ANNUAL REPORT
2016 - 17

ICMR - NATIONAL INSTITUTE OF VIROLOGY
PUNE, INDIA

आय सी एम आर – राष्ट्रीय विषाणु विज्ञान संस्थान, पुणे

- ***Published by:***
  Dr. Devendra T. Mourya
- ***Compiled & Edited by :***
  Dr. P. S. Sathe
  Dr. A. B. Sudeep
  Dr. P. Shil

- ***Printed by: Impressions, Pune - 411016***

NATIONAL INSTITUTE OF VIROLOGY, PUNE
(Indian Council of Medical Research)

Annual Report
2016-2017

Contents

From the Director's desk **5**

Abbreviations **7**

Institutional Committees **9**

Performance Indicators **13**

Research Groups

Maximum Containment Facility 19

Human Influenza 31

Avian Influenza 39

Hepatitis 45

Encephalitis 57

Dengue and Chikungunya 63

Enteric viruses 71

Diagnostic Reagents Facility 79

Medical Entomology & Zoology 83

Measles 89

Epidemiology 95

Electron Microscopy & Pathology 103

Bioinformatics and Data Management 107

Animal House Facility 115

Diagnostic Virology 119

Bacteriology 123

Translational Research 125

Field Units

Gorakhpur Unit 131

Bengaluru Unit 141

Kerala Unit 145

Research Support Groups

Library & Information Centre 151

Maintenance 157

M.Sc. Virology

Administration **159**

राजभाषा रिपोर्ट **161**

List of Publications **165**

From Director's Desk

I am pleased to present the annual report of ICMR-National Institute of Virology (NIV), Pune for the year 2016-17. The year has been hectic for the institute due to the scare of Zika virus (ZIKV) as well as the avian influenza (H5N8) outbreak in New Delhi. NIV standardized diagnostics for ZIKV detection and also provided hands-on training to personnel from ICMR institutes, VRDL network and other state laboratories for detection of ZIKV in human samples and mosquitoes. Large numbers of retrospective and prospective (blood and mosquito) samples were screened at NIV, which were tested negative for dengue and Chikungunya to determine the prevalence of ZIKV in India. Extensive laboratory studies were carried out in mosquito vector, Aedes aegypti to determine its replication potential, vector competence and interaction with other arboviruses vectored by the mosquito.

NIV investigated an outbreak that caused unusual mortality among domestic, wild and semi-feral birds in New Delhi during November 2016. Though, suspected initially as H5N1 outbreak, NIV investigation resulted in the confirmation of H5N8 virus as the etiological agent responsible for the outbreak. NIV continued to be active not only in developing diagnostic reagents for highly pathogenic viruses viz., Ebola, Crimean Congo hemorrhagic fever, Kyasanur Forest disease, Nipah, Yellow fever etc. but also strengthened the diagnostic capacity of scientific and technical staff of National laboratories as well as WHO SEAR countries by providing hands-on training.

NIV has always remained in the forefront of ICMR for its contributions to science either in the form of scholarly publications or in the development of technologies. Last year, six technologies were transferred to private companies for production of diagnostic kits against viruses of public health importance in India. During the year, four more ELISA (Anti-CCHF Human IgM and IgG ELISA, Anti-KFD Human IgG ELISA and Anti-Measles IgM) and three PCR based technologies (KFD real time RT-PCR, KFD nested RT-PCR and duplex real time PCR for dengue and Chikungunya) have been standardized & evaluated for performance. The newly developed ELISA kits showed stability from 12 to 18 months at 2-8sC. Despite the hectic activities, basic research has shown an upward trend as evidenced by the number of high quality publications (n=79) in journals of repute with an average impact factor of >3.0.

Outbreak investigations and diagnosis of referred samples of vector-borne and other viral diseases of public health importance remained the mainstay of NIV's contribution to the Nation. NIV Scientists, with the support of its three field units located at Gorakhpur, Bangalore and Alappuzha, have investigated and successfully identified the etiological agents and helped timely management of outbreaks. The number of samples referred to NIV has also shown an upward trend; 37,000 referred samples were tested and given diagnosis in time to save the precious lives

NVBDCP the nodal agency that monitors vector borne diseases in India along with other National laboratories depend solely on NIV for the diagnostic kits viz., Dengue, Chikungunya and Japanese encephalitis due to consistently high Quality of kits. During the reporting period, NIV has matched the demand and supplied approx. 10,000 kits to different laboratories in India and WHO SEAR countries.

NIV is also involved in human resource development by organizing workshops and trainings to health professionals in India and Southeast Asian countries. During the year NIV provided hands on training to health care professionals of ICMR and other national laboratories in the diagnosis of ZIKV, Yellow fever virus etc. The RCVDL group (Apex laboratory for VRDL) of NIV provides month long hands-on training in diverse areas viz., serology, cell culture, molecular biology, epidemiology etc. routinely to scientists and staff of viral diagnostic network laboratories across the country to strengthen diagnostic capabilities. Training to work on highly pathogenic viruses in containment facilities have been provided to scientific and technical staff in India and Southeast Asian countries. In addition, NIV scientists provided support to WHO-SEAR countries in establishing diagnostic capabilities.

MSc Virology course conducted at NIV remains a coveted course of the Pune University due to its uniqueness and remains a challenge to the prospective students. Every year, 20-25 students pass out with M.Sc. (Virology) degree after acquiring enough experience in virological techniques to work independently in any International laboratory. NIV relentlessly believes in scientific progress of the country and helps researchers from other educational institutes by providing free access to its core facilities like electron microscopy, bioinformatics, animal house, library etc.

The three field units of NIV are well established with all the infra structure and provide diagnostic support to their home states as well as neighboring states. Bengaluru Field unit has played a commendable role in polio eradication in India. It now acts as a sentinel laboratory for diagnosis of measles, mumps and rubella viruses under the WHO program. The Gorakhpur and Kerala units are also active in providing diagnostic support to AES infections and viral outbreaks in the respective states.

As the Director of NIV, I congratulate my fellow scientists for their sincere efforts to make NIV a flagship institute of ICMR. It is indeed incomplete if I do not appreciate the administrative, technical and engineering staff for their relentless support extended during the year. Last but not the least; I extend my sincere gratitude to Dr. Soumya Swaminathan, Secretary, DHR & Director General, ICMR and the administrative staff of ICMR & DHR for their whole hearted support and cooperation.

(Dr. D. T. Mourya)

Abbreviations

AES	:	Acute encephalopathy syndrome
BSL-4	:	Biosafety Laboratory Level 4
CCHF	:	Crimean Congo Hemorrhagic Fever
CHPV	:	Chandipura virus
CHIKV	:	Chikungunya virus
DENV	:	Dengue virus
DST	:	Department of Science and Technology, Government of India
ELISA	:	Enzyme Linked Immunosorbent Assay
HCV	:	Hepatitis C virus
HRV	:	Human rotavirus
KFD	:	Kyasanur Forest Disease
miRNA	:	mitochondrial RNA
NiV	:	Nipah virus
NoV	:	Noroviruses
RNA	:	Ribonucleic acid
RT-PCR	:	Real Time RT-PCR
SEARO	:	South East Asia Region of WHO
SERB	:	Science and Engineering Research Board, Dept of Science and Technology, Govt of India
VRDL	:	Virological Research and Diagnostics Laboratory
WHO	:	World Health Organization
ZIKV	:	Zika virus

Institutional Committees

Scientific Advisory Committee

Dr. S.P. Thyagarajan Professor of Eminence & Dean (Research), Sri Ramachandra University Porur, Chennai - 600 116, Tamil Nadu	**Chairman**
Dr. A. P. Dash Vice Chancellor, Central University of Tamil Nadu Thiruvarur - 610 101, India	**Member**
Dr. Randeep Guleria Professor, Department of Medicine, All India Institute of Medical Sciences, Ansari Nagar, NEW DELHI - 110 029	**Member**
Dr. Shekhar Mande Director, National Centre for Cell Science, NCCS Complex, SP Pune University Campus, Ganeshkhind, PUNE - 411 007.	**Member**
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, Delhi-110054	**Member**
Dr. S. Mehendale Director, National Institute of Epidemiology, R-127, 3rd Avenue, Tamil Nadu Housing Board, Ayapakkam, Chennai-600077.	**Member**
Dr. S.Venkatesh Director, National Centre for Disease Control,Directorate General of Health Services, 22, Sham Nath Marg, New Delhi-110 054.	**Member**
Dr. R.K.Singh Director, Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, UP	**Member**
Dr. Mohan Jadhav Director, Health Services, Directorate of Health Services, 8th Floor, Arogya Bhavan, St.George's Hospital Compound, P.D'Mello Road, Mumbai-400001	**Member**
Dr. V.S. Padbidri D-18, Gita Society, Synagogue Street, PUNE 411 001.	**Member**
Dr. Praveen Malik Director, Chaudhary Charan Singh,National Institute of Animal Health, Department of Animal Husbandry, Dairying and Fisheries, Min of Agriculture and Farmers Welfare, Government of India, SH 57, Baghpat,Uttar Pradesh-250609	**Member**
Dr. Rashmi Arora, Scientist G & Head ECD, ICMR New Delhi	**ICMR Representative**
Dr. Nivedita Gupta, Scientist E, Division of ECD, ICMR, New Delhi	**ICMR Representative**

Human Ethics Committee

Name & Designation	Role
Dr. Sheila Bhave Consultant in Pediatric Research, Department of Pediatrics, KEM Hospital Research Centre, Pune - 411011.	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. Shashikala Sangle, M.D	Clinician
Dr. Aarathi Nagarkar, Ph.D.	Social Scientist
Dr. Vikram Padbidri, Consultant, (Microbiology & Infection Control), Department Of Microbiology & Infection Control	Basic Medical Scientist
Mr. T Krishnamurty, B.Com., LLB.	Legal Expert
Mr. Malsidha B. Shelke, B.A., MSW	Person From Community
Dr. Anuradha Tripathy, Ph.D	Biological Scientist
Dr. Yogesh Gurav, MD	Medical Scientist

Animal Ethics Committee

Name & Designation	Role
Dr. Mangesh Shamrao Kamble C-901, Aarohi Project, Sr. No. 123, Susgoan, Tal. Mulshi, Dist PUNE - 411 021 drmangesh.kamble@gmail.com	Main Nominee
Dr. Balasaheb Siraskar Principal, SVNHT's College of B. Pharmacy, Rahuri Factory, Pin: 413706, Dist. Ahmednagar svnhtcop@gmail.com	Link Nominee
Dr. Ramanamurthy Boppana Scientist F & In charge, Animal House, National Centre for Cell Science, PUNE-411 007 raman@nccs.res.in	Scientist from outside the Institute
Shri. R P. Kulkarni B-5, Building C, Anjira Sankul Vidyanagar, Karad-Masur Road, Karad Tal., Dist. SATARA – 415124	Socially Aware Nominee
Dr. Jayati Mullick, Scientist E & Group Leader Avian Influenza Group, National Institute of Virology, 130/1, Sus Road, Pashan, PUNE - 411 021 jayati_mullick@hotmail.com	Chairperson (Biological Scientist)
Dr. Dilip Rewa Patil Scientist In Charge, Animal House Facility, NIV. dipupatil@yahoo.com	Member Secretary
Dr. P.S. Shah Scientist E & Group Leader, Diagnostic Reagent Facility, National Institute of Virology, PUNE - 411001. paresh17@hotmail.com	Scientist from biological discipline
Dr. Sreelekshmy Mohandas Scientist B, Translational Research Group, National Institute of Virology, Microbial Containment Complex, 130/1, Sus Road, Pashan, PUNE - 411 021 sreelekshmy88@gmail.com	Veterinarian
Dr. Virendra Kumar Meena Scientist B, Electron Microscopy Group, National Institute of Virology, 20-A, Dr Ambedkar Road, Post Box 11, PUNE - 411001 viren27091988@gmail.com	Scientist from biological discipline

Institutional Biosafety Committee

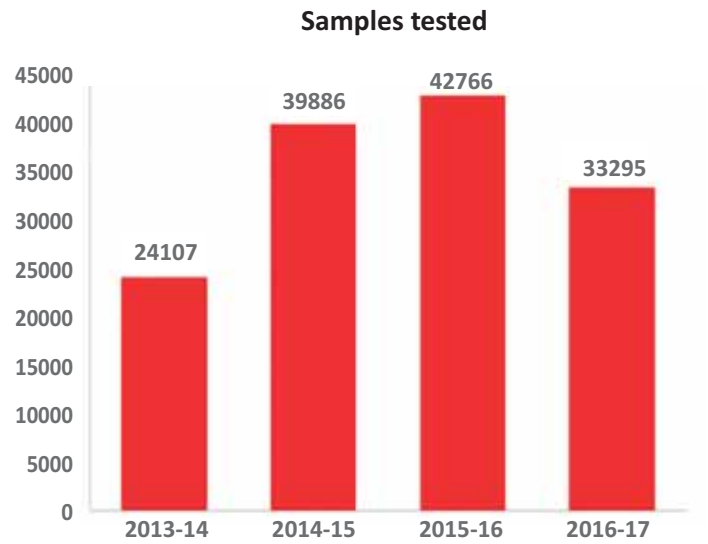
Name & Designation	Role
Dr. D.T. Mourya, Director, National Institute Of Virology, 20-A, Dr.Ambedkar Road, Pune-411001.	Chairman
Dr. Kavita Lole, Scientist 'E', Group Leader, Hepatitis Division, National Institute of Virology, Microbial Containment Complex, 130/1, Sus Road, Pashan, Pune-411021.	Member Secretary
Dr. Arvind Sahu, Scientist 'G', National Centre For Cell Science, SP Pune University, Ganeshkhind, Pune-411007.	[DBT Nominee]
Dr. V. Ghole, Retd. 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, Bengalore-560029.	Veterinary Faculty Expert, Member
Dr. Yogesh Gurav, Scientist'D' National Institute of Virology, Microbial Containment Complex, 130/1, Sus Road, Pashan, Pune-411021.	Medical Faculty Expert, Member
Dr. Tejeswini Deshmukh, Scientist 'B', National Institute of Virology, Microbial Containment Complex, 130/1, Sus Road, Pashan, Pune-411021.	Molecular Biology expert, Member

Performance Indicators

A. Public Health Contributions

Total number of samples tested	33295
Total number of Diagnostic Kits supplied	9145
Patents filed	02
Publications	79

B. Samples Tested Over The Years



C. Publications Over The Years

D. Academics

M.Sc Virology

NIV conducts M.Sc. Virology under the IBB Program of the Savitribai Phule Pune University. Every year 22 students are admitted and NIV provides Hostel facilities, 24 hrs WiFi and library facilities.

Ph.D. Program

NIV is affiliated to Savitribai Phule Pune University.

Total number of recognized guides	18
Number of students pursuing Ph.D	22
Number of students awarded Ph.D	07

E . List of ICMR Awardees

Dr. B.V. Tandale
Basanti Devi Amir Chand Prize (2014)
Indian Council of Medical research, New Delhi

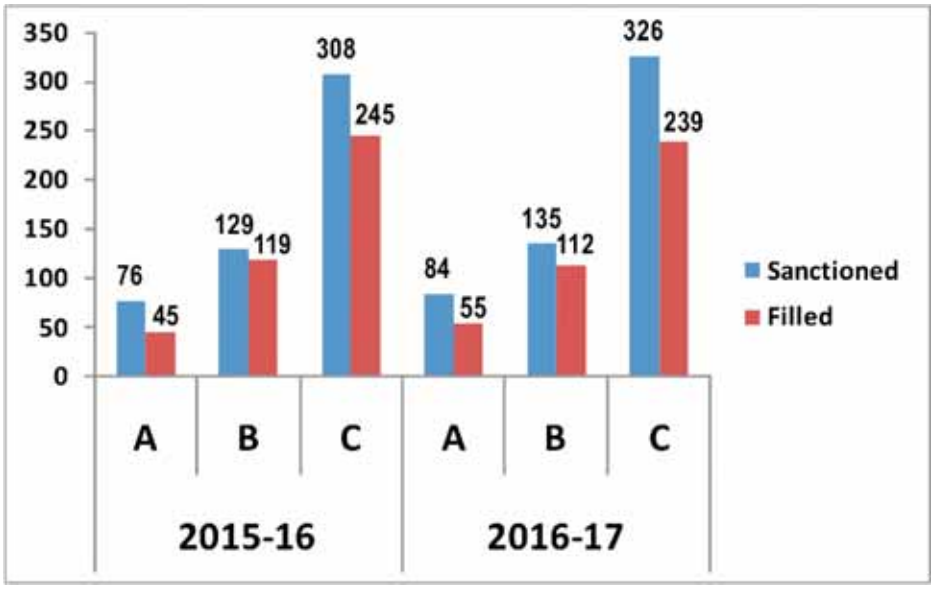
Dr. G. Sapkal
G. K. Kamat Oration appreciation award for the year 2017
by Fergusson College, Pune (8th February 2017).

F. List of distinguished visitors

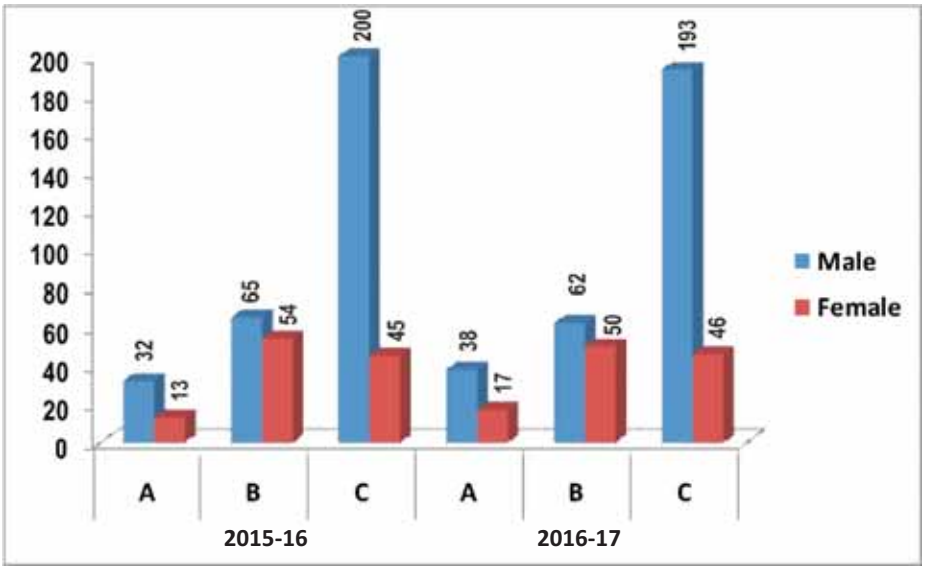
- Dr. Soumya Swaminathan, The Secretary, Department of Health Research, MoHFW, Govt. of India & Director General, Indian Council of Medical Research, V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi
- Dr. GS Grover, Scientist G (Retd.) Head, Safety Management, NCL, Pune
- Dr. Stuart Nichol, Viral Special Pathogens Branch, Centers for Disease Control & Prevention, Atlanta, Georgia 30333, USA
- Dr. John Klena, Centers for Disease Control & Prevention, Atlanta, Georgia 30333, USA

STAFF DETAILS

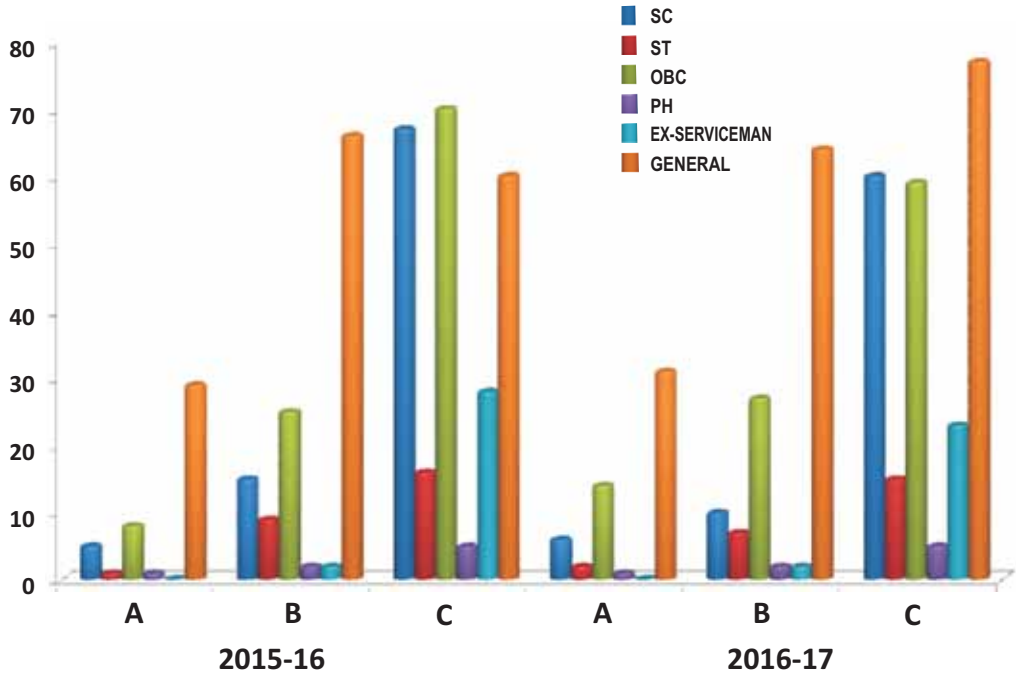
(i) Groupwise Staff Strength



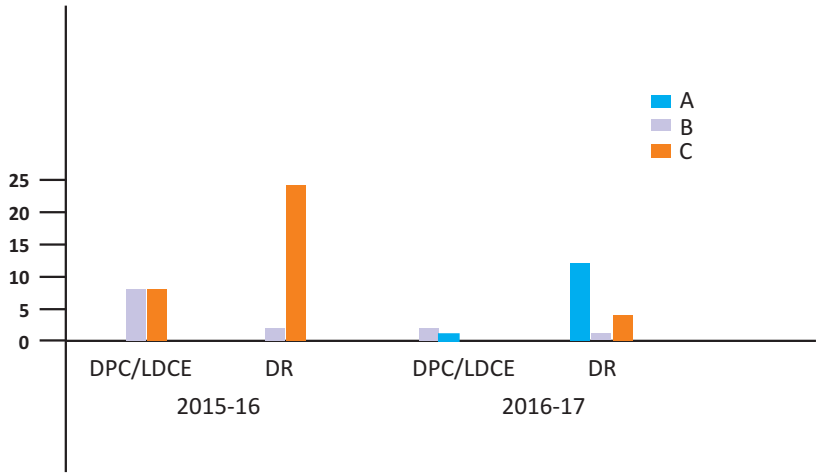
(ii) Groupwise Gender Distribution



(iii) Groupwise category distribution

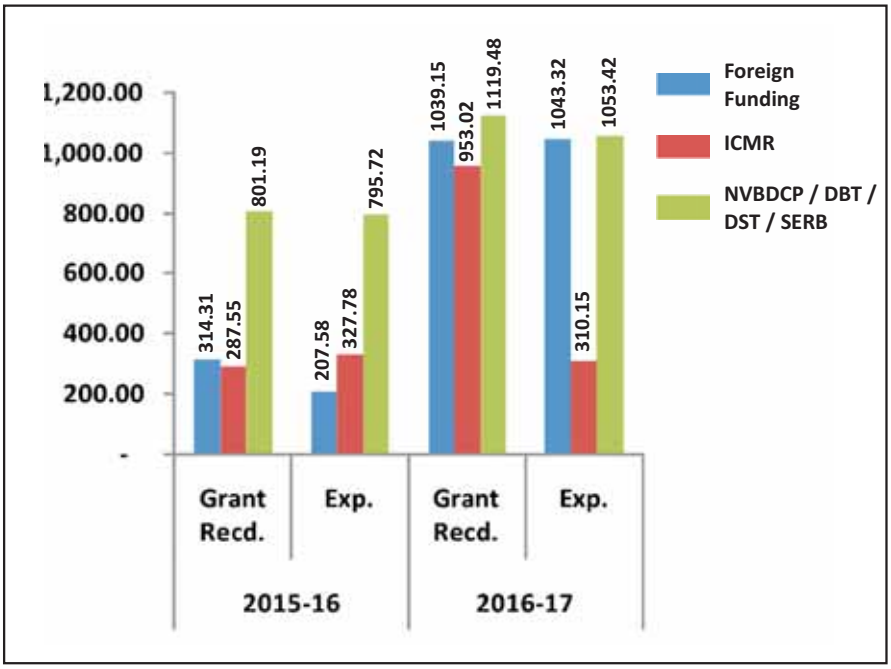


(iv) Groupwise categories filled

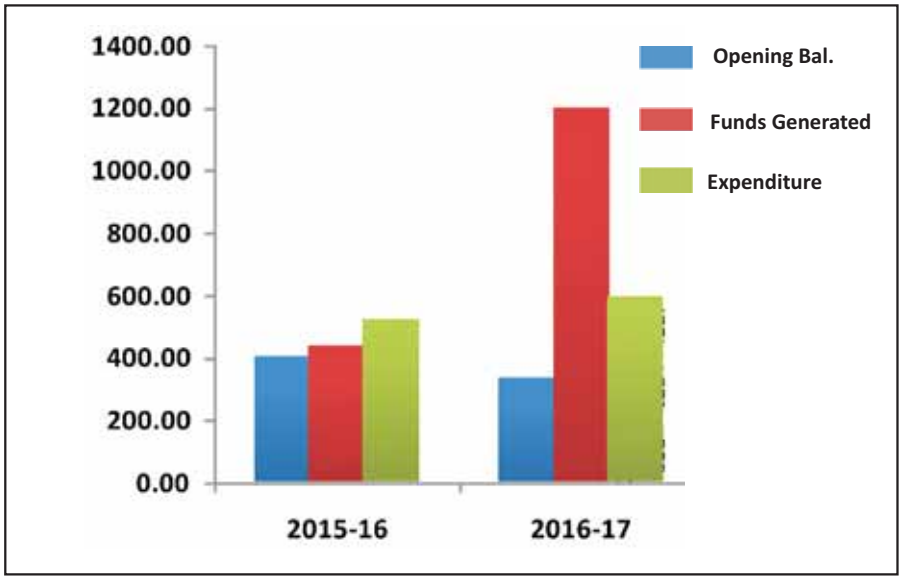


FINANCIAL INDICATORS

(I) Funds received

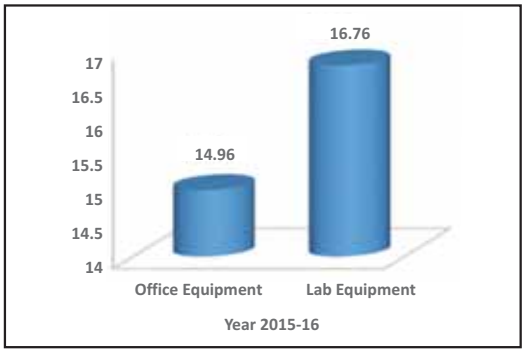


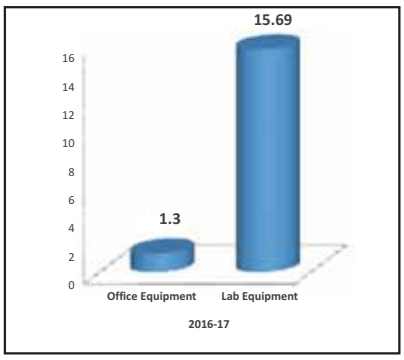
(ii) Funds generated



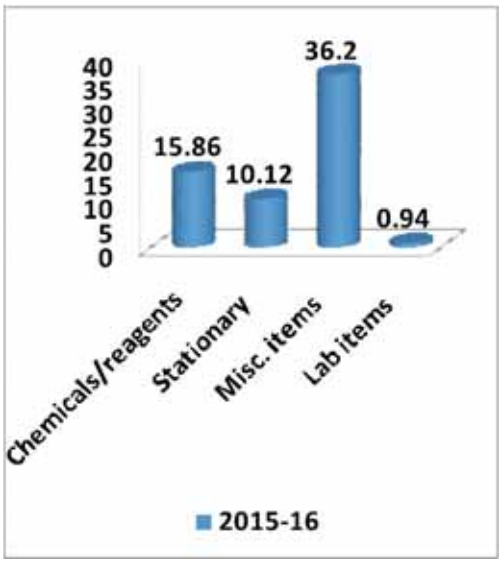
FINANCIAL INDICATORS

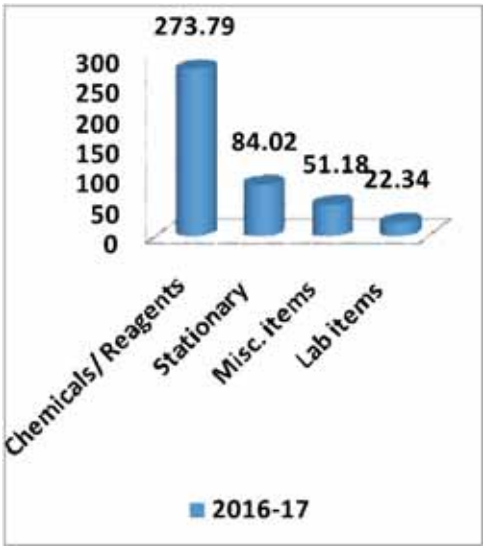
Details of equipment procured for different groups (Rupees in lakhs)





Details of material procured (Amount in Lakhs)





Maximum Containment Facility Group

Scientific staff

Dr. Yadav PD	Scientist E (yadavpd@icmr.org.in)
Dr. Sahay Rima	Scientist B (dr.rima.sahay@gmail.com)

Technical Staff

Mr. Upadhyay C	Technical Officer (A)
Dr. Jain R	Technical Officer (A)
Mr. Lakra R	Technical Assistant
Mr. Sarkale P	Technical Assistant
Mrs. Majumdar T	Technical Assistant
Mrs. Bhave S	Personal Assistant
Mrs. Patil S	Technical Assistant
Mr. Shende U	Technician-'B'
Mr. Thorat S	Laboratory Attendant (2)
Mr. Gopale S	Technician-'A'
Mr. Aacharya M	Technician-'A'
Mr. Chopade G	Laboratory Attendant (2)
Mr. Holepppanavar M	Laboratory Attendant (2)

Project Staff

Dr. Shete A	Scientist 'C'
Dr. Patil D	Scientist 'B'
Dr. Chaubal G	Scientist 'B'
Dr. Kumar V	Scientist 'B'
Dr. Pardeshi P	Scientist 'B'
Ms. Pailwan M	Section Officer
Mr. Kore P	Technician-C
Mrs. Melag S	Technician-C
Ms. Chopde Y	Technician-C
Mr. Bagmare K	Technician-C
Mr. KadamM	Project Technician III
Mr. Pacharne N	DEO -CDC Project
Mr. Gondane Y	Technical Officer-A (Engineering)
Mr. Patil P	Technical Officer-A (Engineering)

MCL 1503: Study of KFD viremia and antibody kinetics in patients and disease progression in monkey model

Investigators: Yadav PD, Patil D, Shete A, Viswanathan R, Gurav YK & Mourya DT

Funding agency: Intramural [2016-2018]

Due to the spread of KFD in newer regions of Karnataka, Kerala, Tamil Nadu & Maharashtra, it became imperative to develop a tool for early detection & diagnosis of KFD infection. Multiple aspects of KFD disease progression like, persistence of viremia, anti-KFD IgM, IgG antibodies and biphasic fever is still not clearly understood. An exploratory study was initially planned in Karnataka during the KFD season However, due to KFD outbreak of Sindhudurg district, Maharashtra state during 2016; Sindhudurg district was selected as a new study site and accordingly IHEC approval was obtained for collection of samples from KFD patients at different time points to monitor the viremia and presence of anti-KFD antibodies).

Work done: Out of 152 KFD positive cases (either by real time RT-PCR or anti-KFD IgM ELISA); 70 cases were identified for this study for further follow up for 1 year (January 2016 to February 2017) and beyond. So far, 316 samples from 70 patients have been processed for detection of KFDV RNA, anti-KFD IgM and IgG antibodies. Data is being analyzed.

MCL 1602: Vector Competence Study of Zika virus in Indian strain of *Aedes aegypti* mosquitoes

Investigators: Mourya DT, Gokhale MD, Yadav PD, Sapkal GN & Majumdar T

Funding agency: Intramural [2016-2018]

It has been documented that Zika virus (ZIKV) is transmitted to humans by bite of different mosquitoes *viz. A. africanus, A. luteocephalus, A. furcifer,* and *A. taylori. A. aegypti* and to a lesser extent *A. albopictus.* All these mosquito have been linked with nearly all known Zika virus outbreaks. However, there is no information available on the susceptibility of Indian strain of *A. aegypti* to Zika virus. Considering, the risk of introduction of ZIKV in India through *A. aegypti* mosquitoes, the present study was carried out to determine the susceptibility of *A. aegypti* to ZIKV with co-infection by DENV and CHIKV. Mosquitoes were experimentally infected with ZIKV and super-infected with either DENV or CHIKV. Replication of these viruses in mosquitoes was confirmed by real time RT-PCR and Immunofluorescence assay (IFA).

Work done: Indian strain of *A. aegypti* was found to be susceptible to Zika virus. Infection and super-infection was determined by studying individual head squashes and pooled mosquito bodies by IFA and real time RT-PCR. Infection of mosquitoes with CHIKV followed by ZIKV showed positivity for both viruses; primary infection of ZIKV followed by DENV showed dual positivity, however only ZIKV RNA could be detected from pooled mosquito bodies. Primary infection with DENV followed by ZIKV showed positivity for both viruses. Primary infection with ZIKV followed by CHIKV also showed positivity for both the viruses. Transmission of the Zika virus to suckling mice was confirmed by detection of ZIKV by real time RT PCR. Further study is in progress.

MCL 1308: Nationwide serosurvey of Crimean Congo hemorrhagic fever virus (CCHFV) in domestic animals and Epidemiology, risk factors and sero-prevalence of CCHF infection among humans in rural population in Gujarat (NIV-ICAR- Gujarat).

Investigators: Yadav PD, Gurav Y, Sathe P, Sharma G (IVRI, Mukteswar), Raval D (Commissionerate of Health, Gujarat), K Upadhyay (B J Medical College, Ahmadabad)

Funding agency: Extramural (ICMR)[2015-2017]

Objective 1: To study the epidemiology, risk factors and seroprevalence of Crimean-Congo Hemorrhagic Fever (CCHF) infection among humans in rural population in Gujarat

To control the spread of CCHF, it is important to understand the epidemiological profile of the disease, its spread and the high-risk population and risk behavior.

Seroepidemiological survey was planned in all 33 districts of Gujarat state. A total of 4635 human samples were received from 33 districts of Gujarat State. Samples were categorized as Group A: Subject from CCHF affected house/care taker of CCHF case), Group B: Neighborhood contact, C: Animal handler, D: General population, E: Farmer, F: Abattoir house worker, G: Veterinary worker, H: Health care worker)

An indigenous anti-CCHF Human IgG ELISA was developed by BSL-4 laboratory. A total of 2932 samples from 14 districts of Gujarat state were screened till March 2017.

To understand the persistence of IgG in CCHF positive cases from the year 2013 (8 cases from Amreli), 2015 (2 cases from Amreli and 1 case from Morbi), 2016 (1 case from Amreli) were collected as category A. All the 12 human samples showed detectable levels of anti-CCHF IgG antibodies.

Presence of IgG in 8 cases of Amreli (2013) suggests persistence of anti-CCHF human IgG antibodies for approximately 3 years.In addition two samples from Rajkot (Category B) and one each from Morbi (Category A) and Panchmahal (Category F) also showed presence of anti-CCHF IgG. Positivity in abattoir workers indicates that they are specifically prone to zoonotic infections than other individuals.

Objective 2: Screening of livestock samples for CCHFV specific IgG antibodies

Data on presence of CCHF virus specific IgG antibody in serum samples of livestock (Cattle, Buffalo, Sheep & Goat) screened from 22 states and 1 Union territory of India has been already reported in the earlier annual report of 2015-16.

Subsequently, 208 cattle samples were received from Gujarat (118), Bhopal (20), Himachal Pradesh (12), Madhya Pradesh (10), Odisha (10), Rajasthan (10), Uttarakhand (18) and West Bengal (10).

Work done: Cattle samples from Gujarat (8/11) and Bhopal (14/20) showed detectable level of anti-CCHF IgG antibodies. Furthermore, sheep and goat samples from Bhopal (10/20) and Gujarat (2/5) showed detectable levels of anti-CCHFV IgG antibodies. These results indicate possible exposure of livestock to CCHF through infected ticks.

MCL 1407: Establishment of facility for production of standard virus positive controls for diagnostic PCRs and RT-PCRs tests for the important public health viral diseases

Investigators: Yadav PD, Sapkal GN, Sudeep AB

Funding agency: Extramural Project (DHR) [2016-2019]

The objective of this project is to prepare and supply inactivated prototype strain of virus stable at 2-8sC as positive controls for molecular diagnosis to the Virus Diagnostic laboratories (VDL) from different parts of the country.

Protocol for preparation of positive control for different viruses was standardized. The Dose & period of gamma radiation for inactivation of different viruses has already been standardized.

The virus stock (positive control, Quantity: 100 ml) and respective cell control (negative control, Quantity: 50 ml) were prepared at Diagnostic Virology group. Both the stocks were gamma irradiated for 6hr (25kGy) in liquid conditions at BSL-4 laboratory.

Complete inactivation was confirmed by two blind passages in susceptible cell lines. After confirmation of inactivation, the positive control and negative control stock was aliquoted and lyophilized at BSL-4 laboratory and inventory of the stock was maintained.

Positive controls for Chikungunya, Japanese encephalitis, West Nile and Zika viruses were

prepared. Preparation of virus controls for Rubella, Measles and Mumps are in process.

MCL 1303: Testing and rectification of different components of the BSL-4 facility & preparation of basic documents

Investigators: Mourya DT & Yadav PD

Funding agency: Intramural [2015-2017]

Re-validation of BSL-4 laboratory: The BSL-4 laboratory was shut down from 16th August – 30th September 2016 for preventive maintenance, calibration & validation. All the activities were planned and mock drills were performed. During the shutdown period, preventive maintenance of bio-safety doors, inlet and exhaust plenums, biosafety cabinets, autoclaves, BLED Plant and tissue digester etc. was done. The certified laboratory calibrated these.The experts committee (Dr. AD Desai, Dr. DT Mourya, Dr. PD Yadav, Dr. J Mullick and Er. AB Khare) examined all the documents for calibration and validation.

MCL 1304 Preparation of reagent for highly infectious diseases

Investigators:Yadav PD & Shete A

Funding agency: Intramural [2013-2016]

1. **Preparation of reagents for diagnosis of Yellow fever**

   Considering, yellow fever outbreak in Angola, Democratic Republic of the Congo and Uganda in 2016, NIV, Pune took initiative to develop reagents for diagnosis of yellow fever.

   Work done: Yellow fever virus (17D) was grown Vero CCL-81 cells. Virus stock and cell control were gamma irradiated at 25kGY. Inactivation was confirmed by blind passaging in Vero CCL-81 cells. Gamma irradiated virus and control stocks were concentrated, filtered and lyophilized for further use. Real time RT-PCR and RT-PCR assays was standardized targeting NS3 and envelope region of Yellow fever viral genome.

   NIV is WHO Collaborating Center for arboviruses and hemorrhagic fever reference and research. To deal with the emergency preparedness and/or response to Yellow fever (on request from World Health Organization (WHO), India), Yellow Fever real time RT-PCR was provided to SEAR countries. Six VRDL laboratories and NCDC (Delhi) staff were also trained and reagents were provided.

2. **Preparation of reagents for diagnosis of Zika virus:**

   Zika a Flavivirus is closely related to dengue. Cases of Zika have previously been reported from Africa, southern Asia and the Pacific Islands. In 2015 and 2016, Zika virus outbreaks were reported throughout the tropical and sub-tropical areas of the western hemisphere (Mexico and Puerto Rico).

Work done:

***In vitro* propagation of Zika virus:**

The ZIKV strain (MR-766 strain, GenBank ID DQ859059) was obtained from European Union and European Virus Archive goes Global (EVAg). Stock virus was prepared in Vero *CCL-81* and *Vero E6* cells infected at a multiplicity of infection (MOI) of 0.01. ZIKV was titrated in Vero *CCL-81* using standard protocol. $TCID_{50}$ titer of Zika Virus in Vero CCL81 is $10^{5.6}$/ml

***In vivo* propagation of Zika virus**

Zika virus stock was prepared by intracerebral inoculation of infant CD1 mice. Infected mouse brain suspension was prepared in phosphate buffers containing 1.25% bovine serum albumin. To raise hyper immune serum, mouse brain suspension was used.

Development of PCR assays for Zika virus

With the view of preparedness to deal with the disease, RT-PCR & real time RT-PCR was standardized targeting "envelope and NS5" region of Zika virus genome. The sensitivity of the assay was determined by serial dilution of clonal plasmid of Zika virus (Strain: MR766). The assay could detect up to 61 copies of Zika specific plasmid DNA in case of RT-PCR. The developed reagents were sent for internal & external validation. The results of validations were concurrent with results obtained at BSL4 laboratory.

A total of 523 KFD negative samples and 1286 CCHF negative samples (retro-spective samples) were screened for Zika virus by real time RT-PCR and all samples were found to be negative.

Supply of reagents for Zika diagnosis

Zika virus reagents of indigenously developed real time RT–PCR/RT-PCR were provided to various WHO-SEAR countries, VRDL network laboratories across the country and NCDC, New Delhi.

3. Development of indigenous single step RT-PCR for detection of KFD virus

A primer set (Sequence) designed from NS5 region of KFDV considering P9605 strain as template showed very high sensitivity & specificity for KFDV (no cross reactivity with Zika, Dengue, Chikungunya, CCHF, Nipah, Yellow fever, Japanese encephalitis and West Nile virus). No false positive results were seen for 104 samples (human, monkey and tick positive and negative samples) that were tested using this assay. Comparison of sensitivity of the assay with real time RT-PCR and nested RT-PCR assays, the current single step RT PCR assay could detect up to 100 copies of viral RNA as compared to real time RT-PCR (10 copies) and nested RT-PCR (10^4 copies). Thus the assay is more sensitive than the nested RT-PCR but less sensitive than real time RT-PCR (Table. 1).

Table 1:Diagnostic sensitivity of KFD single step RT-PCR as compared to other molecular methods.

KFDV RNA Copies	Diagnostic Sensitivity of single step RT-PCR	Ct Value (By real time RT-PCR)	Diagnostic Sensitivity of Nested step RT-PCR
10^6 copies	Positive	17.12	Positive
10^5 copies	Positive	21.51	Positive
10^4 copies	Positive	25.4	Positive
10^3 copies	Positive	29.46	Not detected
10^2 copies	Positive	34	Not detected
10 copies	Not detected	37.3	Not detected
1 copy	Not detected	No Ct	Not detected

4. Development of real time RT-PCR for detection of Nipah virus

Work done: Nipah virus from autopsied brain tissue of a case from the Nadia 2007 outbreak (GenBank accession number: FJ513078) was used in this study. A highly specific N gene based indigenous Nipah real time RT-PCR that could detect up to 4 copies of in-vitro transcribed RNA was reported in the earlier annual report.

The developed reagents were sent for internal & external validation to the institutes of national repute. Results of the Internal and external validation were concurrent with the results obtained at BSL4 laboratory.

5. Translation of technologies

Work done: BSL-4 laboratory has standardized three more ELISA (CCHF Human IgM ELISA, CCHF Human IgG ELISA & KFD Human IgG ELISA) and two PCR based assays (KFD Real Time RT-PCR and KFD nested RT-PCR).

Stability of ELISA assays (KFD human IgM, CCHF Human IgM, KFD human IgG, CCHF cattle IgG, CCHF Sheep -goat IgG, and CCHF Human IgG) was monitored for 18 months at 4 degrees. Stability of CCHF Human IgG & KFD Human IgG ELISA kit at 4sC was completed for 12 months (Figure 1).

6. Transfer of technologies to Industry:
Three ELISA technologies were transferred to Zydus Cadila (KFD Human IgM ELISA, CCHF Sheep/Goat IgG ELISA and CCHF Cattle IgG ELISA) under the translation program of DHR /ICMR. The test protocol for KFD IgM ELISA has been approved by DCGI, Government of India.

7. **NABL accreditation:**

Investigators: Yadav PD & Shete A

Three laboratories at NIV; viz., Human Influenza, Avian Influenza and Maximum Containment Laboratory (BSL-4) have fulfilled the requirements of lab accreditation policies and procedures of ISO/IEC 17025:2005. The accredited laboratories have shown technical competence for reliable testing for viral detection as per the International Standards. The scope & services of the Maximum Containment Laboratory (BSL-4) included in NABL accreditation:

1. Anti-CCHF Human IgM & IgG ELISA: By NIV kit and Commercial kit
2. Crimean Congo hemorrhagic fever (CCHF) Real time RT-PCR: detection of CCHF Viral RNA from human, animal and ticks
3. Kyasanur Forest Disease (KFD) Real time

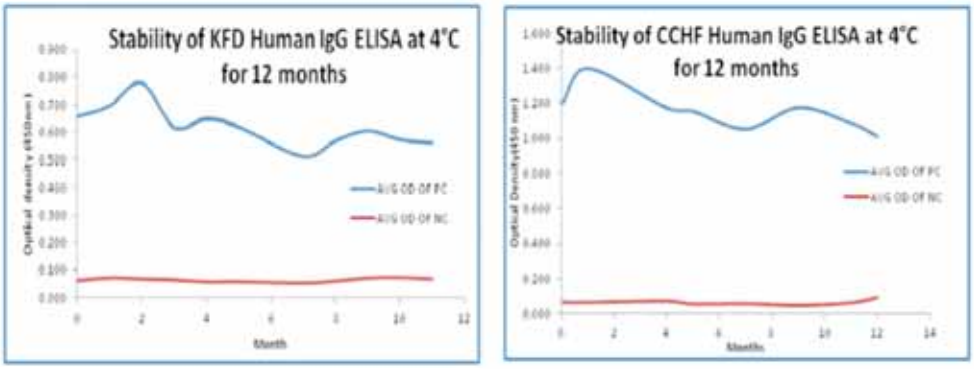


Figure 1: Stability of KFD Human IgG ELISA and CCHF Human IgG ELISA kits

RT-PCR: detection of KFD Viral RNA from human, animal and ticks

4. Anti-KFD IgM ELISA: detection of IgM antibody in Human sample

MCL 1301: Provide diagnostic for referred samples for viral hemorrhagic fever & unknown etiology and outbreak investigation.

Investigators: Yadav PD, Shete A, Patil D, Jain R, Patil S & Majumdar T

Funding agency: Intramural [2013-2017]

1. **Occupational exposure of cashew nut workers to Kyasanur Forest disease at Sattari taluka, Goa**

Work done: During April–June 2016, blood samples of 76 human cases with fever, body ache and myalgia were referred from District Surveillance Unit, Belgaum district, Karnataka State to National Institute of Virology, Pune, India for laboratory diagnosis. All the cases referred from Belgaum had travel history to Keri village, Sattari taluk of Goa State in connection with cashew nut harvesting.

Cases were screened using Real time RT-PCR or IgM ELISA. KFD was confirmed in 13 out of 76 cases by Real time RT-PCR or IgM ELISA. No case fatality was recorded. Envelope gene sequence of positive human samples from Belgaum showed highest identity with sequences of KFD human case and monkey from Goa (>99.98,>99.99%) (Figure 2). KFD positivity was also recorded among human and monkeys from Sattari taluk, Goa during the same period. The finding proves that these cases might have been infected during Cashew nut harvesting from KFD affected Keri village, Sattaritaluk, Goa. This is the first report of KFD due to occupational exposure.

Our findings of KFD positivity in monkeys and human cases from Sattari taluk corroborate with KFD positivity among human cases from Belgaum, Karnataka.

2. **Screening of suspected specimens for Kyasanur Forest Disease virus**

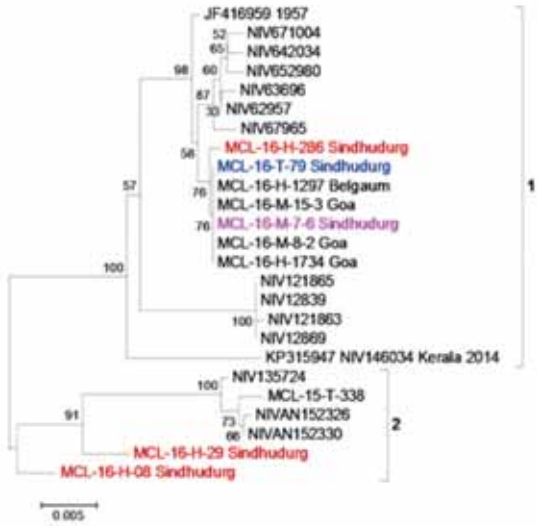


Figure 2: Phylogenetic tree of envelope gene of KFD cases from Belgaum, Karnataka with other representative KFD strains from India

Work done: A total of 394 clinical specimens of suspected KFD cases were referred from Karnataka (263), Goa (7), Gujarat (1), Jammu & Kashmir (49), Jharkhand (2), Madhya Pradesh (22), Meghalaya (2), Telangana (2), Nagaland (1), Uttar Pradesh (10) and Odisha (34) were tested for KFD virus. 54/394 cases were positive by Real Time RT-PCR, and IgM ELISA.

In continuation with KFD diagnostic support; Serum samples of 259 suspected KFD cases of 33 villages of Dodamarg taluk, Sindhudurg district, Maharashtra were referred to NIV, Pune for screening of KFD from local health authorities during April 2016- March 2017. All human samples were tested for KFD using either Real time RT-PCR or IgM ELISA. Forty-eight cases (10 different villages) were positive for KFD either by Real time RT-PCR or IgM ELISA. The results were communicated to the concerned health authorities for necessary action.

Further, necropsy samples of 25 monkeys were received from Goa (12), Karnataka (10) and Maharashtra (1) and tested. A total of 9 monkeys were found to be positive from Goa (6), Karnataka (2) and Maharashtra (1) by KFDV Real

Time RT-PCR

In addition, 337 tick pools were referred from Karnataka (256), Kerala (55), Maharashtra (29) and Goa (1). Only one tick pool from Maharashtra was positive for KFDV by Real Time RT-PCR.

3. **Diagnosis on sample of suspected Yellow fever case, a resident of Liberia**

   Clinical sample of suspected Yellow fever case was referred from Integrated Disease Surveillance Project unit, Kerala State for laboratory confirmation. The patient was resident of Liberia and travelled to India with halt at Doha (UAE) during transit.

   The sample was negative for CCHF, Ebola, Marburg, and Ebola Zaire by Real time RT-PCR as well as for Lassa fever and Yellow fever using RT-PCR. The sample did not show cytopathic effect in Vero cells. The result was communicated to the concerned health authorities.

4. **Screening of suspected specimens for Crimean Congo Hemorrhagic fever (CCHF)**

   Clinical samples of 202 suspected cases of CCHF were referred from different districts of Gujarat (67), Jammu & Kashmir (50), Kerala (1), Madhya Pradesh (22), Maharashtra (3), Meghalaya (2), Orissa (34), Telangana (5), Jharkhand (2), Nagaland (1) and Uttar Pradesh (10) states.

   Eleven cases from Gujarat were positive either by CCHF Real Time RT-PCR or IgM ELISA

   Furthermore, none of the 84 tick pools referred by Gujarat State was positive for CCHF real time RT-PCR.

5. **Screening of suspected specimens for Hanta virus**

   Clinical specimens of 10 suspected VHF cases were referred from Maharashtra (7), Gujarat (1), Telangana (1) and Karnataka (1) states were tested for Hanta virus using Nested RT-PCR and IgM ELISA. All samples were negative for Hanta virus

6. **Complete genome sequencing of KFD viruses**

   Kyasanur Forest disease virus (KFDV) was first recognized in 1957 in the Kyasanur Forest of Shimoga District, Karnataka State, India. However it is a major public health problem in Karnataka, Kerala, Maharashtra and Goa. In-spite of vaccination programs, large numbers of cases were positive for KFDV and a spillover of KFD cases has been noted. Phylogeography studies for KFDV are important to genetically characterize the recently circulating KFD viruses and understand the dispersal pattern of the virus within Karnataka and to newer geographical regions.

   KFDV positive-sense RNA genome is approximately 11 kb in length and encodes a single 3416 amino acid polyprotein that is post-translationally cleaved into a total of 3 structural (C, M and E) and 7 nonstructural (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) proteins.

   Total 28 KFD Isolates obtained from human samples, tick samples and monkey were amplified and sequenced. The objective of this study is to determine the complete genome sequence and it genetic variability and relationship with other tick borne flaviviruses.

   Full genome sequencing of 8 isolates has been done and sequencing of 20 isolates is in process. Overall the study is important for the characterizing and identifying KFDV phylogeographic distribution across the country.

   Percentage nucleotide identity (PNI) and percentage amino acid identity (PAI) between the sequences analyzed. Within mean group distance between Indian isolate for amino acids is 0.007with S.E=0.001 and that of isolates near Saudi Arabia is 0.005 with S.E=0.001. Similarly mean group distance between Indian isolate for nucleotide is 0.019 with S.E=0.001 and that

of isolates near Saudi Arabia is 0.007 with S.E=0. It could be inferred that the KFD isolates from India have genetic difference with respect to the Alkhurma hemorrhagic fever virus (AHFV) and altogether forms a different lineage. It could also be observed that there is more variation in the percentage nucleotide identity within Indian subgroup than the AHFV.(Figure 3)

7. Immunization program for the staff during the year 2016-2017

As part of the routine immunization program, 22 laboratory personnel were immunized during 2016-2017 against KFDV and Hepatitis B virus.Pre-samples (before vaccination) were taken from all the vaccines.

BSL- 4 facility is designated as Polio Essential Facility (for containment of Poliovirus) by Ministry of Health and Indian Council of Medical Research, New Delhi under Global Polio Eradication and End game Strategy. Hence, it becomes mandatory to vaccinate all the staff in BSL-4 facility with polio vaccine as per the Global Action Plan-III guidelines. A total of 59 staff members of NIV, Pune were

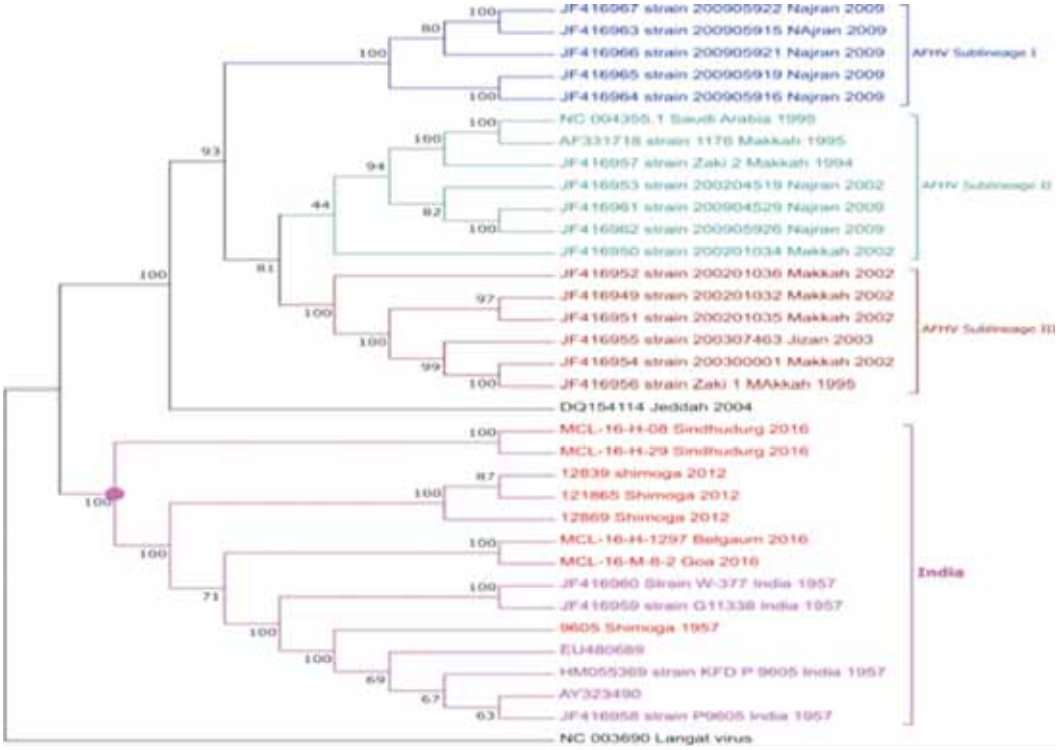


Figure 3: Phylogeny based on neighbor-joining analysis of AHFV and KFDV CDS sequences. phylogenetic tree is created using a bootstrap of 1000 replication as the test for phylogeny. Langat virus is an out-group added to the tree. Indian strains chosen for the study are marked in red color

administered Polio vaccine (OPV) and (IPV) in April and May 2016 respectively. Pre and post vaccination blood were sent to EVRC, Mumbai.

All vaccines had detectable levels of anti-polio antibodies, even before the current vaccination. The individuals with low titer against all three serotypes were vaccinated

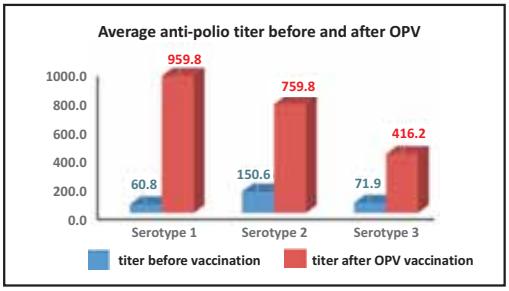


Figure 4 : Anti-polio antibodies detected post vaccination

using OPV vaccine. A titer of = 8 was considered as protective. (Figure 4)

MCL 1404: Multi-site epidemiological and virological survey of Nipah virus: Special emphasis on Northeast Region of India

Investigators: Yadav PD, Gokhale MD, Biswas D, Khan S (RMRC, Dibrugarh)

Funding: Extramural (ICMR) [2015-2017]

Objective 1:

Survey of Nipah virus (NiV) in bat and human populations in Northeast region of India

Work done/result: During the year, 388 clinical specimens from encephalitis cases, referred by RMRC, Dibrugarh, were screened by real time RT-PCR / NiV virus specific IgM. Out of all tested, 3 samples were positive for Nipah viral RNA & virus specific IgM antibodies.

Additionally, 1733 pig serum samples received from Mizoram were tested for NiV viral RNA but none of the samples was positive.

Conclusion: Proactive surveillance is necessaryin the northeast region as Nipah and Tioman, a new virus (Family: Paramyxoviridae) are active in the region.

Objective 2:

Development of Nipah IgM/ IgG ELISA using recombinant antigen for screening human, pigs and bat samples

Work done: Nipah N gene was cloned in pET28a vector and transformed in BL21 DE3 cells. The protein specifically reacted with the Nipah positive sera (IgM or IgG). The recombinant protein retained its activity even when stored at 4°C with 20% glycerol for 45 days. Recombinant Nipah N protein was tested using anti-Nipah IgG positive pig, bat and human sera as well as respective known negative sera. The results correlated well with the CDC (USA) ELISA. The recombinant protein is being concentrated & purified. Further work is in progress.

MCL 1504: Enhancing Biorisk mitigation awareness in public health community and creating laboratory networks for enhanced diagnostic capabilities to deal with surveillance and outbreaks of Viral Hemorrhagic Fever and Respiratory Illness diseases

Investigators: Mourya DT, Yadav PD, Sapkal GN, Mullick J

Funding: Extramural (GHSA, CDC, USA) [2015-2020]

There is a need for enhancing laboratory capabilities & preparedness to deal with outbreaks. The proposal has five different modules of teaching and training programs for management, scientific, technical, hospital health care worker and laboratory engineering personnel. NIV, Pune would function as the apex-training center.

Objective 1: Increasing awareness about Biorisk management and engineering controls for safe

operating of biomedical laboratories and control of infection in the public health settings

Work done: On-site trainings/meetings were organized at various establishments in the country. More than 300 participants were trained during the activity (Refer performance indicators – Human Resource Development).

To strengthen the VHF network laboratories, CDC Trioplex rRT-PCR kit (500 Rxn) were supplied for screening suspected VHF cases. The validation report of CDC Trioplex rRT-PCR was satisfactory and so the centers were allowed to use CDC Trioplex rRT-PCR kits.

Verification panel provided by CDC, USA was supplied to all 7 VHF Laboratories & 10 VDLs as a part of Quality Assurance Program. Results have been submitted to International Trioplex Verification Panel box for scoring. (eocevent191@cdc.gov)

Samples of suspected VHF cases (n=1542) were screened for Dengue, Chikungunya and Zika virus at VHF network laboratories during April 2016-March 2017. Nearly 22% of samples (n=335) were positive for dengue and 14% samples were positive for Chikungunya (n=212) whereas none was positive for Zika.

The Dengue, Chikungunya & Zika negative samples referred from network laboratories were also negative for CCHF and KFD at NIV, the Apex laboratory. In addition, clinical specimens from suspected VHF cases referred by different states were tested for different viral agents (Figure 5a, b). Figure 6 represents total samples tested by BSL-4 Laboratory

NIV Pune is a WHO Collaborating Center for arboviruses and hemorrhagic fever reference laboratory, as a part of PTP program, WHO had sent a PTP panel for the detection of Arboviruses (Dengue, Chikungunya, and Zika viruses) and yellow fever virus by Real time PCR. The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) coordinated PTP. NIV Pune participated in the PTP program and reports were found to be satisfactory.

