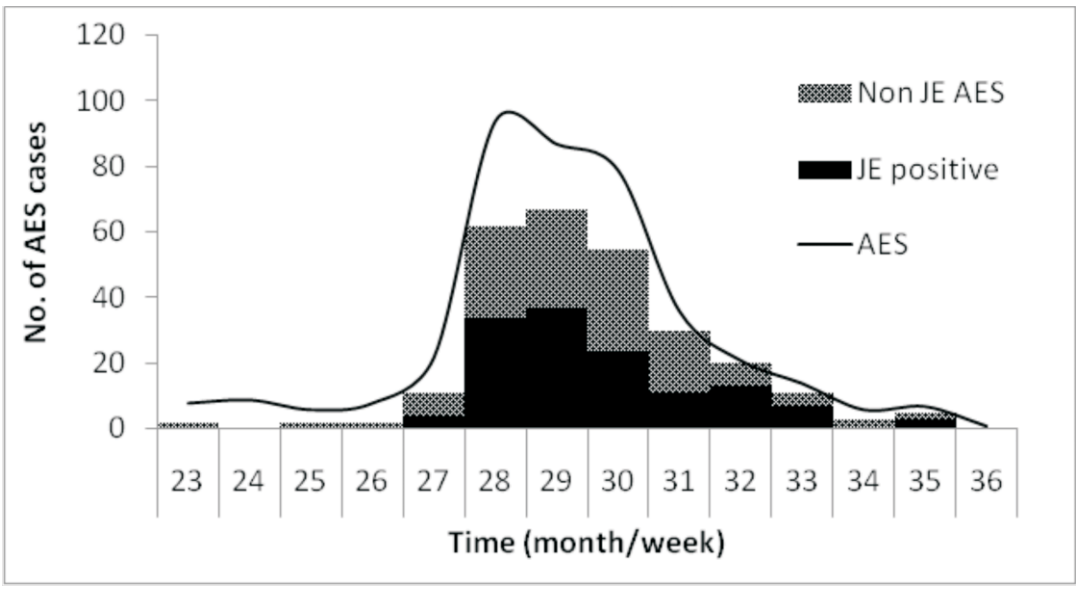

Figure 1: Distribution of AES cases showing JEV etiology in relation to time

4. Hepatitis E outbreak in Wai Taluka, Satara, Maharashtra

AR Deoshatwar, KS Lole, BV Tandale & MS Chadha

NIV team has investigated a hepatitis outbreak during 10th-11th December, 2014 in Wai taluka as per the request of Taluka Health Officer. The first case was reported on 1st November and the last case on 16th December and the total number of cases recorded were 176. All the cases were investigated as per the acute viral hepatitis case definition. Ninety two percent comprised of males of which 66% were between the age group of 20-40 yr. The minimum age of patients reported was 20 years and no pregnant woman was affected.

An uncovered well was the only source of water for hundreds of people in the market. Most of the people visiting the market committee were males. All the symptomatic males in that village reported to have the history of visiting the market committee for trade.

A total of 168 blood samples, 13 stool samples and 14 water samples were collected. Anti Hepatitis E IgM was detected in 64 (84.2%) of the 76 symptomatic and 25 (27.5%) of the 91 asymptomatic subjects. Water samples collected from Bavdhan and Bhuinj village were found to be contaminated with HEV.

The outbreak was caused by HEV. The virus was similar to the one in circulation in other parts of the state. Two different foci of infection were likely. Contamination of unprotected well supplying water to the Wai market committee was probably the cause of the outbreak. Prevention and control measures were suggested to the local health authorities.

EPD1002: Community-based surveillance of viral diseases/syndromes in Janata Vasahat in Pune city, Maharashtra

YK Gurav, BV Tandale, MS Chadha, PS Shah, P Shil, SD Chitambar, V Gopalkrishna & AR Deoshatwar, R Bharadwaj[a], V Dohe[a] & S Pole[a] [a]BJ Govt. Medical College, Pune]

Funding: ICMR Project Duration: December 2013-November 2015

Following the initiation of the project in December 2013, community-based surveillance activities of viral diseases/syndromes in a slum area in Pune city were continued. Demographic data is collected and is being entered in demographic surveillance software. The total study population under surveillance is 31,024.

A total of 837 Influenza like illness (ILI) cases were reported (April 2014-March 2015). Rise in influenza activity was recorded in February 2015. Community incidence was 1.7 per 1000 person per week in week number 7 (year 2015). Throat swabs were collected from 337 (40.2%) ILI cases and viral or bacterial etiology could be detected in 115 (31.4%) cases. Influenza virus etiology could be detected in 89 (28.3%) cases-[Influenza A(H1N1) in 61, Influenza A(H3N2) in 22 and Type B in 6] (Figure 2) and bacterial etiology in 26 [Group B Haemolytic *Streptococci* (11), Group G *Streptococci* (7), *Streptococcus pyogens* (6), *Staphylococcus aureus* (2)].

Among 245 dengue like-illness cases, blood samples were collected from 94 cases and 21 cases tested positive for anti dengue IgM antibodies. Four cases tested positive for Leptospira (aged between 12-19 yrs).

Acute diarrheal disease (ADD) cases reported among children aged d" 6yrs (n=102). Stool samples were collected from 27 ADD cases and rotavirus etiology was detected in 6 cases. ***E. coli***

was detected in 23 cases and one case each of Shigella flexneri and Shigella sonnei were detected.

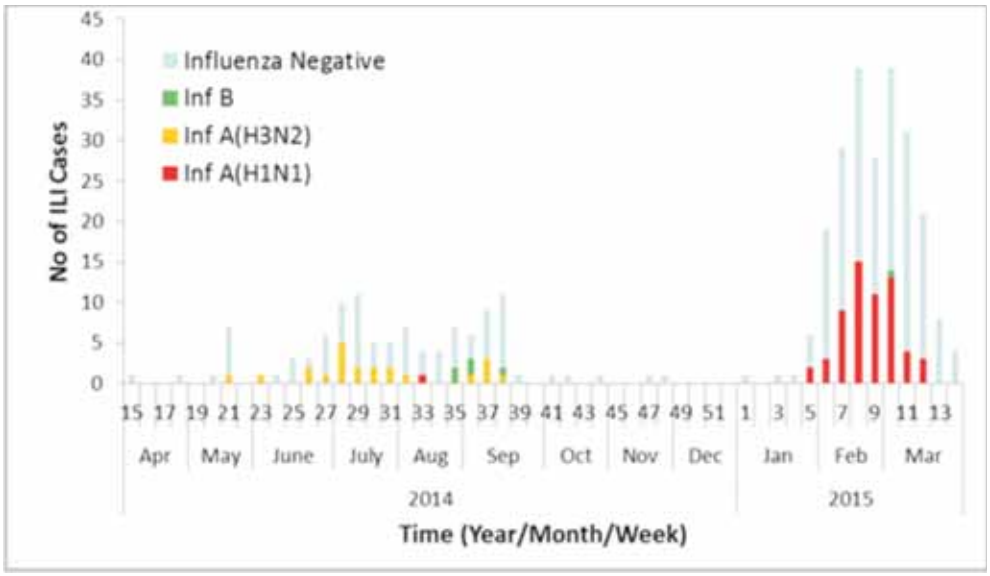

Figure 2: Weekly distribution of influenza like illness cases showing influenza virus etiology (April 2014 to March 2015)

EPD1003: Multi-centric hospital-based surveillance of acute encephalitis syndrome for viral etiology among children in selected districts of Maharashtra and Andhra Pradesh

BV Tandale, YK Gurav, VP Bondre, V Gopalkrishna, GN Sapkal (NIV Pune); M Qazi, (GMC, Nagpur), R Narang (MGIMS, Sewagram & RK Rao (KMC, Warangal)

Funding: ICMR Project Duration: December 2013–November 2016

The project aims to establish and undertake surveillance for viral diagnosis of AES hospitalizations among children. Surveillance is needed to understand the clinical and epidemiological aspects and likely factors for endemic occurrence/outbreaks. It includes estimation of incidence of AES along with etiological contributions of JE and CHP viruses. NIV Pune is a reference laboratory required to coordinate project activities at the three sites viz-GMC, Nagpur, MGIMS, Sewagram and KMC, Warangal.

Clinical investigations were undertaken by the site investigators. Clinical specimens were collected and tested at the site laboratory for JE IgM by MAC ELISA and CHPV RNA by PCR. These specimens were retested for JE, CHPV and additional viruses at the reference laboratory for quality control. Additionally, stool/rectal swabs were collected from the enrolled cases and matched controls for Enterovirus RT-PCR testing. Referred autopsy specimens were also considered for histo-pathological studies.

Training of investigators and recruited project staff was undertaken at NIV Pune. Surveillance was initiated from December 2014 along with diagnostic testing for JE by IgM ELISA by the site laboratories. During December 2014 to March 2015, the number of enrolled AES cases included-

13 cases (KMC, Warangal), 11 cases (GMC, Nagpur) and 1 case (MGIMS, Sewagram). All the specimens were negative for JE IgM ELISA testing at 3 site laboratories (Figure 3). CHPV RNA by RT-PCR would be considered after PCR laboratory set up. Reports were communicated to the hospitals/physicians along with sharing to the State health officials.

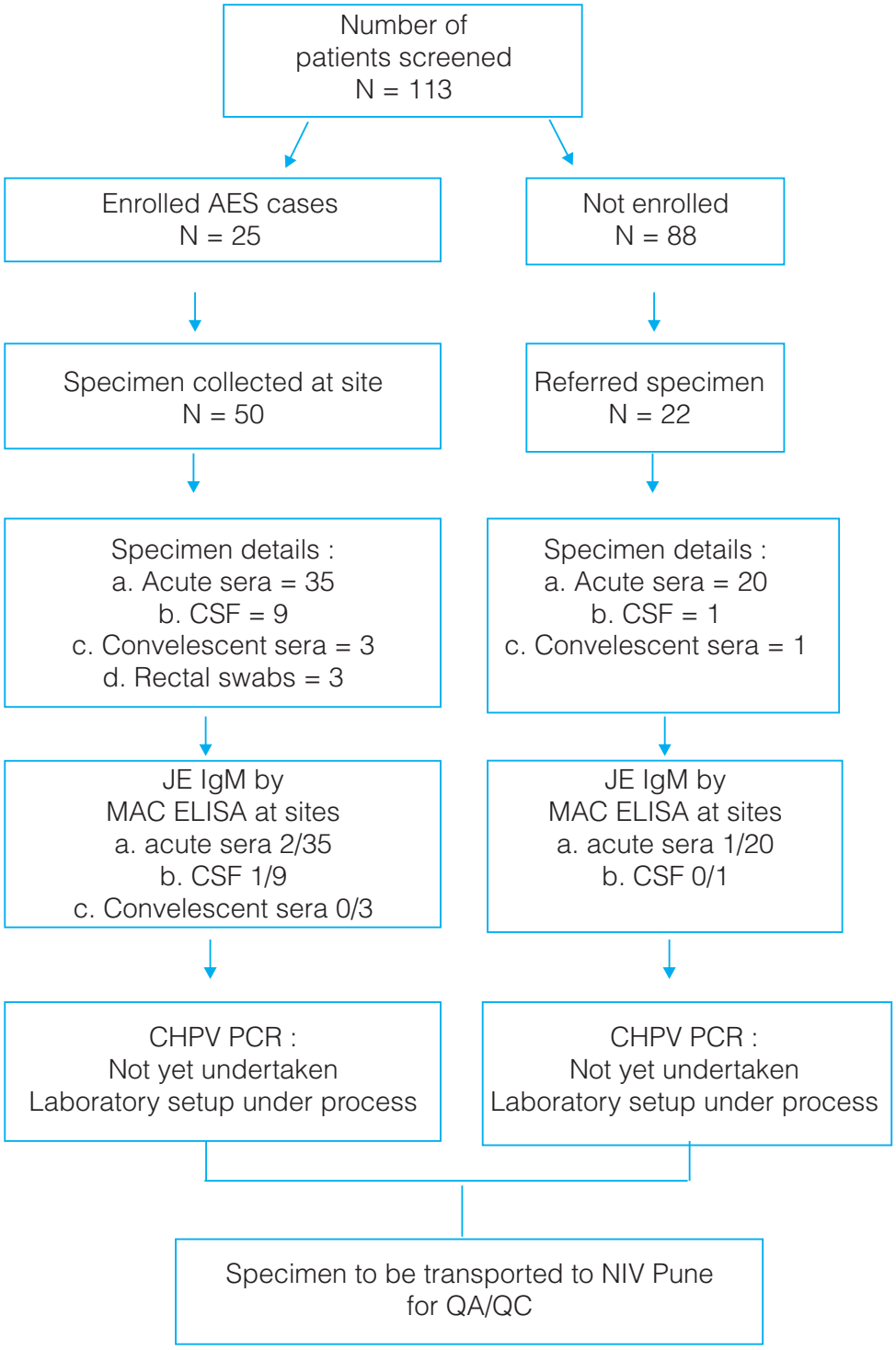

Figure 3: Flow diagram showing the number of cases and specimens at different stages of the study

ELECTRON MICROSCOPY AND HISTOPATHOLOGY GROUP

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ELECTRON MICROSCOPY AND HISTOPATHOLOGY GROUP

Core Facility Activity

Applied high-end transmission electron microscopy services were provided to both in-house researchers and scientists from different National research institutes in the area of imaging and analysis. A total of 436 samples were analyzed. These included negative staining, cryo imaging, analysis of ultrathin sections, etc. Participation in routine EQ quality Assurance Program was also carried out.

Characterizing the role of dengue virus NS1 protein on vascular endothelial physiology

A Basu & K Ghosh, NIIH Mumbai

Funding: DBT (Extramural) Project Duration: 2014-2017

HRTEM studies on dengue 2 virus NS1

The non structural protein 1 (NS1) of dengue viruses is a 150 Kd protein that plays a dual role in both virus replication biology and potentially altering host cell physiology. While a significant amount of knowledge about its structure has been gained from recent studies, the role of NS1 protein in pathophysiology of dengue virus infection remains incompletely understood. In the first phase of the planned study we expressed dengue 2 virus NS1 protein in a mammalian expression system. High-resolution transmission electron microscopy of the NS1 protein showed a defined structure. Interestingly, when compared with the NS1 proteins of Kysanur Forest Disease virus (KFD) (collaboration with Dr DT Mourya, BSL4 lab) and West Nile virus, the KFD NS1 protein was seen to be drastically different with very well organized structures. Further imaging studies are ongoing to characterize the 3D macromolecular structure.

Response of endothelial cells to exogenous exposure to dengue 2 virus NS1 protein

Human vascular endothelial cells were exposed to various doses of the purified dengue 2 virus NS1 protein and the effect on cell morphology, toxicity and adhesion was studied. Primary evidence suggests that the dengue viral NS1 protein does not have acute cytotoxic effects per se on the growing vascular endothelial cells but can alter adhesion molecule expression and physiology. Further studies are ongoing to characterize these in detail.

Ultrastructural changes in host cells infected with Malsoor, a novel bat *Phlebovirus*

A Basu, S Prasad, PD Yadav & DT Mourya

Funding: DBT (Extramural) Project Duration: 2013-2014

Transmission electron microscopy studies to examine the effect of Malsoor virus (MV) on host cells and replication events of the virus were studied in detail. Vero CCL81cell line infected with the MV showed evidence of distinct cytopathic effect from the 2nd day post infection (PI). This was observed as rounding of cells with increased granularity and progressive detachment of the cell sheet. Immunofluorescence assay (IFA) for MV antigens was positive by the 2nd day PI. Detection of MV nucleic acids was also positive by PCR from the 2nd day PI. (data not shown). The ultrastructural changes were distinct in MV infected cells. These included detection of cytoplasmic and extracellular multilamellate structures (or myelin bodies-MB) as a consistent finding in most of the cell profiles observed (Figure 1) and presence of cytoplasmic rod shaped paracrystalline inclusion

bodies (IB) in a fraction of cells (25 % approximately) (Figure 1). The ultrastructure of majority of the dying cells was suggestive of necrosis while very few apoptotic bodies could be seen. Extracellular virions were also observed maturing from the infected cells (Figure 1). Interestingly, the evidence of autophagic engagement was noticeable in approximately 25-30% of the cells examined. This was seen as formation of typical autophagosomes and multivesiculate structures. Morphogenesis of MV was imaged associated closely with the golgi complex. Extracellular virus particles were seen at moderate frequency and budding viruses imaged within golgi compartments in multilayered vesicles. A significant number of cells showed necrotic ultrastructure.

A notable observation from the sequence alignment studies of the G1 and G2 proteins coded by the M gene segment shows the presence of an N-linked glycosylation site at a conserved position between the Heartland, SFTS and MV. This site was seen to be present in Sand fly Fever (Naples)-like virus but lost in Rift Valley Fever virus.

Another very interesting point that stands out from the present study is that although the overall morphology and morphogenesis events of MV as imaged by transmission electron microscopy is consistent with the literature on Bunyavirus replication. The virus was unusually small in size as compared with other Bunyaviruses. This is consistent with an earlier report of another bat Bunyavirus, Kaeng Khoi, isolated from Chaerephon plicata bats from Cambodia. Importantly, whether this has any association with the presence of N linked glycosylation sites seen shared with the Heartland and SFTS virus and could be a virulence marker is not clear. The public health importance of MV remains incompletely understood and studies are in progress to address this issue. This is the first report on the detailed ultrastructure of this novel phlebovirus and forms the basis of further studies to explore the functional biology of this virus through more correlative and cryo electron microscopy approaches.

