FROM THE DIRECTOR'S DESK

It is indeed my pleasure to present the annual report of NIV for the year 2015-16. This year NIV has entered into translational research, a priority mission objective of ICMR. During the year, memorandum of understanding for six technologies has been signed with private companies for production of diagnostic kits and immunotherapeutics. Technology for production and marketing of hepatitis E human ELISA and Crimean Congo hemorrhagic fever sheep /goat IgG ELISA, Kyasanur Forest Disease (KFD) IgM ELISA and Chandipura virus human IgM ELISA technologies have been transferred to Zydus Cadila, Ahmedabad after fulfilling their requirements satisfactorily. More technologies have been proposed to ICMR for transfer to industry. Two patents of public health importance have been filed for new inventions.

Outbreak investigations and diagnosis of referred samples remained a major activity during the year. The year witnessed the outbreak of KFD in Sindhudurg district of Maharashtra, causing a public health alert. NIV investigated the outbreak and confirmed the etiological agent as KFD virus by demonstrating virus isolation from monkey, human and tick samples. NIV organized seminars and workshops to create awareness among health care workers and affected/at risk population in the region.

Investigation of hepatitis E outbreak in Himachal Pradesh has been unique as the NIV team set up a field laboratory and screened blood and water samples providing onsite diagnosis. The source of infection was identified and state health authorities were advised to take necessary measures. NIV also investigated a hepatitis outbreak in Bathinda district of Punjab and confirmed the etiological agents as hepatitis B and hepatitis C viruses by laboratory diagnosis. An outbreak of gastroenteritis, which affected approx. 2000 people in Bhimashankar Sahakari Sakhar Karkhana, Pargaonwas also investigated and the etiologic agent identified as Rotavirus. More than 40,000 referred clinical samples from suspected viral infections throughout the country were tested during this reporting period.

Activity of Influenza A (H1N1) pdm09 virus was detected in Maharashtra state. Avian Influenza surveillance showed ring recovery of wild and migratory birds in Pune district. Antibodies to Nipah virus (NiV) were demonstrated in bat population of Assam and West Bengal. Simultaneously, Japanese encephalitis (JE) negative acute encephalitis syndrome cases were found positive for NiV IgM antibodies.

NIV remained the mainstay in the diagnosis of arboviral infections for National Vector Borne Diseases Control Program and other National laboratories engaged in the diagnosis of JE, dengue and chikungunya. During 2015-16, NIV supplied around 7,000 ELISA kits to these agencies in India and over 100 kits to WHO SEAR countries. This year, NIV (BSL-4) became the Essential Facility for poliovirus containment as per the recommendation of national task force for containing wild type strains and vaccine strains of polio type-2.

In addition to diagnostic services and basic and applied research, NIV contributed to human resource development by organizing workshops and trainings for laboratory and health professionals in India and Southeast Asian countries. Resource Centre for VRDL network at NIV provides hands-on training in diverse areas including biosafety, outbreak investigation, serological and molecular diagnosis of viral infections, routinely to scientists and staff of VRDLs across the country. Apart from India, NIV also provides technical expertise and consultation to countries like Nepal, Sri Lanka, Bhutan, Timor Leste, Myanmar, and Bangladesh as part of WHO-SEARO program.

NIV has received a grant from Global Health Security Agency to provide training on Biorisk management in laboratory and hospital settings. This program will also create a network of laboratories for strengthening capacity of laboratory diagnosis of viral hemorrhagic fever and viral respiratory infections. The program has been initiated and training conducted in Delhi, Bangalore and Sindhudurg district (Maharashtra). Post-graduate course in virology, conducted at NIV, affiliated to Savitribai Phule Pune University remains a coveted course of the university. NIV believes in scientific progress of the country and collaborates with researchers from other institutes across the country.

As part of preparedness and emergency response, guidelines for Ministry of Health for diagnosis of suspected Zika virus infection, prepared reagents and provided training to ten identified VRDLs in the country. In addition, three ICMR laboratories have been trained for mosquito surveillance. The algorithm for diagnosis of suspected ZIKV infection has been provided and the VRDLs are testing dengue and Chikungunya negative samples for ZIKV. Samples screened by NIV and all centres were negative for ZIKV. NIV has also provided reagents to these laboratories, in addition to NCDC, New Delhi and WHO SEAR countries.

The three field units of NIV have also shown excellent performance by supporting public health in their respective states. The Bengaluru unit played a major role in the eradication of poliovirus in India and also acts as a sentinel laboratory for measles, mumps and rubella viruses. NIV continues to flourish in the field of publication, with papers in peer reviewed journals including, Nature, New England Journal of Medicine, Proc Natl Acad Sci USA, PLoS Medicine, Emerging Infectious Diseases etc.

As the Director of NIV, I congratulate my fellow scientists for the achievements made during the year and extend all possible support to excel in the coming years. I take this opportunity to thank the administration team of NIV for excellent management of administration and logistic support. I also thank my engineering team for their devoted services in the maintenance of NIV equipments, which are vital for research infrastructure. I extend my sincere gratitude to Dr. Soumya Swaminathan, DG ICMR and Secretary, DHR for her keen interest in NIV activities and wholehearted support. I take the opportunity to thank the officers and staff of ICMR and DHR for their support and cooperation.

(DT Mourya)

ABBREVIATIONS

AES	:	Acute encephalopathy syndrome
BSL-4	:	Biosafety Laboratory Level 4
CCHF	:	Crimean Congo Hemorrhagic Fever
CHPV	:	Chandipura virus
CHIKV	:	Chikungunya virus
CPE	:	Cytopathic effect
DENV	:	Dengue virus
DST	:	Dept of Science and Technology, Government of India
ELISA	:	Enzyme Linked Immunosorbent Assay
HCV	:	Hepatitis C virus
HRV	:	Human rotavirus
H	:	Hour
KFD	:	Kyasanur Forest Disease
miRNA	:	mitochondrial RNA
NiV	:	Nipah virus
NoV	:	Noroviruses
PI	:	Post infection
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, Gov. of India
VRDL	:	Virological Research and Diagnostics Laboratory
WHO	:	World Health Organization
ZIKV	:	Zika virus

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Name & Designation	Role
Dr. Dileep Kadam, Professor & Head, Department of Medicine, B J Medical College, Pune-411001.	Chairperson
Dr. Devendra T Mourya, Director, National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune-411001.	Member Secretary
Dr. Babasaheb V Tandale, Scientist 'E', National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune-411001.	Member Coordinator & Basic Medical Scientist
Dr. Sheila Bhave, Consultant in Pediatric Research, Department of Pediatrics, KEM Hospital Research Centre, Pune-411011.	Clinician
Dr. Arun Risbud, Scientist 'F', (Retd.) National Aids Research Institute, 73, 'G' Block, MIDC, Bhosari, Pune-411026.	Basic Medical Scientist
Dr. Vikram Padbidri, Consultant, (Microbiology & Infection Control), Department of Microbiology & Infection Control, Jahangir Hospital, Pune 411 001	Basic Medical Scientist
Dr. (Mrs.) Seema Sahay, Scientist 'E', National Aids Research Institute, 73, 'G' Block, MIDC, Bhosari, Pune-411026.	Social Scientist
Adv. Milind Heblikar, Advocate, Office No.E-202, Business Court, S.No-707, Mukundnagar, Pune-411037.	Advocate
Mr. Shrikrishna Deshmukh, A/P Makhai, Tal-Shirur, Dist.Pune-412208.	Person from Community

INSTITUTIONAL ANIMAL ETHICS COMMITTEE (IAEC)

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

INSTITUTIONAL BIOSAFETY COMMITTEE (IBSC)

Name & Designation	Role
Dr. D.T. Mourya, Director, National Institute Of Virology, 20-A, Dr.Ambedkar Road, Pune-411001.	Chairman
Dr. Kavita Lole, Scientist'E' & Group Leader, Hepatitis Division,National Institute of Virology, 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

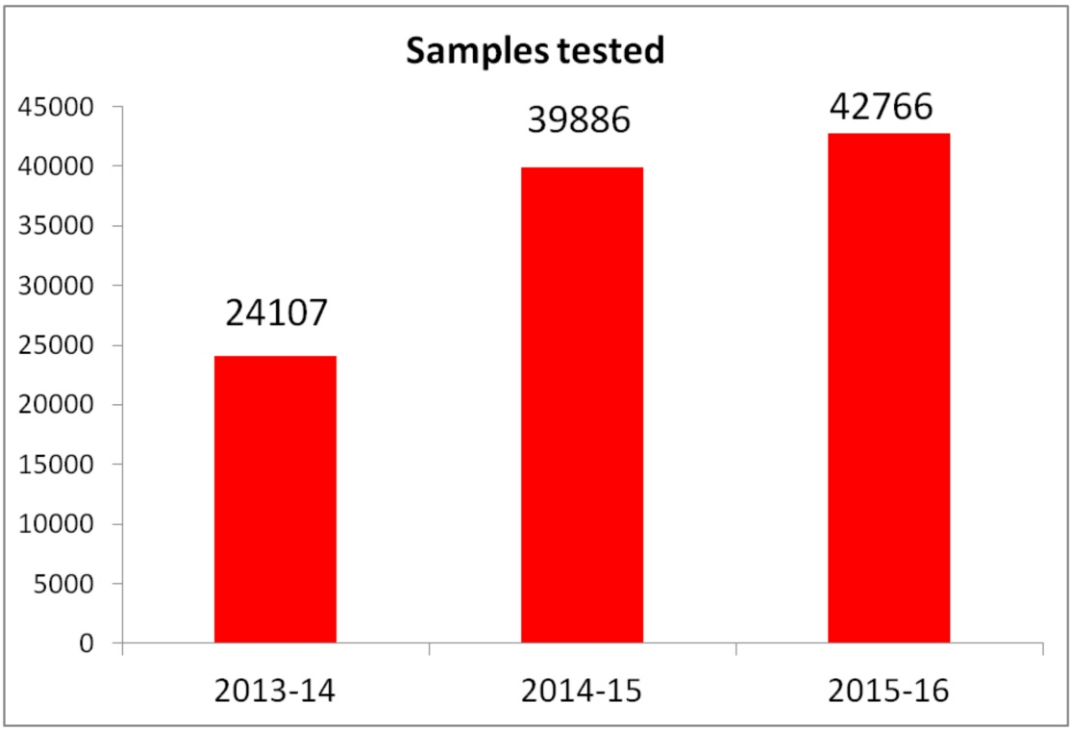
PERFORMANCE INDICATORS

Service to the Nation

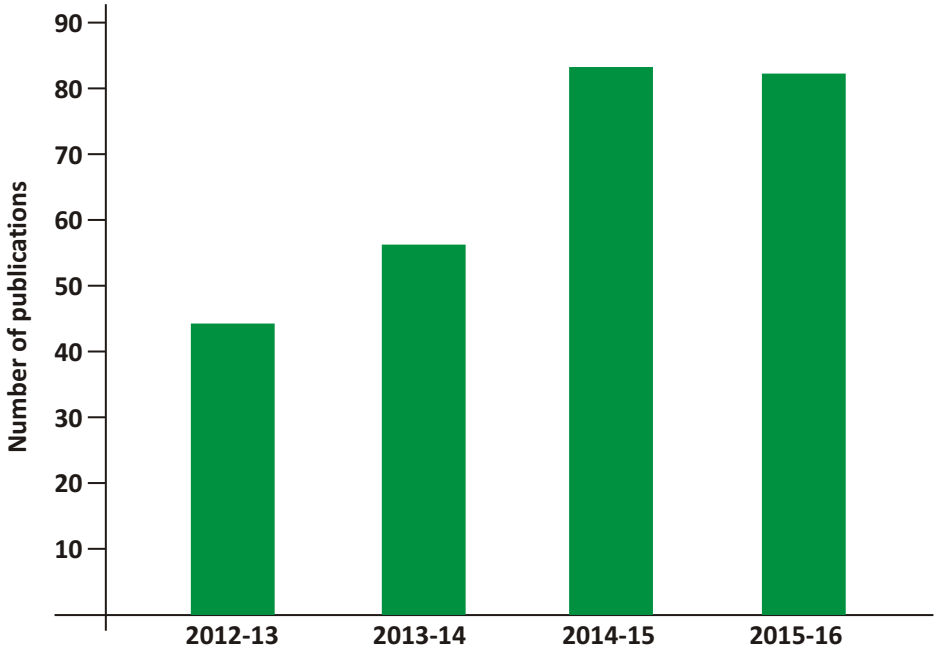
A. PUBLIC HEALTH CONTRIBUTIONS

● Total number of outbreaks investigated (Major)	04
● Total number of samples tested:	42766
● Total number of diagnostic kits supplied to various hospitals/centres:	6994
● Products/Technology developed and transferred to industry	03
● Patents filed	02
● Number of publications (Average Impact Factor 3.641)	82

B. REFERRED AND OUTBREAK SAMPLES TESTED OVER THE YEARS



C. PUBLICATIONS OVER THE YEARS



D. ACADEMICS

M.Sc. Virology

The National Institute of Virology (NIV) conducts MSc Virology course under the IBB program of Savitribai Phule Pune University (formerly University of Pune). The intake is 22 students per batch and NIV faculty take care of the theory and practical courses as per university guidelines.

Students are housed in NIV hostels at Pashan and are provided with Wi-Fi, Internet etc. They are also given 24 h access to NIV library and Information centre.

Ph.D. Program

NIV is affiliated to Savitribai Phule Pune University, Pune and other universities in the state to award Ph.D. degree in various disciplines *viz.,* Biotechnology, Microbiology, Zoology and Basic Medical Sciences (interdisciplinary sciences).

Number of recognized guides	17
Number of students pursuing PhD	28
Scholars awarded PhD during 2015-16	01

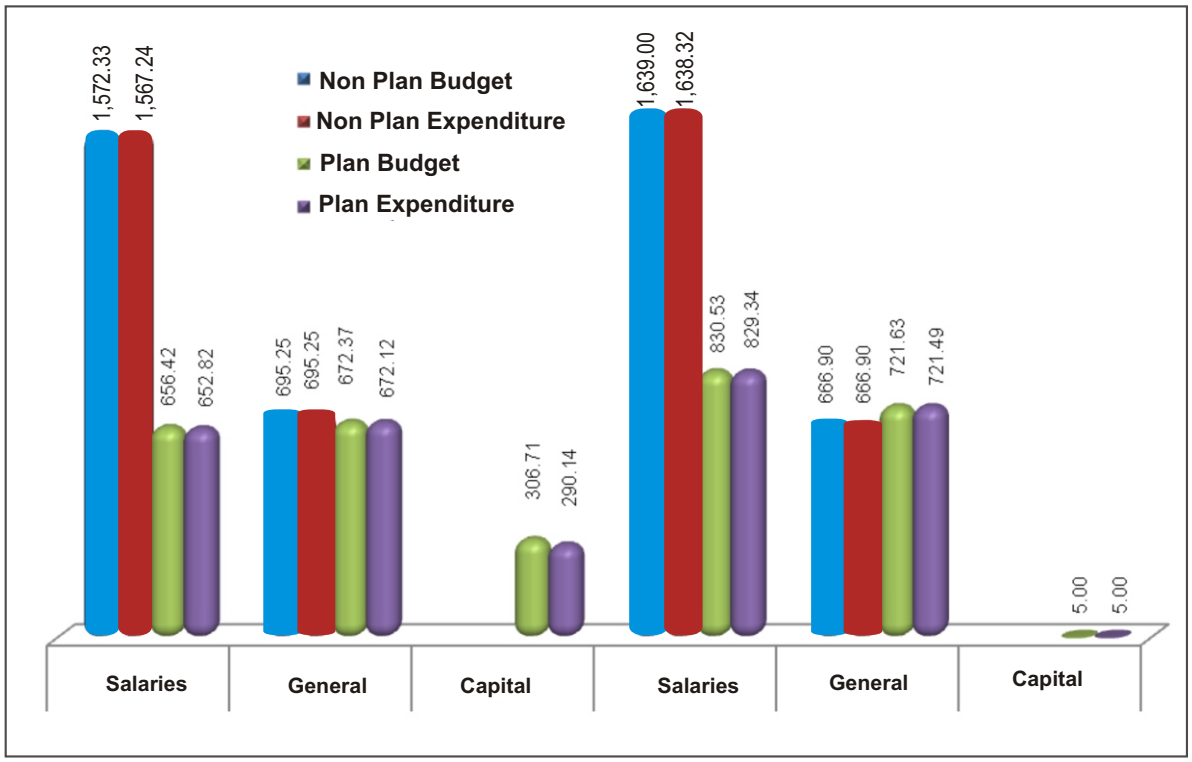
E. LIST OF TRAININGS & WORKSHOPS CONDUCTED
(April 2015 - March 2016)

Title & Resource person(s)	Period & Venue
Workshop on collection, handling, storage of specimens in Microbiology (Virology) Laboratories; Cold-Chain Maintenance, dangerous goods declaration & shipment of specimens. (Dr. SD Pawar)	6-8 April, 2015 NPHL, Kathmandu, Nepal.
WHO Training program on Biosafety level 3 laboratory for PHL, Bhutan on Engineering aspects. (Dr. DT Mourya, Dr. PD Yadav and Mr. Khare)	13th-18th April 2015 NIV, Pune
WHO-SEARO Regional Workshop on "Virological techniques in Influenza and other Emerging Viruses"(Dr Chadha, SD Pawar & Dr VA Potdar)	11-15th May 2015 NIV, Pune
Biosafety preparedness to handle infectious materials in laboratory and hospital setting at Krishna Institute of Medical Sciences, Karad (Dr. D.T. Mourya,Dr. P.D. Yadav, Dr. AM Shete, Dr. DY Patil, Mr. P Kokate &Mr. Kumar Bagmare)	13th-15th May 2015 KIMS, Karad
Molecular Techniques for dengue and chikungunya (Dr. Cecilia D, Dr. PS Shah, Dr. D. Parasher, Dr. K. Alagarasu)	22nd June to 10th July 2015, NIV, Pune
WHO Regional workshop on Quality Management System, biosafety & biosecurity practices in laboratories in the WHO South-East Asia Region, Pune, India, (Dr. SD Pawar)	24-28 August 2015 NIV, Pune
Molecular characterization of influenza viruses(Dr VA Potdar)	5th - 9th October 2015 Dept of Virology, Institute of Epidemiology, Disease Control and Research & National Influenza Centre, Bangladesh
Workshop for Ornithology students, MES College, Pune. (Dr. SD Pawar & Dr. MD Gokhale)	10th January, 2016 NIV, Pune
Susceptibility of Influenza Viruses to Neuraminidase Inhibitors using NI Assay & Sequence Analysis (Dr. J Mullick & Dr. SD Pawar)	9-12 February 2016 NIV, Pune
Biosafety preparedness to handle potential bio-hazardous material in laboratory settings (Dr. DT Mourya, Dr. PD Yadav)	19th-20th March 2016 NDTL, New Delhi

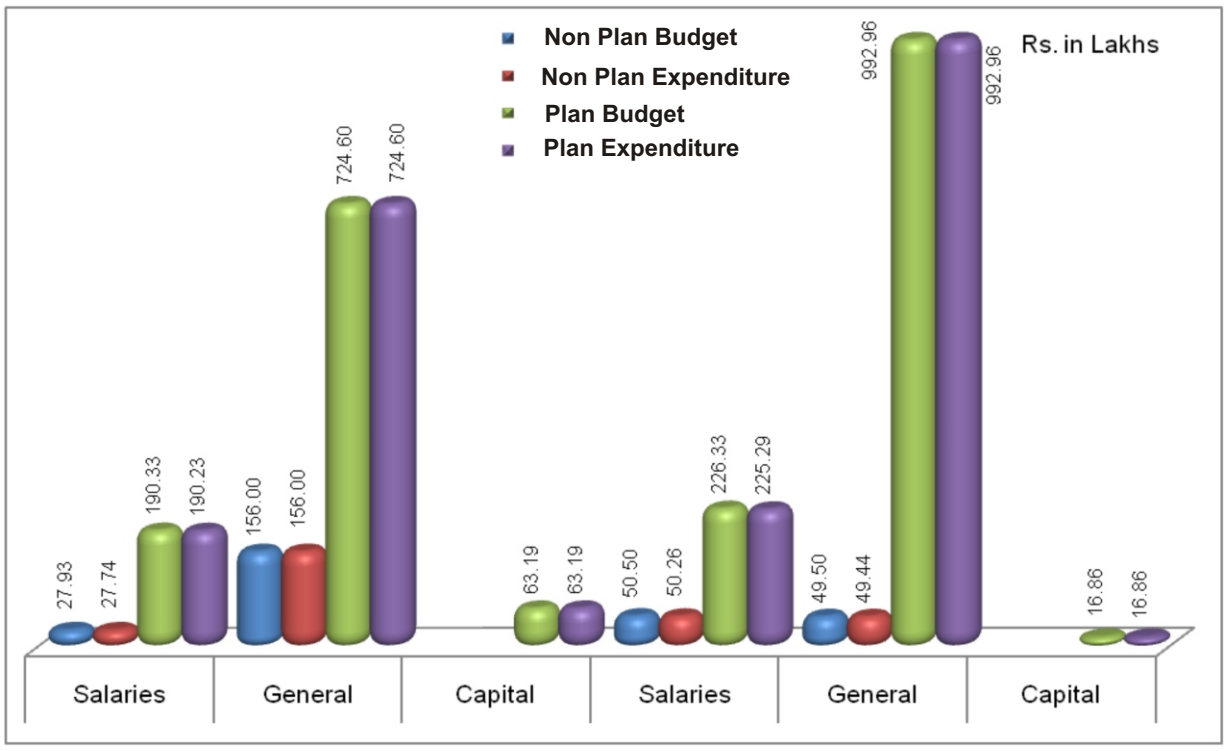
FINANCIAL INDICATORS

1. BUDGET DETAILS (in lakhs)

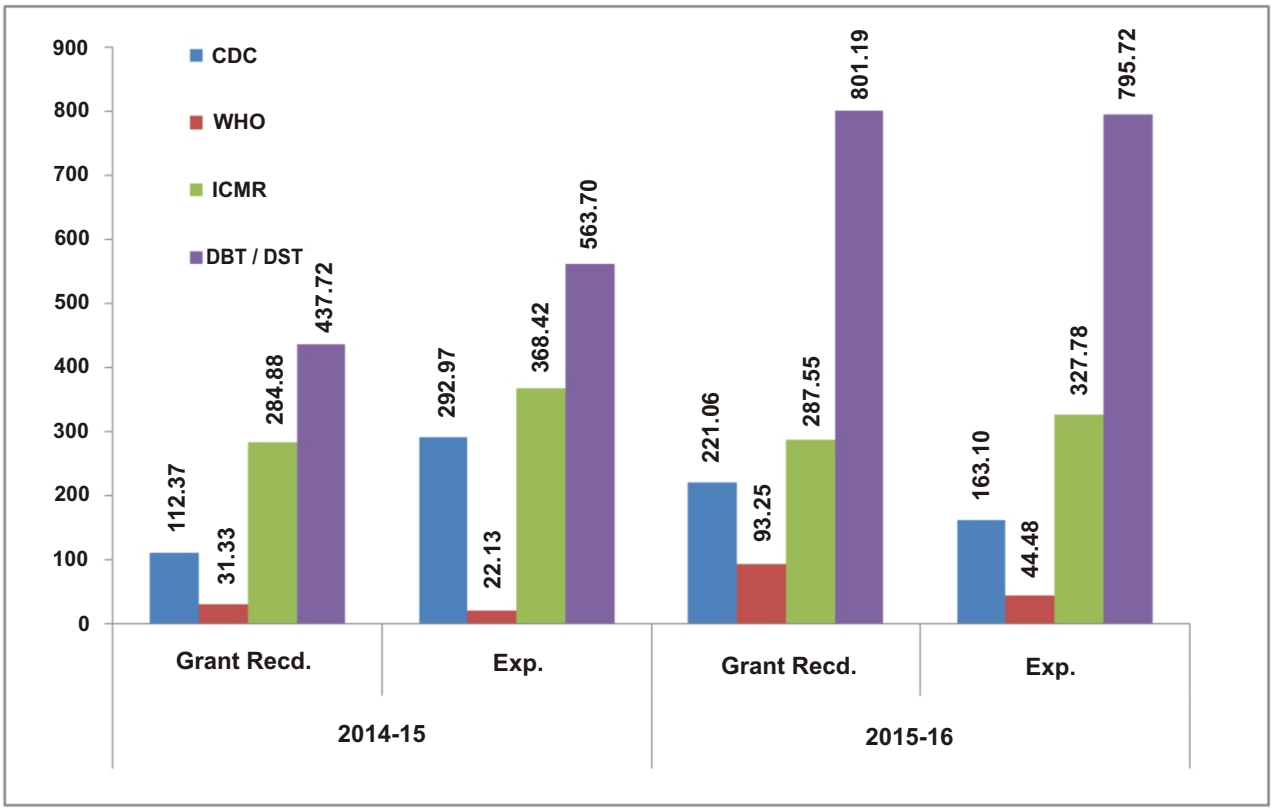
Budget NIV, Pune



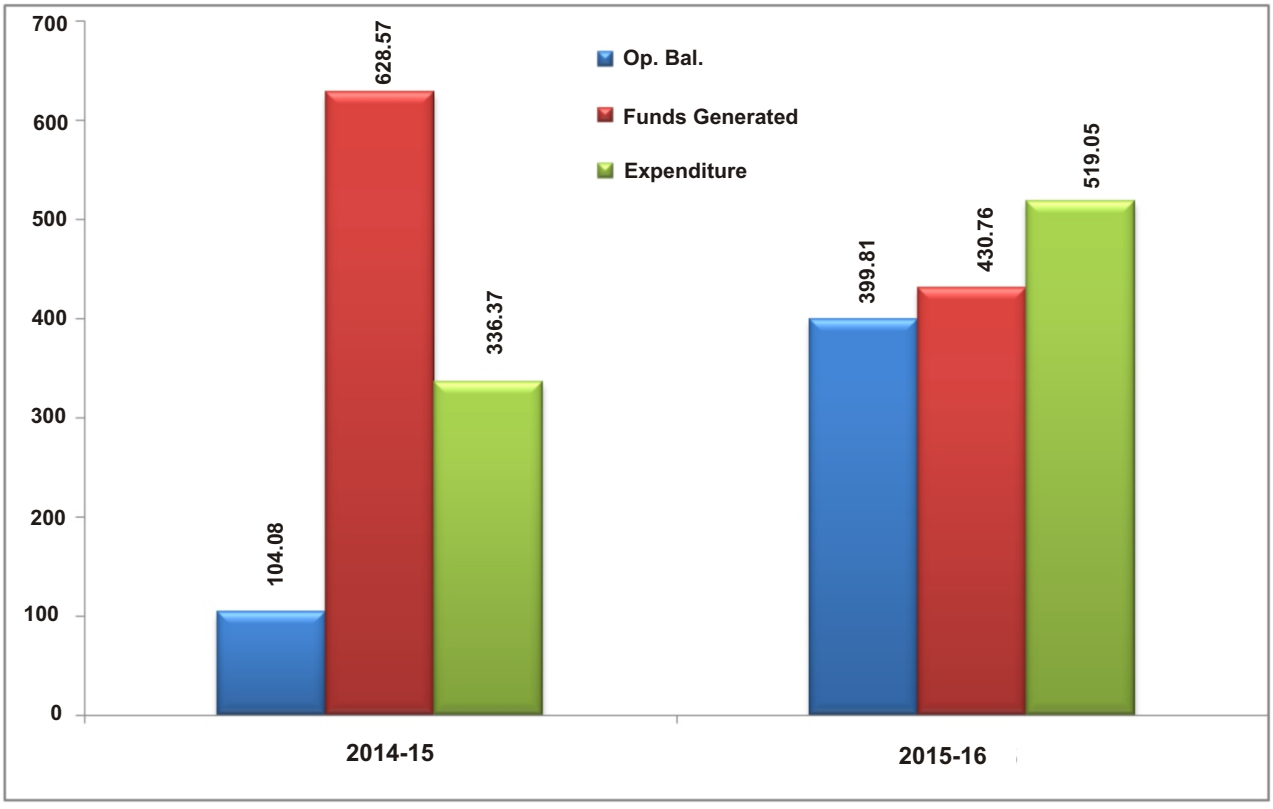
Budget MCC, Pune



Budget - Project Account (Rs. in lakhs)



Budget - Generated Funds



RESEARCH GROUPS

MAXIMUM CONTAINMENT LABORATORY

Scientific staff

Dr. DT Mourya	Director & Scientist 'G'
Dr. PD Yadav	Group Leader & Scientist 'D'

Technical Staff

Dr. Jain R	Technical Officer
Mr. Upadhyay C	Technical Assistant
Mr. Sarkale P	Technical Assistant
Mrs. Majumdar T	Technical Assistant
Mrs. Patil S	Technical Assistant
Mr. Lakra R	Technical Assistant
Mrs. Zawar D	Technician-'C'
Mr. Shende U	Technician-'B'
Mr. Thorat S	Technician-'B'
Mr. Gopale S	Technician-'B'
Mr. Aacharya M	Technician-'A'
Mr. Chopade G	Lab Attendant
Mr. Holepppanavar M	Lab Attendant

Project Staff

Dr. Shete A	Scientist 'C'
Dr. Patil D	Scientist 'B'
Dr. Chaubal G	Scientist 'B'
Dr. Yadav V	Scientist 'B'
Mr. Kore P	Technician-'C'
Mrs. Melag S	Technician-'C'
Mrs. Bargat A	Technician-'C'
Mrs. Rale T	Technician-'C'
Mr. Bagmare K	Technician-'C'
Mr. Kadam M	Technician-'C'
Mrs. Chopade Y	Technician-'B'

Engineering Staff

Mr. Gondane Y	Technical Officer-A
Mr. Patil P	Technical Officer-A

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

***Investigators**: PD Yadav, AB Sudeep, M Gokhale, SD Pawar, P Sarkale, A Shete, V Kumar, and DT Mourya*

Duration: 2015-17

Funding agency: Extramural (ICMR)

Brief background

Siliguri and Nadia districts of West Bengal witnessed outbreaks of Nipah virus (NiV) during 2001 and 2007. Comparison between the N-gene sequences of the Siliguri samples and the N-gene sequences from NiV samples isolated in Bangladesh (2004) and Malaysia (1999), a nucleotide identity of 97.5% was observed. *Pteropus giganteus* bat species is suspected as the reservoir host for this virus in India. Currently, there is no information available on the presence or seasonality of this virus in pigs although we have documented evidence of NiV activity in different parts of West Bengal and NE region. It is therefore felt essential to monitor the states adjoining Bangladesh *viz.,* West Bengal, Assam and Sikkim to determine the prevalence of this virus in humans and animals.

Work objectives 2015-16

- Serosurvey of NiV specific IgG antibodies in bats and pigs in NE region for "serosignature" pattern profiling.
- NiV isolation attempts from Pteropus bat and pig samples as well as from PCR positive human samples

Summary of work done

A total of 107 *P. giganteus* bats were trapped from Jalpaiguri and Cooch Behar districts of West Bengal and Dhubri district of Assam during three field visits conducted between March-December 2015. All the bat tissue specimens were tested for NiV by real-time RT-PCR. Samples tested positive for NiV were inoculated in hamsters and Vero CCL81 cells for virus isolation. In addition, 740 bat tissues that were tested negative for NiV were inoculated in Vero CCL81 cells for possible isolation of other viruses.