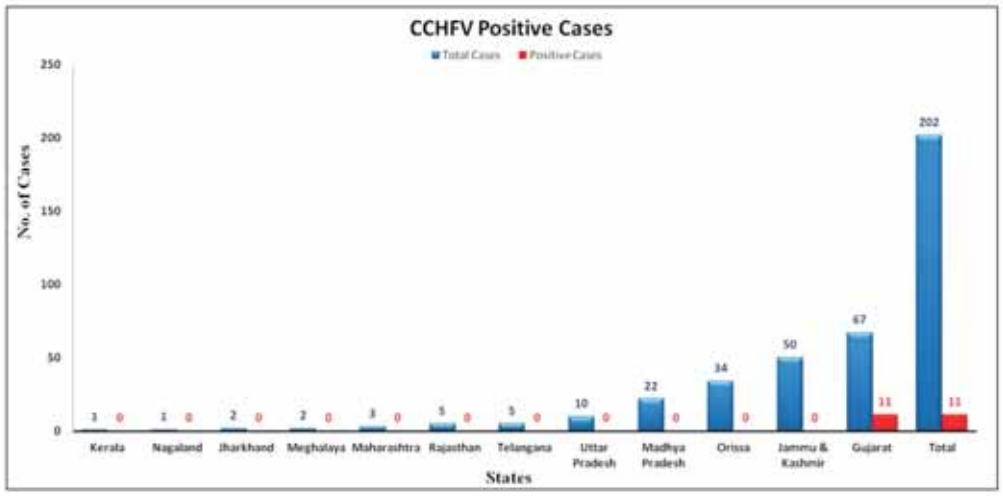


Figure 5a: State-wise details of CCHF Positive Cases

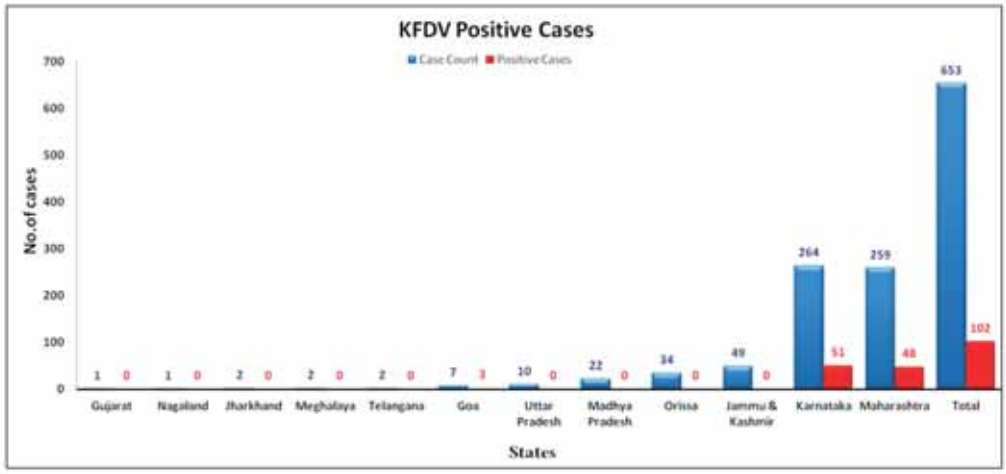


Figure 5b: State-wise details of KFD Positive Cases

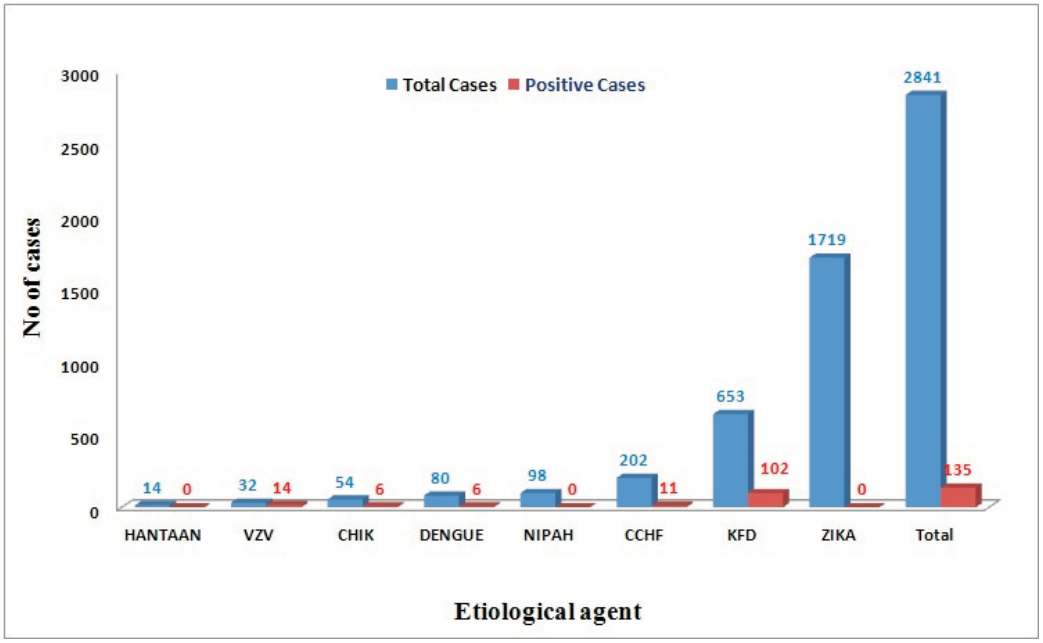


Figure 6: Total samples tested at Maximum Containment Laboratory

Influenza Group

Scientific Staff

Dr. MS Chadha	Scientist 'G' & Group leader
Dr. VA Potdar	Scientist ‘C’
Dr. ML Choudhary	Scientist 'C'
Dr. SD Bhardwaj	Scientist ‘B’

Technical Staff

Mrs. SM Karambalekar	Technical Officer (A)
Mrs. VC Vipat	Technical Assistant
Mrs. SY Jadhav	Technician ‘C’
Mr. HK Kengale	Technician ‘C’
Ms. A Vaidyanathan	Technician ‘C’
Mr. AS Awhale	Technician ‘B’
Mr. VN Autade	Technician ‘B’

Project Staff

Mr. DD Hinge	Technical Assistant (Since Nov 2016)
Mrs. TS Rale	Technical Assistant (Since Feb 2017)

INF 1601: Diagnostic Services/Outbreak Investigation

Investigators: Chadha MS, Potdar VA & Choudhary ML

Funding Agency: Intramural [2015-2020]

Clinical samples referred by different clinics/ hospitals across Maharashtra for laboratory diagnosis of influenza A(H1N1)pdm09

Objective: To provide laboratory diagnosis for suspected H1N1 cases.

Work done: Screening of 790 clinical samples by Real time RT-PCR showed that 13% were positive (105/790) for A(H1N1)pdm09, 3% (24/790) for A(H3N2) and 1% of samples were positive (9/709) for influenza B virus (Figure 1). Influenza A(H1N1)pdm09 positivity for March was 32%. Isolation attempts in MDCK cell line yielded 48 isolates ((H1N1)pdm09: 33; H3N2: 4; Influenza B: 11)). HA gene analysis showed that 2016-17 isolates were grouped in clades 6B and 6B.1. Clade 6B was similar to A/California/07/2009 vaccine components with P83S, S185T and E374K amino acid change whereas clade 6B.1 was found similar to A/Michigan/45/2015 which is northern hemisphere 2017 vaccine components with S64N, S162N and I216T signature amino acid change (Figure 2). Phylogenetic analysis of HA gene of 11 influenza B isolates from 2016 showed that nine isolates belonged to Victoria V1A clade and was similar to B/Brisbane/60/2008, 2016-17 vaccine components.

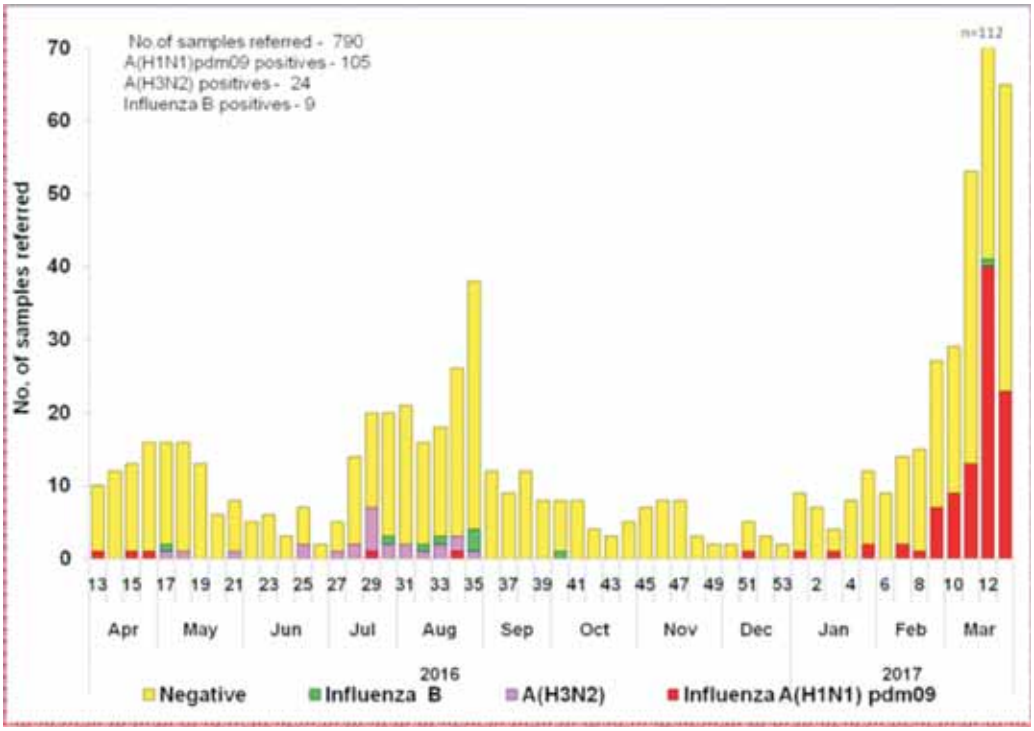


Figure 1 : Influenza activity in referred clinical samples

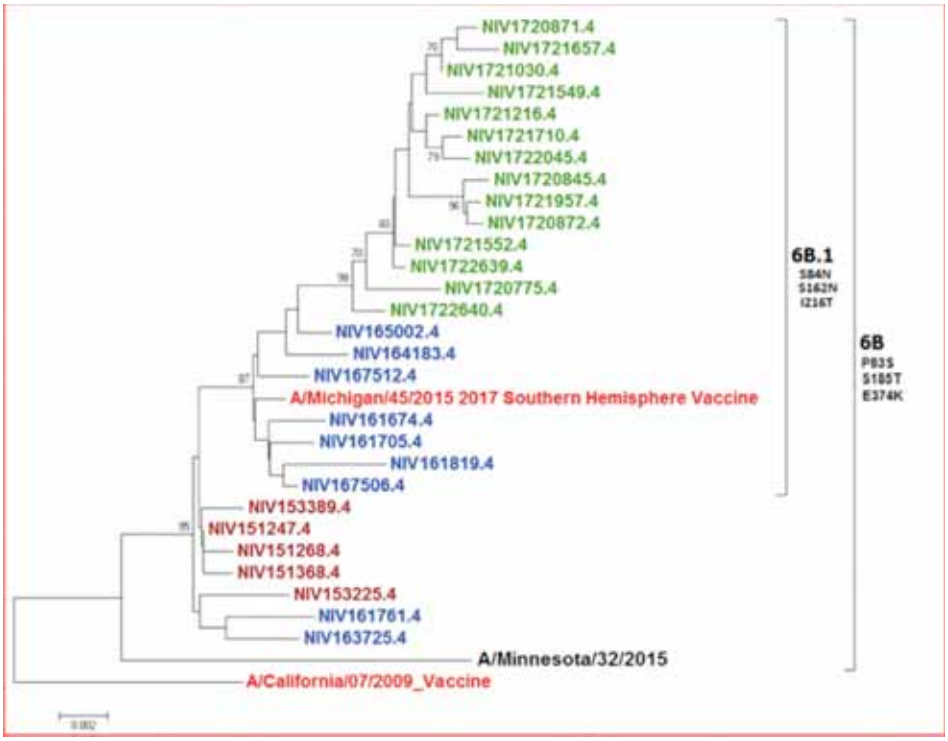


Figure 2 : Phylogenetic analysis of influenza A/H1N1pdm09 HA gene of 2016-17 (n=23)

INF 1201: Association of respiratory viruses with pneumonia among hospitalized children below the age of 5 years.

Investigators: Chadha MS, Potdar VA & Choudhary ML

Funding Agency: Intramural [Duration: 2014-2017]

Severe Acute Respiratory Infection (SARI) is a major cause of morbidity and death among children. Pneumonia, a form of acute respiratory infection of lungs, is the single largest cause of death among children worldwide. Respiratory viral infections (RVIs) contribute to approx. 50% of community-acquired pneumonia (CAP) in children, 90% of infant bronchiolitis seeking medical attention as well as to exacerbations of 90% of asthma in children.

Objectives: To analyze association of various respiratory viruses with pneumonia among hospitalized children (I 5 yrs.), in India.

Work done (2014-17): Nasal/throat swab samples from 1012 patients (643 male and 369 female) patients were tested by real time RT-PCR for Influenza A(H1N1)pdm09, A/H3N2, Influenza B, respiratory syncytial virus (RSV) A&B, meta-pneumovirus, parainfluenza virus (PIV) 1-4, rhinovirus and adenoviruses . RSV was detected in rainy season and winter months while rhinovirus was detected throughout the study period (Figure 3). RSV was the most predominant among the viruses detected (39.5%) followed by Rhinovirus (13.8%), PIV (4.6%), Influenza (4.2%), Adenovirus (4%) and HMPV (1.5%).

INF 1501: Creating laboratory network for enhancing diagnostic capabilities for surveillance, outbreaks and epidemic

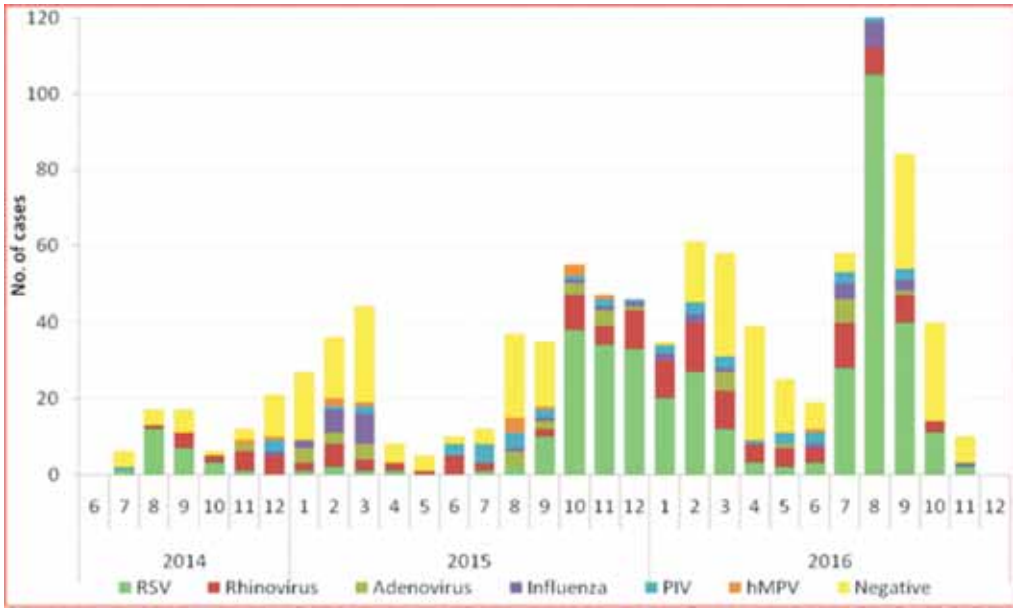


Fig 3: Week wise distribution of positivity for different viruses in patient serum

investigations of high-risk group of viral pathogens causing respiratory infections

Investigators: MS Chadha, VA Potdar & ML Choudhary

Funding Agency: GHSA-CDC [2015-2020]

Emerging and reemerging respiratory tract infections and the increasing antimicrobial resistance pose a challenge for diagnostic and control strategies worldwide. Several new viral respiratory track infectious agents mainly of zoonotic origin with high epidemic potential have emerged in the recent decades.It has thus become necessary to enhance the diagnostic capacity to identify pathogens early to take necessary control measures.

Objectives: Epidemiological and virological surveillance network for influenza and non-influenza respiratory viruses, viz., RSV, hMPV, PIV 1-4, adenoviruses, rhinoviruses etc. in India.

Work done: As a regional site, 320 clinical throat/nasal swabs collected from acute respiratory infections (ARI) and 479 from SARI patients were screened for different respiratory viruses by duplex real time PCR. Results indicated 21.3% (170/799) were positive for different respiratory viruses (Influenza A(H1N1)pdm09 (8.8%), PIV (5.5%), rhinovirus (2.6%), hMPV (1.8%), influenza B (1.2%), adenovirus (0.8%) and RSV (0.5%) (Figure 4).

INF1602: Antiviral Susceptibility profiling for influenza viruses in India

Investigators: VA Potdar & MS Chadha

Funding Agency: Extramural (DHR) [2016-19]

Monitoring influenza viruses for antiviral susceptibility is important for clinical management of patients. Unexpected emergence and global spread of oseltamivir resistant seasonal A(H1N1)pdm09 viruses were seen in late 2007. Indiscriminate use of

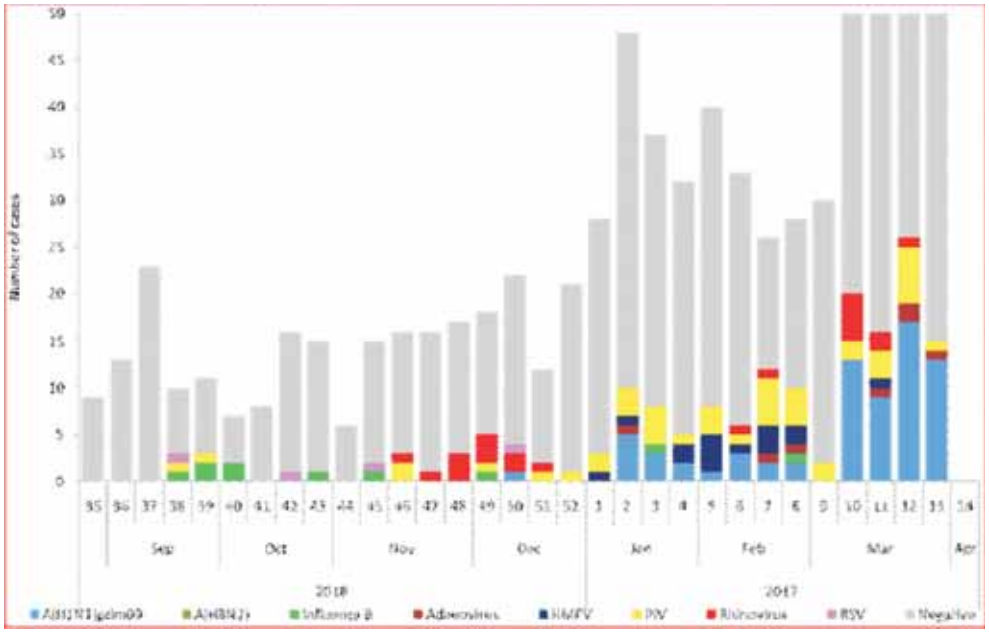


Figure 4: Weekly distribution of respiratory viruses (Sep 2016-Mar 2017) (ARI+SARI combined)

Neuraminidase Inhibitors (NAIs) to control pandemic influenza may create increasing selective pressure and help the emergence and spread of drug-resistant influenza viruses

Objectives: To identify known and compensatory mutations in influenza isolates to determine the sensitivity of neuraminidase inhibitor drugs.

Work done: During the reporting period, 61 A(H1N1)pdm09 clinical samples and 26 solates (Pune and Chennai) were assessed byallelic discrimination real time PCR for H 274Y mutation and all were sensitive. Using 70 isolates of A/H3 (2013-2016) & 62 A(H1N1)pdm09 (2014-2016) from Maharashtra, Jammu & Kashmir and Madhya Pradesh baseline IC 50 values for NAI drug (oseltamivir carboxylate) were generated by phenotypic assay. The normal IC 50 range for A(H1N1)pdm09 was 0.01 – 35.6 and 0.1–11.3 nM for A(H3N2). All H3 viruses were sensitive while two A(H1N1)pdm09 viruses of 2015 were resistant to NAI drug with reduced IC 50 values 209 and 163nM respectively.

INF1603: WHO pilot study on RSV surveillance based on the Global Influenza Surveillance and Response System

Investigators: Chadha MS, Potdar VA, Choudhary ML & GuravYK

Funding Agency: Extramural (WHO) [2016-2019]

RSV is a leading cause of acute lower respiratory tract infection in children. RSV surveillance will provide epidemiological and virological information as well as the disease burden to support RSV vaccine development. The WHO Global Influenza Program (GIP) is establishing RSV surveillance based on the influenza surveillance platform, the WHO Global Influenza Surveillance and Response System (GISRS).

Objectives:

- To establish the feasibility of RSV surveillance built on the GISRS platform

- To develop laboratory capacity for RSV diagnosis
- To determine age and risk groups for severe RSV disease and RSV seasonality
- To assess the feasibility of FluNet and FluID for reporting RSV data

Work done: Under this project, 799 clinical samples were collected from OPD as well as hospitalized cases of all age groups and screened for RSV by real time PCR. RSV was detected in 4 (0.5%) samples and the data has been uploaded on FluNet.

INF1604: Tracking community mortality due to respiratory syncytial virus in collaboration with University of Colorado and MAHAN Melghat

Investigators: Chadha MS & Potdar. VA

Funding Agency: Extramural (Bill & Melinda Gates Foundation) [2016-2019]

RSV is a major cause of morbidity and mortality in children in developing countries. There is insufficient data from India on RSV associated mortality in infants and children below the age of

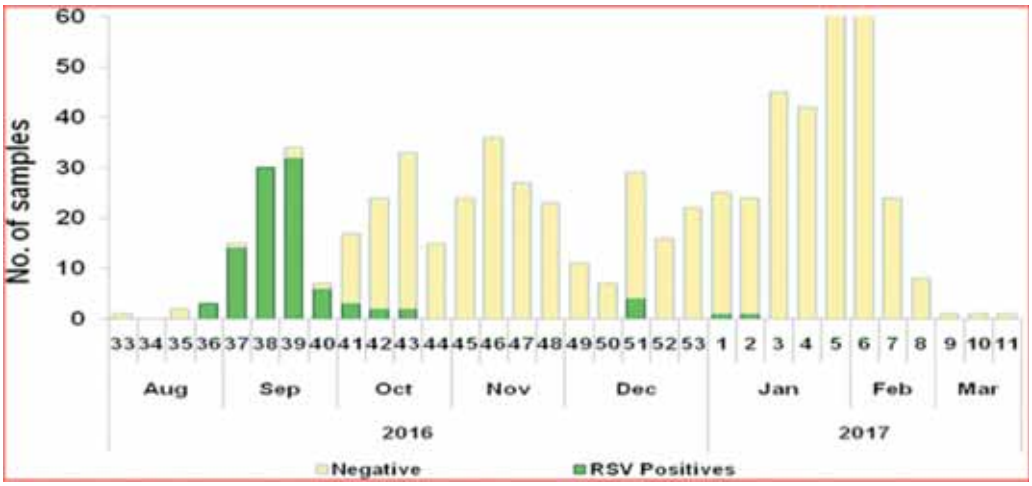


Figure 5: RSV positivity in blood samples collected from Melghat area

Samples tested for different respiratory viruses during 2016 – 2017 :

Virus	No. of samples tested	No. of samples positive
Influenza A/H1N1pdm09	2701	238
Influenza A/H3N2	2701	24
Influenza B	2701	19
RSV	1911	299
Rhinovirus	1911	144
Adenovirus	1911	24
Parainfluenza	1911	105
HMPV	1911	19

Avian Influenza Group

Scientific Staff

Dr. Jayati Mullick	Scientist-E & Group Leader
Dr. Shailesh D. Pawar	Scientist-D

Technical Staff

Mr. Aniruddha V. Jamgaonkar	Technical Officer (B) (Till February 2017)
Mrs. Sadhana S. Kode	Technical Officer (A)
Mr. Sachin S. Keng	Technical Assistant
Mr. Shrihari K. Waghmare	Technician C
Mr. Dinesh K Singh	Technician C
Mr. Javvaji P N Babu	Technician A

Project Staff

Mr. Milind M Thube	SRF (CSIR)
Ms. Rewati Kasbe	SRF (UGC)
Ms. Deeksha S Tare	JRF

AVI 1001: Avian Influenza outbreak investigation and diagnostic services

Investigators: Mullick J &Pawar SD

Funding Agency: Intramural [Ongoing (Service Project)]

(i) Investigation of highly pathogenic avian influenza (AI) H5N8 outbreak in wild and migratory birds in New Delhi.

Unusual mortality in common geese, pelicans, painted storks, spot-billed ducks and crows (domestic, wild and semi-feral birds) were reported in New Delhi during November, 2016.

Objectives: Investigation of bird mortality in New Delhi

Work done: Tracheal swabs and cloacal swabs from dead birds, fecal droppings and water specimens from different locations in New Delhi were negative for AI-H5N1 virus by real time RT PCR. However, specimens collected from Sanjay Zeel, Shaktisthal and bird droppings from Hauz Khas Lake, Deer Park were positive for AI-H5N8 virus. Partial HA and NA gene sequencing and BLAST analysis suggested similarity to the HA and NA genes of AI A/duck/Eastern China/S1109/2014(H5N8) virus. The receptor specificity and antiviral susceptibility studies showed that the virus is specific for avian species and susceptible to oseltamivir carboxylate. Sequence analysis of the whole genome of three isolates revealed >99% nucleotide identity with the AI-H5N8 virus in HA, NA and NS1 gene segments, >98% identity with AI-H3N8 virus in the PB2, PA and M gene segments and >98% identity with AI-H4N6 and AI-H6N2 viruses in PB1 and NP gene segments, respectively.

(ii) Investigation of highly pathogenic AI viruses in bird population in Mumbai

Background and Work done: Initial investigation of outbreak of the HPAI-H5N1 virus in birds in Ahmedabad, Gujarat in January 2017 revealed that the birds were procured from the Crawford market, Mumbai. Fifty samples (16 Tracheal, 16 cloacal, 02 bird droppings and 16 water specimens sourced from bird cages) were collected from eight avian species viz., Guinea fowls, turkeys, domestic ducks, manila ducks, quails, fowls, blue rock pigeons and parrots.

Results: All the specimens were negative for AI-H5N1 virus by Real-time RT-PCR. However, low pathogenic AI-H9N2 & Newcastle-disease virus was isolated from nine specimens.

(iii) Potential of avian and mammalian erythrocytes for concentration of avian influenza viruses from environmental specimens

Use of chicken erythrocytes for virus concentration from water has recently been reported.

Objectives: Comparison of avian and mammalian erythrocytes with the novel virus precipitation method developed at NIV for concentration of AI viruses from water

Work done: Erythrocytes from chicken, turkey, goose, guinea pig and horse were used for concentration and evaluation of AI-H9N2 virus by real-time RT-PCR and 50% egg infectious dose (EID_{50}) method and compared with the novel virus precipitation method developed at NIV.

Results: Virus precipitation method for AI H9N2 virus was superior to turkey and chicken erythrocytes. The yield in turkey and chicken erythrocytes was $10^{2.34}$/0.2ml, and $10^{1.58}$/0.2 ml. respectively, whereas the yield in the precipitation method was $10^{3.10}$ EID_{50}/0.2ml. The minimum virus detection limit by erythrocytes and virus precipitation method was $10^{2.5}$ EID_{50}/ml and $10^{1.25}$ EID_{50}/ml, respectively.

AVI 1301: Survey of avian influenza in wild birds during the winter migratory season in Maharashtra

Investigators: Pawar SD, Pande S & Mullick J

Funding agency: Intramural [2013-2016]

In view of the recurring outbreaks of avian influenza (AI) viruses in India, AI virus surveillance was conducted at sites, which are known for the arrival of wild and migratory birds. Attempts were made for isolation of virus.

Objective: Avian Influenza surveillance in Pune and Satara districts

Work done: Fecal droppings, water samples and environmental specimens were collected (n=278) from seven locations in Pune *viz.* Bhigvan, Jejury, Kumbhargaon, Dhumalwadi, Pimpri and Warje and one location (Lonand) in Satara District. The specimens belonged to 23 avian species from 13 families of wild and migratory birds. Two hundred specimens were processed for virus isolation in *in ovo* system.

Results: All the Specimens tested negative for AI viruses. 78 specimens are being processed.

AVI 1302: Operation and maintenance of High Containment Laboratory.

Investigators: Mullick J, Pawar SD & Khare AB

Funding Agency: Intramural [Ongoing (Service project]

Biosafety Level 3 (BSL3) laboratory is a containment facility that provides safe environment to the working personnel and community while handling highly infectious pathogens. Since it is a National Facility, it has been used by ICMR and other institutions to work with infectious agents. Timely maintenance is needed to ensure the availability of the facility for emergencies.

Work done: During the year, the laboratory has been extensively used to handle highly pathogenic viruses, viz., AI-H5N1, AI-H5N8, influenza A H1N1pdm09, vaccinia, Zika and polio viruses by NIV scientists. The facility was also made available to the Enterovirus Research Centre, Mumbai staff during January to March, 2017 to handle clinical samples collected during a poliovirus serosurvey as part of the surveillance of Global Polio Eradication program. Specialized BSL3 trainings were imparted to NIV staff, as well as the ERC staff before they were allowed to work inside the facility and bio-safety trainings to M.Sc. virology students.

AVI1601: Susceptibility of avian influenza viruses isolated from India to neuraminidase inhibitor antiviral drugs

Investigators: Pawar SD & Mullick J

Funding Agency: Intramural [2016-2019]

(i) Assessing the susceptibility of highly pathogenic avian influenza H5N1 viruses to oseltamivir using embryonated chicken eggs

In an earlier study, the use of embryonated chicken eggs (*in ovo*) to determine the susceptibility of low pathogenic avian influenza H9N2 viruses to oseltamivir carboxylate (OC) was reported. However there is no data available on susceptibility of Highly Pathogenic Avian Influenza (HPAI) (H5N1) viruses.

Objectives: To determine oseltamivir susceptibility of the Indian HPAI H5N1 from poultry, using chicken eggs as a model.

Work done: Neuraminidase inhibitor assay (NAI) was performed to test the susceptibility of avian influenza viruses to oseltamivir carboxylate as per the standard protocol. *In ovo* antiviral assays were carried out using 10-day-old embryonated chicken eggs. The viruses were treated with 14 µg/ml of OC and inoculated in the allantoic cavity; 50% egg infectious dose (EID_{50}) and 50% egg lethal dose (ELD_{50}) were calculated using the Reed and Muench method.

Results: OC treatment showed significant drop in HA and EID_{50} titers as compared to the untreated virus controls (Figure 1).

Conclusions: *In ovo* method is the most appropriate indicator of the susceptibility of HPAI H5N1 viruses to oseltamivir than ELD_{50}.

(ii) Susceptibility of highly pathogenic avian influenza H5N1 viruses isolated from India to antiviral drug, oseltamivir carboxylate (OC)

India reported more than 121 outbreaks of HPAI H5N1 viruses in poultry since 2006. As vaccines are not available, antiviral therapy is crucial for prophylaxis, treatment and management of human infections. The neuraminidase inhibitors (NAI) like oseltamivir and zanamivir are the drugs

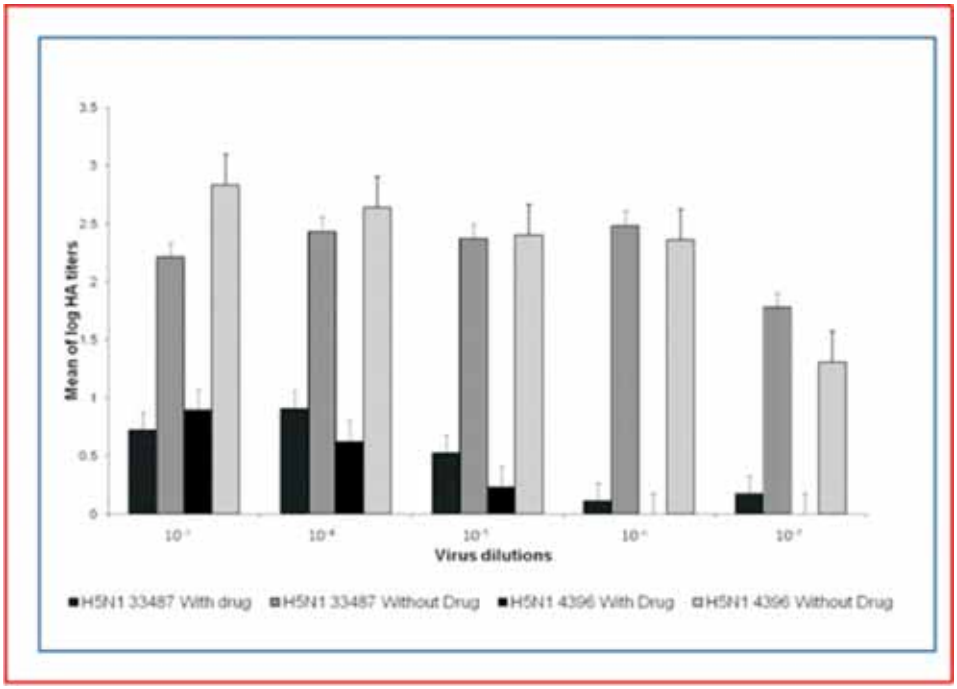


Figure 1. Comparison of mean log HA titers of H5N1 viruses with and without drug

of choice. Amino acid substitutions in NA gene of H5N1 viruses have been shown to be associated with the reduced susceptibility with oseltamivir and zanamivir. There are however no studies on the susceptibility of AI viruses to oseltamivir and zanamivir from India.

Objectives: To determine the susceptibility of AI H5N1 viruses to oseltamivir.

Work done: The NA gene sequencing of 25 Indian isolates of HPAI H5N1 (clades 2.2 and 2.3.2.1) were susceptible to OC except one (presence of E99A as well as N275S substitutions) that is known to confer reduced susceptibility to oseltamivir. Further analyses to correlate the results of sequencing with the NAI assay and *in ovo* studies are in progress.

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

Investigators: Mullick J, Thube MM, Pawar SD & Shil P

Funding Agency: Intramural [2012-2017]

HPAI H5N1 viruses continue to pose a serious threat to human health globally. The HPAI H5N1 Indian isolates (2006 to 2010) belonged to the clade 2.2. H5N1 virus isolated from crows and poultry in 2011 were phylogenetically distinct (clade 2.3.2.1) and also antigenically divergent from the previous one. Type I interferons are secreted as host-antiviral responses and influenza NS1 protein acts as a principal IFN antagonist. Different strains are heterogeneous in their capacity to activate host responses.

Work done: IFN responses by two distinct strains of HPAI H5N1 was studied in the human A549 lung epithelial cells. Growth kinetic studies have shown that the H5N1 strain of the 2.2 clade replicated faster ($p<0.05$) than the 2.3.2.1 clade. No significant difference was observed in the expression of the IFN-b RNA (real-time RT-PCR), however, there was a significant difference in the expression of the pattern-recognition receptor gene (MDA5), innate-immune response genes

(ISG-15) and innate restriction factor (Mx1) (Figure 2). Immunoblotting of the infected cell extract with anti-NS1 antibody showed higher level of NS1protein in the H5N1 2.3.2.1 clade virus at 4 hrs. and 24 hr. PI.

Conclusion: Further studies on structural differences of NS1 and the inhibitory action of NS1 on IFN-b production will pave way in understanding the mechanism.

AVI 1006: Role of complement during Influenza infection (In collaboration with NCCS).

Investigators: Sahu A (NCCS), Anujrattan A (NCCS), Pawar SD and Mullick J (NIV)

Funding Agency: Intramural [2010-2015, extended]

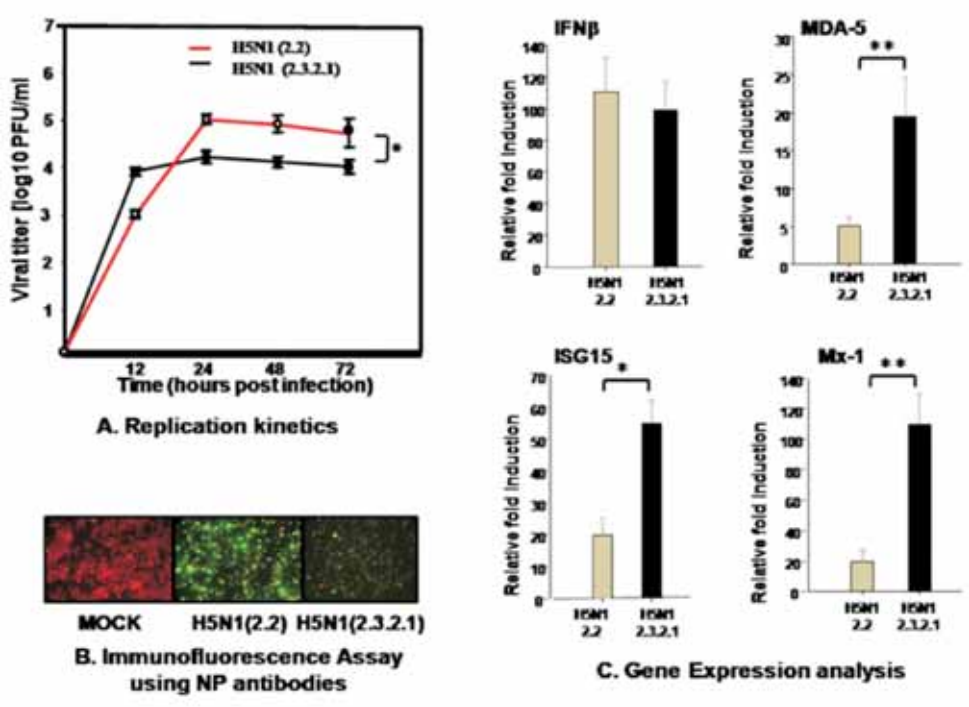


Figure 2. Replication kinetics of H5N1 viruses of two different clades in A549 cells and differential expression of innate-immune response genes. A) Monolayers of A549 cells (1.5 ×10^5 cells) were infected with each of the viruses at 0.01 MOI, culture supernatants were collected at 2, 12, 24, 48 and 72 h post-infection and determined titre of supernatants by plaque assay in MDCK cells (p<0.01). B) Immunofluorescence assay of mock and infected A549 cells using the NP antibodies. C) Innate-immune response against H5N1 viruses in A549 cells using Real time RT-PCR.**

$C3aR^{-/-}$ and $C5aR1^{-/-}$ mice. Conforming to our receptor antagonist data, $C3aR^{-/-}$ mice showed heightened susceptibility to the A(H1N1)pdm2009 virus infection, while $C5aR1^{-/-}$ mice showed complete recovery (Figures 3A & B). $C5aR1^{-/-}$ mice recovered better than the WT mice.

Conclusion: These data clearly suggest that C3aR signaling plays a major role during pandemic influenza 2009 virus infection.

NABL (National Accreditation Board for Testing and Calibration Laboratories) Accreditation of Human Influenza, Avian Influenza and Maximum Containment Laboratories as per ISO/IEC 17025:2005

Pawar SD (Quality Manager)

Three laboratories at NIV; *viz.*, Human Influenza, Avian Influenza and Maximum Containment Laboratories (BSL-4) have fulfilled the requirements of laboratory accreditation policies and procedures of ISO/IEC 17025:2005. The accredited laboratories have shown technical competence for reliable testing for viruses as per the International Standards.

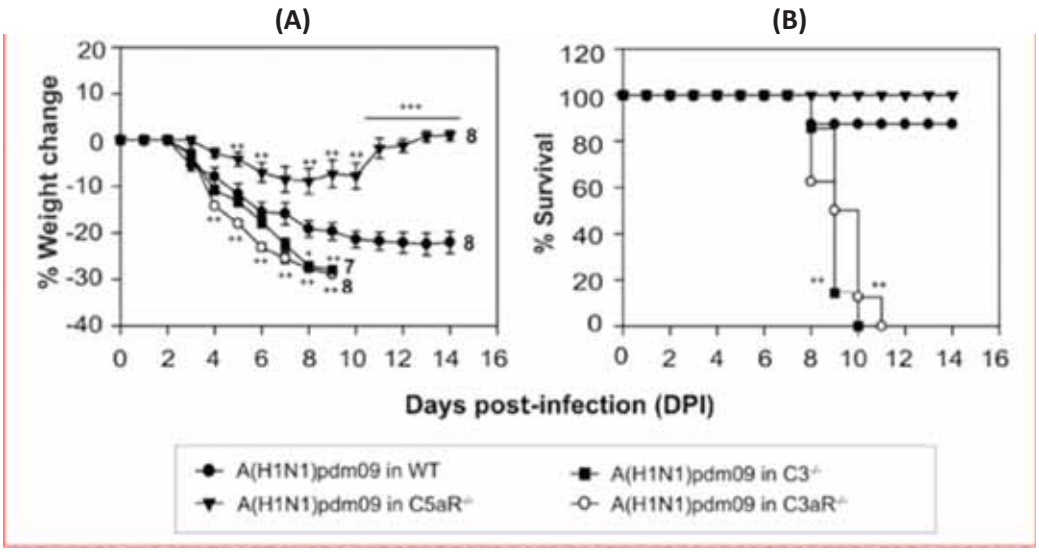


Figure 3. C3a signaling is primarily important for generating protective immune response against the A(H1N1)pdm09 virus. $A(H_1N_1)$pdm09 virus infection in $C3aR^{-/-}$ and $C5aR1^{-/-}$ mice in Balb/C background. A) Percentage of body weight loss during the infection in $C3aR^{-/-}$, $C5aR1^{-/-}$ and $C3^{-/-}$ mice in comparison to WT (Balb/C) mice (*p <0.05, **p < 0.02 and ***p < 0.001). B) Percentage of survival during the infection in $C3aR^{-/-}$, $C5aR1^{-/-}$ and $C3^{-/-}$ mice in comparison to WT mice (p < 0.002).**

Hepatitis Group

Scientific Staff

Dr. (Mrs.) Kavita S. Lole	Scientist E
Dr. (Mrs.) Anuradha S. Tripathy	Scientist E

Technical Staff

Mrs. Ashwini Y. Ramdasi	Technical Assistant
Mr. Satish S. Ranawade	Technical Assistant
Mrs. Supriya L Hundekar	Technical Assistant
Miss Neeta C. Thorat	Technical Assistant
Mr. Vasant Walkoli	Technical Assistant
Mr. Prakash B. Jawalkar	Technical Assistant
Mr. Shirish V. Vaidya	Technician C
Mr. Prasad Babar	Technician C
Mr. Pranit Ayachit	Technician B
Mr. KD Ramaiah	Technician B
Mr. PD Sarje	Technician A

Ph.D. Students

Ms. Shruti Kulkarni	CSIR-SRF
Mr. Subrat Thanapati	UGC-SRF
Mr. Swapnil Desai	CSIR SRF
Ms. Neha Bhise	UGC JRF
Ms. Meenal Sharma	ICMR JRF
Mr. Bhukya Prudhvi Lal	DBT JRF

Project Staff

Mr. Pankaj Aher	Research Assistant
Ms. Priya Ayyer	Research Associate

HEP1307: Evaluation of role of miR122 in hepatitis E

Investigators: Haldipur B & Lole K

Funding: Intramural [2013-2016]

Background

MiR-122 is involved in diverse aspects of hepatic functions, hepatocyte growth and differentiation, neoplastic transformation, lipid metabolism, iron homeostasis and liver development. miR-122 influences hepatitis B virus and hepatitis C virus replication in liver cells. Molecular mechanisms of liver pathology and clinical disease in HEV infection are not yet clear. Given the important role of miR-122 in liver pathobiology, we are investigating possible role of miR-122 in HEV replication and pathogenesis.

Objectives: To understand the involvement of miR-122 in HEV replication

Work done and results

It was previously reported that genotype 1 (HEV genome) has highly conserved target sites for miR-122 and/or miR-122*. It was also seen that cellular levels of miR122 directly influence replication efficiency of HEV in hepatoma cells. During this year, mutant HEV replicons were constructed by site directed mutagenesis to alter miR122 target sites. It was observed that mutations in the highly conserved miR-122 target sequence (CACTCC) in RdRp encoding region results in drastic decrease in virus replication. Further, depletion of miR-122 levels in cells (Figure 1), using miR122 inhibitors (LNA), resulted in significant reduction in HEV replication.

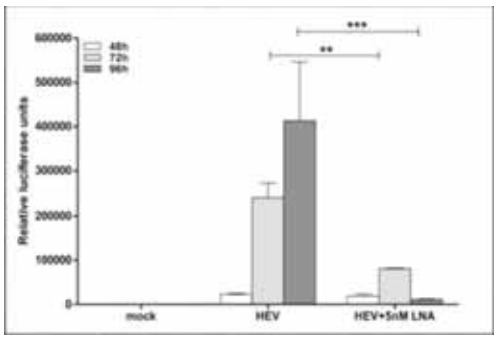


Figure 1: Anti-miR-122 LNA suppresses HEV replication in human hepatoma cells:

Summary: Our study indicates that miR-122 facilitates HEV replication possibly via direct interaction with its target site in HEV genome. The positive role of miR-122 in viral replication presents novel opportunities for antiviral therapy and management of Hepatitis E.

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

Investigators: Lole KS

Funding: Extramural (DBTBT/PR7413/MED/29/672/2012) [2013- 2017]

Interferon a and ribavirin based therapy is in use for more than 20 years against HCV. HCV has been a difficult virus to study since it grows poorly in cell culture. It displays very high sequence variations. Several new antivirals are being introduced for hepatitis C, none of the drugs/therapies is found to be universally effective against all HCV genotypes. Genotype and quasispecies impact response to therapy. Genotype 3 is most prevalent in India and there is an urgent need to find better anti-HCV drugs. Hence there is a need for genotype 3 based replicon system.

Objectives

- Development of HCV genotype 3 infectious clone
- Development of HCV sub genomic replicons

Work done: Multiple (20) sub genomic replicons were developed by introducing Neo, Rluc and DsRed reported genes. Poly U tail was extended from 16 to 60 nucleotides to improve replication competence. Cell culture adaptive mutations

were introduced in the constructs. Replication competence of the full genome and various sub genomic replicons was checked in Huh7.5 cells, however, none of the clones replicated efficiently in Huh7.5 cells. Huh7.5 cells stably expressing SEC14L2 protein were developed to see whether it improves replication. There is however very low-level replication of sub genomic replicons, detected only by RT-PCR. These HCV replicons probably need additional adaptive mutations for efficient replication in cells.

Summary: HCV replicons developed in the current study are not replicating efficiently.

HEP1316: Investigating the role of papain like cysteine protease in hepatitis E virus replication (initiated with intramural funds in 2013, extramural fund sanctioned in 2015)

Investigators: Lole KS & Desai S

Funding: Extramural (SERB/F/3675/2015-16) [2015-2018]

Generally, virus encoded proteases are involved in viral polyprotein processing in positive sense RNA viruses. These proteases are also known to target cell signaling/ antiviral pathway proteins and help virus to evade host antiviral responses. We have recently demonstrated deubiquitination activity of hepatitis E virus (HEV) papain like cysteine protease (PCP). It is likely that HEV PCP may have dual role during virus infection-ORF1 polyprotein (pORF1) processing and down regulation of the cellular antiviral response. It is not yet known whether HEV ORF1 polyprotein undergoes post-translational processing during replication. Current study aims to understand role of HEV PCP during HEV replication.

Objectives

- Analysis of ORF1 protein maturation in HEV infected hepatocytes
- To study the significance of deubiquitination activity of HEV PCP during virus replication

Work done: The protease domain encompasses 440–610 amino acid residues in the 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 such as alpha and rubella virus. To establish cell line persistently expressing HEV proteins, pSK-HEV-2 sub genomic Neo replicon was developed. S10-3 cells (a clonal cell line of Huh7) were transfected with transcripts obtained from the clone and cells were maintained on medium containing 500- 300µg/ml G418 to select Neo expressing cells. Colonies surviving after 4-week selection were expanded in medium containing 500 µg/ml G418 & cryopreserved for further analysis. Cells processed for the detection of HEV RNA by real-time PCR showed 107 HEV RNA copies/1000ng total cellular RNA confirming presence of HEV replicon in the selected cells. These cells will be used as model to study HEV ORF1 polyprotein processing during the course of proposed work. To analyze ORF1 polyprotein processing we need probing antibodies to detect processed ORF1 protein domains. Helicase and methyl transferase proteins from the ORF1 polyprotein were expressed and purified and balb/c mice were immunized with 20 and 50 µg protein/ dose using Freund's complete adjuvant for the first dose and incomplete adjuvant for the 2^{nd} and 3^{rd} dose. At every step blood samples were collected from the retro-orbital route and samples were analyzed for presence of antibodies in ELISA.

Summary: Antibodies were developed against methyltransferase and helicase domains of HEV ORF1. HEV sub genomic replicon with Neo gene was developed.

HEP1317: Development of virus like particles of hepatitis A virus, development of diagnostic assay and testing them as an immunogen in mice

Investigators: Lole KS & Hundekar S

Funding: Intramural [2014-2017]

Increase in number of susceptible adolescents/ young adults, especially in urban and semi urban areas in India and HAV outbreaks in the recent past have indicated need of hepatitis A vaccination in these susceptible individuals. HAV grows slowly in cell culture and commercial vaccines are not cost effective. Adaptation of HAV to tissue culture requires multiple cell culture passages. Hence, alternative methods for obtaining HAV proteins (recombinant HAV VLPs) for use as candidate vaccines or for diagnostic assays have been explored..

Objectives: Development of VLPs in Baculovirus expression system

Work done: HAV capsid (VP2, VP3 and VP1-2A) encoding regions were cloned in pET15b vector for protein expression. All 3 proteins were successfully expressed, however, levels of VP2 and VP1-2A were significantly low. For insect cell expression, capsid (VP1-2A) and protease (3C) encoding regions were cloned in pFastbac dual vector (PH3Cp10P12A), recombinant Baculovirus was developed and protein expression was done in Sf9 cells. A low-level protein expression was seen at 72 hrs.post infection in the cell supernatants. On concentration and sucrose gradient centrifugation, it was observed that HAV proteins are getting separated at the boundary of 20-30% sucrose layers of linear gradient. These results indicated that HAV proteins are probably assembling as VLPs.

Summary: Preliminary experiments using Baculovirus system indicated that HAV structural proteins are probably forming VLPs in insect cells but expression levels were very low.

HEP1304:Study of T regulatory cells in Hepatitis E infection

Investigators: Tripathy A & Rathod S

Funding: Intramural [2013-2017]

Elevated levels of transforming growth factor-b1 (TGF-b1) and its positive correlation with Foxp3 expression in hepatitis E patients have indicated involvement of TGF-b1 in hepatitis E pathogenesis.

Objective:

To determine polymorphisms in TGF-b1 gene,

HEP1302: Immunological memory in Hepatitis E infection

Investigators: Tripathy A & Kulkarni S

Funding: Intramural [2013-2016]

Infection with Hepatitis E is usually self-limiting. In acute infections, the host immune system mounts a protective response against the pathogen, leading to its clearance and subsequent generation of immunological memory against the pathogen. Whether immunity to Hepatitis E is long lasting is being debated and re-infection is suspected to be a common phenomenon. To understand this, studies on immunological memory against HEV infection is needed. As long-term maintenance of memory responses is the basis of most vaccine development studies, this study was designed to assess the longevity of memory response (1) in mice immunized with hepatitis E vaccine candidate developed at NIV, Pune. (2) in naturally infected hepatitis E patients after 1-26 years of primary exposure.

Objectives:

1) To study immunological memory in individuals infected with hepatitis E virus 1-26 years ago.

2) To assess immunological memory in mice immunized with hepatitis E vaccine candidate developed at NIV, Pune.

Work done: Acute hepatitis E patients from outbreaks in Shimla, Himachal Pradesh and Roha, Maharashtra (n=35), recovered individuals from hepatitis E from Bhor, Maharashtra (n=24) and apparently healthy control individuals (n=41) from similar settings were the study population for this project. The acute samples were cases of primary HEV infection as confirmed by HEV-

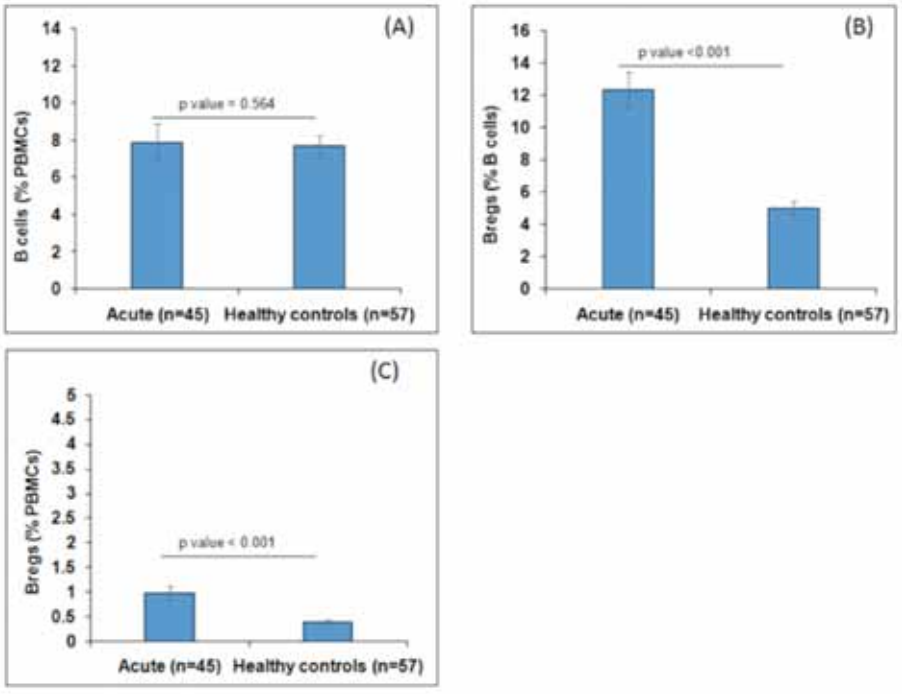


Figure 2: Percentage frequencies of (A) B cells [%PBMCs] (B) Bregs [% B cells] and (C) Bregs [% PBMCs] in HEV acute patients (n=45) and healthy controls (n=57)

specific IgG avidity assay. Flow cytometric analysis of immune cells of study subjects for immunological memory cell markers revealed that memory B and Tc cells were lower in recovered individuals compared to controls; whereas memory Th cells were higher in recovered individuals compared to acute patients. Percentages of CD4+ CD8+ cells were higher in acute patients compared to recovered individuals and controls. On analyzing the central (TCM) and effector (T_{EM}) subsets of memory T cells, percentages of CD4+ T_{CM}, CD8+ T_{CM} and CD8+ T_{EM} were higher in recovered individuals compared to controls. Expressions of HEV specific IFN-g and TNF-a on Th and Tc cells were comparable among all the study groups. B cell ELISPOT assay was carried out to assess the functionality of memory B cells in individuals recovered from both clinical and subclinical HEV infection Anti-HEV IgG1 secreting B cells (ASCs) were higher in patients recovered from clinical infections compared to those recovered from sub clinical infection and acute HEV patients (Figure 3).

Summary: The current data demonstrate presence of functional memory B cells with a stronger anamnestic response in recovered individuals with a history of clinical infection compared to those recovered with a history of sub clinical infection.

HEP1501: Antiviral agents against hepatitis E virus

Investigators: Lole KS & Bhise N

Funding: Intramural [2015-2018]

Hepatitis E virus (HEV) mostly causes acute self-limiting infections. In some patients HEV infection may progress to fulminant liver failure and in pregnant women 20-25% mortality is reported. This has been observed only for genotype 1 infections. However, recent reports of chronic HEV infections have shown the need of antiviral treatments to inhibit virus replication for protecting liver from damage.

Objective: To see the effect of helicase and polymerase inhibitors on HEV replication

Work done: We plan to use replicon system for screening of known antiviral drugs against HEV. To improve replication efficiency of the current HEV replicon attempts are being made. Current HEV sub genomic replicon in use has 16 A residues in the 3'noncoding region. Length of poly A tail was decreased by deleting 3 and 6A residues. The tail length was increased by adding 3A and 6A residues to extend the poly (A) tail to 19 and 22 A respectively. Deletion of 3 A residues reduced HEV replication significantly while removal of additional 3 A residues completely inhibited HEV replication, indicating requirement of at least 13-16 poly A residues in 3'NCR for successful replication. There was about 2-fold increase in the expression levels of the reporter gene when 3 poly A residues were added (+3, 19 A residues). Addition of 6 A residues (22 A) in the A tail further increased expression levels by 3-folds. This construct with 22 A residues in 3'NCR will be useful tool for antiviral screening.

For the development of in vitro enzymatic assay, expression and purification of replicase protein (both genotype 1 & 4) was optimized using ProBond Purification System (denaturing conditions). Replicase activity of the recombinant protein was determined by in vitro assay using a32P-ATP and 3'NCR as the template (Figure 4).

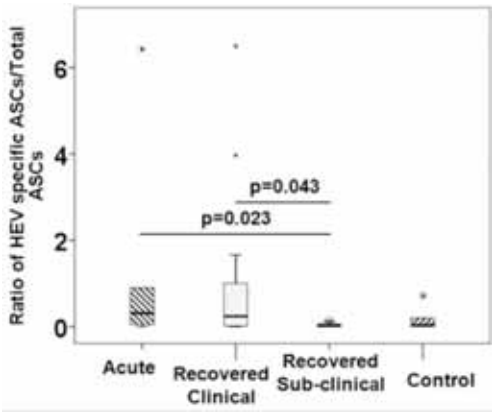
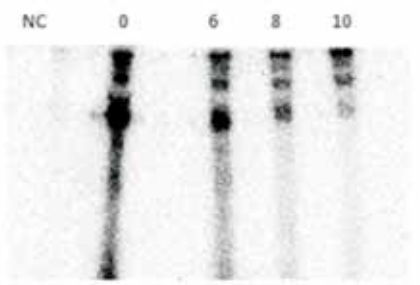


Figure 3: HEV specific ASCs as percent of total IgG ASCs

Length of poly A tail dis not affect polymerization efficiency of replicase enzyme. Polymerization activity of type 4 HEV replicase was found to be inhibited by commercially available antivirals such as Ribavirin tri-phosphate, Psammaplin A and Benzimidazole and respective LD50 values of these drugs in human hepatoma cells, S10-3, were found to be 67 µM, 10µM and 46 µM.

Summary: Radioactive signals from RNA products can be easily detected and quantified but these assays are not suitable for developing high-throughput system for screening of inhibitors. Hence, we plan to develop non-radioactive replicase assay using phosphate based calorimetric method consisting of malachite green molybdate as color developing reagent.

Figure 4: Inhibition of HEV polymerase by Benzimidazole:



HEP1602: Cellular antiviral responses against hepatitis B virus

Investigators: Lole K & Bhukya PL

Funding: Intramural [2016-2019]

Hepatitis B virus (HBV) is a noncytopathic hepatotropic virus and causes both acute and chronic liver infections. It is known that the host immune responses are the main determinants of hepatocellular injury during HBV pathogenesis. Mutations in pre core (PC) and basal core promoter (BCP) regions of HBV genome have been implicated in various liver associated clinical complications with high mortality rates. In 2009, an outbreak of hepatitis B with high mortality occurred in Sabarkantha district, Gujarat state in India with 89 deaths among 456 cases studied. Unusually high mortality was seen among the hospitalized patients due to abrupt fulminant hepatic failure. These findings along with mutations in precore and basal core promoter region seen only in Fulminant hepatic failure (FHF) cases needed in depth analysis.

Objectives:

1) To develop full genome clones of genotype D1 wild type and mutant HBV virus containing PC and BCP mutations and checking their replication competence in cell culture system

antibodies and for HBsAg. Analysis and compilation of the results is ongoing.

HEP1604: Screening of blood donors from Pune city for Occult Hepatitis B infection

Investigators: Tripathy A & Puranik S

Funding: Intramural [2016-2018]

Hepatitis B virus (HBV) infection is an important global public issue. Despite mandatory screening for HBsAg by ELISA for over 20 years, transfusion-associated HBV (TAHBV) continues to be a major problem in India. It has been demonstrated that in some HBsAg-negative individuals, HBV continue to replicate. Thus the absence of HBsAg in the blood of apparently healthy individuals does not ensure lack of circulating HBV. Persistent low levels of HBV DNA in serum and liver tissues after HBsAg clearance observed during acute self-limited or chronic HBV infection has brought about the concept of "occult" HBV infection, indicating the presence of HBV DNA in the absence of detectable HBsAg. Occult HBV infection (OBI) refers to the presence of HBV DNA in the absence of detectable hepatitis B surface antigen.No such data is available from western India. Detection of HBsAg in blood is a diagnostic marker for infection with HBV. In the blood banks screening for HBsAg is carried out routinely to detect HBV infection. It is possible that, donors with occult HBV infection, who lack detectable HBsAg might have exposure to HBV infection and are a potential source of HBV infection. Hence, the aim of this study is to determine the presence of HBV DNA among HBsAg negative blood donors from Pune city and to assess the magnitude of occult HBV infection in these subjects.

Objective: To determine the frequency of occult HBV infection in blood donors of Pune city..

Work done:Volunteer blood donors from Pune city are the study population. Till date, 1026 blood samples from blood donors negative for HBsAg have been screened for OBI. Analysis and compilation of the results is ongoing.

HEP1009: Study of the involvement of host factors in Chikungunya infection (initiated with intramural funds, extramural funding sanctioned in 2017)

Investigators: Tripathy A, Ganu M & Kulkarni S

Funding: Extramural ICMR (grant no: VIR/75/2013/ECD-1) [2017-2020]

Outcomes in Chikungunya infection could be attributed to the host factors and/or viral factors. There has been an increasing interest in the understanding of the role of genetic factors in the pathogenesis of various diseases. Variable cytokine production by different individuals is attributed to polymorphisms within the regulatory regions or signal sequences of cytokine genes. The TNF-a gene is responsible for

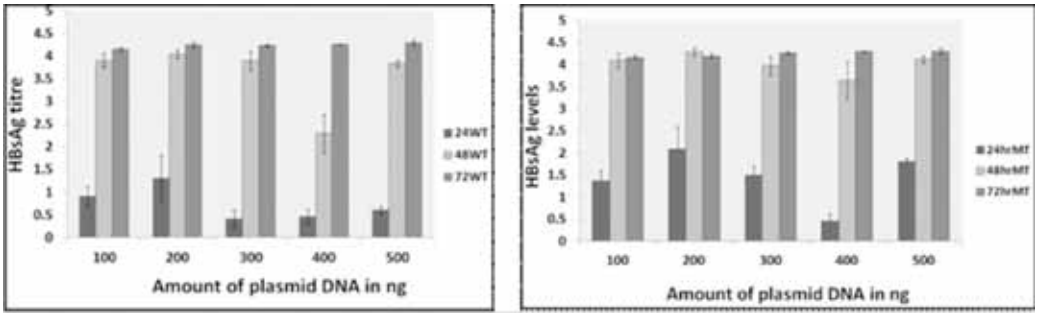


Figure 5a, b : Replication of HBV clones in hepatoma cells

the production of the pro-inflammatory Th1 cytokine. Similarly, IFN-?, the other antiviral Th1 cytokine involved in Chikungunya infection plays a crucial role in protection from viral infections. Though, substantial evidence indicates that IgG antibodies to CHIKV play a role in protection from Chikungunya, the involvement of biologically different functional differences among the IgG subclasses and their association with the disease status has not been studied. Therefore, this study was undertaken to understand the pathogenesis of CHIKV infection to elucidate the specific host responses in acute, recovered and in long-lasting chronic conditions that may help to distinguish patterns between patients who either "recovered" or suffer from "chronic" infection.