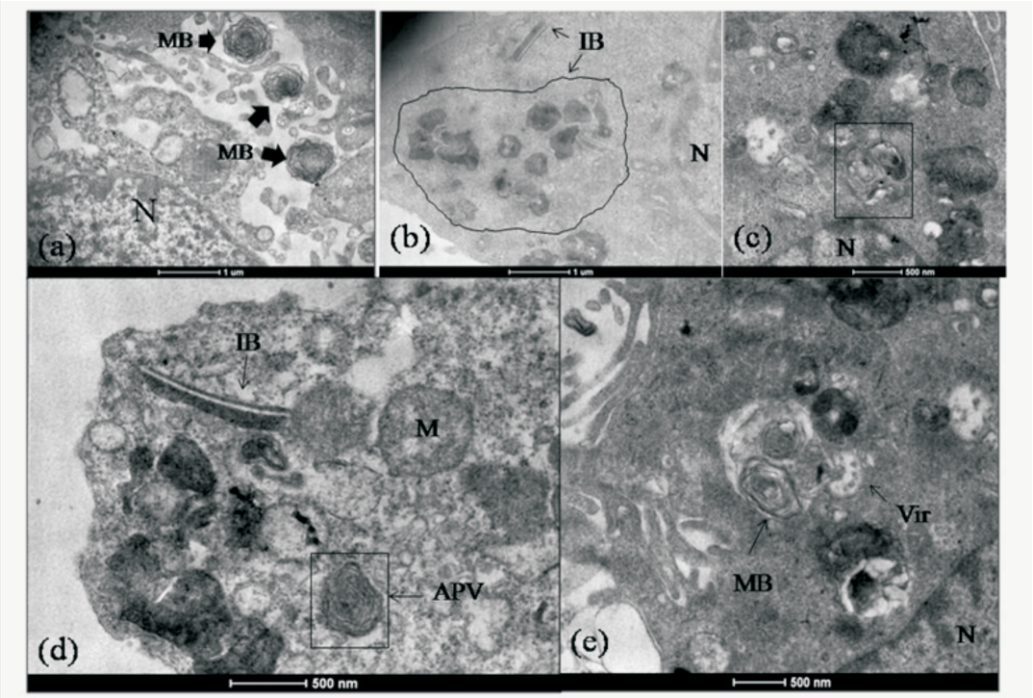

Figure 1: Ultrastructural changes in host cell infected with Malsoor virus. (a) cytoplasmic myelin-like bodies suggesting inclusion structures; (b-e) areas of autophagic vesicles. MB-myelin body; IB-inclusion body.

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MICROARRAY GROUP

MAC1401: Transcriptome analysis in response to low pathogenic avian Influenza virus infection and a comparison with highly pathogenic avian influenza virus (HPAI H5N1) infection

AK Chakrabarti, G Pasricha, U Solanki & SD Pawar

Funding: Intramural Project Duration: 2014-2017

Continued evolution of avian influenza viruses and its capacity to transform into virulent form is a serious concern across the globe. The interspecies transmission of influenza virus from avian to mammalian hosts (influenza A H5N1, H9N2) continues to occur and poses a persistent threat for humans. In this connection it is important to carry out studies to understand the biology of low pathogenic avian influenza A virus (LPAI) and a comparison of LPAI with the highly pathogenic H5N1 influenza viruses (HPAI). Influenza A H9N2 is circulating for the last several years and already posed threat of pandemic while influenza A H11N1 is a very rarely isolated low pathogenic avian influenza virus. Therefore a comparative analysis between the LPAI viruses and further comparison with HPAI will be an interesting aspect to study the host response to influenza infection.

Influenza A H11N1 viruses were grown in MDCK cell lines. Virus infection was checked by direct immunofluorescence assay and virus titre was determined by plaque assay (Figure 1). Plaque assay was performed at different time point post infection to study the growth kinetics of influenza A H11N1 virus. Growth curve analysis of H11N1 virus and comparison with another LPAI indicated that H11N1 grew well in MDCK and A549 cell lines and show typical growth kinetics like other influenza viruses.

Unique Eurasian-American genetic reassortment of H11N1 influenza virus has been described earlier. Few of the genes have much similarity with HPAI. To get an insight of the implication of the special genetic reassortment, host gene expression study has been performed. The study in these aspects is ongoing and need further analysis of more number of genes.

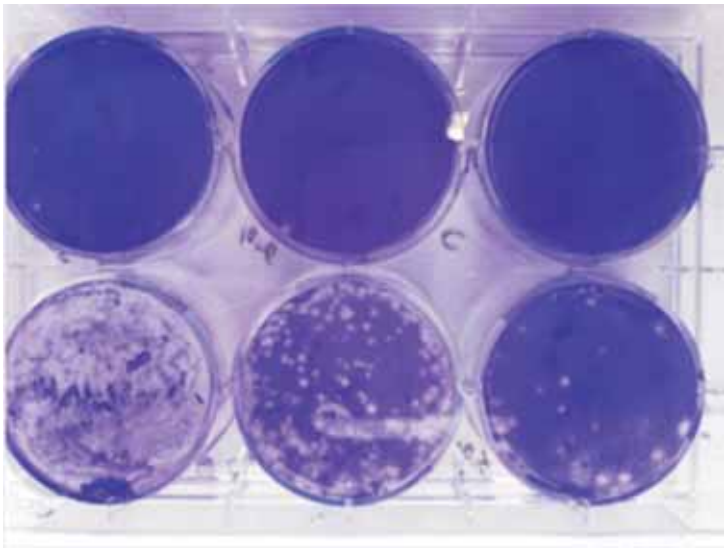

Figure 1: Plaque Assay of influenza A H11N1 viruses. MDCK cells were grown to monolayer and infected with different dilutions of H11N1 virus. Infected cells overlayed with top agar (0.75% LMP agarose in VGM) showed appearance of plaques at 60 hr post infection. Appearance of 25 plaques at 10^4 dilutions showed the titer of that particular stock as $2.5x10^6$ pfu/ml.

MICROARRAY GROUP

MAC1101: Biological property of PB1 F2 gene from different subtypes of influenza viruses.

AK Chakrabarti & G Pasricha

Funding: Intramural Project Duration: 2012-2015

PB1F2 is the 11th protein of influenza A virus which was identified and characterized as a non-structural protein more than a decade ago. It is encoded by the +1 alternate open reading frame within the PB1 gene and composed of 90 or 87 amino acids with a molecular weight of 10.5 kDa. Since its discovery, varying sizes and functions of the PB1F2 protein of influenza A viruses have been reported (Figure 2). However there is no clarity about the pathogenicity determinants of this highly variable protein. The effects of the protein on the host range from being minimal to deleterious, either by increasing the number and level of expression of activated genes linked to cell death or intensifying the activity of genes causing uncontrolled inflammation or deregulating the innate immune responses of the host. Selection of PB1 gene segment in the pandemics, variable size and pleiotropic effect of PB1F2 intrigued us to analyze this protein in various influenza A viruses.

Highly pathogenic avian influenza virus (H5N1), low pathogenic avian influenza virus H9N2 and H11N1, human influenza virus (H1N1) were cloned and the multiple functions of this protein were analyzed. Our data indicated that PB1F2 proteins have a typical helix-loop-helix structure except for H1N1 which showed a small β sheet structure in between α-helices. Structurally, PB1F2 of avian influenza viruses varied from each other in the length of their alpha-helices. We observed strain specific variability in apoptotic ability of the PB1F2 protein expressed from different influenza A viruses. PB1F2 has been implicated in possessing proinflammatory properties and the ability to intensify viral pathogenicity. PB1 protein from H5N1 influenza virus showed highest proinflammatory properties in inducing proinflammatory cytokines.

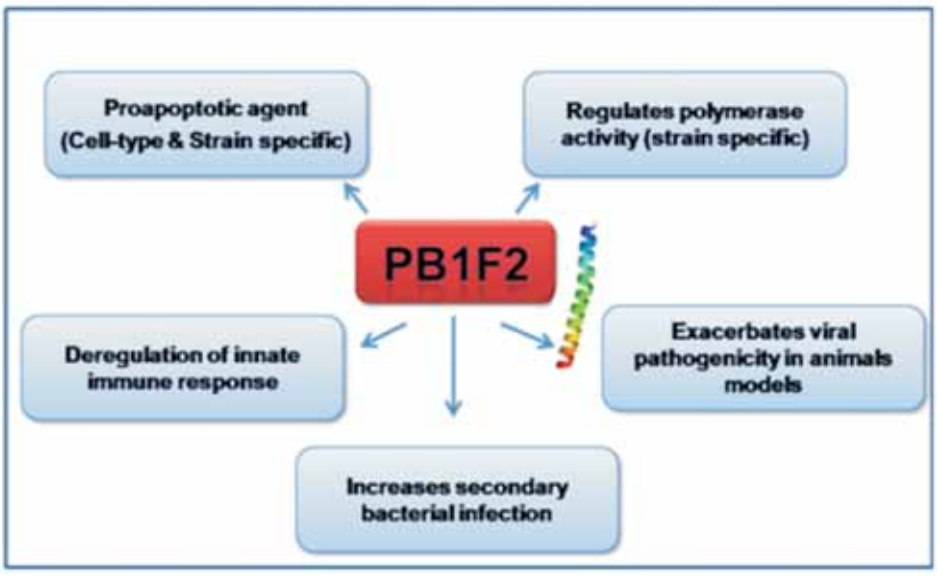

Figure 2: Multifunctional role of PB1 F2 protein of influenza A viruses

MICROARRAY GROUP

MAC1401 Determination of Apoptotic ability of Nucleoprotein of Avian Influenza A virus in human lung epithelial cells (M Sc project)

J Shinde, A Auti & AK Chakrabarti

The nucleoprotein (NP) of the influenza A virus is a structural protein composed of 498 amino acid with a molecular weight of 56 kDa. It is encoded by segment 5 and is a comparatively conserved protein which encapsidates the virus genome to form a ribonucleoprotein (RNP) particle for the purposes of transcription and packaging. Functions of the NP are mediated through interaction between the virus and host cell processes and it is known to interact with several viral proteins such as PB1, PB2, M1 and NP itself. It is mainly involved in viral genome trafficking and protection. The NP is known to contribute to viral pathogenesis, but its role in virus-induced host cell death is not clearly known. The project has been undertaken to unravel the role of NP of highly pathogenic H5N1 avian influenza virus and low pathogenic H11N1 influenza virus in the human lung epithelial cell line.

Nucleoprotein of highly pathogenic avian influenza A H5N1 and low pathogenic H11N1 viruses were cloned in the mammalian expression vector. Confirmed clones after sequence analysis were further used for the transfection experiments. Protein expression was analyzed by direct immunofluorescence assay and its role in apoptosis in transfected cells is being evaluated by TUNEL assay. Host gene expression pattern in response to NP is underway.

Host response to influenza A virus infection is a result of interactions between several viral and hundreds of host proteins. The study performed so far on NP indicated that NP alone is capable of inducing host genes to facilitate viral infection. Detailed study on host response to NP is ongoing with addition of more NP from other influenza subtypes (H1N1).

MAC1402: Cloning of NS1 protein of seasonal and pandemic H1N1 Influenza A virus in mammalian expression vector (M Sc project)

P Jadhav, A Deshpande, G Pasricha & AK Chakrabarti

Non-structural (NS1) protein of the Influenza A viruses is an extensively studied multifunctional protein which is commonly considered as a key viral component to fight against the host immune responses. Even though there have been a lot of studies on the involvement of NS1 protein in host immune responses ambiguities still remain regarding its role in apoptosis in infected cells. Interactions of NS1 protein with the host factors, role of NS1 protein in regulating cellular responses and apoptosis are quite complicated and further studies are still needed to understand it completely.

Origin and rapid spread of pandemic (2009) H1N1 virus (pH1N1), its pathogenicity, especially its ability to grow faster in human lungs and ability to enhance secondary complications presents an opportunity to study virus host interaction to understand the biology of pH1N1 virus infection and examine virulence factors. NS1 protein of influenza A viruses is considered as a key viral protein which contributes in viral pathogenesis. This study has been undertaken to study NS1 proteins of influenza viruses from different subtypes.

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NS1 genes of seasonal H1N1 and pH1N1 influenza virus were cloned in mammalian expression vector. Positive clones confirmed by sequencing were further used for protein and gene expression analysis. Expression of NS1 protein was detected by Western Blot analysis (Figure 3). Assessment of differential expression of selective host immune genes in response to the NS1 protein of seasonal and pH1N1 were performed by Real-time RT- PCR.

Preliminary results indicated that NS1 protein of both pandemic and seasonal H1N1 viruses are capable of influencing host immune responses and possess necessary functionality to support apoptosis in host cells. Further study is needed to confirm the role of the NS1 protein in regulation of viral infection and to justify its subtype specific difference.

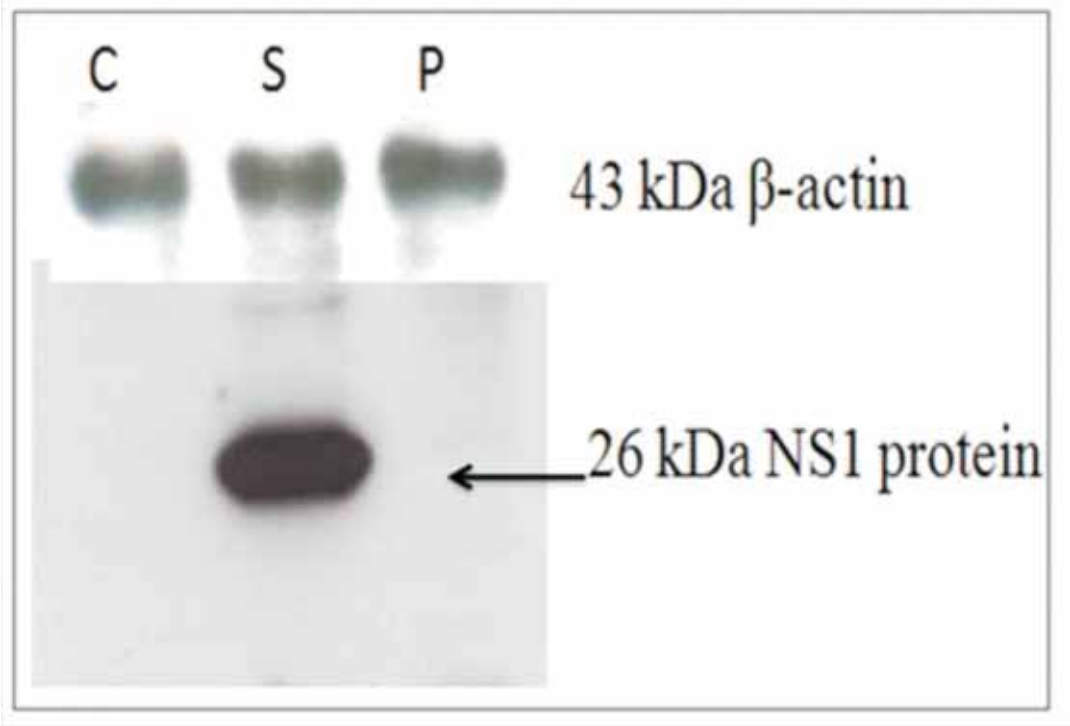

Figure 3: Western blot analysis of NS1 protein expression in transfected cells. Equal amount of cellular proteins isolated (10 ìg) from transfected and control cell extracts were separated by 12.5% SDS-polyacrylamide gel electrophoresis. Proteins transferred to Hybond-C membrane were probed with specific monoclonal antibodies against Influenza A-NS1.C. Control Cells (untransfected); S. Cells transfected with NS1 clone of seasonal H1N1 virus; P. Cells transfected with NS1 clone of pandemic (2009) H1N1 virus

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BIOINFORMATICS AND DATA MANAGEMENT GROUP

BDM1001: Core facility services

SS Cherian, P Shil, AM Walimbe, K Vijayasimha & Jadhav SM

Regular services to the various experimental groups at NIV and MCC, Pashan, in the areas of statistical data analyses of epidemiological and serological data, bioinformatics sequence and structure analyses, mathematical modeling, etc. were provided.

Management and maintenance of computers, servers, LAN and internet services at NIV, Pune and MCC, Pashan was done. The connectivity between NIV, MCC and the 3 field units has been established and the AIMS software is running in all the field units. Back-up of AIMS and LIMS (Laboratory Information Management System) data has been maintained. Maintenance and upgradation of the indigenously developed pay-roll system has been carried out. The NIV website is maintained and re-designing of the web pages and incorporation of online database for research publications was done. McAfee antivirus provided by ICMR has been re-installed for another 3 years. The antivirus server is being maintained on a regular basis.