Nine organ samples (Liver/spleen and kidney) of 8 bats [5 from Cooch Behar and 3 from Dhubri] tested positive for NiV RNA. Twelve tissue samples (liver/spleen and kidney) of bats (1 bat from Cooch Behar and 11 bat from Dhubri) were found to be positive by partial nucleocapsid gene specific RT-nPCR (100bp). Sequences showed 99% similarity with *P. giganteus*-derived NiV nucleoprotein sequences from India (AFJ513078) and Bangladesh (AY988601).

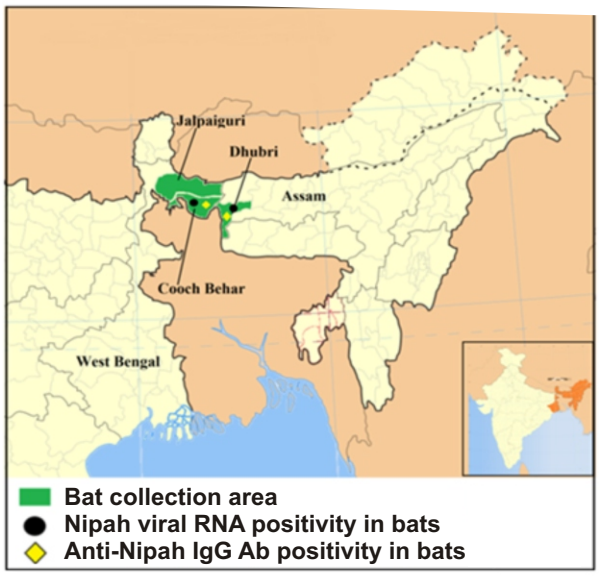


Fig 1: Geographic locations of Nipah virus positivity in *P. giganteus* bats in Northeast India

However, they are divergent from Malaysian strains. Four serum samples (3 from Dhubri and 1 from Cooch Bihar) out of 63 *P. giganteus* bats tested positive for anti-NiV IgG antibodies (antibody titer 1:100) by anti-NiV IgG ELISA (Figure 1). Throat swabs, rectal swabs and urine samples were tested negative for NiV RNA. Among the collected bat samples, nine real-time RT-PCR positive bat samples (liver/spleen and kidney) were inoculated in hamsters and Vero CCL81 cells for virus isolation. Neither clinical signs nor presence of the virus in organs (liver/spleen, kidney, brain) could be detected in virus inoculated hamsters upto 12 days. Real-time RT-PCR studies for two consecutive passages could not detect the virus in serum of the inoculated hamsters. Similarly, no cytopathic effect (CPE) could be observed in Vero CCL81 cells inoculated with organ suspensions of inoculated hamsters despite five consecutive passages.

Among those samples that tested negative for NiV and were inoculated in cell lines, CPE was observed in one of the cultures inoculated with bat liver sample collected from Dhubri. The agent was

isolated in the same cell line and identified as Tioman virus (TioV), a Paramyxovirus. This is the first isolation of TioV from India. Three bat samples; one from Cooch Bihar and two from Dhubri were also tested positive for TioV RNA by RT-PCR. Genomic analysis revealed 88% homology with the nucleoprotein gene and 90% homology with phosphoprotein gene of the TioV isolate from Malaysia (Bat/2002/AF298895). Disease causing potential of the virus to humans is still unknown.

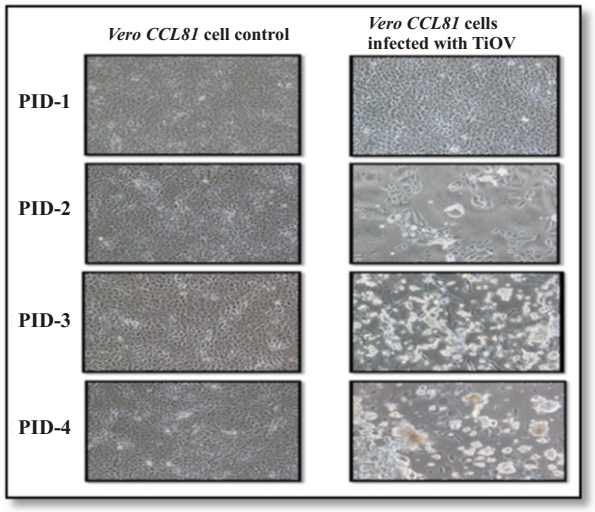


Figure 2 : Tioman virus propagation and cytopathic effects in Vero CCL-81 cells at different post infection days (PID)

Susceptibility of six vertebrate cell lines [Vero E6, *Pipistrellus ceylonicus* bat embryo cells, BHK-21, Porcine Stable (PS) and Vero-CCL81] to TioV was studied and all the cell lines were found susceptible to the novel virus. TioV stock was prepared in Vero CCL81 cells, and two ELISA assays were developed for detecting anti-TioV antibody from bat and swine serum. Using the optimized assay, 41 bat sera samples collected from Cooch Bihar and Siliguri were screened for anti-TioV IgG antibodies but all tested negative. Using anti-TioV optimized swine IgG ELISA assay, 320 pig samples collected from Mizoram was screened but none of the samples tested positive for anti-TioV IgG antibodies.

Findings/summary

The presence of NiV among *Pteropus* bats from West Bengal and Assam was confirmed in the current study. The present study also reports the isolation of TioV from *Pteropus giganteus* bats in India. This is the second report of TioV isolation apart from Malaysia (Chua et al., 2001). Partial sequences of TioV phosphoprotein and nucleocapsid gene revealed that TioV strains from India and Malaysia are from one lineage, while Menangle, Tuhoko, Achimota and Sosuga viruses formed a separate clade.

MCL 1304: Preparation of reagents for highly infectious diseases

***Investigators**: PD Yadav, A Shete, T Majumdar, R Jain, P Sarkale, P Kore, G Chaubal & DT Mourya*

Funding agency: Intramural

Period: 2014-2016

Work objectives:

- Development of molecular diagnostic tests for Zika virus
- Detection of KFD virus (KFDV) specific antibody using recombinant KFD NS1 protein based ELISA
- Standardization of anti-Ingwavuma (INGV) human IgG antibody ELISA assay
- Development of real-time RT-PCR for detection of Nipah virus

(i) Development of molecular diagnostic tests for Zika virus

Brief background:

Zika virus (ZIKV) is an emerging mosquito-borne virus that was first identified in Uganda in 1947 in rhesus monkeys during investigation of sylvatic yellow fever. Outbreaks of ZIKV have been recorded in Africa, the Americas, Asia and the Pacific subsequently. During the large scale outbreaks in French Polynesia and Brazil in 2013 and 2015 respectively, national health authorities reported potential neurological and autoimmune complications associated with ZIKV infection.

Work Objectives:

In view of WHO declaring Zika viral disease a global public health emergency, the first objective for BSL-4 laboratory was to revive an isolate of ZIKV that was deposited with NIV virus repository in the early 1950s. The second objective was to develop diagnostic tests for diagnosis of ZIKV.

Summary of work done:

Attempts to revive the stored virus from NIV repository were futile as the virus could not be revived despite making efforts to replicate it in mosquito, tissue culture and infant mice. However, the viral RNA was cloned and positive controls were generated for RT-PCR and real-time RT PCR assays. The BSL-4 laboratory has established diagnostic RT-PCR (NS5 region) and real time-RT PCR (envelope region) assays based on references provided by CDC.

Findings/summary

Diagnostic real time assay was optimized for ZIKV and supplied for training of 10 VRDL laboratories across the country as part of the preparedness for probable ZIKV outbreak.

(ii) Detection of KFD/specific antibody using recombinant KFD NS1 protein based ELISA

Brief background:

Assuming that non-structural protein (NS1) of KFDV may be a potential biomarker for early diagnosis of the disease, this project was aimed at expression of KFDV NS1 protein in bacterial expression system for use in capture ELISA.

Work Objectives:

- Development of NS1 antigen capture ELISA for early diagnosis of KFD

Summary of work done:

KFDV NS1 gene was cloned in pET28a vector. The expressed and purified rNS1 protein was used to raise anti-NS1 antibodies in mice and rabbits. ELISA using anti-NS1 antibodies raised in mice (for coating) and biotinylated anti-NS1 antibodies raised in rabbit (for capture) was standardized. A total of 107 samples that were KFDV RNA positive & IgM negative (38), RNA & IgM positive (10), RNA negative & IgM positive (14), RNA negative & IgM, IgG positive (6), RNA & IgG positive (1), RNA, IgM, IgG positive (3), IgG positive (3) Samples negative for KFD (32) were used in the study. The assay was found to be specific for KFDV NS1 protein and could detect up to 1µg rNS1 protein when spiked in negative serum. However, only 2 (2/38) RNA positive and IgM negative samples showed positivity in the assay.

Findings/summary

NS1 antigen capture ELISA developed though showed positivity in a few samples, could not be used as a diagnostic tool for early detection of KFDV.

(iii) Standardization of anti-Ingwavuma (INGV) human IgG antibody ELISA assay

Brief background:

There has been two isolations of INGV in the past from Karnataka; one from a pig and the other from a human case. In 2003, patient samples received from Bangalore field station [2001-2003] showed positivity to INGV by IFA. Partial characterization of genome of this virus showed that INGV isolated from India has difference at nucleotide and amino acid levels with reported INGV isolates. Looking at the requirement to characterize the virus, it is required to develop molecular [RT-PCR, qRT-PCR] and serological diagnostic tests [antigen detection IFA and ELISA] for detection of INGV from clinical specimens.

Work Objectives:

- **Standardization of anti-INGV human IgG antibody ELISA assay**

Summary of work done:

Tissue culture raised INGV antigen (FCS free) was concentrated and used at dilutions from 1:100 to 1:800 for optimization of anti INGV human IgG ELISA. Sample dilutions of 1:200-1:600 and conjugate dilutions from 1:8000 to 1:12000 were used for checkerboard titration of the INGV antigen. Various sample diluents and post coat buffers were also tried for optimization. Indigenously developed anti INGV human IgG ELISA is having 1:200 INGV coating antigen, 1:500 sample dilution and 1:10000 anti human IgG conjugate. Retrospective human sera samples were screened with the optimized ELISA to check the presence of INGV IgG antibodies.

Findings/summary

Anti-INGV human IgG antibody ELISA developed which can be used for screening of samples.

(iv) Development of real-time RT-PCR for detection of Nipah virus

Brief background

Due to less sensitivity of the existing real time PCR assay for the Indian isolate, it is difficult to quantify the virus and establish a relationship between the virus titre and disease severity. Also there are chances of giving a false negative result for samples having low virus titre. Hence the need to develop a real time PCR assay specific for the isolates of NiV from the 2007 Nadia outbreak in India (GenBank accession number – FJ513078.1).

Work Objectives

- Developing an indigenous, sensitive real-time RT PCR for Nipah virus detection

Summary of work done

For standardizing real-time RT-PCR, primers were designed and N gene of NiV was amplified by RT-PCR using source material (autopsied brain tissue from Nadia 2007 outbreak; GenBank accession number: FJ513078). The N gene was cloned in pGEMT Easy vector and *in-vitro* transcribed using Riboprobe *in-vitro* transcription systems. The real time RT-PCR primers could detect up to 4 copies of *in-vitro* transcribed NiV RNA. This assay was found highly specific to NiV.

Findings/summary

An indigenous, sensitive real-time RT PCR for NiV detection was developed for screening of samples.

e. Translation of the ELISA technologies

***Investigators**: PD Yadav & DT Mourya*

Funding agency: Intramural

To support national public health programs, BSL-4 Laboratory group has developed several ELISA kits for diagnosis of KFD and Cremian Congo hemorrhagic fever (CCHF). The following 3 ELISA technologies were transferred to Zydus Cadila, Ahmedabad and are in the process of commercialization:

1. Anti-CCHF Sheep and Goat IgG ELISA
2. Anti-CCHF Bovine IgG ELISA
3. Anti KFD Human IgM antibodies ELISA

Figure 3 : NIV, Pune (ICMR) participated in The Festival of Innovation (FOIN) - 2016 held at Rashtrapati Bhavan during 12th to 19th March, 2016

Laboratory standardization of the following technologies is complete and is ready for technology transfer (Fig 3).

1. Anti KFD human IgG antibody ELISA
2. Anti-CCHF human IgG antibody ELISA
3. Anti-CCHF Human IgM ELISA
4. KFD Real time RT-PCR
5. CCHF real time RT-PCR
6. KFD RT-PCR

MCL1406: Serological survey for exploring human infections with Malsoor virus (MV) in Mahabaleshwar area, Maharashtra.

***Investigators**: DT Mourya, P D Yadav, A Deoshtwar, BV Tandale, A Shete, P Kokate, R Lakra & R Jain*

Funding agency: Intramural

Period: 2014-2015

Brief Background:

During a survey of bats in the year 2010, two virus isolations were made from *Rousettus leschenaultii* species of bats from Malsoor Village of Mahabaleshwar, Maharashtra.

The virus isolates were confirmed as Malsoor virus (MV), genus *Phlebovirus*, family *Bunyaviridae*. Close relatedness of MV to SFTSV and heartland viruses makes it imperative to screen this virus in human population.

Work objectives:

- Development of a PCR based diagnostic test for detection of Malsoor virus
- Serosurvey of Malsoor virus among *Rousettus leschenaultii* bat and human population in Mahabaleshwar, Maharashtra

Summary of work done:

a. Development of a PCR based diagnostic tests for detection of MV

During 2011 to 2014, NIV has carried out a survey of bats to detect the presence of highly infectious pathogens *viz.*, Ebola, Marburg, Nipah and other possible emerging viruses in different parts of the country. A novel virus, Malsoor virus (family *Bunyaviridae*, genus, *Phlebovirus*) was isolated from *Rousettus* bats from Mahabaleshwar, Maharashtra during the period. Though its potential to cause infections in humans is unknown, an attempt was made to develop diagnostics against the virus to determine the prevalence of the virus in bats and humans. Liver/spleen samples of 69 bats were screened for MV; 19 bats were found to be positive by nested RT PCR and 3 bats were positive by real time RT-PCR. Sequence analysis of MV showed highest homology with known sequences of *Phleboviruses* and found to be distinctly close to Heartland and SFTS viruses.

Findings/summary

A PCR based diagnostic test for detection of MV was developed which is useful in early detection of the virus. MV positivity was recorded in bat population from Mahabaleshwar by nested RT PCR.

b. Serosurvey of Malsoor virus among *R. leschenaultii* bat and human population in Mahabaleshwar, Maharashtra, India

Looking at the disease causing potential of the viruses belonging to genus *Phlebovirus*, a preliminary investigation was conducted among human population residing adjacent to Robber's Cave (The place of virus isolation) to determine the seroprevalence of MV. Prior permission and approval of Institutional human ethical committee (IHEC) were obtained to initiate this study and samples were collected from villagers staying close to the caves as well as a village 22 km away from the caves. Among 174 participants, three persons had a history of epitaxis and another three had a history of fever. No other major illnesses were reported from the area. Gamma inactivated MV antigen and Vero CCL81 TCF were used as positive and negative controls in the study. In-house developed ELISA was used for screening bat and human samples.

Findings/summary

Using the indigenously developed anti-Malsoor IgG ELISA seroprevalence of MV among bats population from Mahabaleshwar was recorded. Results indicated presence of anti-Malsoor IgG antibodies in 7 out of 19 bats while none of the human samples tested positive for Malsoor virus specific IgG antibody.

MCL 1301: Outbreak investigation and laboratory diagnostic support for referred samples of viral hemorrhagic fever and other unknown etiology

***Investigators**: PD Yadav, A Shete, D Patil, R Jain, S Patil & DT Mourya*

Funding agency: Intramural

Period: On-going project

Brief background:

Diagnostic tests have been established for many hemorrhagic fever viruses *viz*., CCHFV, Hantaan virus, NiV, Marburg virus, Ebola virus, KFDV, Poxvirus etc. These tests are being continuously refined and used for testing referred samples.

Work Objective:

BSL-4 laboratory provides diagnostic support for referred samples of viral hemorrhagic fevers and other unknown etiologies as and when needed. Secondly, the laboratory provides kits and reagents for diagnosis as required.

Summary of work done

(i) KFD in Maharashtra, Goa and Karnataka:

During January 2016, an outbreak of KFD was confirmed for the first time in Dodamarg taluk, Sindhudurg district, Maharashtra. KFD positivity was confirmed in 8 villages of Dodamarg taluk with highest positivity in Ker village (7%). Four tick pools and necropsy samples of a deceased monkey were

tested positive for KFDV by real time RT-PCR. Four out of nine cases referred from Belgaum, Karnataka who had a history of travel to Ker village were also found positive for KFD by real time RT-PCR. Necropsy samples of 15 out of 21 monkeys received from Goa were also found positive for KFDV (Fig 4).

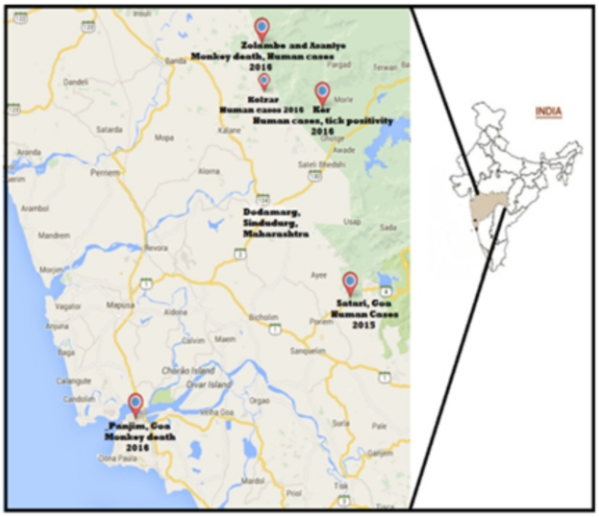


Figure 4 : Geographic location of KFD positivity among human, monkeys and ticks in Dodamurg taluka, Maharashtra, India

Serosurvey was conducted for detection of anti KFDV IgG antibodies in the KFD affected areas to determine exposure to KFDV in the past and also to determine the status of asymptomatic ratio with cases. During the serosurvey, 942 samples were collected from KFD affected villages and a control village and screened for anti-KFD IgG antibodies. Out of the 751 samples screened, 40 were positive for anti-KFD IgG. KFD IgG positive samples were also screened for anti KFD IgM and 21% samples tested positive. Screening of the remaining samples is in progress.

Findings/summary

This study reports the first confirmed outbreak of KFD in Dodamarg taluk, Sindhudurg district of Maharashtra. Confirmed cases were the residents of Ker, Zolambe, Kolzar and Asaniye villages of Dodamarg taluk. KFDV positivity was also recorded among dead monkeys and tick pools. These findings supports the hypothesis that this virus is prevalent in many areas especially in Western Ghats covering the hot spot zone running with forest and a possible existence of KFDV in a monkey-tick-human cycle.

Miscellaneous studies:

(i) A non-fatal case of CCHF from Gujarat with travel history to Muscat, Oman

In January 2016, an Indian traveller who returned from Muscat, Oman presented with fever, hemorrhagic signs and altered sensorium to a hospital in Gujarat and the sample was referred to NIV, Pune for CCHF diagnosis. CCHF viral RNA was detected in serum sample of the patient on 3rd day of illness using real time RT-PCR. The report was immediately communicated to health authorities. Sequence of S segment of the sample revealed 99% homology with isolate from Zahedan 2007 (gb|KC867274.1). CCHFV was isolated from the sample in Vero cells.

(ii) Diagnosis of suspected hemorrhagic fever samples referred from Nepal through WHO

Three human sera samples of suspected hemorrhagic fever cases were referred to NIV, Pune from National Influenza centre, and Molecular diagnostic unit of National Public Health Laboratory, Kathmandu, Nepal through WHO. These samples were tested for CCHF, KFD, Nipah (Real time RT-PCR and IgM ELISA), Rickettsia (Real time PCR, PCR and IgM ELISA), Hantan (RT-PCR and IgM ELISA), Chicken pox (PCR), Leptospirosis (Real time PCR and PCR), Measles, Rubella and Dengue (IgM ELISA).

Two samples were found to be positive for Rickettsia by IgM ELISA of which one was also tested positive for Measles by IgM ELISA. However, positivity with Measles IgM ELISA, *Rickettsia prowzaki* IgM ELISA, *Rickettsia conori* IgM ELISA and *Rickettsia typhi* IgM ELISA suggest cross reactivity, thus no conclusion could be drawn from these tests. Moreover, most of these kits are commercial kits where specificity and sensitivity is still not reliable.

In addition, 25 human serum samples of suspected hemorrhagic fever cases were also referred to NIV, Pune through WHO for NiV diagnosis. The samples were tested for Nipah virus by RT-PCR, IgM & IgG ELISA and all tested negative.

(iii) Diagnosis of suspected hemorrhagic fever samples referred from Bhutan through WHO

One hundred and thirty one human sera samples of suspected hemorrhagic fever cases were referred to NIV, Pune through WHO country office, Bhutan. The

samples were tested for Nipah virus IgM & IgG ELISA and found negative.

(iv) Diagnostic support and trainings for SEARO countries

Two kits each of CCHF IgG Sheep/Goat ELISA, CCHF IgG Cattle ELISA, CCHF Human IgM ELISA and KFD Human IgM ELISA kits were provided to Public Health Laboratory, Bhutan. Hands on training were also imparted to the staff of PHL for using the kits.

(v) Screening of suspected encephalitis samples referred from Dibrugarh, Assam

Human serum samples (n=183) of suspected encephalitis cases were referred to NIV Pune by RMRC, Dibrugarh for identification/confirmation of the etiologic agents. The samples were screened for anti-NiV IgM & IgG antibodies using CDC kit and 25 samples tested positive for IgM. Three (3/26) samples that showed OD above the cut-off for NiV, were falso ound positive for NiV RNA. However, virus could not be isolated from the sample.

Findings/summary

Nipah virus activity was detected in Dibrugarh and adjoining places in Assam

Detection of Kyasanur Forest Disease virus in tick population in forest area of Kerala

***Investigators**: PD Yadav, P Shil, R Balasubramanian & DT Mourya*

Funding agency: Intramural

Period: 2015-2016

Brief Background:

In view of the KFD positive human cases, KFD confirmed monkey deaths and IgG antibody positivity in Kerala State, it was essential to monitor the temporal and spatial risk of its expansion/spread to these areas. Therefore, this study was proposed to survey tick population in forest sites in Wayanad and Malapuram districts for KFD activity which would help in determining the cause of the recent upsurge and emergence of KFDV in these areas.

Work Objectives:

- Studies on tick distribution and relative abundance at selected forest sites in Wayanad and Mallapuram district
- To detect KFDV virus activity in ticks by PCR methods and virus isolation
- To generate spatial distribution map of host-seeking ticks present on vegetation
- KFDV E gene analysis to understand the introduction of KFDV in Kerala state

Work done:

To determine the prevalence of KFDV in ticks, 27 tick pools were collected from the KFD risk areas of Wayanad and Malappuram districts of Kerala. All the pools were homogenized using Geno-grinder, extracted RNA and tested for KFDV using Real time RT-PCR. One tick pool was tested positive for KFD viral RNA, which was sequenced with other recent KFDV isolates obtained from Karnataka and Kerala. The flavivirus E protein is the major immunogen for this genus of viruses and is also involved in cell receptor attachment and membrane fusion. The structural architecture of the viral E protein is more critical to proper viral function. Complete E gene was sequenced to predict B-cell epitopes and compared envelope glycoprotein (E proteins) of the recent ones with old strains of KFDV (KFDV E-protein sequences obtained from the NCBI database) using bioinformatics tools. Multiple sequence alignment was performed considering E-protein sequences from tick-borne encephalitis and Japanese encephalitis viruses as outliers. Pairwise sequence comparisons of the ectodomains for all the possible pairs in the data set were performed. Epitope predictions were carried out for selected strains using *in silico* techniques. The 3D structure prediction is predicted shown in Fig 5.

Findings/summary

Present study confirmed a new focus of KFDV activity among tribals in a reserve forest in the Malapuram district of Kerala, India. KFDV E protein analysis revealed that there were no drastic changes in the structure or antigenicity of the E proteins in the isolates from the recent outbreak.

MCL1407: 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**: DT Mourya, PD Yadav, GN Sapkal& DY Patil*

Funding agency: Extramural (ICMR)

Period: 2014-2017

Brief background:

Understanding the demand from different parts of country and also from the upcoming VRDL laboratories, production of positive and negative controls in the form of inactivated antigen required for diagnostic tests was initiated. This will be useful in patient management for providing early diagnosis at primary health care centers and in understanding the disease burden. Hence, this project is designed for the indigenous development of positive and negative inactivated virus controls to fulfill the demand of laboratories of India and Southeast Asian countries.

Objectives:

- Preparation of standard positive controls of viruses for molecular tests of public health importance.

Work done:

Protocols for positive and negative control production were standardized. Virus stocks (positive control, Quantity: 100 ml) and respective cell control (negative control, Quantity: 50 ml) will be prepared at VRDL and the stocks will be gamma irradiated for 6hr in liquid conditions at BSL-4 laboratory. One ml of the virus stock will be tested at VRDL for gamma inactivation confirmation by standard procedure. After confirmation, the positive control and negative control stock will be aliquoted, frozen/lyophilized at BSL-4 laboratory and proper inventory will be maintained. Virus controls for CHIKV, JEV and WNV were prepared. Preparation of virus controls for Rubella, Measles and Mumps are in progress.

Findings/summary

SOP for preparation of virus controls have been standardized and stocks of CHIKV, JEV and WNV were prepared.

Laboratory diagnostic services on referred samples

The number of samples tested in the BSL-4 laboratory and the results for different viruses are given in Fig 5-8.

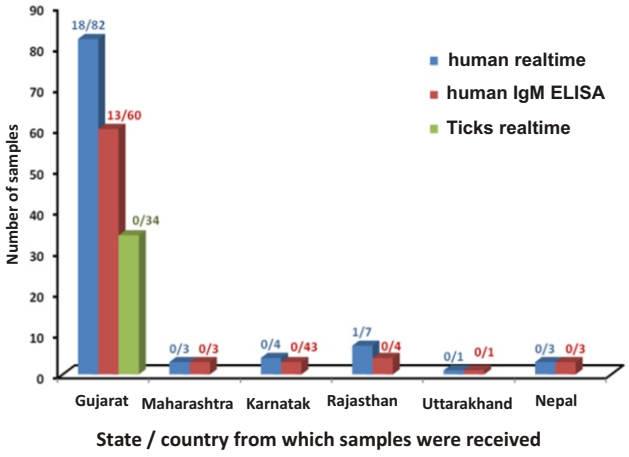


Fig 5: CCHF virus diagnosis

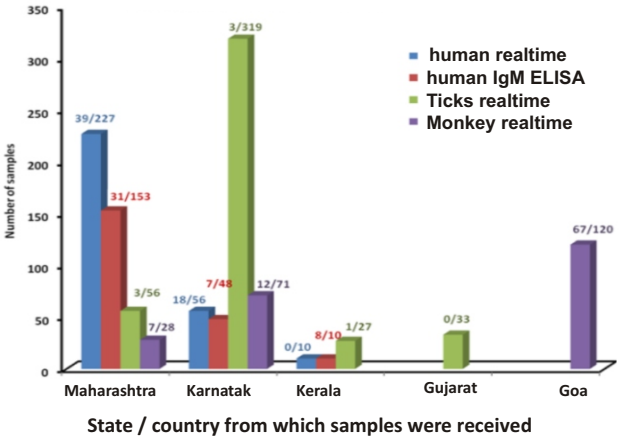


Fig 6: KFD virus diagnosis

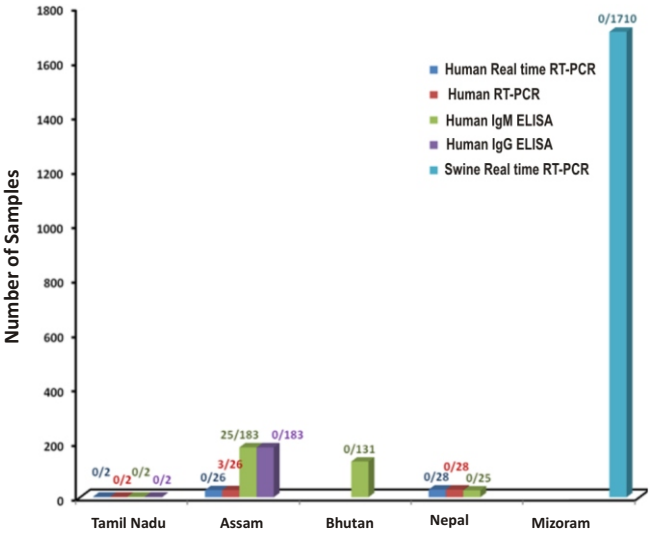


Fig 7: Nipah virus diagnosis

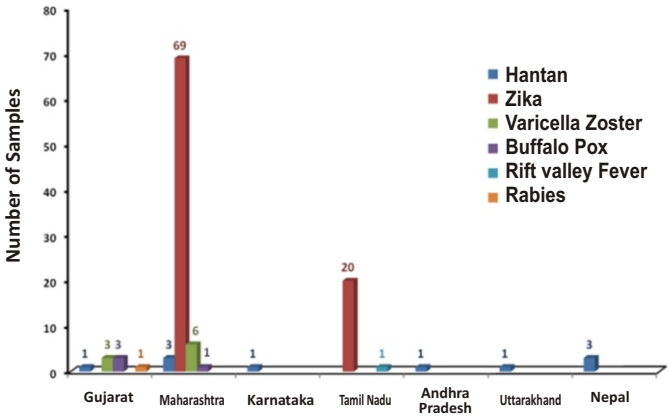


Fig 8: Diagnosis of other pathogenic viruses

INFLUENZA GROUP

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INF1001: Diagnostic Services/Outbreak Investigation

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

Funding Agency: Intramural

Project duration: operational on-going diagnostic services

Work done

Screening of 2606 clinical samples referred by Maharashtra State health authorities for Influenza virus diagnosis showed 19% positivity (497/2606) for A(H1N1)pdm09; 1.3% for A(H3N2) and 2.1% for Type B influenza virus by real time RT-PCR (Fig 1). Attempts were made to isolate virus from 93 samples in MDCK cell line and 52 yielded isolates [46 A(H1N1)pdm09 and 6 Type B Yamagata].