Objective: Assess the involvement of regulatory T cells in Chikungunya infection

Work done: The involvement of regulatory T (Treg) cells in Chikungunya is unexplored. In the current study, the frequencies and functionality of peripheral Treg and T effector (Teff) cells were assessed in 39 acute, 45 chronic Chikungunya arthritis patients, 38 individuals recovered from Chikungunya and 37 healthy controls. The role of Treg cells was also studied in 25 rheumatoid arthritis (RA) patients whose symptoms closely mimic chronic Chikungunya arthritis. Lower frequency of Treg cells in acute and chronic Chikungunya arthritis patients compared to recovered and controls, comparable Treg cells among recovered and controls indicated reduction of Treg cells to be associated with ongoing Chikungunya infection and normalization of Treg cells with resolution of disease (Fig 6A). Teff cells were higher in acute compared to chronic Chikungunya arthritis patients (Figure 6B). Higher TGF-b expression on Teff cells in acute patients compared to controls, higher TGF-b secretion in acute compared to chronic patients and higher IL-10 secretion in recovered individuals compared to all studied groups indicated the involvement of TGF-b in acute Chikungunya and IL-10 with recovery. Treg as well as Teff cells were elevated in RA patients compared to both chronic Chikungunya patients and controls suggesting an altogether different mechanism of Treg-mediated pathology in RA (Figure 6 A and B).

Conclusions: Overall, this study indicates an association of Teff cells and TGF-b with acute Chikungunya infection & Treg cells and IL-10 with recovery.

HEP1211: Studies on the involvement of natural Killer cells and cytokine signaling inhibitors in Chikungunya virus infection

Investigators: Tripathy A & Thanapati S

Funding: Intramural [2012-2016]

The chronic Chikungunya arthritis symptoms closely mimic the rheumatoid arthritis (RA) symptoms, thus making it difficult to distinguish between these two clinical entities.

Objective: To characterize NK ($CD3^{-}CD56^{+}$) and NK-like T ($Cd3^{+}CD56^{+}$) cell responses in patients with chronic Chikungunya arthritis and RA.

Work done: Phenotype and functions of NK and NK-like T cells repertoire were assessed in 56 chronic Chikungunya arthritis, 26 RA patients and 82 controls using flow cytometry. TNF-a and IFNg-secreting NK-like T cells were high in both chronic arthritis patients than in controls. Percentage of TNF-a^{+} NK cells was higher in RA patients than in controls. Percentage of perforin^{+} NK cells was low in both chronic arthritis patient groups. Among the patient groups, expressions of perforin^{+} and IFN-g^{+} NK-like T cells were higher in RA. Overall, our data show reduced frequency of NK-like T cells, lower expression of perforin^{+} NK, higher expression of TNF-a^{+} NK-like T and IFN-g^{+} NK-like T cells as the markers of chronic arthritic diseases (Figure 7) .

Summary: In the absence of any specific treatment for chronic Chikungunya induced arthritis and promising results of anti-TNF-a therapy against RA, current data may form the basis for future in vivo studies and has scope as possible therapeutics against Chikungunya.

HEP1601: Outbreak Investigation and

providing diagnosis to referred samples

Investigators: Lole KS & Tripathy A

Funding: Intramural [Ongoing]

(i) Water testing for Shimla, Himachal Pradesh

An outbreak of hepatitis E was reported from Shimla, Himachal Pradesh, in January, 2016 with 1109 suspected cases. NIV has investigated the outbreak (reported last year). Though immediate control measures were undertaken after detection of HEV and HAV from the water, to prevent future outbreaks, water quality is being monitored by the local health authorities.

Work done: Virus detection is being done at NIV by concentrating the water followed by reverse transcription PCR. Results of a representative set of samples are shown in Figure 8. Overall virus positivity in the water samples indicated that there is a cyclic contamination of the water source in winter months (Jan and Feb) in the area (Table 1). These reports are submitted to the public health authorities immediately after analysis for further actions.

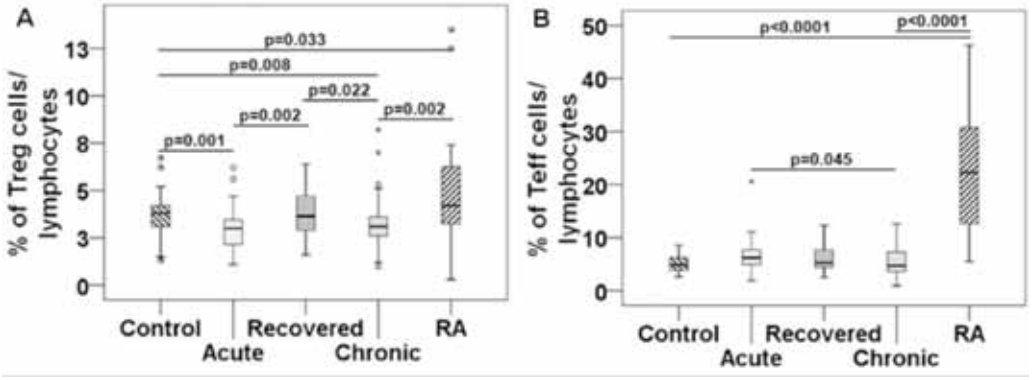


Figure 6: Frequencies of Treg and Teff cells in Chikungunya and RA patients

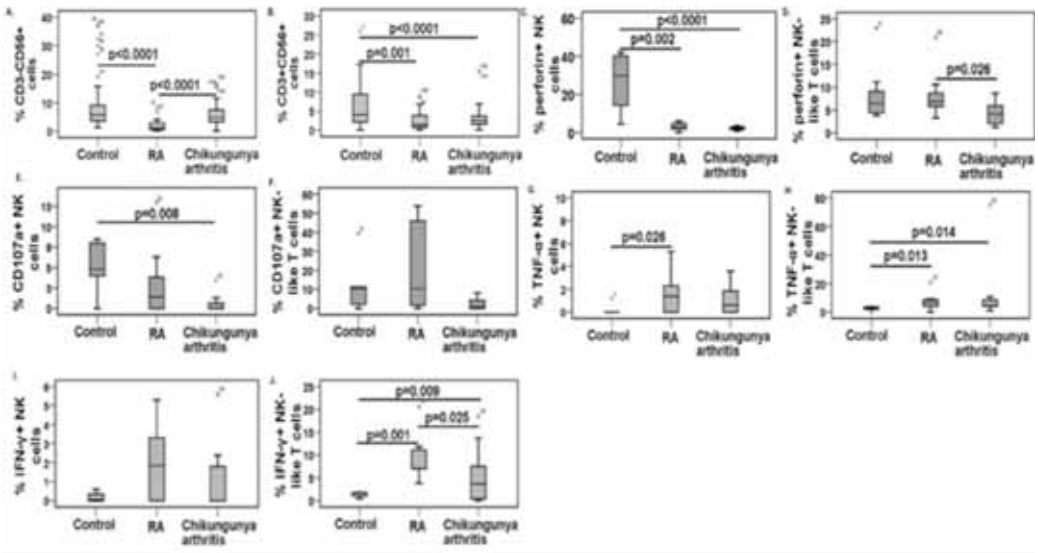


Figure 7: Distribution of NK, NK-like T cells and the proportion of NK, NK-like T cells expressing effector molecules.

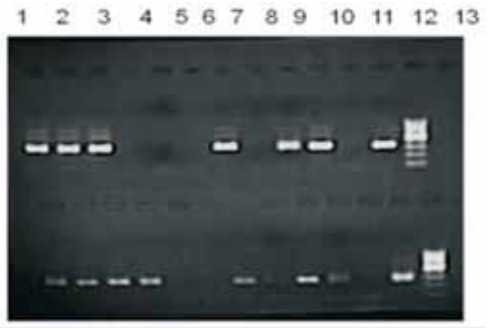


Figure 8: Detection of HAV and HEV from Shimla water samples:

Table 1: Month wise positivity for HEV RNA and HAVRNA in Shimla water

Month	No. of samples	HEV RNA (positive/ total samples)	HAV RNA (positive/ total samples)
Jan, 2016	6	4/6	4/6
Feb, 2016	7	7/7	Not done
March, 2016	17	2/17	1/17
April, 2016	23	0/23	0/23
May, 2016	7	0/7	0/7
June, 2016	2	0/2	0/2
July, 2016	8	0/8	0/8
Aug, 2016	7	0/7	0/7
Sept, 2016	2	0/2	0/2
Oct, 2016	0	0	0
Nov, 2016	6	0/6	0/6
Dec, 2016	2	0/2	0/2
Jan, 2017	30	7/30	3/30
Feb, 2017	10	0/10	0/10

- **A total of 728 products from the drug controller of India were tested for HBsAg and HCV RNA and reports were submitted**

Encephalitis Group

Scientific Staff

Dr. MM Gore	Scientist G and Group Leader (Superannuation on 31st Jan 2017)
Dr. RG Damle	Scientist C

Technical Staff

Mrs. VS Bhide	Technical Officer (A)
Mr. B. Y. Kadam	Technical Officer (A)
Mrs. SA Mahamuni	Technical Assistant
Mrs. V. Sankararaman	Technical Assistant
Dr. D. V. Pavitrakar	Technical Assistant
Mr. V. K. Jadhav	Technician C
Mr. D. K. Butte	Technician C
Mr. A. Venkataramanaiah	Technician B

Project Staff

Ms. S. Mundhra	SRF
Ms. D. N. Mali	JRF

ENC1301: Service Project: Providing Laboratory diagnosis for suspected encephalitis outbreak and referred samples.

Investigators: Gore MM, Damle RG

Funding Agency: Intramural [2016-17]

Diagnostic support provided for AES outbreak

1. **Gaya, Bihar: July 2016**

   Twelve clinical samples from 7 AES cases consisting of 7 sera, 2 CSF and 1 sample each of urine, throat swab and nasal swab were referred from Anurag Narayan Magadh Medical College Hospital, Gaya, Bihar. JE IgM antibodies were detected in MAC ELISA in acute and convalescent sera of one patient who had a history of fever for 5 days. Chandipura virus RNA was detected by RT-PCR in acute sera of two AES patients hospitalized with sudden onset of fever for 1 day leading to death. All other samples were tested negative for Flavivirus, CHPV, HSV and Enterovirus. Results indicated sporadic JE virus transmission in the area and also a likely role of CHPV as etiological agent associated with acute encephalitis among children in the region.

2. **Shirur, Maharashtra; August 2016**

   Investigated 10 pediatric cases referred by Taluka Health Officer, Shirur, Maharashtra for Chandipura and JE virus. Seven paired and three acute phase serum samples were tested in MAC ELISA for JE and CHPV and RT-PCR for CHPV. All the samples were negative for CHPV in both the tests; three acute phase sera and 2 convalescent sera showed indeterminate reaction in MAC ELISA for JEV.

3. **Malkangiri, Odisha; October 2016**

   Clinical samples (30 Sera and 1 CSF) in early phase of illness were received from 30 pediatric cases from District Hospital, Malkangiri, Odisha in October 2016. The samples were subjected to JEV and CHPV virus and IgM antibodies. In JEV IgM ELISA 12 samples were positive, 2 negative and 13 showed indeterminate reaction. 27 serum samples collected during the convalescent phase from the same patients were tested by JE MAC ELISA and 20 cases tested positive.

4. **Diagnosis of referred human clinical specimens:**

   During 2016-17, a total of 421 cases (374 Referred and 47 Outbreak) comprising 595 (270 serum and 325 CSF) specimens were received. State wise distribution and samples tested are given in Table 1. JEV positivity was detected in referred cases 28/374 (7.48%); Maharashtra (n= 16), Gujarat (n=10) and one case each from MP and Rajasthan. Acute phase samples were tested for JEV by RT-PCR, all 7 samples (Serum 2; CSF 5) tested were negative.

   180 samples out of 421 (Referred 133 and 47 outbreak) cases were screened for CHPV IgM capture ELISA and 115/180 were tested in CHPV RT-PCR. Six cases were positive, five for anti CHPV IgM and one case was positive in RT-PCR.

   Additionally, depending on clinical history or request by physician, cases were also screened in molecular diagnosis for Entero virus (EV) (n=7), HSV (n=90), and Flavivirus (n=79). None of the cases tested positive.

5. **Virus isolation from clinical specimens:**

   Acute phase samples, serum and CSF samples were inoculated in Vero&BHK-21 cell lines for virus isolation. Details of the acute phase samples used: Shirur, Maharashtra (1 CSF, 9 sera), Malkangiri, Odisha (9 CSF, 23 sera), Gaya, Bihar (one each of CSF, serum, urine, RS, TS) and Vadodara (2 CSF, 2 Serum). No virus could be isolated from any of these samples.

Table 1: Processing of Referred Samples

State	Name of the test performed	Total samples tested		Positive	
		Serum	CSF	Serum	CSF
Maharashtra	JE MAC ELISA	122	199	12	05
	CHPV MAC ELISA	44	57	00	01
	CHPV RT- PCR	42	40	00	00
	HSV PCR	18	55	02	04
Gujarat	JE MAC ELISA	84	86	10	03
	CHPV MAC ELISA	83	61	03	01
	CHPV RT- PCR	36	44	01	00
	HSV PCR	11	17	00	00
Other states (MP, Tamil Nadu, Karnataka, Puducherry)	JE MAC ELISA	14	15	01	01
	CHPV MAC ELISA	08	09	00	01
	CHPV RT- PCR	05	06	00	00
	HSV PCR	03	07	00	01

Note: None of the referred samples was positive for JEV RT-PCR, Flavivirus RT PCR or Enterovirus PCR.

Additionally, isolation of virus was attempted in infant CD1 mice using fifteen clinical samples; 2 CSF and 6 sera from Beed, Maharashtra and 6 CSF and 1 serum from Gujarat and Maharashtra. No virus could be isolated.

Four sand fly homogenates from Panchmahal, Gujarat were inoculated in BHK-21 and three blind passages were made which did not yield any virus isolation.

6. **Neutralization test: Testing of Human sera for JEV, WNV and CHPV antibodies**

As part of diagnostic services single serum samples from CHPV endemic region Udaipur, Dahod and Andhra Pradesh showed presence of anti CHPV neutralizing antibodies indicating past exposure to the virus. In paired sera from Malkangiri, Odisha, 6/27 pairs showed neutralizing antibodies to JEV, of which fourfold rise in NT titer was observed in only one pair and one pair also neutralized WNV (1:20).

7. **Pig sera for in vitro neutralization test (JEV):** Eight pig sera received from Indore, MP in January 2017. Two sera showed very high JEV neutralizing antibody titers (=1:1280). All eight sera were negative for JEV RT PCR.

Genetic characterization of the glycoprotein G of Chandipura viruses in India with emphasis on an outbreak of 2015

Investigators: Damle RG, Walimbe AM, Cherian S

During June-August 2015, CHPV activity was investigated after a sudden increase in the

number of encephalitis cases in different districts of Gujarat state. In diagnostic RT-PCR, followed by sequencing of the product yielding partial G gene (370nt), CHPV was detected in 11/45 cases (7/39 sera and 5/33 cerebrospinal fluid, CSF). CHPV was reported from Vadodara (n=6), Ahmadabad (n=3) and Kheda (n=2). CHPV could be isolated from a 1 serum of a three year old male from Ahmadabad and its full G gene was sequenced (KX790789).

During this period, sequence, phylogenetic and selection pressure analyses of G gene sequences was done to understand mutations that have taken place from the 1965 prototype and trends ofadaptive evolution; if any. Several unique mutations were noted in the G gene sequence of the Gujarat 2015 strain. Of the total of eight unique mutations in strain KX790789, five were localized in N-terminus 22 amino acid residues, which comprised the signal peptide. Phylogenetic study including other available sequences of strains from different geographical locations and isolation years (1965- 2015), indicated the relatedness of the 2015 strain to a group of the CHPV prototype strain of 1965 and the earliest outbreak strains of 2003. Analyses of selection pressure in the G gene, revealed positively selected sites within the signal peptide region and putative CHPV epitopes. These results indicate a probable role of G protein-based immune selection and underline the need for continued surveillance to monitor genetic and antigenic variations in the CHPV.

ENC 1701: Development and validation of monoclonal antibody (MAb) based antigen capture ELISA for detection of Chandipura virus infection in hospitalized encephalitis patients.

Investigators: Damle RG

Funding Agency: Intramural

MAbs were developed against an Indian strain (Andhra Pradesh, 2003) of CHPV. With very good fusion efficiency, 300 hybrids were obtained & of which 264 hybrids were screened in indirect antibody detection ELISA. 10/264 hybrids were positive for anti CHPV antibodies. Cloning of positive hybrids yielded 35 CHPV positive clones. 11/35 clones were shortlisted for the further characterization.

Characterization of MAbs with respect to isotype, protein specificity and ability to neutralize CHPV in vitro was done. All Mab clones were of IgG isotype. Three clones required sub cloning. In Western Blot, MAbs reacted with G protein (Mol wt. 69-72kd) of CHPV. CHPV neutralization test showed that 8/11 MAbs could neutralize CHPV in vitro. IgG is being purified, biotinylated to standardize Antigen capture ELISA from clinical samples.

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

Investigators:Bondre VP and Mali DN

Funding: Intramural [2015-2018]

Membrane fusion is the essential step in flaviviral replication. Low pH mediated conformational change in envelope protein mediate the fusion of viral membrane. Histidine residues are known to play crucial role as putative pH sensors during virus-cell membrane fusion. Hence, studies to understand the pH-sensing role of H residues conserved among JEV and neurotropic Flavivirus envelope (E) protein is undertaken. This membrane fusion study will be carried out by replacing H residues with alanine or asparagine by site directed mutagenesis in virus like particles (VLP) as a model system.

Recombinant plasmid having insert spanning from 390-2478 was developed and sequence confirmed. Transfection of recombinant plasmid was done in pre seeded BHK-21 cells. Transfected cells and cell soupwere harvested at different time intervals (24, 48, 72, and 96 hr.) post transfection. Immunofluorescence assay (Figure1) was done on harvested cells while cell soup was used for ELISA and western blotting

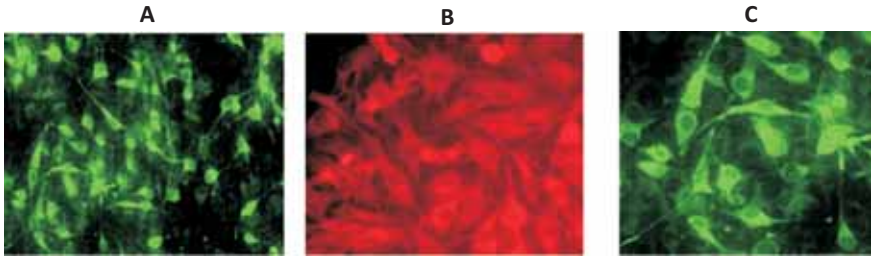


Figure 1: JEV (0945054) infected BHK-21 cells as positive control (A), Mock cells as a negative control (B), Expression of E protein in transfected BHK-21cells (C)

confirmation. TEM analysis will be done to confirm the expression of E protein in VLP form.

Result-Maximum expression was observed in cells harvested at 24 and 48 hrs. post transfection.

Detection of CHIKV antigen from infected mosquito in C MAb based antigen cap ELISA

Investigators: Damle RG, Gokhale MD & Parashar D

Funding Agency: Institutional Funding [One year]

Aedes aegypti mosquitoes were inoculated via intra-thoracic route with CHIKV and harvested on 10th PID. A suspension of 5infected and uninfected mosquitoes, triturated and sonicated (3 min, 5 sec ON/OFF), was used as source of CHIKV/normal antigen in the test. Sonication gave higher P/N ratio 11.00 as against 7.25 given by trituration. To mimic the field conditions different pools of infected and uninfected mosquitoes were made and employed as source of CHKV antigen in the assay. CHIKV was detected even from 2-3 infected mosquitoes in pool of 10, as against test cut offP/N values = 10.0 (Table-2).

Further, CHIKV intrathoracic inoculated mosquitoes were harvested on day 1-10 post infection and tested in both ELISA and real time PCR, the results were comparable. Further studies on membrane fed mosquitoes are in progress. Detection of CHIKV from triturated infected mosquitoes would be of immense help considering the field application of this test

Validation of kits: During the reporting period, 10 batches of JE MAC ELISA kits were evaluated.

HEPATITIS GROUP

Scientific Staff

Dr. (Mrs.) Kavita S. Lole	Scientist E
Dr. (Mrs.) Anuradha S. Tripathy	Scientist E

Technical Staff

Ms. Ashwini Y. Ramdasi	Technical Assistant
Mr. Satish S. Ranawade	Technical Assistant
Ms. Supriya L Hundekar	Technical Assistant
Ms. Neeta C. Thorat	Technical Assistant
Mr. Vasant Walkoli	Technical Assistant
Mr. Prakash B. Jawalkar	Technical Assistant
Mr. Shirish V. Vaidya	Technician C
Mr. Prasad Babar	Technician C
Mr. Pranit Ayachit	Technician B
Mr. K.D. Ramaiah	Technician B
Mr. P.D. Sarje	Technician A

Research Fellows

Ms. Bangari Haldipur	UGC-SRF
Ms. Shruti Kulkarni	CSIR-SRF
Mr. Subrat Thanapati	UGC-SRF
Mr. Swapnil Desai	CSIR JRF
Ms. Neha Bhise	UGC JRF
Ms. Meenal Sharma	ICMR JRF
Mr. Bhukya Prudhvi Lal	DBT JRF

Project Staff

Mr. Pankaj Aher	Research Assistant
Ms. Priya Ayyer	Research Associate

HEP1310: Development of HCV genotype 3 replicons and infectious molecular clones: identification of adaptive mutations and under-standing their biological mechanisms.

***Investigators**: K Lole & V Arankalle*

Duration: 2013-2016

Funding Agency: DBTBT/PR7413/MED/29/672/2012)

Background

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 replication, proliferation and antiviral testing. Genotype 3 is the predominant HCV genotype circulating in India and availability of infectious cDNA for genotype 3 would be very useful for developing antiviral testing platform as well as pathogenesis studies.

Objectives:

- Development of HCV genotype 3 infectious clone
- To develop HCV sub genomic replicons

Findings

Cloning and mutagenesis of genotype 3a infectious cDNA clone was done in the previous year. However, there was no replication when tested in cells. On comparative analysis of sequences of other genomic regions in the previously reported HCV replicons of genotype 3 as well as other genotypes of HCV, it was realized that our clones had relatively short poly U region (16 nucleotides) in the 3'UTR. Hence, we extended the poly U tail up to 60 nucleotides and tested replication efficiencies of these clones. These changes, however, could not improve the replication.

When HCV replicates successfully in cells, presence of neo gene in the replicon enables establishment of a stable cell line after 3-4 weeks of G418 selection. As we were not getting sufficient cell number after three weeks for any other confirmatory experiments, we decided to do analysis at early time points after cell transfection. For that, cells were harvested at early time points (72 h - 7 day post transfection) and processed for immunoblotting using anti-Neo, anti-NS3 and anti-NS5a antibodies. However, it was not possible to detect any of these proteins, further confirming very low replication efficiency of the clones even at early time points.

It was decided to add one more reporter gene in the replicon, which will enable us to detect the low level replication of these replicons in cells. Hence, we developed dual reporter sub genomic replicons (with Neo and Renilla luciferase (RLuc) reporter genes downstream of the 5'NCR) using Gibson assembly method. To confirm expression of Rluc gene in the replicon, one microgram transcripts were tested using rabbit reticulocyte lysate based *in vitro* translation system followed by dual luciferase reporter assay. The replicon RNA showed 250000 luminescence units (LU) as compared to 140 LU for control neo replicon confirming successful expression of the RLuc gene from the replicon. However, when Huh 7.5 cells were transfected with these *in vitro* transcripts, they failed to show any Rluc activity.

Summary

Our preliminary testing experiments showed that SEC14L2 expressing Huh 7.5 cell line does not help in improving replication competence.

HEP1302: Immunological memory in Hepatitis E infection

***Investigators**: A Tripathy, V Arankalle & S Kulkarni*

Funding Agency: Intramural

Background

Hepatitis E is a self-limiting disease caused by HEV. Whether primary infection with HEV confers protective and lifelong immunity to individuals is not well established.

Objective

- To study the immunological memory responses against HEV and to understand the immune correlates of protection.

Findings

A memory B cell functional assay was optimized. In brief, memory B cell ELISPOT assay was done using PBMCs isolated from 6 acute hepatitis E patients and 6 anti-HEV negative healthy controls. Anti-HEV

antibody secreting cells (ASCs) were significantly higher in acute patients compared to healthy controls ($p<0.05$) as shown in Fig 1. Effector functions of memory Th and Tc cells were also assessed by intracellular staining with IFN-γ and TNF. No significant difference was observed between acute hepatitis E patients and healthy controls. Memory Tc cells in acute patients showed expression of IFN-γ, but not TNF. The control individuals didn't express any of the two key cytokines. IFN-γ and TNF secreting memory Th cells were detected in both acute hepatitis E patients as well as in healthy controls.

In parallel, longevity of vaccine induced recall responses was studied in mice immunized with a low dose of vaccine candidate. Balb/c mice (n=8) were immunized with 2 doses of rNEp + liposome-based adjuvant 28 days apart and anti-HEV IgG titers (GMT) were determined at regular intervals. At day 120-post immunization, the titers declined to 100. At this time point, a booster dose of candidate vaccine was given. Memory B cells ELISPOT assay was carried out at 6th day post booster dose. Spots were seen in splenocytes from 4/8 immunized mice showing that the memory B cell are functional in 50% of mice even if anti-HEV antibody titers have declined.

Summary

These results suggest that a booster dose can enhance the protective efficacy of the candidate vaccine.

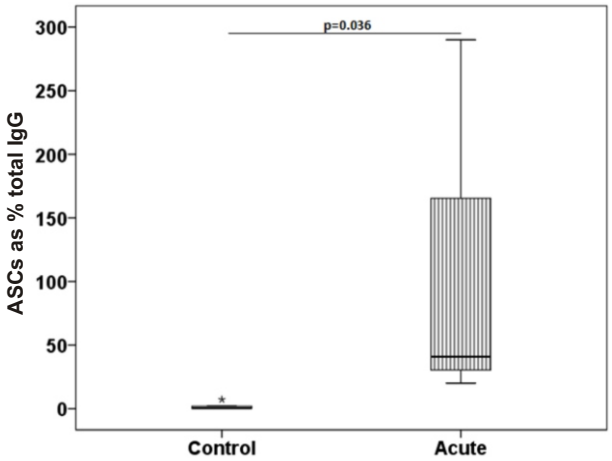


Fig 1: HEV-specific memory B cells response by ELISPOT.

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

***Investigators**: K Lole & S Desai*

Funding Agency: SERB/F/3675/201516

Duration: 2015-2018

Background

The putative papain-like protease (PCP) domain encompasses 440–610 amino acid residues in the 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 PCPs, suggests that HEV encoded protease bears similarity to proteases observed in other positive strand RNA viruses such as alphaviruses and rubella virus. 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. This study aims to characterize PCP of HEV and to understand its role during virus replication and pathogenesis.

Objective

- To study the significance of deubiquitination activity of HEV PCP during virus replication

Findings

We constructed clones of PCP from genotype 1 and genotype 4 viruses that extended into downstream proline rich hinge region (2.4 kb) and macro domain region (2.9 kb). These four proteins were expressed in *E. coli* cells, purified and tested for their enzymatic activities using substrates such as ubiquitin and ubiquitin like modifiers (SUMO, ISG15 and NEDD8) in an *in vitro* assay. The genotype 1 protease showed activity against all tested substrates while genotype 4 protease was unable to hydrolyze any of these substrates indicating significantly different characteristic of this important protein, presumed to have crucial role in HEV pathogenesis.

Summary

Human (genotype 1) hepatitis E virus encoded protease shows deconjugation activity when substrates conjugated to ubiquitin like modifiers are

used. Swine HEV (genotype 4) encoded protease either has very low or no such activity.

Miscellaneous Work

Study of the interaction between non-structural polyprotein of hepatitis E virus and its host cell proteins

***Investigators**: K Lole & N Ojha*

Period: 2013-16

Funding Agency: Intramural

Background

Hepatitis E virus (HEV) ORF1 encodes for non-structural polyprotein with multiple enzyme domains such as methyl transferase, papain like cysteine protease (PCP), macrodomain, helicase and RNA dependent RNA polymerase. Although HEV ORF1 proteins are functionally characterized, their precise role in viral life cycle is still understudied.

Objectives

- To understand the interactions between non-structural polyprotein (nsP) of Hepatitis E Virus and its host cell proteins.

Findings

We carried out yeast-two-hybrid screening of human liver cell cDNA library using baits prepared from HEV ORF1 encoded methylstransferase, protease, macro, helicase and RdRp domains. A total of 155 proteins were identified as potential interacting partners of these domains. The viral proteins preferentially interacted with the proteins of metabolism and energy generation, host immune response and ubiquitin proteasomal pathways. The mTOR and focal adhesion pathways were also targeted by the virus. These interactions suggest that HEV probably utilizes important proteins in

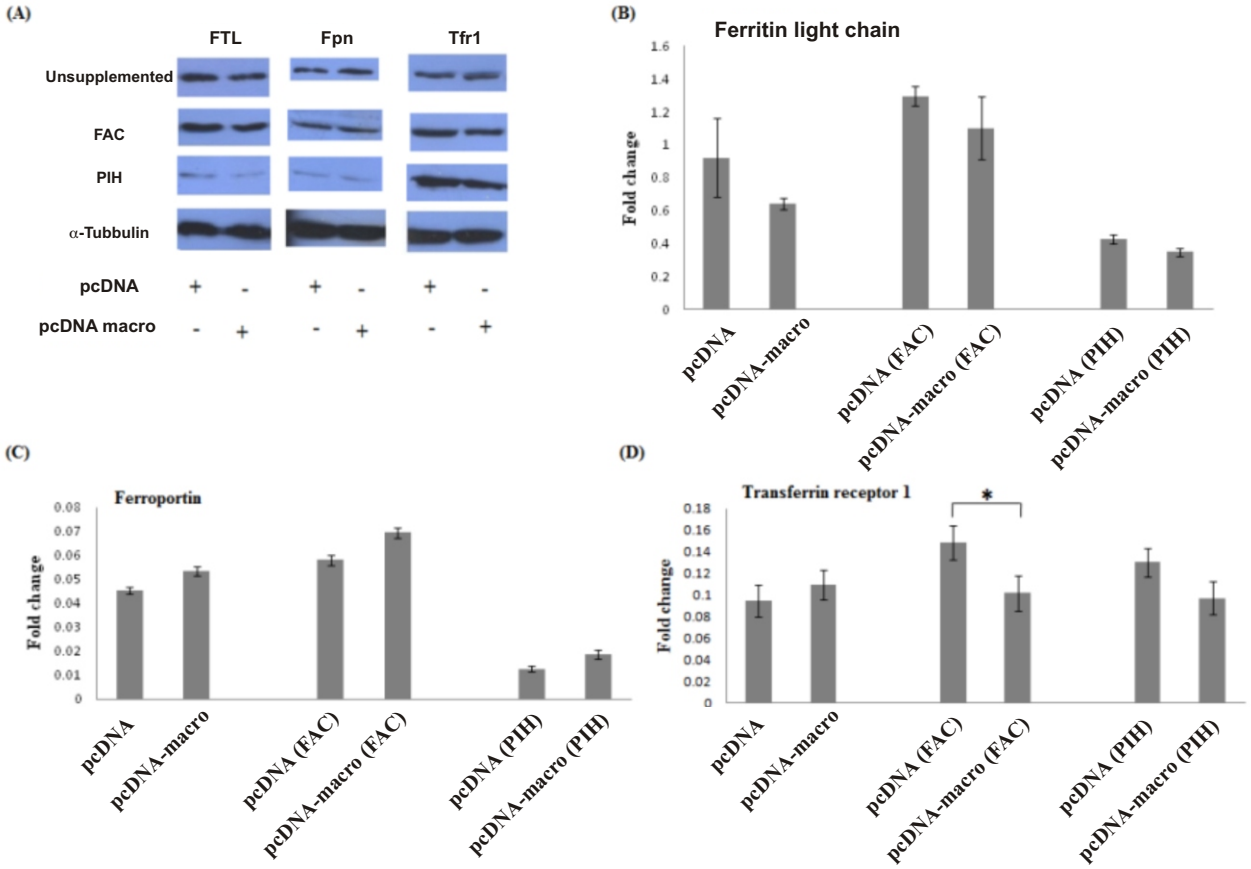


Fig 2: HEV macro domain does not alter expression of iron metabolism proteins (A) immunoblot for ferritin light chain, ferroportin and transferrin receptor 1. Cells were treated with either 100 µM ferric ammonium citrate (FAC) or 100 µM Pyridoxalisonicotinoylhydrazone (PIH) to increase or deplete labile iron pool respectively. (B), (C) and (D) show fold changes in signal intensities for FTL, Fpn and Tfr1 respectively after normalization with á-tubulin.

carbohydrate metabolism, energy generation and iron homoeostasis in the host cells during its establishment. Interactions with cytoskeleton and cellular membrane bound proteins suggested possible role of these proteins in virus trafficking and replication complex formation respectively.

Amongst total HEV-macro domain interacting cDNA clones, human Ferritin Light Chain (FTL) protein clones were found to be the most abundant (30% of the total cDNA clones). We validated this interaction and analyzed significance of this interaction. HEV macro did not affect expression levels of important proteins in iron metabolism such as transferrin receptor 1 (TfR1) and Ferroportin (Fpn) or FTL (Figure 2). It also did not have influence on the labile iron pool or reactive oxygen species in hepatoma cells expressing this protein. However, it significantly reduced secretion of ferritin from cells irrespective of cellular levels of labile iron pool (Fig 3). Brefeldin-A treatment of these cells further reduced secreted ferritin levels indicating secretary pathway independent mechanism of ferritin retention by HEV macro domain. Ferritin is an acute phase protein and secreted in large amounts upon virus infection

Summary

HEV ORF1 domains interact with host proteins. They primarily belong to the metabolic processes, oxidative phosphorylation, mTOR and focal adhesion pathways. These interactions suggest that HEV probably utilizes important proteins in carbohydrate metabolism, energy generation and iron homoeostasis in the host cells and inhibits secretion of ferritin.

Identification and characterization of regulatory elements in the HEV genome

***Investigators**: K Lole & S Mahilkar*

Funding Agency: Intramural

Duration: 2010-2015

Background

The first step in HEV genome replication is synthesis of replicative intermediate RNA (negative sense antigenome) from the genomic RNA. The antigenome then serves as a template for synthesis of two classes of positive sense RNA molecules, genomic (gRNA) and sub genomic (sgRNA). The sgRNA requires RdRp to initiate synthesis from internal promoter (SgP) within the antigenome. The recognition elements that determine template

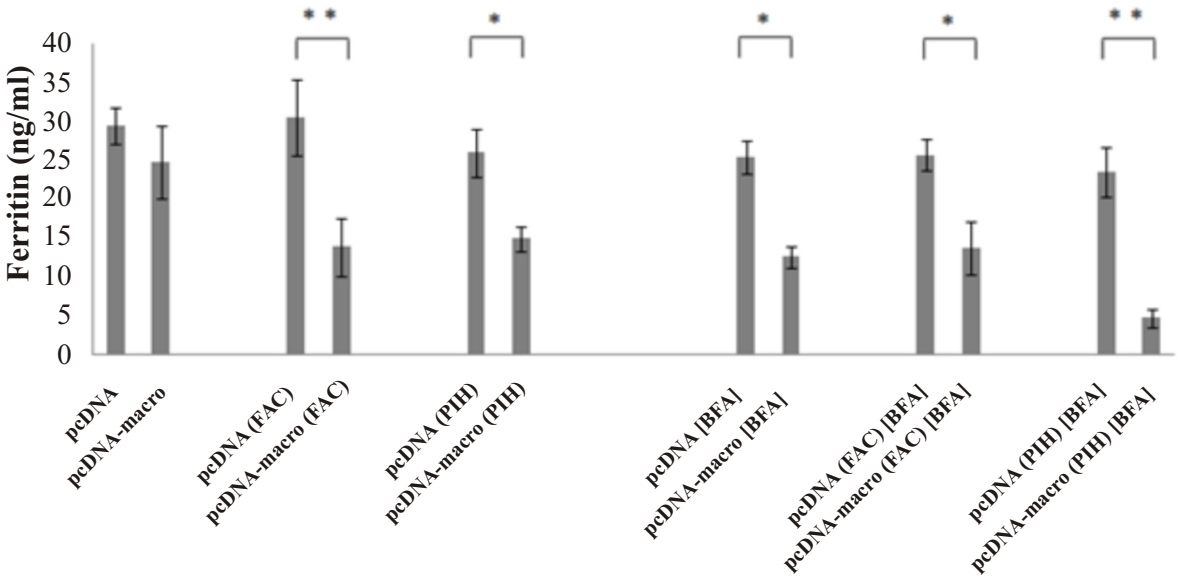


Fig 3. Measurement of ferritin secretion in the presence of HEV macro domain

specificity of HEV-RdRp to ensure amplification of appropriate viral RNA species are yet not well characterized.

Objective

- To identify RNA dependent RNA polymerase binding sites in HEV genome

Findings

To characterize different RNA regulatory elements in HEV genome, we expressed HEV RdRp protein with HIS-tag using bacterial system and analyzed template specificities using different putative cis-regulatory elements in the HEV genome. The enzyme showed highest affinity for the 3' non-coding region (NCR), then the 5'NCR and the putative sub genomic promoter (SgP). The enzyme could co-bind to 3'NCR and putative SgP templates together, as evident from the super shift in binding assay, indicating presence of different binding sites for these elements (Fig 4). Proteomic analysis revealed that the RNA elements share two common peptides for binding, while a third peptide, which is highly conserved across different HEV genotypes, is specific for 3'NCR. We propose that, during the early phases of replication, as negative sense antigenome copies accumulate at the replication site, it probably triggers promoter swapping from 3'NCR to SgP, to favor synthesis of sub genomic RNA and prevents synthesis of genomic RNA. The conserved site for 3'NCR binding could be potential antiviral target and needs further evaluation.

Summary

HEV RdRp showed highest affinity for the 3' non-coding region (NCR) as compared to the 5'NCR and the putative sub genomic promoter region (SgP). The enzyme showed co-binding with the 3'NCR and the putative SgP. These RNA elements shared two closely associated motifs in the enzyme for binding. The 3'NCR interacted with one additional motif, which was found to be highly conserved across different HEV genotypes.

HEP1307: Evaluation of role of miR122 in hepatitis E

Investigators: *V Arankalle, K Lole & B Haldipur*

Funding Agency: Intramural

Background

MiR-122 is a highly expressed, liver-specific miRNA that comprises 70% of the total miRNA population in normal adult hepatocytes. Highly significant positive correlation has been found between serum miR-122 levels and enzymes associated with liver damage, irrespective of the cause. MiR-122 has been shown to stimulate hepatitis C virus replication via interaction with two binding sites in the 5'NCR of the virus genome, while it inhibits replication of hepatitis B virus via interaction with cyclinG1 and p53.

A

SG5096NS (nm conc.)	-	-	10	500	1000	2000
α^{32}P-3PS (nm conc.)	10	10	10	10	10	10
RdRp	-	+	+	+	+	+

B

3PS (nm conc.)	-	-	10	500	1000	2000
α^{32}P-SG5096NS (nm conc.)	10	10	10	10	10	10
RdRp	-	+	+	+	+	+

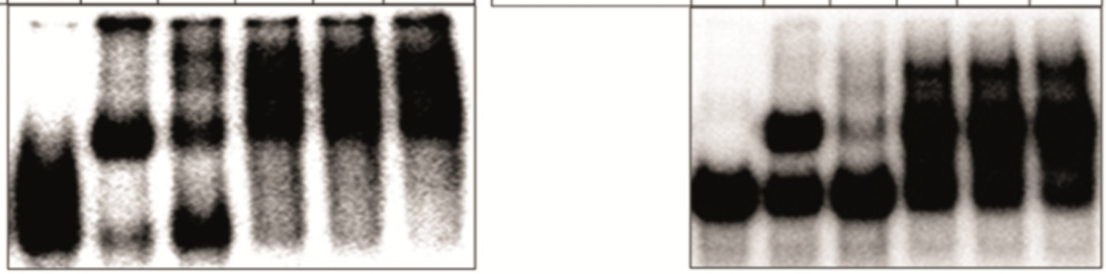


Fig 4: Competition binding assays using 3PS and SG5096NS (SgP) templates (A) Competition between 10nM α-32P-labeled 3PS and 10, 500, 1000, 2000 nm cold SG5096NS SG5096NS templates (B) Competition between 10nM α-32P-labeled SG5096NS and 10, 500, 1000, 2000 nM cold 3PS templates

Objective

- To investigate whether miR-122 levels correlate with hepatic inflammation and active viral replication & HEV viral loads.

Findings

We investigated potential role of miR-122 in HEV replication. *In silico* analysis of HEV full genome sequences representing genotype 1, 2, 3 and 4 viruses was carried out to identify a highly conserved miR-122 binding site in the RdRp encoding region of genotype 1 virus. A hybrid PCR assay was used to confirm presence of this miR122 binding site. To analyze influence of 122 levels on HEV replication, two human hepatoma cells, S10-3 (Huh 7 clonal cell line) and HepG3/C3A and a liver carcinoma cell line, A549, which are known to support HEV replication were used. HEV replication analysis using replicon expressing renilla luciferase reporter showed efficient replication of virus in S10-3 cells, which had high cellular levels of miR122. While, replication was very poor in HepG2/C3A cells which expressed comparatively low miR122 levels. Poor virus replication also correlated with less miR122 levels in A549 cells. Enhancement of cellular miR122 levels in HepG2/C3A and A549 cells improved virus replication efficiencies, while, decreasing miR122 levels resulted in a significant decrease in virus replication in all cell lines.

Summary

These results suggested positive correlation between miR122 level and HEV replication.

Hep 1304: Study of T regulatory cells in hepatitis E infection

Investigators*: A Tripathy & S Rathod*

Funding Agency: Intramural

Background

Pathogenesis of hepatitis E appears to be substantially immune mediated. It is of importance to understand and identify the key molecules regulating the immune response towards recovery. Literature on the role of Regulatory T cells (Tregs) in acute viral infections is limited.

Objective

- Characterization of the specificity, phenotypes and identification of the molecules/factors responsible for enhancement of Treg cells/abrogation in hepatitis E infection. Findings: HEV rORF2p specific (a) Treg frequency, subset analysis and expression of surface and intracellular markers on Tregs and CFSE based functional analysis by flow cytometry (b) key cytokines quantification by multiplex (c) suppressive functional assay in the presence of anti-TGF-α1/anti-IL-10/both antibodies/Trans well insert, were performed on samples from 58 acute patients (AVH-E), 45 recovered individuals from hepatitis E and 55 controls.

Findings

In AVH-E, the increased frequencies of Tregs and Teff cells were HEV rORF2p specific and Treg cells were of effector memory phenotype. Higher expressions of HEV rORF2p stimulated CTLA-4, GITR, PD1L, CD103, CD39, TLR2 and TGF-β1 molecules on Tregs of AVH-E were observed. Tregs produced TGF-α1 and inhibited the secretion of IFN-γ (fig 5). Transwell insert and cytokines blocking assays indicated Tregs mediated suppression in AVH-E patients is majorly TGF-β1 mediated and partly cell-cell contact mediated.

Summary

Overall, we have identified beneficial involvement of HEV specific, functional T regulatory cells and TGF-β1as the regulatory molecule responsible for enhancement of Tregs in self-limiting HEV infection. Therefore, use of TGF-β1as a possible supplement for boosting Treg response in recovery from severe hepatitis E needs evaluation.

(A-C) The plots show the proliferation of CD4+CD25- Teff cells after compilation of data from AVH-E patients, (n=6), recovered individuals (n=6), controls (n=6), performed in duplicate. (D) Teff proliferation in AVH-E patients with or without HEV rORF2p stimulation and one representative overlay plot of AVH-E patients shown (E). † $p<0.05$ comparing with Teff cells only; ‡ $p<0.05$ comparing Teff cells with HEV rORF2p stimulation, & comparing with recovered individuals, @ comparing with control group.

Production of IFN-γ (F), suppressive cytokines TGF-β1 (G) and IL-10 (H) in co-culture supernatants

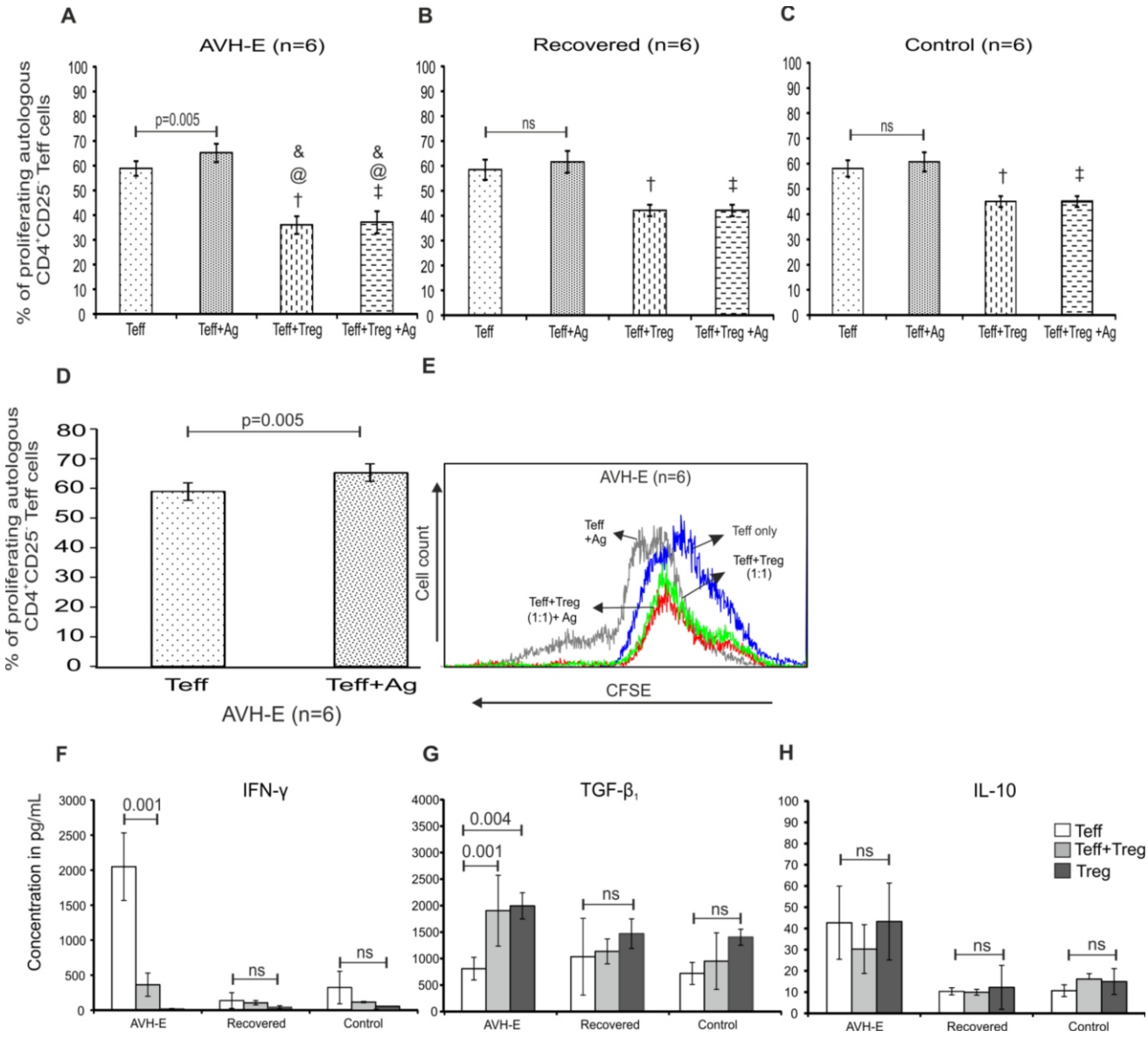


Fig 5. HEV rORF2p specific functional assay and key cytokines in supernatant of functional assay

stimulated with HEVrORF2p in the presence or absence of Treg cells. Data represented as mean ± SD and p-values were calculated using a one-way ANOVA, followed by Tukey's multiple comparisons test.

HEP1313: Study of B regulatory cells in hepatitis E virus infection

***Investigators**: A Tripathy & M Sharma*

Duration: 2015-18

Funding Agency: Intramural

Background

Reports from the Indian subcontinent indicating high mortality in patients with hepatitis E infection has resulted in attempts to understand HEV pathogenesis. Presence of robust antibody response to the ORF 2 region of HEV in recovered individuals from hepatitis E infection has formed the basis for development of vaccine against HEV and has also established the involvement of humoral immune response in HEV pathogenesis. B cells have been

identified as potent regulators of T cell immune responses in studies of autoimmunity, infection and cancer and their functions are not just confined to antibody production. B regulatory cells (Bregs), a subset of B cells have been identified to play a role in viral pathogenesis and they modulate other immune cells primarily by IL-10 cytokine production. We have reported higher levels of IL-10 and TGF-β productions in HEV acute patients compared to recovered individuals and healthy controls and have established its association with immunosuppressive responses in HEV infection. Detection of only TGF-β on the T-regulatory cells in HEV patients suggested that the source of IL-10 needs investigation.

Objective

- To identify/assess the role of B regulatory cells and IL-10 in HEV pathogenesis.

Findings

Forty-five acute HEV patients and 57 healthy controls from Wai, Maharashtra and Shimla, Himachal Pradesh were assessed for Bregs frequencies by staining and flowcytometry. B cells were comparable in acute patients vs. healthy controls whereas Bregs were high in acute patients as compared to controls. HEV specific Bregs were assessed by flowcytometry at 2 time points; 16h and 72h post antigen (affinity purified HEV rORF2p) stimulation in 10 HEV acute patients and 13 healthy controls. B cells and Bregs were comparable in rORF2p stimulated vs. unstimulated PBMCs in healthy controls. In case of acute HEV patients, B cells were comparable, but Bregs were high in rORF2p stimulated vs. unstimulated PBMCs compared to healthy controls.

Summary

The potential involvement of Breg cells in the immunopathology of HEV infection is implicated.

HEP1211: Studies on the involvement of natural Killer cells and cytokine signaling inhibitors in chikungunya virus infection

***Investigators**: A Tripathy & S Thanapati*

Duration: 2012-16

Funding Agency: Intramural

Background

NK and NKT cells are the important sentinels of innate immune responses that play a major role in the control of viral pathogenesis. Inefficient antiviral response of the host due to perturbation in its immune cell (natural killer [NK] cell, T cell, B cell etc.) functions could be a possible reason for virus persistence and/or chronic arthralgia. The role of NK cells (CD3-CD56+)/NKT (CD3+CD56+)-like cells in CHIKV disease progression/recovery remains unclear.

Objective

- To investigate the expression profiles and function of T/NK/NKT-like cells in chronic chikungunya (CHIK) patients and in recovered individuals from chikungunya infection

Findings

Here, we investigated the expression profiles and function of T/NK/NKT-like cells from 35 chronic CHIK patients and 30 recovered individuals. Percentage of NKT-like cells was low in chronic CHIK patients. NKp30+, CD224+, DNAM-1+ and NKG2D+ NK cell percentages were also lower, while those of CD94+ and NKG2A+ NKT-like cells were higher in chronic patients than in recovered subjects. IFN-γ and TNF-α expression on NKT-like cells was high in the chronic patients, while only IFN-γ expression on NK cells was high in the recovered individuals. Furthermore, percentage of perforin+NK cells was low in the chronic patients. Lower cytotoxic activity was observed in the chronic patients than in the controls. CD107a expression on NK and NKT-like cells post anti-CD94/anti-NKG2A blocking was comparable among the patients and controls (Fig 6).

Summary

Deregulation of NKR expression might underlie CHIKV-induced chronicity. IFN-γ and TNF-α expression on NKT-like cells are possibly associated with chronic CHIKV infection, while IFN-γ and NKG2D expression on NK cells are associated with recovery.

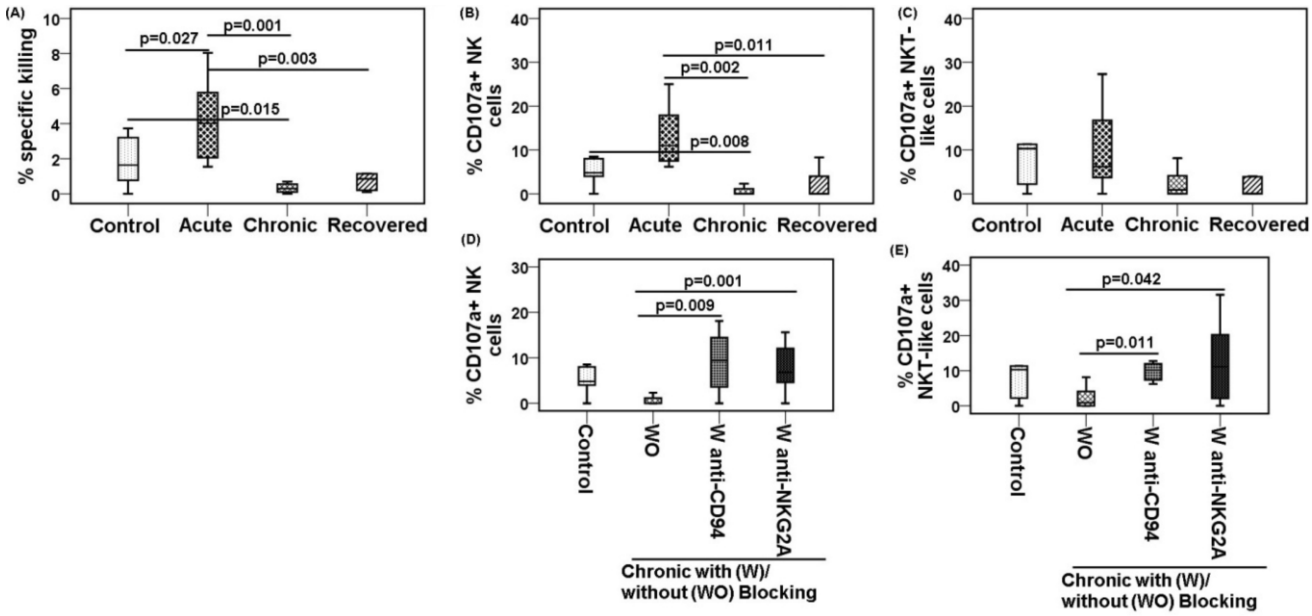


Fig 6: Cytotoxic and degranulation potential of NK/NKT-like cells during chikungunya against target cells (K562). PBMCs (effector) were isolated from whole blood of 12 chronic patients and 12 individuals recovered from chikungunya and were co-cultured with K562 (target) cells at effector/target ratio of 10:1 for 6 h to enumerate the percentage of CD107a on NK/NKT-like cells. Box plots show (A) % specific killing against target cells, (B) CD107a expression by NK cells, (C) CD107a expression by NKT-like cells, (D) CD107a expression by NK cells in the chronic patients before and after blocking with neutralization antibodies and (E) CD107a expression by NKT-like cells in the chronic patients before and after blocking with neutralization antibodies. Mann-Whitney U-test/Kolmogorov-Smirnov test was used for intergroup comparison. p value <0.05 is considered significant. Data of Cytotoxic potential and CD107a+ NK/NKT-like cells for acute and control groups were previously published and are shown here for comparison with chronic and recovered groups.

HEP1501: Laboratory diagnosis support

***Investigators**: A Tripathy & A Deoshatwar*

Funding Agency: Intramural

Duration: On-going

- Hepatitis outbreak in Shimla, Himachal Pradesh:

An outbreak of jaundice in Shimla, HP during Dec15 and Feb 16 was investigated by NIV team and 57 blood samples were collected from early acute IPD or OPD patients of Indira Gandhi Medical College (IGMC), Kamla Nehru Hospital, Deen Dayal Upadhyaya Hospital and General Hospital in Solan district. This included 12 ANC/PNC cases. Water samples from sewage treatment plant, water pumping station, affected and unaffected households and water samples before and after chlorination from Solan were collected. ELISA was set up at the VRDL laboratory in the Microbiology department of IGMC and provided spot diagnosis to all the OPD cases came with jaundice presentation. Forty-six out of 57 patients tested positive for anti IgM HEV antibodies indicating recent infection. More number of males was affected compared to females (68% vs. 32%). Adults in the age group 15-45 years were the worst affected, accounting for 71.4% of the cases. All the 8 water samples collected were found positive for HEV. Comparative analysis of virus sequences from patients and water samples showed 100% identity.

The etiological agent of the current outbreak was confirmed as HEV. Mixing of effluent water from the sewage treatment plant into the Ashwani Khudd water and lapses in the chlorination treatment could probably be the major causes of the outbreak. The people were advised to drink boiled water and advised the local administration to stop water supply through the Ashwini Khudd pumping station until the authorities made sure that water being pumped out is safe for drinking.

Diagnosis of referred human clinical samples:

During the reporting year, referred clinical samples were processed for hepatitis virus diagnosis and genotyping. Details of the tests conducted are given in Table.

Samples processed during the year

Sr. No.	Samples	HAV IgM	HEV IM	HBs Ag	HCV RNA	HCV geno-type	HBV DNA Qual	HBV DNA Quant	HEV RNA	HAV RNA
1	Testing for Drug Controller of India	–	–	709	709	–	–	–	–	–
2	Chronic hepatitis B and C patients	–	–	–	14	21	14	96	–	–
3	Sporadic acute viral hepatitis patients	336	267	69	–			–	4	39
4	Epidemics of viral hepatitis:	72	72	–	–			–	–	–
5	Water samples	–	–	–	–			–	67	–
6	Stool samples								32	–

ENCEPHALITIS GROUP

Scientific staff

Dr. M. M. Gore	Scientist G and Group Leader
Dr. R. G. Damle	Scientist C

Technical staff

Mrs. V S Bhide	Technical Officer (A)
Mr. B Y Kadam	Technical Officer (A)
Dr. D V Pavitrakar	Technical Assistant
Mrs. S A Mahamuni	Technical Assistant
Mrs. V. Sankararaman	Technical Assistant
Mr. V. K. Jadhav	Technician C
Mr. D. K. Butte	Technician C
Mr. A. Venkataramanaiah	Technician B

Project staff

Ms. S. Mundhra	SRF
Ms. D. N. Mali	JRF

ENC1301: Laboratory diagnosis of suspected viral encephalitis

Investigators: *MM Gore, RG Damle, VS Bhide, DV Pavitrakar, SA Mahamuni, V Sankararaman, VK Jadhav and DK Butte*

Funding Agency: Intramural

Duration: 2015-16.

A. Diagnosis of referred clinical samples

During 2015-16, 362 samples from 239 cases of suspected viral encephalitis were received for laboratory diagnosis. Cases were referred from Maharashtra, Gujarat, Karnataka, Madhya Pradesh, and Tamil Nadu states of India along with Puducherry. Japanese Encephalitis Virus (JEV) positivity was detected mainly from samples from Maharashtra while indeterminate results were observed with samples received from Gujarat. Acute phase samples were tested by RT-PCR for Chandipura virus (CHPV) and JEV. All the 33 acute phase samples (Sera 16; CSF 17) were found negative for JEV (Table 1).

Highest CHPV detection was observed in June-August 2015 in samples from Gujarat. Of the 45 cases referred to NIV, 11 (24.44%) were found positive for CHPV by RT-PCR and sequencing. The CHPV positive cases were from Vadodara (6), Ahmadabad (3) and Kheda (2) districts of Gujarat. Sequence information of these 11 CHPV RNA showed 99% homology with an Indian isolate CIN0360, obtained during the 2003 Andhra Pradesh outbreak.

A panel of referred samples were also tested for Herpes simplex virus (n=44), Enterovirus (n=10), Flaviviruses (n=7) and West Nile virus (WNV) (n=2), but none tested positive. For detection of Flaviviruses, Flavivirus consensus primers from NS5 gene were used (described below) whereas for specific detection of WNV, primers amplifying envelope gene were used.

B. Virus isolation from clinical specimens:

As part of routine investigations virus isolation from clinical specimens and their genetic characterization was carried out. 43 samples were used for virus isolation in Vero and BHK-21 cell lines. One serum sample from a 3-year-old boy (NIV No.1511584) from Ahmadabad, Gujarat yielded CHPV isolate. Sequence analysis showed 99% homology with an Indian strain CIN0360. Eighteen sandfly homogenates were also inoculated in BHK 21 cells, but virus could not be isolated.

Four serum and CSF specimens from Vadodara, Gujarat, which were, tested negative for CHPV by PCR and two pig sera received from Belgaum were processed for virus isolation in BHK21/Vero cells. Further studies are in progress.

C. Detection of Chandipura virus in sandflies

Nine pools consisting of 277 adult sandflies from Dahod (1), Panchamahal (4) and Vadodara (4) districts of Gujarat were processed for diagnosis of CHPV. Sandflies were identified as *Sergentomyia* spp. Individual pools were triturated and the clarified suspension was processed for virus

Table 1: Details of Processing of Referred Samples by Encephalitis Group

	JEV MAC-ELISA		CHPV MAC-ELISA		JEV RT-PCR		CHPV RT-PCR	
	Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF
Total samples processed	155	207	82	78	16	17	30	48
Positives	9	5	1	0	0	0	6	6
Indeterminate	21	3	0	0	0	0	0	0
Negatives	125	199	81	78	16	17	24	42

detection and isolation. Two pools from Vadodara and one from Panchmahal tested positive for CHPV RNA by RT-PCR (Fig 1). The sequence showed 99% homology with an Indian strain CIN0360 of Andhra Pradesh, 2003.