BDM1102: A comparison of phylogeography of flaviviruses namely Dengue, Japanese encephalitis and West Nile viruses with emphasis on Indian isolates

SS Cherian & AM Walimbe

Funding: Intramural Project Duration: 2012-2015

Patterns in the spread and epidemiology of viral diseases can be revealed to some extent by comparisons of genomic sequences and advanced phylogenetic and molecular clock analyses. However, the most recent developments in the algorithms for evolutionary dynamics enable examination of the geographic origin of different lineages, using known geographic information of the genomic sequences by implementing character mapping in the Bayesian software that samples time-scaled phylogenies.

The phylogeographic analyses of Japanese encephalitis virus (JEV) were carried out by Bayesian MCMC approach, to understand the global evolutionary dynamics of the disease, with emphasis on Indian genotypes. Analyses of representative E-gene sequences of genotype III (n=197) available in GenBank, showed that the geographical ancestor was predicted to be Japan with state probability of 0.44. The earliest entry to India around 1930s appears to be from Japan to south India from where it spread to northern and north-eastern states of the country. Subsequently, a separate introduction from China is predicted around 1950s to several states of India including Uttar Pradesh (UP). An independent introduction to Assam during 1960s-70s from Japan is also indicated. Phylogeography studies for genotype I (n=203), revealed Vietnam to be the ancestral state though with a low state probability. Two independent introductions to India around 2003 and 2006 to UP and West Bengal from China and Japan, respectively were indicated (Figure 1). Overall, the current study provides insight into the spatial as well as temporal dynamics of JEV.

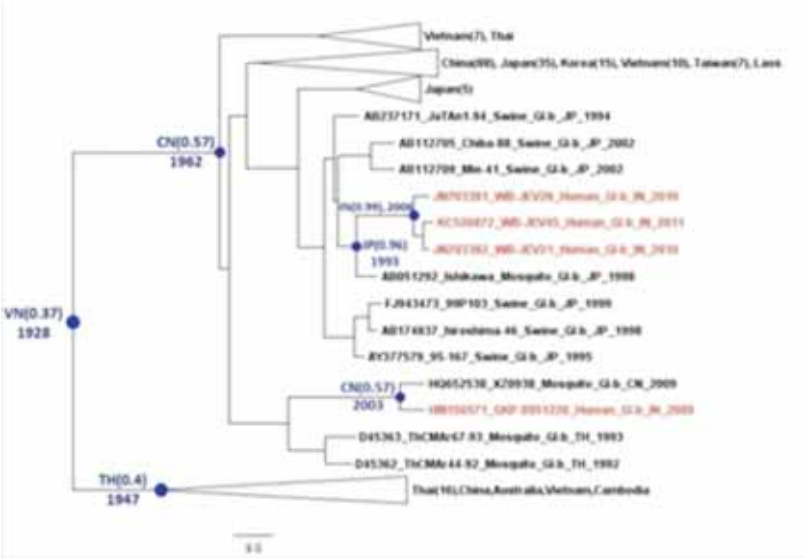

Figure 1: Maximum clade credibility (MCC) tree of the 'E' gene sequences of JEV genotype I. Ancestral states with their probabilities and divergence times are shown at key nodes

BDM1103: Mathematical modeling of cell electroporation for DNA and small molecule delivery.

P Shil, K Alagarasu & BK Achary

Funding: DST Project Duration: July 2013-March 2015

Electroporation is a physical process involving enhanced permeability of biological cell membrane due to application of high voltage electric pulses of very short duration. Since its inception in the 1970s, the technique has found wide range of applications in biotechnology including cancer therapy and anti-viral vaccine delivery. Though considerable efforts have been made to understand the physical process, differences exist between experimental observations and theoretical models due to various approximations.

The present project involves theoretical formulation and numerical implementation of electroporation of a mammalian cell with cholesterol containing membrane. The cell geometry of PS cells and THP-1 cells were determined experimentally and parameters used as inputs in the simulations. Numerical solutions of the equations were carried out in MATLAB. Effective electroporation was evaluated in terms of transmembrane potential, average pore radius, pore count and distribution vs time. Effect of variation in membrane cholesterol content (15-29% mole-fraction) was evaluated in terms of changes in these parameters. It was observed that variation of cholesterol-content resulted in significant alteration of the pore radius, number and distribution of pores on the cell surface for each cell type.

The model was extended to evaluate the uptake of small molecule (Doxorubicin). It was observed that the increase in cholesterol content from 15-29% mole-fraction resulted in 5%

decrease in the molecular uptake (Figure 2). Results indicated a similar trend observed in experiments reported elsewhere. This model can be a useful tool for prediction of electroporation phenomenon in mammalian cells (of various descriptions) at higher magnitude of applied electric fields.

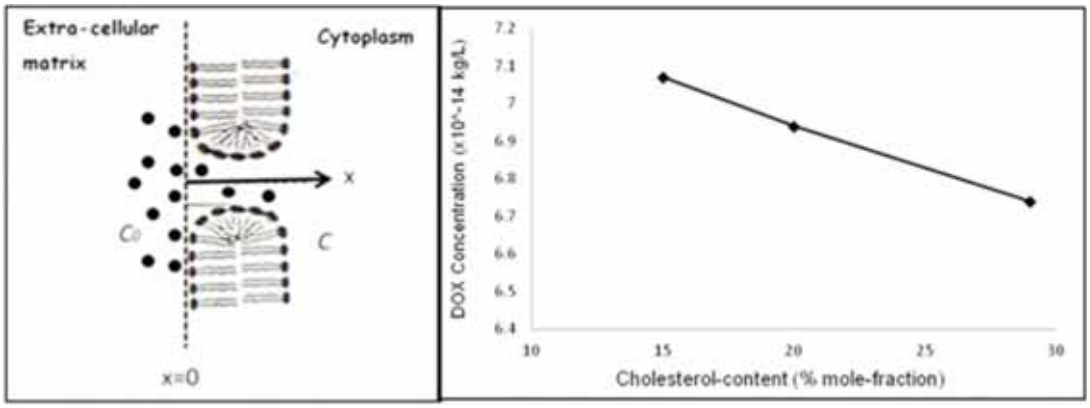

Figure 2: A) Artistic impression of small molecules diffusion through a pore in cell membrane during electroporation. B) Intracellular Doxorubicin (DOX) concentration, C due to electroporation-mediated uptake (numerical solutions) vs. membrane cholesterol-content.

BDM1301: Development of a co-evolution database for major Influenza A proteins and integration of sequence and structure-based tools for correlations to pathogenecity.

Sarah Cherian and Abhisek Behera

Funding: ICMR SRF project Project Duration: 2014-2017

The project aims at developing a co-evolution database based on selected protein segments (HA, NA, PA and PB2) for major influenza subtypes (seasonal H1N1, 2009 pH1N1, H5N1). This database will provide information on marker mutations for pathogenecity known from literature and co-evolutionary mutations obtained from statistical approaches like mutual information, etc also providing the facility for structural correlations. Significant co-evolutionary mutations will also be studied in-depth by modeling and simulation studies to understand the molecular basis.

Secondary mutations occurring in NA and HA in post 2007 H1N1 seasonal strains are reported to compensate for the deleterious effects of low growth capability known to arise as a consequence of the H274Y marker mutation conferring Oseltamivir drug resistance. We studied the molecular mechanism behind the improved viral fitness due to R192K mutation located at the HA receptor binding site. Computational modeling based on the HA protein of A/Tottori/52/2008 (To/08), possessing R192K mutation and wild type strain A/Solomon Island/3/2006 (SI/06), docking and MD simulations with the human receptor, Sialic acid α2-6 Galactose, was carried out. The binding affinity was energetically more favorable in SI/06 than To/08. The reduced binding affinity of To/08 HA with Sialic acid á2-6 Galactose is interpreted as a consequence of the absence of H-bonds with the 190 helix (Figure 3), and abrogation of an intramolecular contact between R192 and E198 both due to gain in relative positional flexibility of K192. Overall, R192K mutation in the HA

strains mediates weaker receptor binding, balancing with weaker substrate-binding affinity noted in the NA of oseltamivir-resistant viruses.

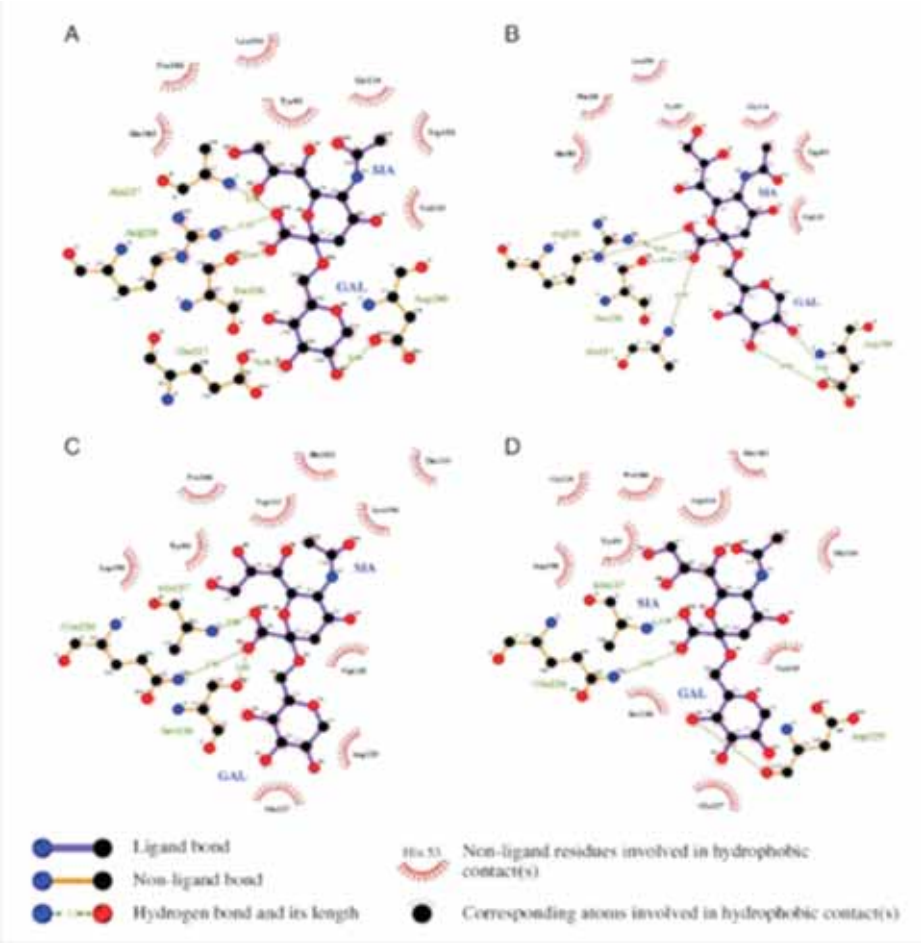

Figure 3: 2D interaction diagrams of Sia-á2,6-Gal with HA of: A). A/Solomon Island/3/2006 at 0 ns; B). A/Solomon Island/3/2006 at 10 ns; C). A/Tottori/52/2008 at 0 ns; D). A/Tottori/52/2008 at 10 ns.

BDM1403: Phylodynamics and molecular evolution of Influenza A Polymerase genes (PB1, PB2 and PA) inferred from large scale sequence analyses and Structural Bioinformatics (ICMR Intramural project; 2014-2017):

SS Cherian & Bhoye D

Though early sequence analyses of the 2009 pandemic H1N1 viruses showed absence of PB2 specific markers associated with virulence and high pathogenicity in mammalian species, it is important to understand the evolution of this gene for acquisition of any such markers over the period of time.

Selection pressure analysis based on PB2 gene sequences of pH1N1 viruses of the period 2009-2013, was therefore carried out. Three codon sites showed evidence of evolving under

positive selection pressure in PB2, of which amino acid 344V/M was found to be in the vicinity of the cap binding pocket. Docking of pre-mRNA cap analog m(7)GTP to pH1N1 PB2 cap binding domain (CBD) structures possessing 344V or 344M and 10 ns MD simulations of the docked complexes indicated that the complex possessing 344M, showed better ligand binding affinity and stability. Analyses of the contacts in the docked complexes revealed that the 344 residue position was not directly involved in inter-molecular contacts, though another mutation I354L in the vicinity of 344M, additively resulted in better positioning of m(7)GTP in the active site and significant increase in the number of H-bond contacts (Figure 4) through displacement of the 424-loop in the PB2 CBD. The acquisition of these co-occurring mutations in the PB2 CBD of pH1N1 viruses may contribute to increased viral fitness by mediating increased efficiency of cap-binding and thus replication competence of these viruses.

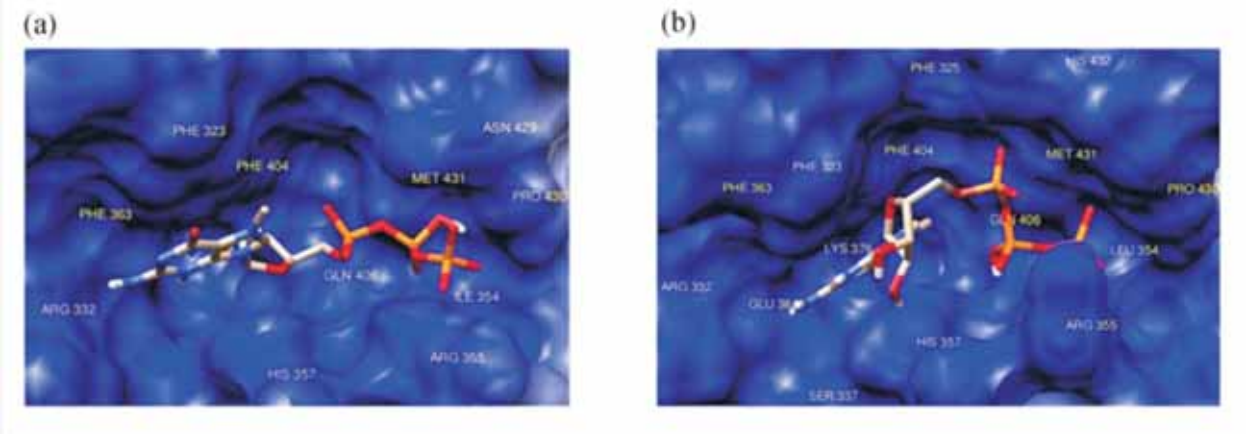

Figure 4: Docked complexes of m(7)GTP with PB2 CBD variants (a) V344 and I354 (b) M344 and L354, of pH1N1 viruses after 10ns molecular dynamics simulations. Contact residues in the form of hydrogen bonds and hydrophobic interactions are labeled.

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DIAGNOSTIC VIROLOGY GROUP

DVG1301: Resource Centre for Virus Diagnostic Laboratories (RCVDL) at NIV, Pune

AC Mishra, GN Sapkal, R Viswanathan, VA Potdar & AB Sudeep

Funding: ICMR (Extramural) Project Duration: October 2013-September 2016

RCVRDL has been developed in response to the recommendation of the Virology Task Force monitoring the VRDL network, with the objective of providing training for different categories of VDL network staff and conducting quality assurance (QA) and quality control (QC) programs for the network laboratories.

During the current year, seven separate training programs were conducted. A broad range of subjects on different aspects of viral diagnostics were covered. Training modules included lectures, demonstrations and hands on practicals. Pre- and post-assessment tests were conducted. Feedback was invited from participants and PIs after circulation of the training modules. This feedback was taken into account while preparing subsequent training modules. So far, 80 staff/faculties from 21centres have been trained (Table 1).

Table1: List of training programs conducted for VRDL Network

S. No	Name of the Module	Date & Duration
1.	Culture based viral diagnosis	26th to 30th May, 2014
2.	Viral Diagnostic PCR	23rd-27th June, 2014
3.	Basic Laboratory Techniques and ELISA for Viral Diagnosis	20th-28th August, 2014
4.	Real Time PCR for Viral Diagnosis	15th-19th December, 2014
5.	Specimen collection, handling and transport	20th January, 2015
6.	Training on Biosafety & Biosecurity	21st-22nd January, 2015
7.	Sequence Analysis and Interpretation	23rd-26th February, 2015

Standard Operating Procedures (SOP) has been prepared for Quality Control and generation of serum panels for the VRDL Network, which has been approved by the ICMR and circulated to the network laboratories. In the first phase, tests included were Dengue IgM and NS1, Chikungunya IgM and Japanese encephalitis IgM. The program has been initiated and 480 samples were received from 10 VRDLs for QC testing.

The Resource Centre has also developed documents and SOPs for the VRDL network for Purchase of Articles/Equipments, list of viruses to be tested by the VRDL network, testing algorithm for viral infections, testing strategy for individual viruses, list of proposed training programs at RCVDL and model specifications for equipments sanctioned for the VRDL network.