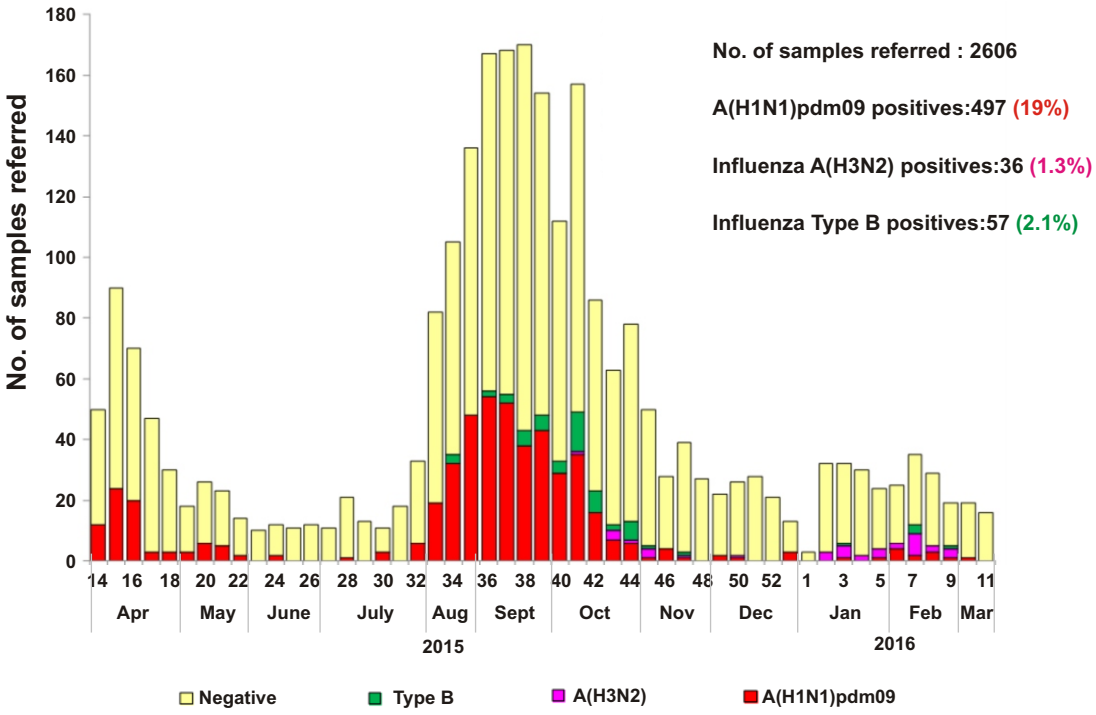

Fig 1: Influenza activity in referred clinical samples 2015-16

Genetic Analysis:

Phylogenetic analysis of 80 HA gene and whole genome analysis of 8 A(H1N1)pdm09 isolates were carried out and were found similar to A/California/07/2009, 2009-2015 vaccine component (Fig. 2). All the virus isolates of 2015 grouped in 6B clade with K163Q, A256T signature mutation. Whole genome analysis did not show any pathogenic markers.

INF1003: Epidemiological and virological monitoring of human Influenza virus infections

***Investigators**: MS Chadha & VA Potdar*

Duration: Ongoing activity, reporting period 2015-16

Funding Agency: Intramural

Five hundred and forty-two specimens collected from influenza like illness (ILI) patients attending various dispensary/hospital outpatient departments in Pune were screened for Influenza viruses and positivity was detected in 33 samples (A(H1N1)pdm09=19; A(H3N2) =4; type B=10) as shown in Fig 3. Virus isolation attempts yielded 4 isolates from 4 PCR positive samples (3-A(H1N1)pdm09, 1-Type B(Y)) in MDCK cell line.

Thirty nine (A (H1N1) pdm09 (n=33), A(H3N2) (n=2)& type B (n=4))isolates and 183 clinical samples

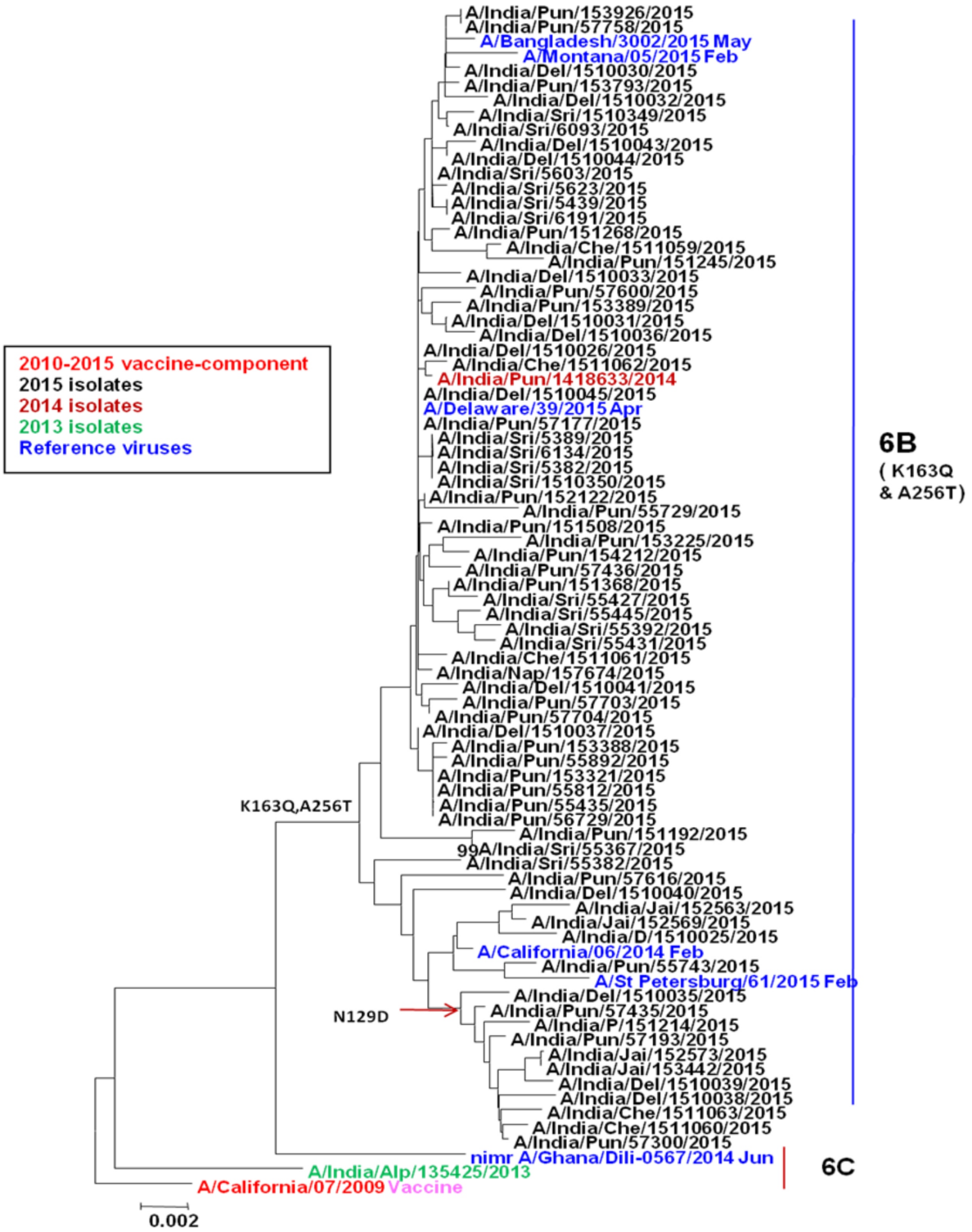

Fig 2: Phylogenetic analysis of Influenza A (H1N1)pdm 09 HA gene 2015-16 (n=80)

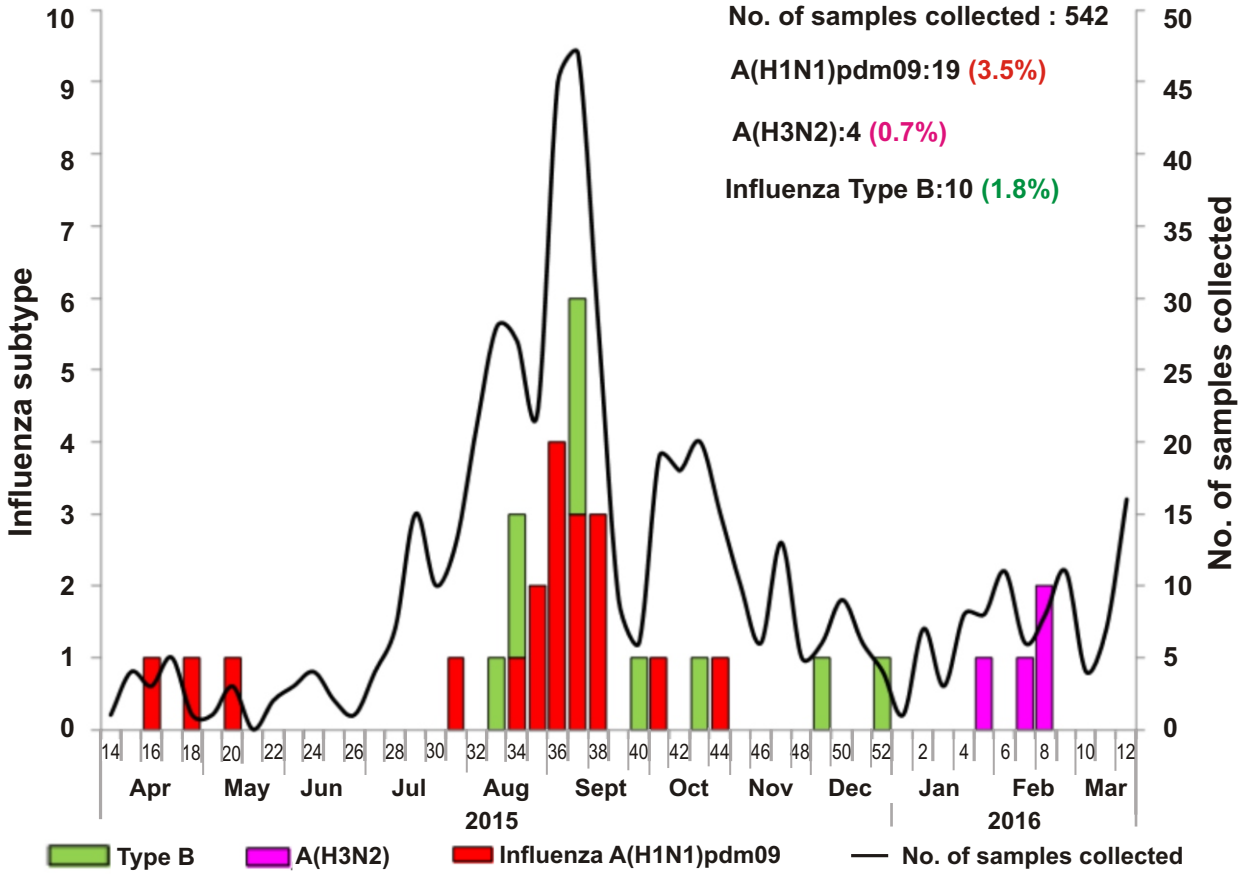

Fig 3: Influenza virus activity in ILI cases, 2015-16

were received from regional centers for reconfirmation. All the samples were concordant in real time RT-PCR. Representative isolates were processed for isolation and 13 were found concordant.

Out of 183 real time PCR positive clinical samples received from Srinagar, 25 samples were processed for virus isolation and 10 (9- H1N1pdm09) and 1- A(H3N2) yielded isolation. The real time PCR results were concordant.

Contribution to Global Influenza Network

Virological data for 15657 samples (3629 A(H1N1)pdm09, 39 A(H3N2) and 137 type B) from the influenza network was submitted to Global Influenza Surveillance and Response System (FluNet). Fourteen isolates were submitted to the collaborating centre at CDC.

WHO External Quality Assessment Program (EQAP)

WHO EQA Panel No 14 containing 10 vials of Triton X-100 inactivated virus were received by NIV in April 2015. All the samples were tested for influenza A(H3), Influenza A(H5), Influenza A(H1)pdm09, influenza B and other less commonly recognized Influenza A viruses. All the results were found concordant. For regional labs, QA/QC panel for isolation and real time PCR were sent and the results were found concordant for both.

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

***Investigators**: MS Chadha & VA Potdar*

Duration: 2013-16

Funding: Intramural

Brief background

Neuraminidase inhibitor (NAI) is effective against type A & type B influenza viruses. Substitution at amino acid H274Y in N1 subtype is known to occur clinically and cause clinical resistance. Depending on the NA subtype {N1:D199N,I223R,N295S, N2: E119V,R292K,N294S,Type B: R150K, D197E/N, I 221T, N294S, G407S}amino acid substitutions are

known to occur leading to reduced susceptibility *in-vitro*, however, their clinical impact is unknown.

Objectives

- Assessing 495 influenza samples/isolates by allelic discrimination for H275Y (A (H1N1)pdm09 positive clinical samples and 33 isolates from the above A (H1N1)pdm09 positives)

Findings

Three isolates showed amplification with 275Y allele, which is marker for resistance. Resistance to NAI is not a common finding in representative influenza A (H1N1) pdm09 isolates during the period 2015-16 from India.

INF12011: Detection of respiratory viruses among children (<5yr) hospitalized for pneumonia

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

Duration: 2014-2016

Funding Agency: Intramural

Background

Pneumonia affects the lungs and is the single largest cause of deaths among children worldwide. Respiratory viral infections are implicated in approx. 50% of community acquired pneumonia (CAP) in young children. Every year pneumonia kills an estimated 1.4 million children under the age of five years, accounting for 18% of all deaths of children under five years old worldwide (WHO Aug 2012).

Work done

Samples collected (April 2015 to 31 March 2016) from pneumonia patients (n=424; 280 males and 144 females) were screened by real time RT-PCR for Influenza A/H1N1pdm09, A/H3N2, Influenza B, Respiratory syncytial virus, Metapneumovirus, (HMPV). Parainfluenza virus 1-4, Rhinovirus and Adenoviruses.

Findings

Of the 424 samples, 337 (79%) tested positive for different viruses *viz.,* RSV A&B: 176 (42%); Rhinovirus: 69 (16.2%); influenza A/H1N1pdm09: 13 (3%); Adenovirus: 20 (4.7%); parainfluenza: 26

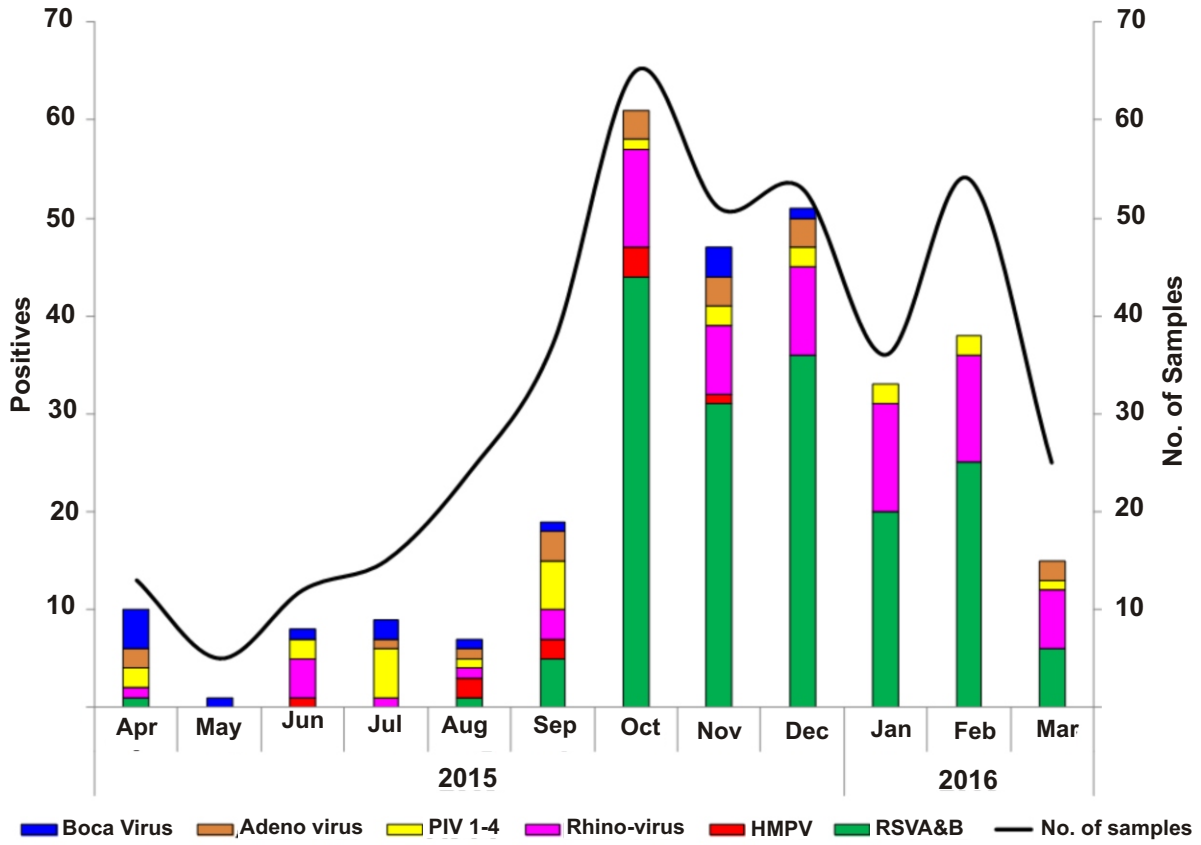

Figure 4: Isolation of respiratory viruses from hospitalized children below 5 years of age with pneumonia

(16.4%), HMPV: 9 (2.1%) and Bocavirus: 20 (4.7%)(Fig 4). Co-infection was seen in 53 samples.

Summary

This study provides information on detection of viruses in pediatric patients with pneumonia. This information will be crucial for making effective policies and planning interventions.

INF1202: Immunogenetics of severe pandemic H1N1pdm09 infections

Investigators**:** *ML Choudhary, K Alagarasu & MS Chadha*

Duration: 2013 -2016

Funding Agency- Intramural

Background

Susceptibility severe influenza A/H1N1pdm09 virus is multi-factorial involving pathogen, host and environmental factors.

Objectives

To determine whether single nucleotide polymorphisms (SNP) in the genes coding for pattern recognition receptors, antiviral response genes, cytokine and chemokine genes are associated with H1N1pdm09 virus severity.

Work done

Blood from 91 severe and 109 mild patients were collected, genomic DNA extracted and studied single nucleotide polymorphisms using PCR or PCR RLFP based methods. Genotyping of SNPs for different genes *viz*., TNFα, IL-10, IFNγ, CD209, IFITM3, MBL are in progress.

Summary

The preliminary data obtained during 2015-16 is under analysis. The proposal is under final review process with DBT.

INF1501: Creating laboratory network for enhancing diagnostic capabilities for surveillance, outbreaks and epidemic investigations of high-risk group of viral pathogens causing respiratory infections

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

Duration: 2015-2020

Funding Agency: CDC, USA

The main aim is to continue the established epidemiological and virological influenza surveillance network and develop capacity for diagnosis of non-influenza respiratory viruses, *viz.*, RSV, human metapneumovirus (hMPV), parainfluenza virus 1-4 (PIV), adenoviruses and rhinovirus in different geographical areas of India. Under the project, six regional centres were established with NIV as the apex institute. Various respiratory virus study protocols, case definition, clinical proforma, test protocol, SOPs and consent forms were developed.

Objective for 2015-16

- During the reporting year the documentation and planning of the project was completed.

Conclusions

The project activities will commence in 2016 and will be reported in next reporting year.

AVIAN INFLUENZA GROUP

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Mr. Anil L. Thormothe	Technician (till 24.06.2015)

AVI 1301: Surveys of avian influenza in wild birds during the winter migratory season, 2013 to 2016 in Maharashtra State

***Investigators**: SD Pawar, S Pande & J Mullick*

Duration: 2013-2016

Funding agency: Intramural

Background

As part of avian influenza (AI) surveillance, NIV, in collaboration with the state forest department and Ela foundation, Pune, conducted ringing and tagging of wild and migratory birds to study their movement, longevity and site fidelity. Birds that were tagged included painted storks, grey herons, black-headed ibis, little cormorants and greater flamingos. These birds were screened and found negative for avian influenza virus/antibodies. A ring was put on their left leg, while their wings were tagged with red-coloured rexine material for spotting from a distance. Each bird was also given a unique identification number.

Findings

In December 2015, two years after tagging, a painted stork returned to the Bhadalwadi irrigation tank in Pune's Indapur tehsil (Fig 1).

The birds were tagged as part of a bird flu monitoring project

Fig 1: Ring recovery of painted storks, which were tagged during AI surveillance in 2014

The stork is one of the 18 endangered and near-threatened species. Two black-headed ibis, also tagged during the exercise, were sighted at the same location. These are the first recorded re-sightings of ringed and wing-tagged painted stork and black-headed ibis from Bhigwan.

Significance

It has been shown that wild and migratory birds play an important role in transmission of avian influenza viruses. India reported outbreaks of high pathogenic avian influenza H5N1 viruses in domestic, wild, migratory birds and poultry. The present study showed that wild and migratory birds came back to the same location in Maharashtra after two years. This showed that in the current scenario of emerging influenza viruses, such studies are useful to understand transmission of avian influenza viruses.

A novel virus precipitation method for detection of avian influenza viruses from environmental samples and its possible application in outbreak investigations

***Investigators**: SD Pawar, SS Keng, AL Thormothe, GN Sapkal, B Anukumar, KS Lole, J Mullick & DT Mourya*

Duration: 2015-2016

Funding: Intramural

Background

In view of the emerging influenza viruses, detection of AI viruses during outbreak investigations and surveillance is utmost necessary. Wild, migratory birds and environmental samples such as droppings of birds and water samples where aquatic migratory birds reside play an important role in virus transmission. Though there are a few methods for AI virus detection from water, they are expensive, cumbersome and time-consuming. The present study reports findings of a novel method for the detection of AI viruses from water.

Objective / Word done

- To develop a novel method for detecting influenza viruses from environmental water samples and to check their viability *in-vitro*.

Tap water from the Municipal Corporation water supply was spiked with known quantity of high pathogenic AI H5N1 and low pathogenic AI H5N1, H9N2, H11N1 and H4N6 viruses. Viruses were then concentrated by precipitation using in-house reagents and detected by real-time RT-PCR. Fresh water from dams and saline water from Arabian Sea were used for spiking with H5N1 virus. Additionally, poultry drinking water (PDW) samples from live poultry markets were also tested. The viability of the virus in the seawater and the precipitate thus obtained was also tested by virus isolation.

Findings

The minimum level of detection was 10 infectious particles (I.P.)/ml for H5N1 virus and 50 I.P./ml for H9N2, H11N1, and H4N6 viruses from fresh tap water, while the minimum level of detection for H5N1 was 10 I.P./ml and 100 I.P./ml from dam water and sea water respectively. A minimum of 260 RNA copies of the H5N1 virus was detected. The virus was re-isolated from seawater and showed its viability. H9N2 viruses were detected from PDW samples from poultry markets, indicating the utility of this method on field.

Summary

In conclusion, the present method is a simple, sensitive, user-friendly and cost-effective procedure, which has potential application for detection of AI viruses from different water sources and other environmental samples during outbreak investigations. The method can also be a good tool for routine surveillance studies.

AVI1302: Operation and maintenance of BSL3 laboratory.

***Investigators**: J Mullick & SD Pawar*

Duration: On-going operational program

Funding: Intramural

Providing safe environment while working with highly infectious agents in the High Containment laboratory also known as Biosafety Level 3 (BSL-3) laboratory is a commitment. We are constantly working to provide uninterrupted functioning for the workers in the laboratory by carrying out routine maintenance. In this tenure, the class II B2 biosafety cabinets were replaced with Class II A2 biosafety cabinets in the lab side and the animal side. The filters of Individually Ventilated Cages, which are used for small animal experiments with infectious agents were replaced. During this period, mice infection experiments with high pathogenic avian influenza virus, pandemic influenza virus and vaccinia virus were conducted. In addition, support was provided to the Hepatitis group for conducting primate experiments with human Parvovirus 4 agent. As part of the M.Sc. Virology course, we are also providing appropriate trainings to the students as a human resource building capacity.

AVI1101: Studies on host-virus interactions of low-pathogenic and high-pathogenic avian influenza viruses.

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

Duration: 2012-2017

Funding agency: Intramural

Background

AI viruses are broadly classified as low pathogenic (LPAI) and high pathogenic (HPAI) viruses primarily on the basis of pathogenesis. Among the many factors responsible for the successful adaptation in the host, one is the ability of the virus to escape the host innate immune response. India has witnessed outbreaks of the high pathogenic H5N1 strains since 2006. The H5N1 virus in the current study caused focal poultry outbreak in Manipur, India in 2007 belonging to the clade 2.2 and thought to have been introduced independently in the country through migratory birds. Further, surveillance studies conducted in the winter migratory season during 2007-2008 resulted in the isolation of a low pathogenic avian influenza H11N1 virus from a migratory bird, a Eurasian-American reassortant strain with the NP and M segments having identity to the H5N1 virus.

Objective

- To characterize the innate immune response of avian influenza viruses in the human lung epithelial cell line (A549).

Findings

Growth kinetics of these viruses showed that H5N1 virus is more virulent than H11N1 in the A549 cells (Fig 2). Gene expression profiling of the innate immune response related genes using real time RT-PCR has shown that H5N1 inhibits the host innate immune response more potently than H11N1. Non-structural 1 (NS1) protein of influenza virus is known to be an important viral protein against the host innate immune response. Sequence alignment and 3D structure prediction and comparison (using Bioinformatics tools) of the NS1 proteins from the two strains revealed nine differences in the amino-acid composition across the effector domain. This prompted us to investigate the ability of NS1 of the

respective viruses in inhibiting the IFN α. Surprisingly, NS1 of H11N1 showed stronger inhibition of IFN α than the NS1 of H5N1, suggesting that the observed higher inhibition of IFN α seen with the H5N1 virus compared with the H11N1 virus ought to be due to other viral proteins. Further studies governing the differential interferon response by these two viruses are underway.

Summary

Our results show that H5N1 virus replicated faster and inhibited the host innate immune response more potently than H11N1 virus. The ability of NS1 to inhibit the host IFN response was however different, which possibly could be due to differences in the amino acid composition that probably bring about localized changes in the surface contour, affecting interaction with other proteins. Together, our studies script for the possible involvement of other viral proteins supplementing NS1 towards the differential interferon response to H5N1 virus and potent NS1 of the H11N1 virus, warranting further structural and protein-interacting studies.

AVI1008: Studies on the inflammatory mediators in osteoclast differentiation.

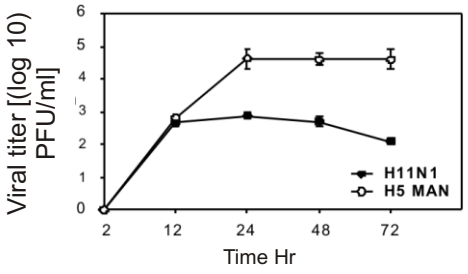
***Investigators**: J Mullick, R Kasbe, MR Wani (NCCS), AB Sudeep & AS Tripathy*

Duration: 2011-2016

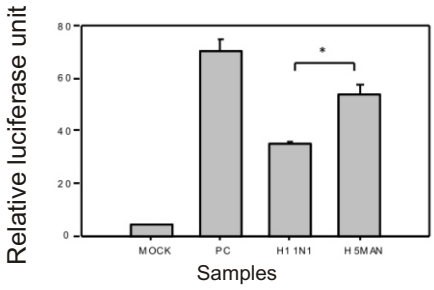
Funding: Intramural

Background

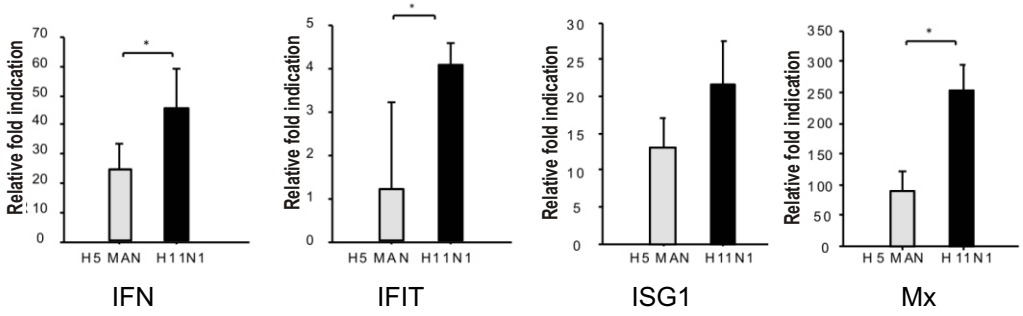
Inflammatory mediators such as interleukins, interferons and complement anaphylatoxins are known to be triggered during viral infections. There are enough evidences of the involvement of complement anaphylatoxins C3a and C5a in dengue virus and Ross River virus (RRV) infection with the later affecting the bone and resulting in severe monocytic inflammation of the bone, joints and skeletal muscle tissues. In addition to the primary role of complement in innate immunity it is also known to play a major role in differentiation. Chikungunya virus (CHIKV), also an arthritogenic virus belonging to the same genus (Alphavirus) as RRV and there exists enough basis to believe that CHIKV also might be modulated by complement.



A. Replication Kinetics of AI viruses



B. NS1 transfection



C. Gene expression analysis

Fig 2: Replication kinetics and gene expression analysis of HPAI & LPAI viruses

Objective

- One of the objectives of the project is to determine whether similar to RRV infection, complement imparts any role in CHIKV infection and to determine its effect in osteoclast differentiation and progression of virus-induced polyarthritis.

Findings

Our earlier results have shown the involvement of the complement pathways in osteoclast differentiation. During this period we have looked into the *in vivo* role of complement in CHIKV infected individuals. We have tested the plasma C3a and plasma C5a levels in the acute CHIK patients by ELISA.

Summary

Our results show significant difference in C3a levels but not C5a levels in acute CHIK patient samples compared to the controls (n=15). Further studies are in progress.

Laboratory Comparison of Influenza Micro-neutralization Assays for A(H1N1)pdm09, A(H3N2) and A(H5N1) Influenza Viruses

***Investigators**: SD Pawar & SS Kode*

Duration: 2015-2016

Funding: Consortium for the Standardization of Influenza Seroepidemiology (CONSISE)

Background

Microneutralization (MN) assay is used to detect antibodies to influenza virus. A laboratory comparison of the 2-day ELISA and 3-day haemagglutination (HA) MN protocols, using A(H1N1)pdm09, A(H3N2) and A(H5N1) viruses, was performed by the CONSISE Laboratory Working Group.

Findings

We standardized both the assays and performed the assay protocols on multiple occasions using different serum panels. Thirteen laboratories from around the world participated. Within each laboratory, serum sample titers for the different assay protocols were compared between assays to determine the sensitivity of each assay and were compared between replicates to assess the reproducibility of each protocol for each laboratory.

Summary

There was good correlation of the results obtained using the two assay protocols in most of the laboratories, indicating that these assays may be interchangeable for detecting antibodies to influenza A viruses included in the study. These assays would be useful to study human-animal interface.

Comparability of neuraminidase inhibition antibody titers measured by enzyme-linked lectin assay (ELLA) for the analysis of influenza vaccine immunogenicity.

***Investigators**: SD Pawar & SS Kode*

Duration: 2015-2016

Funding: Intramural

Background

Neuraminidase-inhibition (NI) antibody titers can be used to evaluate the immunogenicity of inactivated influenza vaccines. We were part of a study conducted through the CONSISE to evaluate the variability of the ELLA.

Findings

We standardized ELLA assay in our laboratory as per CONSISE protocols. NI antibody titers of a set of 12 samples were measured against both N1 and N2 neuraminidase antigens in 3 independent assays. For a sample repeated in the same assay, e”96% of N1 and N2 assays had less than 4-fold difference in titer. Comparison of the titers measured in assays conducted on 3 different days showed that a four-fold difference in titer was uncommon. Titers of the same sera measured in different laboratories spanned 3 to 6 two-fold dilutions (*i.e.,* 8-64 fold difference in titer), with an average percent geometric coefficient of variation (%GCV) of 112 and 82% against N1 and N2 antigens, respectively.

Summary

This study identified background signal and the amount of antigen in the assay as critical factors that influence titer, providing important information towards development of a consensus ELLA protocol.

Surveillance for Avian Influenza H7N9 virus in humans

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

Duration: 2015-2016

Funding: ICMR & Supplemental Funding from CDC, Atlanta, USA under Cooperative Agreement No 5U51IP000333CDC; 2013-2014

Background

An emergence of a novel avian-origin reassortant influenza A (H7N9) virus in eastern China has caused an alarming situation due to severe lower respiratory tract infections in humans. As of October 2, 2014, a total of 453 human infections of H7N9 (resulting in 175 deaths) in China have been reported. Human infections of H7N9 virus have been associated with poultry exposures in live bird markets, although H7N9 virus is not easily transmitted to humans.