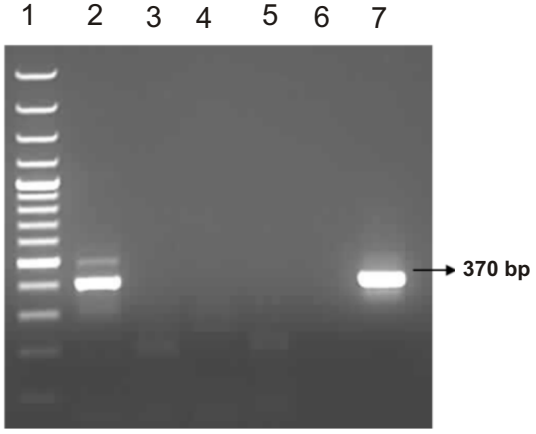


Fig 1: CHPV diagnostic RT-PCR of representative sandfly pools from Panchmahal

Lane 1: DNA ladder, Lane 2: Male sandflies (AA46366-1), Lane 3: Female sandflies (AA46366-2), Lane 4: Male sandflies (AA46367-1), Lane 5: Female sandflies (AA46367-2), Lane 6: Negative control, Lane7: CHPV positive control

Genomic analysis of 2015 CHPV isolates:

Molecular analysis of partial G gene of 12 CHPV isolates from 2015 was undertaken. The dataset comprised of 10 human and 2 sand fly isolates (from the present study) and 24 CHPV sequences downloaded from GenBank. Maximum likelihood tree was constructed for length 286 nt (Fig 2). The reliability of phylogenetic analysis was assessed by bootstrap test with 500 replications. Three groups can be identified, but with very less difference. Eight human and two sand fly isolates from 2015 grouped together. One human isolate NIV 1512525 grouped with earlier CHPV strains of 2003, 04, 07, 10, 12 and 2014. However, one strain (NIV 1511584) formed a separate group with 100% bootstrap support. Further molecular analysis of full G gene of the strains from 2015 is in progress.

D. Standardization of single tube RT-PCR assay for detection of flaviviruses

An attempt to develop a single step polymerase chain reaction with multiple primer sets for different neurotropic viruses was done. These primer sets were capable of detecting at least 12 flaviviruses (JEV, WNV, DENV, YF, SLE, TBE, KFD, Edge Hill,

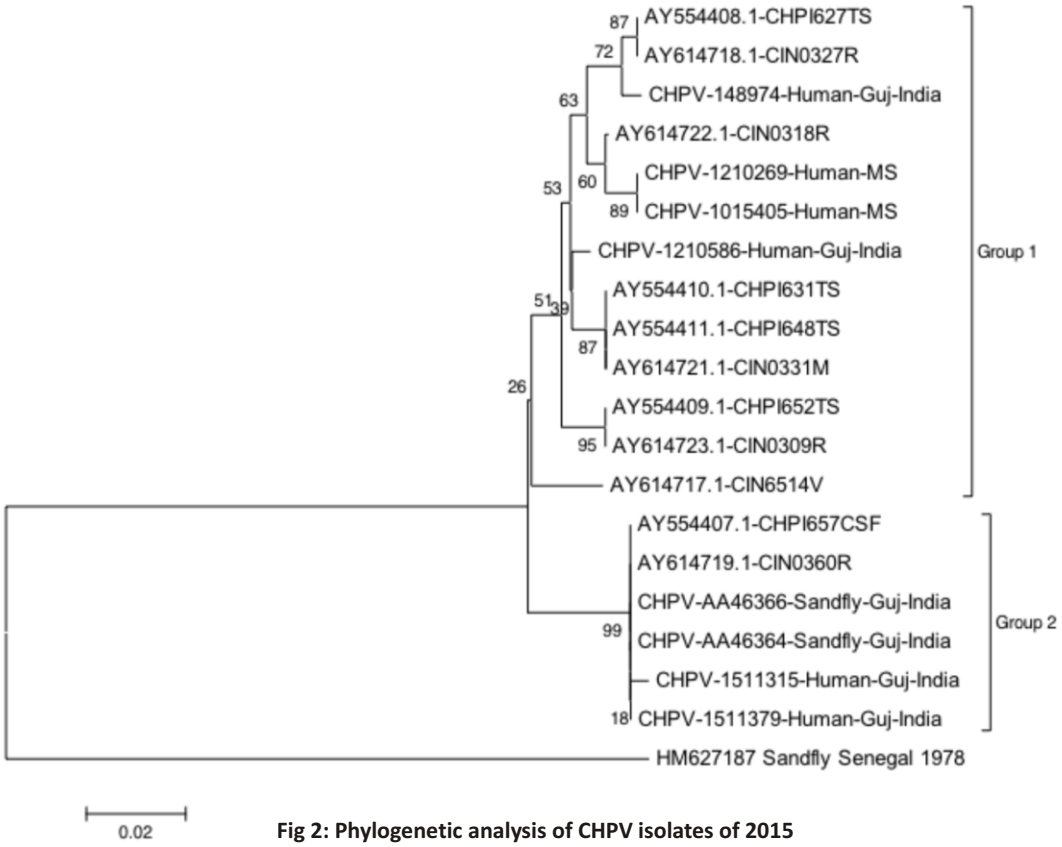


Fig 2: Phylogenetic analysis of CHPV isolates of 2015

Powassan, Ususu, Zika and MVE) were designed using online software tools. Initially, RT-PCR was performed using known positive controls for JEV, WNV and Dengue 1 - 4. Standardization and determination of sensitivity of the assay are in progress with human clinical specimens.

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

***Investigators**: RG Damle, A Patil, V Bhide, SD Pawar, GN Sapkal & VP Bondre*

Duration: 2014 onwards

Funding: Intramural

Background

CHPV is a leading cause of acute encephalitis with high mortality among pediatric population of India. A micro-neutralization ELISA (MN-ELISA) assay was developed for the detection of neutralizing antibodies (N'Ab) against CHPV. This method gives read-out in the form of ELISA optical density (OD) values and has a shorter turn-around time (TAT) as compared to the conventional cytopathic effect (CPE)-based neutralization assay (MN-CPE). The new and conventional assays were run in parallel where known positive and negative human serum samples were used as test controls. The conventional MN-CPE was terminated at 48 h post-infection and stained with amido black, while the MN-ELISA was terminated at pre-determined 18 h post infection for carrying ELISA assay.

Objectives

- To develop rapid micro-neutralization ELISA for detection of neutralizing antibodies against Chandipura virus

Findings

BHK-21 cells were used during the development of the assay using an Indian strain of CHPV. Sequential dilution of serum sample was mixed with 100 $TCID_{50}$ dilution of CHPV and added to $0.5x10^6$cells/ml of BHK-21 and incubated for 1 hr. After 18 h PI cells were washed and fixed with cold acetone (80%). The optimum dilution of detector antibody and Av-HRP was 1:800 and 1:8000 respectively for ELISA. During the development of the assay different parameters such as cell count, dilution of primary and secondary antibodies and time point for the test termination were optimized. The new and conventional assays were run in parallel where known positive and negative human serum samples were used as test controls. In evaluation using 80 clinical samples the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the new test was 100% when compared with the conventional MN-CPE method as a 'gold standard'. The MN-ELISA showed two-fold higher antibody titer in one sample (1:5600 Vs. 1:11240) and one sample was additionally positive than MN-CPE ELISA indicating detection of low-level sero conversion by the new assay.

Summary

The MN-ELISA is rapid, more sensitive and read-out of results is by measurement of OD, which could be more accurate than manual observation of reduction in CPE. This novel test could be used as an alternative to the conventional MN-CPE based assay in sero-surveillance and in vaccine studies in future especially for viruses that do not cause CPE in infected cells

ENC1602: Development of an attenuated Japanese encephalitis virus (JEV) genotype I strain infectious cDNA

***Investigators**: VP Bondre, DV Pavitrakar, RG Damle & SA Mahamuni*

Funding: Intramural

Background

Genotype I (GI) JEV that is replacing GIII virus as the dominant circulating virus in Asia; however, all available JEV vaccines are derived from genotype III viruses. The dominant vaccine for public health administration is attenuated GIII JEV. Preliminary results from studies conducted on the cross-neutralization and protection elicited by GIII JEV vaccines against GI viruses indicated the reduced neutralization potency due to genotype. Thus generating infectious cDNA clone of JEV GI will be instrumental in attempting various studies on defined virus by incorporating defined mutations.

Objectives (2015-16)

- Development of an infectious cDNA clone of JEV GI strain (0945054).

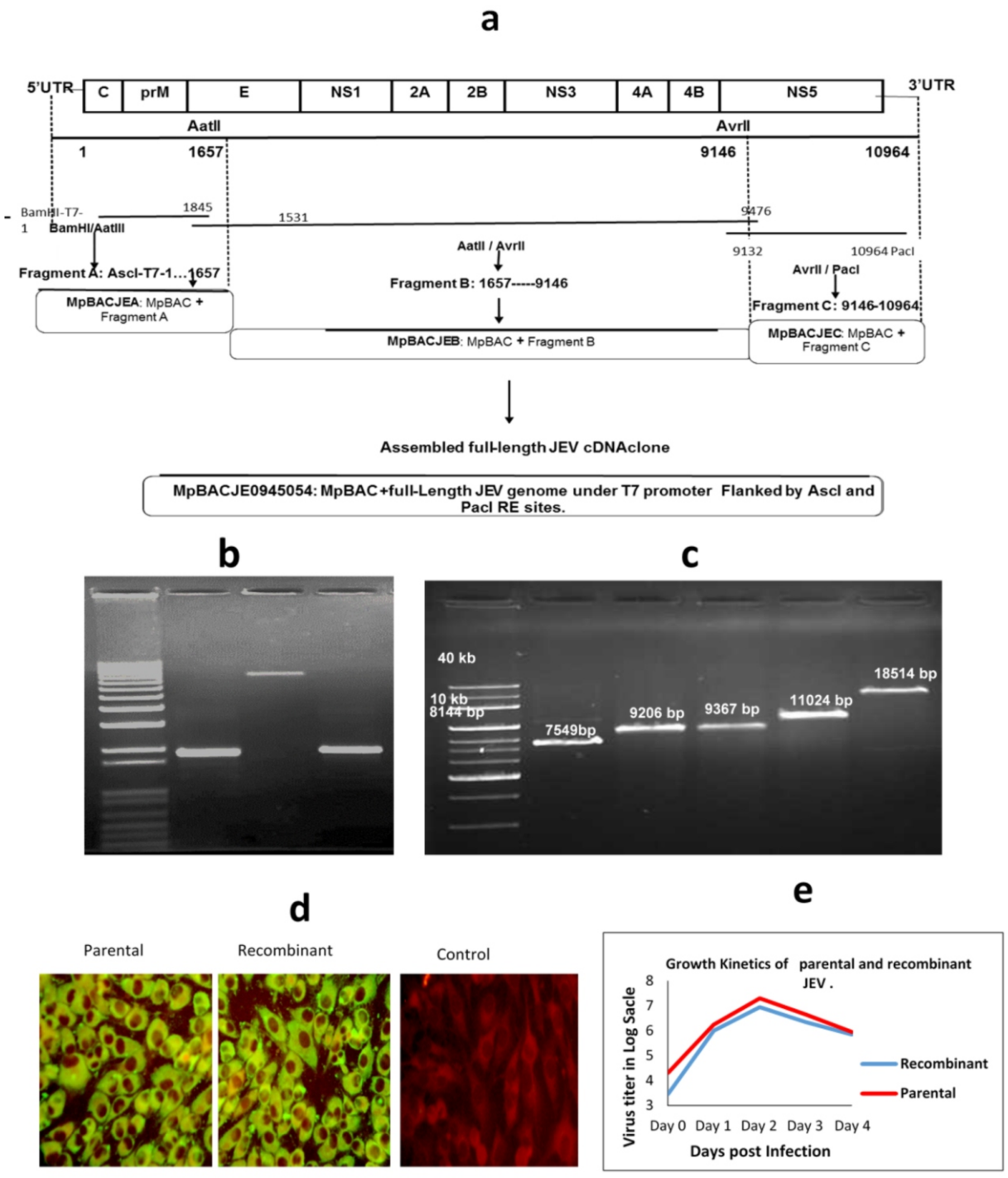


Fig. 3: a) Cloning strategy; b) Amplification of JEV genome: Lane 1: 1 Kb plus DNA ladder, Lane 2 –JEV 2-1845 (1845bp), Lane 3: 1531-9476 (7945bp) and Lane 4: 9132-10964 (1849bp); c) Construction of JEV cDNA clone in BAC vector, Lane 1- 1 Kb Extension ladder, Lane 2- Modified pBeloBAC11, Lane 3-MpBAC+ Fragment A, Lane 4- MpBAC+ Fragment C, Lane 5- MpBAC+ Fragment (A+C), Lane 6: MpBAC + Fragment (A+B+C); d) Immunofluorescence assay for detection of JEV protein after transfection of in vitro synthesized RNA in BHK-21 cells; e) growth kinetics of parental and recombinant virus in BHK-21 cells.

Findings

Complete genome sequence of plaque purified JEV GI isolate 0945054 was carried out. Sequential cloning of three amplified fragments was carried out in modified pBeloBAC11 vector, which resulted in full-length JEV cDNA clone named as MpBAC-JE-0945054 (Fig. 3 a,b,c). It was linearized with PacI and *in vitro* transcribed. Purified resultant RNA transcripts were used for transfection of BHK-21 cells and cultures showing CPE were harvested and stored at 72 h post transfection. Detection of JEV protein in CPE positive cells was shown by immunofluorescence using anti JEV specific MAbs (Fig.3d). Progeny virus was characterized by full genome sequencing after five subsequent passages in BHK-21 cells. Sequence analysis indicated that the recombinant virus resembles the parent virus except for six nucleotide substitutions. Three genetic changes were silent in addition to engineered genetic mutation at 9167 C-T to distinguish between recombinant and parental virus. Whereas three changes led to amino acid substitutions, two are located in E protein (E138K and D477N) and one in the NS1 (H191R) protein. Inoculation of recombinant and parental virus in 2-day old infant CD1 mice through IC route with 100 PFU resulted in 100% mortality. With the recombinant virus, 100% mortality was noted on day 5 while with parental virus, 25% mortality was observed on day 5 and the rest on day 6. In vitro growth kinetic studies with the two viruses showed titer in the range of 106-107.3 PFU/ml during 24-72 hr. post infection, which declined to 105.85 PFU/ml at 96 hr. (Fig. 3e).

Summary

CPE caused by synthetic RNA transcripts in mammalian cells, detection of cell associated viral protein after transfection and recovery of genetic markers in the progeny virus genome marked the successful development of reverse genetics system for JEV GI in BACMID vector.

ENC1305: Role of histidine residues in membrane fusion of Japanese encephalitis virus envelope protein.

***Investigators**: Bondre VP &Mali DN*

Duration: 2015-2018

Funding: Intramural

Background

Membrane fusion is an essential step in flavivirus replication. Low pH mediated conformational change in envelope protein mediate the fusion of viral membrane. Histidine residues are known to play a crucial role as putative pH sensors during virus-cell membrane fusion. Analysis of flavivirus E protein coding regions suggests that five His residues are conserved in accessible region on E protein. While JEV E protein analysis indicated conservation of additional two H residues in accessible region. Hence, studies to understand the pH-sensing role of H residues among JEV membrane fusion study was carried out by replacing H residues with alanine or asparagine by site directed mutagenesis in virus like particles (VLP) as a model system.

Objective

- To generate virus like particles of JEV strain 0945054.

Findings

A fragment spanning partial C-PrM-E region of JEV G1 (0945054) was amplified by RT-PCR and cloned into a 5.4 kb plasmid vector pcDNA 3.1(+) along with initiation codon, stop codon and signal sequence of C protein upstream to T7 promoter for expression. The 7.4 kb plasmid containing the cloned 2 kb JEV genomic insert was transformed in competent *Escherichia coli* bacteria and selected against ampicillin. Cloning of the insert in desired direction was confirmed by restriction digestion with KpnI and EcoRI as well as sequencing (Fig.4)

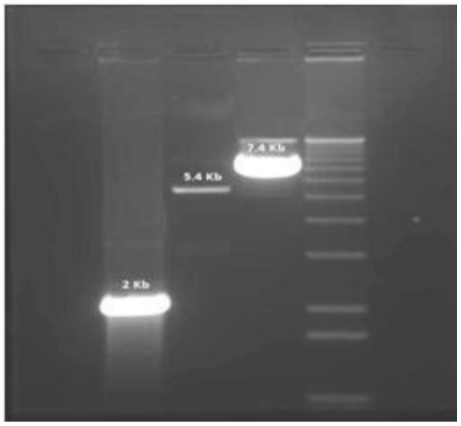


Fig. 4: Restriction digestion of a 7.4 Kb recombinant clone (lane 3) carrying 5.4 KB plasmid DNA (Lane 2) and 2KB viral genomic insert (lane 1)

Transfection and expression of the clone in mammalian cells for generation of VLP is in progress. The selected H residues will be mutated in the construct by site directed mutagenesis and its impact on endosomal membrane fusion will be evaluated further. Recombinant plasmid containing 2 kb JEV genomic insert was obtained. Clones were obtained after transformation of this recombinant plasmid in *Escherichia coli*.

Summary

Transfection and expression of the clone in mammalian cells for generation of VLPs are in progress. The VLPs will be surface labeled and used for compartment specific membrane fusion study.

ENC1304: Studies on neuronal cell damage induced by West Nile virus infection.

Investigators: *VP Bondre & S Mundhra*

Duration: 2013 - 2016

Funding: Intramural

Background

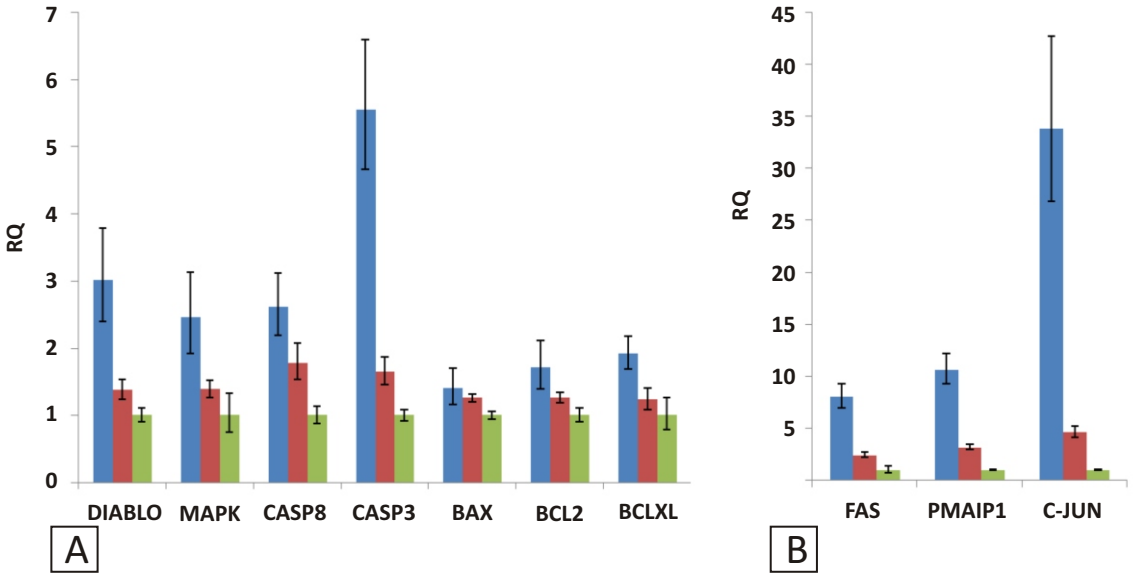
WNV strains vary in their ability to invade CNS and damage neurons resulting in varying degree of outcome. In our earlier studies, we have shown that that virulent strain (WNV68856) induced higher degree of apoptosis than the milder strain (WNV804994). Viruses are known to modify host gene expression, especially the pathways involved in cell death and survival.

Objectives (2015-16)

- Studies on impact of WNV replication on cellular apoptotic gene expression.

Findings

Relative quantitation of gene expression upon infection of SK-N-MC cells (human neuroblastoma) with 1 MOI of both WNV strains was performed by SyBrGreen qPCR. Calculated mRNA copies were normalized against that of housekeeping genes GAPDH and β-actin. A marked up-regulation (>4-fold) in expression of pro-apoptotic genes including FAS(CD95), PMAIP1(NOXA) and c-JUN at 48 h post infection (hpi) in cells infected with WNV68856 was observed. Also, >2-fold up-regulation of SMAC/DIABLO (inhibits Inhibitor of Apoptotic Proteins -IAPs) and Caspase 3 (terminator Caspase of apoptotic cascade) genes was documented during



RQ (Relative expression) values represent the fold change in gene expression in SK-N-MC cells infected with WNV68856 (blue), wnv804994 (red) as compared to uninfected cells (green) at 48 hours postinfection.

Fig 5. Expression profile of cellular mRNA upon infection with WNV68856 and WNV804994 in SK-N-MC cells.

WNV68856 infection. However, the anti-apoptotic BCL2 and BCLXL mRNA levels remained almost similar in both viruses (Fig. 5). Quantification of viral genomic RNA in cell supernatant harvested during different time points (24-72 h) by WNV quantitative RT-PCR indicated higher replication rate of WNV68856. The RNA titre increased from 1.5x1010/mL in WNV68856 and $1.9X10^{9}$/ml in WNV804994 at 24 hpi to a maximum of $9X10^{10}$/ml and $2X10^{10}$/ml respectively at 48 hpi and remained constant till 72 hpi.

Summary

Virulent strain of WNV induced higher up regulation of proapoptotic genes as compared to the lower virulent strain. Though the fold change was significant for DIABLO and MAPK ($p<0.05$), it was highly significant in case of FAS, PMAIP1, c-JUN and Caspase 3 ($p<0.005$). This increase may be a result of higher titre in case of WNV68856 as against WNV804994.

DENGUE AND CHIKUNGUNYA GROUP

Scientific staff

Dr. Cecilia D	Scientist F & Group leader
Dr. D Parashar	Scientist D
Dr. K Alagarasu	Scientist C

Technical staff

Mr. Anand Singh	Technical Officer (A)
Dr. (Mrs.) Rupali V Bachal	Technical Assistant
Dr. (Mrs.) Jayashri A Patil	Technical Assistant
Mrs. Asha Salunke	Technician C
Mr. Mahadeo Kakade	Technician C
Mrs. Ashwini More	Technician C
Mrs. Poonam Patil	Technician C
Mr. H B Supe	Technician B
Mr. S K Pandey	Technician A
Mr. P B Gore	Multitasking staff
Mr. D M Jadhav	Multitasking staff

Project staff

Ms. Sarika Amdekar	Research Scientist (CSIR)
Mr. Himanshu Tillu	SRF (ICMR)
Mr. Ashish Tiwary	SRF (ICMR)

Dengue Virus infection in India: Molecular characterization of dengue viruses circulating in India

***Investigators**: J Patil, M Kakade & C Dayaraj*

Duration: 2015-20

Funding Agency: NIH and intramural

Dengue suspected samples (n=239) received from different parts of the country were tested and 82 were found positive. Serotyping has shown predominance of DENV-2 followed by DENV-4 and DENV-3. Details of the location and serotypes prevalent in the area are shown in Table 1.

Table 1: DENV serotypes circulating in India Jan-Dec 2015
(Breakup of state-wise referred samples will help)

Place	DENV-1	DENV-2	DENV-3	DENV-4
Pune	1	13	6	7
Maharashtra	1	5	3	2
Gujarat		1	5	4
Vellore	2	4	2	1
Delhi		17		4
Vijaynagar, AP	2	1	1	
Total	6	41	17	18

One of the largest outbreaks was reported in Delhi in 2015 with more than 15,000 cases. DENV-2 and DENV-4 were the serotypes detected with DENV-2 being predominant. It was suspected that the DENV-2 circulating in Delhi may be different from those circulating in the rest of India. Therefore, envelope (E) gene of DENV-2 viruses circulating during 2015 in Pune, Delhi and Vellore were sequenced and compared. DENV-2 sequences obtained from clinical samples from the three different cities were found to share 94.8 to 97.3% similarity. Two different lineages of genotype IV were found to be circulating. Full genome sequencing is being undertaken to define the full extent of variation in the viruses.

DENV-4 is believed to be associated with sporadic cases in India, but since 2007 larger numbers of DENV-4 cases are being observed. Based on E gene sequence and phylogenetic analysis of DENV-4 in samples received from Hyderabad, Pune, Tirunelveli, Kerala and Delhi during 2007-15, we found that the circulating DENV-4 belonged to two different lineages of genotype I.

Project No.1306 Characterization of the T regulatory cell response in dengue

***Investigators**: H Tillu, A Tripathy, C Dayaraj*

Duration: 2012-16

Funding Agency: Intramural

Background

Pathogenesis of dengue is immune-mediated. Regulatory T cells suppress immune response and may contribute to better prognosis.

Objectives

- To study the role of Tregs in dengue virus infection.

Work done

Tregs and cytokines were evaluated in a cohort of 90 patients (51 mild,39 moderate) and 27 healthy controls. Frequencies of Tregs, CD4+CD25"Foxp3+ T cells and CD3+, CD3+CD4+ and CD3+CD8+ T cells were enumerated by flow cytometry. Circulating levels of 15 cytokines/ chemokines were measured using Luminex technology and mRNA levels of IL-10 and TGF-â were assessed by real-time RT-PCR.

Findings

Significantly higher frequencies of Tregs were observed in mild cases, especially during post-defervescence. The difference between mild and moderate cases was more evident in secondary infections (Fig. 1). The levels of IL-6, IL-7, IL-8, TNF-á and IL-10 were significantly higher in moderate cases. IL-6 and IL-8 levels correlated negatively with Treg frequencies during post-defervescence and in secondary infections. Higher levels of IL-10 and TGF-â in moderate cases were not reflected by their corresponding mRNA levels. Platelet counts correlated positively with Treg frequencies and TGF-â levels and negatively with IL-10 levels.

Summary

Higher Treg frequencies may favor a beneficial outcome in dengue. Higher cytokine levels may indirectly contribute to disease severity by exerting an inhibitory influence on Tregs. The dichotomy between mRNA and proteins levels for IL-10 and

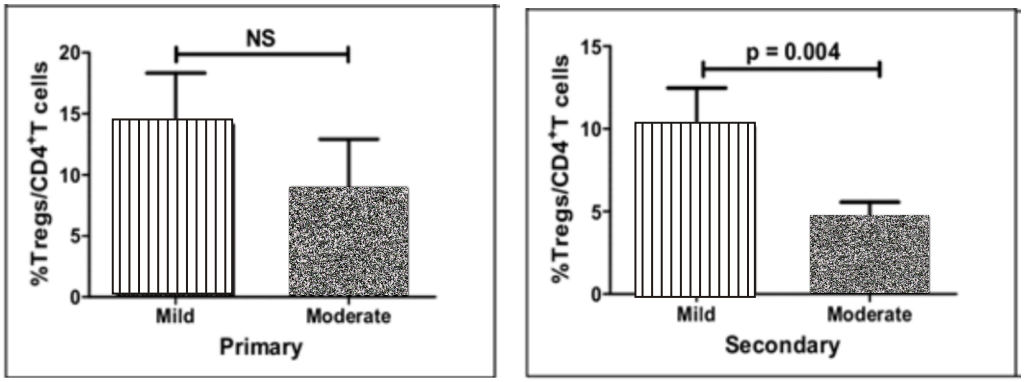


Fig.1. Treg frequencies in mild Vs. moderate dengue cases with primary Vs. secondary infections

TGF-β is suggestive of increased translational efficiency. The ongoing studies will determine the Treg recall response to different viral proteins *in vitro* in PBMC cultures of immune individuals. For this purpose, NS1 and NS3 genes of DENV-2 were cloned and expressed and DENV-2 stocks were semi-purified to represent structural proteins.

DEN 1311: Role of Dengue virus Core protein in viral replication

***Investigators**: A Tiwary & C Dayaraj*

Duration: 2012-16

Funding Agency: Intramural

Background

Core (C) protein of DENV has been shown to localize to the nucleus but the functional significance is not understood. We had earlier shown that the C protein preferentially localizes to the nucleolus in DENV infected mammalian cell lines but not insect cells (C6/36). We demonstrated that the localization of the core protein was dynamic by FRAP analysis, 33% was stably bound and 67% was mobile.

Objectives

- To understand the significance of dynamic subnucleolar interaction of core with nucleolus in dengue replication.

Work done

The nucleolus has three different regions, which are involved in specific steps of ribosome biogenesis, the fibrillar center (FC) and dense fibrillar center (DFC) involved in pre-rRNA transcription and the granular component (GC) involved in formation of

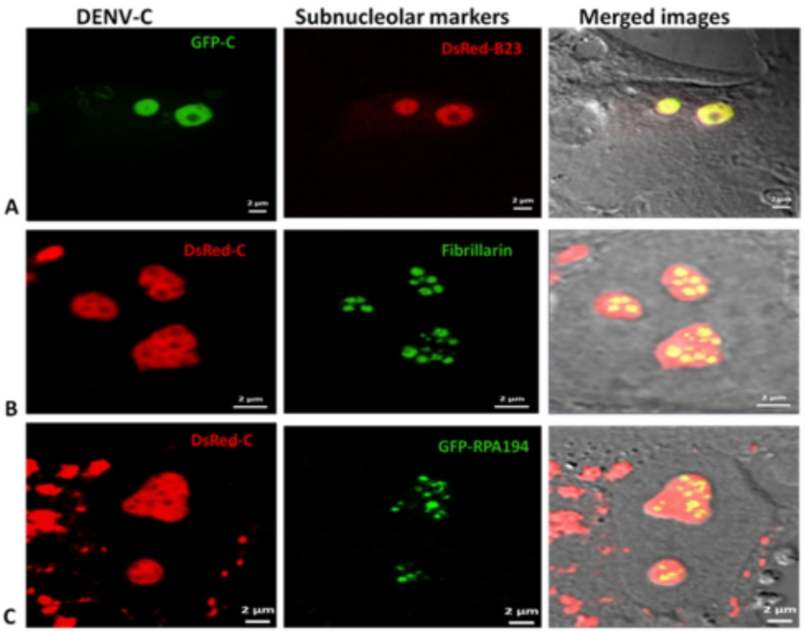


Fig. 2 Co-localization of DENV core protein with B23 in granular component of nucleolus

sub-ribosomal subunits. BHK-21 cells were co-transfected with plasmids expressing GFP or DsRed labeled DENV C protein and plasmids expressing nucleolar markers *i.e.,* DsRed-B23 for GC or GFP-Fibrillarin for DFC or GFP-RPA194 for FC.

Findings

After 24 h of transfection, live cells were analyzed for co-localization. No co-localization was observed with markers for FC and DFC regions. The GFP-C was observed to co-localize with DsRed-B23 in the GC region (Fig.2). B23 has been attributed several functions including regulation of host transcription.

Summary

Therefore, selective localization of DENV C protein may be involved in controlling host transcription and promote virus replication.

Host microRNA response against chikungunya virus infection

Investigators*: S Ambekar, A More & D Parashar*

Duration: 2012-16

Funding agency: DST

Background

Micro RNAs are posttranscriptional regulators (around 22 nucleotides) that bind to complementary sequences in the three prime untranslated regions of target messenger RNA (mRNA) and usually result in gene silencing. Previous studies suggest that miRNAs, which are implicated in rheumatoid arthritis, showed differential regulation in CHIKV infection. The miRNA profile (384 miRNAs) was analyzed in 1BR3 (human fibroblast). Infection with CHIKV resulted in differential regulation of 13 miRNAs (hsa-mir-140, 146a, 146b, 125a, 125b, 155, 15a, 16, 17-3p, 17-5p, 203, 223, 99a). i.e. hsa-mir-140, 146a, 155, 15a, 16 and 125b.

Objectives

- To compare miRNA expressions in chikungunya patients and healthy controls.

Work done:

We collected acute blood samples from nine patients and five healthy controls. Primers were designed for six of these 13miRNAs. PBMC's were separated and total RNA was isolated for real time RT-PCR.

Findings

Results of the present study suggest that the six miRNAs were differentially regulated in Chikungunya patients when compared with healthy controls. Host mir-146a and mir-155 showed significant upregulation.

Influence of Vitamin D on innate immune response to dengue virus

Investigators: *A Salunke & A Kalichamy*

Duration: 2015-17

Funding Agency: SERB (DST, grant # YSSFF/2014/000284)

Vitamin D is a modulator of immune responses. Our earlier study has shown elevated levels of vitamin D in symptomatic dengue cases particularly in secondary DHF cases compared to healthy controls. Moreover, polymorphisms in vitamin D receptor gene have also been shown to be associated with DHF. The objective of the study was to study the effect of vitamin D and vitamin D induced antimicrobial peptide LL-37 on the replication of DENV and innate immune response to the virus. To study the effect of LL-37 on cell proliferation, PS cell monolayers were cultured with different concentrations of LL-37 for 72 h. Cell proliferation was assessed by MTT assay. Concentrations above 100 μg/ml inhibited cell proliferation. To study the effect of pre-treatment of virus with LL-37 on viral replication, DENV-2 was treated with different concentrations of LL-37 (ranging from 20 – 100μg/ml) and allowed to infect PS cell monolayers and cultured for 4 days. The culture supernatant was assayed for viral RNA by real time RT-PCR and infectious particles by plaque forming unit (PFU). The results revealed dose dependent inhibition of the replication of virus by LL-37 (Figure 3). Further studies are in progress.

Referred samples tested (Jan-Dec 2015)

Dengue IgM – 2637 tested, 1260 (47.7%) positive

DENV serotyping – 239 tested, 82 were positive (all four serotypes)

Chikungunya IgM – 586 tested, 252 were positive (44.4%)

CHIKV RNA detection – 65 tested, 12 positive

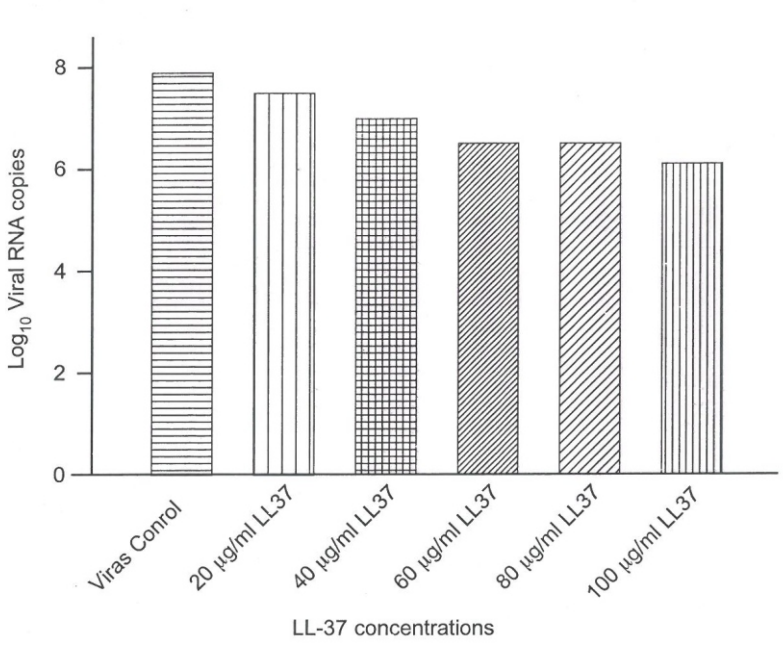


Figure 3: Effect of pre-treatment of DENV-2 with LL-37 on viral RNA levels

ENTERIC VIRUSES GROUP

Scientific staff

Dr. V. Gopalkrishna	Scientist E & Group Leader

Technical Staff

Mr. Tikute SS	Technical Officer (A)
Dr. (Mrs.) Joshi MS	Technical Officer (A)
Mrs. Ranshing SS	Technical Assistant
Dr. (Mrs.) Tatte VS	Technical Assistant
Mr. Jadhav PS	Technical Assistant
Mr. Doiphode RS	Technician C
Mr. Ubale SK	Technician B
Mr. Shinde MS	Technician B
Mrs. Chavan NA	Technician B

Project Staff

Dr. Jain PN	Scientist B
Dr. Ghuge R	Scientist B
Ms. Chothe NS	Technical Assistant
Ms. Borawake K	Technician C
Mr. Jagtap VD	Technician C
Mr. Takale GH	Multi Tasking Staff
Ms. Rongala L	ICMR-SRF
Ms. Kulkarni R	UGC-SRF
Ms. Lasure N	UGC-SRF
Ms. Pradhan G	CSIR-SRF

ENV 1301: Laboratory diagnosis of enteric viruses in referred samples

***Investigators**: V Gopalkrishna, MS Joshi, SS Ranshing & NS Chothe*

Funding Agency: Intramural

Project duration: Ongoing

(i) Acute gastroenteritis outbreak in Bhimashankar, Pargaon, Pune.

An outbreak of acute gastroenteritis at Bhimashankar Sahakari Sakhar Karkhana (Sugar factory), Pargaon with 1789 cases (IPD=321; OPD=1468) was investigated during November-December 2015. Ninety percent of the patients belonged to 15 to 59 year age group and clinical severity score indicated severe disease in 67.8% and moderate disease in 32.2%. Source of infection was thought to be a well situated near Bhîma River, which is the only source of drinking water for the village. Water samples collected from the well and water storage tank, showed presence of >16 coliforms and declared unfit for drinking by state/sub divisional health laboratory. Thirty-two stool samples were collected from patients for determining viral etiology. Group A rotavirus and enterovirus were detected in one sample each, while all the samples tested negative for Norovirus (NoV), Adenovirus (AdV) and Group C rotaviruses. Eight specimens showed positivity for Group B rotavirus (GBR) by RT-PCR and 7 specimens showed typical RNA migration pattern of GBR (4-2-1-1-1-1-1) by RNA-PAGE. Seventeen clinical specimens were analyzed by electron microscopy and 3 showed rotavirus particles. Further investigations are in progress.

A. Acute gastroenteritis in Miraj, Sangali: a retrospective study.

***Investigators**: V Gopalkrishna NS Chothe & PR Patil*

Cases of acute gastroenteritis were reported from Miraj and rural areas of Sangli district (Maharashtra) during November 2014 and NIV received 33 faecal specimens for diagnosis and characterization. Three of the 33 samples (9%) tested positive for rotavirus by ELISA. Positive samples subjected to multiplex PCR for VP7 (G) and VP4 (P) gene typing revealed the presence of G1P(8) genotype. All the 33 samples were further tested for detection of NoV, Astrovirus (AstV), Aichivirus (AiV), AdV, GBR, Enterovirus (EV) and Parechovirus (HPeV). Three tested positive for NoV and HPeV; one for AstV; two for EV and 5 for GBR positivity. Samples were negative for AdV and AiV. Genotyping of EV and HPeV positive samples were carried out using VP1 gene. Sequencing of EV, HPeV and GBR amplicons revealed the presence of EV-99 and HPeV-1 and HGBR.

ENV1302: Assessment of genetic diversity in group A rotavirus isolates

***Investigators**: VS Tatte & V Gopalkrishna*

Funding Agency: Intramural

Background

The genetic diversity amongst group A rotaviruses has been reported earlier. This information is very crucial in understanding both disease epidemiology as well as for intervention strategies.

Objectives 2015-16

- To isolate common and unusual GARV strains in cell culture and to determine their genogroups.

Work done

Three G9P [4] RV strains isolated earlier from Pune region in MA-104 cell line were further passaged and confirmed by VP7 and VP4 gene based multiplex PCR. Cell culture supernatant detected VP7-G9 and / or G2 and VP4-P[4] genotypes. Multiplex PCR for the VP7 and VP4 genes of the clinical samples selected for isolation confirmed unusual G-P combination (G9P[4]) of RV strains. Sequencing and phylogenetic analysis of the VP6 and NSP4 genes of the RV isolates was carried out.

Findings

Phylogenetic analysis of the VP6 gene of the three isolates showed clustering in the I2 genotype. All the three isolates showed 88.1-97.3% nucleotide identity and 81.6-99.1% amino acid identity with the prototype strain DS-1. With the reference strains from I2 genotype 73.7-97.3% nt and 79.6-99.1% aa identity was noted. Within the strains 0.3-0.6% nt / 0.0-9.9 % aa divergence was noted (Fig 1). Phylogenetic analysis of the NSP4 gene of the two isolates showed clustering in the E2 genotype for the NSP4 gene and showed 84.0-94.7% nucleotide identity 93.9-95.3% amino acid identity with the prototype strain DS-1. With the reference strains from I2 genotype 80.8-96.5% nt and 82.4-97.1% aa identity was noted. Within the strains 1.3% nt / 4.0% aa divergence was noted.

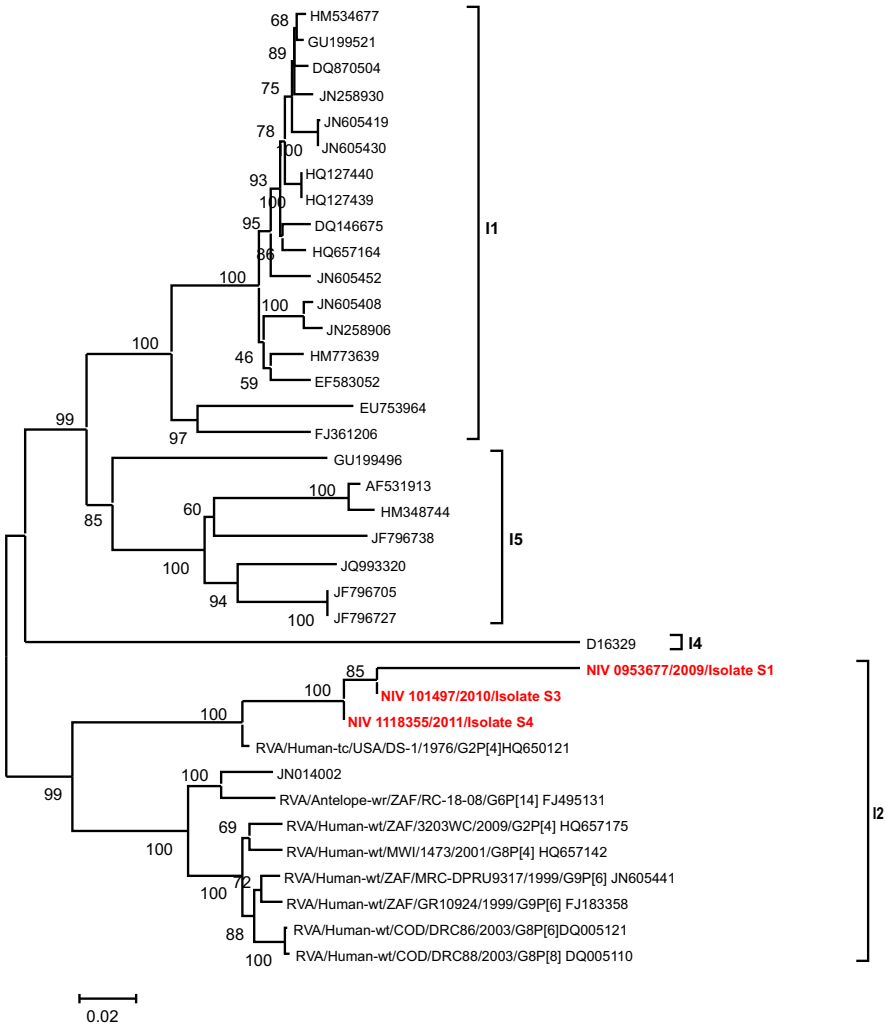


Fig 1: Phylogenetic tree based on the nucleotide sequences of VP6 gene (792-1130 nt) of cell culture isolates of G9P[4] rotavirus strains . The strains of the present study are highlighted in red

Summary

This preliminary analysis shows that wild types group A rotavirus strains in circulation shows distinct genetic diversity. Sequencing of other RV genes (VP1-VP3, NSP1-NSP3, NSP5) of the isolates and isolation of rotavirus strains with unusual G-P combinations are in progress.

ENV 1305: Development of immunoassays for detection of Norovirus infection using recombinant Norovirus GII.4 capsid proteins

***Investigators**: R Kulkarni, K Lole & SD Chitambar*

Funding agency: Intramural

Project duration: 2013–16

Background

Noroviruses (NoV) are found as important etiologic agents for viral gastroenteritis in children. NoV Genogroup II genotype 4 (GII.4) has been identified as the predominant genotype in human infections of NoV in India. However, the major lacunae have been the lack of reliable diagnostic assays.

Objectives

- Development of immunoassays for detection of Norovirus infection using recombinant Norovirus GII.4 capsid proteins

Work done

Acute phase serum specimens (n=191) collected from children (≤5 years) hospitalized for acute gastroenteritis in Pune were tested and 98 (51.3%) were found positive with 61, 34 and 3 having IgG, IgG-IgA and IgG-IgA-IgM respectively. Histoblood group antigen (HBGA)-blocking antibodies were detected in

33 of the 54 positive specimens. IgG and blocking antibody prevalence and titer varied with age and was found lowest among infants (6-23 months).

Summary

Testing of the corresponding acute phase fecal specimens for NoV RNA indicated that antibody-positive children, suggesting past Norovirus exposure, showed significantly lower fecal NoV RNA detection rate than antibody-negative children.

ENV 1306: Diversity of non-Rota enteric viruses in patients with acute gastroenteritis

***Investigators**: N Lasure & V Gopalkrishna*

Funding agency: Intramural

Project duration: 2013-16

Objective:

- Molecular detection and characterization of Sapovirus, human Bocavirus and salivirus strains in outbreaks (retrospective) of gastroenteritis

Background

Association of non-Rota enteric viruses in acute gastroenteritis has been reported among infants and young children in our earlier studies, Sapovirus (SaV), human Bocavirus (HBoV) and salivirus has been detected at a frequency of 0.9-10.2%, 6% and 2.4% respectively in children hospitalized with acute gastroenteritis suggesting their possible etiology. However, no such studies have been conducted in outbreaks of gastroenteritis in western India.

Objectives

- Molecular characterization and genetic diversity of SaV, BoV, SaliV's in outbreaks of acute gastroenteritis.

Work done

During the year, acute clinical samples collected during the Solapur gastroenteritis outbreaks of 2010 (253) and 2011 (47) were screened for the presence of SaV, HBoV and salivirus using a PCR targeting the RdRp-Capsid junction region for SaV (~420bp), VP1/VP2 region for HBoV (~575bp) and 2C region for salivirus (~354bp).

Findings

SaV was not detected in the 2010 and 2011 outbreak samples However, HBoV was detected in 4.3% (2/47) of the 2011 Solapur outbreak samples. Co-infections with other enteric viruses such as rota, noro, sapo, astro, adeno, aichi, sali, entero and parecho were not detected. HBoV was detected in children aged between 18 to 40 months. Two of the HBoV positive strains belonged to HBoV3 genotype and shared >99% amino acid identity between themselves and 96.7-99.8% with HBoV3 strains detected previously in Pune.

Summary

The study indicates prevalence of HBoV3 in sporadic as well as outbreak cases. Although HBoV was detected in outbreaks of acute gastroenteritis, rotavirus was found to be the major etiological agent.

ENV 1307: Pathogenesis of Coxsackie virus A-16 associated with hand, foot and mouth Disease (HFMD) in neonatal mice

***Investigators**: S Tikute & V Gopalkrishna*

Study period: 2013-16

Funding: Intramural

Background

HFMD is a childhood viral infection. Cases were reported earlier from southern and eastern parts of India. Coxsackie virus A-16 (CVA 16) was found as the major etiological agent. However, pathogenicity of CVA-16 in HFMD has not been reported from India.

Objective

- To study the pathogenicity of CVA-16 infection using neonatal mouse model.

Findings

In continuation to the earlier study, different organs *viz.*, brain, heart, liver, lung, kidney, spleen, intestine and skeletal muscles collected from CVA16 strain inoculated CD1 mice were homogenized in MEM. RNA was extracted and subjected for RT-PCR (5'NCR) to detect the virus. Twenty-four samples from experimental groups *i.e.,* of day 1, 3, 5, 7, 11 and day 13 were found positive for EV. The EV

positive samples were subjected for VP1 genotyping, which showed 98% homology with CVA-16 of Yamagata strain (AB772003).

ENV 1308: Molecular characterization of non-polio enterovirus strains from patients with acute flaccid paralysis (AFP)

***Investigators**: L Rongala, S Cherian & SD Chitambar*

Funding agency: Intramural

Project duration: 2013-2016

Background

Coxsackievirus-B3 (CV-B3) of enterovirus-B (EV-B) species is known to cause varied spectrum of infections from asymptomatic to fatal in humans. Complete genomes of CV-B3 strains isolated from AFP cases and their asymptomatic contacts from different regions have not been reported from India.

Objectives

- To examine the genomic variations among strains of the same NPEV genotype related to diverse infection sequelae from no symptoms to minor febrile illness to irreversible paralysis.

Findings

Phylogenetic analysis of complete VP1 gene sequences of global CV-B3 strains classified Indian CV-B3 strains into genogroup GVI, along with strains from Uzbekistan and Bangladesh and into a new genogroup, GVII. Genomic divergence between genogroups of the study strains was 14.4% with significantly lower divergence (1.8 %) within GVI than that within GVII (8.5%). The strains from both AFP cases and asymptomatic contacts, identified mainly in the coastal Karnataka and Kerala, belonged to the dominant genogroup GVI, while the GVII strains were recovered from AFP cases in north interior Karnataka. All the strains carried inter-genotypic recombination with the structural region similar to reference CV-B3 strains and 5' non-coding regions and non-structural regions closer to other enterovirus B types. Domain II structures of 5' non-coding regions, described to modulate virus replication were predicted to have varied structural folds in the two genogroups and were attributed to differing recombination patterns. Complete genomes of CV-B3 strains isolated from AFP cases and their asymptomatic contacts from different regions were determined for the first time in India

Summary

The study indicated two distinct genetic lineages of CV-B3 strains circulating in India.

ENV-1309 & 1310: National hospital based rotavirus surveillance network (referral & peripheral site)

***Investigators**: V Gopalkrishna, P Jain & R Ghuge*

Funding agency: ICMR (Extramural)

Project duration: 2013-2016

Background:

The study involves a national hospital based surveillance of rotavirus disease and strains among children <5 years for determination of the age, seasonal distribution and prevalent G-P types at multiple centres in India. NIV Pune is one of the referral centers and represents the west zone of India comprising four peripheral sites, *viz.,* Pune (KEM Hospital, Bharati Hospital and Shaishav Clinic); Mumbai (LTMGH & MC), Ahmadabad (BJMC) and Surat (SMIMER) and RMRC Belgaum with two clinical recruitment sites (CRSs) (JNMC, Belgaum and KIMS Karad).

Work done and findings:

A total of 1457 stool specimens collected during the year from Pune (304), Mumbai (156), Ahmadabad (110), Surat (381), Karad (314) and Belgaum (192) were screened. Rotavirus positivity was detected in 459 specimens in a range of 21.15%- 43.75% from all sites with a mean value of 31.5%. The highest rotavirus positivity (35.5%- 44%) was noted in 7-12 month age group in all CRSs except Mumbai where the age group of 0-6 months was found to be more predominant (42.4%). Over all, rotavirus positivity was found more during winter (35.7%) and post monsoon (37%) seasons though it varied between different CRSs. Of the 459 rotavirus strains, genotyping of VP7 (G) and VP4 (P) genes was carried out for 146 strains.

Summary

The data showed the predominance of G1P [8] strains (28.8%) followed by G9P [4] (24%), G2P[4] (20.5%) and G3P[8] (15.1%) in circulation. G9P [8], G12P[11], G1P[6], G12P[6], G2P[6], G9P[6] and mixed strains were detected, though at low levels (0.7% - 2.7%).

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

***Investigators**: SD Chitambar & GR Ghalsasi (Venky India Ltd, Pune)*

Funding Agency: Venky's India Ltd. Pune

Duration: 2013-2016

Summary

In continuation to the earlier work, IgYs against cell culture adapted human rotavirus (HRV) types 1, 3 and 9 were prepared, checked purity and evaluated their activity against rotavirus infection in mice. HRV-2 and HRV-4 are being propagated in cell culture for generation of IgYs.

ENV 1501: Identification and molecular characterization of group C rotaviruses in humans and animals with acute gastroenteritis from western India: a retrospective study

***Investigators**: MS Joshi & V Gopalkrishna*

Funding Agency: Intramural

Project Duration: 2015-2018

Background

Molecular 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 bovine species respectively. GCR is globally distributed in sporadic and epidemic cases of gastroenteritis patients in all age groups. However, role of GCR in causing gastroenteritis in both humans and animals is unknown and not reported from India.

Objective

- To detect and characterize GCR in patients with acute gastroenteritis.

Findings

Retrospective stool specimens collected from sporadic (n=147, Pune, Aurangabad) and outbreak (n=253) (Sholapur, Mumbai, Miraj) cases of acute gastroenteritis during 2006-2014 were screened for Group C rotavirus (GCR) RNA using partial VP6 gene based RT-PCR assay followed by nucleotide sequencing and phylogenetic analysis. The GCR detection rate was observed to be 8.3 and 0.68% among outbreak and sporadic cases respectively. Among the outbreaks investigated, rural outbreaks (n=3) showed detection rate between 8.4% and 19.0% while urban outbreaks showed 1.6%. The age distribution analysis showed occurrence of GCR mainly (76.9%) in patients of pediatric group. Phylogenetic analysis of the strains demonstrated two clusters within I2 genotype of VP6 gene as one with the rural region outbreak strains and the other with a mixture of rural and urban strains (Fig 2). The strains of the latter cluster need to be studied further due to their unique nucleotide and amino acid substitutions.

Summary

This is the first study to demonstrate GCR infections in outbreaks of gastroenteritis in India.

ENV 1502: Development of a multiplex PCR assay for detection of viral pathogens associated with diarrheal diseases

***Investigators**: VS Tatte & V Gopalkrishna*

Funding Agency: Intramural

Project Duration: 2015-2018

Background

Diarrhoea caused due to viral pathogens is a major public health problem. Recently, several novel enteric viruses *viz.*, Aichi virus, enteroviruses, parechoviruses, Sali/klassie and HBoV have been found to be associated with acute gastroenteritis in addition to rotaviruses, Noro and enteric adenoviruses. Hence, development of a simple, rapid and cost effective multiplex PCR for simultaneous detection of the enteric pathogens in diarrhea cases is essential for providing rapid diagnosis.

Objective

- To develop multiplex PCR assay for detection of enteric viruses associated with diarrheal diseases.

Findings

In continuation to the previous years work, specimens showing positivity for a single virus were selected for further standardization of multiplex PCR for 9 different enteric viruses. Multiplex PCR in three tubes *i.e.,* tube No. 1 containing Aichivirus,

Sapovirus and human Parecho virus; tube No. 2 containing Boca, Rota and Astro viruses and tube No. 3 containing Entero, Adeno and Noro viruses showing different band sizes was further standardized. Clinical specimens were tested for 6 different enteric viruses using the standardized multiplex PCR. Screening of remaining clinical specimens is in progress.

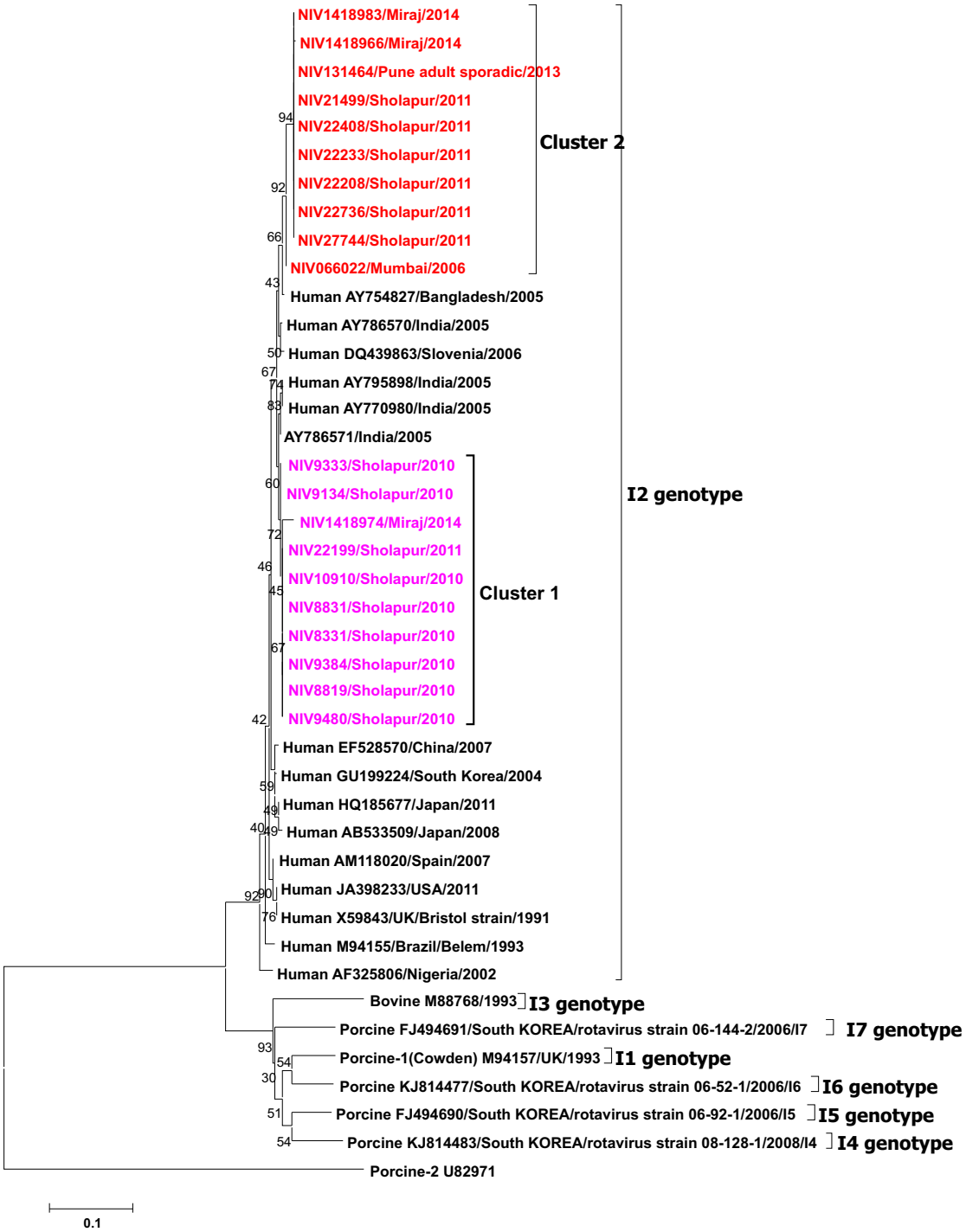


Fig 2: Phylogenetic tree constructed based on the partial nucleotide sequences of VP6 gene (372bp) of GCR strains. The strains of the present study are shown as Cluster 1 (pink color) and Cluster 2 (red color). The reference strains are indicated by accession numbers followed by the country name and year. Scale indicates genetic distances.

DIAGNOSTIC REAGENT FACILITY

Scientific Staff:

Dr. Paresh Shah	Scientist-E& Group Leader

Technical Staff:

Mr. T. L. Gangadhararao	Technical Officer-A
Mr. Sujeetkumar Belani	Technical Officer-A
Mr. Kishor Kshirsagar	Technical Assistant
Mr. Anant Deshpande	Technical Assistant
Mr. Ashok Kamble	Technical Assistant
Smt. Shobha Kulkarni	Technician-C
Mrs. Reva More	Technician-C
Ms. Snehal Veer	Technician-C
Mrs. Anjana Ugale	Technician-B
Mrs. Purvi Patel	Technician-A
Mr. Mukesh Chavan	Technician-A
Mr. Sanjay Jadhav	Multi Tasking Staff

Project Staff

Dr. Kanchankumar Patil	Scientist-B
Mr. Sudipta Dhani	Technical Officer-A
Mrs. Rupali Jagtap	Technical Assistant
Mrs. Swati Totad	Technical Assistant
Mrs. Jyotsna Khade	Technical Assistant
Ms. Sapna Gawhale	Technical Assistant
Ms. Aparna Rakhe	Technical Assistant
Ms. Anuja Satvekar	Technical Assistant
Mr. Tushar Raut	Technical Assistant
Mrs. Shweta Chondhe	Technician-C
Mr. Prashant Gore	Technician-A

DRF 1501: Scaling up of facilities for production of diagnostic kits/ reagents for detection of JE, DEN & CHIK virus IgM antibodies

***Investigators**: Paresh Shah*

Funding agency: NVBDCP

Project duration: Ongoing

Production & supply of MAC ELISA diagnostic kits to SSHs and 15 national laboratories (Apex) engaged in the diagnosis of Japanese encephalitis (JE), dengue (DEN) and chikungunya (CHIK). The kits are being supplied as per the need of the sentinel centers during outbreaks and post outbreaks.

During 2015-16, 6994 MAC ELISA kits were supplied to SSH and Apex labs under the national program as well as to WHO SEAR for onward transmission to neighboring countries (Table 1). Kits were provided to >500 sentinel centers identified in the country as per their requirements. During non-outbreak period also, the kits were in demand either due to outbreaks in neighboring areas or for surveillance and hence kits were supplied throughout the year (Fig 1).