DIAGNOSTIC VIROLOGY GROUP

Figure 1: Training programs organized for the VRDL Network

DVG1402: Generation and characterization of monoclonal antibodies against Kyasanur Forest disease virus

GN Sapkal, PD Yadav, S Vidhate, GR Deshpande, O Kaduskar & DT Mourya

Funding: Intramural (approved for DHR Fund) Project Duration: 2014-2016

Since the first record of the KFD in Shimoga district of Karnataka, India during 1957, recurring epidemics of KFD were recorded every year. Despite routine vaccination, recent reports suggested an increasing number of cases in several districts of Karnataka. This has warranted an urgent need for developing a rapid diagnostic system for KFD virus detection in the infected patients. Since the use of polyclonal antibodies in diagnostic assays reduce the assay sensitivity, a monoclonal antibody (MAb) based assay has been designed. Generation of KFDV hybridoma was initiated using gamma inactivated (GC5000 facility) KFDV as the antigen for immunization. ELISA using inactivated KFDV was also optimized with the known positive (mouse positive) and negative immune sera for screening antibody secreting hybridoma. A total of 78 hybrids were screened for anti_KFDV antibody secretion and six (IH4, III C1, I A2, II H3, I G2, III D2) anti KFDV secreting MAbs were generated and characterized for their reactivity in ELISA, Immunofluroesence assay (IFA) and Western blot. All the MAbs were found to be positive by ELISA and IFA. Western blot analysis indicated that the MAbs are reactive against the envelop protein of KFDV. The utility of these MAbs needs to be evaluated in the diagnostic IgM ELISA.

DIAGNOSTIC VIROLOGY GROUP

DVG1403: Infection Dynamics of Congenital Cytomegalovirus in Neonates in Pune, Maharashtra

(In collaboration with KEM Hospital & Research Centre and Bharati Hospital)

R Viswanathan, GN Sapkal & S Vidhate

Funding: Intramural Project Duration: 2014-2017

Cytomegalovirus (CMV) is the most prevalent cause of congenital infection, affecting 1-2% of all live births. It is the leading infectious cause of mental retardation and sensorineural deafness. The study has the following objectives: determination of seroprevalence and reactivation of CMV in antenatal mothers, determination of prevalence of congenital CMV in neonates born at or referred study sites and follow up of the affected babies. Approval has been received from Institutional Ethics Committee of both the centres and the project has been initiated at two study sites. As the study involves collection of large number of samples from a vulnerable population, Dr DY Patil Medical College has also been approached for collaboration. Training of project members, validation of case report form, Patient Information Sheet (PIS), Investigation Consent Form (ICF), SOPs has been done. So far 88 maternal sera have been collected after counseling and taking informed consent out of which 84 were found to be anti-CMV IgG positive by ELISA. Majority (69.3%) had very high titre of antibodies. Collection of specimens from clinically suspected cases of congenital CMV infection has been initiated. One baby with suspected congenital CMV infection was detected as anti-CMV IgM positive.

Necessary laboratory techniques have been adapted/standardized for virus isolation from the clinical samples. This includes cell culture techniques, virus titration and immunofluorescence assay. Virus pools and cell stocks have been prepared. Procurement of reagents and consumables for molecular analysis is underway and preliminary laboratory work has been initiated.

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ANIMAL HOUSE GROUP

Breeding, supply and maintenance of laboratory animals.

DR Patil & SV Nipunage

Funding: Intramural Project Duration: Ongoing

The mandate of Animal house group is to maintain, breed and supply quality laboratory animals for research in compliance with the CPCSEA regulations. The animal facility is currently housing and breeding seven different strains of laboratory mice in individually ventilated caging system for in-house supply. During the report period, a total of 2828 animals including 2806 mice, 3 rabbits, 9 guinea pigs and 10 rhesus monkeys were supplied to the institutional scientists against 29 approved research projects. In addition, blood (724 ml) from different species of laboratory animals as diagnostic reagent for various assays was supplied to the institutional scientists.

The quality control (QC) programme at the mouse breeding facility included bi-annual testing of consumables like mouse feed, bedding and drinking water for microbial and chemical quality, annual testing of mice stool samples for parasitological examination and screening of mice for genetic purity and presence of murine viruses.

CPCSEA registration has been renewed until March 2017 by CPCSEA, New Delhi and the Institutional Animal Ethics Committee (IAEC) that was newly reconstituted. During the report period two meetings of IAEC were held for project evaluation in which, 17 projects were reviewed and approved by the committee.

Ten rhesus monkeys were procured under CPCSEA approved extramural research project and the quarantine procedure was accomplished. As a part of the quarantine procedure, chest X ray, intra-dermal tuberculin tests (three consecutive tests at 15 days interval), hemogram and clinical biochemistry were performed. Simultaneously, prophylactic antiparasitic, antibiotic treatment was given and pre-conditioning of animals was done. Annual health monitoring programme for the rhesus monkeys under rehabilitation (N=42) was also conducted during which, hemogram, tuberculosis testing, chest X ray, stool examination for parasites was carried out.

As an additional responsibility, the animal house group maintained records pertaining to biomedical waste of different categories generated at the NIV and submitted the annual report for the period 1.1.2014 to 31.12.2014 to the Maharashtra Pollution Control Board (MPCB) as mandatory compliance.

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Dr. Mittal M	Associate Professor, Pediatrics
Dr. Srivastava DK	Professor & Head, Social & Preventive Medicine

NIV, GORAKHPUR UNIT

GKP1001: Diagnostic services for AES Patients for anti-JE IgM

MM Gore, AK Pandey, H Deval, N Kumar, BR Misra, SP Behera, KP Kushwaha, M Mittal, G. Patil & DK Srivastava

Funding: Intramural Project Duration: 2008-ongoing

NIV continued the work on providing diagnosis of AES cases admitted to Nehru Hospitals & BRD Medical College, Gorakhpur. During 2014, most of the cases (72%) were admitted during July-October (Figure 1). As documented in the earlier years, 73.5% of the total AES cases were from four districts of eastern Uttar Pradesh viz, Gorakhpur, Kushinagar, Mahrajganj and Deoria. Another 10% of the cases were from the adjoining districts of Bihar while the remaining 16.5% of cases were from other eastern 10 districts in UP. Japanese encephalitis diagnosis was provided on a day to day basis using standard IgM capture ELISA. Out of 2208 AES cases, only 101 could be confirmed as JE. However, Bihar JE positivity was documented higher (8.8%) than that of UP (3.7%). Age distribution in JE and Non JE cases was similar to the pediatric group contributing 88% of AES cases. In both JE and non JE cases, the highest number of cases was from age group of 1-10 (Figure 2).

Diagnosis of patient and initial analysis of AES cases admitted in BRD Medical College forms the basic information being explored by various agencies of Governmrnt of India for planning better control strategies. Analysis of AES incidence has led to designing of newer insights in possible leads for preventive measures.

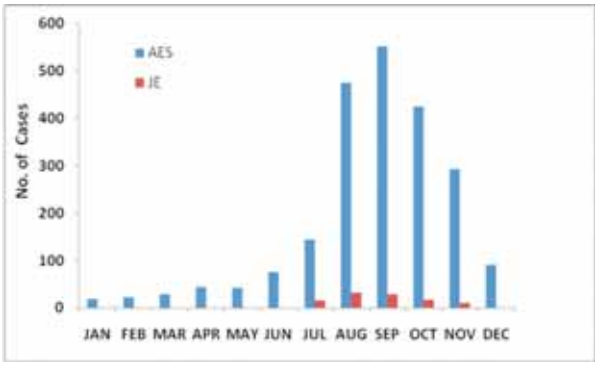

Figure 1: Monthly distribution of AES and JE cases during 2014.

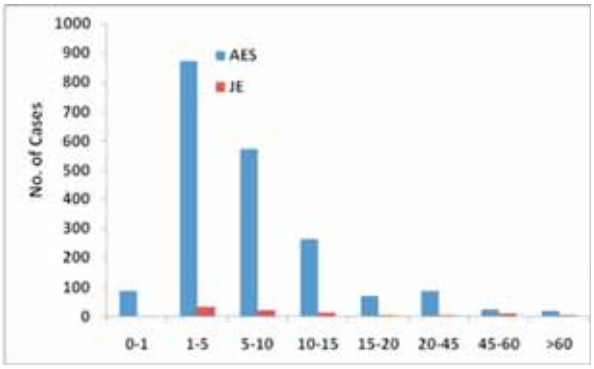

Figure 2: Age distribution within JE and non-JE encephalitis cases admitted in BRD Medical College.

GKP1201: Epidemiology and dynamics of transmission of JE/AES in Eastern part of UP and implementation of appropriate interventions

MM Gore, BR Misra, R Gupta, A Thakur, V Janardan, BV Tandale & A Rai

Funding: ICMR (Extramural) Project Duration: 2012-2015

JE is a serious health problem in India especially in the north eastern states. JE virus is maintained in nature by a complex cycle that involves pigs as the amplifying host and mosquitoes

as the vectors. Gorakhpur and its adjoining districts are highly endemic for JE since its first appearance in this region in 1978. Hence, it is necessary to estimate the prevalence of JE infection in amplifying host during summer and rainy season in this endemic area. Accordingly, blood collection was performed from piglets twice in a month to detect appropriate time of seroconversion in all the selected blocks during summer and starting of rainy season. Seroconversion in piglets was not observed during summer (i.e. April month) in 2014, except in Deoria block (Table 1). Fourth sample collected from Belghat and Chargawan block showed low level of seroconversion in the month of July. Delayed monsoon during 2014 might have had an impact in the outcome.

Table 1: Seroprevalence of JEV in amplifying host during summer and rainy season

		Samples collected					Samples tested (NT pos/tested) Pos >20			
Block	Month	1st	2nd	3rd	4th	Total	1st	2nd	3rd	4th
Belghat	April		3			3		0/3		
	May	15	10	2		27	15-May	10-Jan	0/3	
	June		5	15	10	30		5-May	15-Jul	10-Feb
	July	5			5	10	5-May			4-Jan
Campierganj	April	5	4	3		12	5-Feb	4-Jan	4-Feb	
	May	12	10	9	6	37	10-May	0/15	7-Apr	0/6
	June	8		10	10	28	8-Jan		10-Jun	0/10
	July	1	1		5	7	1-Jan	0/1		0/5
Chargawan	April	9	7		4	20	9-Jan	0/7		0/4
	May	12	10	9	6	37	12-Apr	10-Apr	9-Mar	5-Feb
	June	2	5	12	10	29	2-Jan	0/5	12-Jan	10-Feb
	July	1	2	2	4	9	1-Jan	2-Feb	0/2	4-Feb
Khorabar	April	9		6	4	19	9-Jan		6-Jan	0/4
	May	5	14	9	6	34	5-May	14-Aug	9-Aug	5-Jan
	June			5	14	19			5-May	0/14
	July	9				9	0/9			
Bhathat	April	5		1	4	10	0/5		0/3	0/4
	May	7	7	5	1	20	7-Feb	0/7	0/5	0/1
	June		2	4	7	13		2-Jan	0/4	0/7
	July	12				12	12-May			
Deoria	April	3	7		1	11	3-Mar	7-Jun		1-Jan
	May	9		7	4	20			7-Feb	4-Apr
	June		9	3	3	15		9-Apr	3-Feb	0/3
	July				2	2				0/2

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Seroconversion was observed only at Chabutra Tiwari, Parsauni, Manikapar and Kusmauni village of Belghat, Campierganj, Chargawan and Deoria blocks, respectively. This showed the focal activity of JEV transmission rather than uniform activity. As a local practice during May ground water is also used for preparation of paddy seedling before the rainy season, this leads to substantial mosquito breeding activity. As mosquito survival is restricted in the dry season, generalized JE activity is not documented at the sites of studied blocks except Chabutra Tiwari, Parsauni, Manikapar and Kusmauni villages.

This study suggests that seropositivity for JE directly depends on the rain fall that facilitates breeding of the JE vector. During 2014, late seroconversion in amplifying host was related with delayed monsoon. Hence, while planning strategies of intervention for amplifying host, it is necessary to consider the impact of climate on the transmission to get unbiased results.

GKP1302: A Study on mosquito biodiversity and circulation of arboviruses in endemic areas of acute encephalitis syndrome in Eastern Uttar Pradesh.

BR Misra, MM Gore, R Srivastava & K Satish

Funding: ICMR (Extramural) Project Duration: 2014-2017

AES has emerged as one of the serious public health problems in Gorakhpur. The area harbors vast range of mosquito species capable of transmitting different vector borne infections. Hence, it is essential to investigate the distribution of mosquito species and viruses transmitted by them for better implementation of prevention and control strategies in AES endemic area.

Jungle Ayodhya Prashad, Mahesara and Siktour villages of Gorakhpur district were selected for dusk collection of adult female mosquitoes. Monitoring of mosquito prevalence was undertaken with the help of mechanical aspirators. After identification, mosquito pools were subjected to flavivirus (NS5) RT-PCR to detect flavivirus presence. Positivity of mosquito pools containing 25 mosquitoes of a species expressed as number of infected mosquito per thousand of tested mosquito termed as minimum infection rate (MIR).

A total of 13,220 mosquitoes were collected from field sites that included 52% *Culex* species, 23.4% *Anopheles* spp., 6.3% *Armigeres* spp, 5.5% Mansonia spp, and 5.2% Aedes spp. in 144 man-hr from Gorakhpur district. Density of *Cx. quinquefasciatus* was highest in winter and density of *Cx vishnui* group especially *Cx. tritaeniorhynchus* was highest in the rainy season.

MIR of flavivirus per 1000 mosquitoes was 1.03 in *Cx. quinquefasciatus,* 0.75 in *Cx. gelidus* and 0.37 in *Cx. tritaeniorhynchus*. The *Cx. tritaeniorhynchus* showed maximum positivity in August while *Cx.gelidus* and *Cx. quinquefasciatus* showed positivity in pools collected durng the winter season.

This study illustrates the impact of climatic seasonality on the circulation of flavivirus in nature and suggests that summer season is suitable for introduction of intervention strategies against mosquitoes due to very low circulation of the virus.

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GKP1006: Identification of etiological agent(s) of AES in eastern UP.

MM Gore, AK Pandey, H Deval, Niraj Kumar, Asif, Sanjeev, G Patil, J Ansari, M Mittal & KP Kushwaha

Funding: Intramural Project Duration: 2010-2015

During 2014, efforts to detect viruses in AES cases continued. Virological diagnosis in the form of anti-JE IgM ELISA and PCR for Herpes simplex virus (HSV), Cytomegalo virus (CMV) and Varicella zoster virus (VZV) was carried out as per clinical diagnosis. Out of 2208 AES cases only 101 could be confirmed as JE, based on IgM positivity.

A total of 343 CSF specimen collected during acute illness were tested by PCR for HSV, CMV, VZV along with bacteria ***Streptococcus*** pneumoniae, ***Neisseria meningitides*** & ***Haemophilus influenzae.*** Four CSF (1.4%) were found to be positive for HSV, two (0.5%) for VZV and one (0.025%) for ***S. pneumoniae.*** CSF collected from 10 non AES cases were also tested for HSV by PCR and one was found to be positive.

GKP1101: Development of antibody detection test for diagnosis of enterovirus encephalitis

MM Gore, GP Patil & K P Kushwaha

Funding: Intramural Project Duration: 2011-2014

Viral diagnosis in AES cases can be performed using multiple standard assays either by detecting viral antigens, genome or by detecting anti-viral IgM antibody in the CSF/serum. Hence, IgM capture ELISA in the clinical sample (CSF/serum) to detect anti-EV IgM antibody may prove to be useful. Indigenously developed anti-EV IgM ELISA was used for detection of anti-EV IgM antibodies in serum and CSF of AES patients. The study indicated that the test can be used for diagnosis after further validation. The EV PCR showed limited positivity (2-3%), probably due to the delayed (5-7days) admission of AES patients for treatment.

During 2014, 741 CSF and 891 serum samples from AES cases admitted in the BRD medical college were tested for detection of anti EV IgM antibodies. The cut off value (P/N) was taken 2.5 for CSF while 5 for serum samples. A total of 99/741 (13.36%) CSF samples were tested positive for anti-EV IgM antibody while one of the CSF sample was positive for both JE & EV IgM antibodies. In serum, 129/891 (14.47%) samples were tested positive, out of which 4 samples also showed JE IgM positivity. IgM positivity was higher (73.7%) in the pediatric age groups (1-10) (Figure 3a). The EV IgM positivity was higher during the months of August-September (Figure 3b). Validation of the EV IgM kit is in progress.

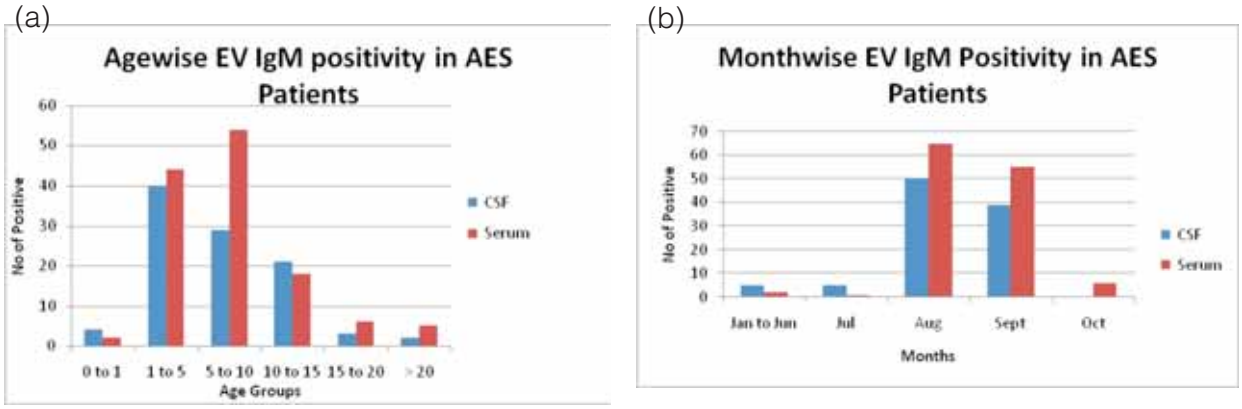

Figure 3: Monthwise and agewise EV IgM positivity in AES patients.