Objectives

- Considering the proximity of Northeastern regions of India with China and likelihood of introduction of this virus, the present proactive exploratory study was conducted to detect AI H7N9 virus infections among poultry workers as a high-risk group in Western and North-Eastern India during 2014.

Findings

AI H7N9 virus infections among poultry workers at live poultry markets and farms in Pune (Western India), West Bengal and Assam (North-Eastern India) was planned and executed with the approval and support of the local Municipal and State health departments. The areas from Western and Northeastern states of India were selected as outbreaks of AI have been reported from these states. Also the Northeastern states have close proximity to Southeast-Asian countries and China. The study was approved by the Institutional Human Ethics Committee of the National Institute of Virology using guidelines laid down by the Indian Council of Medical Research for Research on Human Subjects. Written informed consent was obtained from individual study participants.

Five hundred and forty poultry workers were enrolled in the study during January to November 2014. The age group of poultry workers ranged from 15-79 years with the median of 30 years. The samples were collected from Pimpri-Chinchwad Municipal Corporation and Pune Municipal Corporation areas in Pune; Siliguri, Alipurduar, Dhupguri-Jalpaiguri, Kalimpong-Darjeeling, West-Bengal and Dibrugarh, Assam. Serum samples were tested by haemagglutination inhibition (HI) and MN assays as per WHO recommended protocols for the detection of antibodies against H7N9 virus. Twenty eight throat and nasal swab samples were collected from individuals with ILI and tested for influenza A virus using Real-Time RT-PCR.

Summary

All the samples tested negative for influenza A virus by real-time RT-PCR. The samples were also negative for antibodies against H7N9 virus by both HI and MN assays.

AVI1006: Role of complement during Influenza infection.

***Investigators**: A Sahu, A Anujrattan, SD Pawar & J Mullick*

Duration: 2010-2015

Funding: Intramural

Background

The pandemic influenza A(H1N1) 2009 virus has caused significant morbidity and mortality worldwide emphasizing the need to study the host factors influencing its control. Earlier the complement system has been shown to provide protection during the seasonal influenza virus infection though the role of the individual complement pathways in coping with influenza was not studied. Earlier we have shown that: i) the pandemic influenza A(H1N1) 2009 virus is susceptible to the classical pathway (CP), but not to the alternative pathway (AP) unless coated by antibodies; ii) both the pathways play important roles in controlling the *in vivo* infection as revealed by infection studies in various complement knockout mice (C3-/-, C4-/-& FB-/-); iii) infection was more severe in C3-/-mice, which was evident from more virus load in the C3-/-mice; iv) blocking of C3a and/or C5a receptor signaling in WT infected mice using receptor antagonists resulted in significant mortality and v) passive antibody administration

increased the survival in C3-/- infected mice.

Objective

- To determine role of the local complement in influenza infection.

Findings

During this tenure in this NCCS-NIV collaborative project adoptive transfer studies were performed wherein, naïve splenocytes from WT mice or C3-/- mice were transferred to C3-/- mice, challenged with the virus and monitored for weight loss and survival. Results showed that adoptive transfer of WT splenocytes in C3-/- mice increased their survival proving the role of the local complement in controlling the influenza infection (Fig 3). Our results indicate that cooperation between the CP and AP result in efficient direct neutralization of the pandemic influenza virus.

Summary

Our data reveal the importance of cross talk between the CP and AP that provides sufficient trigger (C3b deposition and C3a/C5a production) required for efficient protection against the pandemic influenza virus infection.

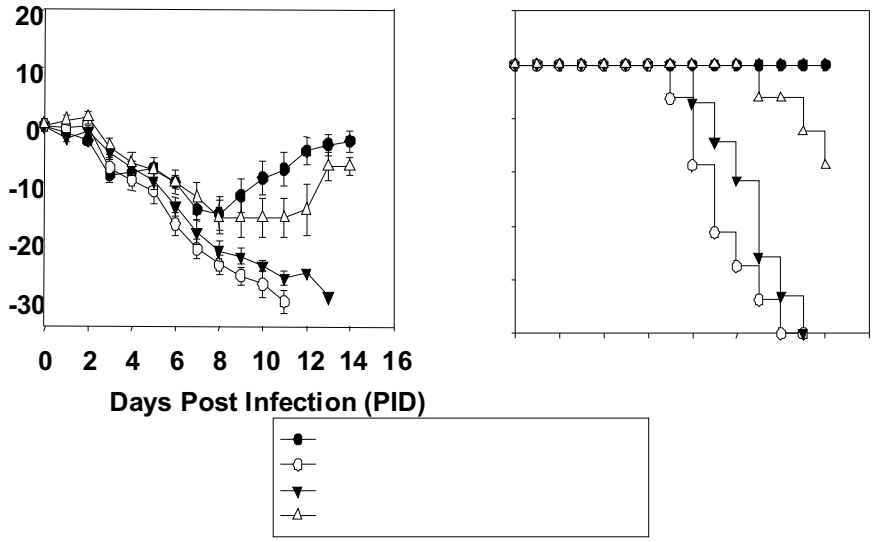


Fig 3: Role of local complement in controlling influenza infection. Adoptive transfer of naive splenocytes from WT or C3-/- mice to C3-/- mice, followed by intranasal challenge with 450 $TCID_{50}$ of the virus. Body weight and survival of the mice was monitored for 14 days post-infection.

HEPATITIS GROUP

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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.

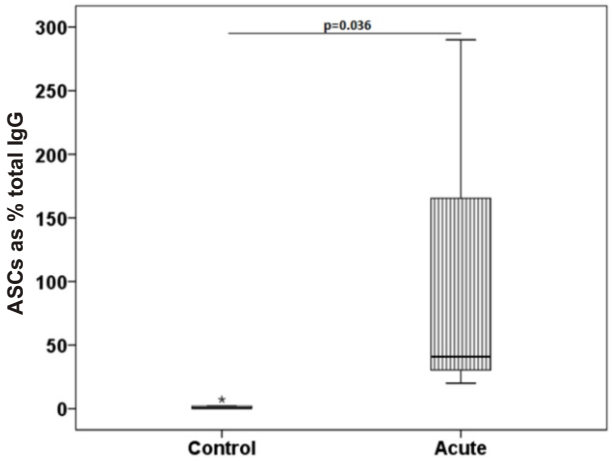

Fig 1: HEV-specific memory B cells response by ELISPOT.

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

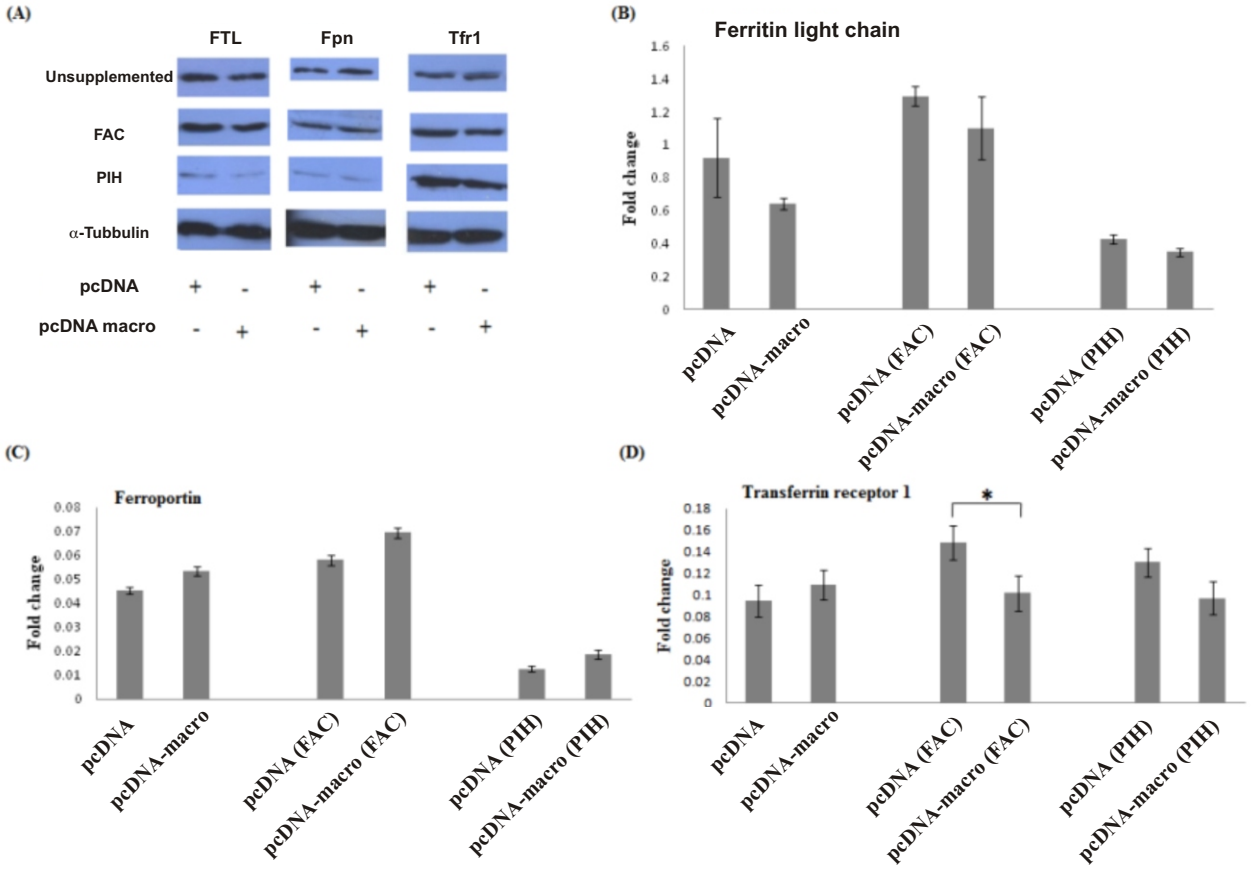

Fig 2: HEV macro domain does not alter expression of iron metabolism proteins (A) immunoblot for ferritin light chain, ferroportin and transferrin receptor 1. Cells were treated with either 100 µM ferric ammonium citrate (FAC) or 100 µM Pyridoxalisonicotinoylhydrazone (PIH) to increase or deplete labile 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

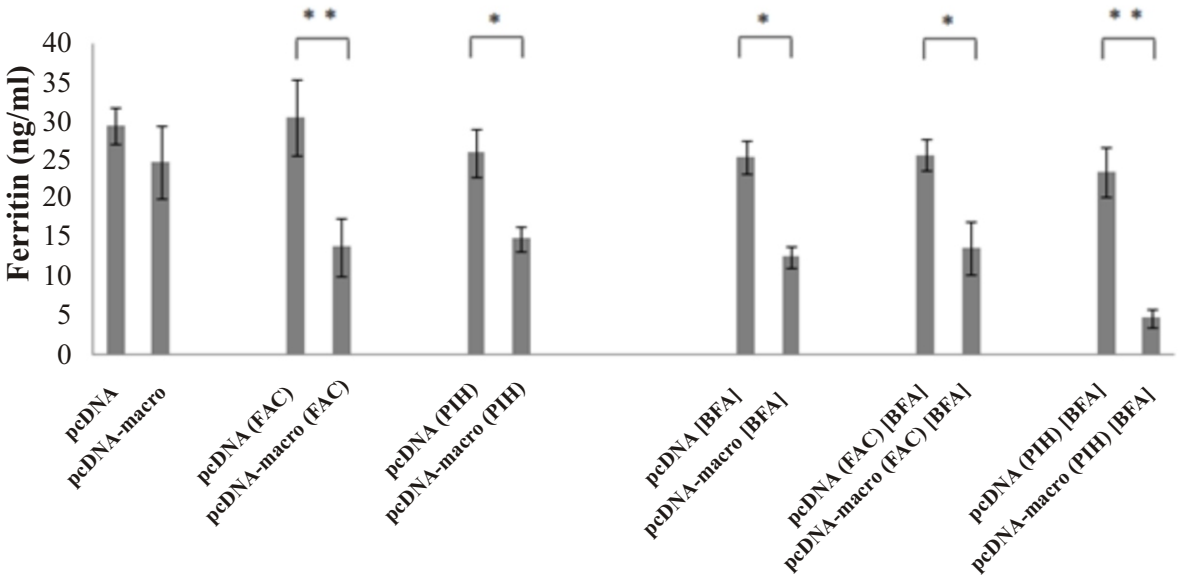

Fig 3. Measurement of ferritin secretion in the presence of HEV macro domain

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	-	+	+	+	+	+

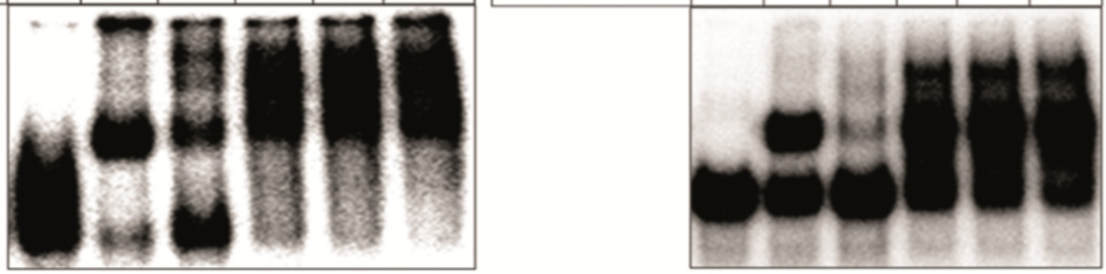

Fig 4: Competition binding assays using 3PS and SG5096NS (SgP) templates (A) Competition between 10nM α-32P-labeled 3PS and 10, 500, 1000, 2000 nm cold SG5096NS SG5096NS templates (B) Competition between 10nM α-32P-labeled SG5096NS and 10, 500, 1000, 2000 nM cold 3PS templates

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

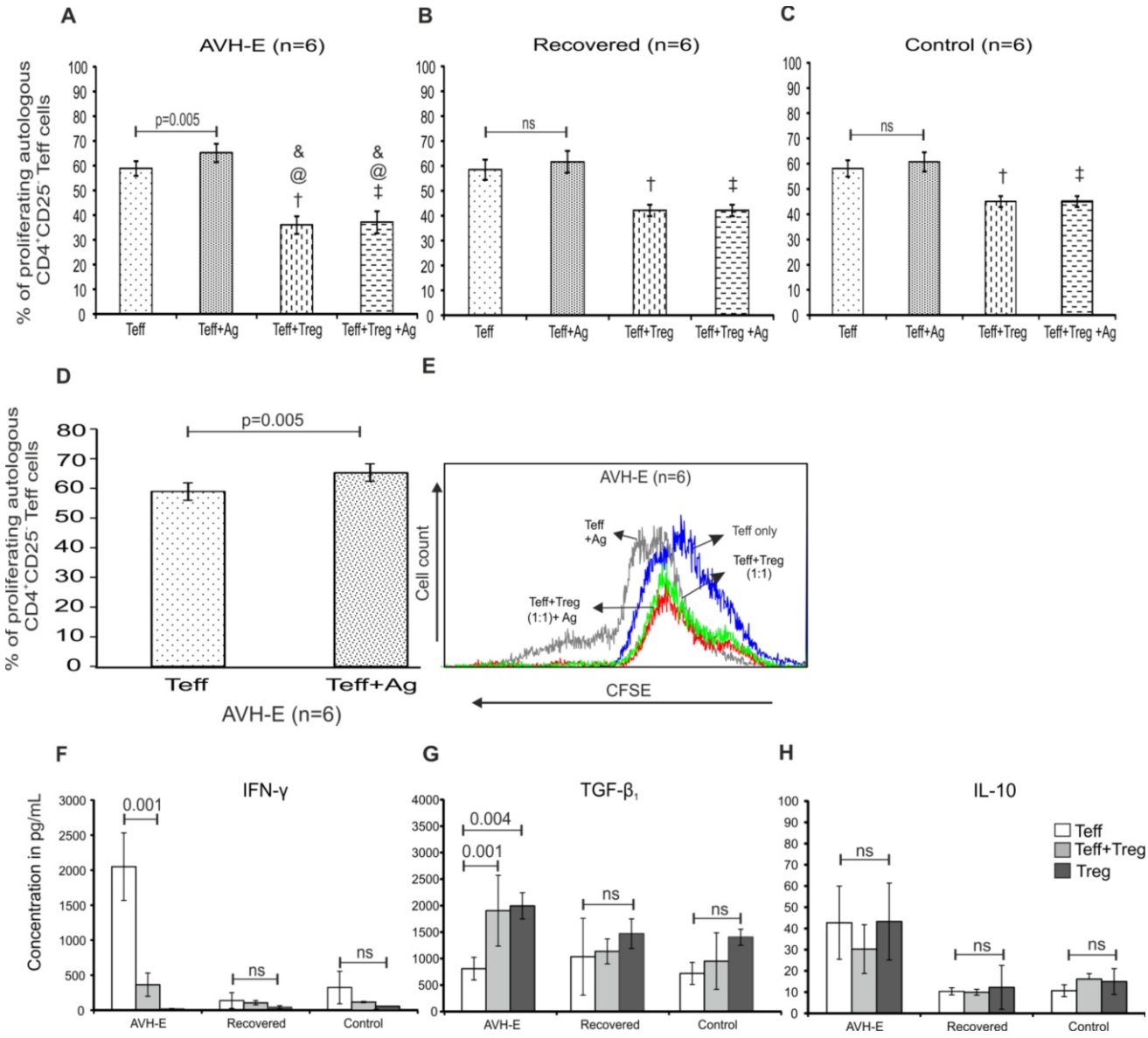

Fig 5. HEV rORF2p specific functional assay and key cytokines in supernatant of functional assay

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.

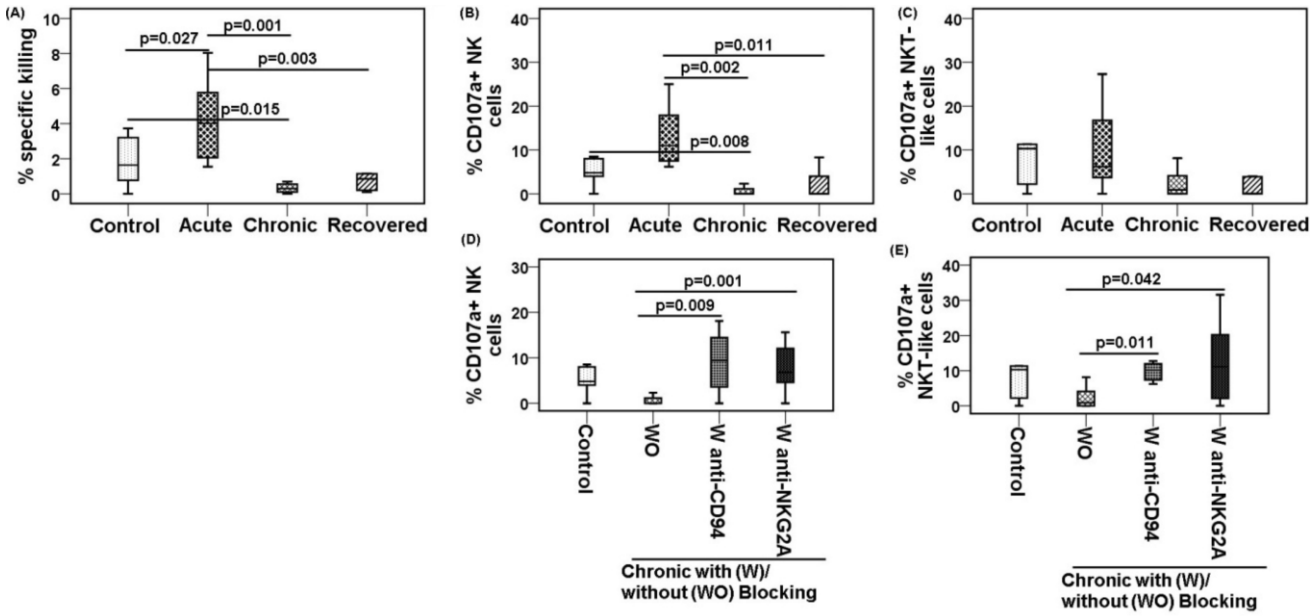

Fig 6: Cytotoxic and degranulation potential of NK/NKT-like cells during chikungunya against target cells (K562). PBMCs (effector) were isolated from whole blood of 12 chronic patients and 12 individuals recovered from chikungunya and were co-cultured with K562 (target) cells at effector/target ratio 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	–

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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.

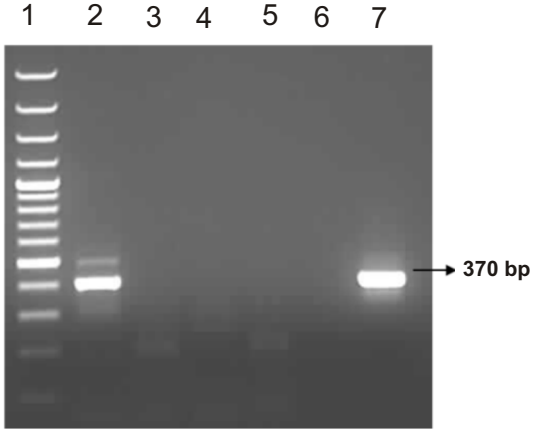


Fig 1: CHPV diagnostic RT-PCR of representative sandfly pools from Panchmahal

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,

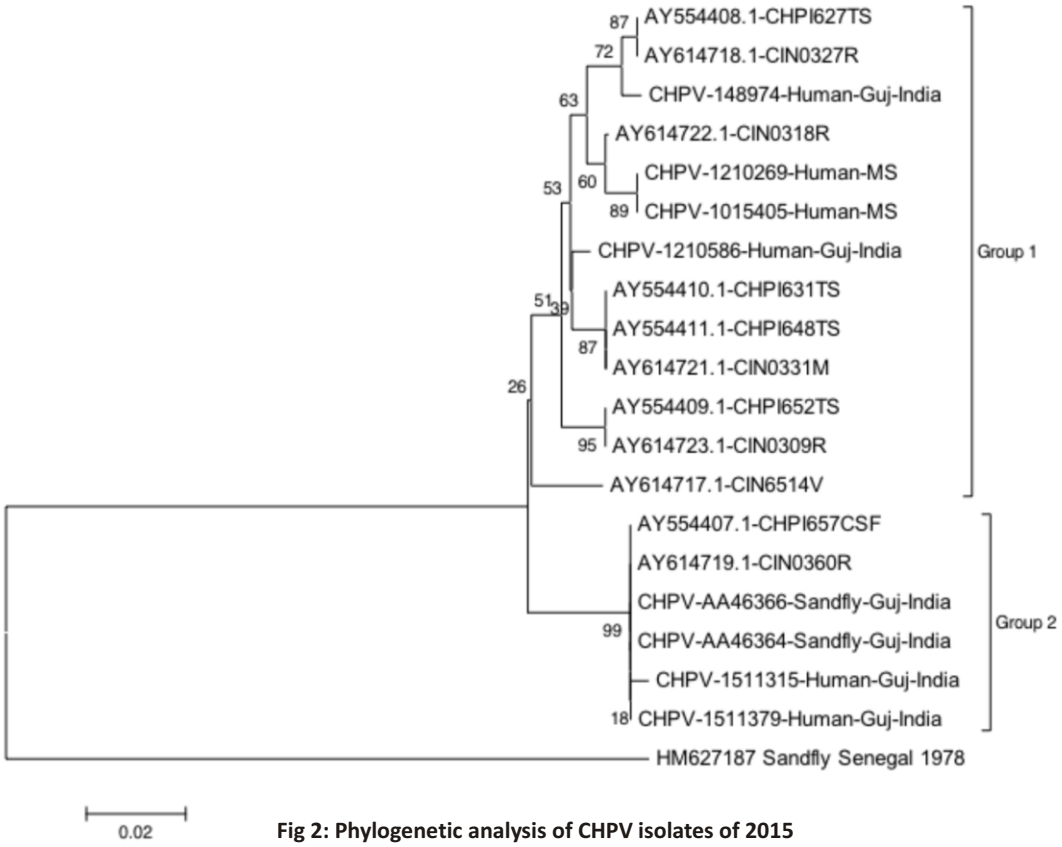


Fig 2: Phylogenetic analysis of CHPV isolates of 2015

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).

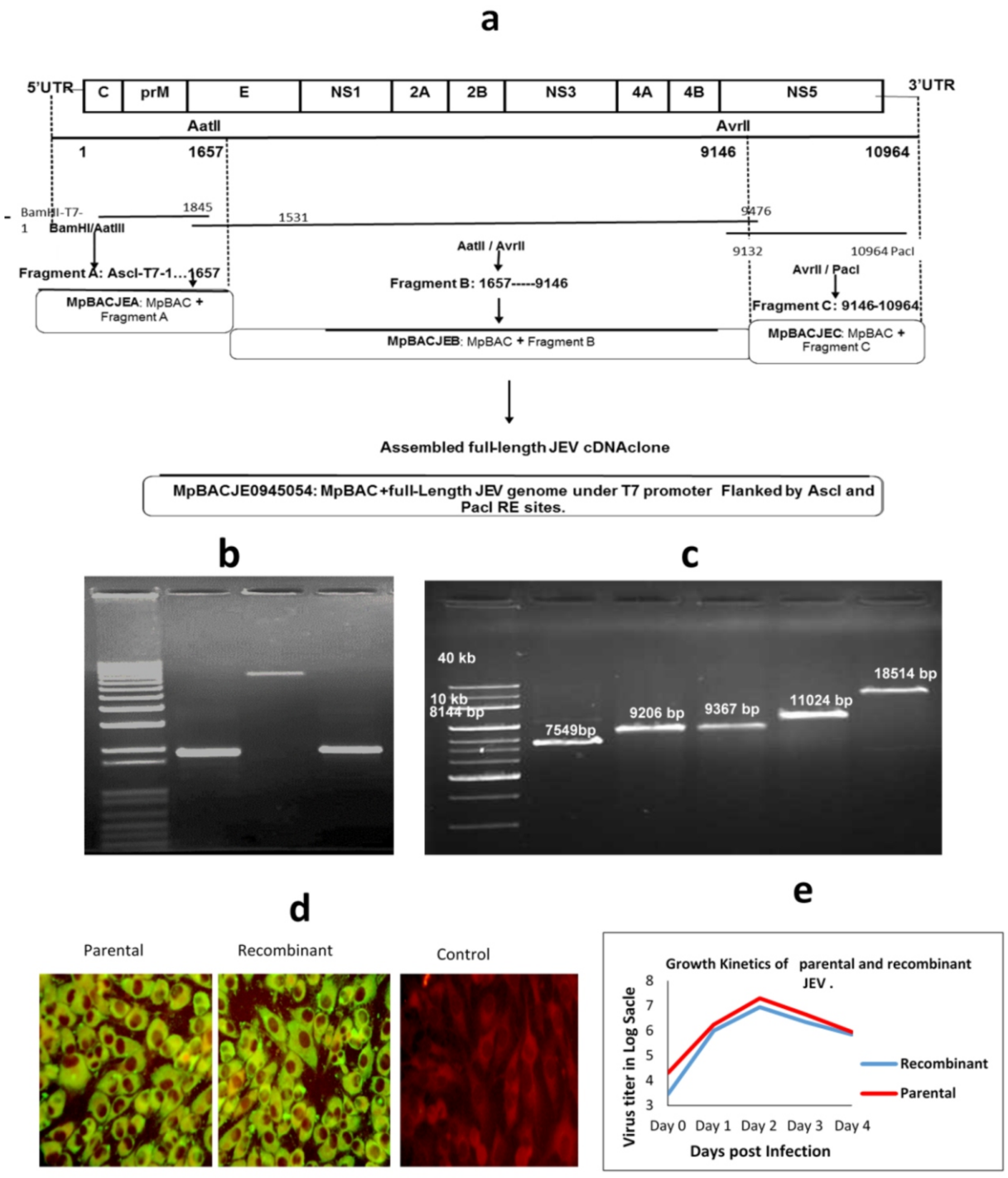


Fig. 3: a) Cloning strategy; b) Amplification of JEV genome: Lane 1: 1 Kb plus DNA ladder, Lane 2 –JEV 2-1845 (1845bp), Lane 3: 1531-9476 (7945bp) and Lane 4: 9132-10964 (1849bp); c) Construction of JEV cDNA clone in BAC vector, Lane 1- 1 Kb Extension ladder, Lane 2- Modified pBeloBAC11, Lane 3-MpBAC+ 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)

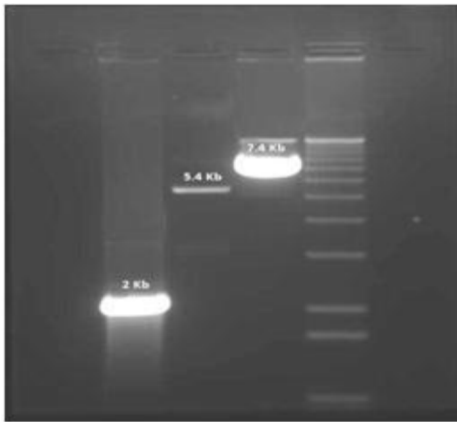


Fig. 4: Restriction digestion of a 7.4 Kb recombinant clone (lane 3) carrying 5.4 KB plasmid DNA (Lane 2) and 2KB viral genomic insert (lane 1)

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

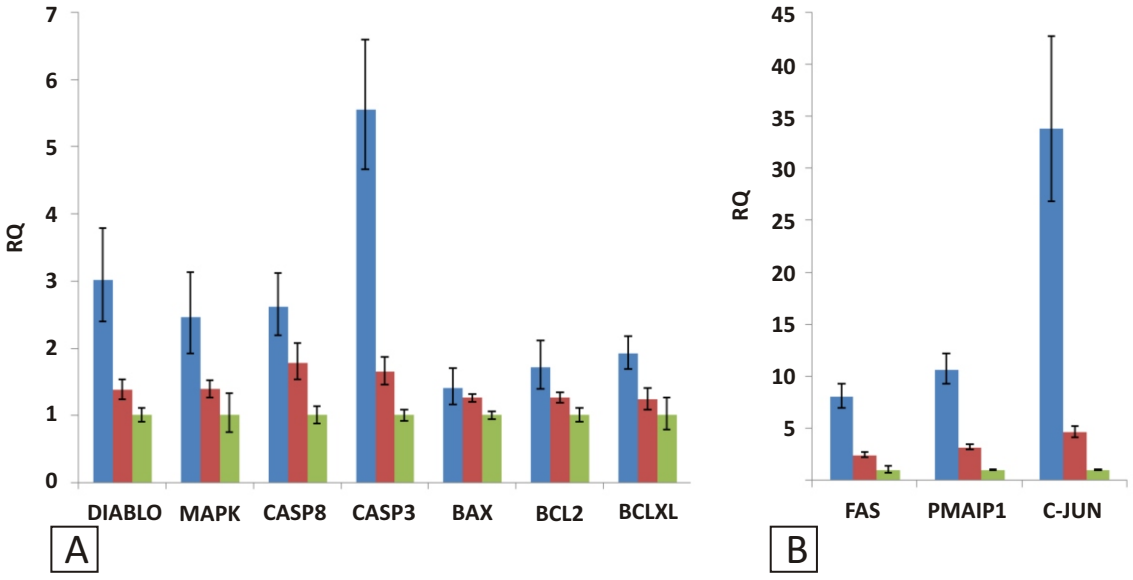
WNV strains vary in their ability to invade CNS and damage neurons resulting in varying degree of outcome. In our earlier studies, we have shown that that virulent strain (WNV68856) induced higher degree of apoptosis than the milder strain (WNV804994). Viruses are known to modify host gene expression,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