DRF 1502: Development of JEV/ WNV combo IgM Capture ELISA test

***Investigators**: Paresh Shah*

Funding agency: Intramural

Project duration: 2015-17

Increased West Nile virus (WNV) activity with fatalities has been reported from Assam and Kerala recently. Diagnosis of WNV is done through virus isolation and sequence analysis which is time consuming and expensive. Moreover, it is always

Table 1: Supply of MAC ELISA kits in 2015-2016

Supplied to	JE	DEN	CHIK	TOTAL
National laboratories and sentinel centers	418	5410	1044	6872
WHO-SEAR, New Delhi	102	10	10	122
Total	520	5420	1054	**6994**

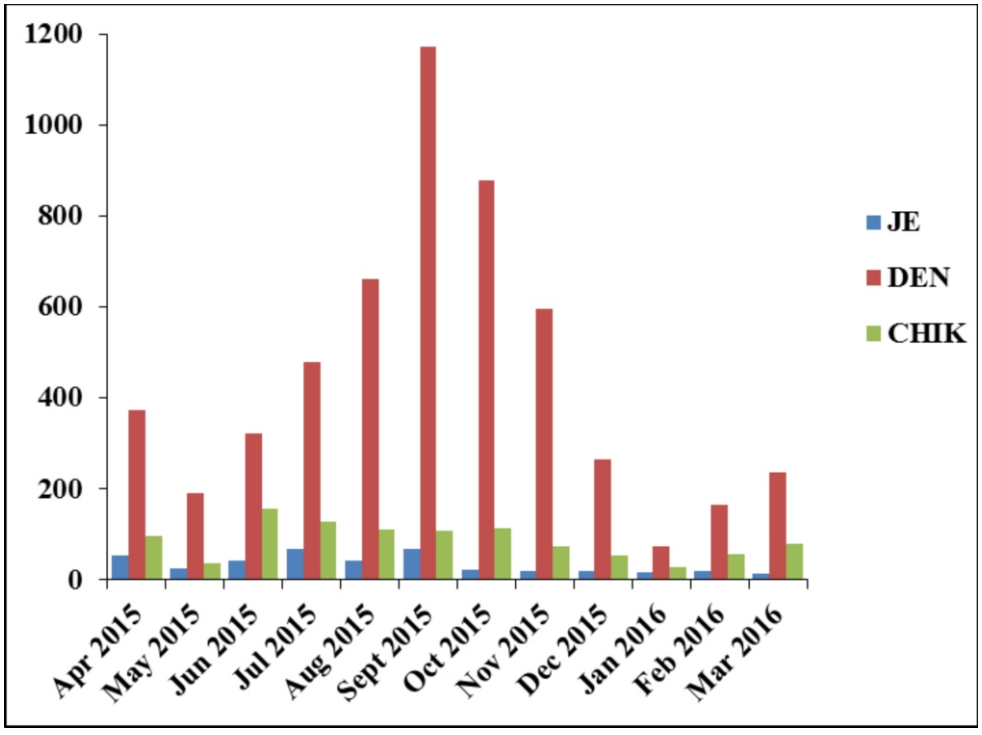


Fig 1: Month-wise supply of MAC ELISA kits under the National Program

difficult to diagnose JEV and WNV infection using serological tests. An attempt was therefore made to develop a combo kit for diagnosis of WNV and JEV. During the year, a new JEV/WNV combo ELISA kit has been standardized using limited archived WNV positive samples (positive by micro Neutralization test). It has also been compared with Commercial ELISA kit (In-Bios) and found satisfactory. The ratio of OD (WNV Ag)/ OD (JEV Ag) of all the WNV positive samples were found to be in the range of 1.5 – 3.9 confirming WNV positivity. Validation of the Combo ELISA with more number of WNV positive and negative samples is underway.

Training (hands on and class room) for SSHs:

Imparted hands on training to perform the MAC ELISA kits and the analysis of results to staff and medical officers of newly identified SSHs in Pune to enhance the diagnostic capabilities.

MEDICAL ENTOMOLOGY AND ZOOLOGY

Scientific staff

Dr. Sathe PS	Scientist 'F' & Group Leader
Dr. Sudeep AB	Scientist 'D'
Dr. Gokhale MD	Scientist 'C'

Technical staff

Mrs. Mavale MS	Technical Officer (A)
Mr. Aher RV	Technical Assistant
Mr. Khude PD	Technical Assistant
Mr. Ghodke YS	Technical Assistant
Mr. Sonawane PA	Technical Assistant
Mr. Ingale VS	Technician-'C'
Mr. Lekhraj KN	Technician-'B'
Mr. Dhaigude SD	Technician-'B'
Mr. Kunal Nikalje	Technician-'A'
Mr. Meliya MZ	Multi Tasking Staff

ENT1601: Entomological studies during viral disease outbreaks.

***Investigators**: Gokhale MD & Sudeep AB*

Funding Agency: intramural operational

Project Duration: ongoing

Kyasanur Forest Disease (KFD) virus Survey

Background & Objectives

KFD cases were reported from certain villages of Sindhudurg district of Maharashtra during January 2016. The villages are surrounded by tropical evergreen forest with thick undergrowth of bushes and shrubs and local inhabitants visit the forest daily to fulfill their needs and requirements. Since ticks are the vectors of KFD virus, a survey was undertaken in the affected villages to determine the species of ticks involved in the transmission of the virus in the area.

Work done

Vector studies were undertaken at the Dodamarg Taluka in between 23 and 28 February 2016. Larval and adult stages of ticks were collected from 7 locations; 5 affected areas that have reported human cases and monkey death in the very recent past and 2 areas from where no human /monkey cases have been reported. In all 1388 tick individuals were collected, identified and pooled based on their genus/species status, sex and stage. The details of the pools are as follows *viz. Haemaphysalis spinigera* (23 pools/1030individuals), *H turturis* (6 pools/61 individuals), *Amblyomma spp* (5 pools/5 individuals), *Boophilus spp* (4 pools/9 individuals), *Dermacentor spp* (3 pools/283 individuals). The tick pools (n=41) were tested for the detection of KFD virus by real time RT-PCR technique at BSL-4 lab.

Findings

Two tick pools (One from Ker village and the other from Talkat forest) tested positive for KFD virus.

ENT1302: Bionomics of Phlebotomine sandflies in Nagpur division of Maharashtra and Andhra Pradesh and determination of their role in transmission of Chandipura virus and other AES causing agents.

***Investigators**: Sudeep AB, Gokhale MD & Harsh Pawar (University of Pune).*

Funding: Intramural

Project Duration: 2014-2016

Background

Cell lines from sandflies are very important for studying host-virus interactions of human pathogenic viruses as sandflies are intermediate vectors in the ecobiology of several pathogenic arboviruses like Chandipura virus (CHPV). However, there are no reports of the protein profile of these cell lines and developing such information will help in better understanding of both virus replication and disease biology.

Objective

- Preliminary proteome characterization of cell lines developed from sandflies

Work done

Proteome of PP-9 cell line, a cell line developed from *Phlebotomus papatasi* sand flies has been mapped using a comparative proteogenomic-based approach.

Findings

The study identified a conserved set of proteins that are shared between the different dipterans. Majority of peptides (3380) mapped to the three frame translated transcript database of *P. papatasi* resulted in the identification of 813 unique proteins. However, careful analysis of the corresponding translated entries by protein homology searches revealed all of the proteins identified in *P. papatasi* were well conserved. The study also identified 1381 unique peptides that mapped to 583 proteins from the four related dipterans *viz.*, *Aedes aegypti*, *Anopheles gambiae*, *Culex quinquefasciatus* and *Drosophila melanogaster*.

Summary

The study resulted in identification of 1,313 proteins in *P. paptasi* based on homology with related sequenced dipterans. The present proteogenomic analysis of *P. papatasi* illustrates the value of high-resolution MS-derived peptide data in mapping the proteome of sand flies. The present study reports the largest catalogue of *P. papatasi* proteome till date and many proteins identified in this study were not reported previously in sand flies.

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

***Investigators:** AB Sudeep, D Parashar & MD Gokhale*

Duration: 2013-16

Funding: Intramural

Background and Objective (2015-16)

Chikungunya virus (CHIKV) has emerged as a major public health problem in India and South East Asian countries. Since no licensed vaccine is available attempts were made to attenuate CHIKV by serial passaging in cell lines. As reported in the last AR, one of the strains has lost virulence completely and during the year, whole genome sequence analysis of the attenuated strain was carried out.

Findings and summary

The attenuated strain showed 99.99% nucleotide identity with original virus strain and the sequence data is being analyzed currently.

Development of ELISA for detection of dengue virus (es) in vector mosquitoes

***Investigators**: MD Gokhale & PS Sathe*

Duration: Preliminary work started

Funding Agency: Intramural

Background

Standardization of a sensitive, specific and cost effective assay to monitor dengue virus infected vector mosquitoes in the field is a complex process. The existing assays like virus isolation in susceptible cell lines, mosquito and mice inoculations, detection of viral RNA are time consuming and need specialized laboratory and experienced technical staff / personnel and are difficult to perform in the rural settings like PHC's. Therefore to address this lacuna and develop better assays is the need-of-the hour.

Objectives

- An attempt to develop NS1 based dengue virus detection in vectors was undertaken.

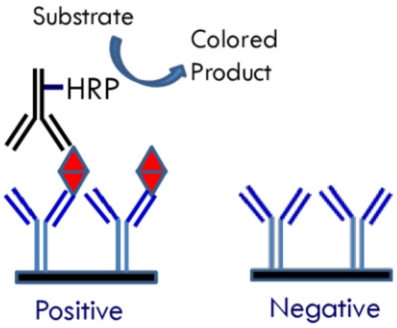
Work done

Aedes aegypti mosquitoes (4-5 days old female mosquito) were infected with dengue virus using intra-thoracic inoculation and membrane feeding method. After the respective incubation period these infected mosquitoes were tested for detection of the presence of dengue NS-1 protein using the commercial NS-1 detection kit.

Findings and summary

The preliminary results suggest that DEN NS-1 protein could be detected in the infected mosquitoes. Further work is underway.

Monitoring NS1 in response to



Schematic Presentation: Dengue NS 1 ELISA

Intra thoracic inoculation – (11th PID)

Virus inoculum	OD	P/N
Dengue 2	3.96	28.3
Dengue 3	3.88	27.7
Dengue 4	0.09	<1.0
CHIK Virus	0.09	<1.0
MEM	0.14	–

Orally fed (Blood & Den-2 Virus) – (14th PID)

Blood Virus (Oral)	OD	P/N
Pool	1.45	22.3
Pool 2	0.76	11.7
Pool 3	0.45	6.92
Pool 4	0.18	2.76
Blood (Mean)	0.065	–

MEASLES

Scientific Staff

Mr. Sunil R. Vaidya	Scientist-E & Group Leader

Technical Staff

Mr. Vijay A. Sonawane	Technical Officer-A
Mrs. Deepika T. Chowdhuri	Technical Assistant
Mrs. Neelakshi S. Kumbhar	Technical Assistant
Mr. Madhukar B. Kamble	Technician-C
Mrs. Sarang S. Kamble	Technician-B
Mr. Roben P. George	Technician-A

MMR 1101: Establishment of rapid and reliable assay for detection of measles, mumps and rubella neutralizing antibody

***Investigator**: SR Vaidya*

Funding: Intramural

Project duration: 2015-16

Background

Plaque reduction neutralization test (PRNT) measures the functional antibody by *in vitro* virus neutralization and is considered the 'gold-standard' for assessing serological correlates of protection. However, PRNT is technically demanding, time consuming, not easy to automate and has limitations for screening a large numbers of sera needed for epidemiological investigations. For large-scale studies, alternative assays that could be performed in 96-well tissue culture plates would be preferred. However, commercially available ELISAs did not differentiate neutralizing and non-neutralizing antibody.

Objectives

- Development of a cell culture-based rapid and reliable immuno-colorimetric assay (ICA) for measles, mumps and rubella viruses and its utility for characterization of immune responses.

Work done

Use of ICA was documented on 35 virus isolates, three vaccine strains and clinical specimens collected from suspected cases of measles and mumps. Furthermore, application of ICA in a neutralization test (*i.e.* focus reduction neutralization test, FRNT) was documented.

Summary

This may be useful for seroepidemiological, cross-neutralization and pre/post-vaccine studies in India.

MMR 1202: Genetic and antigenic characterization of measles, mumps and rubella virus isolates

***Investigator**: SR Vaidya*

Funding: intramural

Duration: Ongoing operational project

Genetic and antigenic characterization of mumps viruses (seven isolates; three genotypes) has been completed. However, full genome sequencing as well as cross-neutralization activity of measles and rubella viruses could not be carried out.

MMR 1201: Routine laboratory activities: outbreak Investigations/ diagnostic services provided to local/state health authorities/ WHO-reference laboratories

***Investigator**: SR Vaidya*

Funding: NIV & WHO India

Duration of project: On-going

During the year, 222 serum samples (Male-130; Female-92) were referred for measles virus diagnosis from 19 hospitals/institutions in Maharashtra and Chhattisgarh. Majority of the suspected cases belong to 0-15 years (n=204, 91.89%). Among the 222 cases, 3 had history of measles vaccination (by documentation or parent's recall) during childhood.

During screening, 130 (58.55%) samples were found positive, 89 were negative and 3 were equivocal for Measles IgM antibody. Of the 18 suspected adult cases (>15 yrs., 12 male & 6 female), 7 were confirmed as measles (6 male &1female) and six as rubella (2 male & 4 female). The measles negative and equivocal serum samples (n=92) on screening for rubella IgM antibody, thirty-five tested positive; 52 negative and 5 equivocal. Measles could not be confirmed in the vaccinated individuals. Laboratory reports were sent to the concerned authorities to undertake necessary measures and weekly/monthly reports were sent to WHO.

During this year, 103 clinical specimens collected from different outbreaks or sporadic cases from the States of Maharashtra (n=32), Gujarat (n=46), Rajasthan (n=2), Dadra & Nagar Haveli (n=9), Diu & Daman (n=2), Madhya Pradesh (n=8), Chhattisgarh (n=2) and Karnataka (n=2) were subjected to measles virus isolation. Altogether, 28 wild type measles strains were isolated using Vero hSLAM cells (Table 1).

Table 1: Details of the measles virus isolates (wild types) obtained during 2015-16

Sr #	Age (y)/ Sex	Place (District, State/ Union Territories)	Specimen	MeV IgM EIA result	MeV RT-PCR	Genotype
1	5/F	Veraval, Gujarat	TS	NA	Positive	D8
2	9.2/F	Sagar, Madhya Pradesh	TS	NA	Positive	D8
3	12/F	Nagpur, Maharashtra	TS	Positive	Positive	D4
4	8/F	Nagpur, Maharashtra	TS	NA	Positive	D4
5	5/M*	Mehsana, Gujarat	TS	NA	Positive	D8
6	4.2/M	Gr Mumbai, Maharashtra	TS	NA	Positive	D4
7	3/M	Bhavnagar, Gujarat	TS	NA	Positive	D8
8	5/M	Gandhidham, Gujarat	TS	NA	not done	—
9	2/M	Bhavnagar, Gujarat	TS	NA	Positive	D8
10	1/F	Gr Mumbai, Maharashtra	TS	NA	Positive	D8
11	2/F	Gr Mumbai, Maharashtra	TS	Positive	Positive	D4
12	2/F	Junagadh,Gujarat	TS	NA	Positive	D8
13	10/M	Silvassa, Dadra & Nagar Haveli	TS	NA	Positive	D8
14	10/F	Silvassa, Dadra & Nagar Haveli	TS	NA	Positive	D8
15	2/M*	Silvassa, Dadra & Nagar Haveli	TS	NA	Positive	D8
16	2.6/M	Jamnagar, Gujarat	TS	NA	Positive	D8
17	12/F	Panna, Madhya Pradesh	TS	NA	Positive	D8
18	6/M	Daman & Diu	TS	NA	Positive	D8
19	5/F	Jamnagar, Gujarat	TS	NA	Positive	D8
20	7/F	Jamnagar, Gujarat	TS	NA	Positive	D8
21	4/M*	Anand, Gujarat	TS	NA	Positive	D8
22	8/F	Bijapur, Chhattisgarh	TS	NA	Positive	D8
23	7/F	Bijapur, Chhattisgarh	TS	NA	Positive	D8
24	4.3/M	Gr Mumbai, Maharashtra	TS	NA	Positive	D8
25	3/M	Sagar, Madhya Pradesh	TS	NA	Positive	D8
26	8.6/M	Jamnagar, Gujarat	TS	NA	Positive	D8
27	1.6/F	Jamnagar, Gujarat	TS	NA	Positive	D8
28	22/M*	Pune, Maharashtra	TS	Positive	Positive	D8

*History of measles vaccination, TS= Throat swab

MMR 1201: Outbreak based measles surveillance in Maharashtra

***Investigator**: SR Vaidya*

Funding: NIV & WHO India

Project duration: ongoing

Background

Maharashtra State has launched outbreak based measles virus surveillance Since October 2012 and sends 3-5 representative serum samples from each suspected outbreak to NIV for confirmation.

Objectives for 2015-16

- During the year, the State health agencies investigated 88 measles suspected outbreaks in 19 districts and sent 416 serum samples (Male-214; Female-202) for laboratory diagnosis of measles/rubella.

Findings

Of the 416 suspected cases, 231 (55.5%) were confirmed serologically at NIV (Table 2). Majority of the cases belong to 0-15 years (95.67%) Rubella IgM positivity was noted in 59 of 185 measles IgM negative and equivocal samples. Measles was confirmed in 31 cases that had a history of vaccination. Of the 18 suspected adults cases (>15 yrs., 3 male &15 female), seven (2 male & 5female) were confirmed as measles and seven as rubella (1 male & 6 female).

Summary

Of the 88 suspected measles outbreaks, 58 were confirmed as measles outbreaks; eight as rubella outbreaks and 15 as mixed outbreaks of measles and rubella. The etiology of 7 outbreaks could not be identified.

Table 2: Laboratory diagnosis performed on referred serum samples

District/No. of outbreaks	Serum samples (n)	MeV IgM Positive	MeV IgM Negative	MeV IgM Equivocal	RuV IgM Positive	RuV IgM Negative	RuV IgM Equivocal
Ahmednagar (1)	5	0	5	0	5	0	0
Akola (2)	10	5	5	0	0	5	0
Aurangabad (1)	2	0	2	0	1	1	0
Bhandara (1)	5	0	5	0	0	5	0
Chandrapur (1)	6	5	1	0	0	1	0
Gadchiroli (5)	30	17	12	1	1	12	0
Greater Mumbai (28)	132	85	44	3	2	44	1
Hingoli (1)	5	4	1	0	1	0	0
Nagpur (3)	16	0	16	0	0	16	0
Nanded (3)	11	3	8	0	1	6	1
Nasik (8)	38	15	22	1	17	6	0
Palghar (3)	15	12	3	0	0	3	0
Pune (1)	4	0	4	0	4	0	0
Raigad (3)	12	3	9	0	8	1	0
Ratnagiri (2)	10	8	1	1	0	2	0
Sangli (1)	5	0	5	0	2	3	0
Thane (21)	95	67	27	1	12	15	1
Washim (1)	5	0	5	0	5	0	0
Yavatmal (2)	10	7	3	0	0	3	0
Total (88)	416	231	178	7	59	123	3

MMR 1201: Measles virus genotyping of referred samples across India

***Investigator**: SR Vaidya*

Duration: Ongoing

Funding agency: NIV & WHO

Eighty-three throat swabs and 79 urine specimens collected from suspected measles cases (n=138) from Maharashtra, Assam, Chhattisgarh, Rajasthan, Andhra Pradesh, Madhya Pradesh, Gujarat, Rajasthan, Daman & Diu, Dadra & Nagar Haveli were referred to NIV for virus detection and genotyping. Studies have shown the circulation of measles virus genotypes D4 (n=4), D8 (n=78) and B3 (n=3) (Table 3). The sequences were deposited in the WHO global measles sequence database (MeaNS/GenBank).

Genotyping of N-gene PCR products:

One hundred twenty seven measles N gene PCR products referred from four national measles laboratories *viz.*, NIV Unit Bangalore (n=33), SGPGIMS Lucknow (n=46), KIPM&R Chennai (n=48) and Government Medical College Guwahati (n=2) were genotyped. Circulation of measles genotype B3 (n=1), D4 (n=3) and D8 (n=123) has been detected and the sequences were deposited by the respective laboratories in the WHO global measles sequence database.

MMR 1201: Laboratory confirmation of rubella infection in suspected measles cases referred during 2010-15

***Investigator**: SR Vaidya*

Funding: NIV& WHO

Table 3: Measles virus genotypes detected from different States/ Union Territories

State	Cases referred	Specimens processed	Measles genotype	GenBank ID
Maharashtra	52	54	10 D8, 1D4	KT283653, KT283654, KU248831, KU248832, KU571704, KX033380, KX033375, KX0333377
Madhya Pradesh	8	8	6 D8	KT283639, KT382313, KU571705 KU571706, KX033376, KX033378
Gujarat	51	51	36 D8, 3 D4	KT283649, KT283661, KT283650, KT283651, KT283652, KT355770, KT355771, KT355772, KT382308, KT382309, KT382310, KT588917 KT588918, KT588919, KT895342, KT895343, KU248834, KU248835, KU382090, KU248833, KU888846, KU888844, KU888845, KU888847 KU888848, KU888841, KU888843, KX033376, KX033379, KU888842
Chhattisgarh	4	4	2 D8	KU382091
Daman & Diu	5	5	3 D8	KT382312
Rajasthan	2	2	2 D8	KT283659, KT283660
Andhra Pradesh	2	2	2 B3	
Dadra & Nagar Haveli	9	9	6 D8	KT283643, KT283644, KT283645
Assam	4	4	2 D8, 2B3	Sequence to be submitted
Total	137	139	**D8=134, D4=4, B3=4**	

Duration: Ongoing

As part of the measles outbreak based surveillance, 4592 suspected measles cases from Karnataka (n=1173), Kerala (n=559) and Maharashtra (n=2860) were referred to NIV Pune and NIV Unit Bengaluru for laboratory confirmation during 2010-15. Serum samples initially screened for measles IgM antibody and samples negative for measles or equivocal samples (n=1954) were screened for rubella IgM antibody. Overall, 62.9% (2889/4592) samples were confirmed as measles while 27.7% (542/1954) were confirmed as rubella and 25.2% (1161/4592) were found negative for both. The measles infection was confirmed in 50.7% of the 1206 vaccinated cases. The distribution of confirmed measles cases for the three states is: 493 (40.8%) for Maharashtra; 90 (7.5%) for Karnataka and 29 (2.4%) for Kerala. Since, 1/3rd of suspected measles cases were laboratory confirmed as rubella, an urgent attention is needed to build rubella surveillance in India. Additional efforts are required to rule out other exanthematous diseases in measles and rubella negatives.

WHO IQA & EQA program

- A panel of serum samples (n=50) sent to KIPM&R, Chennai for re-testing for measles and rubella IgM EIA and the results were found concordant for both the viruses.
- As part of the WHO's external quality assurance, a panel of serum samples (n=20) received from Victorian Infectious Diseases Reference Laboratory (VIDRL), Australia were tested at NIV Pune using standard kits for measles/ rubella IgM antibody detection. Results indicated 100% score for measles/ rubella testing at NIV Pune and VIDRL, Australia.
- As part of the WHO's external quality assurance, a panel of molecular samples (virus coated ten filter paper discs) was dispatched to NIV by Centre for Disease Control and Prevention, USA. Results showed 100% score for measles/ rubella molecular testing (RT-PCR, Sequencing & Phylogenetic analysis).

Table 4: Number of samples tested in the reporting year

Sr #	Test	No. of samples tested
1	Measles IgM ELISA	638
2	Mumps IgM ELISA	2
3	Rubella IgM ELISA	277
4	Measles RT-PCR	137
5	Mumps RT-PCR	1
6	Rubella RT-PCR	35
7	Measles PCR product sequencing	127
8	Rubella PCR product sequencing	3

EPIDEMIOLOGY GROUP

Scientific staff

Dr. Babasaheb Tandale	Scientist E & Group Leader
Dr. Yogesh Gurav	Scientist D
Dr. Avinash Deoshatwar	Scientist C

Technical staff

Mrs. Vasanthy Venkatesh	Technical Officer (A)
Mr. Kailas Gadekar	Technician C
Mr. Rahul Jagtap	Technician B
Mr. Machindra Karanjwane	Technician B
Mr. Avanish Pande	Technician A
Mr. Vishal Khond	Multi Tasking Staff

Project staff

Dr. Sanjay Boddul	Research Associate (till June 2015)
Dr. Nidhi Shrivastava	Research Associate (from August 2015)
Mr. Avinash Shinde	Technical Officer A (till 15th January 2016)
Mrs. Mahalaxmi Mayekar	Technical Officer A (till 15th January 2016)
Mrs. Poornima Khude	Technical Assistant (till 15th January 2016)
Mr. Arvind Bhushan	Technical Assistant (till 15th January 2016)
Mr. Gaurav Telang	Technician C (till 15th January 2016)
Mr. Ravi Uraon	Technician C (till 15th January 2016)
Mrs. Snehal Patil	Technician C (till 15th January 2016)
Mrs. Sandhya Jachak	Technician C (till 15th January 2016)
Mr. Arvind Gaiwale	Technician C (till 15th January 2016)
Mr. Samir Shaikh	Technician C (till 15th January 2016)
Mr. Kalyan Bhandare	Technician C (till 15th January 2016)
Mr. Yogesh Kolap	Technician B (D.E.O) (till 15th January 2016)
Mr. Zubin Khan	Technician B (D.E.O) (till 15th January 2016)
Mr. Vishal Mhaske	Technician B (D.E.O) (till 15th January 2016)

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

Investigators: *YK Gurav, BV Tandale, MS Chadha, PS Shah, P Shil, SD Chitambar, V Gopalkrishna, A Deoshatwar, R Bharadwaj[1], V Dohe[1] & S Pol[1]*

[1]B J Medical College, Pune

Funding: Extramural (ICMR)

Project duration: December 2013 – November 2016

Background

The one of its kind project was undertaken to understand the transmission of viral diseases especially dengue-like and influenza-like illnesses in close-knit communities in India, with high density of population and clustering. This is a prospective community based incidence study in a population living in Janata Vasahat slum in Pune City, Maharashtra. Community based surveillance activities were continued in the area with a population of 31489, including 16380 males and 15109 females.

Objectives (2015-16)

- To monitor the attacks of acute febrile illness in study area and study the contribution of various viral and bacterial agents.

Findings

Influenza-like illness (ILI) cases were reported in 444 patients. The highest community incidence rate of ILI was 0.5 per 1000 persons per week. Throat swabs were collected from 120 (27.1%) ILI cases and Influenza virus etiology could be detected in 23 (19.2%) cases [INF A (H1N1) in 8, Type B in 9, INF(H3N2) in one] (Fig. 1). Bacterial etiology was detected in 8 (6.5%) cases [*Streptococus pyogens* (1), Group G *streptococi* (4), Group A *streptococi* (1) and Group *B streptococci* (2)].

Among 210 dengue-like illness (DLI) cases, blood samples were collected from 95 cases; 14 cases were found positive for anti dengue IgM antibodies and 9 cases for anti CHIKV IgM antibodies. Four cases were tested positive for leptospira (aged 22-62 years). Among 84 acute diarrhea cases in children aged ≤6 years, stool samples were collected from 13 cases. the samples tested negative for rotavirus, but tested positive for *Shigella flexnerni* (1) and *E coli* (9).

Summary

Community surveillance provided important information on seasonality, incidence and etiological contributions of viral and bacterial agents in urban slum population.

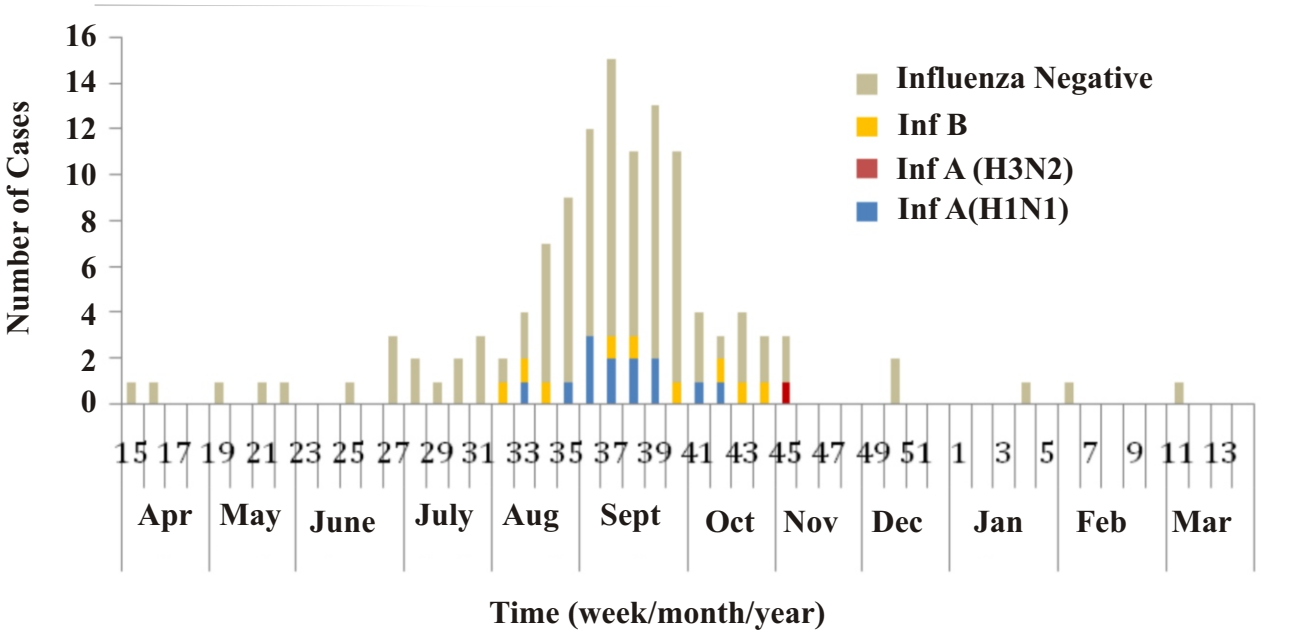


Fig. 1: Weekly distribution of ILI cases with influenza etiology

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

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

Funding Agency: ICMR

Duration: December 2013 – November 2016

Background

NIV acts as the reference laboratory with three peripheral sites *viz.,* GMC Nagpur, MGIMS Sewagram and KMC Warangal. NIV provided equipments to set up PCR faciliites at the peripheral sites and imparted trainings to the project staff. CSF, acute sera and convalescent sera were collected from all AES cases; stool/ rectal swabs were collected from enrolled AES cases along with controls. Ongoing review and guidance was provided during testing at sites. Reports of laboratory testing results were communicated timely to hospitals and health officials.

Objectives (2015-16)

- The project aims to undertake surveillance of AES hospitalizations among children <15 years for detection of viral etiologies; especially JE, Chandipura and enteroviruses.

Findings

During 2015-16, screening of 634 patients with fever and neurological manifestations was done. Among them, 133 were AES cases as per the NVBDCP, of which 110 were eligible for inclusion in the study. Sixty-four provided consent for enrolment [47 cases (KMC Warangal), 11 cases (GMC Nagpur) and 6 cases (MGIMS, Sewagram]. Anti JEV IgM antibodies were detected in 4/60 CSF, 14/101 acute sera and 2/7 convalescent sera. The month-wise distribution of AES cases with virus etiology is given in Fig. 2.

All specimens tested negative for anti CHPV IgM. CHPV RNA was negative for all CSF, but was positive in one acute serum by RT-PCR. All CSF and stool/rectal swabs were negative for enteroviruses by RT-PCR.

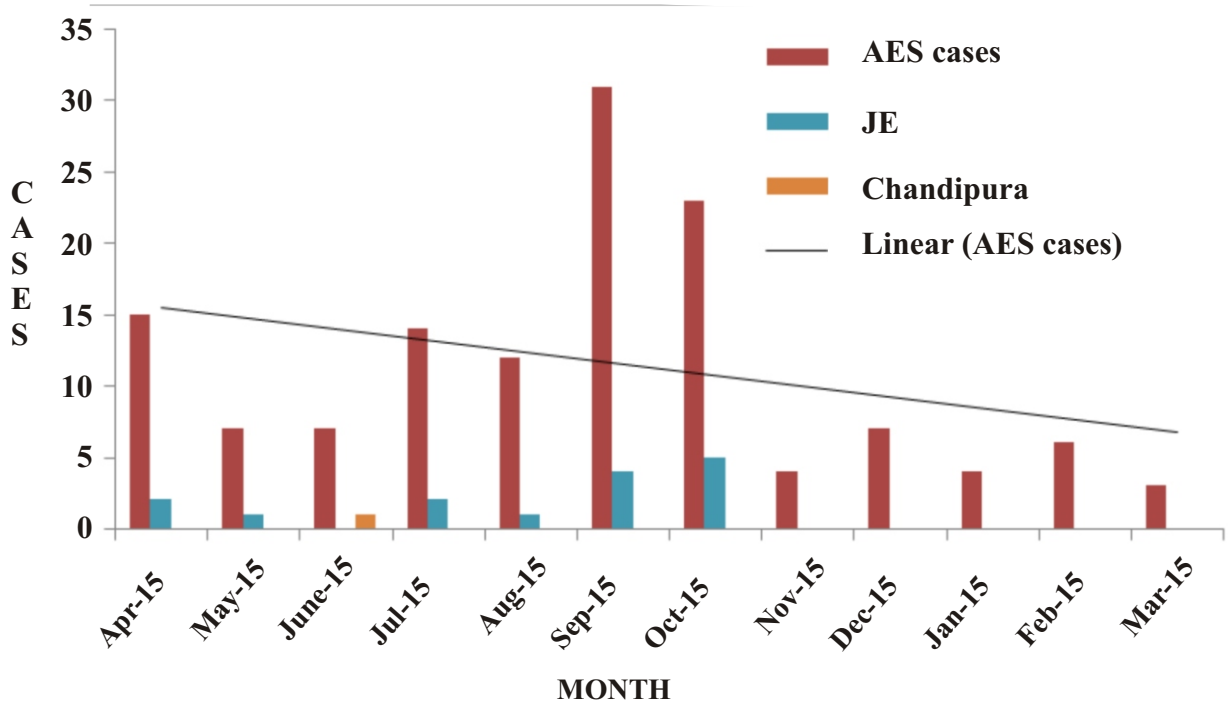


Fig. 2: Month-wise distribution of screened, eligible and enrolled cases with etiology

Summary

Most of the AES and cases occurred during July to October 2015. This signifies the endemicity of JE in the study area during monsoon months.

INVESTIGATION OF OUTBREAKS

(1) Kyasanur forest disease (KFD) outbreak in Sindhudurg, Maharashtra, February 2016

***Investigator**: YK Gurav, PD Yadav, MD Gokhale, R Viswanathan & DT Mourya*

An ongoing outbreak of Kyasanur Forest Disease (KFD) was jointly investigated by NIV and state health department from 23rd January 2016 in Dodamarg, Sindhudurg, Maharashtra. Rise in fever cases with headache and severe myalgia were reported from Ker village (population=300) in the 2nd week of January 2016 (Fig. 3).

Findings:

A total of 196 suspected KFD cases were reported from 10 villages. Male-female ratio was 1:1.2. Majority of the cases were adults who had a history of visit to forest area. Three suspected KFD deaths among adults were reported with co-morbid conditions. The highest attack rate of KFD (7%) was reported from Ker village. Common clinical features among KFD cases were fever, headache and myalgia. No hemorrhagic manifestations were noted.

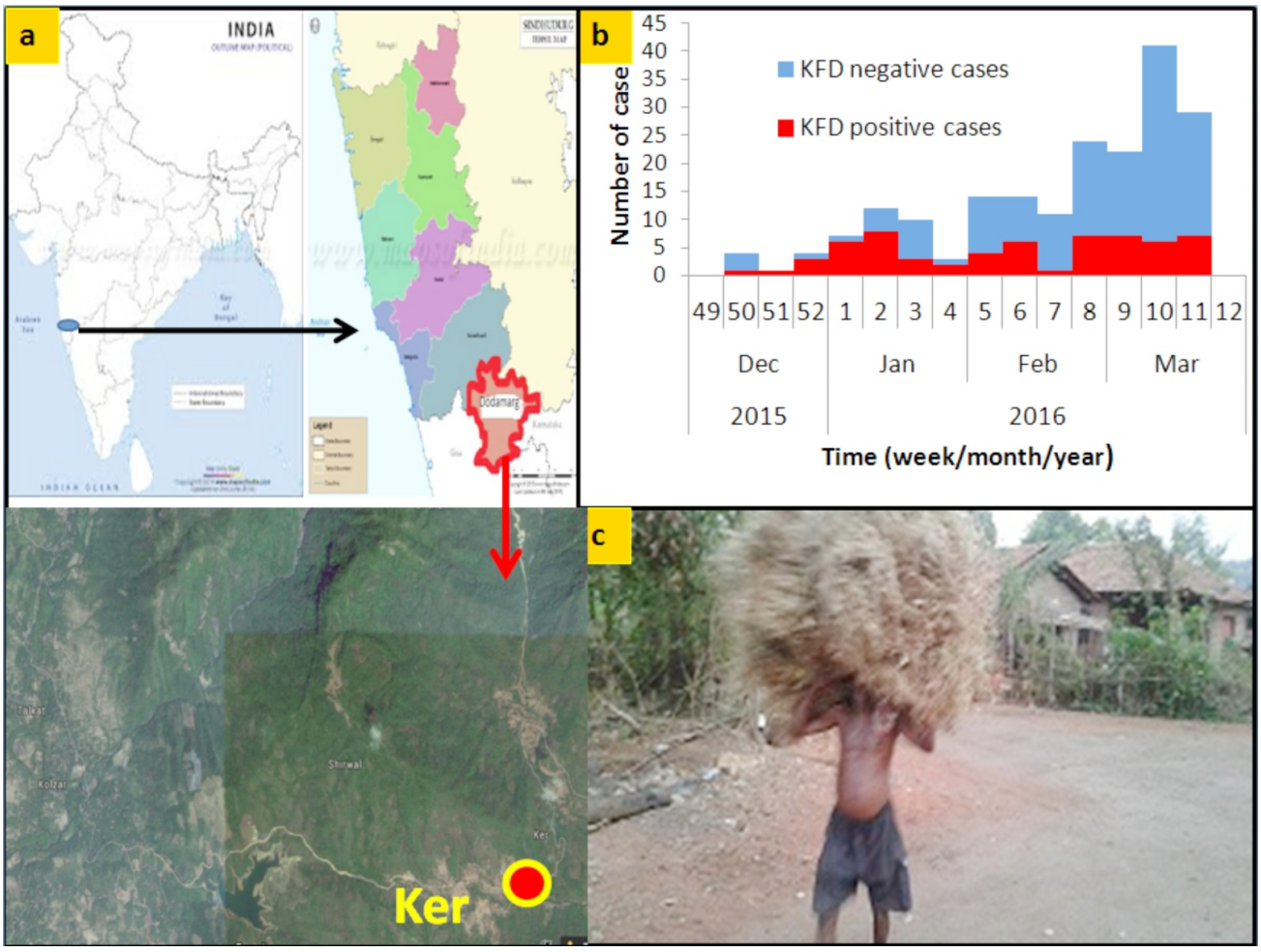


Fig 3: Kyasanur Forest disease cases (a) Ker village, the most affected village (b) KFD and suspected KFD cases during December 2015 to March 2016 and (c) risk behavior among people living in forest area

Sixty two (31.6%) cases were confirmed as KFD by real time RT PCR and anti KFDV IgM. Processing of serosurvey samples for anti KFDV IgG antibodies from the affected (701 sera) and unaffected areas (168 sera) is in progress.

Summary

KFD outbreak was confirmed in Dodamarg Taluka in Sindhudurg district of Maharashtra state.

(2) Hepatitis B and C infections in Malwa region in Punjab

***Investigators**: BV Tandale & K Lole*

Background:

Director of Emergency and Medical Relief, Govt. of India deputed a central team to investigate Hepatitis B/C infections in four districts in Malwa region of Punjab during 18-22 May 2015.

As per the reports made available by the state, Bathinda district reported 225 Hepatitis C and 49 Hepatitis B infections and Mansa district reported 168 Hepatitis C and 21 Hepatitis B infections. Infections had been identified during the screenings of blood donors, pre-surgical screening and screening camps organized in the communities. Hepatitis B vaccination coverage was reported to be 50-80%. Most of the cases were above 18 years of age. There was no gender difference.

Surveys were undertaken in one of the unaffected villages each in Mansa and Bathinda districts in 10% households by systematic random sampling with interviews. During investigation, 117 blood samples were collected and transported to NCDC, New Delhi.

Findings:

Hepatitis B and C infections were confirmed on the basis of available data and test results. Genotyping of Hepatitis C virus showed the prevalence of 1, 3 and 4.

Enteric Viruses Group

Scientific Staff

Dr. V. Gopalkrishna	Scientist-E& Group Leader
Dr. Pradeep Sawant	Scientist-B

Technical Staff

Dr. (Mrs.) Joshi MS	Technical Officer (A)
Mr. Tikute SS	Technical Officer (A)
Mrs. Ranshing SS	Technical Assistant
Dr. (Mrs.) Tatte VS	Technical Assistant
Mr. Doiphode RS	Sr. Technician-II
Mr. Jadhav PS	Technical Assistant
Mr. Shinde MS	Technician C
Mr. Ubale SK	Technician B
Mrs. Chavan NA	Technician B

Project Staff

Dr. Jain PN	Scientist B
Dr. Ghuge R	Scientist B
Ms. Borawake K	Technician C
Mr. Jagtap VD	Technician C
Mr. Takale GH	Multi Tasking Staff
Ms. Chothe NS	Technical Assistant
Ms. Shweta Bhosale	Data Entry Operator
Ms. Rongala L	ICMR-SRF (Ph.D. Awarded)
Ms. Kulkarni R	UGC-SRF (Ph.D. Awarded)
Ms. Lasure N	UGC-SRF (Ph.D. Awarded)
Ms. Pradhan G	CSIR-SRF
Mrs. Nital N. Ganorkar	ICMR-SRF

ENV 1301: Investigation of outbreaks and diagnostic services

Investigators: Gopalkrishna V, Joshi MS, Ranshing SS, Chothe NS, Chavan NA

Funding Agency: Intramural [Ongoing]

Acute gastroenteritis outbreak in Bhimashankar, Pargaon, Pune

An outbreak of acute gastroenteritis at Bhimashankar Sahakari Sakhar Karkhana (Sugar factory), Pargaon, (Maharashtra) with 1789 cases (IPD=321; OPD=1468) was reported from 29th November to 5th December 2015 (reported last year). Stool specimens (n=32) were tested for different enteric viral agents. Rotavirus like particles using electron microscopy, RNA migration pattern by RNA PAGE, RT-PCR and nucleotide sequencing confirmed presence of Group B Rota virus (GBR) in 28%, 72% and 97% of the specimens respectively. Phylogenetic analysis placed all the study strains in genotype N1 of NSP2 gene (Figure1). Comparison of study strains with GenBank genotype N1 strains indicated 98% nucleotide identity. The study highlighted GBR as the major etiological agent associated with the outbreak. Keeping in view of the public health policy and considering GBR virus as a major threat to cause large gastroenteritis outbreaks, such type of investigations from other parts of the country should be carried out.

Genetic characterization of CVA16, CVA6, and EV71 strains identified in Hand, Foot and Mouth Disease (HFMD): Emergence of B1c, E_2, C1 sub-genotypes in India.

Investigators: Ganorkar NN, Patil PR, Tikute SS & Gopalkrishna V

Funding agency: Intramural [Ongoing]

Hand, Foot and Mouth disease (HFMD) is a common childhood disease and caused due to Enterovirus-A (EV-A), EV-B and EV-C species. Among enteroviruses, Coxsackie virus A 16 (CVA-16) and Enterovirus 71 (EV71) are the two major causative agents identified in HFMD. Cases of HFMD were reported from Ahmadabad and Pune from western India. Genetic characterization was carried out to determine the genotypes distribution.

Objective:Molecular characterization of the HFMD cases to understand the genetic diversity of the associated strains.

Findings: A total 158 clinical specimen (Rectal swab/vesicular fluid/ Throat swabs) collected from 64 HFMD cases were included. EV detection was carried out by 5'NCR based RT-PCR, followed by genotyping using VP1/2A junction or VP1, full VP1 gene amplification. The study reports 63.92% (101/158) EV positivity and of these, ninety-four (93.06%) positive enterovirus samples were further typed.CVA16 (61.7%), CVA6 (34.04%), CVA4 and Echo12 (4.3%) strains were identified. All CVA16 Indian strains (n=70) clustered with rarely reported B1c sub genotype, CVA6 (n=43) and EV71 (n=1) strains clustered with sub-lineage E2 and C1 sub genotypes.

Summary: Study highlights emergence of B1c sub genotype of CVA16, E2 sub-lineage of CVA6 and C1 sub genotype of EV 71 strains in HFMD cases and these were reported for the first time from India.

ENV 1501: Identification and molecular characterization of group C rotaviruses in patients with acute gastroenteritis and animals from western India: A retrospective study

Investigators: Joshi MS & Gopalkrishna V

Funding Agency: Intramural [2015- 2018]

Group C rotavirus (GCR) is globally distributed in sporadic and outbreak cases of acute gastroenteritis in all age groups. Studies conducted earlier documented low prevalence of antibodies against GCR in urban and high prevalence in rural populations and evidence of cross species transmission suggests its role as an emerging zoonotic infection in humans. Characteristics of human GCR genome are highly essential to elucidate the genetic diversity and evolution studies. The development of sensitive diagnostic assays for detection of GCR was

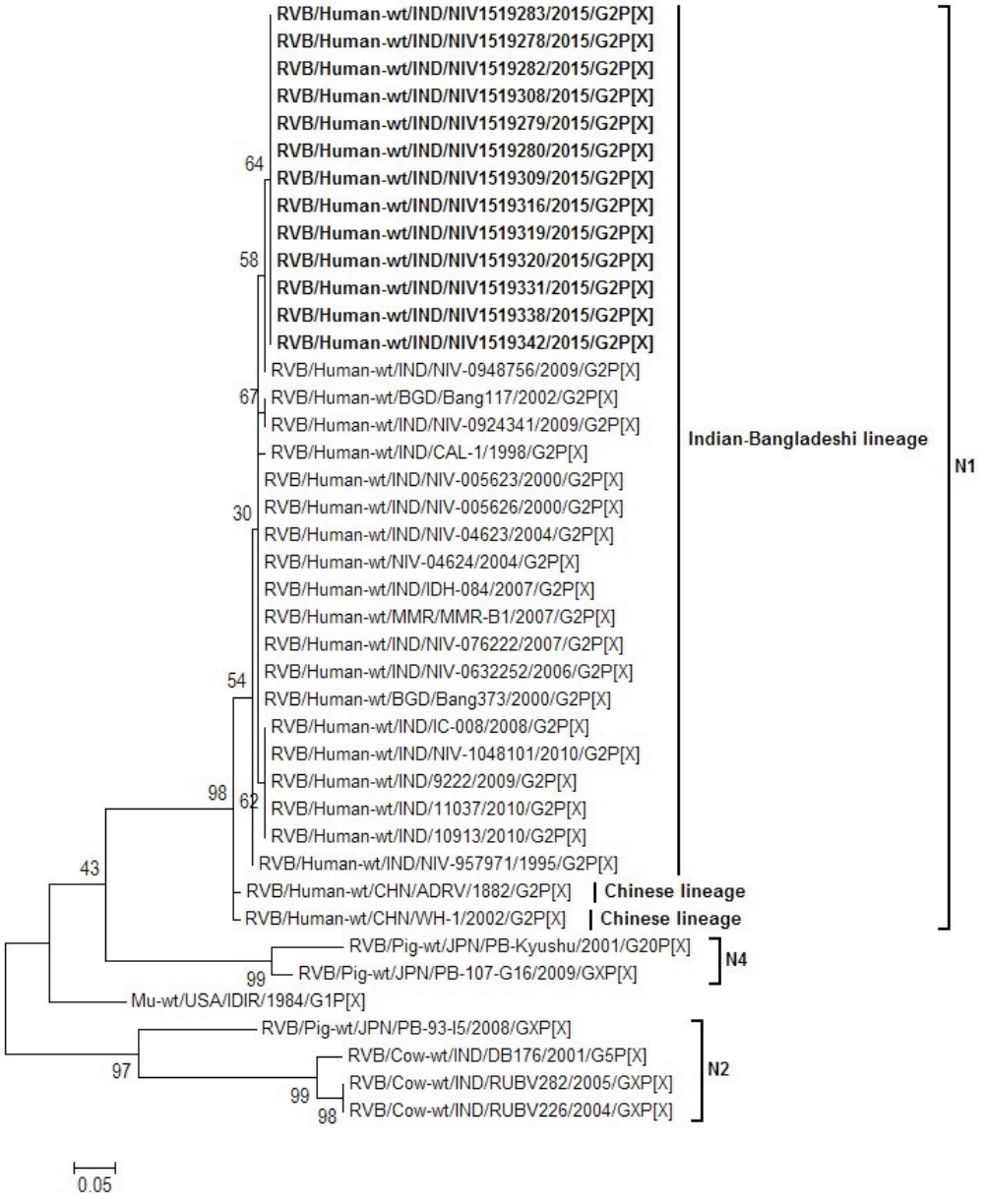


Figure 1: Phylogenetic dendrogram of partial group B rotavirus (GBR) NSP2 gene(112- 432bp). The study strains are indicated in bold letters. The scale represents genetic distance

hampered due to availability of limited numbers of complete genome sequences. The occurrence of GCR in outbreak cases of gastroenteritis was recently reported from India (Joshi et.al 2016). However, the molecular characteristics of the GCR strains circulating in the region are under reported.

Objective: Full genome sequencing and phylogenetic analysis of GCR strains circulating in western India

Findings: Three representative GCR strains from the earlier outbreak of gastroenteritis were included in the study. By using indigenously designed primers, full gene sequencing of VP1, VP2, VP3, VP4, VP6 and VP7 was carried out. The optimization of RT-PCR assays for nonstructural gene segments of human GCR strains is in progress.

ENV 1302: Assessment of genetic diversity in group A rotavirus isolates.

Investigators: Tatte VS & Gopalkrishna V

Funding Agency: Intramural [2013-2018]

Genomic and antigenic analysis of rotavirus strains circulating in a geographic region is essential as the diversity within group A rotaviruses remains a challenge to the efficacy of currently available group A rotavirus (GARV) vaccines.

Objective: To isolate common and unusual group A rotavirus strains in cell culture and to determine their genogroups.

Findings: Of the three G9P[4] RV strains isolated in MA-104 cell line earlier were further passaged and confirmed their presence by Multiplex PCR for VP7 and VP4 genes. It showed unusual G-P combinations, (G9P[4]) of the selected RV strains. In continuation to the earlier work, sequencing and phylogenetic analysis of the NSP4 genes of the rotavirus isolates was carried out. Two GARV isolates showed clustering in the E2 genotype of the NSP4 gene. These isolates showed 84.0-94.7% nucleotide identity and 93.9-95.3% amino acid identity with the prototype strain DS-1. With the reference strains from E2 genotype, 80.8-96.5% nt, 82.4-97.1% aa identity and within the strains 1.3% nt / 4.0% aa divergence was noted.Sequencing of the other (VP1-VP3, NSP1-NSP3, NSP5) genes of the isolates and attempts to isolate other rotavirus strains with unusual G-P combinations is in progress

ENV 1502: Development of a multiplex PCR assay for detection of viral pathogens associated with diarrheal diseases.

Investigators: Tatte VS & Gopalkrishna V

Funding Agency: Intramural [2015-2018]

Diarrhea caused due to viral and bacterial pathogens is a major public health problem in developing countries. Among enteric viral pathogens, Rotaviruses followed by Noro viruses, enteric adenoviruses, Astroviruses, Sapoviruses contribute in causing the disease. Recently, several novel enteric viruses such as Aichi virus, enteroviruses, parechoviruses, Sali/klassie and human Boca viruses have shown to be associated with acute gastroenteritis. Development of a simple, rapid and cost effective multiplex PCR for simultaneous detection of majority of the enteric pathogens in diarrhea cases will be useful to provide rapid diagnoses especially in outbreak situations.

Objective: To develop multiplex PCR assay for detection of enteric viruses associated with diarrheal diseases.

Findings: In continuation to the previous work, eighty-six clinical specimens tested for nine different Enteric viruses (i.e. Rota, Noro, Astro, Adeno, Entero, Sapo, Boca, Human Parecho and Aichi) using monoplex PCR were retested by using multiplex RT-PCR protocol standardized for enteric viruses in three tubes. Clinical specimens (n=86) analyzed by multiplex PCR showed presence of Rotavirus in 28 (32.5%), Norovirus in 1(1.16%), Astrovirus in 2(2.32%), Enterovirus in 5(5.8%), Bocavirus in 1(1.16%), Human Parecho in 1(1.16%) and mixed infections in 40 (46.5%). Both mono and multiplex PCR results showed 93% concordance.

ENV 1601: Identification and Molecular Characterization of Rota and Noroviruses in Neonates admitted at Neonatal Intensive Care Unit (NICU).

Investigators: Ranshing S & Gopalkrishna V

Funding Agency: Intramural [2016-2019]

Nosocomial infections are the important causes of morbidity and mortality in neonates, particularly those born preterm. Infants admitted at neonatal care units are particularly at "high risk" due to their vulnerable condition. Rotavirus (RV) infections and their genotype distribution are well documented in children < 5 years of age. However, no detailed study is available to understand the role, circulation pattern and genotype distribution of Rota and other enteric viruses in neonates admitted at Neonatal Intensive Care Unit (NICU) in Western India.

Objective:To determine the prevalence of Rota and Noro viruses and their genotype distribution among neonates admitted at Neonatal Intensive Care Units (NICUs).

Findings: Premature low birth weight Neonates; admitted at NICUs, were mainly associated respiratory distress syndrome. Stool specimens (n=348) collected from neonates admitted at KEM Hospital NICU (n=214) and SKNMC&GH, (n=92) Pune (Maharashtra) were included in the study. Written informed consents were obtained from parents/guardians. Demographic and clinical data for gestational age, birth weight, gender, clinical presentation, diagnosis, etc. were recorded in case reporting forms (CRF).All the stool specimens were tested for the presence of Group A rotavirus by commercially available ELISA Kit (Meridian Premier® Rota clone®). Rotavirus positivity was detected in 29.63% (72/243) and 21.9% (23/105) of stool samples. Majority (~97%) of the neonates examined were asymptomatic for any diarrheal symptoms. Eighty-three RV positive samples were further subjected to multiplex RT-PCR for VP7 (G) and VP4 (P) genotyping. Rotavirus strains non-typeable in multiplex PCR were typed by sequencing. Nearly 90.36% and 86.74% of the strains were typed for G and P genotypes respectively. A total of 83.33% RVA strains were typed for both the genotypes and 9.6 % strains remained non-typeable for both genes. A single unusual G12P[11] RV genotype was detected in Neonates.

Summary: Study highlights circulation of a single unusual G12P[11]RV genotype in neonates.

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

Investigators: Gopalkrishna V, Lasure N, Ganorkar N, Patil PR

Funding Agency: Intramural [2016-2017]

Association of non-Rota enteric viruses in sporadic and outbreak cases of acute gastroenteritis has been reported worldwide. Viruses such as Calici, Human Boca, Picorna viruses (Entero, Parecho, Sali) have been found associated in acute gastroenteritis. However, no such detailed study is available from western India.

Objective: To detect and characterize Sapo viruses (SaV), Human Boca virus (HBoV), Sali virus, Entero (EVs) and Human Parecho viruses (HpeV) associated with acute gastroenteritis.

Findings: Faecal samples (n=266) collected from children <5years, hospitalized for acute gastroenteritis were analyzed. SaVs were detected at 3.5% of the cases, infections occurred in children <2 years of age with severe infections in 44% and very severe in 33% of the 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% with respective prototype strains. HBoV was detected at 6.1%, 0-24 months age group was found most susceptible. HBoV1, HBoV2 and HBoV3 genotype strains were in circulation. Nucleotide identities ranged between 99.3-100 %, 90.6-98.4% and 95.9-96.1% respectively with their prototype strains. Sali virus was detected by amplification of 2C (354bp) and typing by 3D (688bp) and capsid region (852bp) respectively.Genotyping of the strains

revealed, nucleotide identity between the strains was higher in 3D region (95.6-100%) as compared to capsid region (91.2-100%).Sali virus 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. EV and HPeVs were detected by 5'NCR region and genotyping by VP1 gene amplification. Presence of fifteen genotypes belonging to three different EV species (EV-A:2 [8.3%],EV-B:9 [37.5%] and EV-C:4 [16.7%]) were identified. Six genotypes of HPeVs (HPeV1, HPeV2, HPeV5, HPeV7, HPeV8 and HPeV14) were identified; of these HPeV-1 (61.1%) was predominant. The study documents circulation of HPeV-2 as rarely identified genotype.

Summary: The study highlights circulation and genetic variation of non- Rota enteric viruses in acute gastroenteritis.

ENV-1309 & 1310: National hospital based rotavirus surveillance network (Referral & peripheral site)

Investigators: Gopalkrishna V, Jain P, Ghuge R

Funding agency: ICMR (Extramural) [2013-2016]

The study involves a national hospital based surveillance of rotavirus disease and strains among children <5 years for determination of the age and seasonal distribution and prevalent G-P types at multiple-centres in India. National Institute of Virology (NIV), Pune is one of the Referral centres and represents the west zone of India which includes four Peripheral sites; Pune (KEM Hospital, Bharati Hospital and Shaishav Clinic), Mumbai (LTMGH & MC), Ahmadabad (BJMC) and Surat (SMIMER) and a Regional centre; RMRC Belgaum with two clinical recruitment sites (CRSs) (JNMC, Belgaum and KIMS Karad.

Findings: During NRSN surveillance study conducted at West zone during the year 2016, a total of 1003 diarrheal cases from children < 5 years age were enrolled from different clinical recruitment sites (CRS). Stool specimens (n=872) were collected from all the sites, which includes Pune (n=171), Mumbai (n=66), Ahmadabad (n=56),Surat (n=234), Karad (n=213) and Belgaum (n=132). Reactivity to rotavirus ranged from 13.6-45.6 % of all CRS with a mean value of 29.6%. The highest reactivity was detected at Pune site (45.6%) and lowest (13.6%) at Mumbai with 7-12 months as susceptible age group. Predominance of G1P[8] 33.3%, followed by G2P[4], G9P[4](13.1%), G1P[6] (11.9%), G3P[8] (9.5%), G9P[8], G12P[11], G12P[8], G9P[6], G12P[4], G3P[4] RV genotypes was noted. Mixed infections were detected at low levels (1.2-3.6%).

Summary: The study highlights predominance ofG1P[8] 33.3%, followed by G2P[4], G9P[4](13.1%) RV strains. Also changing pattern in circulation with predominance of G3 P[8] RV strain was observed in Mumbai CRS.

ENV1307: Pathogenesis of Coxsackie virus A-16 (CVA-16) associated with Hand, Foot and Mouth disease in neonatal mice

Investigators: Tikute SS & Gopalkrishna V

Funding agency: Intramural [2013-2018]

Hand, Foot and Mouth Disease (HFMD) is a common childhood viral infection. Among enteroviruses (EV), Coxsackie virus A-16 (CVA-16) and Enterovirus-71 (EV-71) are the EV types majorly associated as etiological agents. Clinical data reported from Asian countries demonstrated that CVA-16 infections do cause neurological complications and leads to fatalities. Therefore it is mandatory to understand the pathogenicity of CVA-16 strains associated with HFMD. However, no such studies are attempted from India where CVA-16 is the major EV type identified and constantly under circulation in HFMD.

Objective: To study the pathogenicity of CVA-16 infection in HFMD using neonatal mouse model.

Findings: In continuation to the previous work, full VP1 gene amplification was carried out in RNA samples extracted from experimental mice (CVA-16 infected) organs.Organs viz. brain, heart, lung, liver, spleen, intestine, kidney and skeletal muscle were collected from CVA 16 inoculated CD1 mice

at different time points i.e. day1, day3, day5, day7, day9, day11 and day13. Sequencing of the VP1 positive amplicons showed 98% nucleotide homology with CVA-16 Yamagata 2011 (AB772003) strain. In addition to this study, retrospective clinical samples collected from HFMD cases were subjected for virus isolation using RD cell-line. CVA-16 virus was isolated from the clinical samples. CVA-16 virus having 105 TCID50/ ml titer was administered separately in one-day-old ICR mice by IP route. Four groups of mice (n=4) were utilized for experimental control group purpose. All experimental and control mice were observed daily for clinical signs and symptoms of disease. Mice were harvested on post inoculation day (PID) 1, 3, 5, 7 till day 15 along with control groups for virus detection. Histopathological study is in progress.

ENV 1401: Development of NIV Rota Virus Antigen capture ELISA Kit

Investigators: GopalkrishnaV, Ranshing S & Sathe PS

Funding agency: Intramural [2016-17]

Rotavirus is the prime cause of severe gastroenteritis leading to a significant mortality in infants and young children worldwide. Studies conducted worldwide on pediatric nosocomial diarrhea have identified rotavirus A (RVA) as the major etiological agent. Isolation of rotaviruses in cell culture directly from human faecal samples is time consuming and difficult, and hence, rotavirus infections are generally identified by the direct detection of antigen(s) in diarrheal samples by immunoassay methods.

Objective: To develop cost effective antigen capture Rota virus ELISA kit.

Findings: This is the first indigenously developed Rota virus antigen captures ELISA kit and is cost effective in Indian setting. Rotavirus ELISA provides qualitative detection of group A rotavirus antigen in human stool samples. It provides presumptive diagnosis of rotavirus diarrhea in acute phase of the disease. It is useful in providing rapid diagnosis during outbreak situation. The sensitivity and specificity of the kit was 97% and 100% respectively. The kit showed stability for 18 months when maintained at 2-8°C.

Diagnostic Reagent Facility

Scientific Staff

Dr. Paresh Shah	Scientist-E& Group Leader

Technical Staff

Mr. T. L. Gangadhararao	Technical Officer-A
Mr. Sujeetkumar Belani	Technical Officer-A
Mrs. Deepika Chawdhary	Technical Assistant
Mr. Kishor Kshirsagar	Technical Assistant
Mr. Anant Deshpande	Technical Assistant
Mr. Ashok Kamble	Technical Assistant
Smt. Shobha Kulkarni	Technician-C
Mrs. Reva More	Technician-C
Ms. Snehal Veer	Technician-C
Mrs. Anjana Ugale	Technician-B
Mrs. Purvi Patel	Technician-A
Mr. Mukesh Chavan	Technician-A
Mr. Sanjay Jadhav	Multi Tasking Staff

Project Staff

Dr. KanchankumarPatil	Scientist-C
Mrs. Swati Totad	Technical Assistant
Mrs. Jyotsna Khade	Technical Assistant
Ms. Sapna Gawhale	Technical Assistant
Ms. Aparna Rakhe	Technical Assistant
Ms. Anuja Satvekar	Technical Assistant
Mr. Tushar Raut	Technical Assistant
Mr. Rohit Ippe	Technician-B,
Mr. Sushant Sable	Technician-A
Mr. Zubair Attar	Technical Officer-A (Engineering)
Mrs. Shweta Chondhe	Technician-C (DEO)
Mr. Prashant Gore	Technician-A (DEO)

Project Title: Scaling up of production of MAC ELISA diagnostic kits (JE, DEN, CHIK) for supply under the national program & to WHO SEAR

Investigators: Shah PS

Work done: During the year, 9145 MAC ELISA kits (JE, DEN & CHIK) were prepared and supplied to more than 550 Sentinel Surveillance Hospitals (SSHs) and 15 Apex Referral Laboratories (ARL) in the country under the National program (Table 1, 2). The kits were also supplied to ICMR institutes, RMRC and VDL as per their requirement. WHO SEAR, Delhi also procured 82 kits from NIV for supply to the SEAR countries.