GKP1407: Detection, isolation and molecular characterization of flaviviruses and other unknown viruses from the AES patients by PBMC co-culture method in Gorakhpur.

H Deval, MM Gore, M Mittal, SP Behera, K Sah, A Thakur, AK Pandey, N Kumar & A Aggrawal.

Funding: Intramural Project Duration: 2014-2017

Japanese encephalitis virus endemic Gorakhpur and adjoining districts of eastern UP and Bihar report an increase in AES cases during July–November every year. In 2014, positivity was documented to be 4.5% by anti JEV-specific IgM capture ELISA. It is difficult to isolate the virus from the patients due to low viremia. The isolation studies in the previous year had adapted different cell lines (Vero, BHK-21, and C6/36) but with limited success. As it is a well established fact that flaviviruses including JEV harbor and proliferate inside the host PBMCs, the phytohemagglutinin (PHA)-stimulated PBMC co-culture method can suitably be explored for isolation of these viruses.

Standardization and establishment of PBMC co-culture was done herein. The whole blood from healthy donors was procured from the Blood Bank, Gorakhnath hospital and the healthy PBMCs were separated by Ficoll-hypaque density gradient centrifugation. Healthy PBMCs were stimulated with 3µg/ml of PHA overnight at 37°C in a tissue culture plate. The activated PBMCs were co-cultured with freshly isolated PBMCs from the AES patients and observed for cytopathic effect (CPE). Three blind passages were carried out for all the samples before they were tested for genomic detection of flaviviruses. A total number of 427 samples were processed for virus isolation by the PBMC co-culture method of which six tested positive which were later confirmed by RT-PCR. Further characterization of one of the six positive isolates revealed that the isolate belonged to the circulating JE genotype III in Gorakhpur. Consequently, CSF from 55 cases was used to isolate the virus by the PBMC co-culture method but was not successful. Microfilaria parasites were detected in three of the PBMC samples during the study (Figure 4).

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Figure 4: Microfilaria parasite detected in PBMCs collected from an AES patient

New Projects:

"Genetics of susceptibility to encephalitis in Japanese encephalitis virus infected children from Uttar Pradesh". The concept proposal has been accepted for submitting detailed project in ICMR task force on neurosciences (extramural funding).

GKP1501: Providing H1N1 diagnostic services by real time PCR in Gorakhpur.

MM Gore, H Deval, SP Behera, AK Pandey, N Kumar, KP Kushwaha & M Mittal

Funding: Intramural Project Duration: 2015 onwards

In response to the emergence of suspected H1N1 cases in B.R.D. Medical College, training was given to Dr. H Deval, in the Influenza division, NIV, Pune from 11th Feb- 16th Feb 2015 to establish the H1N1 virus detection facility at the NIV, Gorakhpur Unit. The lab has been functional since 19th Feb 2015 with trained technical staff starting from sample collection, processing, real time PCR and analysis. A total of 11 samples collected from clinically confirmed influenza cases were processed for lab diagnosis using the standard real time RT-PCR kit. A total of 6/11 specimens were tested positive for H1N1.

GKP1104: Development of uniform clinical guidelines for differential diagnosis and management of JE and non JE cases.

KP Kushwaha, MM Gore, M Mittal, AK Pandey, J Ansari, DK Srivastava, B Sharma, A Mehta, & SK Srivastava.

Funding: ICMR (Extramural) Project Duration: 2012-2014 (completed)

Viral encephalitis is a pressing health problem in India. More than 2000 AES cases per year are admitted in the pediatric wards of B.R.D Medical College, Gorakhpur with about 20% mortality. Approximately, in 75% of the cases, etiology still goes undetermined. The present study aims at development of clinical guidelines based on clinical examination and investigations to differentiate JE from non-JE and development of appropriate treatment guidelines for the same. During the study, AES cases between 1-15 yrs admitted to the pediatric wards of B.R.D Medical College were

studied for two years during the peak seasons. Clinico-laboratory factors, mortality and morbidity patterns of these patients were compared to see the differences in JE confirmed and non-JE cases recorded.

Of the 505 children enrolled, 164 expired, 27 left against medical advice and 313 were discharged. After excluding 27 cases that were positive for other etiologies, 478 were analysed further. JE positive were 31 in number. Features which were significantly different from JE among the non-JE group were swelling over the body, floppiness, tachycardia, tachypnea and 3rd cranial palsy. JE confirmed cases prominently had headache and hypertonia.164 enteroviral cases diagnosed based on anti EV IgM test being developed among the non-JE group presented with significant cardiac involvement in the form of congestive cardiac failure and hypotension, decreased platelet count and cranial nerve palsies (3rd, 6th,7th). Multiorgan involvement led to increased mortality when studied in all AES cases and among non-JE hyponatremia and raised troponin I was also significant for mortality. Cerebral atrophy and edema were features seen in CT MRI studies. 10-14% cases in non-JE group and 4 cases in JE group showed mild to moderate sequale.

From the study, it has become clear that JE is no longer an important cause of encephalitis in Gorakhpur. Undiagnosed non-JE group showed a multiorgan involvement with a prominent cardiac involvement. Etiological diagnosis and focussed studies on cardiac involvement and long term sequale is the need of the hour.

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BNU8801: Etiological and epidemiological investigations on arbovirus infections and laboratory diagnosis in Karnataka and neighbouring areas with particular reference to Dengue, Chikungunya and Japanese encephalitis viruses. (Service Project)

CG Raut

Funding: Intramural and NVBDCP Project duration: Ongoing

Under NVBDCP, the NIV Bangalore Unit is primarily responsible for testing the dengue and chikungunya referred samples from urban and rural parts of Bangalore by IgM ELISA. Also testing of referred samples was undertaken for Japanese encephalitis viral infections from the neighboring hospitals.

During the period, a total of 2792 serum specimens were received for dengue and chikungunya IgM antibodies testing by MAC-ELISA (NIV, Pune Kit). Of the 2743 cases, 1134 (41.34%) cases were tested positive for DEN IgM antibodies and isolation of one dengue virus was achieved from 199 acute sera processed in C6/36 and BHK-21 cell lines (Table 1). Dengue virus infection was observed throughout the year with peak positivity in October and involved all age groups in both sexes. Of the 2422 cases, 560 (23.1%) cases were positive for CHIK IgM antibodies. Eighteen CHIK virus isolations were obtained from 199 acute sera processed in C6/36 and BHK-21 cell lines. Chikungunya virus activity was observed in all the months with peak positivity in January and involved all age groups of both sexes. Bangalore city showed the highest number (1057) of positive cases (45.9%) for dengue and (446) of positive cases (19.9%) for chikungunya IgM antibodies. Dual infection of dengue and chikungunya activity was noticed 10.50% with peak positivity in October.

During the period, specimens received from a total of 49 suspected cases of encephalitis from 6 districts of Karnataka and 1 district each of Andhra Pradesh and Tamil Nadu were screened for JE IgM antibodies. Of these 5 cases were confirmed as JE, 4 from Bangalore urban district and 1 from Bellary district in Karnataka. Occurrence of the cases was observed in the month of December, 2014 and January, 2015.

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Table 1: Month wise IgM detection of dengue, JE and chikungunya cases from Karnataka and neighbouring states

Month	Total Received	Total Tested For CHIK	CHIK IgM Positive	Total Tested for DEN	DEN IgM Positive	Total Tested for JE	JE IgM Positive	Mix Pos CHIK & DEN	Inoculation in C6/36 BHK-21 cell Line	Virus isolation
April	18	18	1	18	6	0	0	3	5	0
May	144	144	28	144	28	2	0	9	41	8
June	182	182	57	182	51	0	0	6	45	10
July	401	373	35	393	167	4	0	18	18	1
August	312	300	21	300	117	6	0	21	9	0
September	339	339	33	339	181	4	1	19	35	0
October	531	476	54	531	256	0	0	50	3	0
November	359	356	42	359	157	1	0	24	3	0
December	208	207	20	208	87	9	4	8	6	0
January	210	20	186	186	61	17	0	16	22	0
February	50	6	47	47	13	3	0	3	6	0
March	38	1	36	36	10	3	0	1	6	0
Total	2792	2422	560	2743	1134	49	5	178	199	19

BNU9702: Studies on Poliovirus

1. Surveillance of Acute Flaccid Paralysis (AFP) cases from Karnataka and Kerala states and parts of Bihar as a part of WHO-SEAR Polio Lab Network in the WHO's Global Eradication of Poliomyelitis Programme. (Service project)

2. Intratypic differentiation of poliovirus isolates from AFP cases received from National Polio Laboratory as a part of WHO-SEAR Polio Lab Network in the WHO's Global Eradication of Poliomyelitis Programme.

CG Raut

Funding: WHO Project duration: Ongoing

Acute flaccid paralysis (AFP) surveillance for Global Eradication of Poliomyelitis Programme was initiated by the Government of India in collaboration with the WHO/WHO-SEARO/WHO-NPSP in India since June, 1997. The NIV Bangalore Unit is responsible for processing all stool specimens from Karnataka State since 1997, Kerala state since September 2007, Bareilly, Badaun and Gorakhpur sub-divisions of Uttar Pradesh since July 2007 and Bhagalpur and Gaya sub-divisions of Bihar since June 2013. In the Global Polio Eradication Initiative (GPEI), AFP is defined as sudden onset of weakness and floppiness in any part of the body in a child <15 years of age or paralysis in a

person of any age in whom polio is suspected. Any case, meeting this definition undergoes a thorough investigation to determine if the paralysis is caused by wild poliovirus or not.

During the period, a total of 10,421 stool specimens from 5240 AFP cases were processed for virus isolation (Table 2). The result of one sample from Karnataka could not be confirmed due to toxicity of the sample on the cell cultures.

- Reporting of results within 14 days for the above samples were 97.75%.
- No wild poliovirus was isolated from any of the cases tested during the period. Last wild poliovirus isolated at NIV, Bangalore was in the month of January 2010 from Uttar Pradesh. Karnataka and Kerala states are free from wild poliovirus from 2007 and 2000, respectively.
- During the period 20 stool specimens were received from contacts, 10 each from Karnataka and Kerala states. The results of the contact specimens are L20B positive-2(vaccine virus), NPEV-1 and 17 negative.

Real Time RT-PCR intratypic differentiation (ITD) assay and vaccine virus serotype wise VDPV assay were performed for ITD of polioviruses following WHO protocols and CDC supplied kits. ITD Laboratory was accredited by the WHO for the year 2013-14.

A total of 381 poliovirus isolates were tested for ITD identification (Figure 1). Results of 381 isolates from AFP cases are P1 vaccine virus (131), P2 vaccine virus (60), P3 vaccine virus (101), poliovirus vaccine mixtures (58), NPEV (16), NEV (0) and Discordant (15).(Table 2).

Table 2: State wise and month-wise details of AFP cases/samples received from April 2014 till March 2015

State	AFP Cases	Total samples	L20B+ve (PV+NPEV)	POLIO VACCINE	NPEV by PCR	NPEV	Negative	Polio + NPEV
KARNATAKA	1019	2040	37	36	1	252	1750	0
KERALA	390	784	8	7	1	68	708	0
BIHAR	3778	7499	323	314	18	1657	5509	10
Others	53	98	2	2	0	20	76	0
TOTAL	5240	10421	370	359	20	1997	8043	10

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Table 2 : Monthwise AFP samples tested from April 2014 to March 2015

Month	Total samples	L20B pos	Polio Vaccine	NPEV BY PCR	NPEV	Negative	Polio+ NPEV
Apr	684	33	32	1	126	525	0
May	745	18	15	5	167	558	2
Jun	850	25	28	0	364	458	3
July	987	19	22	0	361	604	3
Aug	1058	6	5	2	277	774	1
Sept	1224	11	6	5	213	1000	0
Oct	892	9	7	2	134	749	0
Nov	912	51	51	0	117	744	0
Dec	786	28	28	0	65	693	0
Jan	790	36	35	1	54	699	0
Feb	744	58	58	0	43	643	0
Mar	749	76	72	4	76	596	1
Total	10421	370	359	20	1997	8043	10

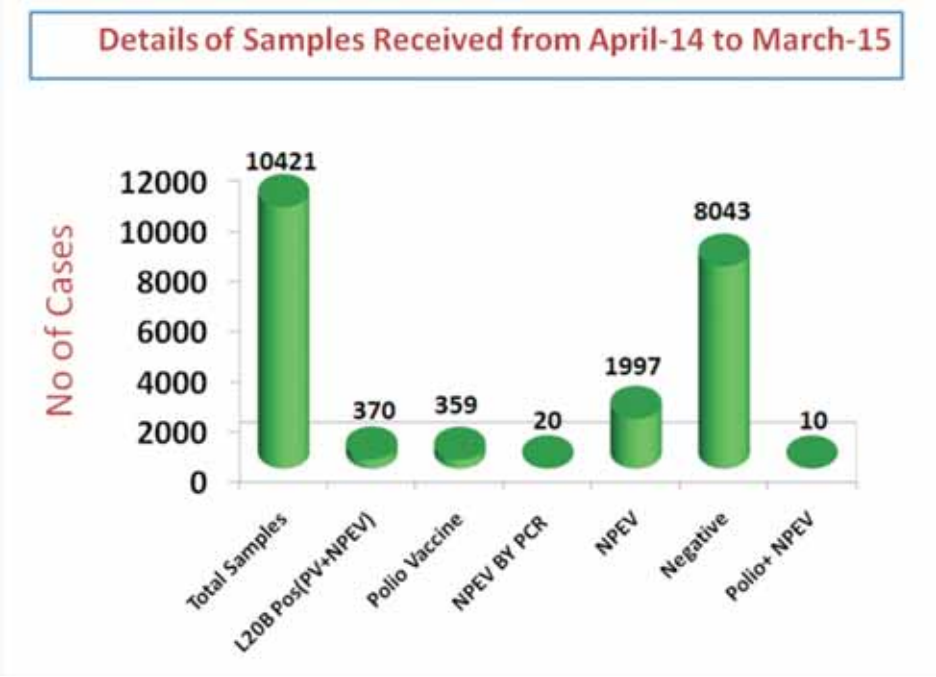

Figure 1: Samples received from April 2014 to March 2015

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BNU0603: Surveillance of Measles and Rubella cases from Karnataka, Kerala and West Bengal as part of WHO-SEAR Measles Laboratory Network in the WHO's Global Measles Elimination Programme (Service Project).

CG Raut

Funding: WHO Project duration: 2006-Ongoing

Surveillance of measles outbreaks was initiated by the Government of Karnataka in collaboration with Government of India and the WHO/WHO-SEARO/WHO-NPSP since June 2006 with NIV Bangalore Unit as Measles/Rubella serological diagnosis and virus isolation laboratory in the WHO-SEAR Network. Surveillance of measles outbreak in Kerala was included since November 2007.

During the period a total of 407 serum specimens from 83 outbreaks investigated were received from 21 districts of Karnataka, 9 districts of Kerala and 1 from Lakshadweep. Of these 228 (56.0%) were positive for Measles and 74 (18.1%) for Rubella while 106 (26.0%) tested negative for both. Of the 83 outbreaks investigated 56 (67.4%) were Measles, 14 (16.8%) were Rubella, 10 (12.0%) were mixed for both Measles and Rubella and 3 (3.6%) were negative for both (Table 3).

Measles laboratory was fully accredited by WHO after onsite review visit by the WHO's expert in January 2015. WHO Measles and Rubella proficiency test panel was passed with 100% score in September, 2014. All laboratory accreditation conditions were fulfilled.