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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

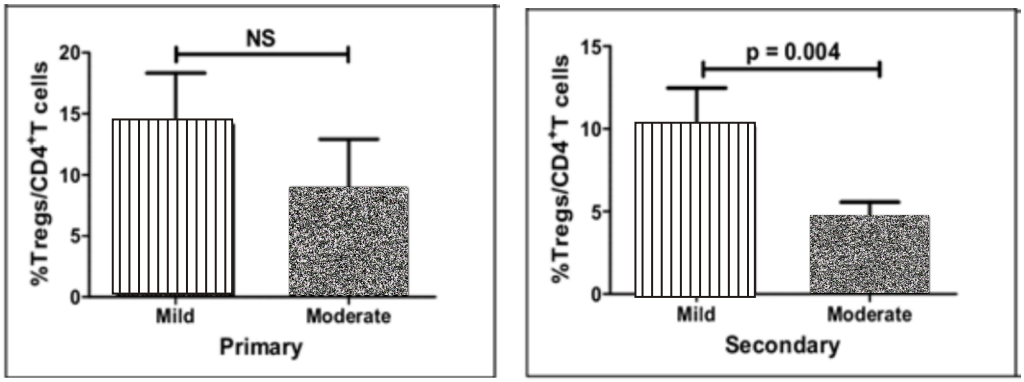

Fig.1. Treg frequencies in mild Vs. moderate dengue cases with primary Vs. secondary infections

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

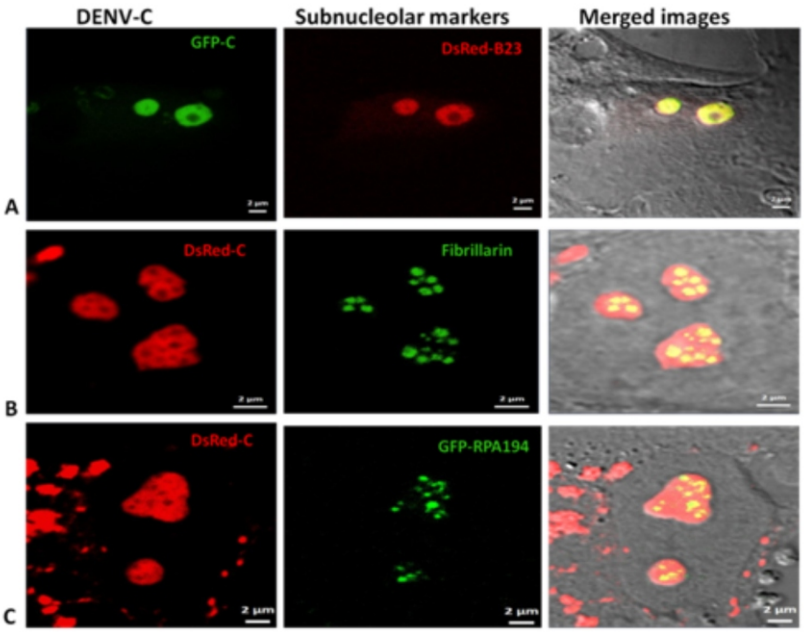

Fig. 2 Co-localization of DENV core protein with B23 in granular component of nucleolus

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

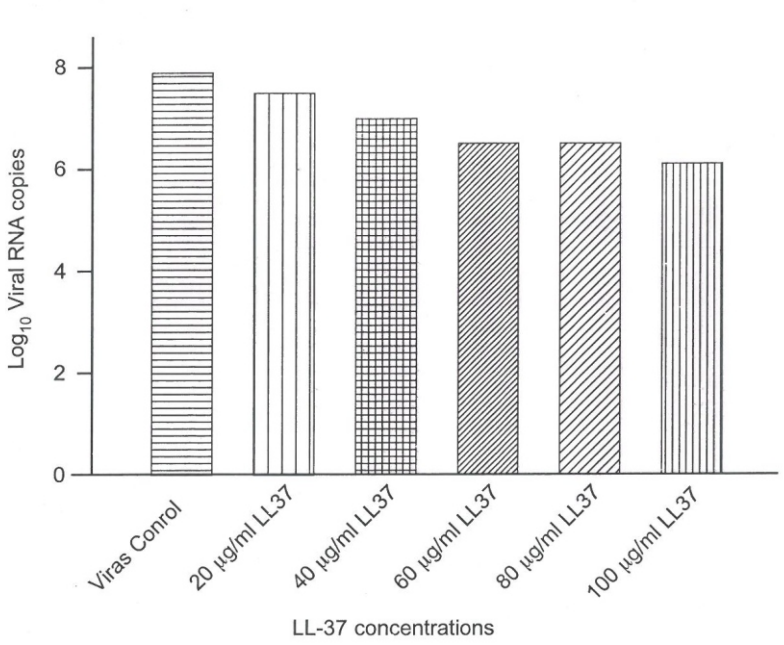

Figure 3: Effect of pre-treatment of DENV-2 with LL-37 on viral RNA levels

ENTERIC VIRUSES GROUP

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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.

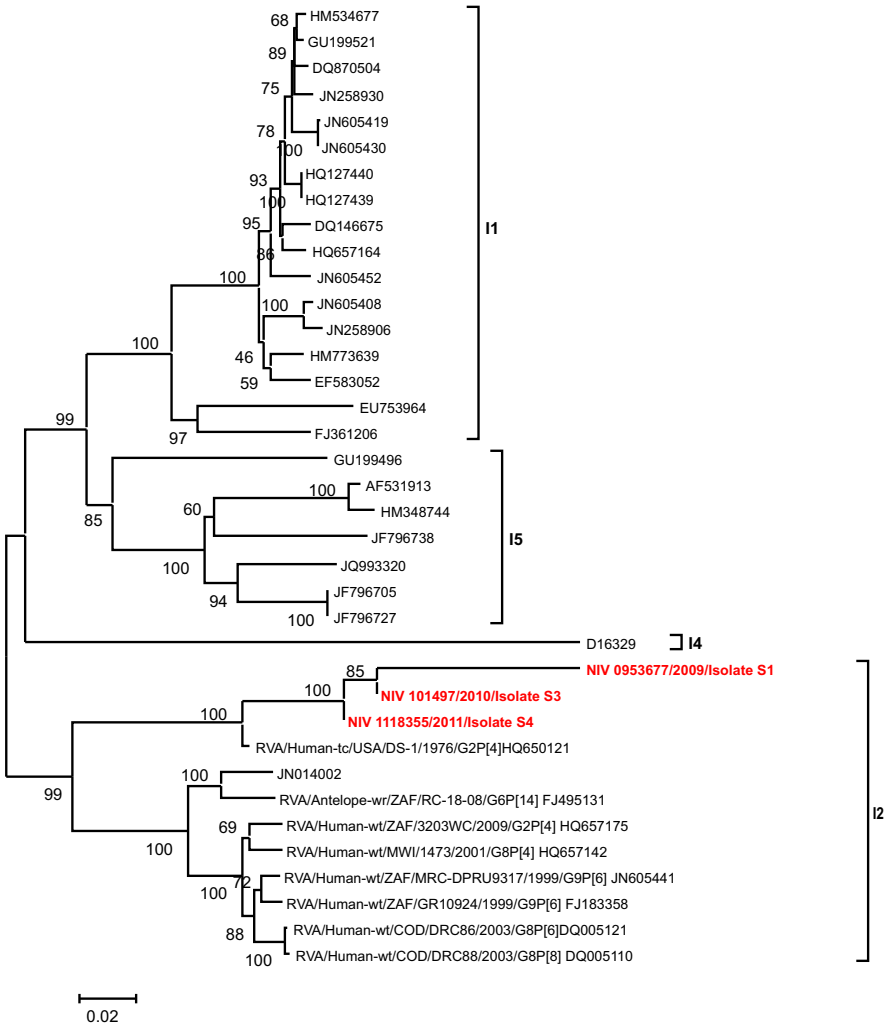

Fig 1: Phylogenetic tree based on the nucleotide sequences of VP6 gene (792-1130 nt) of cell culture isolates of G9P[4] rotavirus strains . The strains of the present study are highlighted in red

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.

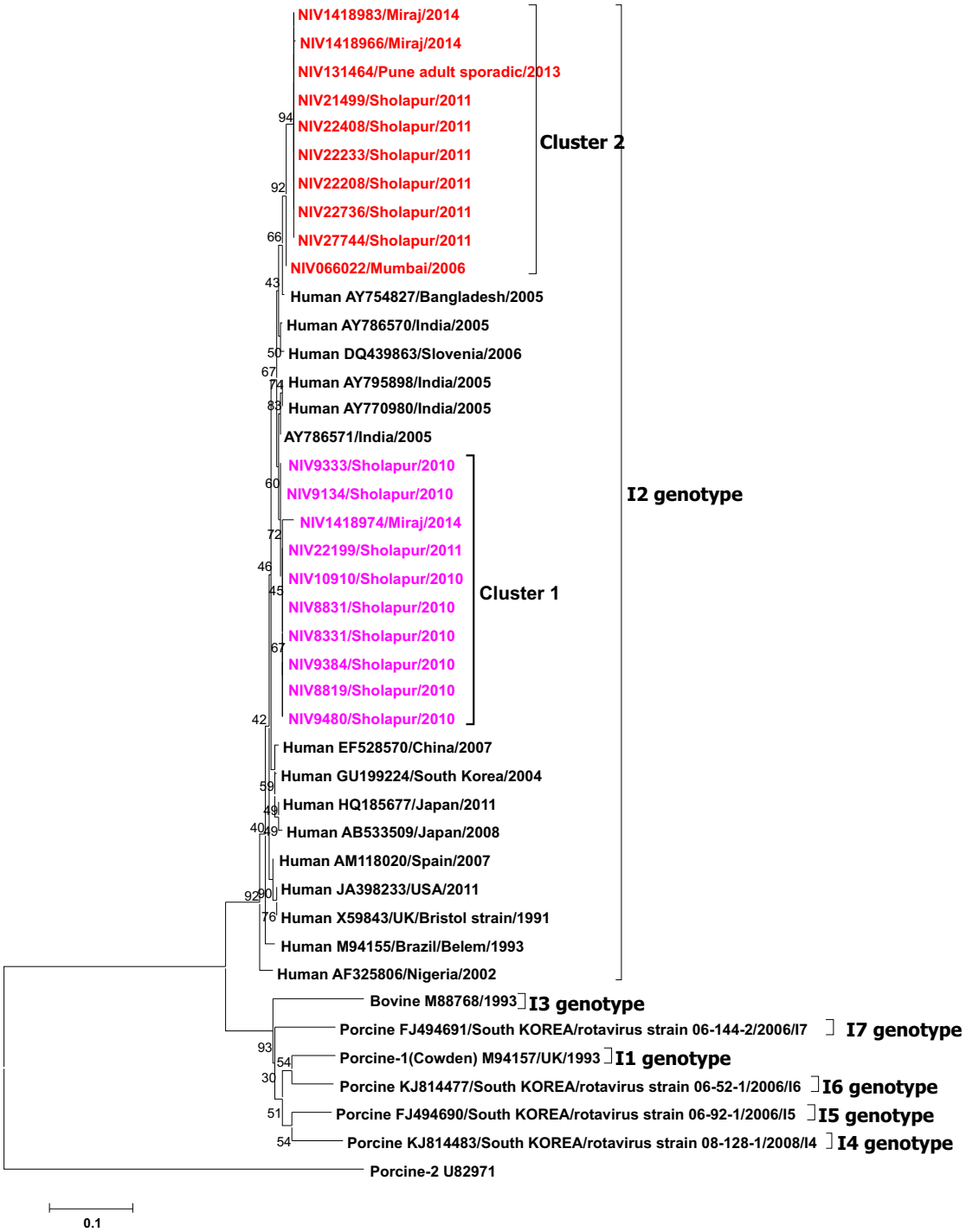

Fig 2: Phylogenetic tree constructed based on the partial nucleotide sequences of VP6 gene (372bp) of GCR strains. The strains of the present study are shown as Cluster 1 (pink color) and Cluster 2 (red color). The reference strains are indicated by accession numbers followed by the country name and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

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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**

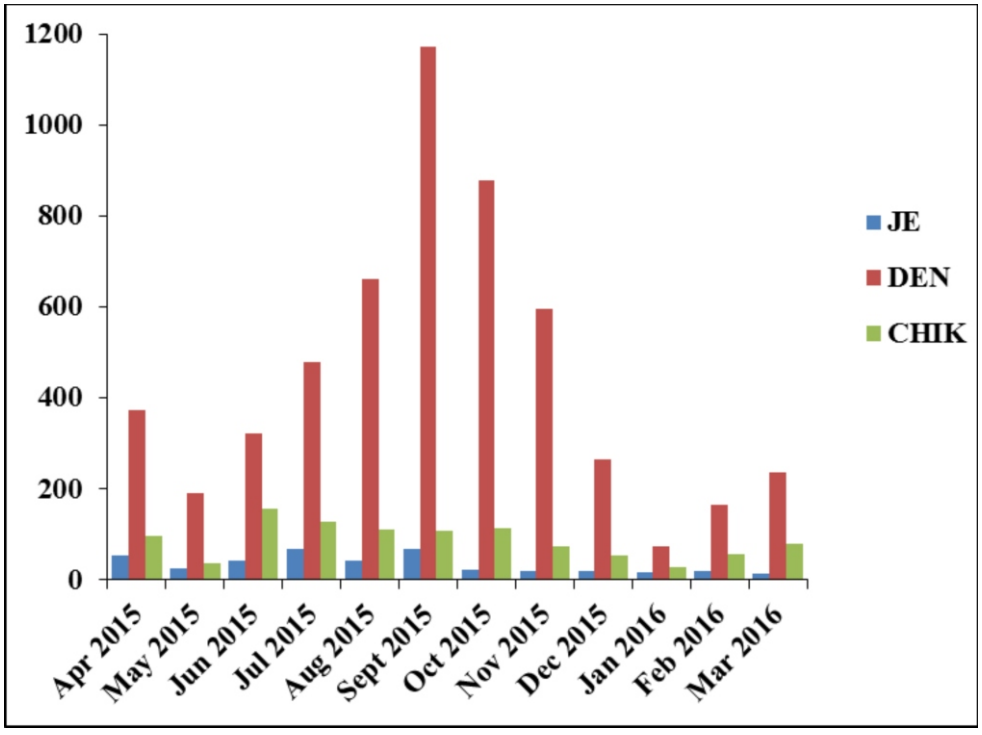

Fig 1: Month-wise supply of MAC ELISA kits under the National Program

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

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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.

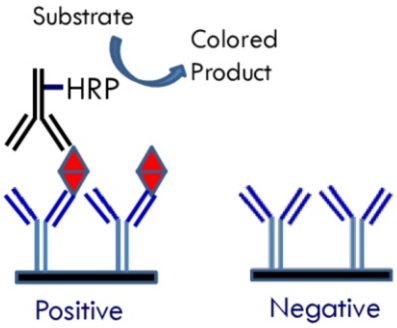
Work done

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

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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

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Mr. Vishal Khond	Multi Tasking Staff

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Mr. Kalyan Bhandare	Technician C (till 15th January 2016)
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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]*

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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.

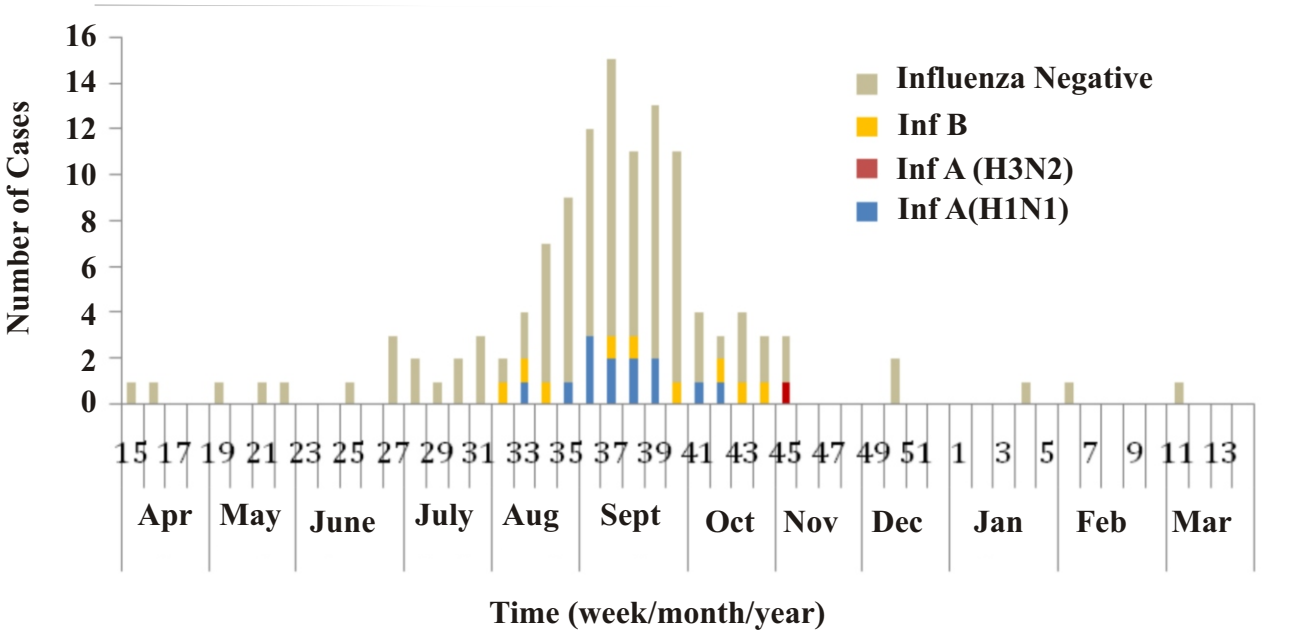

Fig. 1: Weekly distribution of ILI cases with influenza etiology

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.

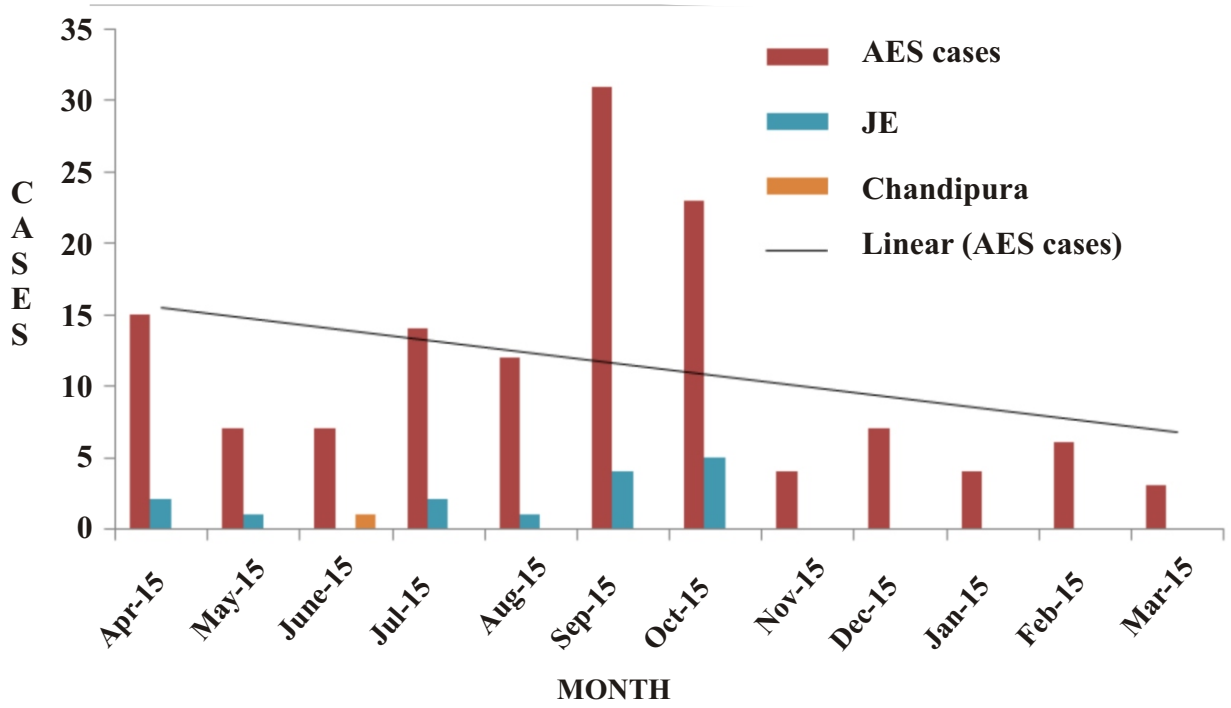

Fig. 2: Month-wise distribution of screened, eligible and enrolled cases with etiology

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.

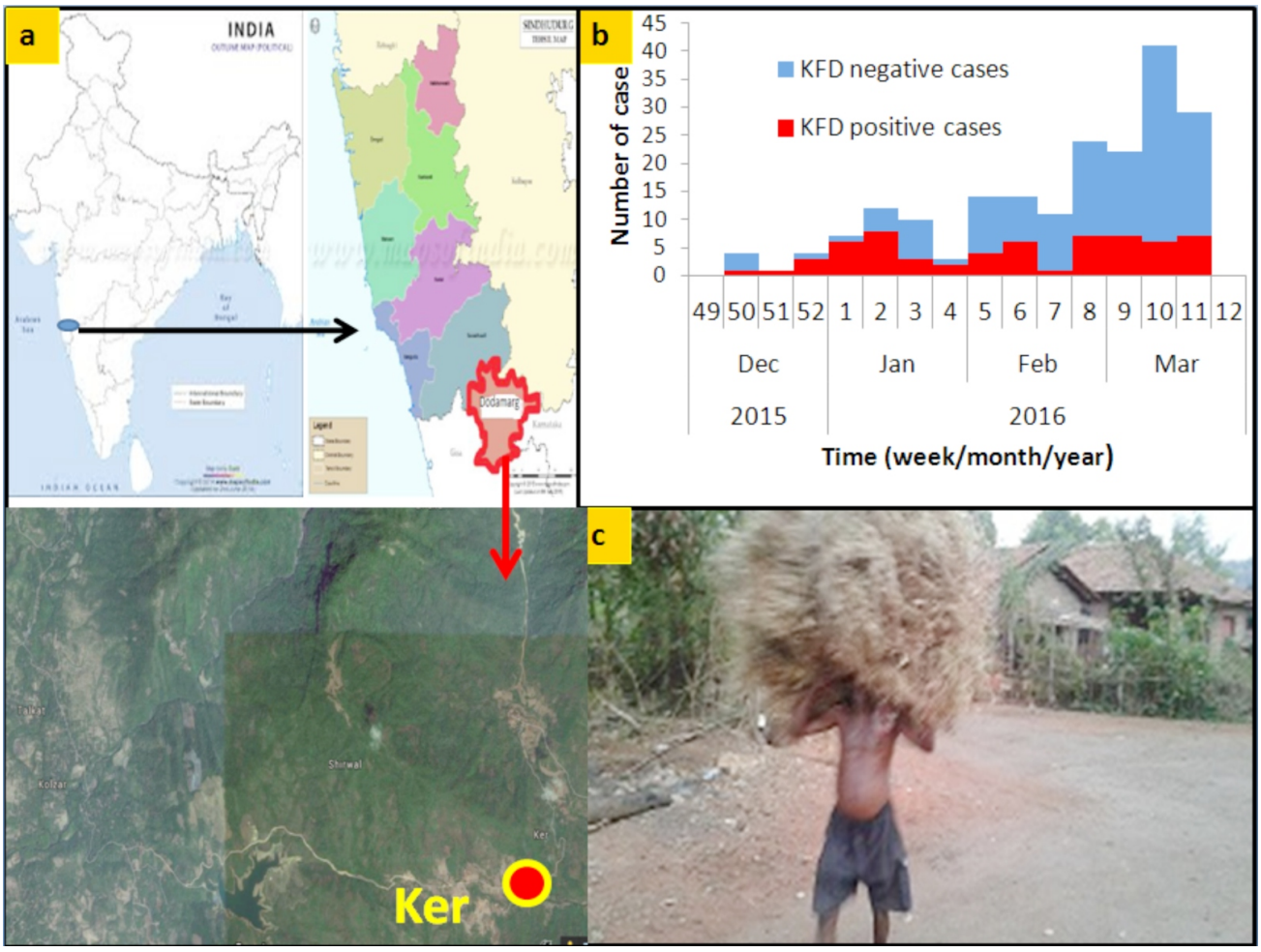

Fig 3: Kyasanur Forest disease cases (a) Ker village, the most affected village (b) KFD and suspected KFD cases during December 2015 to March 2016 and (c) risk behavior among people living in forest area

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.

ENCEPHALITIS GROUP

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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.

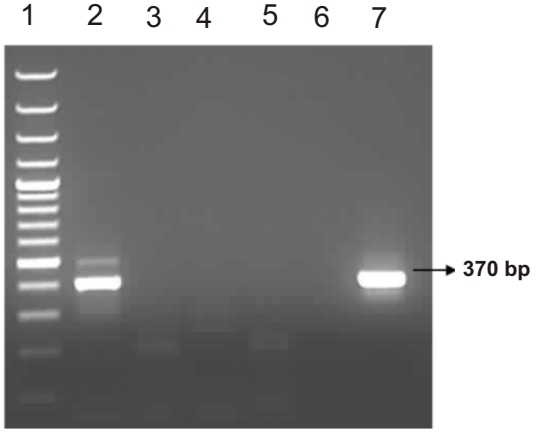


Fig 1: CHPV diagnostic RT-PCR of representative sandfly pools from Panchmahal

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,

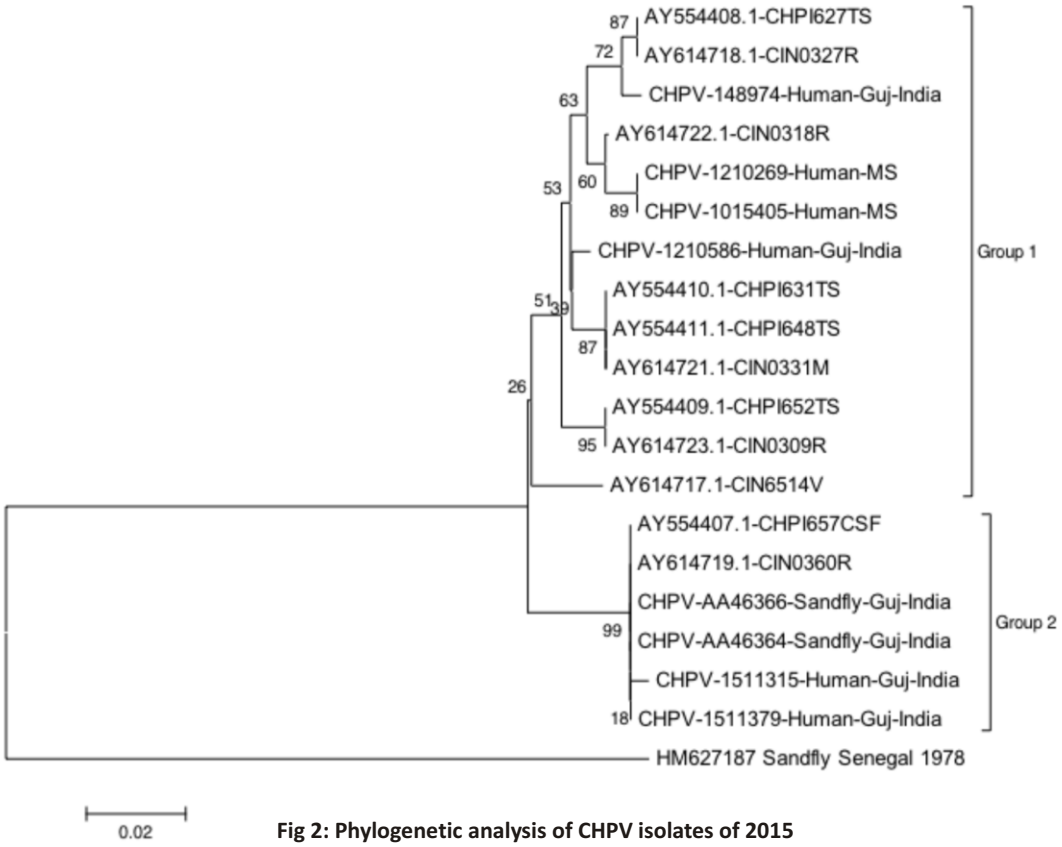


Fig 2: Phylogenetic analysis of CHPV isolates of 2015

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).