Table 1: Supply of MAC ELISA kits in 2016-2017 (April 2016 to March 2017)

Agency	JE	DEN	CHIK	TOTAL
National Program	539	6141	2383	9063
WHO-SEAR, New Delhi	80	–	2	82
Total	**619**	**6141**	**2385**	**9145**

Table 2: Sentinel Surveillance Hospitals (SSHs) recognized in each state and the details of kits supplied during 2016-17 under the national program.

Sr. No.	State & UT	No. of SSH	No. of Kits Supplied			
			JE	DEN	CHIK	Total
1	Andaman Nicobar	3	–	26	3	29
2	Andhra Pradesh	19	5	357	39	401
3	Arunachal Pradesh	7	3	5	7	15
4	Assam	13	105	111	30	246
5	Bihar	7	39	80	45	164
6	Chandigarh	1	4	63	22	89
7	Chhattisgarh	4	4	30	2	36
8	Delhi	33	13	773	653	1439
9	Goa	3	2	27	13	42
10	Gujarat	33		319	119	438
11	Haryana	25	4	87	152	243
12	Himachal Pradesh	7	–	1	–	1
13	Jammu & Kashmir	10	–	18	1	19
14	Jharkhand	5	11	22	12	45
15	Karnataka	32	32	514	295	841
16	Kerala	30	2	462	79	543

Sr. No.	State & UT	No. of SSH	No. of Kits Supplied			
			JE	DEN	CHIK	Total
17	Madhya Pradesh	36		214	82	296
18	Maharashtra	37	26	660	250	936
19	Manipur	2	9	3	1	13
20	Meghalaya	3	7	12	9	28
21	Mizoram	2	1	7	2	10
22	Nagaland	2	1	10	1	12
23	Odisha	36	25	141	7	173
24	Puducherry	5		47	11	58
25	Punjab	22	–	232	110	342
26	Rajasthan	27		210	119	329
27	Sikkim	2		4	2	6
28	Tamil Nadu	30	49	494	63	606
29	Telangana	23	7	224	40	271
30	Tripura	2	9	10	10	29
31	Uttar Pradesh	37	122	509	166	797
32	Uttarakhand	7	–	16	4	20
33	West Bengal	33	59	453	34	546
	Total		539	6141	2383	9063

Medical Entomology & Zoology

Scientific Staff

Dr. Sathe PS	Scientist 'F' & Group Leader
Dr. Sudeep AB	Scientist 'D'
Dr. Gokhale MD	Scientist 'C'

Technical Staff

Mrs. Mavale MS	Technical Officer-'A' (Till Jan 2017)
Mr. Aher RV	Technical Assistant
Mr. Khude PD	Technical Assistant
Mr. Ghodke YS	Technical Assistant
Mr. Sonawane PA	Technical Assistant
Mr. Ingale VS	Technician-'C'
Mr. Lekhraj KN	Technician-'B'
Mr. Dhaigude SD	Technician-'B'
Mr. Kunal Nikalje	Technician-'A'
Mr. Meliya MZ	Multi Tasking Staff

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 (initiated in 2013, got extramural funding in 2017 for a period of two years).

Investigators: Sudeep AB & Gokhale MD

Funding: Extramural (ICMR) [2017-19]

Chandipura virus (CHPV), a negative stranded RNA virus (Vesiculovirus:Rhabdoviridae) is endemic in Central India causing high mortality among children. Sand flies are designated as the vector of CHPV. Since the virus was found active in high temperature areas, a study was carried out to determine the stability of CHPV at different temperatures.

Objectives: Stability of Chandipura virus at different temperatures

Work done: The virus retained its infectivity for eight weeks when stored at 4°C in cell culture medium (MEM with 2% FBS), egg allantoic fluid and sand flies. However, marginal loss in virus titer was observed in cell culture fluid (MEM) and allantoic fluid stored at 37°C (Figure 1). Complete loss of infectivity was seen in infected sandflies stored at 37°C in a week (Figure 2). CHPV RNA could be detected in infected sand flies for 13 weeks irrespective of storage at 4°C and 37°C (Figure 3). Exposure to UV light for 15 min did not inactivate the virus completely. Retaining virulence at 37°C for a week is a concern as the virus could pose a challenge while handling clinical samples or patients in hospitals/research laboratories. RNA persistence in dried sand flies will have positive significance in surveillance studies where maintenance of cold chain is a concern.

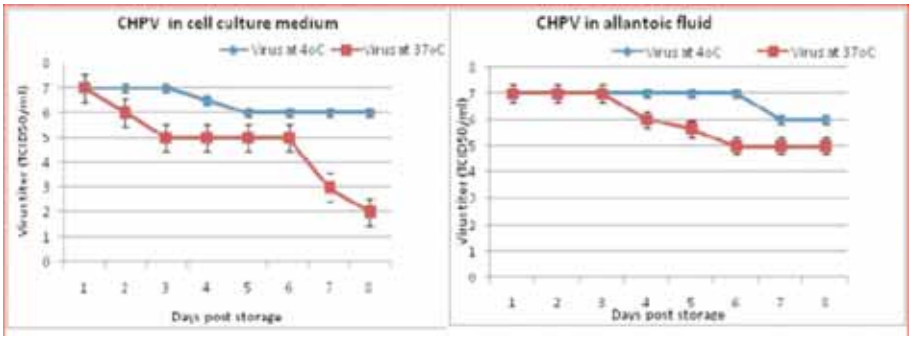


Fig. 1 : CHPV stability in cell culture medium and chick allantoic fluid stored at different temperatures

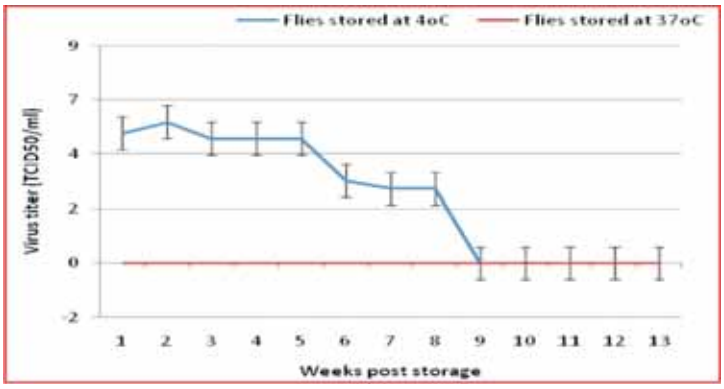


Figure 2: Loss of virulence of CHPV in sand flies stored at different temperatures

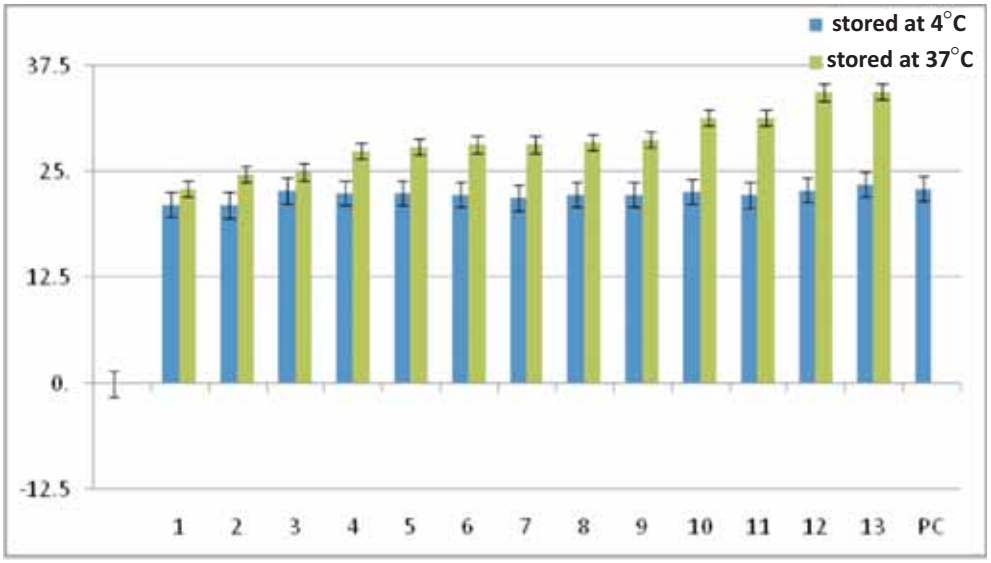


Figure 4: RNA persistence of CHPV in sand flies stored at different temperatures

ENT 1702: Vector biology of certain Culex mosquitoes in relation to Chittoor, Ingwavuma, Umbre viruses etc. (Family Bunyaviridae) from India (Pilot study)

Investigators: Sudeep AB & Gokhale MD

Funding: Intramural [2014-2016]

Studies on Chittoor virus: Chittoor virus (CHITV) has been isolated in India repeatedly from mosquitoes and once from pigs. Neutralizing antibodies to CHITV were detected in humans and a large number of domestic animals viz., horse, sheep, goats, mules, donkeys, camels, cattle etc. Studies have shown that CHITV is antigenically close to Batai virus, which involve in genetic re-assortments and cause severe hemorrhagic diseases in humans. Repeated isolations from mosquitoes and pigs as well as detection of neutralizing antibodies in humans and other vertebrates are suggestive of active circulation of CHITV in India. Though CHITV has been isolated from several mosquitoes, no data is available on the vector competence of these mosquitoes.

Work done: Replication potential and vector competence of *Culex quinquefasciatus* and *Culex tritaeniorhynchus,* two widely prevalent mosquitoes in India to CHITV was studied. Both the mosquitoes replicated the virus to titers of 6.3 and 5 $\log_{10}$ $TCID_{50}$/ml respectively and maintained the virus for over 20 days (Figure 4). CHITV was detected in saliva of both the mosquitoes on 8^{th} day post infection (PI) and demonstrated experimental transmission to infant mice on 11^{th} day PI. Though, no outbreaks involving humans have been reported from the country yet, the

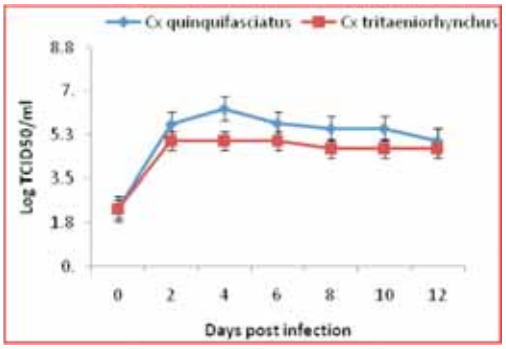


Figure 4: Growth kinetics of CHITV in two Culex spp. Mosquitoes

prevalence of the virus, availability of vertebrate hosts for amplification, increasing populations of competent mosquitoes, pose a serious threat to public health.

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

Investigators: Sudeep AB, Parashar D & Gokhale MD

Funding: Intramural [2013-2016]

Aedes vittatus mosquitoes are known to play an important role in the maintenance and transmission of viruses of public health importance *viz.,* Yellow fever virus (YFV), dengue virus (DENV), Chikungunya virus (CHIKV) and Zika virus. Since the mosquito is highly prevalent in India, its susceptibility to some of the important arboviruses apart from the viruses listed above has been carried out.

Objective 1: Susceptibility of *Aedes vittatus* mosquitoes to certain viruses of public health importance in India:

Work done: Preliminary studies have shown that the mosquito replicates Japanese encephalitis (JEV), West Nile (WNV) and CHPV. Though JEV is found to be replicating in the mosquito, studies could not convincingly demonstrate their vector competence due to the absence of the virus in saliva upto 12 days PI. On the contrary, high degree of WNV replication was found in the mosquitoes with rapid dissemination to wings, legs and salivary glands as early as 6th day PI. WNV was detected in saliva with a titer of j3 log10 TCID50/ml on 6th day PI with a progressive increase on subsequent days PI (upto 12th day PI) demonstrating its vector potential. Similarly, the mosquitoes replicated CHPV to high titers. Further studies are in progress.

Objective 2: Vector competence of *Aedes vittatus* mosquitoes to ECSA strain of Chikungunya virus:

Work done: Earlier studies have shown the vector competence of *A. vittatus* mosquitoes to Asian and West African strains of CHIKV successfully. A study was therefore carried out to determine the competence of the mosquito to replicate and transmit ECSA strain of CHIKV, which is currently circulating in the country. Studies using parenteral inoculation have shown replication of the virus and vector competence of the mosquito to transmit the virus. Studies with natural route of infection (oral feeding) are being carried out.

MCL 1701: Vector competence of Indian strain of *Aedes aegypti* mosquito to Zika virus

Investigators: Gokhale MD & Mourya DT

Funding Agency: Intramural [2016-2018]

Zika virus (ZIKV) was first identified in monkeys in 1947 and remained in a sylvatic mosquito-monkey cycle in Africa. Geographical expansion of the virus was observed since 2007 first to Oceania and subsequently to Brazil and other parts of South and North America with severe clinical manifestations. ZIKV is transmitted to humans, mainly through *A. aegypti* mosquito. The present study was carried out to determine the susceptibility of Indian strain of A. aegypti to ZIKV and the effect of co-infection with DENV and CHIKV on ZIKV replication. Studies were also conducted to determine transovarial transmission (TOT) and transmissibility of ZIKV from mosquitoes to suckling mice. Preliminary studies have shown that Indian strain of A.aegypti is susceptible to ZIKV and replicated the virus to high titers. Based on IFA and Real time RT-PCR data in co-infection experiments, it was concluded that ZIKV may have a relative advantage in replication dynamics over DENV and CHIKV. Transmission of ZIKV by infected mosquitoes to suckling mice was confirmed by appearance of clinical signs and presence of viral RNA in different organs.However, TOT could not be demonstrated.

ENT 1703: Development of ELISA for detection of dengue Virus (es) in mosquitoes

Investigators: Gokhale MD& Sathe PS

Funding Agency: Intramural [2016-2018]

Commercially available Dengue NS1 Ag MICROELISA is designed for *in vitro* qualitative detection of Dengue NS-1 antigen in human serum and plasma and is being used routinely for screening blood samples. Attempts were made to use the Dengue NS-1 detection kit for the detection of the dengue virus in the laboratory infected *A. aegypti* mosquitoes. From the preliminary studies, it was evident that Dengue NS-1 protein could be detected in the parenterally infected mosquitoes at 4th day post infection (PI) while, in oral fed mosquitoes detection was evident from 8 day PI.

ENT 1704: Development of ELISA for the detection of Chandipura Virus (CHPV) in arthropods

Investigators: Sathe PS, Sapkal GN& Gokhale MD

Funding: Intramural [2017-2018]

Chandipura virus (CHPV) has been causing very severe outbreaks of encephalitis in central India and has been classified as an important emerging human pathogenic virus. The virus affects the pediatric group and cause death within 24 hr. of commencement of symptoms. Sand flies belonging to Phlebotomus genus are the incriminated vector of CHPV. An attempt was made to develop an ELISA to detect CHPV in sandflies and other arthropods. Different species of arthropods *viz., A. aegypti, Anopheles stephensi, Culex quinquefasciatus, C. tritaeniorhynchus* and *Sergentomyia punjabensis* were infected with CHPV (NIV034627) by intrathoracic inoculation and membrane feeding techniques and used to standardize the ELISA. In the study, the ELISA successfully detected the CHPV antigen in all the infected species. Further standardization is in progress.

Measles Group

Scientific Staff

Sunil R. Vaidya	Scientist-E & Group Leader

Technical Staff

Mr. Vijay A. Sonawane	Technical Officer-A
Mrs. Deepika T. Chowdhury	Technical Assistant (till 30/ 09/ 2016)
Mrs. Neelakshi S. Kumbhar	Technical Assistant
Mrs. Madhukar B. Kamble	Technician-C
Mrs. Divya R Bhattad	Technician-C
Mr. Sarang S Kamble	Technician-A
Mr. Roben P George	Technician-A

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

Investigator: Vaidya SR

Funding Agency: Intramural [Ongoing]

Objective (i) Diagnosis of measles/rubella in referred samples

During the year, 177 serum samples (male-106; female-71) received from 20 hospitals/ institutions were processed for measles and/or rubella IgM antibodies. Results revealed presence of measles virus specific IgM antibodies in 49.15% samples whereas 4% of samples showed detectable levels of rubella IgM. Age wise distribution of measles showed 63.27% suspected cases belonged to 0-15 year age group (n=112) while the rest belonged to >15 years. Measles vaccination history (by documentation or parent's recall) during childhood was not available for any of the cases.

(ii) Measles outbreak among medical students in AFMC Pune

AFMC Pune referred 28 suspected measles samples and 28 contact samples for laboratory diagnosis of measles and rubella during 17th October and 12th November 2016.Measles IgM antibodies were detected in 8 of 28 suspected cases and 2 of 22 contacts. Measles virus RNA was detected in 8/26 suspected cases and 2/2 contacts. Rubella virus RNA was detected in 2/9 suspected cases. Though 15 samples were processed for measles virus isolation, only one isolate could be obtained.Sequence analysis revealed circulation of measles genotype D8 and rubella genotype 2B.

(iii) Mumps outbreak investigation in the tribal population

A cluster of parotitis cases were reported from a tribal village (Vansda) in Dadra and Nagar Haveli, India during 2016 and 19 serum samples were collected from 11 suspected cases and 8 contacts. Mumps infection was confirmed by the presence of both mumps specific IgM and IgG antibodies in 8 of the 11 suspected cases. Majority of the suspected mumps cases were children except four adults.

Another cluster of cases with fever and parotitis was observed in the Galonda and Silli villages of Dadra and Nagar Haveli, during October 2016 and January 017.Serum samples were collected (n=81) from 42-suspected cases and 39 close contacts. Screening of the samples confirmed mumps infection in 69% suspected cases and 30.8% close contacts. Majority of the suspected mumps cases were between 5-15 years except three adults. Characteristic of the outbreak was that none of the cases showed mumps associated clinical complications.

(iv): Outbreak based measles surveillance in the State of Maharashtra

Work done: Between April 2016 and Mar 2017, the State health agencies investigated 94 suspected measles outbreaks in 26 districts and 481 serum samples (male-239; female-242) were referred to NIV for laboratory diagnosis of measles/ rubella. Majority of suspected cases belonged to 0-15 year (95.42%) age group. Measles virus specific IgM was detected in 274 cases (56.96%) whereas Rubella virus specific IgM in 48 (23.18%) cases (Table 1). Among the suspected measles cases, though 45 had history of measles vaccination (by documentation or parent's recall) during childhood, 15 tested positive for measles IgM probably indicates primary vaccine failure. Among the suspected adult cases (>15 yrs., 13 male & 9 female), six had confirmed measles (four male & two female) and two had rubella IgM (both females). The laboratory study confirmed 61 as measles outbreak; eight as rubella outbreak and 19 due to both while the etiologic agent could not be confirmed in 6 outbreaks (total 94 suspected outbreaks)

Table 1 : Laboratory diagnosis performed on referred serrum samples

District (No. of outbreaks)	Serum (n=) samples	Measles Virus IgM			Rubella Virus IgM		
		Positive	Negative	Equivocal	Positive	Negative	Equivocal
Ahmednagar (3)	9	7	2	0	0	2	0
Akola (3)	15	13	1	1	0	1	1
Amravati (2)	9	6	2	1	0	3	0
Aurangabad (3)	13	8	5	0	1	4	0
Beed (5)	22	18	4	0	0	4	0
Bhandara (2)	10	5	5	0	0	5	0
Buldhana (1)	2	1	1	0	0	1	0
Chandrapur (3)	15	7	8	0	0	8	0
Dhule (5)	29	22	7	0	0	6	1
Gadhchiroli (1)	7	3	4	0	1	3	0
Gondia(6)	30	18	12	0	2	9	1
Gr. Mumbai (20)	98	47	50	1	17	34	0
Hingoli (5)	40	11	28	1	6	21	2
Jalgaon (3)	15	13	2	0	0	2	0
Kolhapur (1)	5	5	0	0	0	0	0
Nagpur (3)	15	5	10	0	8	1	1
Nanded (2)	14	5	8	1	1	8	0
Nandurbar (2)	10	9	1	0	0	1	0
Nasik (9)	43	31	11	1	6	6	0
Parbhani (5)	19	6	11	2	2	11	0
Pune (1)	5	4	1	0	0	1	0
Solapur (1)	8	1	7	0	0	7	0
Thane (2)	9	7	2	0	1	0	1
Wardha (1)	7	0	7	0	1	6	0
Washim (3)	16	10	6	0	0	6	0
Yavatmal (2)	16	12	4	0	2	2	0
Total (94)	**481**	**274**	**199**	**8**	**48**	**152**	**7**

MMR1402: Genetic & antigenic characterization of measles, mumps & rubella virus isolates

Investigator: Vaidya SR

Funding: Intramural [2014-2017]

Objective: Characterization of measles/rubella virus isolated from referred samples

During the year, 107 clinical specimens collected from different outbreaks/ sporadic cases from Maharashtra (n=59), Gujarat (n=42) & Madhya Pradesh (n=6) were processed for measles virus isolation. Fifteen wild type measles strains were isolated in Vero hSLAM cells and were genotyped (Table 2). The samples were obtained from 56 males and 52 females and 92 cases belonged to the 0-15 yr. age group while the rest belonged to =15yrs.

Table 2: Details of the measles virus isolates (wild types) obtained during 2016-17

SNo	Age (Year) / Sex	Place(District, State)	Specimen	MeV IgM EIA	MeV RT PCR	Genotype
1	7/F*	Jamnagar, Gujarat	TS	NA	Positive	D8
2	5/M	Bhavnagar, Gujarat	TS	Positive	Positive	D8
3	6.4/M	Morbi, Gujarat	TS	NA	Positive	D8
4	5/F	Morbi, Gujarat	TS	NA	Positive	D8
5	7.3/F	Palghar, Maharashtra	TS	NA	Positive	D8
6	19/F*	Pune, Maharashtra	TS	Negative	Positive	D8
7	2.6/F	Bhavnagar, Gujarat	TS	NA	Positive	D8
8	10/M	Indore, Madhya Pradesh	TS	NA	Positive	D8
9	7/M	Indore, Madhya Pradesh	TS	NA	Positive	D8
10	6/M	Indore, Madhya Pradesh	TS	NA	Positive	D8
11	6/F	Una, Gujarat	TS	Positive	Positive	D8
12	8/F	Jamnagar, Gujarat	TS	Positive	Positive	D8
13	5/F	Jamnagar, Gujarat	Urine	NA	Positive	D4
14	7/M	Jamnagar, Gujarat	TS	NA	Positive	D4
15	13/M	Gondia, Maharashtra	Urine	NA	Positive	D8

*History of measles vaccination

Objective (ii): Genotyping of measles virus isolates collected during the year

Work done: Fifty-eight (29 males & 29 females) throat swabs and 140 urine specimens received from Gujarat, Madhya Pradesh, Maharashtra and Rajasthan were sequenced. Phylogenetic analysis revealed the presence of D4 (n=11) and D8 (n=71) genotypes. The sequences were deposited in the WHO global measles sequence database i.e. MeaNS/Genbank.

In addition, 154-PCR products (measles N gene/ rubella E1-E2 gene) referred from four national measles laboratories i.e. NIV Unit Bangalore (n=21), SGPGIMS Lucknow (n=65), KIPM&R

Chennai (n=32) and Government Medical College, Guwahati (n=36) were sequenced. Circulation of measles genotype D4 (n=6) and D8 (n=123) was confirmed. One PCR product received from NIV Bangalore unit was positive for rubella and sequence analysis revealed rubella genotype 2B strain. All sequences were deposited in the WHO global measles sequence database (MeaNS & RubeNS) and GenBank by the respective laboratories.

Miscellaneous studies:

(i) Chickenpox outbreak investigation in the tribal population (Dadra and Nagar Haveli)

Clinical specimens (serum or urine or throat swabs or blister swabs) from 37 suspected cases and 19 contacts from the tribal population of Dadra and Nagar Haveli; collected during chicken pox outbreak, were referred to NIV Pune India for laboratory confirmation.Thirty suspected cases and one contact was laboratory confirmed for Chickenpox either by IgM EIA or VZV DNA PCR.Two of nine PCR positive products were sequenced.

(ii) Support provided for in-house measles IgM ELISA kit development

Laboratory support was provided to Virus Diagnostic Laboratory (VDL) of the institute for development of measles IgM ELISA kit by providing one virus isolate and a panel of serum sample (n=100). Subsequently, the in-house developed ELISA and commercial kits were evaluated in the Measles group using a panel of 50 sera & results communicated to VDL, NIV.Also participated in ELISA validation work conducted in the WHOs four national laboratories, results communicated to the concerned authorities.

Service Project: Total samples received and tested for Measles Mumps and Rubella duringthe year as a part of service project. (Table 3).

Table 3:Number of samples tested in the reporting year (2016-17)

Sr No	Test	Number of samples tested
1	Measles IgM ELISA	638
2	Measles IgG ELISA	89
3	Mumps IgM ELISA	197
4	Mumps IgG ELISA	197
5	Rubella IgM ELISA	277
6	Rubella IgG ELISA	163
7	VZV IgM ELISA	57
8	VZV IgG ELISA	57
9	Measles RT-PCR	230
10	Mumps RT-PCR	54
11	Rubella RT-PCR	115
12	Measles PCR product sequencing	154
13	Rubella PCR product sequencing	2
14	VZV DNA PCR	37

Epidemiology Group

Scientific Staff

Dr. Babasaheb Tandale	Scientist E & Group Leader
Dr. Yogesh Gurav	Scientist D
Dr. Avinash Deoshatwar	Scientist C

Technical Staff

Mrs. Vasanthy Venkatesh	Technical Officer A
Mr. Kailas Gadekar	Technician C
Mr. Rahul Jagtap	Technician B
Mr. Machindra Karanjwane	Technician B
Mr. Avanish Pande	Technician A
Mr. Vishal Khond	Multi Tasking Staff

Project Staff

Mrs. Mahalaxmi Mayekar	Technical Officer A
Mrs. Sandhya Jachak	Technician C
Mr. Kalyan Bhandare	Technician C
Dr. Nidhi Shrivastava	Research Associate
Mrs. Poornima Khude	Technical Assistant

EPD 1002: Community-based surveillance of viral diseases / syndromes in Janata Vasahat in Pune city, Maharashtra

Investigators: Gurav YK, Tandale BV, Chadha MS, Shah PS, Shil P, Gopalkrishna V, Deoshatwar AR, Bharadwaj R, Dohe V and Pol S

Funding agency: Extra mural (ICMR) [Dec, 2013 – Nov, 2016]

To understand the transmission of influenza like illness and dengue like illness in close knit communities, surveillance studies were carried out in Janata Vasahat, a slum area in Pune district.

Objectives: The prospective community based surveillance of viral diseases in Janata Vasahat, **Pune.**

Findings: Attacks of acute febrile illness were monitored along with assessment of different clinical syndromes like influenza like illness (ILI), dengue like illness (DLI) and acute diarrheal diseases (ADD) among children aged ≤ 6 years for association of viral etiological agents. During April-November 2016, 174 ILI episodes were recorded in the community (population of 31489). One influenza type B case was confirmed from 33 ILI cases. Specimens were collected from 59/ 103 (57.3%) DLI cases. Viral etiology could be confirmed in 26 cases; dengue (13), Chikungunya (9) and co-infection of dengue and Chikungunya (4). Dengue cases, reported during rainy season (June-October) were mainly adults (7) (Figure. 1). Incidence of DLI in August was 4 per 1000 persons per week.

Summary: Surveillance of fever, ILI, DLI has helped to have a baseline data on the incidence of viral and bacterial disease syndromes and seasonality in the community. Contribution of bacterial etiology was relatively low among ILI and DLI

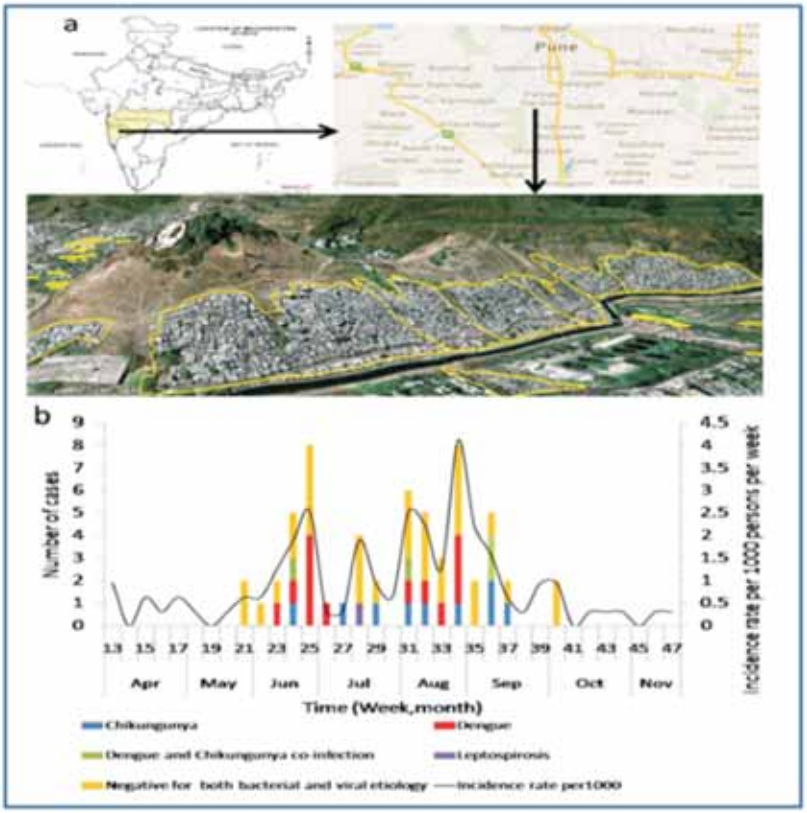


Figure. 1 a) Study area in Janata Vasahat Puneb) distribution of dengue like illness and contribution of viral and bacterial etiology in year 2016

cases. Among the ADD cases, bacterial etiology was high as compared to enteric virus etiology. None of the children received vaccine against rotavirus in the community and there is a need to carry out vaccination in the slums, though the incidence is low at the moment.

EPD 1003 Multi-centric hospital-based surveillance of acute encephalitis syndrome for viral etiology among children in selected districts of Maharashtra and Andhra Pradesh

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

Funding Agency: Extramural (ICMR) [Dec, 2013 – March, 2017]

NIV is the reference laboratory with three peripheral sites viz., GMC Nagpur, MGIMS Sewagram and KMC Warangal. ELISA and PCR laboratory facility was set-up at the peripheral sites and hands-on training provided.

Objective: Surveillance of AES among hospitalized children (<15 years) especially JE, Chandipura and enteroviruses.

Findings: 159/1287 patients were AES cases, of which 110 were eligible to be included in the present study. Sixty-nine provided consent for enrollment (36 cases, KMC Warangal; 32 cases, GMC Nagpur; 1 case, MGIMS, Sewagram). Anti JEV IgM antibodies were detected in CSF (3/106), acute sera (17/181) and convalescent sera (2/14). For CHPV, anti CHPV IgM was detected in 4 acute sera and no CSF sample was positive for CHPV RNA. All CSF, stool/rectal swabs tested negative for enteroviruses by RT-PCR. High concurrence in JE IgM was observed. Most of the AES cases were reported during July to October 2016. JE cases were reported mostly in September while CHPV incidence occurred in July 2016. Month-wise distribution of AES cases with virus etiology is depicted in Figure 2.

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

Investigators: Tandale BV, Gurav YK & Deoshatwar AR

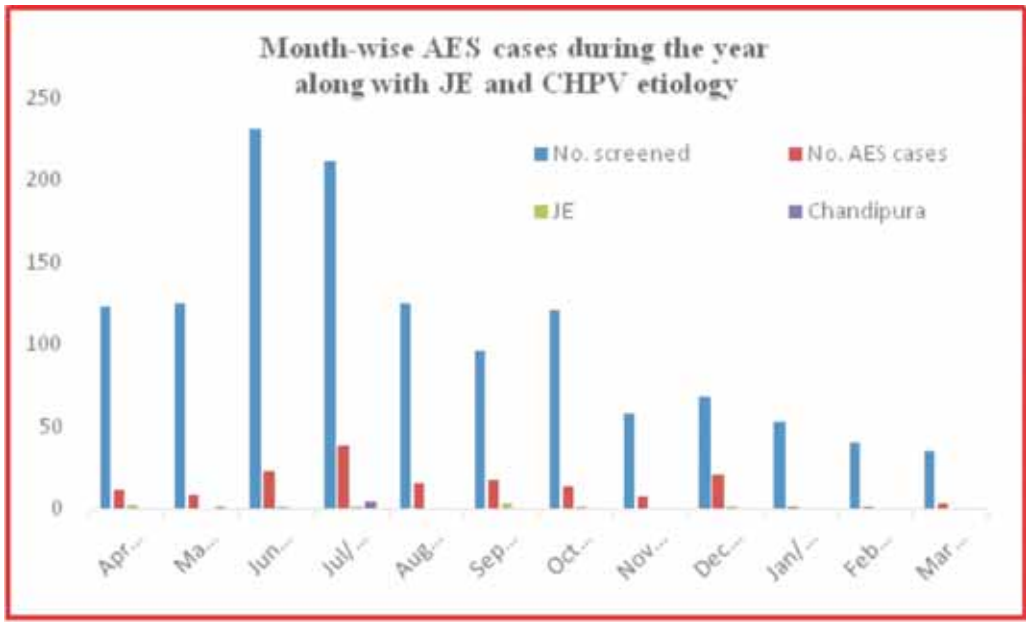


Figure. 2: Month-wise distribution of screened, eligible and enrolled AES cases with viral etiology

Funding agency: Intramural [Ongoing]

(i) Acute encephalitis syndrome among children in Gaya division, Bihar: An outbreak of AES was reported in June 2016 from Gaya division, Bihar with cases mainly from Gaya (9), Navada (2) and Aurangabad (1). The age group of the affected ranged from 2-10 years and eight deaths were reported. No clustering of cases was observed. All the cases were negative for JE according to the Microbiology department of ANMCH, Gaya. In the subsequent week, 14 more cases were admitted in ANMCH, of which 10 expired, 3 left against medical advice and 1 discharged. Seven samples were also tested by RMRIMS, Patna for JE (IgM ELISA and PCR), Leptospira (RDT IgM and IgG), Chikungunya (IgM), Scrub Typhus (IgM), West Nile Virus (PCR) and HSV 1 & 2.Only HSV 1 & 2 etiology could be established, while two cases tested equivocal for JE IgM by MAC ELISA. To take stock of the situation, a central team of doctors was formulated by Secretary Health, Govt. of Bihar

Objective: To determine the etiological agent responsible for the AES outbreak

Work done: BV Tandale along with Central Team visited Gaya district during 2nd to 6th July 2016 and checked the details. Clinical features of the patients were found compatible with AES. Children presented with high-grade fever for 18-24 hr. duration with convulsions and altered sensorium leading to rapid onset of coma. Twelve samples from 7 patients (2 CSF, 6 acute sera and one sample each of convalescent serum, urine, throat swab and rectal swab) were brought to NIV Pune for additional testing. Anti JE IgM antibodies were detected in acute and convalescent sera of one recovered AES patient while Chandipura virus RNA was detected and confirmed by sequencing of genome in acute sera of two cases hospitalized with sudden onset of fever. rapid coma and death. Rest of the CSF and serum samples were found negative for Flavivirus, Chandipura, HSV and Enterovirus genome.

(ii) Kyasanur Forest Disease outbreak in Sindhudurg, Maharashtra, in January-May 2016

Investigators: Gurav YK, Yadav PD, Gokhale MD, Viswanathan R, Mourya DT

An outbreak of Kyasanur Forest disease (KFD) was reported from Dodamarg, district Sindhudurg, Maharashtra since January 2016. NIV continued investigation of KFD in Sindhudurg and adjoining districts in collaboration with state health department, till July 2016.

Objectives: To investigate extent of outbreak and contribution of virus agent

Findings: Among 2306 suspected KFD cases from 32 villages (30439 population) in Dodamarg, rise in suspected KFD cases was reported since January 2016, peaked during March, and then declined gradually from April 2016 (Figure 3) Kyasanur forest disease viral (KFDV) etiology could be identified in 130 cases. KFD death cases were reported withcase fatality ratio 4.5% (7/155).

Among KFD cases, high age specific attack rate (8.4 per 1000 persons) was observed among old adults (aged 40-59 years). Majority of KFD cases were involved in activities like working in cashew nut farm (79.8%), cashew nut fruit collection (76.6%). KFD confirmed cases presented with fever (100%), headache (93.1%), weakness (84.6%), and hemorrhagic manifestations (28.5%). Biphasic fever was recorded in 16.9%, (22/130) KFD cases. Serosurvey in Ker village suggested clinical to sub clinical ratio as 6:1. In KFD affected area, seroprevalence of anti KFDV IgG antibodies ranged from 17.4% to 1.2 % suggests the continuous exposure of villagers to KFD infection and population had been exposed for KFD infection even before the present KFD outbreak. It has helped for preparing strategy of KFD vaccination.

(iii) Kyasanur forest disease outbreak in Banda, Sindhudurg, Maharashtra (2017)

Investigators: Gurav YK, Sahay Reema, Yadav PD, Mourya DT

Till 22nd March 2017, among 182 suspected KFD cases (from 12 villages coming under Banda PHC), 60 KFD cases were reported with 7 deaths. Among

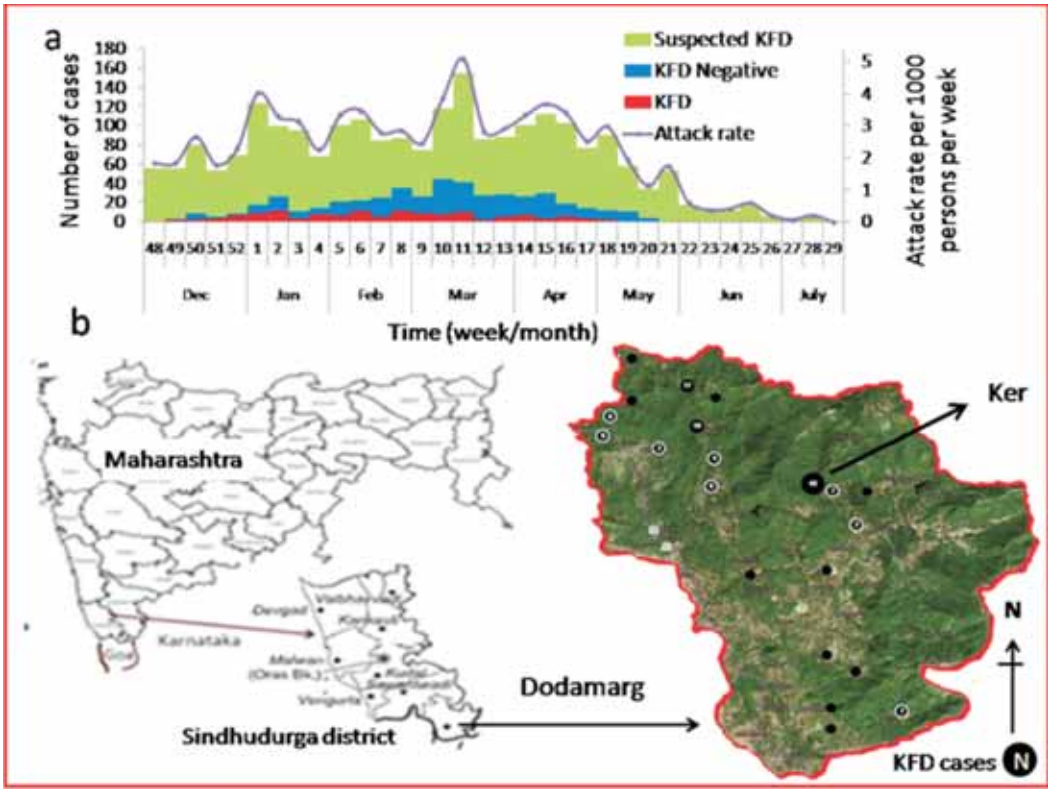


Figure 3. (a).Monthwise distribution of KFD cases (b) Dodamarg, Sindhudurg, Maharashtra

blood samples collected from 25 suspected KFD cases, during the investigation, 15 cases were laboratory confirmed for KFDV by detection of KFDV IgM antibodies (14) and KFDV real time RT-PCR (1). All KFD cases had history of tick bites. Majority of KFD cases (25, 41.7%) were in age group 45 to 59 years with attack rate (43.5%). Majority of KFD cases (38, 63.3%) with attack rate of 71.0% and 4 deaths were reported from Satmatwadi village (Fig 3b). Common clinical features amonglaboratory confirmed KFD cases (60) were fever (100%),headache (83%), body ache (62%), chills(47%), vomiting (37%), weakness (35%), (diarrhea (15%), hemorrhagic manifestation (10%) and abdominal pain (3%). All KFD death cases had co-morbid conditions. The risk behaviors identified amongst KFD cases were visit to the forest. Necropsy samples from a dead monkey and a tick pool was tested for KFDV by Real-time RT-PCR. The tick pools was found to be negative for KFDV, however necropsy tissue sample was positive for KFDV. Kyasanur forest disease is now spreading to newer areas in Sindhudurg district.

(iv) Investigation of Hepatitis E outbreak, Roha, Maharashtra April 2017

Investigators: Deoshatwar AR, Lole KS

An outbreak of hepatitis was reported from Roha Taluka, dist. Raigad and as per the request from state health team, Director NIV has deputed a team.

Objectives The aim was to investigate outbreak with possible etiology and source of infection.

Work done: A total of 124 blood samples and 24 stool samples were collected. These samples include nine blood samples and eight stool

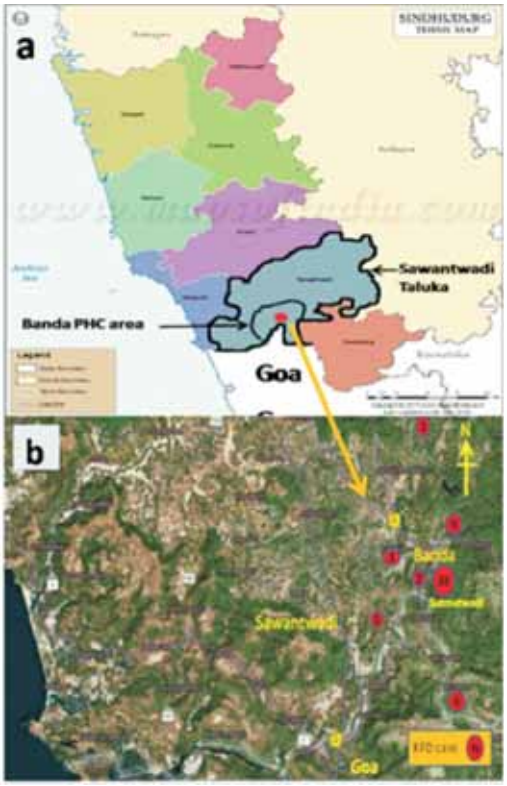


Figure 4) Study area in Sawantwadi taluka b) distribution of Kyasanur Forest Disease cases (N=60) in Banda primary health center in Sindhudurg district.

samples collected from patients admitted to the SDH Roha. Water samples were collected from pre and post-treatment stage at the water treatment plant, two affected households in Dongri Mohalla and one affected household from Lower Mohalla. House to house survey for identifying early stage and mild disease was conducted in the affected areas [around 250 households].

Results: Findings were suggestive of an HEV Outbreak. Majority of the affected people were adults. Ten ANC cases were identified in the area; one was admitted to a hospital but was improving. Nine serum samples and eight stool samples were positive for HEV infection. Dongri Mohalla [100 households] was the worst affected area than lower Mohalla [150 households]. A water line is divided at the Dhavir temple road and supplies water to these Mohallas. No cases seen before the division of this line. Observations suggested contamination of water supply line in this area. The Hepatitis group at NIV was able to isolate virus from the stool samples collected during this investigation.

Summary: The outbreak was confirmed to be caused by Hepatitis E virustransmitted due to water contamination.

(v) Japanese Encephalitis outbreak in Malkangiri, Odisha

Investigators: Gurav YK, Damle R, Cecilia D, Lole KS

Epidemiologist from NIV Pune participated in the meetings and field investigation between 2 and 7 November, 2016.

Objective: The aim was to investigate encephalitis outbreak (Oct, 2016) with high case fatality in children, Malkangiri, Orissa

Findings: Children with acute CNS disease showed clinical features of encephalopathy. From Malkangiri district hospital, clinical samples from 29 admitted fever cases (age 2-13 years) with or without other symptoms (vomiting, diarrhea etc.) and an acute encephalitis syndrome (AES) case (2 year, female) samples were collected which included, acute sera (30), blood clot (20) and a CSF. Acute sera and CSF specimens were tested for virus detection and IgM antibodies to JEV, Dengue, CHPV, HAV and HEV. Convalescent sera (27) for JEV and DENV and acute sera (20) were also tested for alkaline aminotransferase (ALT).Anti JEV IgM antibodies (10 cases) and anti DENV IgM antibodies (2 cases) were detected. No viral etiology (JEV, CHPV, DENV, HAV and HEV) was detected in a 2 year AES case, however ALT was found to be raised.

Summary: Japanese encephalitis and dengue infection were identified among the fever cases.

(vi) An outbreak of Hepatitis A in Nellikuzhy, district Ernakulum, Kerala

Investigators: Gurav YK, Retheesh Babu G, Lole KS

Investigation of hepatitis like outbreak in Nellikuzhy (district Ernakulum), was conducted during 18th-25th December 2016

Findings: Cases were mostly reported from Nellikuzhy Panchayat (ward No. 13) and adjoining areas in Ernakulum district, Kerala in November 2016. Among the 385 suspected cases, majority (66.2%) belonged to the age group of 20-39 years. Most of the suspected cases (87%) gave a history of consumption of food and cold drinks from a newly opened hotel in Nellikuzhy. Blood samples were tested for detection of anti HAV and anti HEV IgM antibodies. Among 46 suspected hepatitis cases 21 (45.6%) were positive for anti-HAV IgM antibodies and none for anti-HEV IgM. Environmental soil sample was also negative for HAV and HEV by RT-PCR.Water samples from wells (8), tank (1) and ice water samples from 2 fish markets collected from Nellikuzhy and 2 well water samples from adjoining control area (Ayvana) were negative for HAV and HEV by RT-PCR. Majority (90%) population in the area consumes water from their own wells. Health education was given to the population for prevention of further spread of hepatitis.

Summary: Hepatitis A outbreak was confirmed in Nellikuzhy and adjoining area. Likely source of outbreak was the food and cold drinks served from the newly opened hotel in Nellikuzhy.

Electron Microscopy & Pathology Group

Scientific Staff

Dr. Atanu Basu	Scientist F & Head	
Dr. Virendra Meena	Scientist B	

Technical Staff

Mr. R.M. Kolhapure	Technical Officer	
Mr. U.B. Umrani	Technical Officer	(Virus Repository)
Mrs. Shobha Gangodkar	Technical Officer	
Mr. S.K. Deshpande	Technical Assistant	(Virus Repository)
Ms. Sharada Prasad	Technician	
Mr. Raju Rahurkar	Technician	(Virus Repository)
Mr. S.R. Mate	MTS	
Smt. S.S. Mohol	MTS	(Virus Repository)
Mr. U.S. Gadekar	MTS	

Core facility activities and External Quality Assurance (EQA)

High-resolution electron microscopy application including specimen processing was provided to both in-house investigators and researchers from other National Laboratories. These included negative stained imaging and conventional ultrathin section examinations and cryo microscopy techniques. A total of 246 samples were studied. Routine virus morphodiagnosis EQA was undertaken and completed successfully.

EMP1301: Characterization of the effect of Dengue virus (DENV) NS1 protein on vascular endothelial cells

Investigator: Dr. Atanu Basu

Funding: Extramural, Dept. of Biotechnology DBT [2014-17]

Effect of exogenous NS1 on endothelial gene expression

DENV NS1 is a 48kDa viral non-structural protein that has role in both virus replication and host responses. This protein has a unique property of existing in soluble monomers and states of higher organization. Importantly, presence of circulating NS1 in dengue-infected patients has been correlated with severe disease outcomes. Recent studies have also shown that exogenous exposure of DENV NS1 can lead to release of vasoactive cytokines from macrophages and alter endothelial glycocalyx functions through heparin engagement. However, the exact mechanism of these effects remains incompletely understood. We examined the direct effect of exposure of DENV NS1 (all four serotypes) on endothelial gene expression using custom tailored arrays that represent major genes relevant to endothelial physiology including adhesion, inflammation and signaling. These arrays were custom tailored with a commercial manufacturer. The primary research question was whether any specific pattern of response could be identified through NS1 exposure.

Cultured endothelial cells were exposed to 5 µg of DENV NS1, cells harvested after 12 and 24 post exposure and cDNA synthesized from quality controlled extracted cellular RNA used to hybridize for array analysis. Experiments were done in triplicates and data analyzed using appropriate statistical processing.

Findings: When compared with controls there were significant changes in the expression of several genes (Figure 1). These included genes responsible for capillary tone, adhesion molecules and several chemokines. Current studies are ongoing to verify these changes using a proteome analysis.

In this part of our studies we examined the effect of exogenous DENV NS1 exposure on the endothelial cell cytoskeletal organization and junctional dynamics using correlative microscopy techniques. Using a similar method as described in the previous section, cultured endothelial cells were exposed to the DENV NS1 and post exposure time points imaged after cytoskeletal staining with phalloidin. In separate experiments endothelial cells exposed to NS1 were also imaged using the tapping mode of an atomic force microscope (AFM).

NS1 of all four DENV serotypes showed evidence of altering the cytoskeletal actin organization (Figure 2). The novel feature was acute condensation near junctions and imaging of stress fibres. Interestingly, AFM imaging showed evidence of increase in cell height and stiffness suggesting glycocalyx alterations as suggested earlier. Physiological responses of the cells are being currently investigated with a focus on junctional integrity.

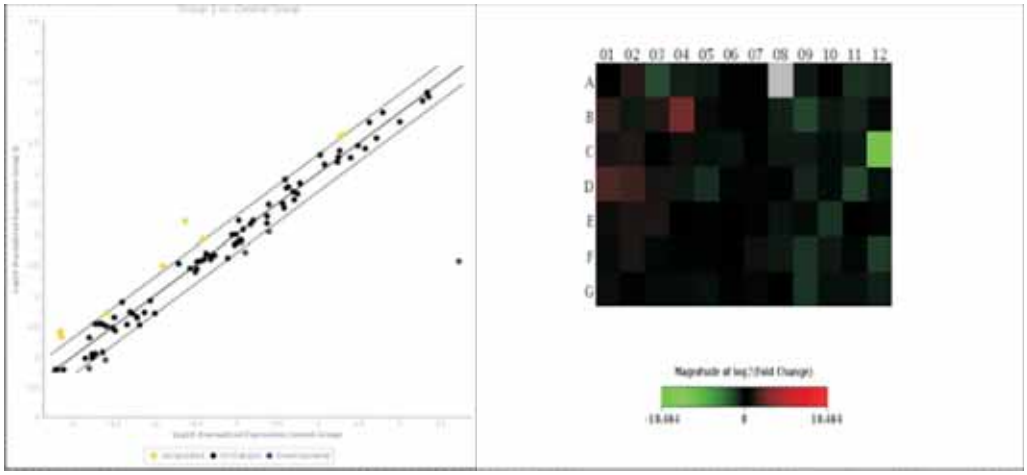


Figure 1: A representative scatter-plot and correlative heatmap showing gene expression changes due to exogenous exposure of DENV 2 on HUVEC cells (unpublished data).

Dengue virus NS1 can alter cytoskeletal morphology and intercellular junctional dynamics

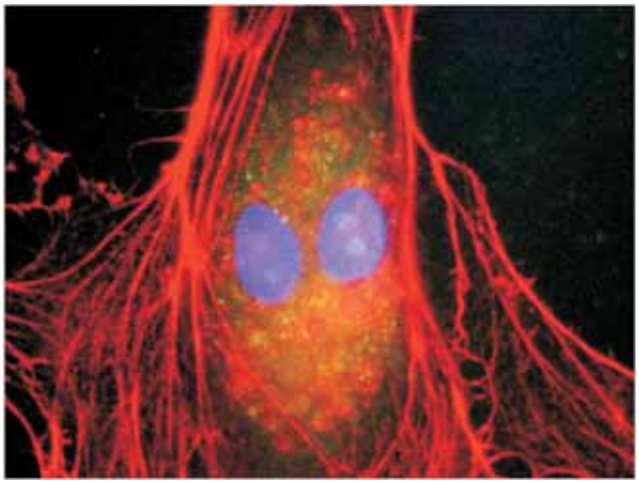


Figure 2: Confocal imaging of DENV2 NS1 (Green) bound to endothelial cells and altered cytoskeletal morphology showing distinct stress fibres and junctional changes.

Bioinformatics And Data Management

Scientific Staff

Dr. Sarah Cherian	Scientist F & Group leader
Dr. Pratip Shil	Scientist C (shilpratip@gmail.com)

Technical Staff

Mr. A.M. Walimbe	Technical Officer B
Mr. Vijayasimha K	Technical Officer A
Mr. Santosh Jadhav	Technical Assistant
Mr. N V Gujar	Technician - B
Mr. Avinash Patil	Technician-A

Project Staff

Mr. Abhisek Behera	ICMR SRF
Ms. Dipali Bhoye	UGC - RGNF SRF

BDM1001: Core facility services

Investigators: Cherian S, Shil P, Walimbe AM, Simha V& JadhavS

Statistical analyses of epidemiological and serological data, bioinformatics, sequence and structure analyses, mathematical modeling support was provided to various groups within the institute. Maintenance of computers, servers, laptops, printers, computer peripherals, network and internet services was done on regular basis. Regular maintenance of connectivity between NIV, MCC and three field units is being done for data transfer, AIMS, LIMS Software and intercom services. NIV website is being maintained and updated on a regular basis. Technical support has been provided for conferences and workshops held by NIV. The indigenously developed software like payroll, pension, supplementary bills, arrears calculator, computer complaint register etc. are being maintained.

Comparative analyses high and low pathogenic strains of Avian Influenza virus NS1 proteins

Investigators: Thube MM, P Shil & J Mullick

The high pathogenic Avian influenza H5N1 virus showed higher replication rate and induced less IFN-b and 6 ISGs compared to H11N1 virus when grown in the human lung epithelial A549 cells, reflecting generation of 7 differential innate-immune responses during the infection of these viruses. Non-structural 1 (NS1) protein, a major IFN-antagonist, known to help the virus in evading host innate-immune response was compared from both the strains using bioinformatics tools. Analyses revealed differences in the composition of NS1 proteins from the two strains that may have an impact on the modulation of the innate immune response.

The significant differences in the ability of NS1 of the two viruses to inhibit IFN-b prompted analysis of amino-acid variations in NS1 and its structural comparison using bioinformatics tools. Results of pairwise-alignment of H5N1 and H11N1 showed 13 amino-acid variations and a 5 amino-acid (80-84) deletion in H5N1 (Figure 1). NS1 protein binds to the 30-kDa subunit of cleavage and polyadenylation-specificity factor (CPSF30) to regulate cellular mRNA processing leading to general inhibition of the host-antiviral response. Notably, we found N171D (H5N1vs H11N1) within CPSF30 binding-site (144-188) while M111V and T112A in close vicinity of another CPSF30 binding-site (103-106), thus proposing a likely role. Role of the 5 amino-acid residues in IFN-signaling is less plausible as it is a known major determinant of virulence in H5N1, which is not presentin LPAI viruses, including H11N1. Nonetheless, it is not located within the CPSF30 binding-sites of H11N1-NS1.

Figure 1:NS1 protein sequences of H5N1 and H11N1 viruses were aligned and the different domains identified. Residues from 1-73 and 74-230 represent the RNA binding domain and the effector domain of the NS1 proteins, respectively. Residues highlighted in bold font and indicated with asterisk represent differences between H5N1 and H11N1 NS1. The CPSF30 binding sites at residues 103-106 and 144-188 have been shown by black horizontal lines.

3D structure-prediction of the NS1 proteins was obtained using the crystallographic structure of Influenza/A H6N6 NS1 (4OPH.PDB) as template using methods described earlier. The target template sequence identity was 93.33 % for H5N1 and 98.70% for H11N1. The minimized energy of NS1 from H5N1 and H11N1 was -9967.9kJ/mol and -10103kJ/mol, respectively. Superimposition of the 3D structures indicated a root- mean-squared-deviation of 0.22Å involving 760 backbone atoms, implying that the proteins have high structural similarity in terms of backbone fold.

Scrutiny of H11N1-NS1 sequences from GenBank and H5N1 strains (2006-2011) revealed N171 in majority of H5N1 viruses and D171 in all H11N1 viruses. The presence of charged aspartic-acid residue in H11N1 may increase the binding affinity for CPSF30 to NS1, explaining the enhanced IFN-b suppression. Hence, N171D and other differences between H5N1 NS1 and H11N1 NS1 and polymerase proteins may be responsible for the altered functional efficiency reflected by the differential response in inhibition of IFN-b promoter activation.

Bioinformatics analyses of KFDV Envelope glycoprotein from emerging outbreaks

Recent outbreaks of KFDV in Maharashtra have raised queries regarding the changing nature of viral proteins that might have facilitated its spread to newer areas.

We used bioinformatics tools to compare sequences, predict B-cell epitopes, 3D structures and compare envelope glycoprotein (E proteins) between the old strains of KFDV and those from emerging outbreaks.

Work done: Analyses based on the envelope glycoproteins (E-protein) revealed that there is no change in strains isolated from the recent outbreaks (99.2%) to the prototype strain (P9605).No drastic changes could be seen in the structure (3D fold), antigenicity or functional sites on the E proteins of recent strains (Figure. 2). The new strains are identical in terms of B-cell epitopes (antigenicity), 3D structure and surface properties (electrostatics).

Comparative analyses of Zika and Dengue virus Envelope proteins

Investigators:Shil P, Yadav P, Balasubramanian R & Mourya DT

Cross-reactivity in diagnostic ELISA is a major problem and is frequently observed between Zika and dengue viruses.In this context, an investigation into the composition of envelope glycoprotein (E-protein) of ZIKV with other flaviviruses is necessary as E-protein is the main target for host antibodies.

Bioinformatics analyses reveal similarity between E protein of ZIKV and dengue viruses. ZIKV E-protein amino acid sequence has been compared (using bioinformatics tools) with other flaviviruses viz. dengue type 2 (DEN2), MVEV, Japanese encephalitis (JEV), West Nile (WNV),

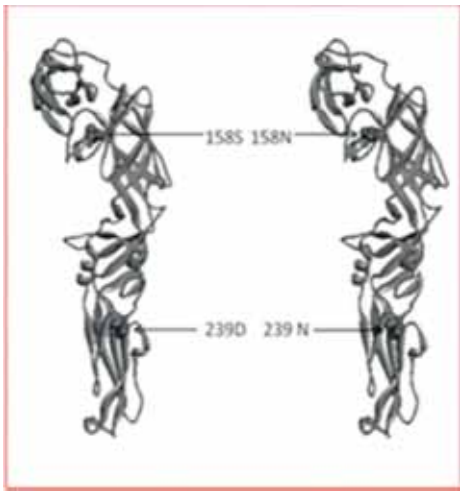


Figure 2:Predicted 3D structures of P9605 and MCL-15-T-338 indicating the mutations

KFDV, Tick-borne encephalitis (TBEV), Yellow Fever (YFV) and St. Louis encephalitis (SLE). Comparison of all possible pairs of sequences was obtained from ISHAN package (Table 1).

Analysis of the predicted B-cell epitope revealed the occurrence of three antigenic regions with >80 per cent identity of amino acid composition with DENV sequences. Of these, the epitope 20-WVDVVLEHGGCVTVMAQ-36 in ZIKV corresponds to the epitope 18-GSWVDIVLEHGSCVTT-33 in DEN2 and highly conserved in other dengue serotypes. This epitope occurs in domain I of 3D structure of flavivirus E-protein (DEN2, 1OAN.pdb numbering). The epitope 285-SSGHLKCRLKM-295 in ZIKV corresponds to the epitope 278-GNHMFAGHLKCKVRM-288 in DEN2 and occurs in domain I extending towards the hinge region, which connects the domain I with domain III (Figure 3). The epitope 104-GCGLFGKGSLVTCAKFACSK-123 in ZIKV corresponds to 104-CGLFGKGGIVTCAMFT-120 in DEN2 and is located in the domain II. The occurrence of these B-cell epitopes on ZIKV having =80%identity of composition with DENV may be the reason for the reported cross-reactivity in serological tests. In the natural dimeric arrangement of E-protein on viral membrane, these epitopes are exposed for interaction with host immune system.

Hence, similarities at the molecular level of the E-proteins may be the possible explanation of cross-reactivity.