Table 3: District wise IgM ELISA results from Karnataka, Kerala and Lakshadweep

Province & District	Measles Test Done	Measles Positive	Measles Negative	Rubella Test done	Rubella Positive	Rubella Negative	Both Negative
Bagalkot	10	4	6	6	5	1	1
Bangalore (u)	9	8	1	1	0	1	1
Belgaum	25	6	19	19	15	4	4
Bellary	27	7	20	21	4	17	16
Bijapur	22	10	12	12	7	5	5
Chamarajnagar	19	13	6	7	0	7	6
Chickaballapur	20	15	5	6	1	5	4
Chitradurga	7	1	6	7	0	7	6
Dakshin kannada	10	7	3	3	0	3	3
Davanagere	5	4	1	1	0	1	1

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Province & District	Measles Test Done	Measles Positive	Measles Negative	Rubella Test done	Rubella Positive	Rubella Negative	Both Negative
Dharwad	5	0	5	5	5	0	0
Gulbarga	10	9	1	1	0	1	1
Hassan	5	0	5	5	3	2	2
Kodagu(coorg)	15	11	4	4	1	3	3
Kolar	7	0	7	7	5	2	2
Mandya	10	4	6	7	4	3	2
Raichur	3	2	1	1	0	1	1
Shimoga	5	5	0	0	0	0	0
Tumkur	11	7	4	4	0	4	4
Udupi	6	4	2	3	0	3	2
Uttar kannad	24	12	12	12	6	6	6
Karnataka total	**255**	**129**	**126**	**132**	**56**	**76**	**70**
Alappuzha	4	4	0	0	0	0	0
Kannur	29	27	2	2	0	2	2
Kasaragod	5	1	4	4	4	0	0
Kollam	10	5	5	6	1	5	5
Malappuram	44	35	9	9	6	3	3
Palakkad	15	12	3	3	0	3	3
Thiruvananthapuram	18	3	15	15	5	10	10
Thrissur	11	7	4	4	0	4	4
Wayanad	10	5	5	5	0	5	5
Kerala total	**146**	**99**	**47**	**48**	**16**	**32**	**32**
Lakshadweep	6	0	6	6	2	4	4

Laboratory diagnosis of Measles and Rubella cases by RT-PCR Assay:

A total of 182 cases from 80 outbreaks were investigated from 8 provinces (Assam, Karnataka, Kerala, Lakshadweep, Manipur, Tripura, Uttar Pradesh and West Bengal) of India. Of these 50 (27.47%) were tested positive for Measles and 15 (8.24%) positive for Rubella (Figure 2).

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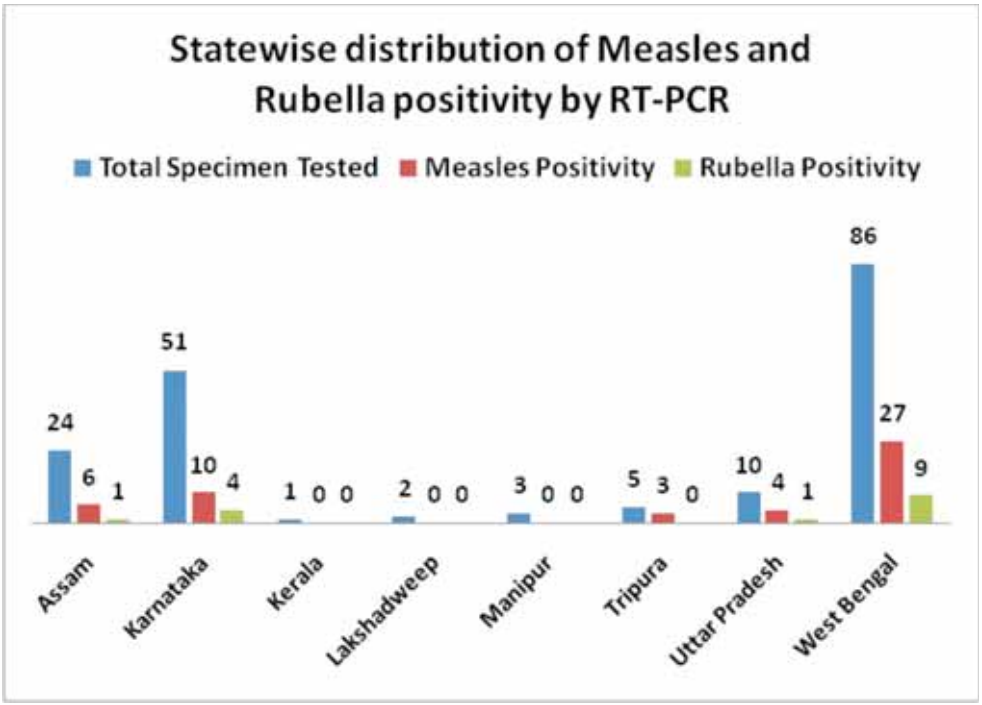

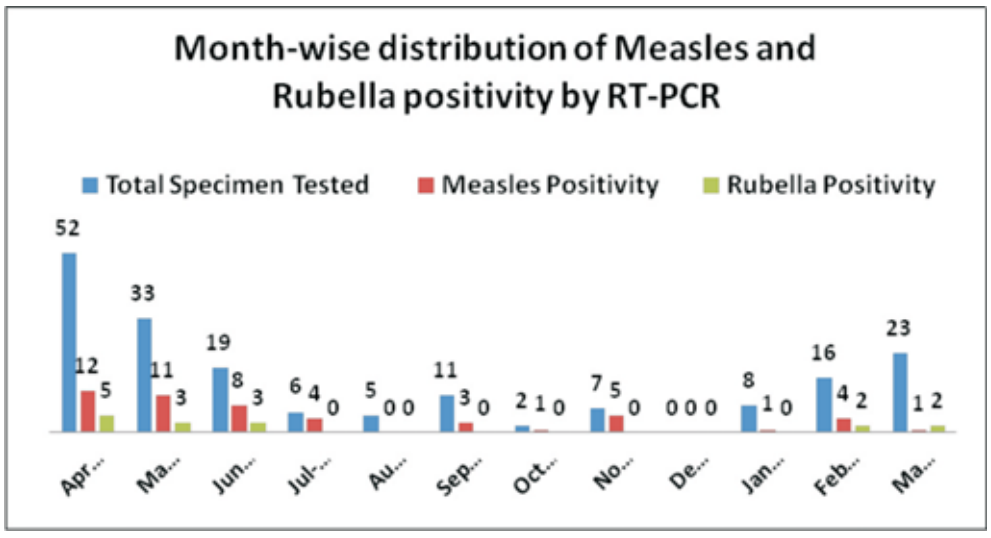

Figure 2: Month-wise and State-wise sample distribution for Measles/Rubella and positivity by RT-PCR assay.

Haemorrhagic conjunctivitis outbreaks in Chennai and Bangalore due to enterovirus- CA24:

CG Raut

Funding: Intramural Project duration: Ongoing

Two subsequent outbreaks of hemorrhagic conjunctivitis were reported by the local media in Chennai and Bangalore in November 2014 and December 2014, respectively. The NIV Bangalore Unit investigated the outbreaks and collected conjunctival swabs and transported to the laboratory

in cold chain for laboratory tests. The outbreaks affected all the age groups in both the genders with bilateral hemorrhagic conjunctivitis. Molecular tools were employed to detect the suspected virus genome and also processed samples for virus isolation in different cell lines. Real time PCR for enterovirus detected positivity in 48 and 81samples from Chennai and Bangalore, respectively and found the involvement of Coxsackie A-24 by sequencing. Virus isolation in HeLa, A459, RD and BHK-21 cell lines yielded 49.2%, 49.1%, 20% and 27.1% positivity respectively.

Molecular Diagnosis of referred samples.

CG Raut & DP Sinha

Funding: Intramural Project Duration: Ongoing

During the period 307 referred specimens were tested for viral diagnosis using RT-PCR. Results of the tests are summarized in Figure 3.

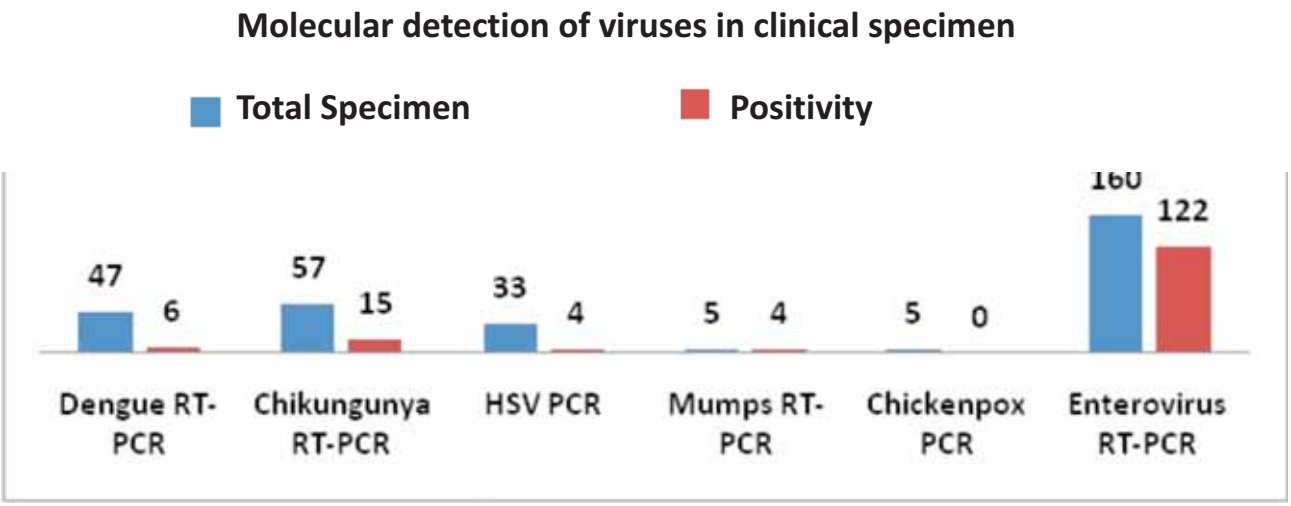

Figure 3: Sample distribution for viral diagnosis and positivity by RT-PCR assay.

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Mr. Sutar DV	Technician-'B'

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KU1101: Multisite virological and epidemiological monitoring of Influenza, phase II

B Anukumar

Funding: ICMR-DHHR (Extramural) Project Duration: September 2010-September 2014

Influenza surveillance in the community is an important tool for conducting surveillance to identify emerging/reemerging strains, unusual epidemiological trends and defining seasonality. The objective of the project was to carry out surveillance of human influenza virus in the Kerala state. The influenza virus surveillance was carried out in 7 sentinel hospitals, including General hospital, primary health centers and medical college in Alappuzha district of Kerala state. A total of 293 nasal/throat swab specimens were collected from patients with influenza-like illness. Samples were initially screened for the presence of influenza type A and B. The influenza type A positive samples were further sub-typed into seasonal H1, H3 and pandemic H1. Thirty two (10.92%) samples tested positive for influenza type A and 1 (0.34%) for influenza type B. The sub-typing results showed the presence of 31 cases of seasonal A(H3N2) and 1 A(H1N1)pdm09. Selected A(H3N2) positive samples (15) were processed for virus isolation in MDCK cell line and all yielded isolation which was further confirmed by real time RT-PCR. The study concluded the activity of seasonal A(H3N2) in the region especially during the rainy season (Figure 1).

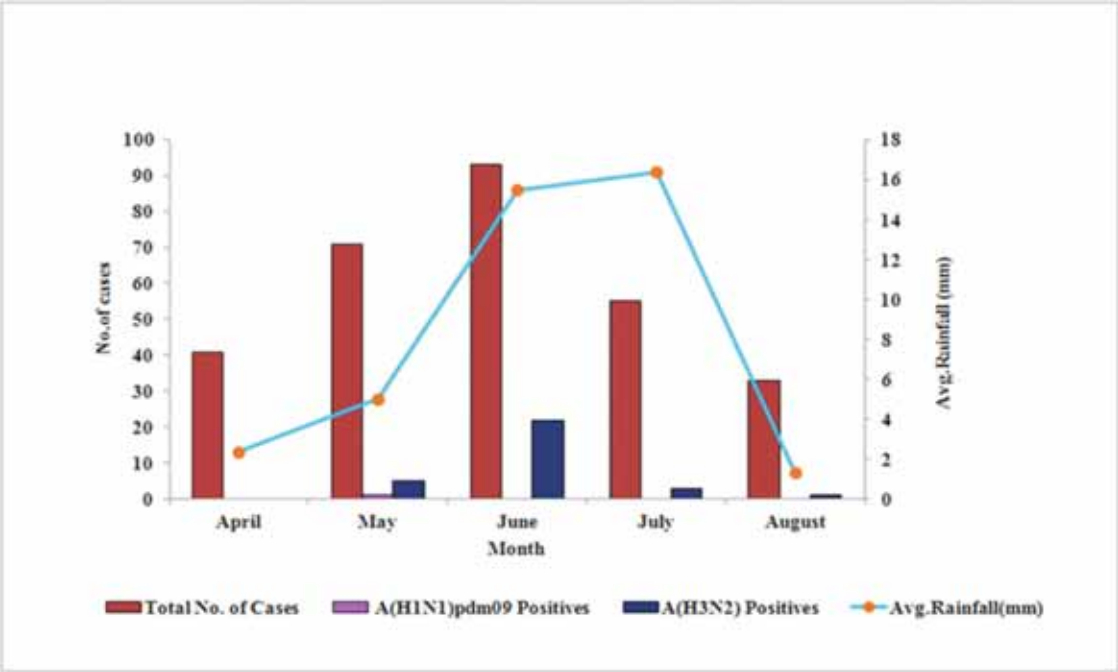

Figure 1: Monthwise distribution of influenza like illness cases, influenza A(H1N1)pdm09 and A(H3N2) positive cases. The blue line indicates the average monthly rainfall (mm) in this region.

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KU1107: Development of NS1 protein capture ELISA for detection of West Nile virus infection

B Anukumar

Funding: Intramural Project Duration: 2013-2015

Viral proteins produced during virus infection are a target for documenting acute infection and diagnostics targeting the viral antigens are available in the market. Detection of Dengue non-structural protein 1 (NS1) in patient sera is one of the universally available method to identify the early stage of infection. The aim of the study is to develop NS1 capture ELISA for WNV to detect the early infection. In order to produce the WNV NS1 specific antibodies, NS1 and envelop (E) proteins were expressed in the baculovirus expression system, confirmed by immuno fluorescent assay and standardized the protein purification method. The expressed recombinant protein has a polyhistidine-tag at the N - terminal end which can bind to the nickel (Ni) residues. Thus, Ni-NTA affinity column was used for protein purification. The expressed proteins were released from the Sf9 cells by lysing the cells with guanidinium-HCl. The proteins were purified under denatured condition in the presence of urea. The bound proteins were eluted from the column using imidazole. The purity of the protein was checked in SDS-PAGE and found acceptable for further downstream applications (Figure 2).

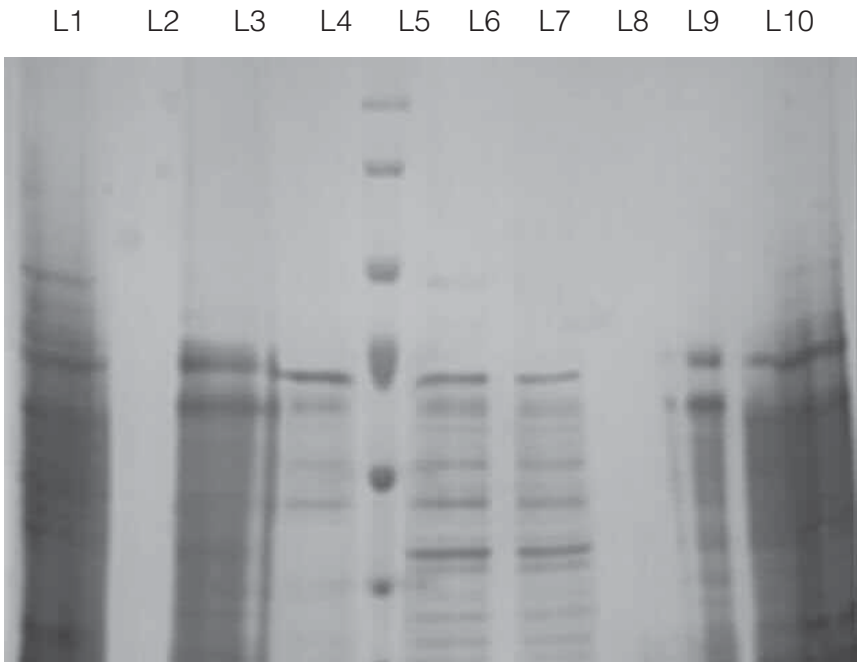

Figure 2: SDS-PAGE analysis of the purified West Nile virus NS1 and E proteins. The separated proteins in the gel were determined by silver staining. L1: whole Sf9 cell lysate (WNV E protein), L2: column wash, L3: unbound, L4: eluted E protein, L5: Protein marker, L6 & L7: Eluted NS1 protein, L8: column wash, L9: unbound, L10: whole cell lysate (WNV NS1 protein).

NIV, KERALA UNIT

KU1302: Virus-host protein-protein interaction: a preliminary study to identify putative host protein(s) interacting with Chandipura virus

B Anukumar & Kavathekar VK

Funding: Intramural Project Duration: 2013-2016

Chandipura virus (CHPV) is a single segmented negative sense RNA virus (Mononegavirales) in the genus Vesiculovirus, family Rhabdoviridae. Earlier studies on mice have shown that intra-peritoneal injection of CHPV led to neurologic dysfunction within 48 h PI. Significant and consistent lesions were observed in the central nervous system and included necrosis of neurons and ependymal cells. Recent studies in mice have demonstrated that a right foot pad injection of Chandipura virus causes progressive viral replication in all nervous tissues. So far, there has been no significant effort to understand CHPV interactions with neurons and its proteins. This study aims to profile the interaction of the whole virus and individual protein components of CHPV with the neuronal proteins. This year, CHPV nucleocapsid (N) gene was cloned into the mammalian expression vector pcDNA 3.1+. The vector has a C-terminal poly His-tag and N-terminal Myc-tag to pull down the protein using affinity column. Expression of N protein was checked in Neuro 2a cells and confirmed by immunofluorescent assay (Figure 3).