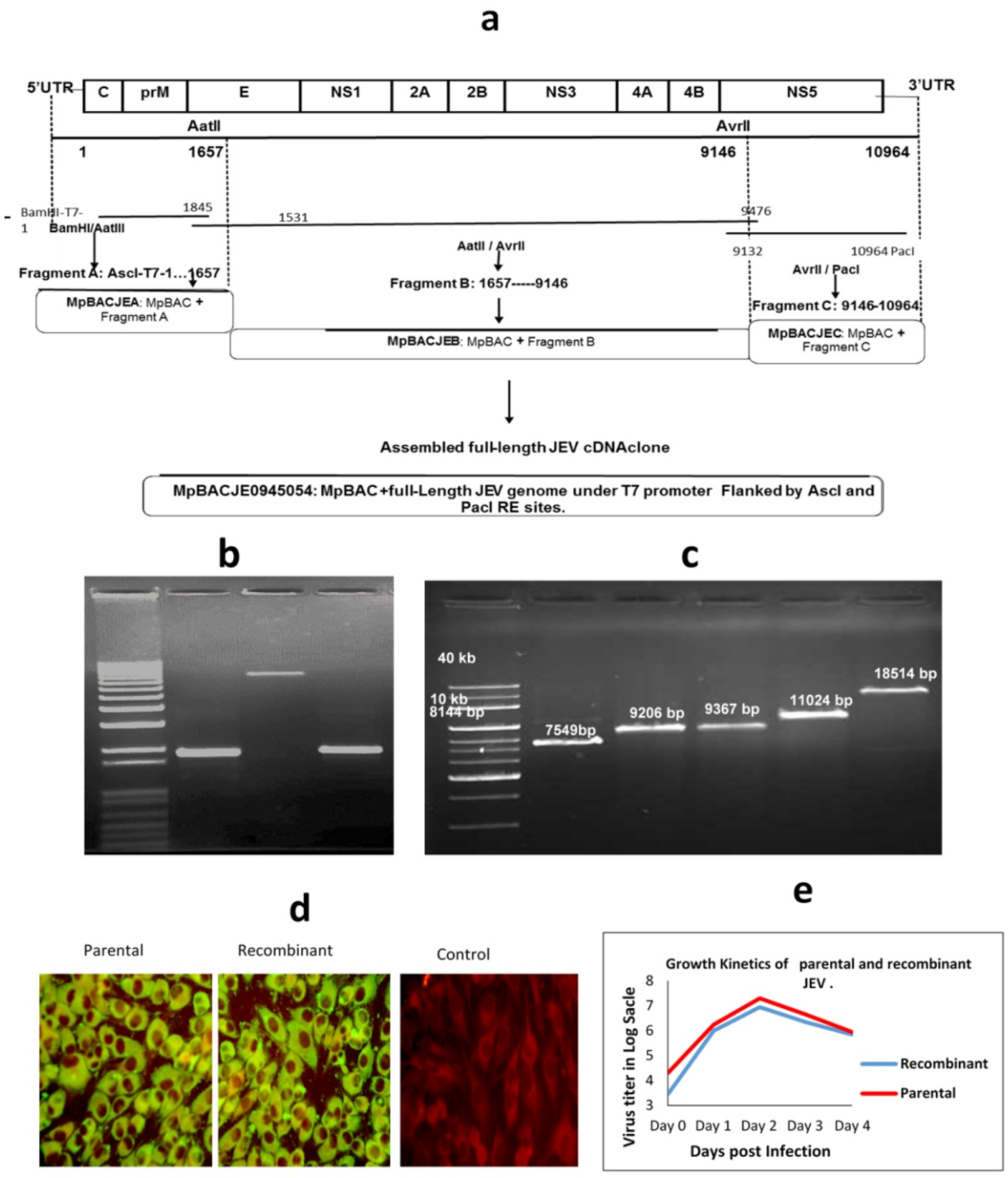


Fig. 3: a) Cloning strategy; b) Amplification of JEV genome: Lane 1: 1 Kb plus DNA ladder, Lane 2 –JEV 2-1845 (1845bp), Lane 3: 1531-9476 (7945bp) and Lane 4: 9132-10964 (1849bp); c) Construction of JEV cDNA clone in BAC vector, Lane 1- 1 Kb Extension ladder, Lane 2- Modified pBeloBAC11, Lane 3-MpBAC+ 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)

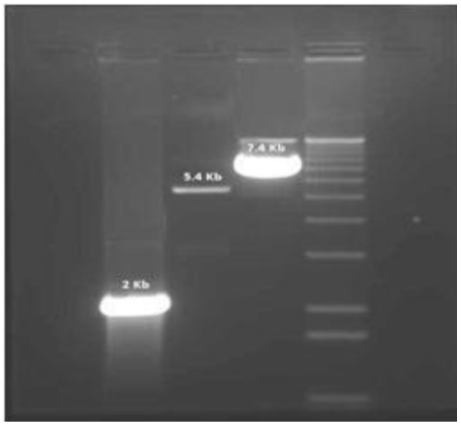


Fig. 4: Restriction digestion of a 7.4 Kb recombinant clone (lane 3) carrying 5.4 KB plasmid DNA (Lane 2) and 2KB viral genomic insert (lane 1)

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

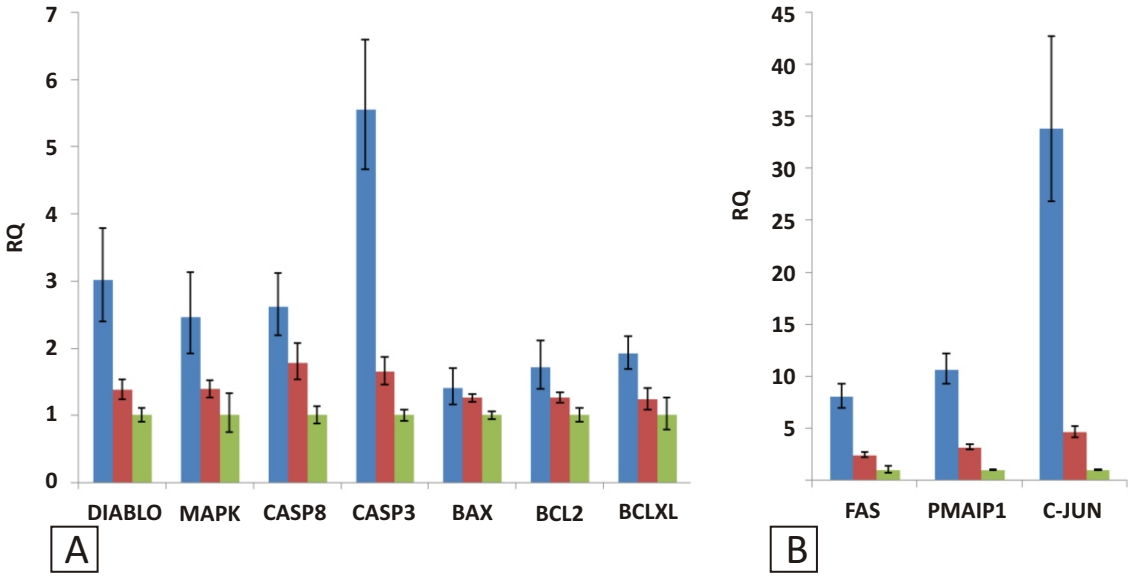
WNV strains vary in their ability to invade CNS and damage neurons resulting in varying degree of outcome. In our earlier studies, we have shown that that virulent strain (WNV68856) induced higher degree of apoptosis than the milder strain (WNV804994). Viruses are known to modify host gene expression,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

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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

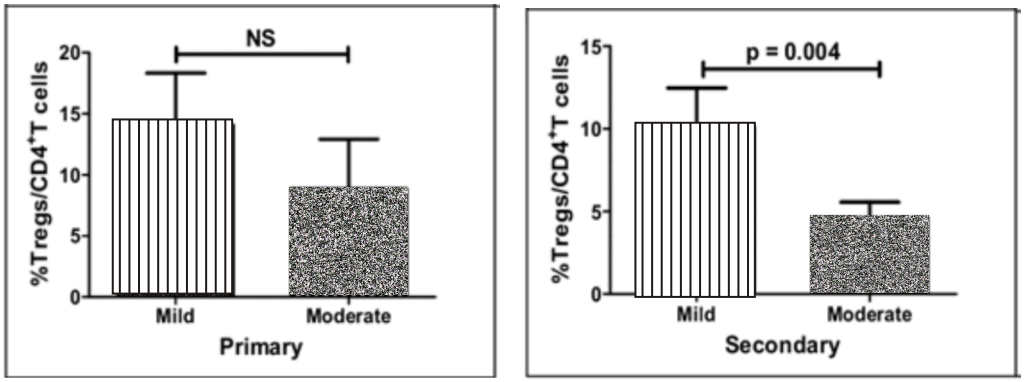

Fig.1. Treg frequencies in mild Vs. moderate dengue cases with primary Vs. secondary infections

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

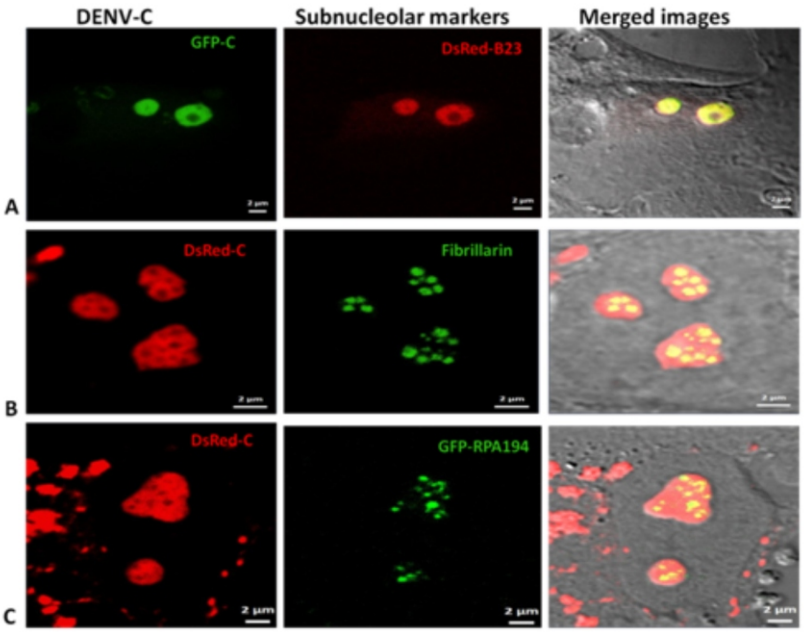

Fig. 2 Co-localization of DENV core protein with B23 in granular component of nucleolus

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

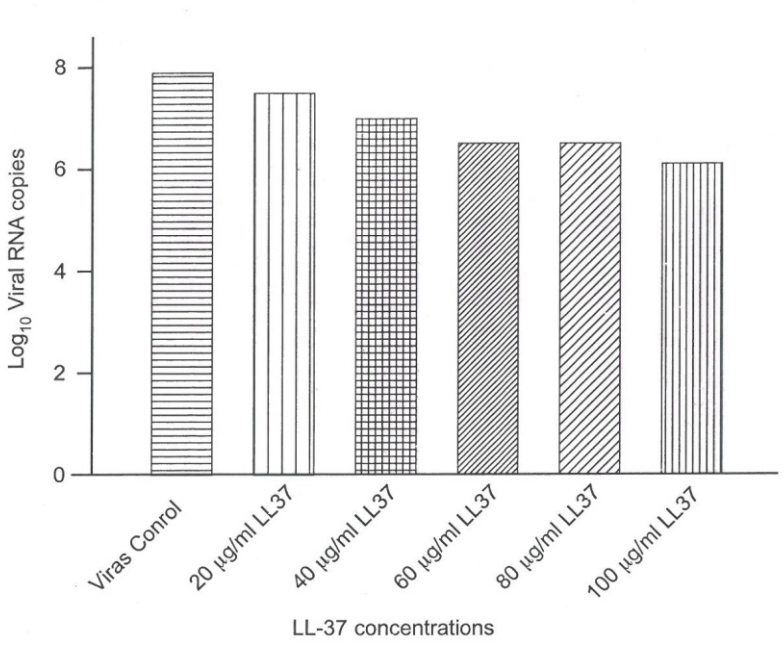

Figure 3: Effect of pre-treatment of DENV-2 with LL-37 on viral RNA levels

ENTERIC VIRUSES GROUP

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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.

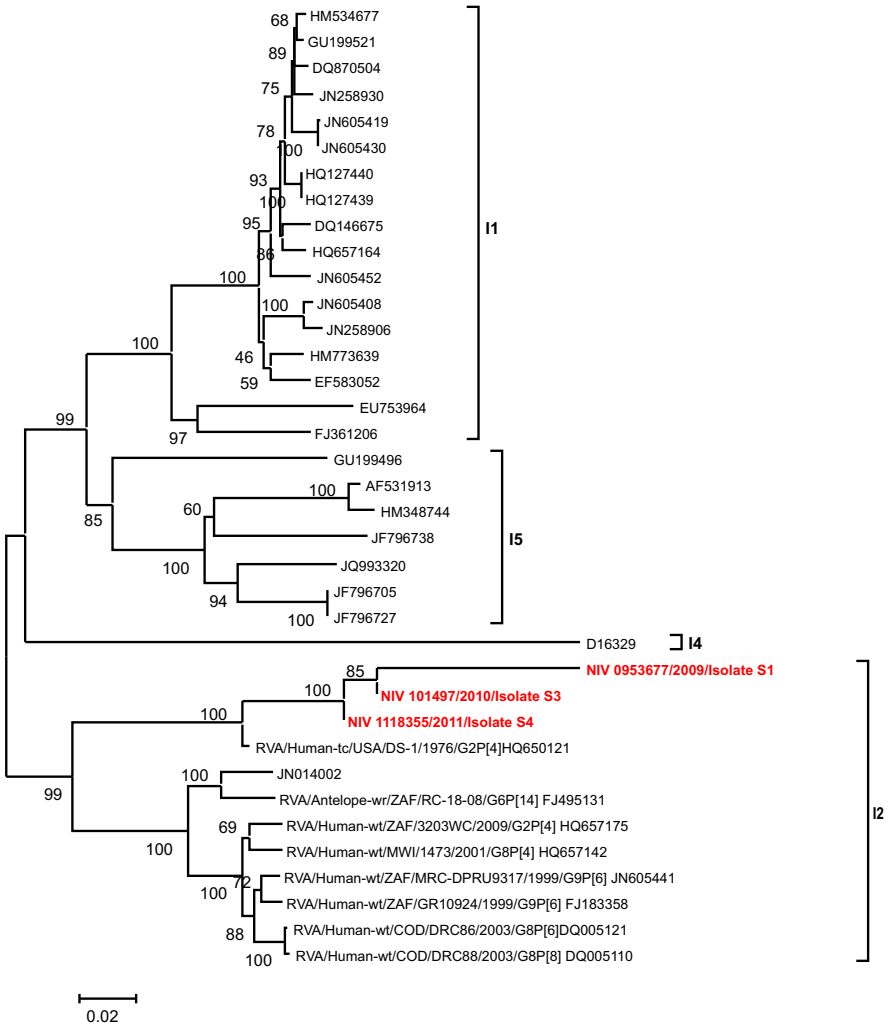

Fig 1: Phylogenetic tree based on the nucleotide sequences of VP6 gene (792-1130 nt) of cell culture isolates of G9P[4] rotavirus strains . The strains of the present study are highlighted in red

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.

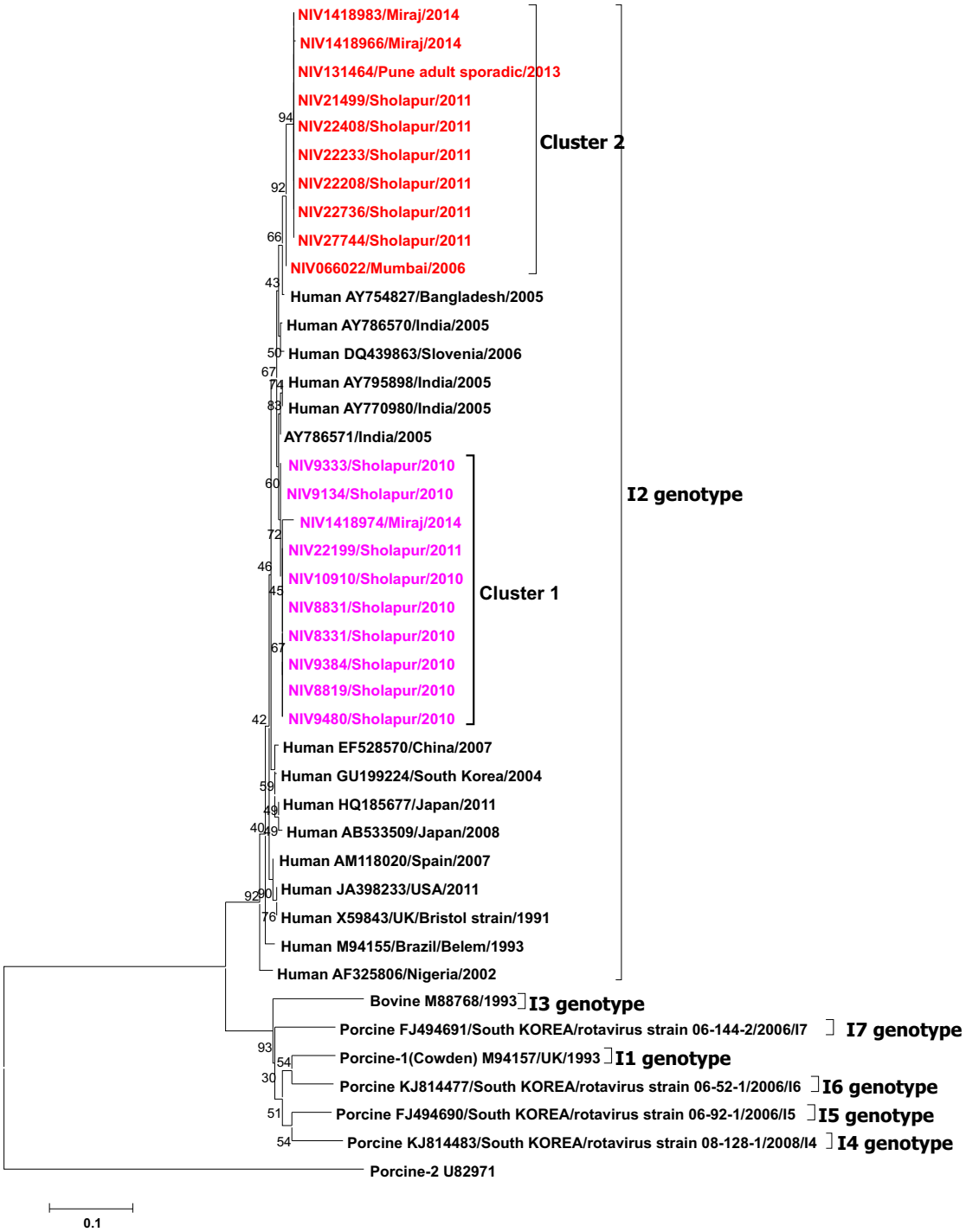

Fig 2: Phylogenetic tree constructed based on the partial nucleotide sequences of VP6 gene (372bp) of GCR strains. The strains of the present study are shown as Cluster 1 (pink color) and Cluster 2 (red color). The reference strains are indicated by accession numbers followed by the country name and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

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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**

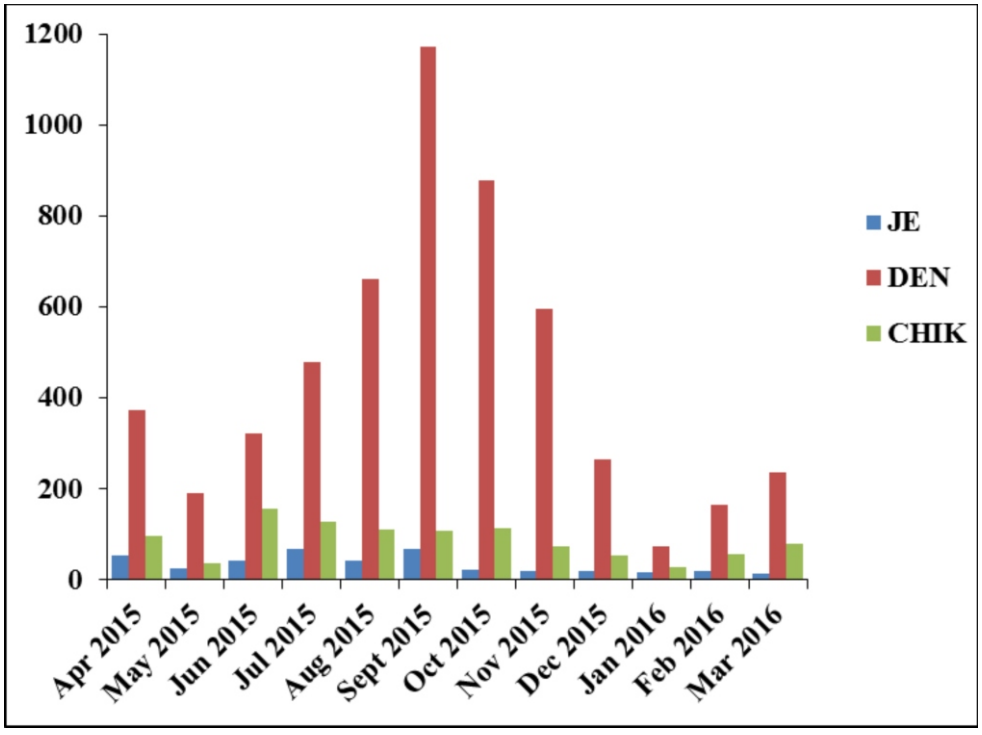

Fig 1: Month-wise supply of MAC ELISA kits under the National Program

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

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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.

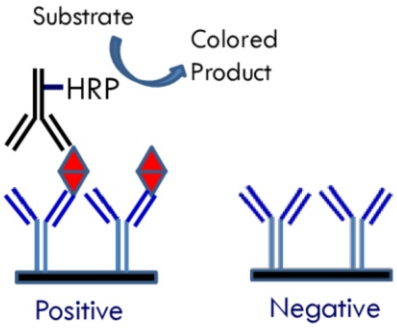
Work done

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

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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

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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.

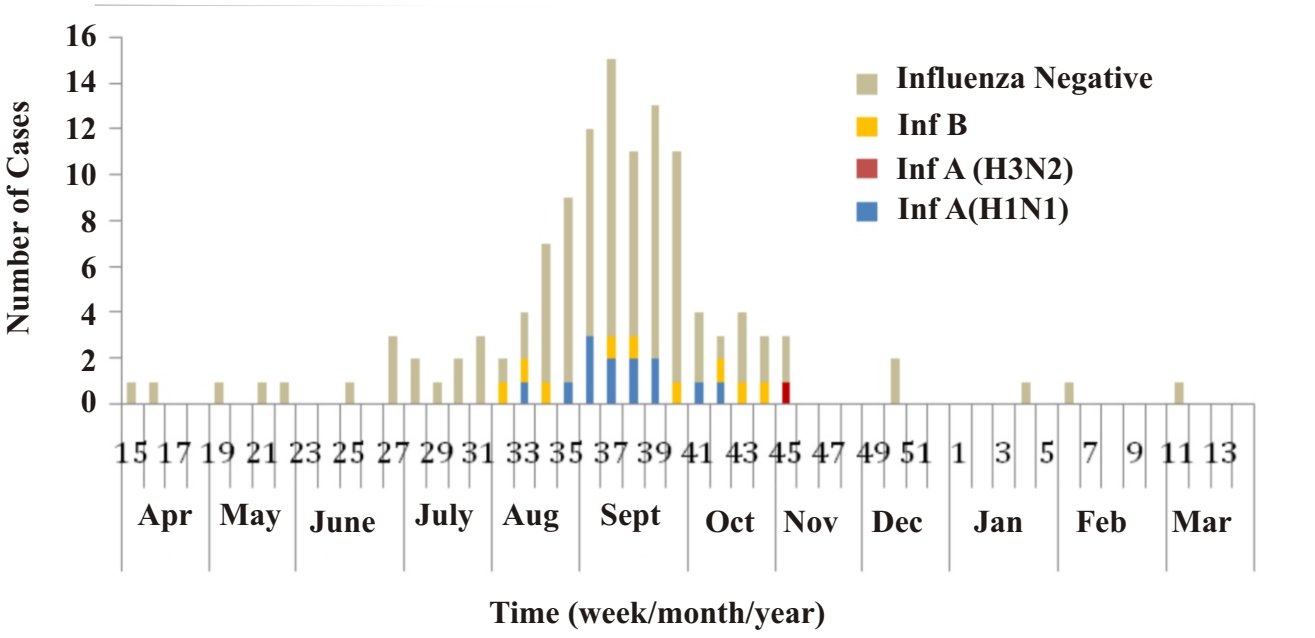

Fig. 1: Weekly distribution of ILI cases with influenza etiology

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.

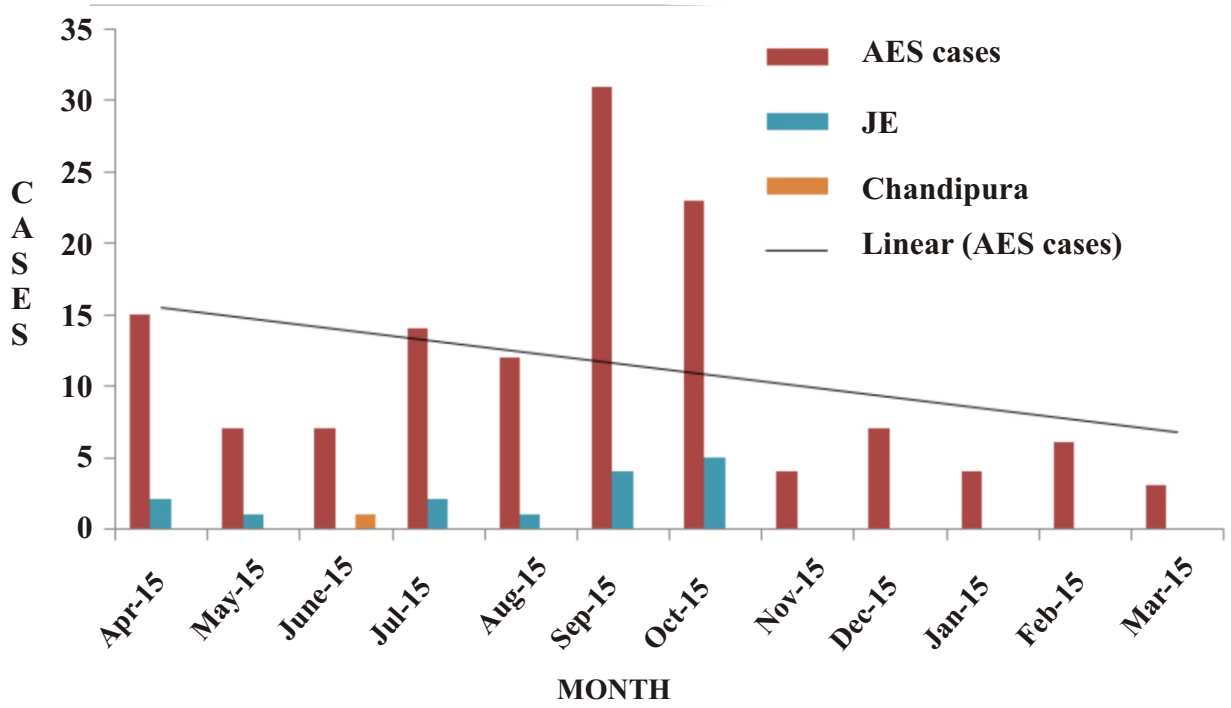

Fig. 2: Month-wise distribution of screened, eligible and enrolled cases with etiology

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.

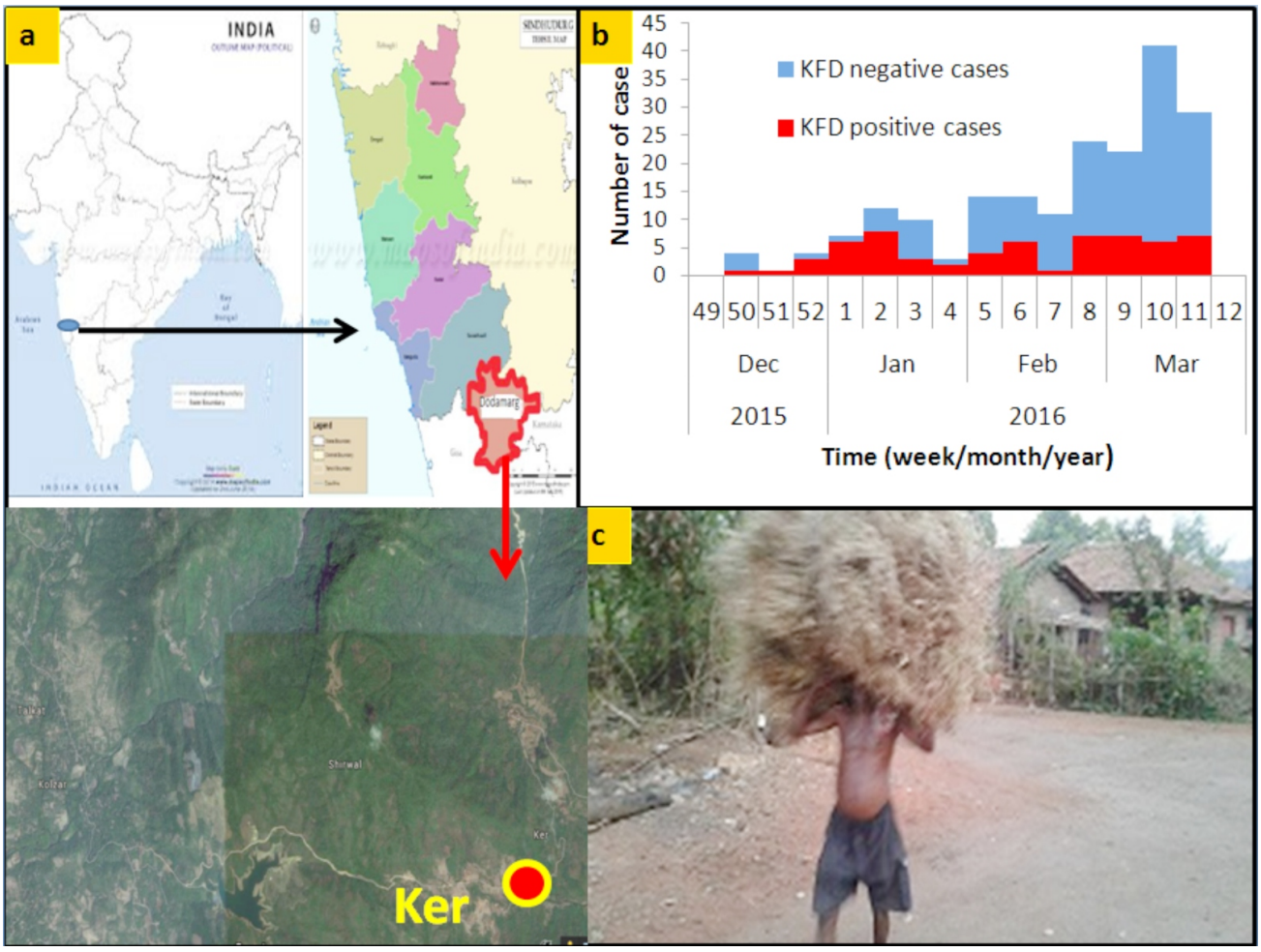

Fig 3: Kyasanur Forest disease cases (a) Ker village, the most affected village (b) KFD and suspected KFD cases during December 2015 to March 2016 and (c) risk behavior among people living in forest area

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.

ELECTRON MICROSCOPY & PATHOLOGY GROUP

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Mr. Mate SR	Multi Tasking Staff
Mr. Gadekar VS	Multi Tasking Staff

Project staff

Dr. Jain P	DBT Post-doctoral Fellow

Research Fellow

Ms. Tadkalkar N	CSIR-SRF

Core Facility Support

Core facility support application for high resolution TEM in areas of morph diagnostic imaging and ultra-thin sectional analysis was provided to various research groups. Support was extended also to researchers from NCL, NCCS in macromolecular, cellular and nano-particle imaging technology. External quality assurance runs for rapid virus morph diagnosis was also completed in the capacity of a reference laboratory.

Characterization of dengue virus NS1 protein towards understanding its role in endothelial pathophysiology

***Investigators**: A Basu, K Ghosh (NIIH) & S Shetty (ICMR)*

Duration: 2015-18

Funding: extramural (Task Force project, DBT)

Background

Earlier studies from our lab had shown that DENV2 virus could affect physiology of endothelial cells in-vitro. However, the cellular and molecular basis of this remains incompletely understood. Furthermore, the absence of proper animal models also hinders studies in exploring virus-endothelial interactions. *In-ovo* models of chicken chorioallantoic membrane has evolved as an important tool for various applications in vascular biology. Here we attempt to use this model to study effects of dengue virus NS1 protein and infection per se on *in-vivo* angiogensis.

Objectives

- The role of DENV2 NS1 on angiogenic development in chicken chorio-allantoic membrane was carried out.
- Studies on effect of exogenous DENV 2 NS1 on endothelial cells.

Work done: objective 1

Four to six day old embryonated eggs were inoculated with various concentrations of thrombin, carboxymethylcellulose in initial experiments to optimize the technique. Subsequently, different doses of VEGF were used to study the angiogenic potential of all different morphological classes of blood vessels (small, medium, tertiary). Purified dengue 2 virus NS1 expressed in a eukaryotic cell was then inoculated into the CAM using the optimized protocol and the CAM harvested at 10th day. Morphometric analysis of the blood vessels under low power scan was carried out as described earlier and quantitated using a software. Samples of blood vessels were also processed for SILAC based proteome imaging for examining the basic differentiation signaling molecules that is ongoing.