Table 1: Percentage identity of amino acid composition between flavivirus E-proteins

	ZIKV	DEN2	JEV	WNV	YFV	TBEV	KFDV	MVEV	StLU
ZIKV	100								
DEN2	54.2	100							
JEV	51.7	46.4	100						
WNV	51.2	46.1	75.3	100					
YFV	40.5	43.7	40.9	39.2	100				
TBEV	38.7	38.8	37.7	39.6	39.2	100			
KFDV	36.3	36.6	36.6	38.1	35.7	78.2	100		
MVEV	51.0	45.7	77.1	76.2	40.9	38.7	36.8	100	
StLU	51.4	47.8	66.8	70.0	42.1	41.1	38.6	70.5	100

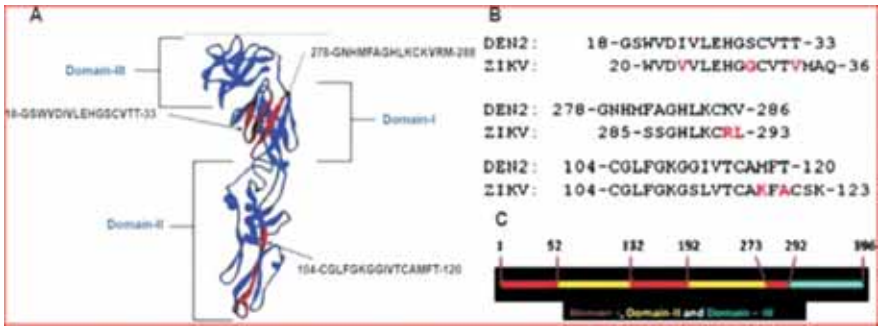


Figure 3: Positions of three most similar predicted B-cell epitopes on 3D structure of DEN2 E-protein (1OAN.pdb). 3D structure visualization and rendering of images was performed in Discovery Studio viewer 3.1. (B). Comparison of epitope compositions, DEN2 verses ZIKV, with differences marked in Red font. (C). Domain classification of dengue virus E-protein (1OAN.pdb) with numbers denoting amino acid order in the sequence.

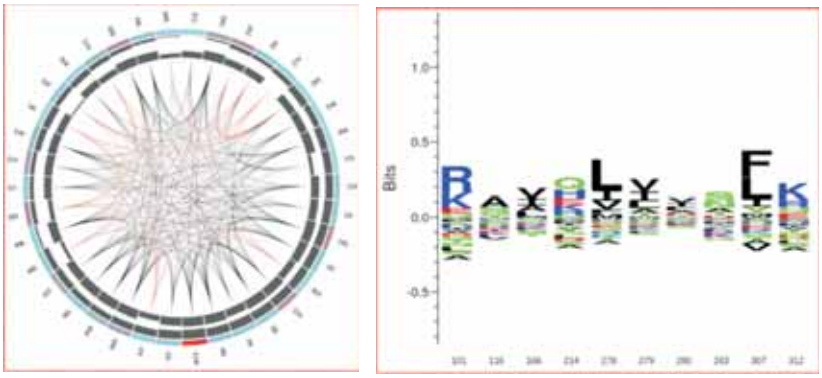


Figure.4. (a) Mutual information network showing the co-evolved residue pairs in M2 of seasonal Influenza A/H1N1 viruses. Red edges represent top 5%. (b) Sequence logo of selected nodes of M1/M2 proteins showing frequency of occurrence of amino acids

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

Investigators:Cherian S & Behera A

Funding: ICMR (Grant #2014-20880)[2014-2017]

This database aims at providing information on co-evolutionary mutations obtained from the implementation of tools based on statistical approaches like correlation coefficient, mutual information, etc. that infer correlated evolution from multiple sequence alignment. Co-evolutionary mutations are frequently seen in intra-protein segments as well as inter-protein segments of the virus. For example, mutations T82K, K141E and R189K in Hemagglutinin (HA) are found to have coevolved with the neuraminidase (NA) drug resistance mutation H274Y in seasonal H1N1 viruses of the period 2004-2009. On the other hand, the M2 protein, of influenza A viruses, is the target for adamantane drugs, amantadine and rimantadine. The most prevalent adamantane-resistance M2 mutations are S31N, along with V27A, G34E, L26F, that are located along the inside rim of the M2 ion channel pore. In this study, we used the MISTC (Mutual Information Server to Infer Coevolution) server to detect possible intra-domain and inter-domain co-evolution sites based on the matrix (M1 and M2) protein. For the latter study, the M1 and M2 sequences were concatenated strain-wise before using MISTIC.

Based on a dataset of representative M2 sequences (n=701) of seasonal H1N1 (1918-2016), it was found that positions 55F/L and60K/Q in the cytoplasmic tail of M2 coevolved with the drug resistant markers S31N and V27A respectively (Figure 4a). In case of concatenated M1 (252aa) and M2 sequences (n=1009), position 101R/K within the nuclear localization signal (101-RKLKR-105) of M1 was found to have coevolved with the S31N (283 position on concatenation) marker (Figure. 4b). The role of these newly identified co-evolving positions need to be further studied.

BDM1403 Phylodynamics and molecular evolution of Influenza A Polymerase genes (PB1, PB2 and PA) inferred from large-scale sequence analyses and structural Bioinformatics

Investigators:Cherian Sarah & Bhoye D[Duration: 2014-2018]

Work done: UGC Grant No: F1-17.1/2012-13/RGNF-2012-13-ST-MAH-34083

The N-terminal domain of the PA subunit (PA-Nter) contains the active site of the endonuclease and recent findings suggest that PA gene mutations have been linked to increased virulence. Specifically, the F35L mutation in PA results in a higher polymerase activity compared with the PA- wild type protein in pH1N1 viruses. In a mouse-adaptive pH1N1 virus, it was found that the F35L PA mutation also had a synergistic effect on the Hemagglutinin (HA) mutations, D222G and K163E.

The objective of the present study was to understand the effect of PA-F35L mutation on viral fitness, specifically the nature of specific binding of uridine and adenosine nucleotide monophosphates including the metal binding aspects in the endonuclease domain active site of the pH1N1virus.

Modeling the 3D-structure of the pH1N1 PA-Nter domain and docking of endonuclease substrate ligand, Uridine-5'-Monophosphate (UMP) molecular dynamics (MD) simulation studies of the docked complexes for the two observed PA variants was done. Results were suggestive of the UMP ligand affinity improves marginally in the mutant strain, due to additional favorable contacts with Lys34 and Lys134 in the endonuclease domain cavity (Figure. 5). Further studies with the other endonuclease ligands, adenosine-5'-Phosphate (AMP), guanidine monophosphates (GMP) etc. needs to be done.

BDM 1501: Molecular Characterization of the Measles Virus genotypes circulating in India (1996-2014) using phylodynamics and structural bioinformatics analyses

Investigators: Cherian S, Walimbe AM & Vaidya S

Funding: Intramural [2015-2018]

Measles virus (MeV) is serologically a monotypic virus but genetically distinguished into 24 genotypes. To further our understanding of the molecular evolution of the endemic genotypes D4 and D8, selection pressure studies of the hemagglutinin (H) gene, the main target for neutralizing antibodies, was carried out. Four mutant viruses when analyzed for changes in their receptor binding affinities, by docking of Signaling Lymphocyte Activation Molecule (SLAM) and nectin4 receptors, showed a marginal change in binding affinities. In vitro studies of the mutants revealed that the viruses grew well on Vero/hSLAM and MCF7 cells expressing the SLAM and nectin4 receptors respectively in comparison to Vero cells containing CD46 receptor. Single amino acid changes, N481Y or S546G, in the H determine its ability to utilize the CD46 receptor. Hence, molecular docking studies of CD46 with MeV-H possessing Y481N/D were carried out to understand the in-depth molecular mechanism by which MeV-H residues define CD46 binding

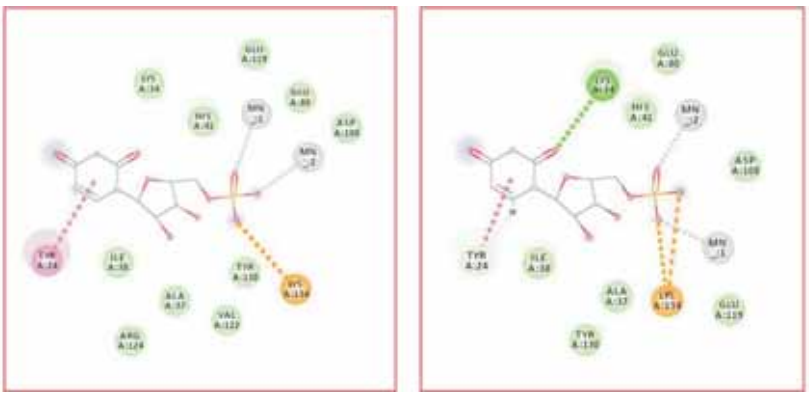


Figure. 5: Contacts formed by the ligand with the PA-Nter endonuclease domain at the end of 20ns MD simulation (a) UMP with PA-F35 (b) UMP with PA-L35

affinity.

It was found that loss in either of the hydrogen bond (H-bond) contacts (MeV-H:481–CD46:65, MeV-H:546–CD46:63) in the central contact region prevented efficient CD46 binding. Y481N could form the specific H-bond, while G546S H-bond could be formed only in conjunction with Y481.Both the H-bonds are noted in the Edmonston vaccine strain that can utilize CD46 as a receptor while loss in 1 of the H-bonds is noted in Indian strains (Bijapur'06 and Perambalur'07) possessing Y481N/D mutation with the S546G mutation (Figure. 6).

The understanding may provide a basis for predicting mutations that could alter MeV-H-CD46 binding efficiency and thus alter viral tropism.

Mathematical Epidemiology

1) Mathematical modeling of viral epidemics

Investigators: Shil P

Mathematical models to describe transmission and propagation of diseases have gained momentum over the last hundred years. Formulated mathematical models are currently applied to understand the epidemiology of various diseases including viral diseases viz., Influenza, SARS, measles, etc. With the emergence of advanced computing tools, designing mathematical models and generating simulations (numerical solutions) have become feasible and there is an enormous scope to study boththe epidemiology of viral diseases through transmission dynamics of outbreaks and evaluating or predicting the effects of interventions and vaccinations. The influenza pandemic of 2009 and the Ebola epidemics of 2014-15 have generated renewed interest in mathematical modeling of epidemics, considering its implications to Public Health. Review of the various mathematical models and their applications in the study of virus driven epidemics was published.

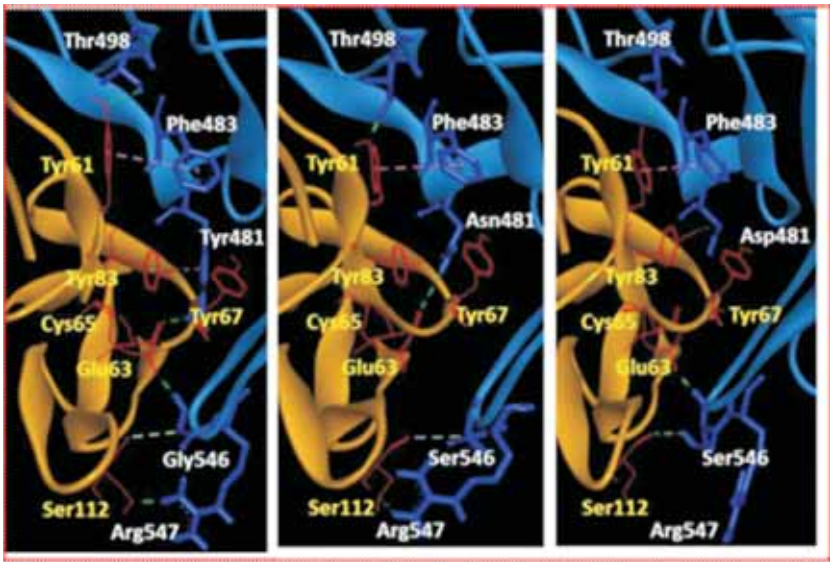


Figure. 6: 3D views of the MeV-H–CD46 docked complexes for strains (i) Edmonston(ii) Bijapur'06 (iii) Perambalur'07. All H chains are indicated in blue ribbon, and CD46 chain in goldenrod ribbon (A) Close-up in the central contact region showing the hydrogen bond (H-bond) interactions, and other interacting residues involving MeV-H residues located in the ß4 and ß5 blades of the ß-propeller fold. The H-bonds are indicated as green dashed lines while hydrophobic contacts (= 5Å) are in pink dashed lines

2) Mathematical Epidemiology: Investigations on outbreaks due to Influenza H1N1 pdm09 in urban slum under surveillance between 2012 and 2015

Investigators: Shil P, Gurav Y, Tandale BV&Chadha MS

To understand the pattern of transmission of influenza in Pune city, community-based surveillance of ILI was undertaken since 2011 in Janata Vasahat slum. Outbreaks due to influenza A(H1N1)pdm09occurred twice, once in February 2012 and another in February 2015. The epidemiological parameters required for disease propagation during the outbreaks were estimated by mathematical analyses of data.

The growth rate of the epidemic (r) was calculated from the estimates of cumulative number of confirmed infections (y) the effective reproduction number R was computed. The transmission rate (ß) and doubling time (the time period in which the size of the outbreak doubles) were determined. Graph plotting and mathematical calculations were performed using the MATLAB® software package. In year 2012, a total of 499 ILI cases were reported between 6 February and 29 April 2012. Based on the growth of the cumulative confirmed cases for the first 30 days (Fig. 7a), the intrinsic exponential growth rate (r) was found to be 0•1121 per day. Assuming the mean incubation period to be 1•5 days and the mean infectious period as 4 days, R was estimated to be 1•30. This indicated a moderate transmission of influenza A (H1N1) pdm09 in the population. Also, considering the total residing population 29 797, the transmission rate (ß) was estimated as 1•09 × 10-5 per day. The doubling time of the epidemic was estimated to be 2•88 days.

A total of 377 ILI cases were reported in February 2015 outbreak. Considering the growth of cumulative confirmed cases for the first 16 days (Figure. 7) the intrinsic exponential growth rate (r) was 0•1743 per day. Assuming all other parameters for influenza A(H1N1)pdm09 to remain the same (as in 2012), the R was estimated to be 1•64. This indicated a slightly higher transmission in the population compared with 2012. The transmission rate (ß) was estimated as 1•37 × 10-5 per day. The doubling time of the epidemic was estimated to be 2•44 days.

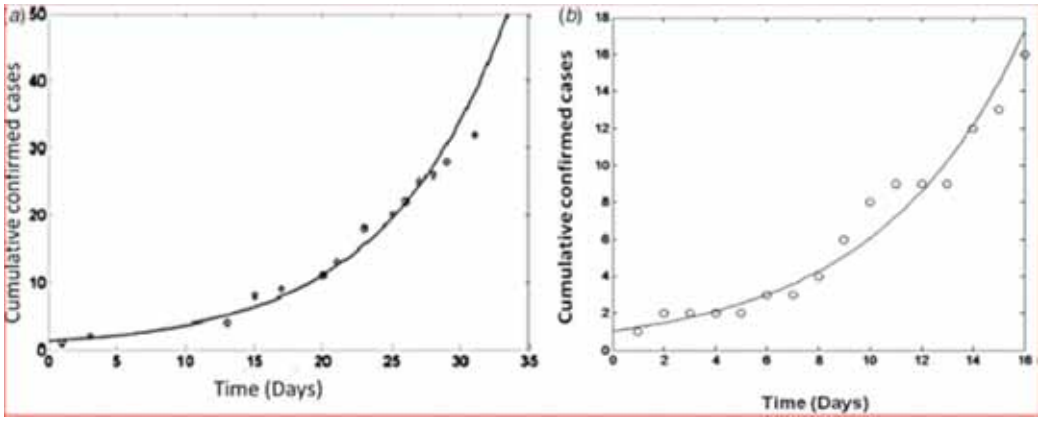


Figure 7: Cumulative growth of confirmed cases of influenza A(H1N1)pdm09 with time. Data plot and curve fitting in MATLAB for (a) year 2012 (data set: 21 February–22 March 2012) and (b) year 2015 (data set: 1 February–21 March 2015).

Animal House Group

Scientific Staff

Dr. D.R. Patil	Scientist D & Group Leader

Technical Staff

Mr. S.V. Nipunage	Technical Officer (A)
Mr. H.L. Chakankar	Technical Assistant
Mr. M.P. Rajarshi	Technical Assistant
Mr. S.N. Fulari	Technical Assistant
Mr. R.H. Chavan	Technician C
Mr. R.J. Sarpatil	Technician C
Mr. S.M. Doke	Technician C
Mr. V.B. Kalangade	Technician B
Mr. N.B. Bhandekar	Technician B
Mr. M.B. Kamble	Technician B
Mr. A.N. Shitole	Technician B
Mr. A.B. Sunsuna	Multi Tasking Staff (MTS)
Mr. A.V. Kondayya	MTS
Mr. A.D. Dalvi	MTS
Mr. V.P. Sasane	MTS
Mr. S.B. More	MTS
Mr. D.L. Nagayya	MTS
Mr. T.S. Ramayya	MTS
Mr. R. Venkatayya	MTS
Mr. A. Thimanapalli	MTS

Project Staff

Mr. A.S. Shinde

Mr. M.S. Parshuram

AHG 1501: Breeding, supply and maintenance of laboratory animals.

Investigators: Patil DR & Nipunage SV

Funding Agency: Intramural [Ongoing (service project)]

Large and small animal facilities at the Institute are registered with CPCSEA under "Research for education and breeding for in house use" vide Registration No. 43/GO/ReBi/SL/99/CPCSEA. The registration of the Institute is renewed in March 2017 and is valid till March 2022. IAEC of the Institute was reconstituted simultaneously.

Seven strains of mice (Inbred: BALB/c, C57BL/6, DBA/2, C3H, Outbred: CD-1, Immunodeficient: CD-1 nu/nu and BALB/c nu/nu) are currently bred and maintained in the IVC system and transported in filter top cages. All mouse-handling procedures at the breeding facility are done in the laminar airflow cabinets and soiled cages are changed in specially designed cage change stations. Health status of animals is ensured by routine observations and various quality checks carried out in live animals and consumables. Controlled environmental conditions are strictly ensured with animal room temperature maintained between 22 ± 2°C, relative humidity between 45-55 %, 12:12 hr. light dark cycle and 15-16 air changes per hour with 100% fresh air.

Objectives:

To breed, maintain and supply quality laboratory animals for in house research

To ensure regulatory compliance in animal breeding and experimentation

To provide logistic and technical support for animal experiments

To provide training with regard to animal breeding and experimentation

Work done: During the report period, 2725 mice and 950 ml blood from different species of animals were supplied to institutional scientists against 36 IAEC approved research projects (Table 1, 2). Other laboratory animals viz., guinea pig, rabbit, fowl, turkey, goose are procured from CPCSEA authorized sources as per requirement. Rhesus monkeys are under post experimentation rehabilitation. Records are maintained at the facility as per CPCSEA requirements, which include, livestock register, breeding records, pedigree, microbial and genetic monitoring of animals, staff health records, consumables testing records, individual project files and form D (animal utilization), AMC of equipment. Annual facility inspections by IAEC were carried out in February (old committee) and March 2017 (Newly reconstituted committee) and reports submitted to CPCSEA.

Microbial & Genetic Monitoring of mouse strains:

Testing of seven mouse strains by ELISA for 4 pathogens (Minute Virus of Mice, Lactate Dehydrogenase Elevating Virus, Mouse Hepatitis Virus &Mycoplasma pulmonis) and by PCR for 3 pathogens (Sendai, Pneumocystis Carinii, Tyzzer's Organism) was undertaken at ACTREC, Navi Mumbai and all mouse strains were negative for all the seven rodent pathogens thereby indicating SPF status.

Genetic Monitoring of four inbred mouse strains by PCR for 6 markers (D2Mit15, D5Mit18, D6Mit274, D8Mit88, D15Mit175, and D19Mit177) was undertaken at ACTREC, Navi Mumbai and found to be non-contaminated.

No abnormalities were detected in mouse stool samples tested at Veterinary Diagnostic Centre & Allied Services, Pune for examination of physical, microscopic, parasites and occult blood.

• Random serum samples from 28 female BALB/c mice were negative for maternal anti rotavirus antibodies by ELISA when tested at Venky's (India) Limited, Pune

Table – 1:Supply of Laboratory Animals to NIV Scientists (numbers)

CD1 Mice Infants*	CD1 Mice Adults	BALB/c Mice Adults	C57BL/6 Mice Infants*	C57BL/6 Mice Adults	Total Number
2083	83	495	24	40	2725

(* with mothers)

Table – 2:details of the supply of blood to NIV Scientists (quantity in ml)

Rabbit	Guinea pig	Fowl	Turkey	Monkey	Goose	Total (ml)
124	49	234	451	55	37	950

Monkey Health Monitoring:Husbandry and veterinary care was provided to rhesus monkeys under rehabilitation at the institute. Comprehensive health monitoring was conducted for individual animals. The tests were conducted through NABL accredited laboratory, which included, Complete Blood Count, Differential Count, Peripheral Blood Smear Examination, hepatic profile, kidney profile, lipid profile, Chest X-ray, intra-dermal tuberculin test. Primate facility inspection and verification of records was done by IAEC during the year. Besides institutional care program for primates, services of consultant veterinarian were also sought. All the test results were found to be within the normal literature range for the species.

IAEC Activities:

•Three mandatory annual inspection meetings of IAEC were organized on 15.4.2016, 27.2.2017 and 30.3.2017. During the meetings, inspection of the facility and verification of various records pertaining to animal breeding and experiments at the institute was conducted by the committee and the report was submitted to CPCSEA, New Delhi.

Two meetings of IAEC were conducted on 26.5.2016 & 10.2.2017 for evaluation of animal experimentation projects. During the meetings, 14 animal experimentation projects (10 new and 4 extensions) were reviewed by the committee. Form D (animal utilization form) and progress reports of ongoing animal experiments were reviewed by the committee. Two projects involving use of large animals were recommended by the IAEC for onward submission to CPCSEA for approval.

CPCSEA, New Delhi renewed the registration and reconstituted the Institutional Animals Ethics Committee of National Institute of Virology, Pune for the period from 10.03.2017 to 09.03.22 List of revised IAEC attached herewith

Diagnostic Virology Group

Scientific Staff

Dr. Gajanan N Sapkal	Scientist D & Group Leader
Dr. Rajlakshmi Viswanathan	Scientist C

Technical Staff

Mr. Shankar M. Vidhate	Technician B
Mr. Suresh Kamble	Multi Tasking Staff

Project Staff

Dr. Gururaj R Deshpande	Scientist C
Dr. Kiran Munne	Scientist C
Dr. Suji George	Scientist C
Dr. Reshma U Shejwalkar	Scientist B
Mr. Ojas Kaduskar	Technical Assistant
Ms. Reshmi Krishnan	Technical Assistant
Mr. Nitin Mule	Technician C
Mrs. Kirtee Khutwad	Technician C
Mrs. Sarita Raut	Technician C
Mr. Shivshankar Gaikwad	Technician C
Mr. Venkatesevarlu Dongari	Multi Tasking Staff

DVG 1301: Resource Centre for Virus Diagnostic Laboratories (RCVDL) at NIV, Pune

Investigators: Sapkal GN, Viswanathan R & Potdar VA

Funding Agency: ICMR [2017-2020]

(i) Resource Centre for Virus Diagnostic Laboratories: RCVRDL has been established in response to recommendation of virology task force monitoring the VRDL, network with an objective to provide training for VRDL network staff and conducting quality assurance (QA) and quality control (QC) programs.For the first two years, training modules on different aspects of virology were conducted.During the third year, consolidated module was introduced as suggested by DHR and VRDL monitoring committee, proposed modification of the program to expedite the VRDLs role in sample testing. In the current year the same pattern was followed. Sixty-nine participants from 18 centers were trained in different aspects of Biosafety, Outbreak Investigations, ELISA, PCR etc. This included two state Nodal Officers, 10 PIs, 5 Co-PIs, 15 Scientists, 11 Research Assistants and 24 other staff. Training modules included lectures, case studies, demonstrations and hands on practicals. Pre and post assessment tests were conducted; feedback was taken into account while preparing subsequent training modules.

(ii) Response to national emergency: Following the directive of Secretary, DHR and MoH&FW, preparedness training on laboratory diagnosis of Yellow Fever was conducted for 05 laboratories.

(iii) Quality control program for VRDLs: During the year, 846 samples received from VRDL network were tested (Dengue IgM 342, Dengue NS1-91, Chikungunya IgM-184, JE IgM-167, Measles IgM-35, and HAV IgM-27) and overall concordance was 85%.

(iv) Supply of reagents and kits: In the current year, RCVRDL has taken up the responsibility of coordinating supply of reagents for diagnosis of influenza and other respiratory viruses. During March 2016 to October 2016, 2500 reactions for 4 targets were provided to 6 VRDL centers. From November 2016 onwards to 31 March 2017, 4000 reactions for 7 targets were provided to 13 VRDL centers.

(v) Zika virus diagnosis: In response to the directive from Secretary, DHR to strengthen 23 identified VRDLs, training program for ZIKV diagnosis was conducted. In the first phase, staff of 10 identified VRDLs was trained for diagnostic testing of human samples and 3 ICMR laboratories for mosquito surveillance. The topics covered included global scenario and preparedness of India for ZIKV diagnosis, basic concepts in ZIKV diagnosis, specimen management, packing and transport, biosafety, principles and steps of ZIKV RT PCR and Quality Control & Trouble Shooting.

In addition, 2712 Dengue and/ or Chikungunya IgM negative samples received from VRDLs were tested for ZIKV by real Time RT PCR at NIV and all were negative.

DVG 1402: Generation and characterization of monoclonal antibodies developed against Kyasanur Forest disease virus.

Investigators: Sapkal GN, Deshpande GN, Yadav PD & Mourya DT

Funding Agency: ICMR [03 years]

Monoclonal antibodies (MAbs) reactive against KFD were generated to replace the existing KFD IgM ELISA. MAbs of IgG isotypes were further up scaled and purified, biotin labeled and optimized for reactivity. Further studies are in progress.

DVG 1403: Infection Dynamics of Congenital Cytomegalovirus in Neonates in Pune, Maharashtra

Investigators: Viswanathan R & Sapkal GN (in collaboration with city hospitals

Funding: Intramural [Duration: 03 years]

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 sensor neural deafness.

Objective: Determination of CMV seroprevalence in antenatal mothers and congenital CMV in neonates

Work done: During the year, samples from 283 pregnant women were screened and 99% showed anti CMV IgG. One newborn baby tested positive for CMV. Methodology for screening of urine samples is optimized and initiated.

Screening of 1000 samples from newborn babies has resulted in getting 5 CMV positives by real time PCR. Further, eleven babies with suspected congenital CMV infection were confirmed positive by laboratory testing (ELISA, PCR). All babies are under follow up with collaborators.

DVG 1602: Development of serodiagnostic assays for detection of anti ZIKV antibodies

Investigators: Sapkal GN, Yadav PD & Deshpande GN

Funding: Intramural [3 years]

ZIKV infection can be diagnosed in acute phase (<5 days) using PCR. However, there are no commercial kits (Approved or validated) available for sero-diagnosis. Anti ZIKV IgM antibodies can be detected after 5 days post infection.

Work done: Zika standard strain required for assay development was procured from ATCC and immune sera was generated in mice (Balb/c). Further standardization of virus nutrilization assay is in progress.

Congenital Rubella Syndrome (CRS) surveillance in India.

Investigators: Sapkal GN, Viswanathan R, Deshpande GN, George S.

Funding Agency: ICMR [Duration: 05 years]

In India, CRS burden is not known. In view of the proposed introduction of rubella vaccine and immediate need to set up CRS surveillance, the Ministry of Health and Family Welfare has requested ICMR to establish surveillance for CRS, which will provide a baseline estimate of disease burden and monitor the impact and progress made by rubella vaccination over a period of time. This multicentric project has been initiated in six centers across India and is coordinated by National Institute of Epidemiology, Chennai. RCVRDL, NIV, Pune is the laboratory partner for laboratory SOP development, training, troubleshooting and QA/QC. RCVRDL will also perform PCR of throat swabs from all centers and genotyping of positive samples.

SOP for sample collection, transport and laboratory testing has been developed and shared with all centers. Laboratory training was held on 16th-17th November 2016 for 14 participants from six sites. The program included lectures, demonstrations and hands on practical. Diagnostic testing (ELISA, PCR) and genotyping (PCR) have been standardized. Quality control program has been initiated. Till date 95 serum samples from five centers have been tested. Overall agreement of 98% was observed for anti rubella IgM with commercial kit and 91% with WHO recommended kit. Twenty-eight throat swabs out of 58 were positive by PCR (48%).Genotyping and sequencing is in progress.

Bacteriology
(New group established in 2016)

Scientific Staff

Dr. Rajlakshmi Viswanathan	Scientist C
Dr. Anuj Kumar	Scientist B

Technical Staff

Mrs. Savita Dhurandhare	Technician B
Mr. Rajendra Khedkar	Multi Tasking Staff

BACT1601: Diagnostic Services

Respiratory and diarrheal diseases are of diverse etiology, including viral and bacterial pathogens. Viral diagnostics for these diseases are well established at NIV. However, over the years there has been a concern over the correlation of virological findings with bacterial findings and it has been felt that determination of bacterial etiology will contribute significantly to the emergence of meaningful data. Therefore it was proposed to develop a bacteriology laboratory at NIV, Pune with the mandate of providing diagnostic support for detection and identification of bacterial etiological agent in respiratory and diarrheal infections.

Following approval of Scientific Advisory Committee, laboratory space of~850 sq. feet was identified on the first floor of the main building at MCC, Pashan. Renovation is being done with distinct spatially separated work areas for specimen receiving and processing, specimen inoculation and culture characterization, washing and decontamination, sterilization/packing and media preparation. Molecular diagnostic laboratory is remodeled. Purchase of consumables and essential equipment is ongoing.

Panel of respiratory and diarrheal agents to be included in diagnostic work up has been identified and SOPs for are being written and finalized. Conventional PCR is standardized for detection of *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae.*

Translational Research Group (TRG)

Dr. D T Mourya	Group Coordinator
Coordinators	
Dr. Kavita S. Lole	Scientist E
Dr. Gajanan N. Sapkal	Scientist D
Dr. Sudeep A.B.	Scientist D
Scientific Staff	
Dr. Tejaswini M. Deshmukh	Scientist B
Technical Staff	
Mr. Vijay M. Ayachit	Technical Officer - A
Mr. Bipin N. Tilekar	Technical Officer
Mrs. Rashmi S. Gunjikar	Technical Officer
Mrs. Rashi Srivastava	Technical Assistant

Characterization of hepatitis E virus specific antigen-antibody interactions in vitro

Investigator: Tejaswini Deshmukh

Funding Agency: ICMR [2017-2018]

Hepatitis E (HE) has a major share in stressing public health amongst other infectious diseases occurring in India. Hepatitis E virus (HEV, causative agent of HE) associated frequent epidemics of varying magnitudes and sporadic cases persistently challenge the public health infrastructure from almost every part of this developing country. Though antibody based protection is well documented against HE, a complete understanding of the associated humoral immune response is still lacking. Absence of a robust cell culture system has greatly affected characterization of specific antibodies induced post natural/experimental infection or immunization using conventional methods. It becomes imperative to characterize anti-HEV humoral immune response for development of better diagnostic and vaccine targets. Utility of ORF2 (capsid) protein based ELISA for detection of HEV infections has been reported earlier. Immunogenicity of ORF2 gene and/or protein based vaccine candidates using different adjuvants and employing different approaches has been assessed/evaluated in mice and monkeys. Specific IgG subclasses in different phases of HEV infection were also reported.

Objectives: Present project was proposed to further characterize anti-HEV humoral immune response in terms of quality (affinity maturation/avidity) and quantity, simulate/validate HEV binding assay and characterize ORF2/T1NE proteins using chaotrope based avidity assays and kinetic binding assays employing SPR technique. The measured specific antibody characteristics will be correlated with phase of infection, type of infection (primary/re-infection) and antibody response associated with protection.

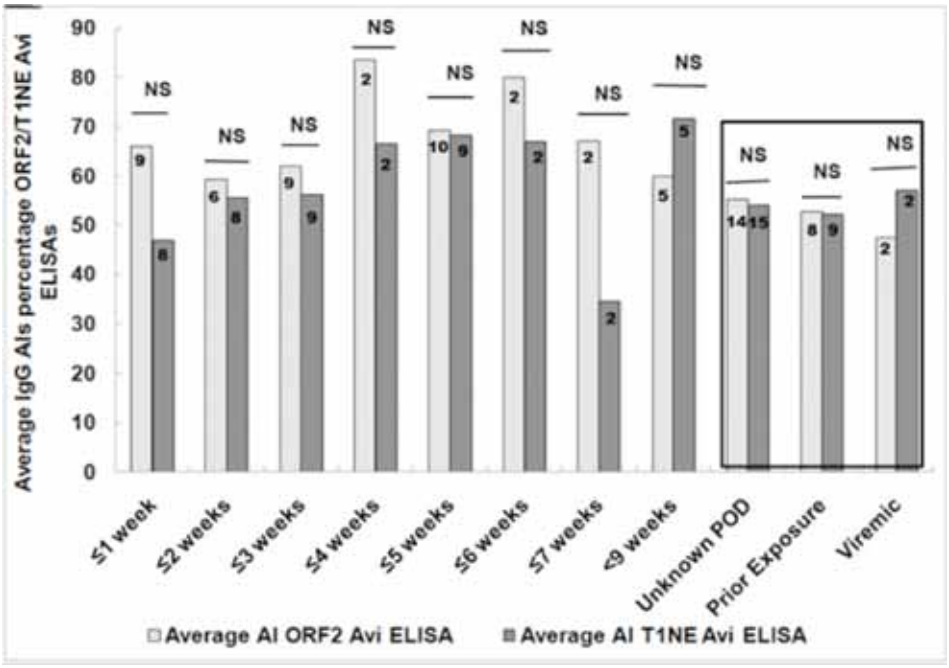


Figure 1:Changes in mean Avidity Index (AI) values in both ORF2 and T1NE (neutralizing epitope region protein within ORF2 protein) protein based avidity ELISAs (ORF2/T1NE Avi ELISAs) with increasing post-onset day (POD) up to <9 weeks. Last three groups with unknown PODs are enclosed in rectangular box. Unknown POD samples are from outbreak, apparently healthy volunteers (Prior Exposure) and viremic patients. NS- not significant.

TRG 1601: Evaluation of certain compounds for inactivation and stabilization of viruses of public health importance.

Investigators: Mourya DT, Sapkal GN, Yadav PD, Gunjikar R, Khutwad K, Tilekar B, Chadha MS

Funding Agency: Intramural [2016 -2018]

Outbreaks due to respiratory viruses such as SARS (2003), avian flu (2005) and the H1N1 pandemic (2009), H7N9 (2012) have been experienced. Several epidemics of newer zoonotic viral infections have emerged from interactions between human and animals *viz.* Crimean Congo hemorrhagic fever (CCHF), Kyasanur forest disease (KFD). Ebola virus has threatened to the public health system of country while a disease like dengue is endemic to India.

The high risk associated clinical samples obtained for diagnostics necessitates their handling and processing in containment specialized laboratories. These facilities would lead to a reduction in the occupational exposures to pathogenic material and ensure safe environment by facilitating early detection of high-risk groups of emerging infectious diseases. Safe means of transporting samples suspected of containing virulent agents to specialized high-level containment laboratories for analysis is a major issue that hampers timely diagnosis.

Objectives

- To develop, a rapid, reliable, and simple method for the complete inactivation of various viruses by using chaotropic agents.
- To assess the combinations for infectivity and stability of different viral titers for selected families of viruses.
- To study the effective use of selected formulations on field conditions.

Table 1: *In vitro* & *in vivo* inactivation of virus using composition of selected inactivation formulation.
(I =Inactivated virus)

Family	Virus Controls		No. of Days after inactivation								
		0	1	2	3	4	5	6	7	8	VC
	JEV	I	I	I	I	I	I	I	I	I	106.5
Flaviviridae	KFDV	I	I	I	I	I	I	I	I	I	105.5
	DEN	I	I	I	I	I	I	I	I	I	105.5
Alphaviridae	CHIKV	I	I	I	I	I	I	I	I	I	106.5
Rhabdoviridae	CHPV	I	I	I	I	I	I	I	I	I	107
Bunyaviridae	CCHFV	I	I	I	I	I	I	I	I	I	102.5

Work done: All inactivated viruses were tested by serological (Antigen capture& IgM Capture ELISA) and Molecular diagnostic methods (Real Time PCR).Viruses could be diagnosed by both methods indicating, no interference of inactivation formulation in diagnostic testing.

These samples can be used for subsequent diagnostics. These inactivation protocols will facilitate the diagnostic examination of these emerging and reemerging viruses by applying standard laboratory conditions at BSL-2 level. In order to achieve the objectives, further, inactivation experiments were conducted with following viruses. (Table 1)

Figure 2A: Antigen capture ELISA

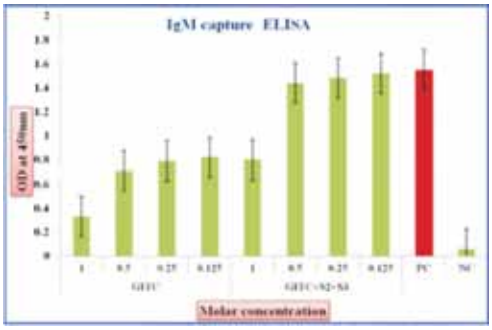


Figure 2B: IgM capture ELISA

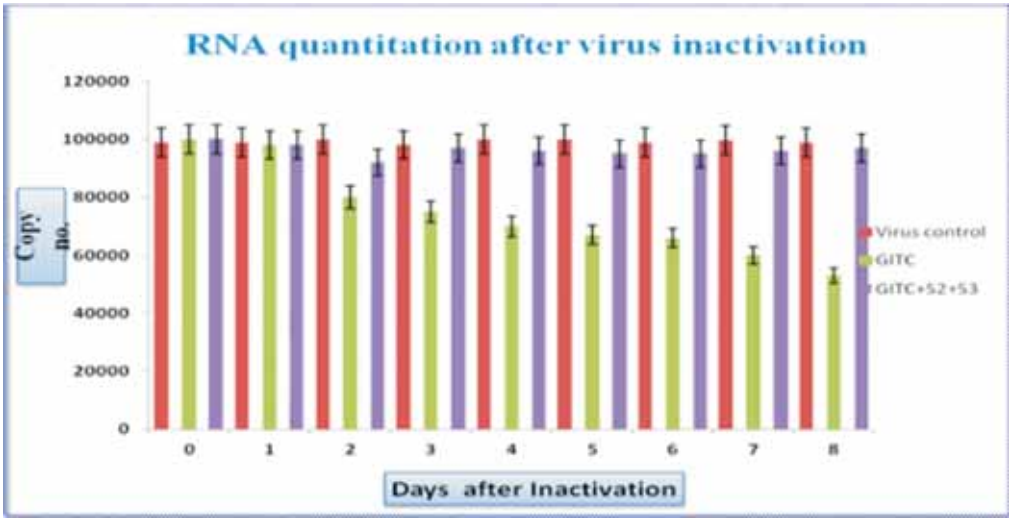


Figure 2C: RNA quantitation by Real time PCR

Figure 2C): Results of qPCR assay : Viral RNA was extracted, and then qRT-PCR assays were performed for CHPV/CHIK/JEV. Viral copies in samples with and without treatment with inactivating agent determined and expressed as RNA copies /ml. Considering the maximum period required for transport of viruses from remote locations, inactivated viruses were kept at room temperature and 4°C up to 8 days. Subsequently virus diagnosis was done by serological and molecular diagnostic methods and results were reproducible.

1. **Effect on hematological (Hb, TLC, DC, plate let count & coagulation profile) & biochemical parameters (LFT, RFT, Markers of tissue damage & serum electrolytes):** Healthy volunteers blood (n=40) was collected in presence of inactivating formulations & tested. No significant differences were observed in the parameters in comparison with untreated controls. These results indicate that the formulations can also be used for hematological & biochemical analysis which is a routine requirement for patients with viral infections.
2. **Field Studies:** To understand the utility of virus inactivation formulations in outbreak situations, all samples from Chikungunya (n=289) & dengue (n=63) outbreak in Pune were collected & tested in the presence of inactivation formulation and compared with untreated sample. Also hematological & biochemical parameters of treated & untreated (n=54) samples were compared. No significant differences in the observed results indicated that the formulation can be used in outbreak situations.

TRG-1701: Establishment of facility for production of positive controls of important public health viruses for diagnostic PCRs and RT-PCRs tested for virus diagnostic laboratories

Investigators: GN Sapkal, Yadav PD, Sudeep AB, Tilekar B, Vidhate S, Srivastava R & Mourya DT,

Funding: DHR [2 Years]

To deal with emerging viral diseases, Department of Health & Research (DHR) has launched a scheme in 12th five-year plan to establish of virology of 160 laboratories (VRDLs) throughout the country. These VRDLs require positive standard viruses to use as controls in molecular diagnostic tests. Distribution of viruses is not in the current mandate of the institutes. Handling of several viruses by various VRDLs for producing positive controls of their own for PCRs has two major issues; one is possibility of contaminations and second biosafety and biosecurity becomes major issue in many of these laboratories. Moreover, providing several infectious and pathogenic viruses can lead to issues like contamination and biosafety, as many of these laboratories may not be able to handle at this stage.

To overcome these problems, NIV has taken this initiative to develop small facility dedicated to answer these problems.

Objective: To establish facility for production of positive controls of important public health viruses for diagnostic PCRs and RT-PCRs.

Work done: During this period, TRG, NIV has started generation of the positive controls for following viruses. (Table 2) *In vitro* stock preparation, gamma inactivation & its confirmation has been done by qPCRs or qRT-PCRs for the following viruses. Further work is in process.

Products Developed

Development of inactivated vaccine against Japanese encephalitis virus

Investigators: Sapkal GN, Ayachit VM, Tilekar B

Objective: To develop inactivated vaccine against Japanese encephalitis virus

Work done During this period, 5 days, hands on training on the following aspects has been imparted to HBL Biotech, (Chennai) staff at TRG, NIV on *in vitro* culture & characterization of Japanese encephalitis virus (JEV) i.e.;

a) Standard procedures of cell culture
b) *In vitro* propagation of JEV
c) JEV virus stock preparation by in vitro
d) JE virus titration
e) JE neutralization test with positive & negative serum
f) Plaque reduction neutralization test

Further, HBL Biotech, (Chennai) has scaled up the JEV vaccine seed culture and performed titrations. Presently, NIV, Pune has received the seed culture of JEV and is working on whole genome sequencing to further characterize.

Table 2 : List of virus positive controls developed

Virus Name & NIV ID
Chandipura virus (034627)
Japanese Encephalitis virus (733913)
WestNile (804994)
Chikungunia virus (061573)
Mumps virus (ov-14-037)
Measles(1520640)
Rubella virus(12195-m-09-060-2)
Cox. B3 virus
Rota virus
Dengue-1 (16007)
Dengue-2 (803347)
Dengue-3 (059826)
Dengue-4 (642069)

A. Development of Immunotherapeutics for dengue virus infection

Investigators: Sapkal GN, Tilekar B, Srivastava R, & Maurya DT

Dengue is a mosquito-borne flavivirus disease that has spread to most tropical and many subtropical areas. The disease is caused by four closely related viruses, the Dengue viruses 1-4. There is no specific therapeutics and prevention is currently limited to vector control measures. A dengue vaccine would therefore represent a major advance in the control of the disease and is considered a high public health priority.

Objectives: To develop and screen for potential neutralizing mice monoclonal antibodies against dengue virus.

Work done: Mice monoclonal antibodies quantification by Plaque Reduction Neutralization test and further characterization by Immunofluorescence assay, Indirect ELISA as well as purification using biochemical methods has revealed important insights for development of therapeutic antibodies in collaboration of Zydus Cadila Pvt. Ltd. Ahmedabad.

B. Progress on ELISA Kit production at M/s Zydus Cadila

During the previous period, the following ELISA diagnostic kits were transferred to the Zydus Cadila Pvt Ltd. Ahmadabad.

- Detection of anti Chandipura virus IgM for human
- Monoclonal antibody based antigen capture ELISA for detection of Japanese encephalitis virus from mosquito
- ORF2 protein based ELISA for diagnosis of hepatitis E virus

In order to scale up these kits on pilot scale at Zydus Cadila, Ahmedabad, reagents were supplied for 50 kits each. Also the training on knowhow and trouble shoot was imparted to staffs. Further the test kits produced at Cadila were tested for Quality control at NIV. The kit batch performance was satisfactory.

Patents filed

- Composition for precipitation of avian viruses and a method thereof (Indian Patent filed Application Number 201623016520 dated May 12, 2016).
- Composition for virus inactivation with serological and nucleic acid integrity for diagnostic analysis (Indian Patent filed Application Number 201621031989 dated Sept 20, 2016).

National Institute Of Virology, Gorakhpur Unit

Scientific Staff

Dr. Vijay P. Bondre	Scientist E & OIC
Dr. Hirawati Deval	Scientist C

Technical Staff

Mr. N. M. Rao	Technical Officer-A
Dr. Niraj Kumar	Technical Officer-A
Mr. Gajanan Patil	Technical Assistant
Mr. Vanka Janardhan	Technical Assistant
Mr. Kamlesh Sah	Technician C
Mr. Ravishankar Singh	Technician C
Mr. Vishal Nagose	Technician C
Mrs. Arati Waghmare	Technician C
Mr. Sanjeev Kumar	Technician B
Mr. Asif Kavathekar	Technician B

Administration Support

Mr. Amol Lohbande	Lower Division Clerk
Ms. Jyoti Kumari	Multi-tasking staff
Mr. Sharvan Kumar	Multi-tasking staff

Maintenance & Support Staff

Mr. Ashish Chaudhary	Technical Assistant (Engg. Support)
Mr. Jitendra Kumar	Technician B (Engg. Support)
Mr. Chandrashekar Singh	Staff car driver (Grade II)

Project Staff

Dr. A. K. Pandey	Scientist C
Dr. B. R. Misra	Scientist B
Dr. S. P. Behera	Scientist B

Collaborators

Dr. Mahima Mittal, Head, Dept. of Pediatrics BRD Medical College
Dr. Mahim Mittal, Head, Dept. of Medicine, BRD MC and Nehru Hospital, Gorakhpur,
Dr. Murrhekar M, Director-in-charge, National Institute of Epidemiology, Chennai
Dr. A K Agrawal, Head, Dept. of Pathology, Gorakhnath Hospital, Gorakhpur.

GKP1001: Diagnostic services to suspected Japanese encephalitis cases from eastern Uttar Pradesh.

Investigators: Bondre VP & Hirawati D

Funding: intramural [Ongoing]

Epidemiology of JE in the AES endemic region: Seven districts of Uttar Pradesh and adjoining areas of Bihar and Nepal are endemic to AES and NIV Gorakhpur unit provided diagnosis to clinically suspected cases (samples) referred from Baba Raghav Das Medical College, BRD Medical College and other tertiary care centers.

Work done: A total of 3402 clinical specimens (CSF and blood/serum) collected from 1899 cases were screened for anti-JEV antibodies using IgM ELISA kit and 173 cases (9.1%), mainly pediatric group (89.5%), were found positive. Almost all the JE cases (171/173) hospitalized during 2016 were from rural areas of eastern UP, mainly from Kushinagar (37), Gorakhpur (32), Siddharthanagar (21), Maharajganj (16), St. Kabirnagar (13), Deoria (10), Basti (04) districts along with a few cases from adjoining districts of Bihar (32) and Nepal. JE activity was documented during May-December months with highest number of cases during August-October 2016. Independent of the number of cases admitted during each outbreak season, the pattern of seasonal distribution of JE cases during last 6 years is largely similar (Fig. 1). Socio-economically almost 95% of the parents of JE cases belong to the lower to upper lower class (up to 10 score by Kuppuswamy scale March, 2016 based on education, occupation and family income) while 5% belongs to the lower middle class.

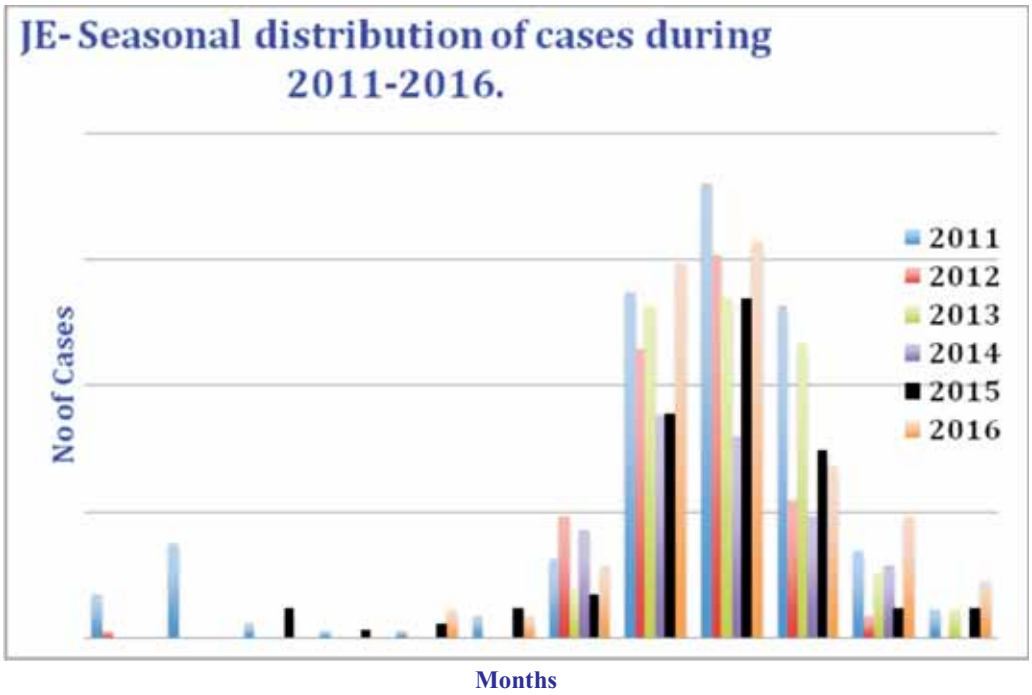


Fig 1. Seasonal distribution of JE cases reported in AES endemic area of UP during 2011-16.

Clinical investigations of Japanese encephalitis: Clinically all the JE cases documented fever as the first clinical symptom along with headache (17% cases), vomiting (44% cases) and abdominal pain (6% cases). Among the specific signs of neurological involvement included altered level of consciousness (100%), up rolling of eyeballs (81%), generalized tonic clonic seizure (41%), Glasgow coma scale = 7 (22%), limbic hypertonia (63%), extensor plantar (63%) and exaggerated deep tendon reflex (11%). Symptoms of the meningeal involvement (neck rigidity in 17% and Kerning's sign in 8%) as well as hepatomegaly (20%) and splenomegaly (3%) were recorded in small proportion of cases. Hepatic involvement was confirmed by raised levels of SGPT (>45 IU/L) in 18% cases. Thrombocytopenia (<100,000 cells/mm3) was noted in 21% JE cases. Raised serum urea levels (>40 gm. / 100 ml) in 34% and creatinine levels (>1 gm. / 100 ml) in 12% cases are suggestive of renal involvement. In about 5-10% recovered JE cases, neurological and physical disabilities were documented for longer duration.

GKP1502: Etiologic investigations of human clinical specimens collected from acute encephalitis syndrome (AES) cases from Eastern Uttar Pradesh.

Investigators: Bondre VP, Hirawati D, Mishra BR & Mittal M

Funding: Intramural [2015-2017]

In the absence of clinical recommendations all clinical specimens were investigated for differential diagnosis of multiple AES associated etiologies based on patient's clinical history, seasonality, age group, window period between onset of symptoms to specimen collection, hematological and biochemical findings.

Clinical Outcome in AES: Overall mortality in the hospitalized AES cases was 27% which is 2% higher than 2015. Among the 1965 cases, ~4% were clinically diagnosed as meningitis, 2.1% as meningoencephalitis and 1% as encephalopathy in addition to respiratory failure (15.9%), pneumonia (1.6%), aspiration pneumonia (1.4%), peripheral vesicular failure (1%), coagulopathy (0.5%) and renal disorder (0.5%) at the time of admission. Case wise clinical data analysis confirmed fever as the onset of illness in 97.3% with a mean of 7.3 days before hospitalization. The mean duration of appearance of altered sensorium was 1.9 days before hospitalization indicating that the mean duration of prodromal phase was <5.4 days in AES. Neurological examination showed severe brain damage in 17.9% of cases, moderate in 73.6% and mild brain damage in 8.5% cases. Abnormal tone and deep tendon reflexes were observed in 59.8% and 28.8% patients respectively. Disruption of corticospinal tract was recorded in 68.8% of AES cases patients. Meningeal sign was noticed in 15.3% of cases. Other general clinical features observed were headache (16.3%), vomiting (49.5%), normal vesicular auscultation (99.9%), abdominal tenderness (3.8%), hepatomegaly (34.6%) and splenomegaly (6.6%). Raised SGOT / SGPT (markers of hepatic involvement)/serum urea were documented in 64.7, 35.4 and 37.9% cases respectively. Associated etiology based analysis of cases suggested marked differences in one or other clinical, biochemical or radiological parameters, which may form the base of overall diagnosis of AES in India.

Summary: Clinical specimens from 1726 out of 1965 hospitalized AES cases were investigated. Cases showing rash, elevated levels of C-reactive protein, thrombocytopenia, low blood sugar, raised TLC counts, multi-organ involvement were tested for anti-Orientia tsutsugamushi IgM antibodies and genome, Enteroviruses, alphaviruses, flavivirusesetc. by PCR/RT-PCR using generic primers.Samples were also tested for a battery of bacterial infections including Streptococcus pneumoniae, Neisseria meningitides, Heamophilus Influenzae etc.by PCR assay. Definitive diagnosis was achieved in 784/1899 (41.28%) and in majority of cases O. tsutsugamushi and JE was detected (Table 1).

Table 1: Diagnosis for AES etiologies in human samples collected from AES cases during 2016

SN	Etiologies	Specimens	Assay(s)	Outcome	% Positive
1	Japanese encephalitis virus	CSF	IgM ELISA	90/1668	5.39
		Serum	IgM ELISA	153/1736	8.81
2	Dengue virus	Serum	NS1 ELISA	27/400	6.75
		Serum	IgM ELISA	12/412	2.91
3	Orientia tsutsugamushi	CSF	IgM ELISA	148/380	38.94
		Serum	IgM ELISA	230/410	56.09
		CSF	PCR	32/417	76.7
		Blood	PCR	82/438	18.72
4	Other rickettsia	CSF	PCR	1/391	0.25
		Blood	PCR	12/419	2.86
5	Hepatitis A virus	Serum	IgM ELISA	3/45	6.66
6	Hepatitis E virus	Serum	IgM ELISA	1/42	2.38
7	Measles virus	Serum	IgM ELISA	4/72	5.55
		CSF	RT-PCR	0/200	0.5
8	Mumps virus	Serum	IgM ELISA	3/11	27.27
		CSF	RT-PCR	0/200	0
9	Varicella Zoster virus	Serum	IgM ELISA	1/14	7.14
		CSF	PCR	2/366	0.54
		Vesicular fluid	PCR	1/1	100
10	Rubella virus	CSF	RT-PCR	0/200	0
11	Herpes simplex virus 1	CSF	PCR	6/366	1.63
12	Herpes simplex virus 2	CSF	PCR	0/366	0
13	Herpes simplex virus 7	CSF	PCR	1/159	0.62
14	Epstein bar virus	CSF	PCR	3/366	0.81
15	Cytomegalovirus	CSF	PCR	0/366	0
16	Parvovirus P4	CSF	PCR	3/88	3.4
17	Parvovirus B19	CSF	PCR	0/88	0
18	Chikungunya virus	Serum	IgM ELISA	4/63**	6.34
19	Alphavirus (Chikungunya)	CSF	RT-PCR Generic	0/131	0
20	Flavivirus (JE/DEN/WNV/Zika)	CSF	RT-PCR Generic	1/290	0.34 (JE)
21	Enterovirus	CSF	RT-PCR Generic	1/268	0.37
22	Influenza B virus	CSF	RT-PCR	0/131	0
23	Parainfluenza virus 1	CSF	RT-PCR	1/131	0.76
24	Parainfluenza virus 2	CSF	RT-PCR	0/131	0
25	Parainfluenza virus 3	CSF	RT-PCR	0/131	0
26	Parainfluenza virus 4	CSF	RT-PCR	0/131	0
27	Human Metapneumovirus	CSF	RT-PCR	0/131	0
28	Respiratory syncytial virus	CSF	RT-PCR	0/131	0
29	Mycobacterium tuberculosis	CSF	PCR	2/178	1.12
30	Haemophilus influenza	CSF	PCR	2/155	1.29
31	Neisseria meningitidis	CSF	PCR	0/155	0
32	Streptococcus pneumonae	CSF	PCR	2/155	1.29

Notes: * denotes diagnosis not performed at NIV, GKP. ** denotescross-reactive in Dengue IgM ELISA.

Scrub typhus: Since, anti-JE IgM and anti-OTs IgM antibodies were primarily detected in majority of the cases, the comparative analysis was performed (Table-2).

Table 2: Comparison of clinical and biochemical parameters recorded in JE and Scrub typhus cases hospitalized as AES cases during 2016.

Parameters	Outcome in JE cases (n=163)	Outcome in OTs cases (n=244)
Male patients	46	51
Age Mean (Years)	10.5	6.5
Mortality	35.6	19.3
History of Fever	100	100
High Grade Fever	58.2	81.1
Fever History before hospitalization (mean, Days)	7.2	8.2
Headache	16.6	18.1
Vomiting	42.3	58.8
Abdominal Pain	5.5	19.7
Altered Level of Consciousness	100	100
Up rolling of Eye Boll	80.9	84.7
Frothing from Mouth	8.5	12.3
Glasgow COMA Scale = 7	22.2	13.2
Generalized tonic clonic Seizure	40.5	44.4
Limbic Hypertonia	62.6	60.5
Exaggerate Deep Tendon Reflex	10.9	4.7
Extensor Plantar	62.4	71.3
Neck Rigidity	16.7	19.2
Kernig's Sign	8.3	4.8
Non tenderness of Abdomen	98.7	95.4
Hepatomegaly	20	48.7
Spleenomegaly	2.6	11.1
Haemoglobin >10gm/dl	65.9	29.7
Total Leukocyte Count >13000 cells/mm3	45.1	50.2
Platelet Count<1X105 cells/mm3	20.6	31.1
SGOT>45IU/L	37.3	34.4
SGPT>45IU/L	18.4	37.9
Urea>40gm/100ml	33.8	39.5
Creatinine>1gm/100ml	11.9	19.5
CSF Protein (>45 mg/dl)	48.5	35.3
CSF Glucose (>75 mg/dl	19.1	18.2

Between the JE and OTs positive cases, major differences were recorded in the affected age group (lower mean age in OTs cases), prolonged high-grade fever and comparatively delayed hospitalization was documented in OTs cases. Among the associated primary symptoms, GIT signs were more severe in OTs cases as compared to JE cases. Although altered sensorium was documented in all OTs cases but coma and deaths as final outcome was less. All other clinical signs associated with CNS involvement were largely similar to JE. Thrombocytopenia, hepatic involvement, CSF protein level were higher in OTs cases as compared to JE cases. In case of non-JE AES cases (excluding death and LAMA), the duration of hospitalization ranged from 1-82 days (average 10.6 days) while in case of JE cases it ranged from 3 to 70 days (average 13.8 days). Ten AES cases were commonly positive for JE (IgM) and OTs (IgM and PCR) infections and were excluded from the analysis. Although, the Escher (scar resulting from bite of infected mite) were not visible in any of the OTs cases, but different types rash were documented in some of the cases as confirmed by IgM and genome detection (Figure 2).

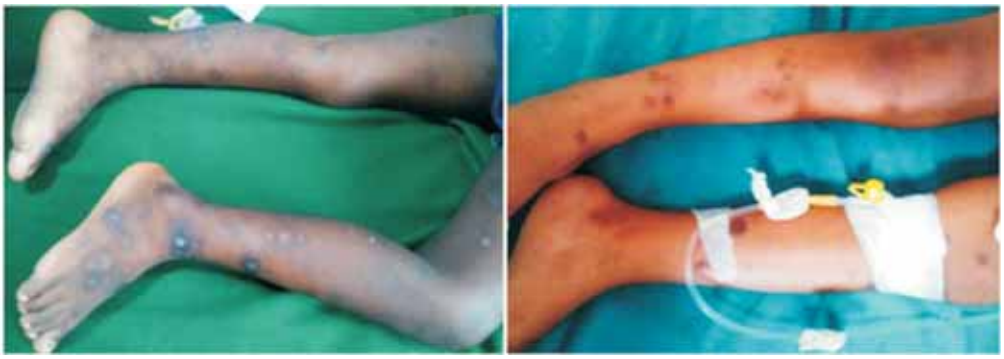


Fig 2: Wound or sore observed in IgM ELISA or PCR confirmed scrub typhus cases

Apart from JE and Scrub typhus, association of different viral etiologies was identified in AES cases. The clinical outcome in other AES associated etiologies are summarized in Table 3.

Table 3. Differential clinical and biochemical patterns documented in non-JE, non-OTs AES cases reported during 2016

Etiologies	Clinical outcome
Measles	Clinically diagnosed as post measles encephalitis. History of high-grade fever (>10 days). Maculopapular rash distributed on the face while pleocytosis recorded in ¾ cases.
Mumps	High-grade fever, vomiting, abdominal pain and tightening of whole body. GCS ranged from 8 to 15; plantar reflex was extensor, TLC and hepatic marker were raised.
Hepatitis A virus	High-grade fever with seizure. Icterus with facial puffiness and pedal edema also observed. Hepatomegaly recorded in 2/3 cases.
Hepatitis E virus	High-grade fever (>7 days), vomiting (4 days), increased tone with normal DTR and extensor plantar reflex. Hepatic markers elevated and normal renal markers. CSF showed pleocytosis with normal glucose and protein levels. No altered sensorium.