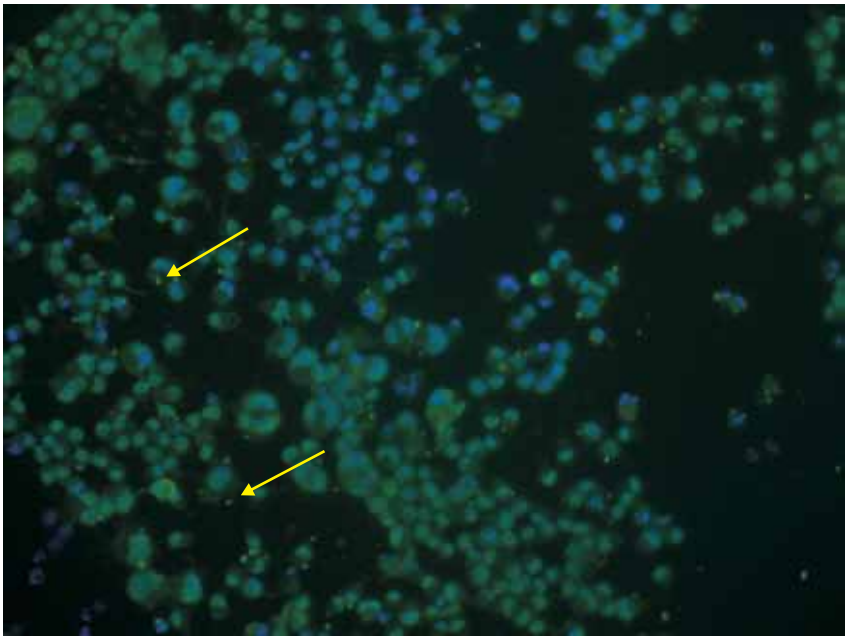

Figure 3: Expression of Chandipura virus nucleocapsid (N) protein in Neuro 2A cells. The 'N' protein expressed in the transfected cell was determined by indirect immunofluorescent assay using mouse anti Chandipura polyclonal sera. The aggregated nucleocapsid protein was seen in the cytoplasm of the cells.

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KU1102: Effect of mutations in chikungunya virus E1 and E2 proteins in virus entry-a virus like particle based approach

B Anukumar & Mathew AM

Funding: Intramural Project Duration: 2014-2017

Mutations in the structural protein genes of Alphaviruses can severely affect critical biological processes like membrane fusion, virus assembly, viral budding, etc. A lot of literature is available about the significance of mutations in E1 and E2 proteins. Chikungunya virus (CHIK) isolates from the recent New Delhi outbreak (2010) have many synonymous and non-synonymous amino acid mutations in the E1 & E2 protein region including unique mutations at positions E1-K211E and E2-V264A. The importance of these mutations is however not known. The role of mutation(s) in viral biology can be studied by producing the infectious cDNA in the laboratory. Besides the infectious cDNA clone, virus like particles (VLP) can also be used to study the role of mutation in the structural genes. VLPs are structurally identical to the wild type virus, but these particles cannot replicate due to the absence of the viral genome. VLPs authentically present viral spikes and other surface components in a repetitive array. In order to produce the CHIKV VLPs, the structural protein genes (capsid (C) -E3-E2-6K-E1) were amplified and cloned into pFastBac1 vector (Baculovirus expression system). The structural cassette was then recombined into Bacmid shuttle vector in order to produce the baculovirus expressing the CHIKV structural genes. The recombinant baculo virus was produced in Sf9 cells and the expression of CHIKV structural protein was confirmed in Western blot using rabbit anti CHIKV polyclonal sera (Figure 4).

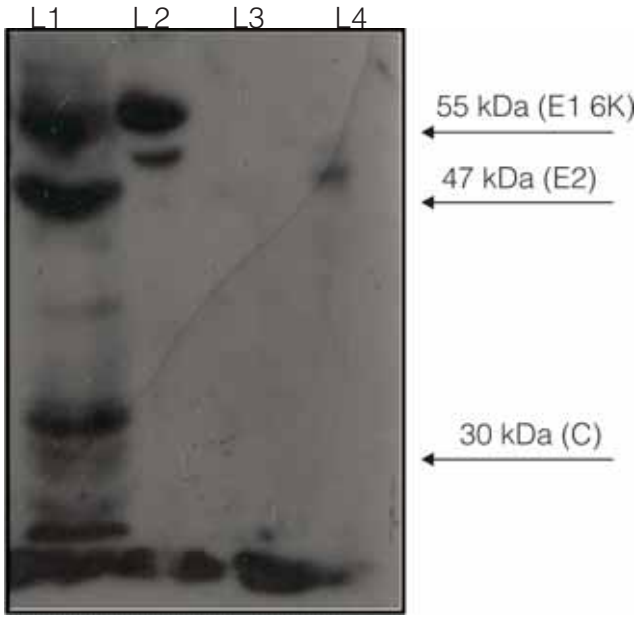

Figure4: Expression of the CHIKV structural proteins determined by Western blot. L1: Recombinant baculovirus infected Sf9 cell pellet, L2: Recombinant baculovirus infected Sf9 cell supernatant, L3: Sf9 cell pellet, L4: Sf9 cell supernatant.

Grade I Virology Laboratory Network

B Anukumar, BV Tandale & R.Balasubramanian

Funding: ICMR (Extramural) Project Duration:2011-2016

The unit can provide a diagnosis of more than 40 different viruses using both serological and

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molecular based diagnostic tests. During this period, 2050 samples collected and/or received were processed for 3828 tests (Table.1).

Table 1 Samples screened for different viruses using various diagnostics

Sl No.	Virus	IgM ELISA	IgG ELISA	Conv ent PCR	Real time PCR	Virus Neut.	Ag. Direct	Virus isolate	Seq.
1	JE	396/9	-	Oct-00	133/0	-	-	-	-
2	Dengue	453/33	-	67/19	May-00	-	-	13/2	27
3	West Nile	-	-	15/0	159/0	-	-	-	-
4	Chandipura	-	-	-	Jan-00	-	-	-	-
5	Chikungunya	252/169	-	-	65/14	-	-	16/11	3
6	HSV 1	-	-	176/1	Jan-00	-	-	-	-
7	HSV 2	-	-	176/1	Jan-00	-	-	-	-
8	HSV 1 & 2	16/0	-	-	-	-	-	-	-
9	HPV	-	-	1-Feb	-	-	-	-	1
10	Rota	-	-	Apr-00	-	-	-	-	-
11	HAV	30/18	-	6-Oct	-	-	-	-	-
12	HEV	30/1	-	-	Jan-00	-	-	-	-
13	Rubella	25/0	-	61/0	-	-	-	-	-
14	Measles	18/0	-	37/3	-	-	-	-	-
15	HBV	May-00	-	Aug-00	-	-	13/2	-	-
16	HCV	May-00	-	Feb-00	-	-	-	-	-
17	Entero virus	-	-	86/10	304/11	-	-	-	12
18	Adeno	-	-	18/1	118/13	-	-	32/21	110
19	CMV	64/0	-	62/5	220/32	-	-	-	-
20	Varicella Zoster	Apr-00	-	1-Feb	Dec-00	-	-	-	-
21	RSV - A	-	-	95/2	Jan-00	-	-	-	-
22	RSV - B	-	-	95/18	Jan-00	-	-	-	19
23	Parvo V	-	-	Apr-00	-	-	-	-	-
24	EBV	60/4	-	17/5	97/4	-	-	-	-
25	Mumps	2-May	-	Jun-00	-	-	-	-	-
26	Rhino	-	-	-	-	-	-	-	1
27	KFD	Mar-00	-	-	Apr-00	-	-	-	-
28	Pandemic A (H1N1)	-	-	-	181/18	-	-	-	-
29	Influenza B	-	-	-	110/2	-	-	-	-
30	H3N2	-	-	-	-	-	-	21/16	-
	TOTAL	1366/236	-	953/73	1414/94	-	13/2	82/50	173

(Total number/total positives)

NIV, KERALA UNIT

KU1110: Entomological studies in Alappuzha district, Kerala state in relation to Japanese encephalitis and West Nile virus vectors

R. Balasurbamanian & B. Anukumar

Funding: Intramural Project Duration: 2012-2015

Studies were conducted to determine the seasonal patterns for abundance of the primary and secondary Japanese encephalitis (JE) and WNV vectors in the area. During this period, adult mosquitoes were collected from the cattle sheds at dusk hours using light trap at monthly intervals and a total of 14,135 female mosquitoes were collected. Thirteen species from six genera viz., *Anopheles, Aedes, Armigers, Culex, Coquillettidia* and *Mansonia* were collected (Table 2). In the study areas, *Cx. tritaeniorhynchus* (63.42%), was predominant followed by Ma. uniformis (11.55%), *Ma. indiana* (8%), *Ar. subalbatus* (7.17%), *Cx. gelidus* (3.19%), *Mn. annulifera* (3.64%) and *Cx. quinquefasciatus* (1.34%). Other species were found to be <1% of the total mosquitoes collected. Direct ELISA was developed for identification of dog, cow, goat, rabbit, rat, mouse, human, and chick blood meals. A total of 90 (39 *Cx. tritaeniorhynchus,* 21 *Cx. quinquefasciatus,* 17 *Cx. sitiens,* 15 *Ma. annulifera*) mosquitoes were processed in which 29 mosquitoes reacted with cow antibody; 16 with human antibody, and 12 for mixed blood meal.

Table 2: Seasonal distribution of mosquitoes collected from different sites in the coastal area of Alappuzha district

Month		Apr. to June	July to Sept	Oct. to Dec.	Jan. to Mar	Total	%
No. of collection		6	3	6	6	6	27
1	*Aedes vexans*	0	0	3	2	5	0.04
2	*Anopheles barbirostris*	10	0	0	7	17	0.12
3	*An. peditaeniatus*	0	4	3	6	13	0.09
4	*Armigeres subalbatus*	24	26	389	575	1014	7.17
5	*Culex bitaeniorhynchus*	0	0	0	7	7	0.04
6	*Cx. gelidus*	68	38	99	246	451	3.19
7	*Cx. quinquefasciatus*	173	0	0	17	190	1.34
8	*Cx. sitiens*	30	18	7	25	80	0.56
9	*Cx. tritaeniorhynchus*	779	7	2869	5310	8965	63.42
10	*Coquillettidia crassipes*	0	11	77	16	104	0.73
11	*Mansonia annulifera*	17	17	154	327	515	3.64
12	*Ma. indiana*	9	9	503	620	1141	8.07
13	*Ma. uniformis*	83	83	534	933	1633	11.55
	Total	1193	213	4638	8091	14135	

NIV, KERALA UNIT

KU1401: Detection of KFD virus in tick populations in forested area of Wayanad district, Kerala state

R. Balasurbamanian, B Anukumar & PD Yadav

Funding: Intramural Project Duration: 2014-2015

Ixodid ticks (Acari: Ixodidae) transmit a wide variety of pathogens including viruses. Kyasanur Forest Disease (KFD) is a tick borne zoonotic disease and has so far been localized only in the Karnataka State. Considering the recent studies confirming an increased risk of KFD in Wayanad district, a survey was carried out to investigate the presence of KFDV in hard tick population during April to May 2014 and January-February 2015 and collected a total of 750 ticks by flag dragging method. A total of 256 domestic animals were examined for ticks and a total of 2900 ticks were collected from 128 infested cattle. Identification revealed the presence of *Rhipicephalus* (56.13%), *H. boophilus* (13.44%), *Haemaphysalis* (29.48%), *Hyalomma* (0.48%) and *Dermacentor* species (0.44%) in the collection (Table 3). A total of 128 pools, each containing 25 ticks were sent to NIV, Pune for KFDV detection. None of the ticks tested positive for KFDV.

Table 3: Prevalence of tick species in cattle of Wayanad district Kerala

S. No.	Genus	Male	Female	Total	%
1	Haemaphysalis spp	555	300	855	29.48
2	Rhipicephalus spp	761	867	1628	56.13
3	Hyalomma spp	12	2	14	0.48
4	Boophilus spp	192	198	390	13.44
5	Dermacentor spp	0	13	13	0.44
	Total	1520	1380	2900	

TRANSLATIONAL RESEARCH GROUP (TRG)

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Dr. Deshmukh TM	Scientist 'B'

Co-ordinators

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Dr. Sapkal GN	Scientist 'D' & Group Leader (DVG)
Dr. Sudeep AB	Scientist 'D' (Entomology)

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Mr. Tilekar BN	Technical Assistant
Mrs. Gunjikar RS	Technical Assistant

TRANSLATIONAL RESEARCH GROUP (TRG)

Translational Research Group (TRG) was constituted on November 21st, 2014 at the Microbial Containment Complex, Pashan.

Funding: Intramural Duration: ongoing

Mandate:

- To support and cater to the proposed MCC facility for supply of quality control positive and negative controls to Viral Diagnosis Laboratories.
- Centralization and long term storage of existing hybridomas.
- To expedite technology transfer process.
- Centralization of patenting technologies.
- Focal point for IPR related issues.
- Providing assistance in trouble shooting R & D and to take various technologies for transfer to the manufacturers.

Laboratory activities:

- Virus stock preparation (cells, suckling mice).
- Virus purification (ultracentrifugation, gradient centrifugation) and viral load determination (plaque assay/real-time PCR).
- Development of controls for routine PCR/real time PCR (in vitro transcribed RNA, inactivated virus, plasmids, etc.).
- Validation of diagnostic tests developed at NIV.
- Storage of cell lines procured from ATCC/other sources.
- Performance testing of diagnostic tests developed at NIV using stabilizers, preservatives.
- Preparation of serum panels to be used as positive and negative controls in different assays.
- Protein expression (bacterial, baculovirus systems) and purification.

Priority tasks proposed:

- Virus stock preparation.
- Development of humanized chimeric mAbs to be used as assay components.
- Hepatitis virus vaccine candidates (E, E+B combined) to be taken ahead for commercialization.
- Development of in-house diagnostics and vaccine candidate against hepatitis A.
- Development of rapid immunochromatographic assay for hepatitis E diagnosis.

TRANSLATIONAL RESEARCH GROUP (TRG)

- Water testing- Detection of waterborne viruses in drinking water.
- Development of combined JE/WN vaccine candidate.

Products developed:

1) Technology for concentration and testing of waterborne viruses in drinking water: Provisional patent filed (Application No. 160/MUM/2015 dated January 16, 2015): Composition for virus precipitation from dilute samples and a method thereof.

2) Development of anti-CHPV IgM antibody detection assay: Meeting to finalize the technology dossiers on September 15, 2014 at ICMR Hqrs., New Delhi.

3) Monoclonal antibody based antigen capture ELISA for detection of Japanese encephalitis virus from mosquito: Meeting to finalize the technology dossiers on September 15, 2014 at ICMR Hqrs., New Delhi.

Research Support Groups

VIRUS REGISTRY

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Mr. Umrani U	Technical Officer-‘A’
Mr. Raju M. Rahurkar	Technician 'B'
Mrs. S.S. Mohol	MTS

VIRUS REGISTRY

Maintencance of Virus Repository and Registry (VRR)

U Umrani, BR Patil & A Basu

The main function of VRR is to provide an interface for receiving and cataloging samples from various organizations that are sent for diagnostic testing at NIV and co-ordinate subsequent test logistics till reporting. This is a central facility. During the period of 2014-15 a total of 19,026 referred samples were handled. In situations of public health emergencies, like the recent episodes of Ebola, KFD, CCHF and H1N1, VRR directly co-ordinated the logistic flow with the maximum containment BSL4 laboratory.

Virus Repository Unit caters to the need of providing reference virus strains to both academic and industrial laboratories as per international/national biosecurity regulatory directives. During 2014-15 a total of 38 reference virus strains were provided to 14 such institutions in the country.

Both these facilities also serve the internal scientists for their research needs like specimen archiving, cataloging and providing logistic issues as needed.