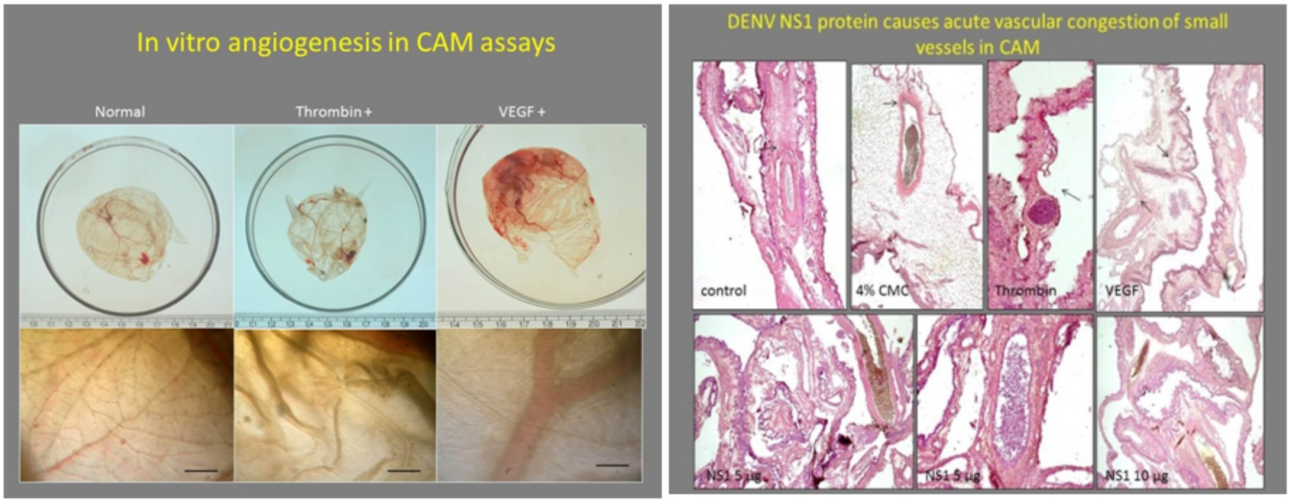

Figure 1: Effect of dengue 2 virus NS1 protein on angiogenesis. The normal CAM shows well-differentiated blood vessels of all types while the thrombin treated (middle) shows aberrations

Findings

Analysis of the data suggests the DENV 2 NS1 protein can suppress the angiogenesis of small blood vessels and lead to significant congestion of medium vessels. Importantly, microscopic pathology showed destabilized medial stroma in tunica media layers of the blood vessels prompting us to investigate the expression of matrix metalloproteases (Figure 1). Both SILAC and in metalloprotease analysis is ongoing.

Summary

DENV 2 NS1 protein may have potential to inhibit in-vivo angiogenesis. Further studies are ongoing.

Exogenous exposure of dengue virus 2 NS1 affects beta catenin expression in endothelial cells

The effect of direct exposure of DENV NS1 protein on endothelial transcriptional factors and adhesion molecules was studied in vitro. Primary human vascular endothelial cells were exposed to purified DENV 2 NS1 and expression of several adhesion/transcriptional molecules assayed using immunofluorescence imaging.

Findings

Exogenous exposure of NS1 DENV 2 NS1 to vascular EC showed differential early down regulation and subsequent transient upregulation of beta catenin, an important cellular regulatory molecule for signaling. Further studies are ongoing to characterize this further.

Summary

DENV NS1 protein can affect endothelial cell transcriptome. High-resolution transmission electron microscopy of human endothelial cells using electron tomography and subcellular reconstruction imaging

A very high-resolution electron tomography imaging was optimized for 3D reconstruction of subcellular structures in endothelial cells. This was carried out in a Tecani 12 Biotwin platform using high tilt series data and the 3D reconstructions done using in-house developed programs. These experiments were done as forerunners towards studying the dengue virus infected cells and effect of non-structural proteins on subcellular ultrastructure, especially on cyto-organelles related to vascular hemostasis.

Findings

This imaging technique has enormous scope in imaging events of virus macromolecule-host interactions at very high resolution in the infection cycle environment.

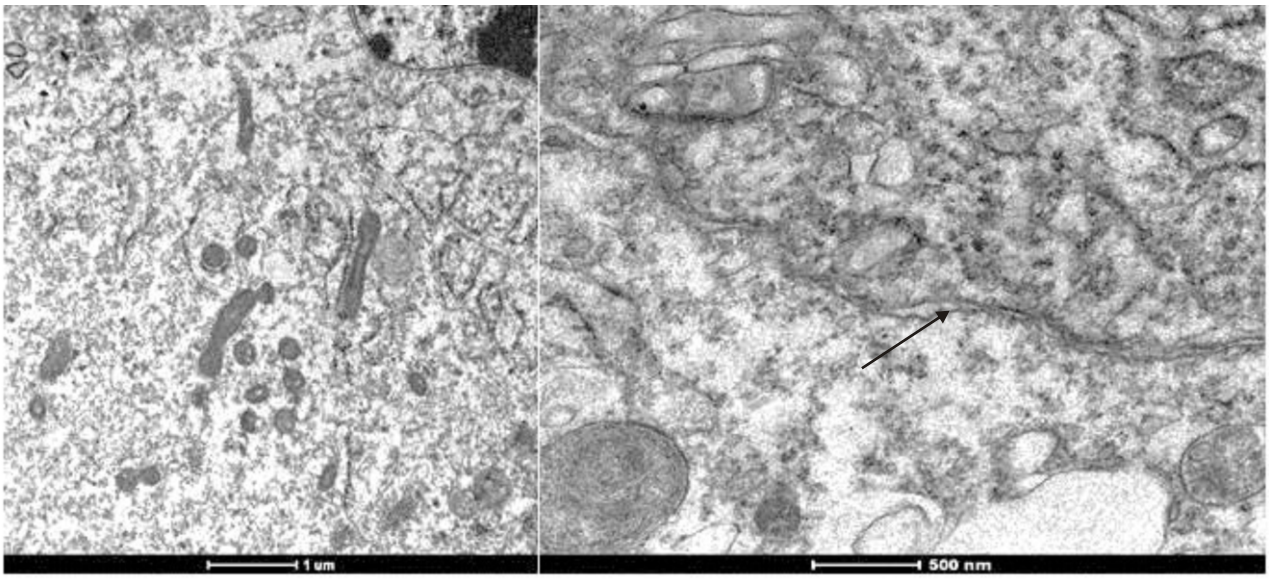

Fig 2: Electron tomographs of vascular endothelial cells imaging very typical Wiebelpalde bodies (a) and junctional morphology (arrows). Magnification bars are built-in.

BIOINFORMATICS AND DATA MANAGEMENT

Scientific Staff

Dr. Sarah Cherian	Scientist F & Group leader
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Mr. Santosh Jadhav	Technical Officer (A)
Mr. N V Gujar	Technician - B
Mr. Avinash Patil	Technician-A
Mr. Sujay Girdavkar	Multi tasking staff

Project Staff

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Ms. Dipali Bhoye	UGC's Rajiv Gandhi Senior Research Fellow

BDM1001: Core facility services

Investigators: *S Cherian, P Shil, AM Walimbe, V Simha, S. Jadhav*

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

Dr. Cherian provides bioinformatics support to other groups in terms of sequence analyses and molecular phylogenetic and evolution. Mathematical modeling (epidemiology) of data and structural bioinformatics support to other groups is provided by Dr. Shil.

Information Technology support to the whole institute is provided by staff of this group. Management and 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 are being done for data transfer, AIMS, LIMS Software and intercom services. NIV website is being monitored on a regular basis. Audio and video conferencing units are maintained on a regular basis. Technical support has been provided for conferences and workshops held by NIV. The internal softwares like payroll, pension, supplementary bills, computer complaint register etc. are being maintained. The problem of McAfee Antivirus was solved with the help of McAfee technical support.

Support to NARI projects

Investigators*: S Cherian, AM Walimbe, S Pandey & R Paranjape*

Phylogenetic and molecular characterization of six full-length HIV-1 genomes of subtype A from India was completed for Dr. R Paranjape.

Support to Enteric viruses group NIV

Investigators*::S. Cherian & R Kulkarni (Enteric Viruses Group)*

(1) Phylogenetic analysis of capsid protein VP1 gene of the GII-4 Noroviruses; "NoVs. Was" carried out.

(2) Support with genomic characterization of coxackie type B3 strains was given to Enteric viruses group (Please refer report EV Group)

Bioinformatics analysis of Avian Influenza NS1 protein

Investigators*: P. Shil, M Thube & J. Mullick (Avian Influenza group)*

The non-structural protein NS1 from high and low pathogenic strains of Avian Influenza viruses has been compared (H5 vs. H11 strains). Sequence based comparison was followed by 3D structure prediction and analyses. Results indicated that there is no major change in the 3D fold of the protein conformations but differences in amino acid compositions at various domains have altered surface contour and surface electrostatics locally. Though predicted protein functional sites (PROSITE) remain unaltered, the mutations may affect interactions of NS1 with other proteins.

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*: S Cherian & A Behera*

Duration: 2014-2017

Funding: ICMR (Grant #2014-20880)

Background

This database aims at providing information on marker mutations for pathogenicity known from literature and co-evolutionary mutations obtained from the implementation of available tools based on statistical approaches like correlation coefficient, mutual information, etc. that infer correlated evolution from multiple sequence alignment. Servers MISTC (Mutual Information Server to Infer Coevolution) and CAPS (Coevolution analysis using protein sequences) were used to identify co-evolved amino acid positions in neuraminidase (NA) sequences of H1N1 viruses.NA evolution occurs by way of antigenic drifts and antiviral resistant viruses emerge and spread due to evolutionary changes in terms of the drug selective pressures. Oseltamivir-resistant Influenza A/H1N1 viruses emerged in 2007-2008 and have subsequently circulated widely with the most common Oseltamivir resistance mutation detected in these viruses as NA-H275Y. Compensatory mutations have also been observed to emerge with the drug resistance mutations.

Objectives

- To examine all resistant/non-resistant strains of 2007-2014 to infer patterns of co-evolutionary mutations using the MISTIC server.

Findings

Results showed that the known compensatory mutations (R194G and D344N) were not found to coevolve with the primary drug resistance mutation H275Y (Fig. 1a), as they had emerged much before the drug resistance marker mutation (Fig 1b), though residue position234 V/M showed co-evolution with 275 H/Y. Notably residues at position 222R/K and 234V/M were found to co-evolve while position 249G/K that is known to have caused NA antigenic drift was found to coevolve with position 275 in the 2007-2014 dataset. The study thus establishes an association between the coevolving residues and molecular functions of the NA protein.

BDM1403: Phylodynamics and molecular evolution of Influenza A Polymerase genes (PB1, PB2 and PA) inferred from large-scale sequence analyses and structural Bioinformatics

***Investigators**: S Cherian & D Bhoye*

Duration: 2014-18

Funding: UGC, Grant No: F1-17.1/2012-13/RGNF-2012-13-ST-MAH-34083

Background

The cap-binding domain of the polymerase basic 2 (PB2) subunit of influenza polymerases plays a critical role in mediating the "cap-snatching" mechanism by binding the 52 cap of host pre-mRNAs during viral mRNA transcription (Fig 2). Monitoring variations in the PB2 protein is thus vital for evaluating the pathogenic potential of the virus.

Findings

Based on selection pressure analysis of PB2 gene sequences of the pandemic H1N1 viruses of the period 2009-2014, we identified a site 344V/M in the

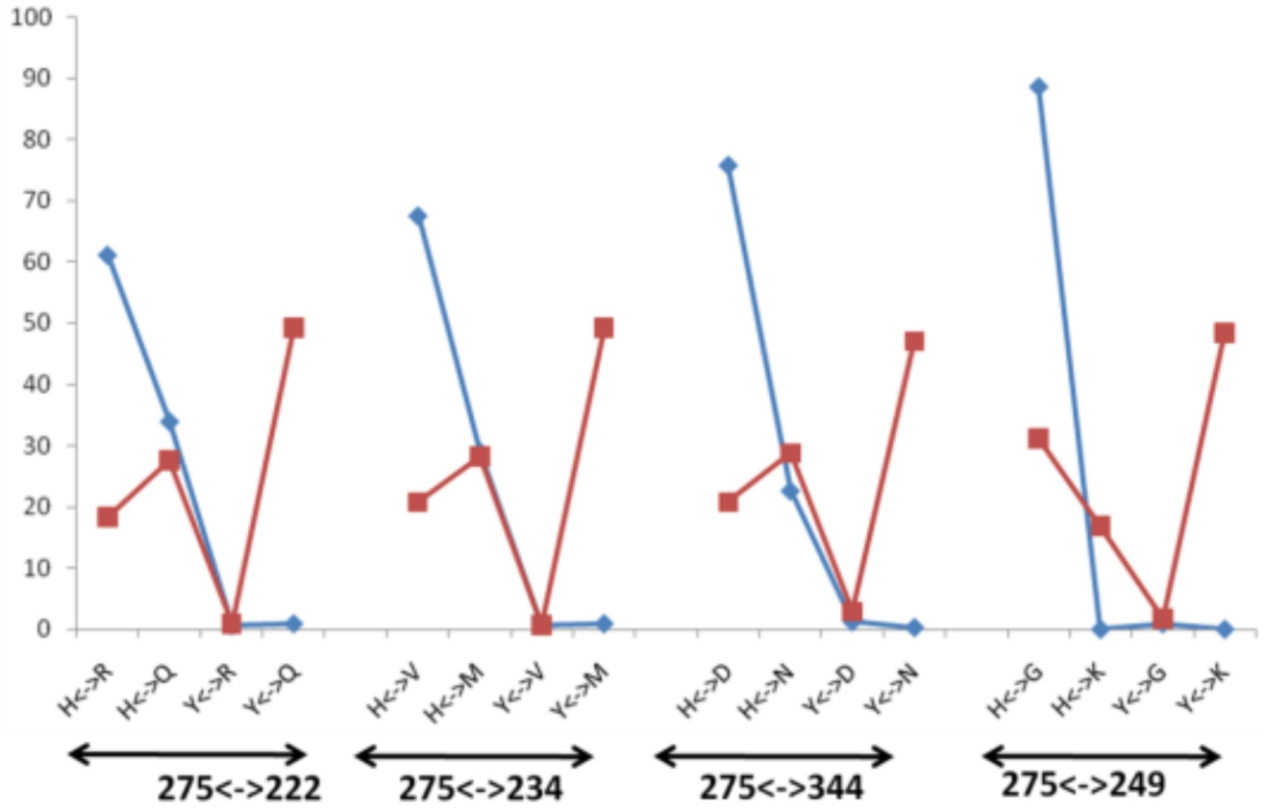

Fig.1. (a) Mutual information network involving residues in the spatial proximity of the NA catalytic residues showing the co-evolved residue pairs inH1N1 viruses of the period 2007-2014 (b) Distribution of residue pairs involving drug resistance marker 275(H/Y) with compensatory residue positions (222, 234, 344) and coevolved residue position 249 in NA sequences of H1N1 viruses of the period 2007-2014

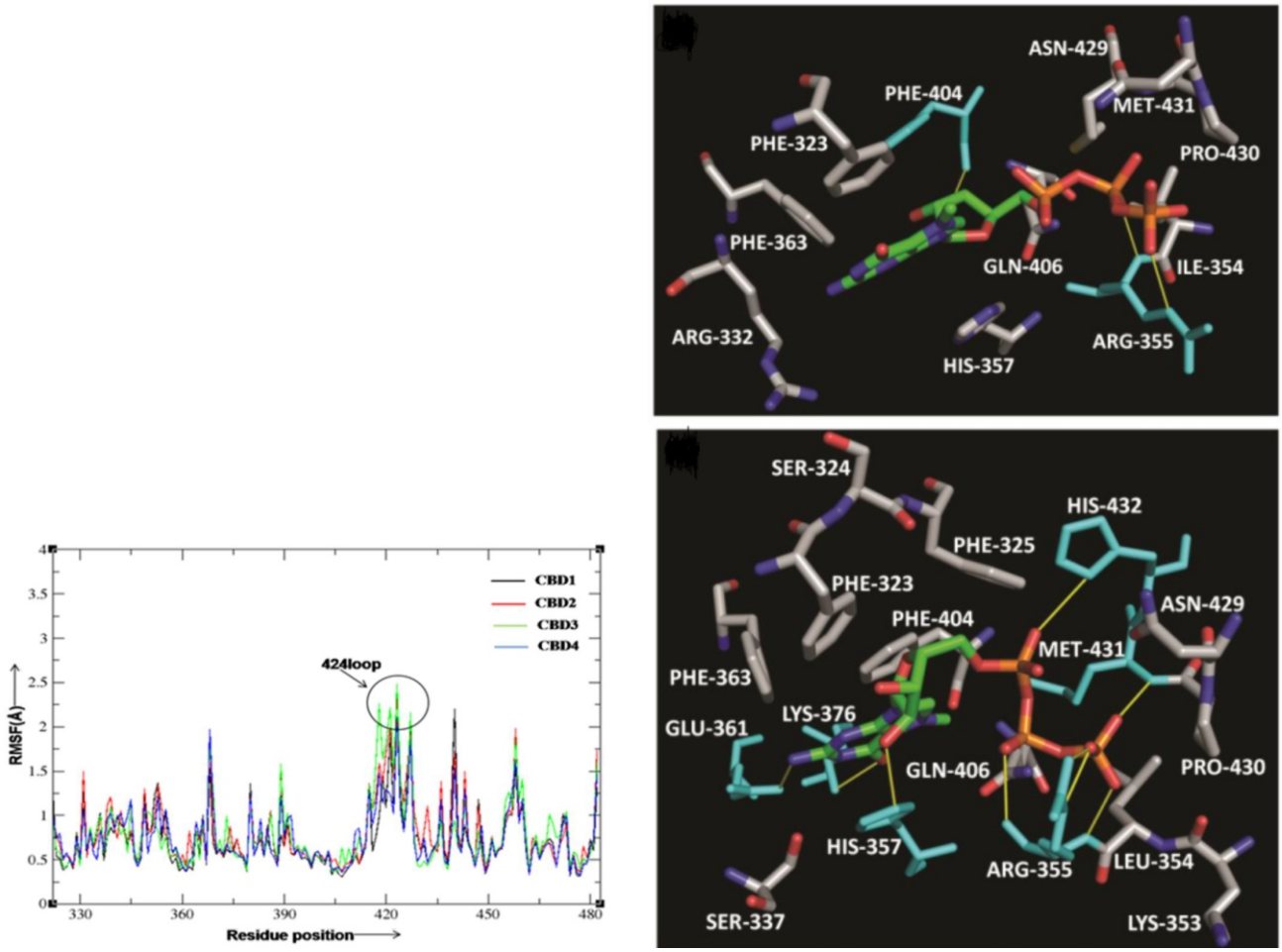

Fig. 2. (A) Overall stability of the four cap binding domain (CBD)-m7GTP complexes over a10 ns MD simulation in terms of RMSF of individual residues of the CBD [V344, I354 (CBD1), V344, L354 (CBD2), M344, L354 (CBD3), M344, I354 (CBD4)] (B) Residues forming H-bonds and hydrophobic contacts in the different PB2 cap binding domain variants [Top: V344, I354 (CBD1); Bottom: M344, L354 (CBD3)] docked with m7GTP. Residues forming the H-bonds are in cyan color while the H-bonds are depicted with yellow lines and residues forming hydrophobic contacts are in grey color.

vicinity of the cap-binding pocket showing evidence of adaptive evolution and another co-evolving residue 354I/L in close vicinity.

Summary

Modeling the 3D-structure of the pH1N1 PB2 cap binding domain, docking of the pre-mRNA cap analog, m7GTP and molecular dynamics (MD) simulation studies of the docked complexes performed for four observed PB2 variants, showed that the complex possessing V344M with I354L, possessed better ligand binding affinity due to additional hydrogen bond contacts between m7GTP and key residues His432 and Arg355 that could be attributed to a displacement of the 424 loop (Fig. 2a) and a flip of the side chain of Arg355most likely due to reduced steric hindrance in the presence of Leu354 (Fig. 2b) respectively.

BDM 1501: Molecular Characterization of the Measles Virus Genotypes circulating in India (1996-2014) using phylodynamics and structural bioinformatics analyses

***Investigators**: S Cherian, AM Walimbe & S Vaidya*

Duration: 2015-17

Funding: Intramural

Background

Measles virus (MV) is serologically a monotypic virus but genetically distinguished into 24 genotypes. In the light of the 2020 target for MV elimination in the country, it is vital to track the national/ international transmission pathways of MV and understand the dispersal trends.

Objectives

- Molecular clock studies of D4 genotype and D8 genotype

Findings

Results of D4 gene sequences (n=206) revealed the divergence time as ~42 years (1972) and all Indian sequences clustered in the "India like" clade showing a time to most recent common ancestor (tMRCA) as ~27 years (1987). For D8 gene sequences (n=254), the tMRCA was identified as ~29 years (1985) indicating a more recent ancestry. The D8 tree was classified in four clades, D8a, D8b, D8c and D8d (Fig. 3).

Summary

Majority of Indian sequences were present in D8a clade. D8a, D8b and D8d and were found to emerge in the 1990s. Mean rate of evolution for both D4 and D8 genotypes was similar (12.791 × 10-4 and 11.25 ×10-4 subs/site/year for D4 and D8 respectively). Thus the studies could help identify the timescales of introduction and evolution of the dominant Measles genotypes in India.

Miscellaneous work

Comparative analyses of RNA polymerase protein subunits PA, PB1 and PB2 from low pathogenic Avian Influenza viruses (H9N2)

***Contributors**: P Shil, J Mullick & R Satange*

Polymerase proteins are important for the replication of AI virus. It is hypothesized that differences in polymerase complex may be linked to efficacy of replication of the virus strains and hence may affect pathogenicity.PA, PB1 and PB2 protein sequences were compared between two strains of low pathogenic AI H9N2 viruses. The two sequences, H9N2 WB (West Bengal 2008) and H9N2 PU (Pune 2011), showed different response in replication kinetics. Sequence alignments and domain mapping showed the occurrence of mutations in the binding interfaces of PA and PB1 subunits. Molecular docking was performed to generate PA-PB1, PB1-PB2

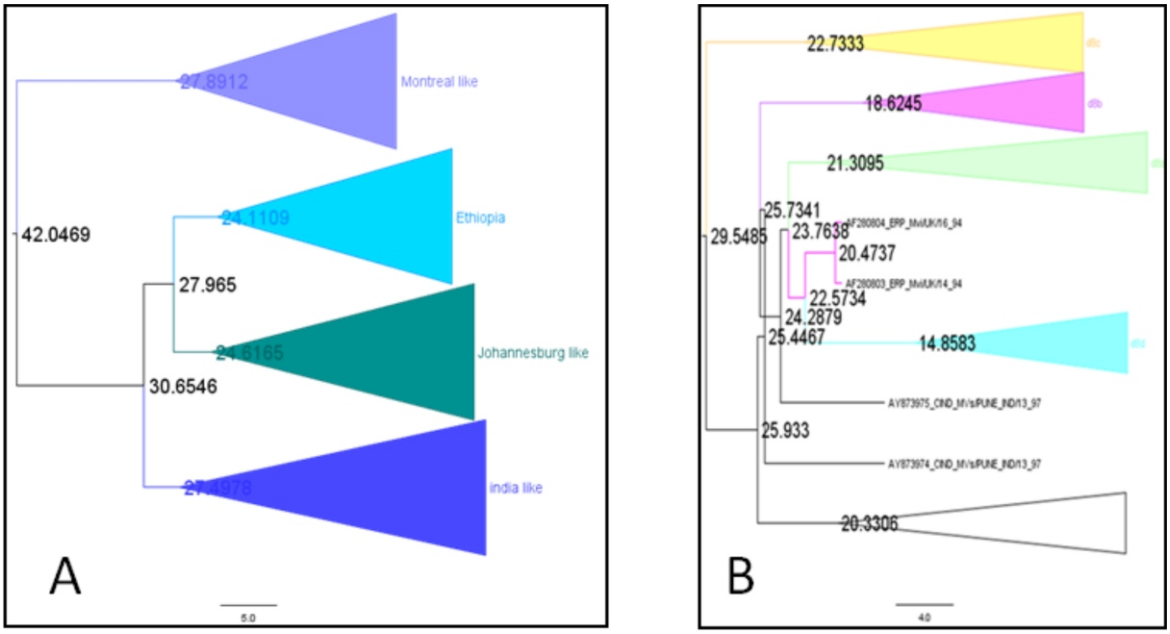

Fig. 3: Maximum clade credibility tree of the 'N' gene sequences of Measles genotypes (a) D4 and (b) D8 showing divergence times at key nodes

complexes from the generated 3D models of the proteins based on hydrogen bonding, hydrophobic interactions, hydropathy analyses etc. From the minimized energy it can be stated that the H9N2 WB PB1-PB2 complex is more stable than that of H9N2 PU. These changes in binding ability and stability of the PB1-PB2 complex may be attributed to: a) mutations D725E, R727A, F730Y in the PB2 binding of PB1 protein; b) differences in electrostatic surface charge distribution between C terminal of H9N2PU and H9N2WB; c) increase in hydrophobicity of H9N2 WB strain. Experimental work involved cloning of the polymerase sub-unit PA from the strains. Based on the outcome efforts to develop functional polymerase assay can be made in future.

ANIMAL HOUSE GROUP

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Support Staff (Adhoc)

Mr. A.S. Shinde

Mr. M.S. Parshuram

AHG1501: Breeding, supply and maintenance of laboratory animals.

***Investigators**: DR Patil & S Nipunage*

Funding Agency: Intramural

Project duration: Ongoing

Animal facilities at the institute registered with CPCSEA (No.43/GO/ReBi/SL/99/CPCSEA), includes, mouse breeding facility, primate enclosure, experimental small animal facilities. Seven different mouse strains (inbred, outbreed and immunodeficient) are bred and maintained in IVC system at the facility for experimentation, while other animals *i.e.* macaques, guinea pig, rabbit, fowl, turkey are procured for experiments from CPCSEA registered suppliers under IAEC approved research protocols. During the year, a total of 2130 laboratory animals were supplied to institutional scientists against 29 approved research projects (Table 1). A total of 910 ml blood from different species of laboratory animals, as diagnostic reagent in various assays was supplied to institutional scientists (Table 2).

Major activities:

(i) Mouse breeding

Mouse breeding program was planned scientifically to minimize generation of surplus animals. Breeding performance parameters and pedigree records were meticulously maintained. Selection of breeders and lines was done carefully to match with the reported breeding data for the strains. Randomly sampled mice from different strains were tested for genetic purity and important rodent pathogens at ACTREC, Navi Mumbai. Mice fecal samples were tested biannually to rule out the presence of any parasites. Biannual testing of consumables like mouse feed, bedding and drinking water for microbial and chemical quality was done to ensure the quality and sterility of supplies.

(ii) Health check up of staff

Health check-up of the staff was conducted through NABH accredited hospital, which included, hemogram, clinical chemistry, ECG, CXR, eye check-up and general check-up by physician. Duties were assigned to the staff, keeping in view the health profile, ergonomic and occupational hazards. Records maintained at the facility includes, livestock register, breeding records, microbial and genetic monitoring of animals, staff health records, consumable testing records, pedigree, individual project files and form D (animal utilization), AMC of equipments etc.

(iii) Maintenance of Primate facility

During the year, 41 rhesus macaques were housed at the eco friendly enclosure for non-human primates at MCC, Pashan. All the necessary tasks pertaining to, crucial aspects in management of primate enclosures, such as husbandry, nutrition, veterinary care, preventive veterinary care, occupational health and safety, enrichment and regulatory compliance were accomplished in scheduled manner. Daily observation and prompt separation of injured /sick animals and their treatment was meticulously followed. Preventive care included prophylactic de-worming treatment and comprehensive health monitoring program through NABL accredited laboratory, which included, complete blood count, differential count, peripheral blood smear examination, hepatic profile, kidney profile, lipid profile, Chest X-ray, intradermal tuberculin test. Results of all the test parameters were found within the literature normal range for the species. Social enrichment for the primates was ensured by careful observations on the compatibility and fighting incidences in the colony. Nutritional enrichment was provided by ensuring supply of seasonally available variety vegetables and fruits and also vitamins and mineral supplements through biscuits and bananas. Regulatory compliance was meticulously followed. Health records of individual animals were maintained. Primate facility inspection and verification of records was done by IAEC during the year and the report was sent to CPCSEA by nominee. Besides institutional care program for primates, services of consultant veterinarian were also sought.

Quarantine procedure for 6 rhesus macaques was completed during the report period and the animals were transferred for experiment in BSL-3 laboratory under the project “Attempts to transmit putative Gorakhpur agent in Rhesus monkeys”.

(iv) Regulatory compliance in animal breeding and experimentation

In the capacity of member secretary, IAEC and in-charge animal facilities, regulatory compliance in animal breeding and experiments at the institute was ensured. Records pertaining to animal

experimentation such as, form B, form D, progress reports, animal requisitions, daily observation register for experimental animals, procedure logs were maintained. Similarly all breeding and supply related records as mentioned above were maintained. Animal facilities were inspected by the committee and various records pertaining to animal breeding and experiments were verified during the mandatory annual inspection meeting and the report was sent to CPCSEA by nominee.

Meeting was organized for animal protocol evaluation, in which, eleven projects were reviewed and approved by the committee. One project involving large animals was recommended by the IAEC, which eventually was submitted to CPCSEA for approval. CPCSEA, New Delhi replaced Shri. Sadhana Jayvant Patil as Socially Aware Nominee in place of Shri Nikunj Sharma.

(v) Biomedical waste records

Animal house group maintained records of quantity of biomedical waste of different categories, generated at NIV groups and submitted annual report for the period 1.1.2015 to 31.12.2015 to Maharashtra Pollution Control Board (MPCB) as mandatory compliance.

Table 1: Supply of Laboratory Animals to NIV Scientists (numbers)

CD1 Mice Infants*	CD1 Mice Adults	BALB/c Mice Adults	C57BL/6 Mice Adults	Total Numbers
1265	203	484	178	2130

(* For annual health check-up)

Table 2: Supply of Blood Samples to NIV Scientists

Guinea pig	Rabbit	Fowl	Turkey	Goose	Monkey	Total Quantity(ml)
83	215	30	355	17	210*	910

(* For annual health check-up)

DIAGNOSTIC VIROLOGY GROUP

Scientific Staff

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Mr. Pravin M Harpale	Technical Assistant (till31st January 2016)
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Mr. Nitin Mule	Technician C
Mrs. Kirtee Khutwad	Technician C
Mrs. Sarita Raut	Technician C
Mr. Shivshankar Gaikwad	Technician C
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DVG 1301: Resource Centre for Virus Diagnostic Laboratories (RCVDL) at NIV, Pune

***Investigators**: GN Sapkal, R Viswanathan & VA Potdar*

Funding Agency: ICMR

Duration: 2013-2016

A. Resource Centre for Virus Diagnostic Laboratories

RCVRDL has been developed in response to recommendation of virology task force monitoring the VRDL network, with an objective of providing training for different categories of VRDL network staff and conducting quality assurance (QA) and quality control (QC) programs for the network laboratories. During the previous two years, training modules on different aspects of virology were conducted. During the current year, DHR and VRDL monitoring committee, proposed modification of the program in order to expedite the VRDLs role in sample testing. Accordingly the training program was restructured into a consolidated module, to involve all the expected VRDL activities including biosafety, outbreak investigation (not conducted during year 2014-15), specimen management, ELISA, PCR and real time PCR (Table:1).

Training modules included lectures, case studies, demonstrations and hands on practicals. Pre and post assessment tests were also conducted. Feedback was invited from participants and PIs after circulation of training modules. This feedback was taken into account while preparing subsequent training modules.

B. Response to national emergency

In response to directive from Secretary, DHR to strengthen 20 identified VRDLs for Zika virus (ZIKV) diagnosis, training program for ZIKV diagnosis was conducted in February 2016 (Table:1). In the first phase, 10 identified VRDLs were 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.

A total of 221 dengue and/ or chikungunya IgM negative samples received from 08 VRDLs were tested for ZIKV diagnosis by PCR at RCVRDL. All the samples tested negative for Zika virus and the results were shared with respective VRDLs and ICMR.