Parainfluenza virus 1	History of low-grade fever, seizure, tightening of body and moderate alteration in normal verbal, eye and motor response as GCS score was 11. Hypertonia, normal DTR and extensor plantar reflex documented. TLC slightly increased and normal CSF parameters.
Enterovirus	History of fever (2 days) with seizure, behavioral abnormality and up rolling of eyeballs. Unconscious at the time of hospitalization with 10 GCS score and normal tone. Meningeal signs absent, pupil constricted and sluggish reactive to light. Highly elevated hepatic markers (SGPT and SGOT >300IU/L). Slightly higher blood urea nitrogen value (23.36mg/dl). CSF protein level increased up to 125.8mg/dl. Death after two days of hospitalization.
Herpes simplex virus 1	High-grade fever (5-6 days), altered sensorium, tightening of whole body and up rolling of eyeballs. Cough in 50% cases while limbic hypertonia recorded in all cases. Hepatic and renal markers normal but pleocytosis commonly seen in all cases. Dermal rash all over body in one case. CFR 60%.
Varicella zostervirus	Low-grade fever, altered sensorium, tightening of whole body and vomiting, low GCS, normal DTR and pleocytosis. CSF glucose levels raised and normal protein levels. GCS >10 in cases, normal DTR, extensor plantar reflex and pleocytosis in 66% cases.
Herpes simplex virus 7	High-grade fever (5 days), altered sensorium, seizure and vomiting. Multiple episodes of loose motions. GCS scale 15, normal eye, verbal and motor responses. TLC >30,000cells/mm3. Pleocytosis with raised CSF protein. Death 6 days after hospital stay.
M. tuberculosis	History of high-grade fever (>10 days) with abnormal behavior, generalized tonic clonic seizure and neck rigidity. Exaggerated deep tendon reflexes (DTR) and plantar reflex not elucidated. Death was final outcome.
S. pneumoniae and Normal H. influenzae blood.	High-grade fever, vomiting, altered mental sensorium and meningitis. total leukocyte count (TLC), neutrophilia in differential leukocyte count of CSF appearance-turbid, increased protein levels (>100mg/dl) with pleocytosis.
Malaria	High-grade fever of 5 – 16 days, vomiting, headache, abdominal pain, altered sensorium 1.5-2 days on admission in 64.4% cases. Hepatic involvement (hepatomegaly) in 42.6% of cases.

GKP1503: Isolation, identification and genetic characterization of viruses isolated from acute encephalitis syndrome cases from eastern Uttar Pradesh.

Investigators: Bondre VP & Hirawati D

Funding: intramural [2015-2017]

Virus isolation is regarded as the 'gold standard' in investigation of viral etiologies. To improve upon the diagnosis of AES, 744 CSF specimens collected from suspected viral encephalitis cases were processed for virus isolation in multiple cell lines viz., Porcine Stable kidney (PS), Baby Hamster Kidney (BHK) 21, and VERO cells. The cultures that showed CPE in P3 were passed for 2 more passages and two isolates were obtained. Characterization of one of the isolates was confirmed as JEV through amplification and sequencing of 810 nucleotide long fragment from the envelope protein. Genetic analysis

using a subset of Gene Bank sequences representing genotype 1–5, suggests that it belongs to genotype 1.

To establish the identity of the other isolate, total nucleic acid extracted and screened for different encephalitic viruses globally known to be associated with AES by PCR. But none could be detected. Since, these virus isolates are obtained directly from the CSF specimens, establishing their identity will be important. Hence, further studies are planned using next generation sequencing platform.

GKP1504: Etiological investigations on non-AES referred cases from Gorakhpur region.

Investigators: Bondre VP & Hirawati D

Funding: Intramural [2015-2017]

Screening of 176 blood samples from dengue suspected hospitalized cases using diagnostic RT-PCR amplifying the C-prM region of all 4 serotypes resulted in the confirmation of 100 cases as DEN-2 infection. Phylogenetic analysis has shown that the strain belonged to the cosmopolitan genotype, which was detected in Gorakhpur and other parts of India in 2015 (Fig 3). Attempts to isolate the virus in PS cell line failed.

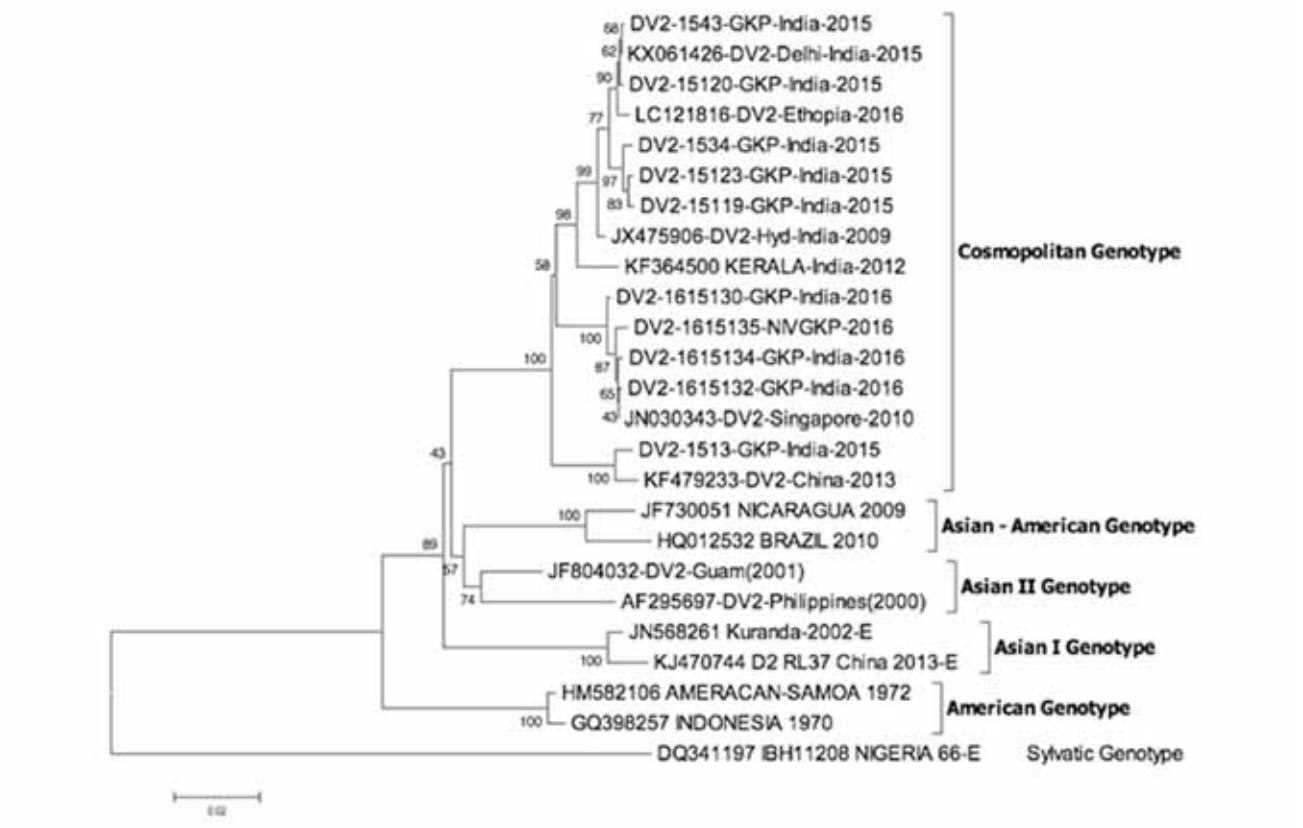


Fig. 3. Phylogenetic analysis of Dengue virus 2 complete Envelope (1500 nts) amplified from cases investigated during 2015 and 2016 in Gorakhpur region.

Apart from Dengue, diagnosis for about 100 clinical specimens referred from different health settings was provided for JE, EV, HSV, VZV, Measles, Mumps and Rubella infections using different diagnostic assays. Among the referred sporadic cases HSV 1 infection was detected in 10/87 (11.49%) and VZV in 5/87 (5.74%) cases. The outcome was communicated to the respective hospitals.

National Institute Of Virology, Bangalore Unit

Scientific Staff:

Dr. Chandrashekhar G. Raut	Scientist – E & Officer-In-Charge
Dr. Ashok M	Scientist-B

Technical Staff

Mr. Hanumaiah	Technical Officer-A
Dr.D. P. Sinha	Technical Officer-A
Mr. A.R. Sable	Technical Officer-A
Mr.Jayaprakash H	Technical Officer-A
Mrs. Asia Thounaojam	Technical Assistant
Mr.Manjunatha MJ	Technical Assistant
Mr.Raju M	Technician-C
Mrs.Prema BM	Technician-C
Mr.Thippeswamy B	Technician-C
Mr.Deepak D. Mali	Technician-C
Mr.H.M.Muninarayanappa	Technician-B
Mr. B.N. Wakde	Technician-A
Mr.Ratnadeep B.More	Technician-A
Mr. Kunal Sakhare	Technician-A
Mr.Naveen G	Technician-A
Mr. Rohit Pawar	Multi-tasking staff (MTS)
Mr. Vaibhav Bichkule	MTS
Mr. Arjun D. Jogangari	MTS (Maintenance)

Administrative Staff

Mr. R.Basavaraju	Upper Division Clerk
Ms.J. Jayajyothi	Upper Division Clerk
Mr. Prashant Dagdu Patil	Lower Division Clerk
Mr. Prem Popat Khandagale	Lower Division Clerk

BNU9702: Studies on poliovirus (as part of WHO-SEAR Polio Lab Network in the WHO's global poliomyelitis eradication initiative)

Investigators: Raut CG, Hanumaiah H & Sinha DP

Funding: WHO [ongoing (since 1997)]

NIV Bangalore unit is a reference laboratory recognized by WHO to conduct surveillance of wild and vaccine strains of poliovirus. During the year, the following studies have been carried out.

Objectives:

(i) Surveillance of acute flaccid paralysis (AFP) cases from Karnataka, Kerala and Bihar (Gaya and Bhagalpur sub-divisions) and Intra-typic differentiation (ITD) of poliovirus isolates from AFP cases received from National Polio Laboratory

During the period, 8371 stool specimens from 4295 AFP cases referred from Karnataka (819), Kerala (377), Bihar (3079) and other states (20) were processed for virus isolation in validated L20B and RD cells. Two hundred and forty five isolates were obtained and were tested for ITD/VDPV by real-time PCR (Table 1). No wild poliovirus or VDPV detected.

No. of isolates tested by rRT-PCR ITD Assay		Isolates tested by rRT-PCR VDPV Assay	Results (ITD) tested by rRT-PCR &ITD Method						
LR/ LLR	RLR/ RRLR		P1 SL	P2SL	P3 SL	Sabin Like Mixture (P1SL + P3SL=51, P2SL+ P3SL = 4, P1SL + P2SL + P3S L = 1)	P1 (Discordant) + P3SL = 2, P1SL + P3 (Discordant) =1	NPEV	NEV
221	24	234	78 (32%)	4 (1.6%)	93 (38%)	56 (23%)	3 (1%)	9 (3%)	2 (0.8%)

(ii) Containment of Polio viruses as per GAPIII/BRM: Polio virus type-2 containing materials are not retained in the laboratory as stool samples (n=11615), chloroform extracts (22076) and viral isolates/TCF tubes (34544) were safely contained/disposed off and the status was declared to the "National Task Force on Polio Containment". Implementation and documentation of 16 elements of GAPIII is in place as the minimum required standards and work is in progress to have the complete and best quality document.

(iii) Environmental sample surveillance for polioviruses: NIV BU Laboratory has been identified for testing of environmental samples from Bangalore STPs by the WHO Polio program. The sample collection site has been identified and processing will commence on receipt of consumables and samples.

BNU 1603: Surveillance of measles in Karnataka and Kerala (as a part of WHO-SEAR Measles Laboratory Network in the WHO's Global Measles Elimination Program).

Investigators: Raut CG, Hanumaiah H & Manjunath MJ

Funding agency: WHO [Ongoing]

Work done: During the reporting period, 1015 samples received from the two states and the union territory of Lakshadweep were processed for IgM antibodies for measles and Rubella as well as virus isolation (Table-2). Measles and rubella IgM antibodies were detected in 342 (38.0%) and 181 (33.0%) samples respectively while 14 and 16 cases were seen as measles equivocal and rubella equivocal. Rest of the samples were negative for both the antibodies. Virus isolation attempts from 49 urine samples yielded 5 measles and one rubella isolates.

Surveillance of dengue, Chikungunya (CHIK) and Japanese encephalitis (JE) viruses in Bangalore city and adjoining areas

Investigators: Raut CG, Manjunath MJ & Hanumaiah H

Funding agency: NVBDCP & NIV [ongoing (since 2010)]

Bangalore city and adjoining areas of Karnataka are endemic to dengue, CHIK and JE viruses as observed by sporadic outbreaks. Sample based surveillance studies have been initiated to understand the prevalence of these viruses.

Work done: During the reporting period, 2229 serum samples were screened for dengue and CHIKV IgM antibodies and found 1021 (45.8%) and 379 (17%) samples positive for the viruses respectively (Table 2). Dengue virus prevalence in Bangalore city was 49% where as that of Chikungunya was 16%. JE cases were detected from Tumkur, Hassan, Chikkaballapura and Bangalore Urban area with a prevalence rate of 4% altogether.

Congenital Rubella Syndrome (CRS) Surveillance in India, as a part of Indian Council of Medical Research (ICMR) program under MoHFW

Investigators: Raut CG & Hanumaiah H

Funding agency: ICMR/MoHFW [going on (since Dec 2016)]

A nationwide program has been initiated to determine the prevalence of congenital rubella syndrome. During the year, 26 samples from 13 cases (16 serum, 8 throat swabs and 2 urine samples) were processed and 61% of the serum samples showed Euroimmune Rubella IgM antibody. In another study, 105 serum samples received from pregnant women also showed Euroimmune Rubella IgM antibody in 87.7% cases (Table 2). Rubella virus was isolated from throat swabs of 2 cases and sequence analysis has shown the circulation of genotype 2B. Further work is in progress.

No. of samples tested: During the year, 11,922 samples from 7647 cases were screened for etiological agent/antibodies according to clinical symptoms. The details are given in Table -2.

Table 2: Number of samples tested (Virus-wise details):

Virus	Samples	No. of cases	No. of samples	Test	Output	
Polio	Stool	4295	8371	Virus isolation	2178 ??	
Measles / Rubella	Serum	905	905	IgM Ab ELISA	Me +ve 342, Eq 14	Ru +ve 181, Eq 16
	Throat swab	52	61	Virus isolation / PCR	PCR +ve 12,VI 3	Rub 1, VI Ru 1
	Urine	44	49	Virus isolation / PCR	PCR +ve 11,VI 2	Rub 1, VI Ru 1
Dengue / Chikungunya	Serum	2018	2229	IgM Ab ELISA	CHIK +ve 379	DEN +ve 1021,
JE	CSF	96	138	IgM Ab ELISA	JE +ve6	
	Serum	108			JE +ve7	
Rubella	Serum	13	16	IgM Ab ELISA / IgG	Rubella IgM 5, Rubella IgG 1	
	Throat swab		8	Virus isolation / PCR	Ru VI 2, PCR Pos 2	
	Urine		2		Ru VI 0, PCR Pos 0	
Rubella in pregnant women	Serum	105	105	IgG Ab ELISA	90 Ru IgG Positive	
Retinitis	Serum, Urine, TS, CS	9	35	Virus isolation / PCR	0	
Mumps	Serum, CSF	2	3	Virus isolation / PCR	2	
Total		7647	11922			

National Institute Of Virology, Kerala Unit

Scientific Staff

Dr. Retheesh Babu G	Scientist C & OIC
Dr. B.Anukumar	Scientist D
Dr. R Balasubramanian	Scientist C

Technical Staff

Mr.Bestin P Joseph	Technical Assistant
Mr.Mayur S Mohite	Technical Assistant (Maintenance)
Ms.Kunjila Konnikara	Technician 'C'
Ms.Sreelekha K.P	Technician 'B'
Mr.Amol B Mun	Technician' B'
Mr.Nikhil. T	Technician 'B'
Mr.Jijo Koshy	Technician 'B'
Ms.Amalmol Peter	Technician 'B'
Mr.Dipak V. Sutar	Technician 'B'
Mrs.Mangala Gangadharan	LDC
Mr.Kiran S Pharande	LDC
Mr.Satish B Maind	LDC
Mr.Harshal Kumthekar	Driver
Mr.Govindsing Patil	Driver
Mr.Ramesh Mane	MTS
Mr.Vikrant Talape	MTS

Project Staff

Ms. Anju Murali	Technical Officer
Ms.Aryadevi P A	Technical Officer
Ms.Sahina S	Technician C
Ms.Aswathy P	Data Entry Operator
Ms.Arathy Nadh V	MTS
Ms.Thanzy Salam	MTS
Ms.Asha Maria Mathew	JRF
Mr.Vishal Kavathekar	JRF

KLU 1301: Virus-host protein-protein interaction: a preliminary study to identify putative host protein(s) interacting with Chandipura virus

Investigators: Balakrishnan A & Kavathekar VK

Funding Agency: Intramural [2013-2016]

The study aims to profile the interaction of individual protein components of Chandipura virus with the neuronal proteins. Host proteins that aid in virus entry, uncoating, transcription, translation and virus budding could be profiled through host-virus interaction studies. Thus host proteins that are target for antiviral therapy might be revealed.

Work done: During the reporting year, CHPV Nucleoprotein (N) gene was cloned in pcDNA3.1 (Mammalian expression vector) and expression was checked in Neuro2a cell line using indirect immunofluorescence assay (IFA) using anti-Chandipura polyclonal antibodies and expression of N protein was confirmed. To validate the interaction of Chandipura viral proteins with the neuronal proteins, the differential gene expression studies in Chandipura virus infected N2a cells with control cells using 2D electrophoresis is evaluated.

KLU 1302: Effect of mutations in Chikungunya virus E1 and E2 proteins in virus entry: a virus like particle based approach

Investigators: Balakrishnan A & Mathew AM

Funding Agency: Intramural [2014-2017]

A Virus like particle of Chikungunya was developed using a Baculovirus Expression System during the earlier stage of the study. Primers were designed for site directed mutagenesis and specific mutations were introduced into the E1 and E2 region. CHIKVpFastBac1 plasmids with the desired mutations were then transformed into DH10Bac cells to get the mutated Bacmids. After PCR confirmation, the Bacmid vectors with mutations were then transfected to Sf-9 cell lines. Proteins present in the cell fraction and medium fraction were analyzed by SDS-PAGE and Western Blot analysis. After the amplification of Baculovirus stock, the infection titers were determined by plaque assay. Then the Sf-9cells were infected with the required MOI of Baculovirus and proteins in the media fraction were collected and PEG precipitated. Discontinuous density gradient ultra-centrifugation was done to purify the VLPs and the presences of mutated VLPs were confirmed using Electron microscopy and western analysis. After the ultra-centrifugation, the VLPs were dialyzed and concentrated. E1 and E2 genes were amplified from P3 Baculovirus stock and sequenced to see the presence of desired mutations in them. Out of the 8 mutations introduced, 7 were present. A study on the binding and attachment of VLPs to cell lines is going on.

KLU1401: Potential species distribution and detection of Kyasanur Forest Disease (KFD) virus in tick population in a forest area of Kerala

Investigators: Balasubramanian R,Yadav PD& Prathiush D (Chief Disease Investigation Office, Palode, Kerala)

Funding Agency:DHR (Extramural) [2016-2019]

Tick survey was conducted to determine species diversity, distribution and relative abundance at Wayanad and Malappuram districts, Kerala. A total of 11614 ticks belonging to 8 species from 4 genera were collected from 60 survey sites and processed to detect KFDV by real time RT-PCR. *Haemaphysalis spinigera* (51.98%) was the predominant species collected during the study, followed by H. turturis (38.75%), *H. bispinosa* (6.22%), *H. kyasanurensis* (1.11%) and Amblyomma species (1.64%). KFDV was detected in two pools of *H. spinigera* collected from Wayanad district.

KLU1501; Community engagement in control of some vector borne diseases: a community based intervention in Alappuzha Municipality

Investigators: Babu RG, Balasubramanian R & Philip S (Prof.& Head, Community Medicine, TDMCH, Alappuzha)

Funding Agency: ICMR (Extramural) [2016-2018]

To prevent infectious diseases and reduce disease burden, there is a need for comprehensive approach, which includes community engagement and their participation formally asserted by WHO (Alma Ata declaration-1978). In addition, entomological, human socio-behavioral factors and the health system's mechanisms were also studied to understand the possible causes of vector borne diseases. Qualitative data was collected from the selected wards of Alappuzha Municipality by conducting 40 "Key Informant Interview", 301 In-depth Interview and 9 FGDs and an entomological survey also conducted in selected wards (Figure 1). Most of the study participants felt that the role as well as response of the government/municipal departments is inadequate. The perception of community members about the need and to engage in community based programs are positive which make the sense that it is a social need too. The formative phase reveals there is a need of a community based intervention program by connecting the community resources. Based on these aspects, intervention program (which is beyond participation) would be done in the second phase of the study.

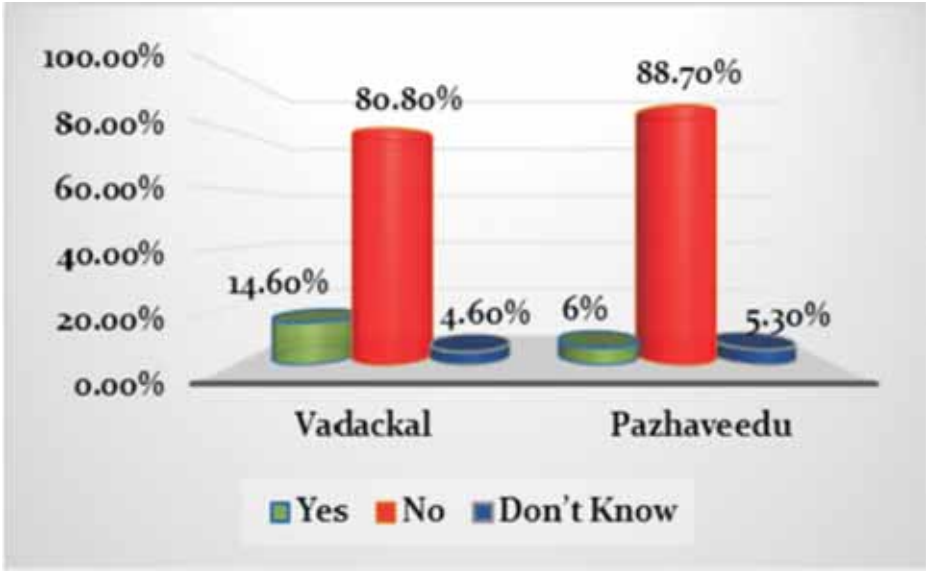


Figure.1–Perception of the community members in Alappuzha Municipal Wards about the role/response of different Govt. Departments in Vector Control Activities

KLU1602: Impact of climate change on mosquito abundance in coastal brackish water and wetlands of Alappuzha district, Kerala

Investigator: Balasubramanian R

Funding: Extramural (DST) [2017-2020]

Mosquito larval survey was conducted in ephemeral water bodies along the coastal areas of Alappuzha demonstrated the presence of larvae of *Culex tritaeniorhynchus, Cx. gelidus, Cx. quinquefasciatus, Cx. biteaniorhynchus* and Aedes albopictus. The salinity levels of the water bodies was up to 10 parts per thousand (PPT) at different times.Based on the field study results, laboratory study was conducted to determine the effects of increased salinity on the development of Cx. tritaeniorhynchus, Cx. gelidus and Cx. quinquefasciatus immatures. High level of emergence (>90%) was observed at 5 PPT in all the three species while decreased emergence was seen as the salinity increased (Figure 2). The study demonstrates the ability of Cx. tritaeniorhynchus, the major vector of Japanese encephalitis in Kerala, to oviposit and breed in brackish water.

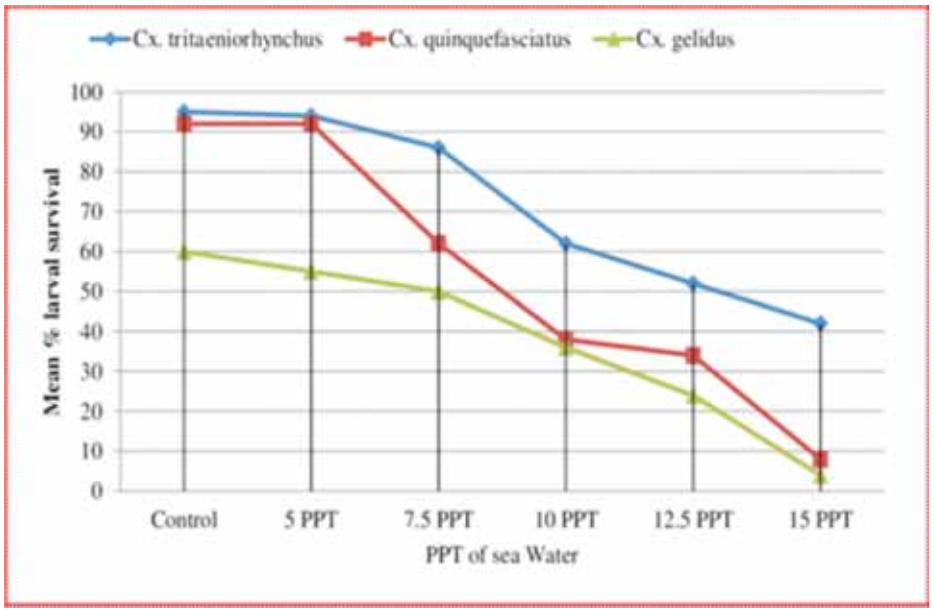


Figure 2- Mean % survival of Culex mosquito larvae at different salinity levels.

Number of Samples Tested (virus-wise details)

The unit provides diagnosis for different viruses using serological and molecular based diagnostic tests. Details of samples and tests are given in Table.1

Table 1: The number of samples screened for different viruses using various diagnostics (Total number/total positives)

S.No	Virus	IgM ELISA	Con PCR	Real time PCR	Virus isolation
1	JE	1/26	–	0/7	–
2	Dengue	22/61	24/41	3/8	–
3	West Nile	–	0/3	0/7	–
4	Chikungunya	0/5	–	-	–
5	HSV 1	–	0/1	-	–
6	HSV 2	–	0/1	-	–
7	HSV 1 & 2	0/8	–	-	–
8	HPV	–	1/20	-	–
9	HCV	–	0/1	-	–
10	Entero virus	–	–	0/2	–
11	Influenza A	–	0/2	29/166	–
12	Influenza B	–	0/2	10/173	–
13	H3N2	–	1/1	10/30	7/7
14	Pandemic A(H1N1)	–	–	26/42	–
15	RSV - A	–	0/9	–	–
16	RSV - B	–	0/9	–	–
17	Rhino	–	0/2	–	–
18	Flavi	–	0/2	–	–
19	Zika	–	0/53	–	–
	POSITIVE /TOTAL	23/100	26/147	84/470	7/7

Library & Information Services

Scientific Staff

Dr. AK Chakrabarti	Scientist C & Library in charge (up to 15.7.2016)
Dr. MD Ghokhale	Scientist C & Library in Charge (w.e.f. 18.7.2016)

Technical Staff

Ms. Vandana Chandere	Assistant Library and Information Officer
Mr. VR Mali	Library and Information Assistant
Ms. Ekta Jain	Library & Information Assistant
Mr. GR Gorayya	MTS

Apprentices

Ms. Jayshree Walhe	(Till 15/11/2016)
Ms. Sharvari Chavan	(Till 19/11/2016)
Mr. Dinesh Tribhan	(From 21/11/2016)

(1) Major activities:

Library continued to cater its services to Scientists, Technical staff, Project staff, M.Sc. Virology & PhD students of NIV as well as scientists and students of other research institutions in Pune.Services provided included support in publication of papers, citation analysis, reference service, Document Delivery Service, literature search, procurement of reprints etc. In addition, books/journals and other related documents were procured through Inter Library Loanfrom other research institutes in Pune, viz., ARI, BJMC, NCCS and Serum Institute of India Pvt. Ltd etc.

The following consortiums for access to full text of journals were managed

- ICMR Consortium for full text access for four journals viz., Science, Nature, Lancet, and NEJM (online).
- J-Gate Plus database, foraccessfor 3488 journaltitleswith access to full text, abstract and on Document delivery Request (DDR) from other ICMR libraries
- ERMED Consortium through National Medical Library, an IP based consortium for access to 243 e-journals from five publishers, i.e.,BMJ Group,Cambridge University Press,Lippincott Williams & Wilkins, Oxford University Press, John Wiley,.

During the year, the following activities were conducted

- Renewal of Magazine and Newspapers
- Carried out Stock Verification of Library documents for the period 2016-17
- Implementation of Barcode System for books and journals is in Process.
- Xeroxing services to scientists.
- Scanning of medically important articles from English and Marathi News Papers daily for ready reference for NIV Scientists through e-mails.
- Prepared bibliographical details of NIV research articles, hyperlinked with full text to upload on NIV Webpage.
- Updated and maintained in-house NIV Scientific Publications form 1953-2016.
- Updated list of NIV Holdings from 1953-2016 for ready reference.

Details of books/journals added to NIV library during the year:

Books	Purchased/Gifts/Gratis	110
	Bound Volumes	NIL
	Annual Reports Received	25
Journals	Print (Gratis)	60
	Online	4
	J Gate	3488
	ERMED Consortium	243
	Loose Issues	491
	Bound Volumes	NIL
Ph.D. Theses		8
M.Sc. Dissertations		22
Others; CDs, Microfilms, Floppies		8

Services Provided:

1.	Newspaper Clippings (sent to Director & Scientists)	2174 Clippings
2.	Inter Library Loan Received and Sent	95
3.	Reference Service + Document delivery Service(P+E)	513
4.	NIV Annual Reports Distributed	74
5.	CAS/SDI (Routinely)	On demand
6.	Citation Analysis	15 Scientists

Trainings conducted:

Figure 1 : A workshop for ICMR Librarians on "Library Management and Information Services in Medical Libraries" was conducted at NIV, Pune during 11-12 August, 2016

Figure 2: A workshop on "Leveraging ICT Tools for Knowledge Management in ICMR Library & Information Centers" was conducted at NIV, Pune during 24th- 25th November 2016.

Maintenance Division

List of Staff

Mr. S.T. Perumal	Technical Officer(C)
Mr. AB Khare	Technical Officer(B)
Mr. Punekar	Technical Officer (A)
Mr. D.R. Kumbhar	Technical Officer (A)
Mr. A. K. Kasar	Technical Officer (A)
Mr. VJ Bhosale	Technical Officer (A)
Mr. N.V. Bhongale	Technical Officer (A)
Mr. A.J. Suresh	Technical Assistant
Mr. G.K. Bagul	Technical Assistant
Mr. R.M. Shukla	Technician-C
Mr. SD Pote	Technician-C
Mr. SN Surbhaiah	Technician-C
Mr. N.S. Dhawale	Technician-C
Mr. V.D. Jagtap	Technician-C
Mr. Y.M. Taru	Technician-C
Mr.B.S. Shelar	Technician-B
Mr. A.B. Kelkar	Technician-B
Mr. S.S. Utale	Technician-B
Mr.S.S. Holkar	Technician-A
Mr. R. S. Gadhave	Technician-A
Mr. D.K. Jagtap	Technician-A
Mr. M.V. Gadhave	Technician-A
Mr. A.M. Pawar	Technician-A
Mr. A.N.Kale	Technician-A
Mr. S.R.Jagtap	Technician-A
Mr. SD Bathe	Technician-A
Mr. Ishte	Technician-A
Mr. Dedunda	Technician-A

Major Contributions by the department:

Maintenance staff of both campus plays an important role in the smooth functioning of the institute by providing un-interrupted power supply as well as keeping all laboratory equipments and installations in good working condition by regular servicing, overhauling and timely repairing of machines. The major contributions from the group in addition to routine work are placed below.

ISO/IEC-17025 certification of laboratories:

- Involved in accreditation process of BSL4, Avian Influenza and human Influenza laboratories as per ISO/IEC 17025: 2005.
- Assisted in preparation of documents and calibration of equipments of these labs.
- Carried out internal audit of Avian Influenza group.

Training and workshop:

- Prepared documents, PPTs, assessments and provided hands on training during CDC workshop.
- Delivered lecture on autoclave, incinerator etc. during Hindi workshop
- Delivered Lecture on Bio safety cabinet, HVAC, Fire and electrical safety, autoclave, Incinerator, engineering control, installation management etc. to MSC student
- Attended meeting at ICMR, Delhi for new BMS system of BSL3 in July 2016.
- Attended workshop on GAP III Implementation at Bangkok
- Visited Gorakhpur Unit for repairing BSC and other equipments

Capital works:

- Proposed 14 capital works during the year amounting to 208.28 lakhs and ICMR has sanctioned all the works and released Rs. 69.50 lakhs as first installment.
- Played an important role in the settlement of accounts with HSCC during the construction of BSL4 laboratory.

Emergency Attended :

- Cold room of 2nd floor of main building broke down in June 2016 due to compressor failure. Restarted the cold room to functionality in record time by MCC staff.
- Freezer Room failure at MCC: Identified the fault and completed the work within 3hr. Reviewed of BSL3 Proposal for Jabalpur alongwith Mr. Perumal as per instructions from ICMR

Other major works carried out by the staff:

- Installation of CCTV cameras at Main gate, Entrance of main building and Admin building as well as near guest house at MCC, Pashan.
- Provision of Access control system for Avian Influenza and DVG department in March 2017.
- Replacement of street lights with LED fittingsin the campus near NIV Guest House
- Replacement of radiator of 500 KVA DG set in place of old faulty radiator with the help of local agency
- Replacement of VFD of exhaust blower of HVAC system in Influenza Division.
- Installation, commissioning and testing of 11 KV HT VCB panels at NIV electrical power house, through state PWD.
- Installation of New water meter near Sump well.
- Replacement of insulated chilled water make-up tank for central air-conditioning plant of new building.
- The SFC documents for the building for NIV, BU were finalized and submitted to ICMR
- The estimate for new building at NIV, GKPR unit has been procured from the CPWD, Lucknow.
- Electrical distribution mapping work in Main building
- Replacement of old AHU at 1st floor ,second floor A wing and ground floor B2 wing completed in Dec 2016
- Repairing of CO_2 incubator of Hepatitis Division at the cost of Rs.1300/- for which M/s Thermo submitted the quotation of Rs. 95793/-.

M.Sc. Virology

List of staff

- Dr. Kavita Lole, Scientist 'E', In-Charge, Academic Cell
- Professor Vikram S. Ghole, Coordinator Academic Cell
- Dr.(Mrs) Tejaswini Deshmukh, Scientist 'B' (from 14 March 2017)
- Mr. Shrinivas S. Bedekar, Technical Officer-A
- Mrs. Neethi Jayaram, Technical Officer (on project)

The 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. Ten batches of students have completed the course successfully until now. In 2016, 21 students passed out and 3 students secured 'O' – Outstanding grade and 5 students secured 'A' – Very Good grade.

Toppers of 2014-16 batch

Rank	Name of the student
1	Ms. Garde Shambhavi Anupam (Gold medalist)
2	Ms. Dixit Kritika

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. The scientific data generated during their dissertation work is published in reputed National and international journals.

The details of our course are available at

1. http://icmr.nic.in/pinstitute/niv.htm
2. http://www.niv.co.in

Administrative Staff At NIV

(As on 31st March 2017)

Dr. R. Lakshminarayanan	Senior Administrative Officer
Mr. P Subramanian	Administrative Officer
Mr. A S Gaikwad	Administrative Officer (Stores)
Ms. S N Ponkshe	Accounts Officer

Section Officer

Mr. J S Rangan
Mrs. V V Shendye
Mrs. A S Deshpande
Mrs. P K Ratnaparkhi
Mrs. A S Palshikar

Private Secretary

Mrs. R K Amale
Mr. B K Wadke

Technical Officer 'A'

Mrs. A V Shendrikar

Assistant

Mr. H S Pasalkar
Mrs. A S Bakare
Mrs. A Mathai
Mrs. S. Srinivasan
Mr. S E Matkar
Mrs. A A Bapat
Mrs. A G Ghorpade
Mr. V C Chavan
Mrs. S S Pathak
Mrs. S P Mulay
Mrs. A R Nair
Mrs. P S Joshi
Mrs. R S Moghe
Mrs. S H Khamkar
Mr A.D. Pardeshi
Mrs. J V Gadre
Mrs. D D Marathe
Mr. P N Jadhav
Mr. J R Kumbhare

Upper Division Clerk

Mr. S R Vasam
Mrs. P B Aher
Mr. K S Galange

Lower Division Clerk

Ms. MJA Shaikh
Mrs. S BChakole
Mrs. T.T. Yadav
Mr.P N Chabukswar
Mrs. M L Rupnar
Mr. A E Matkar
Mr. H D Raut
Mr. V A Bisht
Ms. D S Pisal
Smt.M R Kannalu
Ms. Madhuri S Tandan
Mr. Akram Khan
Ms. Prajakta Bapat
Ms. Sadhana Ubhe
Mrs. Ashwini A Dudhane

Personal Assistant

Mrs. Shibi Jacob
Ms. Shakila Choudhari
Mrs S.M. Bhave

Mrs. S P Bohodkar	**Technical Assistant**
Mrs. S S Dube	**Stenographer**
Mr. B T Chandane	**Technician 'C'**

राजभाषा रिपोर्ट

राष्ट्रीय विषाणु विज्ञान संस्थान
20/ए, डा. आंबेडकर रोड, पुणे- 411 001

भारत सरकार की राजभाषा नीती का अनुपालन करने के लिए संस्थान सदैवकार्यरत है ।

राजभाषा अधिनियम की धारा 3(3) के अंतर्गत जारी होने वाले सभी दस्तावेज अनिवार्य रूप से व्दीभाषी में जारी किए जाते है । केंद्र सरकार राजभाषा नियम 1976 को नियम 10(4) के अंतर्गत कर्मचारीयों ने हिंदी कार्यसाधक ज्ञान प्राप्त कीया है ।

प्रशासन तथा प्रयोगशाला के कर्मचारी कंप्यूटर पर हिन्दी में कार्य करते हैं । प्रयोगशाला के रिपोर्टस हिंदी में भेजने का प्रयास किया जाता है । प्रशासन विभाग का अधिकतम कार्य हिन्दी में किया जाता है ।

राजभाषा कार्यान्वयन समिती की बैठक नियमित रूप से निदेशक महोदय जी की अध्यक्षता में आयोजित की जाती है । नगर राजभाषा कार्यान्वयन समिती की बैठक को संस्थान के अधिकारी उपस्थित रहते है । उसमें कार्यालय में होनेवाले हिंदी कामकाज का लेखा जोखा किया जाता है ।

सभी कर्मचारी राजभाषा हिन्दी से परिचित होने हेतु हिन्दी शिक्षण योजना राजभाषा विभाग द्वारा अमल में लायी जाती है । कार्यालयीन कामकाज हिंदी में करने के लिए प्राज्ञ- 4, प्रबोध-1, प्रविण- 5, पारंगत-23, हिंदी टंकण- 2 , हिंदी आशुलिपि-1 कक्षा के अंतर्गत कर्मचारी ज्ञान प्राप्त कर रहे है ।

हिंदी शिक्षण योजना, पुणे के अंतर्गत कंम्पूटर पर हिंदी मे काम करने के लिए बेसिक प्रशिक्षण कार्यक्रम वर्ष 2016-17 के लिए 7 कर्मचारियों ने प्रशिक्षण प्राप्त कर लिया हैं ।

सदभावना दिवस 22 अगस्त 2016 को मनाया गया इसके अवसर पर सभी कर्मचारियों द्वाराहिंदी सदभावना दिवस की प्रतिज्ञा दिलाई । तथा कौमी एकता सप्ताह, 23 नवंबर 2016 को मनाया गया तथा कर्मचारीयों को प्रतिज्ञा दिलाई ।

संस्थान में. सतर्कता जागरूकता सप्ताह दिनांक 31 अक्तूबर 2016 से 5 नवंबर 2016 तक मनाया गया । संस्थान के सभागृह में राष्ट्रीय सुरक्षा में जनता की भागीदारी इस विषय पर व्याख्याते श्री प्रकाश वाडकर, पी आय, महाराष्ट्र इंटेलिजन्स अकादमी, पुणे द्वारा व्याख्यान का आयोजन किया गया था ।

समितीद्वारा संस्थान में हिंदी को बढावा देने के लिए सितंबर माह के दौरान हिन्दी संगोष्ठी का आयोजन किया गया । संगोष्ठी का मुख्य उद्देश यही है कि हिंदी को बढावा देने के लिए प्रतियोगिताओं को प्रोत्साहीत करें और हिंदी के प्रति अपना स्थान बना लें ।

संस्थान में हिंदी सप्ताह के उपलक्ष्य में दिनांक 26-27 सितंबर 2016 को स्वच्छ भारत-स्वस्थ भारत इस विषय पर हिंदी में कार्यशाला का आयोजन किया गया था । भारतीय आयुर्विज्ञान अनुसंधान परिषद के सभी संस्थानों / केंद्रो के निदेशक महोदयजी से उस कार्याशाला में भाग लेने हेतु प्रतिभागियों के नामांकन करने के बारे में निवेदन किया गया था । निवेदन के अनुसार परिषद के कुल 16 प्रतिभागि तथा संस्थान के कुल 42 प्रतिभागियों ने कार्याशाला में भाग लिय

श्री अजय वाईकर, योग प्रशिक्षक, यशवंत विकास प्रबोधिनी, पुणे इन्होंने अपने व्याख्यान में योग किस तरह हमारे जीवन मे अग्रणीय है तथा उससे हमारे स्वास्थ पर होने वाले अच्छे परिणाम के बारे मे जानकारी दी।

सुश्री सुषमा चावला, वरिष्ठ न्यायिक सदस्य, आयकर अपीलीय अधिकरण मुख्य अतिथि के रूप में कार्यशाला में उपस्थित थी ।

दिवस पर योगा के प्रात्यक्षिक लेते हु श्री संतोष रणदिवे ,योगशिक्षक, पुणे

दि. 21 जून 2016 को संस्थान के सभागृह में अच्छी सेहत के लिए योगा के विषय पर श्री संतोष रणदिवे ,योगशिक्षक, पुणे द्वारा व्याख्यान का आयोजन किया गया था ।

॥ ॥ ॥

List of papers published by NIV scientists

(Apr 2016 - Mar 2017)

1. Alagarasu K. Introducing dengue vaccine: Implications for diagnosis in dengue vaccinated subjects. Vaccine. 2016; 34 (25):2759-61. (IF- 3.413)
2. Arankalle VA, Ramdasi AY. Immune responses in pregnant women presenting with subclinical or clinical HEV infection are different and trimester-dependent: A whole transcription analysis of Peripheral Blood mononuclear cells. Plos Pathogens. 2017 (In Press) (IF- 7.003)
3. Awate P, Yadav PD, Patil D, Shete A, Kumar V, Kore P, Dolare J, Deshpande M, Bagde S, Sakpal G, Gurav Y, Mourya DT. Outbreak of Kyasanur Forest disease (monkey fever) in Sindhudurg, Maharashtra State, India, 2016.J Infect. 2016; 72(6):759-61. (IF- 4.382)
4. Balakrishnan A, Thekkekara RJ, Tandale BV. Outcomes of West Nile encephalitis patients after 1 year of West Nile encephalitis outbreak in Kerala, India: A follow-up study. J Med Virol. 2016 Nov; 88(11):1856-61. (IF- 1.998)
5. Basu A, Yadav PD, Prasad S, Badole S, Patil D, Kohlapure RM, Mourya DT. An early passage human isolate of Kyasanur Forest Disease virus shows acute neuropathology in experimentally infected CD-1 mice. Vector Borne Zoonotic Dis. 2016; 16(7):496-8. (IF- 1.956)
6. Behera AK, Chandra I, Cherian SS. Molecular dynamics simulation of the effects of single (S221P) and double (S221P and K216E) mutations in the hemagglutinin protein of influenza A H5N1 virus: a study on host receptor specificity. J Biomol Struct Dyn. 2016; 34(9):2054-67. (IF- 2.3)
7. Bhoye D, Behera AK, Cherian SS. A molecular modelling approach to understand the effect of co-evolutionary mutations (V344M, I354L) identified in the PB2 subunit of influenza A 2009 pandemic H1N1 virus on m7GTP ligand binding. J Gen Virol. 2016; 97(8):1785-96. (IF- 3.19)
8. Bondre VP, Sankararaman V, Andhare V, Tupekar M, Sapkal GN. Genetic characterization of human herpesvirus type 1: Full-length genome sequence of strain obtained from an encephalitis case from India. Indian J Med Res. 2016; 144(5):750-760. (IF-1.446)
9. Chandra I, Behera, AK, Cherian SS. Identification of potential inhibitors against the human Influenza A virus targeting the CPSF30 and RNA binding domains of the NS1 protein: An E-Pharmacophore approach. Indian J Pharm Educ. 2017; 51(1):25-33. (IF- 0.219)
10. Chatterjee SN, Devhare PB, Pingle SY, Paingankar MS, Arankalle VA, Lole KS. Hepatitis E virus (HEV)-1 harbouring HEV-4 non-structural protein (ORF1) replicates in transfected porcine kidney cells. J Gen Virol. 2016; 97(8):1829-40. (IF- 3.192)
11. Chitamber SD. Use of rabbit antibodies against Norovirus GII.4 Virus- like particles fordiagnosis ofnorovirus infection. Indian J Med Res. 2016 (In Press). (IF-1.446)
12. Damle RG, Jayaram N, Kulkarni SM, Nigade K, Khutwad K, Gosavi S, Parashar D. Diagnostic potential of monoclonal antibodies against the capsid protein of chikungunya virus for detection of recent infection. Arch Virol. 2016; 161(6):1611-22. (IF- 2.255)
13. Damle RG, Patil AA, Bhide VS, Pawar SD, Sapkal GN, Bondre VP. Development of a novel rapid micro-neutralization ELISA for the detection of neutralizing antibodies against Chandipura virus.J Virol Methods.2017; 240:1-6. (IF- 1.508)

14. Devhare PB, Desai S, Lole KS. Innate immune responses in human hepatocyte-derived cell lines alter hepatitis E virus replication efficiencies. Sci. Rep. 2016 May; 6:26827. (IF- 5.228)

15. Gopalkrishna V, Ganorkar NN, Patil PR. Identification and molecular characterization of adenovirus types (HAdV-8, HAdV-37, HAdV-4, HAdV-3) in an epidemic of keratoconjunctivitis occurred in Pune, Maharashtra, Western India. J Med Virol.2016; 88:2100-2105. (IF-1.998)

16. Gopalkrishna V. Adeno virus associated keratoconjunctivitis study. J Med Virol.2016 (In Pres). (IF-1.998)

17. Gore MM. View point: Diagnosis of seasonal viral outbreaks: Preference of antibody assays in contrast to gene detection. Indian J Med Res. 2017 (In Press) (IF- 1.446)

18. Gurav Y, Yadav PD, Mangesh G, Tushar C etal. Kyansanur Forest disease prevalence in Western Ghats proven and confirm by recent outbreak of Maharashtra, India 2016. Vector-Borne and Zoonotic Disease.2017 (In Press) (IF- 1.956)

19. Gurav YK, Bondre VP, Tandale BV, Damle RG, Mallick S, Ghosh US, Nag SS. A large outbreak of Japanese encephalitis predominantly among adults in northern region of West Bengal, India. J Med Virol. 2016; 88(11):2004-11. (IF- 1.998)

20. Hegde NR, Gore MM. Japanese encephalitis vaccines: Immunogenicity, protective efficacy, effectiveness, and impact on the burden of disease. Hum Vaccine Immunother. 2017 22:1-18. (IF-2.146)

21. Ingle NB, Virkar RG, Agnihotri K, Sharma KS, Lole KS, Arankalle VA. Evaluation of liposome, heat-killed Mycobacterium w and alum adjuvants in the protection offered by different combinations of recombinant HA, NP proteins, and M2e against homologous H5N1 virus. Viral Immunol. 2016; 29(8):478-486. (IF- 1.513)

22. Ingle NB, Virkar RG, Arankalle VA. Inter-Clade Protection Offered by Mw-Adjuvanted Recombinant HA, NP Proteins, and M2e Peptide Combination Vaccine in Mice Correlates with Cellular Immune Response. Front Immunol. 2017 9; 7: 674. (IF- 5.695)

23. Jain P, Varanasi G, Ghuge R, Kalrao V, Dhongade R, Bavdekar A, Mehendale S, Chitambar S. Rotavirus Infections in Children Vaccinated Against Rotavirus in Pune, Western India. Indian Pediatr. 2016 8;53(7):589-93. (IF-0.972)

24. Joshi MS, Jare VM, Gopalkrishna V. Group C rotavirus infection in patients with acute gastroenteritis in outbreaks in western India between 2006 and 2014. Epidemiol Infect. 2017; 145(2):310-315. (IF-2.515)

25. Joshi SS, Arankalle VA. Differential immune responses in mice immunized with recombinant neutralizing epitope protein of hepatitis E virus formulated with liposome and alum adjuvants. Viral Immunol. 2016; 29(6):350-60. (IF- 1.513)

26. Koul PA, Mir H, Akram S, Potdar V, Chadha MS. Respiratory viruses in acute exacerbations of chronic obstructive pulmonary disease. Lung India. 2017; 34(1):29-33. (IF-NA)

27. Koul PA, Mir H, Saha S, Chadha MS, Potdar V, Widdowson MA, Lal RB, Krishnan A. Influenza not MERS CoV among returning Hajj and Umrah pilgrims with respiratory illness, Kashmir, north India, 2014-15. Travel Med Infect Dis. 2017; 15:45-47. (IF- 2.192)

28. Kulkarni R, Lole K, Chitambar SD. Seroprevalence of antibodies against GII.4 norovirus among children in Pune, India. J Med Virol. 2016; 88(9):1636-40. (IF-1.998)

29. Kulkarni SP, Thanapati S, Arankalle VA, Tripathy AS. Specific memory B cell response and participation of CD4(+) central and effector memory T cells in mice immunized with liposome encapsulated recombinant NE protein based Hepatitis E vaccine candidate. Vaccine. 2016 Nov 21; 34(48):5895-5902. (IF-3.413)

30. Kumar P, Kshirsagar A, Shil P. Estimation of epidemiological parameters for historical ship outbreaks of Influenza. Natl Med J India. 2017 (In Press) (IF- 0.907)

31. Lasure N, Gopalkrishna V. Clinico-epidemiology and genetic diversity of Salivirus in acute gastroenteritis cases from Pune, Wester India: 2007-2011. Infect Genet Evol. 2016 2; 44:425-430. (IF- 2.591)

32. Lasure N, Gopalkrishna V. Epidemiological profile and genetic diversity of sapoviruses (SaVs) identified in children suffering from acute gastroenteritis in Pune, Maharashtra, Western India, 2007-2011. Epidemiol Infect. 2017; 145(1):106-114. (IF-2.515)

33. Lasure N, Gopalkrishna V. Molecular epidemiology and clinical severity of Human Bocavirus (HBoV) 1-4 in children with acute gastroenteritis from Pune, Western India. J Med Virol. 2017 89(1):17-23. . (IF- 1.998)

34. Maher G, Pradhan G, Shetty S, Ranshing S, Damle A, Chitambar S. Rotavirus Infection in Children with Acute Gastroenteritis in Aurangabad, Central Maharashtra. Indian Pediatr. 2016; 53(7):631-3. (IF-0.972)

35. Mahilkar S, Paingankar MS, Lole KS. Hepatitis E virus RNA-dependent RNA polymerase: RNA template specificities, recruitment and synthesis. J Gen Virol. 2016; 97(9):2231-42. (IF- 3.192)

36. Mourya DT, Gokhale MD, Majumdar T, Yadav PDD, Kumar V, Mavale M. Experimental Zika virus infection of Aedes aegypti: Susceptibility, transmission and co-infections by Dengue and Chikungunya viruses. Parasit Vectors. 2017 (In Press) (IF- 3.234)

37. Mourya DT, Sapkal GN, Yadav PDD. Difference in vector ticks dropping rhythm governs the epidemiology of Crimean-Congo haemorrhagic fever & Kyasanur forest disease in India. Indian J Med Res. 2016: 144(4); 633-635. (IF- 1.446)

38. Mourya DT, Shahani HC, Yadav PDD, Barde PV. Use of hydrogen peroxide vapour & plasma irradiation in combination for quick decontamination of closed chambers. Indian J Med Res. 2016; 144(2):245-249. (IF-1.446)

39. Mourya DT, Shil P, Sapkal GN, Yadav PDD. Zika virus: Indian perspectives. Indian J Med Res. 2016; 143:553-564. (IF- 1.446)

40. Mourya DT, Viswanathan R, Jadhav S, Yadav PD, Basu A, Chadha M. Primary profiling criteria for the differential diagnosis of Crimean Congo hemorrhagic fever. Indian J Med Res. (In Press). (IF-1.446)

41. Mourya DT, Yadav PD. Retrospective analysis of clinical information in Crimean congo hemorrhagic fever patients: 2014-2015. Indian J Med Res. 2016. (In Press) (IF-1.446)

42. Ojha NK, Lole KS. Hepatitis E virus ORF1 encoded macro domain protein interacts with light chain subunit of human ferritin and inhibits its secretion. Mol Cell Biochem. 2016; 417(1-2) :75-85. (IF-2.613)

43. Pandey SS, Cherian S, Thakar M, Paranjape RS. Short Communication: Phylogenetic and Molecular Characterization of Six Full-Length HIV-1 Genomes from India Reveals a Monophyletic Lineage of Indian Sub-Subtype A1. AIDS Res Hum Retroviruses. 2016 May; 32(5):489-502. (IF-1.949)

44. Parashar D, Cherian S. RNA interference as Chikungunya virus therapeutics. Future Virol. 2016 May; 11:321-329. (IF- 0.886)

45. Pawar SD, Jamgaonkar AV, Umarani UB, Kode SS. Seroepidemiology of avian influenza H5N1, H9N2 & Newcastle disease viruses during 1954 to 1981 in India. Indian J Med Res. 2016; 144(3):472-476. (IF-1.446)

46. Pol SS, Dedwal AK, Ranshing SS, Chitambar SD, Pednekar SN, Bharadwaj RS. Prevalence and characterization of rotaviruses in children hospitalized for diarrheal disease in a tertiary care hospital, Pune. Indian J Med Microbiol. 2017.; 35 (1):33-36.

47. Pradhan GN, Walimbe AM, Chitambar SD. Molecular characterization of emerging G9P[4] rotavirus strains possessing a rare E6 NSP4 or T1 NSP3 genotype on a genogroup-2 backbone using a refined classification framework. J Gen Virol. 2016;97(12):3139-3153. (IF- 3.192)

48. Purwar S, Bhattacharya D, Metgud SC, Kumar D, Chitambar SD, Roy S. A cross-sectional study on aetiology of diarrhoeal disease, India.Indian J Med Microbiol.2016; 34:375-9. (IF- 1.006)

49. Rathod SB, Tripathy AS. TGF-ß1 and contact mediated suppression by CD4+CD25+CD127- T regulatory cells of patients with self-limiting hepatitis E. Human Immunology. 2016; 77(12): 1254-63. (IF- 2.127)

50. Rattan A, Pawar SD, Nawadkar R, Kulkarni N, Lal G, Mullick J, Sahu A. Synergy between the classical and alternative pathways of complement is essential for conferring effective protection against the pandemic influenza A(H1N1) 2009 virus infection. PLoS Pathog. 2017; 13(3):e1006248. (IF-7.003)

51. Roy S, Pavitrakar D, Gunjikar R, Ayachit VM, Bondre VP, Sapkal GN. Monocytes and B cells support active replication of Chandipura virus. BMC Infect Dis. 2016 14;16:487. (IF- 2.690)

52. Saha S, Chadha M, Shu Y; Group of Asian Researchers on Influenza (GARI). Divergent seasonal patterns of influenza types A and B across latitude gradient in Tropical Asia.Influenza Other Respir Viruses. 2016; 10(3):176-84. (IF-2.378)

53. Sapkal GN, Sawant P, Mourya DT. Chandipura viral encephalitis - Current status. Special Issue on "Emerging/Re-emerging and Zoonotic Virus Challenges of Developing Nations. O Virol J. 2017 (In Press) (IF- 2.362)

54. Saugandhika S, Sharma V, Malik H, Mohapatra SK, Bondre VP, Kumar S, Mohanty AK, Malakar D. Molecular characterization of IFN-T expressed in buffalo embryonic trophoblasts and expression of recombinant BuIFN-T1a2 and BuIFN-T8 isoforms in *E. coli.* Protein Expr Purif. 2016; 122:8-14. (IF- 1.407)

55. Shete A, Yadav PD, Kumar V, Nikam T, Mehershahi K, Kokate P, Patil D, Mourya DT. Development of PCR based diagnostic tests for detection of Malsoor virus and Adenovirus isolated from Rousettes species of Bats in Maharashtra State of India. Indian J Med Res. 2016 (In Press). (IF-1.446)

56. Srinivas V, Yadav PD, Mittal V, Chaubal G, Chhabra M, Mattoo S, Rai A, Mourya DT. Follow-up of Ebola Patient, 2014-2015. Emerg Infect Dis. 2016; 22(4):748-50. (IF- 6.99)

57. Sudeep AB, Gurav YK, Bondre VP. Changing clinical scenario in Chandipura virus infection. Indian J Med Res. 2016; 143(6): 712-721. (IF-1.446)

58. Sudeep AB, Shil P. Aedes vittatus mosquito: An emerging threat to public health. J Vector Borne Dis. 2017 (In Press) (IF- 0.87)

59. Sudeep AB, Vyas PB, Parashar D, Shil P. Differential susceptibility and replication potential of certain cell lines and one mosquito species to three lineages of chikungunya virus. Indian J Med Res. 2016 (In Press) (IF-1.446)

60. Tare DS, Kode SS, Hurt AC, Pawar SD. Accessing the susceptibility of highly pathogenic avian influenza H5N1 viruses to oseltamivir using embryonated chicken eggs. Antiviral Res. 2016. (In Press) (IF-4.909)

61. Tatte VS, Chaphekar D, Gopalkrishna V. Full genome analysis of rotavirus G9P [8] strains identified in acute gastroenteritis cases reveal genetic diversity: Pune, western India. J Med Virol.2017 Feb 27.doi: 10.1002/jmv.24799. (IF- 1.998)

62. Thounaojam AD, Balakrishnan A, Mun AB. Detection and Molecular Typing of Human Adenoviruses Associated with Respiratory Illnesses in Kerala. Jpn J Infect Dis. 2016; 69(6):500-504. (IF-1.140)

63. Tilarat SM, Vaidya SR, Hamde VS. Mumps outbreak in a tribal.J Med Microbiol.2016 (In Press). (IF-2.269)

64. Tiwary AK, Cecilia D. Kinetics of the association of dengue virus capsid protein with the granular component of nucleolus. Virology. 2017; 502: 48-55. (IF- 3.200)

65. Vaidya SR, Chowdhury DT, Jadhav SM, Hamde VS. Complete genome sequence of mumps viruses isolated from patients with parotitis, pancreatitis and encephalitis in India. Infect Genet Evol. 2016; 39:272-8. (IF- 2.591)

66. Vaidya SR, Chowdhury DT. Measles virus genotypes circulating in India, 2011-2015.J Med Virol. 2017; 89(5):753-758. (IF- 1.998)

67. Vaidya SR, Hamde VS. Is it right time to think of mumps vaccine in India's universal immunization program? Indian Ped. 2016; 53(6): 469-473. (IF-0.972)

68. Vaidya SR, Raut CG, Chowdhury DT, Hamde VS. Complete Genome Sequence of Mumps Virus Isolated from Karnataka State, India. Genome Announc. 2017;5(2). pii: e01429-16. (IF-NA)

69. Vaidya SR, Raut CG, Jadhav SM. Laboratory confirmation of rubella infection in suspected measles cases. J Med Virol. 2016; 88(10):1685-9. (IF- 1.998)

70. Virkar RG, Ingle NB, Arankalle VA. Liposome-adjuvanted recombinant HA and combination of HA, NP and M2e derived from 2.2 clade h5N1 virus confers heterologous protection. Hum Vaccin Immunother. 2016 (In Press) (IF-2.146)

71. Yadav PD, Sarkale P, Patil D, Shete A, Kokate P, Kumar V, Jain R, Jadhav S, Basu A, Pawar S, Sudeep AB, Gokhale M, Lakra R, Mourya D. Isolation of Tioman virus from Pteropus giganteus bat in North-East region of India. Infect Genet Evol. 2016 9; 45:224-229. (IF- 2.591)

72. Yadav PD, Shete A, Bondre V, Patil D, Kokate P, Chaudhari S, Srivastava S, Jadhav S, Mourya D. Isolation and characterization of Oya virus a member of Simbu serogroup, family Bunyaviridae, isolated from Karnataka, India. Infect Genet Evol. 2016 29; 44:122-126. (IF- 2.591)

73. Yadav PD, Shete A, Tandale BV, Deoshwatar A, Mourya DT. Serosurvey of Malsoor virus among Rousettus leschenaulti bat and human population residing nearby Robber's cave, Mahabaleshwar, Maharashtra, India. Indian J Med Res. 2016 (In Press). (IF-1.446)

74. Yadav PD, Patil DY, Shete AM, Kokate P, Goyal P, Jadhav S, Sinha S, Zawar D, Sharma SK, Kapil A, Sharma DK, Upadhyay KJ, Mourya DT. Nosocomial infection of CCHF among health care workers in Rajasthan, India. BMC Infect Dis. 2016; 16:624. (IF- 2.690)

75. Yadav PD, Shete A, Chaubal G, Mourya DT. Review of Bunyaviruses from India.Indian J Med Res. 2016 (In Press). (IF- 1.446)

76. Yadav PD, Sudeep AB, Pawar SD, Sarkale P, Gokhale M, Lakra R, Kumar V, Shete AS, Koakate P, Patil DY, Jadhav SM, Nichol S, Rout M, Pattnaik B, Mourya DT. Presence of Nipah virus in Pteropus giganteus bats in Northeastern India. Indian J Med Res (In Press). (IF- 1.446)

Book Chapters

1. Mourya DT, Yadav PD. Nairobi Sheep Disease virus. In; Dongyou L editor. Molecular detection of animal viral pathogens, CRC Press. 2016, Chapter 60. ISBN 9781498700368.

2. Raut CG, Hanumaiah H, Raut WC. Utilization and assessment of throat swab and urine specimens for diagnosis of chikungunya virus infection. Methods Mol Biol. 2016; 1426: 75-83.

3. Tandale BV, Rajderkar SS, Shrivastava N. Epidemology of Dengue.In; Chakraborty SS, Pal J, Saha B editors. Monograph on Dengue. 2016. p.19-31.

Dr. Soumya Swaminathan
(D.G., ICMR & Secretary DHR)
Visiting BSL - 4 Laboratory

National Institute of Virology
(Indian Council of Medical Research)

20-A, Dr. Ambedkar Road,
Pune - 411 001

Microbial Containment Complex,
Sus Road, Pune - 411021

Phones : 91-20-26006290, 26006390. Fax : 91-20-26122669.
Email : directorniv@gmail.com , Website : www.niv.co.in