प्रतिभागीओं को एन.आय.व्ही. तथा बी.एस.एल.-4 के उपकरण तथा यंत्रसामग्री की जानकारी प्रात्यक्षिक द्वारा अवगत करायी। अत्याधुनिक कार्यप्रणाली के बारे में डॉक्युमेंटरी फिल्म दीखायी गई।

डा. निर्मला मौर्य, निबंधक, राजभाषा दक्षिण भारत हिन्दी प्रचार सभा, चेन्नई का दिनांक 24 दिसंबर, 2014 को बदलते परीपक्ष में हिन्दी और अनुवाद की भूमिका पर व्याख्यान का आयोजन किया गया था।

संस्थान में हिन्दी पखवडा, सप्ताह, कार्यशाला, संगोष्ठी आदी का आयोजन किया जाता है।

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INFORMATION CENTRE AND LIBRARY

Staff

Dr. Singh SN	Senior Library & Information Officer (Till August 2014)
Mrs. Chandere VS	Assistant Library & Information Officer
Dr. Chakrabarti AK	Scientist 'C' & Library in charge (w.e.f. 12th January, 2015)
Mr. Mali VR	Library & Information Assistant
Mr. Gorayya GR	MTS

Apprentices

Mr. Waghmare G	(07-04-2014 to 05-11-2014)
Mrs. Singh J	(01-04-2014 to 30-10-2014)
Mr. Moghe G	(w.e.f. 17-11-2014)
Mr. Shelar O	(w.e.f. 17-11-2014)

INFORMATION CENTRE AND LIBRARY

1. Management of Library & Information Centre

During this year, the library staff provided all the support to the scientists regarding publications and other related subjects viz. providing citations index, procurement of reprints, etc. The library staff also contributed in stock verification of the library and weeding out of old books.

a. Serial (Journal) Control:

Full text online access facility is continued through ERMED Consortium, ICMR Consortium and through J-Gate Plus database. Through NML, DGHS, under ERMED Consortium 289 e-journals were provided with full text online IP based consortium service with six publishers: BMJ Group, Cambridge University Press, Informa Health Care, Lippincott Williams & Wilkins, Oxford University Press, John Wiley. In ICMR Consortium we have full text access for four journals issues: Science, Nature, Lancet, and NEJM. Under J-Gate plus Database we have access to 2226+ journals (with access to full text, abstract and on Document Delivery Request from other ICMR library).

b. Book (Acquisition) Control- Web OPAC LIBSYS based services was continued and automation of all documents received during the year was carried out on real time basis. All the documents available in the NIV Library are accessible to NIV-Library members through intranet. Other activities/applications of LIBSYS-acquisition, serial control, cataloguing-current and retrospective, article indexing, etc. was carried out. A computerized accession register was generated and the traditional manual system has been discontinued. The Information and Library Service of the institute has procured unique and rare collection of different varieties and various kinds of publications printed and digital in the field of virology, viral diseases and allied subjects (Table-1).

Table- 1: Documents added during the year to the Library

S. No.	Description	Quantity
1.	Books- Purchased & Gift/Gratis	56
	Bound Volumes	Nil
	Annual Reports	36
	Reprints	850
2.	Journals: Current	Print: 12
		Online:04
		JGate: 3532
		Gratis/Free-64
	Loose Issues	425
	Bound Volumes	NIL
3.	PhD Thesis	5
	MSc Dissertations	22
4.	Others; CDs, Microforms, floppies, etc	11

c. SDI/CAS and Reference Services provided on demand from our in-house resources, various indexing and abstracting databases. News paper clippings are prepared on daily basis and soft copies are being sent to all scientists of NIV to keep them abridged with the daily news about viral diseases. In addition, e. mails related to library activities viz., List of weekly Arrival (W), List of Current Arrivals of New Books (M) etc are also sent to all the concerns scientists/officials.

d. Inter Library Loan (ILL) and Document Delivery Services (DDS): Books and journals on ILL from local libraries viz., NCL, BJMC, AFMC, NCCS, ARI and other biomedical libraries were procured and provided to NIV scientists on demand basis and Document Delivery Services (DDS) was continued during the year at local, and national levels. NIV library has also continued to extend its information and library services not only to ICMR scientists, researchers and students but also to various colleges and medical institutes/organizations locally (Table 2).

Table-2: Services Provided by NIV library during the year

1.	Books & bound volumes Issued	Staff:	353
		Students:	949
	Books & bound volumes returned	Staff:	555
		Students:	932
2.	Inter Library Loan (ILL) -received and sent)	246 Documents	
3.	Photocopy Services	2562 pages	
4.	Binding	022 documents	
5.	Lamination	060 documents	
6.	Reference services + Document Delivery Services (DDS-Print + Electronic)	380 References	
7.	NIV Annual Reports Sent	45	
8.	SDI/CAS (Routinely)	On demand	
9.	Citation Analysis of NIV Scientists'	23 Scientists	

Publication (April 2014 to March 2015): Published-60 Sent-26

MAINTENANCE DIVISION

Engineering Support Team for NIV, Pune City Campus

Mr. Perumal ST	Technical Officer-C
Mr. Mahadik JM	Technical Officer-A
Mr. Kumbhar DR	Technical Officer-A
Mr. Pacharane JD	Technical Officer-A
Mr. Kasar AK	Technical Officer-A
Mr. Bhongale NV	Technical Assistant
Mr. Suresh AJ	Technical Assistant
Mr. Bagul GK	Technical Assistant
Mr. Shukla RM	Technician-C
Mr. Dhawale NS	Technician-C
Mr. Jagtap VD	Technician-C
Mr. Taru YM	Technician-C
Mr. Shelar BS	Technician-B
Mr. Utale SS T	echnician-A
Mr. Kelkar AB	Technician-A
Mr. Holkar SS	Technician-A
Mr. Gadhave RS	Technician-A
Mr. Pawar AM	Technician-A
Mr. Jagtap DK	Technician-A
Mr. Gadhave MV	Technician-A
Mr. Kale AN	Technician-A
Mr. Jagtap SR	Technician-A

Engineering Support Team for MCC, NIV, Pashan Campus

Mr. Khare AB	Technical Officer-'B'
Mr. Bhosale VJ	Technical Assistant
Mr. Gawate CB	Technical Assistant
Mr. Surbhaiya SN	Technician 'C'
Mr. Pote SD	Technician 'C'
Mr. Bathe SD	Technician 'A'
Mr. Ishte VT	Technician 'A'
Mr. Dedunda IR	Technician 'A'
Mr. Ghogare GR	Technician 'A'(Project)
Mr. Mulla AA	Technician 'C'(Project)

ITI Apprentices

Mr. Raut SS	Electrician
Mr. Chavan SP	Electrician
Mr. Saste BD	Electrician
Mr. Pardeshi AS	Electrician
Mr. Shaikh RA	Electrician
Mr. Kudale SS	Electrician
Mr. Thorat OS	R & AC Mechanic
Mr. Misal SK	R & AC Mechanic
Mr. Chaudhri MS	R & AC Mechanic
Mr. Dhamale TS	Plumber

MAINTENANCE DIVISION

The services of the Engineering Services Division along with ITI Apprentice students have been efficiently utilized throughout the year to keep all equipments and installations of the laboratories in good working condition by carrying out routine preventive/breakdown maintenance works. Regular servicing, overhauling and repairs of machines and equipments were undertaken. A total of 905 jobs of different natures like breakdown, installation, civil/plumbing works, have been completed during the Year.

Maintenance at MCC, Pashan works in three shifts for operation and maintenance of various electrical, HVAC, mechanical installations as well as laboratory equipments and to keep the High Containment Laboratory (BSL3) in proper working condition.

Major work carried out during the Year:

1. Preventive and breakdown maintenance of electrical, electronic, civil and plumbing nature.
2. Servicing & Repairing of 20 HP water pump of NIV Pump House and fabrication the new foundation with 'C' Channel departmentally.
3. Termination of 3 ½ core 240 Sq.mm Al. armoured cable of Essential load at Room No. 5 and Change over panel in the Substation departmentally.
4. Installation of Panel board with MCCB & MCB, termination of Mains supply cable, power and lighting wires, after dismantling the very old conventional ICTP and LDB and BUS duct in the Room No-20.
5. Contributed for shifting the NIV laboratory from the Victoria Hospital campus to the RGICD campus. The new building given for us, being part of Nursing College has to be redesigned for electrical wiring to accommodate our laboratory equipments, intercom system, LAN, essential power supply and required partitions.
6. Design for the new laboratory to be constructed at the plot allotted by the Govt. of Karnataka has been done and co-ordinated for the foundation stone laying ceremony and construction of compound wall along with security cabin & visitor's room.
7. Maintenance division of MCC developed in-house 'Water level indicator for OHT with remote acknowledgement alarm for overflow condition'. The remote alarm acknowledgement is required to reset the alarm from remote location, as the OHT is located at an isolated place.
8. Maintenance, MCC imparted a major role in NABL accreditation as per ISO 17025 for conducting trainings, calibration of equipments of the Avian Influenza, Human Influenza and BSL4 laboratories as well as in preparation of quality manual, procedure manual and application.
9. Maintenance staff attended to all major emergencies of the institute such as breakdown of 4" CI water line in the month of July 2014, BSL4 emergency in August 2014 and freezer room at second floor in the month of September 2014 and rectified them in priority.

NIV ADMINISTRATION

LIST OF ADMINISTRATIVE STAFF AS ON 31st MARCH 2015

S.No.	NAME	DESIGNATION
1	Dr. Lakshminarayanan R	Senior Administrative Officer
2	Mr. Sankaradasan T	Administrative Officer (Stores)
3	Mr. Subramanian P	Administrative Officer
4	Mr. Mazire YK	Accounts Officer
5	Mr. Gaikwad AS	Section Officer
6	MS. Ponkshe SN	Section Officer
7	Mr. Nair NGS	Section Officer
8	Mr. Rangan JS	Section Officer
9	Mr. Pandharkar VP	Section Officer
10	Mrs. Shendye VV	Section Officer
11	Mr. Wadke BK	Private Secretary
12	Mrs. Amale RK	Private Secretary
13	Mrs. Shendrikar AV	Technical Officer-'A'
14	Mrs. Ratnaparkhi PK	Assistant
15	Mrs. Deshpande AS	Assistant
16	Mrs. Palshikar AS	Assistant
17	Mr. Pasalkar HS	Assistant
18	Mrs. Bakare AS	Assistant
19	Mrs. Mathai A	Assistant
20	Mrs. Srinivasan S	Assistant
21	Mr. Matkar SE (MCC Budget)	Assistant
22	Mrs. Bapat AA	Assistant
23	Mrs. Ghorpade AG	Assistant
24	Mr. Chavan VC	Assistant
25	Mrs. Pathak SS	Assistant
26	Mrs. Mulay SP	Assistant
27	Mrs. Nair AR	Assistant
28	Mrs. Joshi PS	Assistant
29	Mrs. Moghe RS	Assistant
30	Mrs. Khamkar SH (MCC Budget)	Assistant
31	Mr. Pardeshi AD	Assistant
32	Mr. Kumbhare JR	Personal Assistant
33	Mrs. Jacob S (MCC Budget)	Personal Assistant
34	Ms. Choudhari SI	Personal Assistant
35	Mrs. Bhave SM	Personal Assistant
36	Mrs Swati Bohodkar	Technical Assistant

36	Mrs. Dube SS	Stenographer
38	Mrs. Marathe DD	Upper Division Clerk
39	Mr. Basvaraju R	Upper Division Clerk
40	Mr. Jadhav PN	Upper Division Clerk
41	Mr. Vasam SR	Upper Division Clerk
42	Mrs. Gadre JV	Upper Division Clerk
43	Miss Jyothi JJ	Upper Division Clerk
44	Mrs. Aher PB	Upper Division Clerk
45	Mr. Galange KS	Upper Division Clerk
46	Ms. Shaikh MJA	Lower Division Clerk
47	Mr. Pote YC	Lower Division Clerk
48	Mrs. S Chakole B	Lower Division Clerk
49	Mrs. Yadav TT	Lower Division Clerk
50	Mr. Chabukswar PN	Lower Division Clerk
51	Mrs. Rupnar ML	Lower Division Clerk
52	Mr. Matkar AE	Lower Division Clerk
53	Mr. Raut HD	Lower Division Clerk
54	Mr. Bisht VA	Lower Division Clerk
55	Ms. Pisal DS	Lower Division Clerk
56	Mrs. Gangadharan M	Lower Division Clerk
57	Smt. Kannalu MR	Lower Division Clerk
58	Mr. Chandane BT	Technician 'C'
59	Mr. Jadhav AJ	Technician 'B'
60	Mr. Titkare MD	Multi Tasking Staff
61	Mr. Kakade SM	Multi Tasking Staff
62	Mr. Karanjawane RB	Multi Tasking Staff
63	Mr. Adgale SR	Multi Tasking Staff
64	Mr. Veer GL	Multi Tasking Staff
65	Mrs. Chabukswar JN	Multi Tasking Staff

M.Sc. VIROLOGY

Staff Members

Dr. Lole KS	Scientist 'E' & In-Charge, Academic Cell
Prof. Ghole VS	Coordinator, Academic Cell
Dr. (Mrs) Damle RG	Scientist 'C'
Mr. Bedekar SS	Technical Officer-'A'
Mrs. Jayaram N,	Technical Assistant (Project)

M.Sc. VIROLOGY

M.Sc. Virology programme was started by the National Institute of Virology in June 2005. This is a unique, need-based and flexible graduate course for developing adequately trained human resource fulfilling the needs of academic, industry and health sectors. The programme is affiliated to the Savitribai Phule Pune University through the Institute of Bioinformatics & Biotechnology.

Since its launch in 2005, the M.Sc. Virology course has been progressing very well. Eight batches of students have completed the course successfully until now. In 2014, 21 students passed out and 5 students secured 'O' – Outstanding grade and 5 students secured 'A' – Very Good grade.

Toppers of 2012-14 batch

Rank	Name of the Student
1	Mr. Banerjee Arinjay (Gold medalist)
2	Ms. Manchanda Aayushi

Mr. Banerjee Arinjay carried out his project work under the Guidance of Dr. Vikram Misra, Western College of Veterinary Medicine, University of Saskatchewan, Canada.

The students who have successfully completed the course are placed/employed in reputed industries and institutes in India, Germany, Nepal, Korea, United Kingdom and the United States of America.

Dissertation work of the following M.Sc. students was included in published papers (2014-15)

1. Alagarasu K, Bachal RV, Memane RS, Shah PS, Cecilia D. Polymorphisms in RNA sensing toll like receptor genes and its association with clinical outcomes of dengue virus infection. Immunobiology. 2015 Jan; 220(1):164-8. (IF:3.18)

2. Cecilia D, Kakade M, Alagarasu K, Patil J, Salunke A, Parashar D, Shah PS. Development of a multiplex real-time RT-PCR assay for simultaneous detection of dengue and chikungunya viruses. Arch Virol. 2015 Jan; 160(1):323-7. (IF:2.282)

The details of our course are available at:

1. http://icmr.nic.in/pinstitute/niv.htm

2. http://www.niv.co.in

3. http://www.unipune.ac.in/pgadmissions.com

राजभाषा रिपोर्ट

भारत सरकार की राजभाषा नीती का अनुपालन करने के लिए संस्थान सदैव कार्यरत है। राजभाषा अधिनियम की धारा 3 (3) के अंतर्गत जारी होने वाले सभी दस्तवेज अनिवार्य रुप से द्विभाषी में जारी किए जाते है। केंद्र सरकार, राजभाषा नियम 1976 के नियम 10 (4) के अंतर्गत कर्मचारीयों ने हिन्दी कार्यसाधक ज्ञान प्राप्त कीया है। प्रशासन तथा प्रयोगशाला के कर्मचारी कंप्यूटर पर हिन्दी में कार्य करते है। प्रयोगशाला के रिपोर्टस् हिन्दी में भेजना का प्रयास किया जाता है। प्रशासन विभाग का अधिकतम कार्य हिन्दी में किया जाता है। राजभाषा कार्यान्वयन समिति की बैठक नियमित रुप से निदेशक महोदय की अध्यक्षता में आयोजित की जाती है। नगर राजभाषा कार्यान्वयन समिती की बैठक में संस्थान के अधिकारी उपस्थित रहते है।

संस्थान के हिन्दी सप्ताह में 19 सितंबर 2014 को हिन्दी कार्यशाला का आयोजन किया गया था। कार्यशाला में ''नयी सरकार की सौ दिन की उपलब्धीयॉ : खास तौर पर आरोग्य सेवा का क्षेत्र में...'' विषय पर वाद-विवाद प्रतियोगिता का आयोजन किया गया था। दिनांक 18 और 19 दिसंबर, 2014 को दो दिवसीय रखरखाव विभाग की हिन्दी कार्यशाला का आयोजन किया गया था। कार्यशाला के लिए भारतीय ''आयुर्विज्ञान अनुसंधान परिषद के संस्थानों में रखरखाव विभाग की अहम भूमिका'' विषय तय किया गया था। परिषद के विभिन्न संस्थानों से आए हुए 17 प्रतिभागिओं और संस्थान के 35 कर्मचारियों ने कार्यशाला में भाग लिया था। कार्यशाला के उपलक्ष्य में बनाई गई पुस्तिका में सभी आयोजित कार्यक्रमोंका सार प्रकाशित कीए गए।

डा. आर. लक्ष्मीनारायणन वरिष्ठ प्रशासनिक अधिकारीजींनी एन.आय.व्ही. द्वारा स्थापित की गई ए.आय.एम.एस. तथा एल.आय.एम.एस. संगणक प्रणाली के बारे में पॉवर-पॉईंट द्वारा सभी को अवगत कराया।

प्रतिभागीओं को एन.आय.व्ही. तथा बी.एस.एल.-4 के उपकरण तथा यंत्रसामग्री की जानकारी प्रात्यक्षिक द्वारा अवगत करायी। अत्याधुनिक कार्यप्रणाली के बारे में डॉक्युमेंटरी फिल्म दीखायी गई।

डा. निर्मला मौर्य, निबंधक, राजभाषा दक्षिण भारत हिन्दी प्रचार सभा, चेन्नई का दिनांक 24 दिसंबर, 2014 को बदलते परीपक्ष में हिन्दी और अनुवाद की भूमिका पर व्याख्यान का आयोजन किया गया था।

संस्थान में हिन्दी पखवडा, सप्ताह, कार्यशाला, संगोष्ठी आदी का आयोजन किया जाता है।

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