C. Quality control program for VRDLs

Quality control program for the VRDL network is ongoing. A total of 583 samples were tested (Dengue IgM 206, Dengue NS1-102, CHIK IgM-64, JE IgM-165, Measles IgM-40, HCV & HBsAg-06). Overall concordance of 80% was observed when compared with VRDL test results. Discordance (20%) observed in quality control testing was mostly, due to the use of other than the recommended kits for VRDLs. The Resource Centre has developed documents for VRDL network: "ICMR/DHR Information booklet for the network of virus research and diagnostic laboratories (VRDLs)" and resource material for all training modules.

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

***Investigators**: GN Sapkal, GN Deshpande, PD Yadav, SM Vidhate, O Kaduskar & SA Gaikwad.*

Duration: 2015-18

Funding: Extramural, DHR.

Background

KFD is a public health problem and endemic in a few districts of Karnataka, but showing geographical expansion to neighboring states. Despite routine vaccination, recent reports suggested an increasing number of cases, necessitates the need of developing a rapid, sensitive and specific diagnostic system for detection in infected patients. The utility of polyclonal immune sera in diagnostic assays may reduce the sensitivity of the assay whereas monoclonal antibodies (MAbs) will reduce lot to lot variation and increase the sensitivity and specificity. Hence the current study.

Objectives

- To generate and characterize monoclonal antibodies against KFDV
- Replace polyclonal antibody based probes with monoclonal antibody in KFDV diagnosis

Work done

(i) KFDV Stock Preparation

Experiments with live KFDV were performed in the BSL-4 facility. Mouse brain suspension of KFDV stock was prepared, inactivated by gamma-radiation and infectivity was checked by inoculating the virus suspension on a baby hamster kidney cells for 3 blind passages. After confirmation of the loss of virulence, the stock was handled at BSL-2 facility for further experiments.

(ii) KFDV Hybridoma generation

The inactivated KFD virus stock was used for female BALB/c mice (3-4 week old) immunization. On day 0, 0.5 ml of inactivated virus was administered through Intraperitoneal (i.p) inoculation along with Fruends complete adjuvant. This was followed by three doses (one week apart) of inactivated KFDV mixed with Fruends incomplete adjuvant. A booster of 20μg of purified KFDV antigen was administered on day 28 intravenously. The mouse was sacrificed for fusion on day 32.

The monoclonal antibody (MAb) secreting hybrids were generated using the spleen cells from immunized animals and fusing them with the myeloma (SP2/O) cells as per the method described earlier (Sapkal et al, 2011). The hybridoma cells were cloned by limiting dilution and screened using ELISA on purified KFDV antigen. Pristane-primed BALB/c mice were injected with hybridoma cells to obtain high titer MAbs in ascetic fluid.

The specificity of MAbs on KFDV protein was characterized by western blotting and further by immunofluorescence assays (IFA) using standard protocols. The isotype of MAbs were determined using a commercial kit.

(iii) KFDV Enzyme linked immunosorbent assay (ELISA) optimization

KFDV ELISA using inactivated KFDV antigen was optimized with known positive and negative sera for replacing polyclonal antibody based probes with monoclonal antibody in KFDV diagnosis.

Findings

Four IgG MAbs secreting hybridomas were generated and found to be reactive with KFDV Envelop protein (E protein). The specificity and reactivity was confirmed by ELISA, western blot and IFA. These MAbs were purified, labeled and replaced in the existing KFD IgM ELISA.

Summary

In the earlier period, 6 anti KFDV MAbs generated were found to be of IgM isotype. Hence, with few modifications in the immunization protocol, another four anti KFDV MAbs were developed during this period and all four MAbs were found to be IgG isotypes. Also they were reactive with KFDV Envelop protein (E protein). These MAbs were purified, labeled and replaced in the existing KFD IgM ELISA. Further optimization work is in progress.

DVG 1403: Infection dynamics of congenital cytomegalovirus in neonates in Pune, Maharashtra

***Investigators**: R Viswanathan, GN Gajanan,*

Funding: Intramural

Duration: 2015-2018

Background:

Cytomegalovirus (CMV) is one of the most prevalent causes of congenital infections affecting 1-2% of all live births. It is the leading infectious cause of mental retardation and sensorineural deafness. Mother-to-child CMV transmission may be due to primary maternal infection during pregnancy or following non-primary infection. There is no data regarding prevalence of congenital CMV from western India. Also, high rates of congenital CMV infection have been consistently demonstrated in populations with a high seroprevalence suggesting the role of re-activation.

Objectives

- To determine Seroprevalence and reactivation of CMV in antenatal mothers at study site
- To determine prevalence of congenital CMV in neonates born at study site
- To determine CMV etiology in clinically suspected cases of CMV; 3 weeks age referred to study site.
- Follow up of infected babies for clinical spectrum of disease

Work Done

Recruitment, sampling and follow up of pregnant women are ongoing. So far 165 maternal sera have been collected after counseling and taking of informed consent. Urine samples for detection of viral DNA have been collected and methodology for processing is being optimized. Follow up samples of 25 babies have been collected. Standardization of real time PCR for screening of congenital CMV infection has been done. Newborn screening has been initiated at three study sites (n=469). Samples of clinically suspected cases of congenital CMV infection are referred for testing by ELISA and real time PCR.

Findings

Out of 165 pregnant women, 163 (98.7%) were anti CMV IgG positive. all of which were negative for CMV. All follow up babies (n-= 469) screened so far has been found negative for CMV, but five babies with suspected congenital CMV infection were detected as anti CMV IgM positive. Further confirmation is ongoing. All the five babies are under follow up with clinical collaborators.

Summary

Majority of women tested are seropositive to CMV. Screening of healthy newborns was found negative for CMV. CMV antibodies were detected in five clinically suspected babies.

TRANSLATIONAL RESEARCH GROUP

Co-coordinators

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Dr. Kavita S. Lole, Scientist E and Group Leader, HEP

Dr. Gajanan N. Sapkal, Scientist D and Group Leader, DVG

Dr. AB Sudeep, Scientist D, Medical Entomology& Zoology

Scientific staff

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Mrs. Rashmi S. Gunjikar	Technical Assistant
Mrs. Rashi Srivastava	Technical Assistant

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

***Investigators**: T Deshmukh & K Lole*

Hepatitis E is a major public health problem endemic to India. Components of in house HEV diagnostic ELISA showed limited stability. Hence a new ELISA in the form of a ready-to-use kit (NIV HEV IgM ELISA) has been developed and optimized to TMB, replacing OPD substrate. Assay limits were reset using sensitivity and specificity serum panels (n=90 each). Performance testing of the ELISA was carried out at 4°C, 25°C and 37°C for 33 weeks (48 ELISA kits were prepared for this purpose). Performance of kit was acceptable up to ~5 weeks post preparation at 25°C and 37°C. At 4°C, kit performance was acceptable throughout the study period. Performance of NIV HEV IgM ELISA was compared with commercial MP Diagnostics ELISA (Singapore) and concordance, sensitivity, specificity were estimated using 75 serum samples. A concordance of 93.3% (”=0.86, P<0.01) was noted between the two assays with NIV assay showing sensitivity of 95% and specificity of 92%. In use performance testing of the ELISA was performed with kits stored at 4°C between July-November, 2015 using 118 referred patient’s serum samples and 6 kits. A single kit was used over a period of 7 to 20 days and for 4 to 8 times; performance was found acceptable and satisfactory.

Evaluation of certain compounds for inactivation and stabilization of viruses of public health importance

***Investigators**: DT Mourya, GN Sapkal, PD Yadav, R Gunjikar, R Shejwalkar, K Khutwad*

Emerging and re-emerging viral infections are important public health concern worldwide. Viruses with epidemic potential often take a heavy toll of human and animal life by rapidly spreading across borders. They have emerged in the past globally including India thus imposing a huge burden on healthcare services. India has also witnessed several such viral outbreaks of public health importance that remained undiagnosed. Outbreaks due to respiratory viruses such as SARS (2003), avian influenza (2005) and the H1N1 pandemic (2009), H7N9 (2012) have been experienced. While dengue is endemic to India, several epidemics of newer zoonotic viral infections *viz.,* CCHF, KFD etc are showing geographic expansion.

The high risk associated with clinical samples obtained for diagnostics necessitates their handling and processing in specialized containment 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. The containment laboratories are complex, very expensive to operate and needs to be staffed by expert personnel. Indian Council of Medical Research (ICMR) network and the institutes supported by the Department of Health Research (DHR) have already established sixteen BSL-2/BSL-3 laboratories to deal with many pathogenic agents of public health importance (Mourya et al., 2014). However, 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.

We propose a rapid, reliable and simple method for the complete inactivation of various viruses by using chaotropic agents. These samples can be used for subsequent diagnostics. These inactivation protocols will facilitate the diagnostic examination of these emerging and re-emerging viruses by applying standard laboratory conditions at BSL-2 level.

Chaotropic agents in various combinations and concentrations were used in the study. The non-toxic concentrations of chaotropic agent along with stabilizers were assessed for loss of infectivity in tissue culture. The time kinetics study was performed to check the loss of virus infectivity in baby hamster kidney cells and presence of viral genome was detected using RT-PCR.

Following laboratory adapted viruses were used for the experiments. Viruses were obtained from Virus Registry, National Institute of virology, Pune. These viruses were propagated in BHK-21 cell line. Titration of the virus stock was carried in BHK cells by plaque assay. The virus titer was determined as plaque forming units per milliliter (pfu/ml) or Tissue culture infective dose 50($TCID_{50}$/ml) to calculate multiplicity of infection (MOI) in infection experiments.

Table: Proposed list of viruses to be inactivated

Family virus	Representative level	Containment
Flaviviridae	Japanese encephalitis (JEV)	BSL-2
	Kyasanur Forest Disease (KFD)	BSL-3
Alphaviridae	Chikungunya (CHIK)	BSL-2
Rhabdoviridae	Chandipura (CHPV)	BSL-2

Chaotropic agents with different stabilizers showed inactivation of above mentioned viruses (Fig 1). The laboratory experiments with spiked virus in gel tube and EDTA tubes also showed virus inactivation. Further the viruses could be detected in PCR indicating no interference in diagnostic detection. The detection of CHPV diagnostic PCR is depicted (Fig 2)

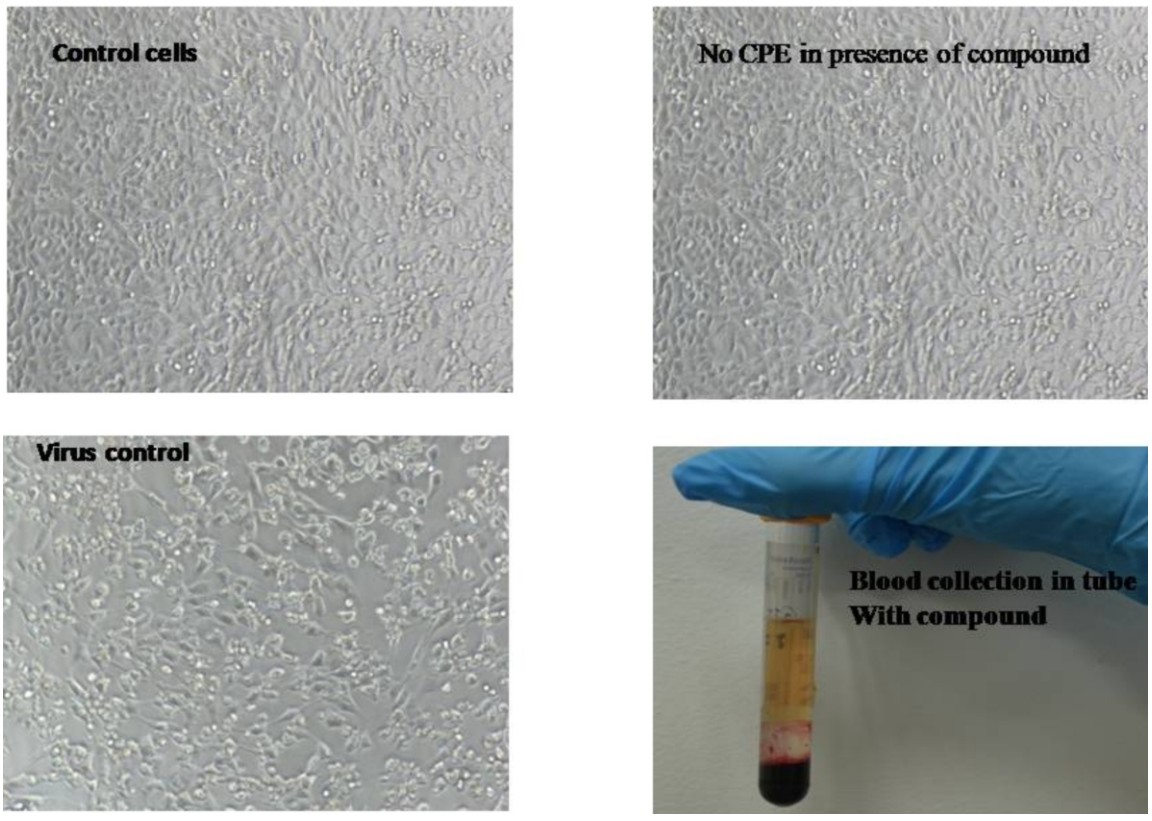
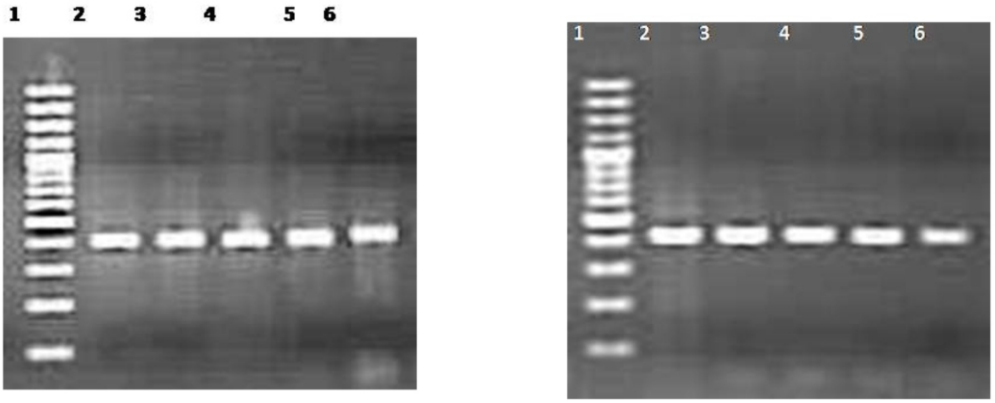


Fig 1: Loss of virulence of virus in the presence of compound



Lane	Sample (Expt at 4°C)
1	100 bp ladder
2	G+2+3 OHr
3	G+2+3 8Hr
4	G+2+3 24 Hr
5	G+2+3 48 Hr
6	Virus control

Lane	Sample (Expt done at RT)
1	100 bp ladder
2	G+2+3 0Hr
3	G+2+3 8Hr
4	G+2+3 24 Hr
5	G+2+3 48 Hr
6	Virus control

Fig 2: Diagnostic PCR showing the reactogenicity of inactivated CHPV

Few combinations of inactivating agents tested at laboratory levels were selected for manufacturing vaccutainers. The ethical committee, Jahangir hospital, Pune has approved the proposal. Further studies are in progress.

Products developed

A. Three ELISA kits were developed for viruses of public health concern and transferred to M/S. Zydus Cadila, Ahmedabad

- 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 kit for diagnosis of hepatitis E

B. Technology for development of inactivated vaccine against Japanese encephalitis virus

HBL Biotech, (Chennai) has established a GLP facility at TICEL Biopark for manufacturing viral vaccines. In this connection NIV was requested to transfer a candidate Japanese encephalitis virus vaccine (JEV) strain for inactivated vaccine development. A MEMORANDUM OF UNDERSTANDING is signed with HBL Biotech for development of JEV. The technology for inactivated JEV vaccine and a candidate vaccine strain was transferred to HBL, Chennai.

Diagnostic Reagent Facility: Generation of anti-Chikungunya E reactive Monoclonal antibody (MAb)

GN Sapkal, PS Shah, SM Vidhate, GN Deshpande, Ojas Kaduskar, S Gaikwad

In-house IgM capture enzyme-linked immunosorbent assays (ELISA) developed by NIV is one of the major diagnostic tools for CHIKV diagnosis in the country. In order to assist in trouble shooting of anti-Chikungunya (CHIK) IgM ELISA, five new monoclonal antibodies reactive against E1/E2 protein was generated. The utility of these MAbs were successfully replaced in the existing ELISA kits.

Development of anti West Nile virus monoclonal antibodies against non structural protein 1 (NS1)

GN Sapkal, B. Anukumar, GN Deshpande, SM Vidhate, K Khutwad, R Gunjikar

Flavivirus NS1 is a non-structural glycoprotein that is expressed on the cell surface and secreted into the extracellular space. Specifically in WNV, NS1 expression starts at early time points *i.e.,* between 16 h to 24 h PI. In the present work three anti WNV NS1 MAbs were generated. The results indicated that MAbs can also detect native NS1 of WNV and reactive in ELISA. So, to identify the reactivity of generated MAbs with surface expressed NS1, immunofluorescence assay was performed and observations indicated strong reactivity (Fig 3). These MAbs will be used for development of WNV NS1 capture ELISA.

Patents Filed

Technology for concentration and testing of waterborne viruses in drinking water.

Complete patent filed (Application No. 160/MUM/201): Composition for virus precipitation from dilute samples and a method thereof. The Patent of addition for detection of Avian Influenza viruses using same technology is drafted and ready for submission.

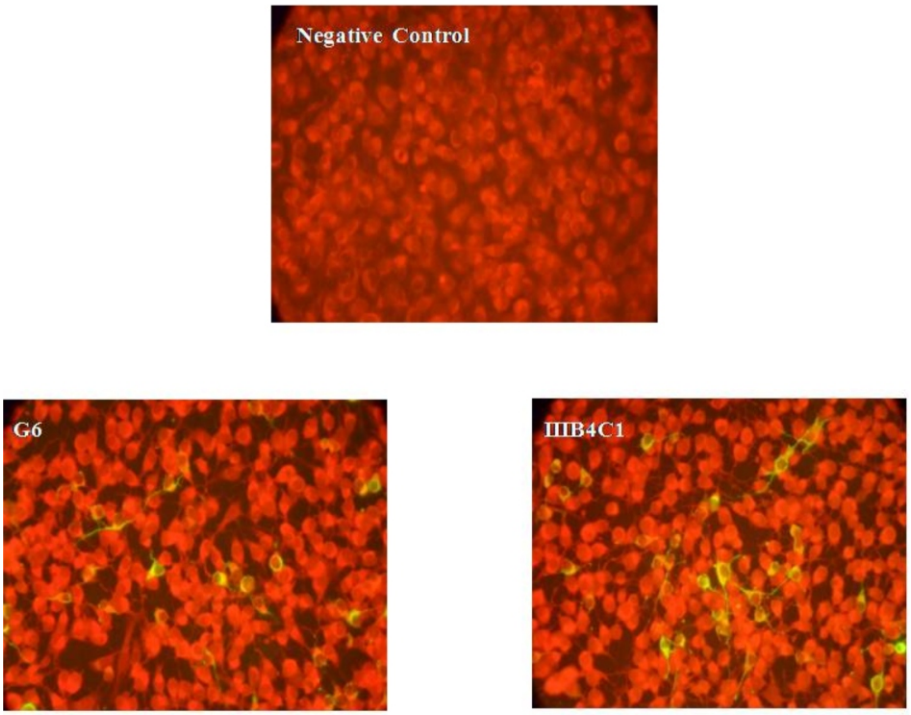


Fig 3: Reactivity of anti WNV NS1 MAbs in surface immunofluorescence

GORAKHPUR UNIT

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Dr. A. K. Agrawal, Head, Dept. of Pathology, Gorakhnath Hospital, Gorakhpur.

GKP1001: Diagnostic services for suspected Japanese encephalitis cases from Eastern Uttar Pradesh.

***Investigators**: VP Bondre, G Patil, V Nagose, D Hirawati, V Janardhan, K Sah, NM Rao, A Waghmare, AK Pandey and M Mittal*

Funding: intramural

Duration: Ongoing

During 2015, a total of 1760 clinically suspected acute encephalitis syndrome (AES) cases were hospitalized in BRD Medical College, Gorakhpur. Clinical specimens (CSF and blood) collected from 1292 cases were screened for JE infection using IgM ELISA kit. Antibodies were detected in 129 cases (9.98%). Both CSF and sera were positive from 46 cases while only CSF or sera were tested positive from 12 and 71 cases respectively.. Highest number of JE cases was reported from Kushinagar district followed by Gorakhpur, Deoria, Maharajganj, Siddharthanagar, Kabirnagarand adjoining districts of Bihar. Though JEV activity was documented throughout the year in eastern UP, maximum number of JE cases was documented during August – October 2015 (Fig. 1). All the JE positive cases belong to the rural settings and the average duration between onsets of symptoms to hospitalization was higher (8 days) in pediatric cases than the adults (5-6 days). More severe neurological complications were documented in JE cases than overall AES cases. Hepatic involvement was documented in a subset of pediatric JE cases which was absent in adults while meningeal signs were recorded in higher number of adult cases Radiological investigations of available JE cases revealed substantial involvement of bilateral thalamus, basal ganglia and cerebral parenchyma. Diagnostic services for referred JE and AES cases from Gorakhpur, Maharajganj, Pipraich, Campiarganj, Gola and Bhathat blocks hospitalized in District and Army Hospitals, Gorakhpur was provided. Altogether, JE was documented in <10% AES cases.

GKP1502: Etiologic investigations inhuman clinical specimens collected from acute encephalitis syndrome (AES) cases from Eastern Uttar Pradesh.

***Investigators**: VP Bondre, SP Behera, D Hirawati, BR Mishra, K Sanjeev, NM Rao, K Neeraj, A Kavathekar & G Patil*

Funding: Intramural

Duration: 2015-2017

Background and objective

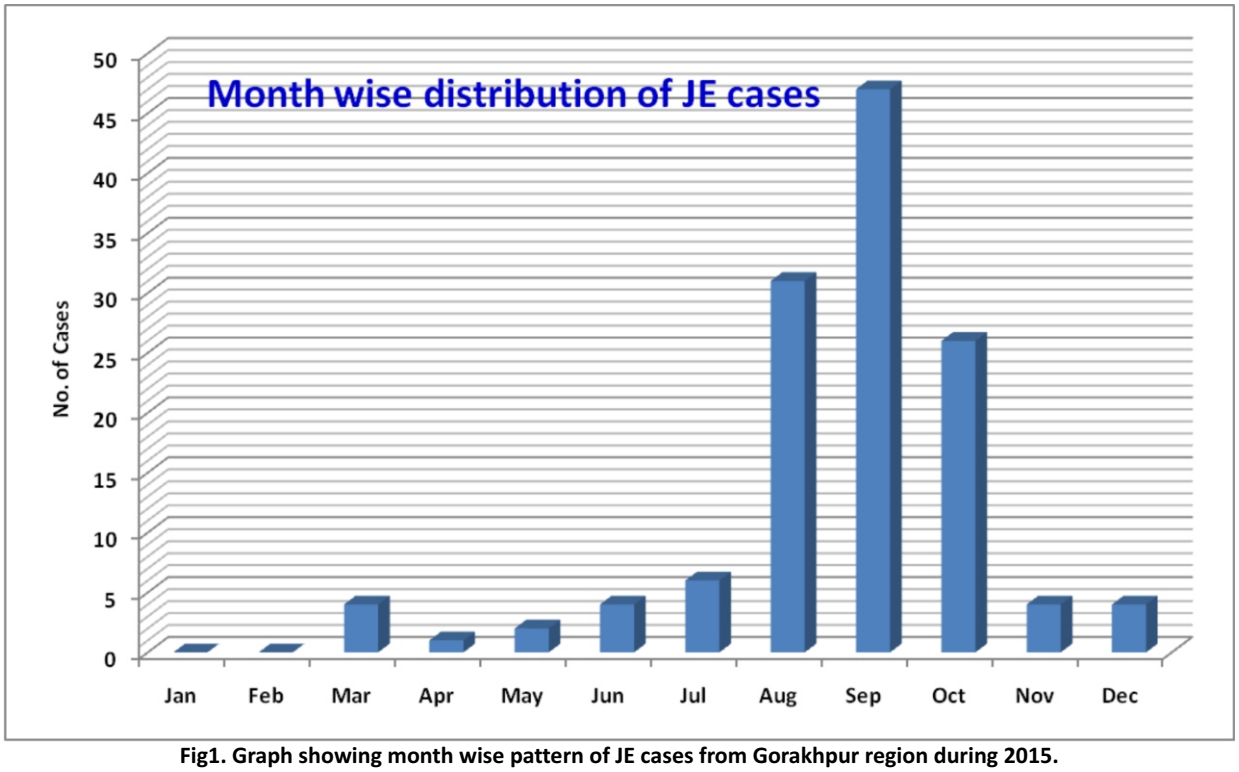


Fig1. Graph showing month wise pattern of JE cases from Gorakhpur region during 2015.

Etiology of AES remains unknown. While several factors like suspected infections have been suggested, the present study was undertaken for in-depth examination of the possible causes of AES in eastern UP.

Work done

During 2015, clinical specimens (CSF and blood, only CSF or only blood) from 1292 AES cases were investigated for detection of anti-JE IgM antibodies. Based on clinical history, seasonality, age group, window period between onset of symptoms to specimen collection, hematological and biochemical findings, the JE negative specimens from 772 AES cases (610 CSF and 200 blood clot / sera from 610 cases and only sera from another 162 AES cases) were screened to detect other viral and non-viral etiologies associated with CNS infections. Processing of anti-JE IgM antibodies negative sera from 450 AES cases with neuronal involvement, rash, thrombocytopenia and raised liver markers resulted in the detection of anti-dengue IgM antibodies in 3/450 AES cases.

Clinical specimens from cases suspected of viral encephalitis were processed to detect neurotropic virus infections. The CSF specimens were tested by RT-PCR / PCR for diagnosis of Enteroviruses, flaviviruses and alphaviruses using generic primers while specimens were individually tested for diagnosis of Herpes simplex virus 1, 2, 6, 7 and 8, Varicella Zoster virus and dengue virus. Specimens were also tested for diagnosis of Measles, Mumps, Rubella, Parvo (B19 and P4), Bagaza and Zika viruses.

Specimens were also tested for a battery of bacterial infections *viz*. *Streptococcus pneumonia, Neisseria meningitides, Heamophilus influenza* by PCR. CSF and blood clot specimens from patients having history of fever for >8 days, multiple organ failure, elevated levels of renal and hepatic markers and increased C-reactive protein were tested for diagnosis of *Orientia tsutsugamushi* by PCR. The

Table 1: Summary of findings of AES cases .

Sr. #	Organism	Specimen	Assay	No of samples	No. +ve & tested % positivity
1.	Herpes simplex virus 1 & 2	CSF	PCR	337	6 (4.37%)
2.	Herpes simplex virus 6, 7 & 8	CSF	PCR	136	Nil
3.	Varicella Zoster virus	CSF	PCR	180	6 (3.33%)
4.	Enterovirus	CSF	RT-PCR	156	Nil
5.	Mumps virus	CSF	RT-PCR	400	Nil
6.	Measles virus	CSF	RT-PCR	400	Nil
7.	Rubella virus	CSF	RT-PCR	400	Nil
8.	Parvo virus B19	CSF and Sera	PCR	200 (CSF and Sera)	4 (1 CSF, 3 serum)(2%)
9.	Parvo virus B4	CSF and Sera	PCR	200 (CSF and Sera)	25 (8 CSF, 17 sera) (12.5%)
10.	Zika Virus	CSF	RT-PCR	60	Nil
11.	Bagaza virus	CSF	RT-PCR	80	Nil
12.	Flavivirus Generic	CSF	RT-PCR	400	12 (3%)
13.	Dengue virus	Sera	NS1 ELISA	209	12 (5.74%)

results of lab tests of AES cases are given in Table 1. In addition to it, diagnosis of clinical specimens by different lab tests including CSF culture detected tubercular meningitis (15), Klebsiella spp (03), Staphylococcus aureus (13), S. pneumonia (04), Entercoccus spp (01), Acinetobacter spp (04), Citrobacterspp (01), Gram + cocci (01) and Candida spp (02).

Summary

A broad spectrum of different organisms could be detected in the specimens tested. However, none of these can be conclusively associated as etiologic agent for AES. Further studies are ongoing.

GKP1503: Epidemiological and clinical correlation of acute encephalitis syndrome cases with JE, non-JE viral and other AES associated etiologies from eastern Uttar Pradesh.

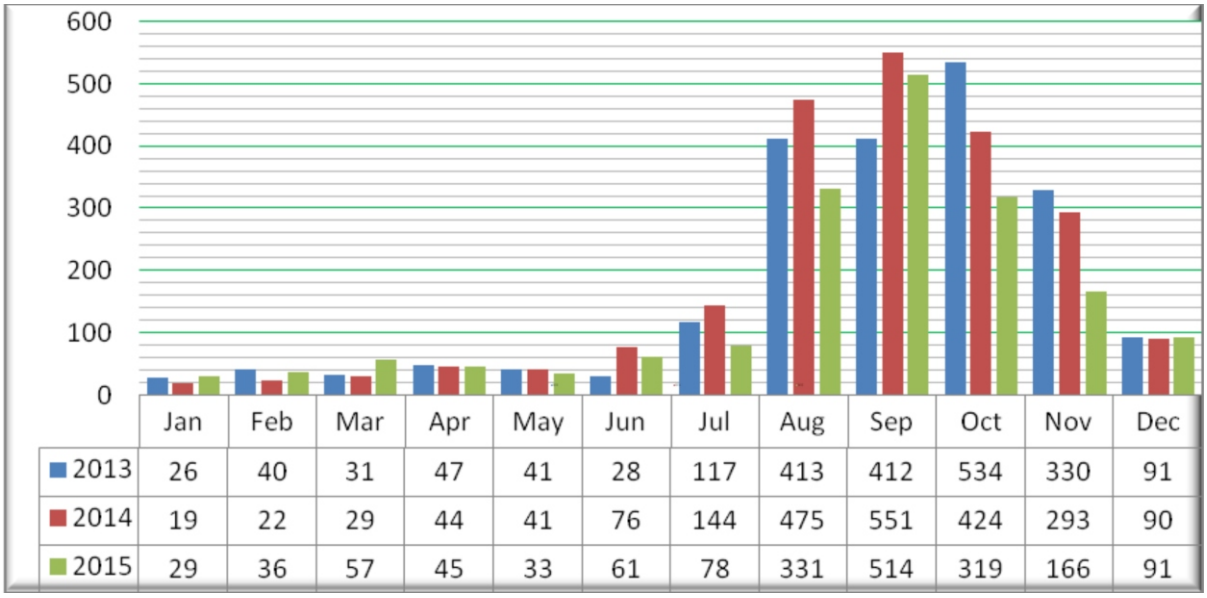
***Investigator**: VP Bondre, BR Mishra, M Mittal, K Neeraj, A Waghmare, A Kavathekar, V Nagose, RS Singh & D Hirawati*

Funding: Intramural

Duration: 2015-2017

Among the 1760 AES cases investigated during 2015, 1581 (90%) cases were children < 15 years of age and approx. 80% of them were hospitalized during July-November 2015. Despite substantial climatic variations documented in the region during this year, the seasonality pattern of AES cases hospitalized during the last 3 years (2012-15) was almost similar (Fig 2). Average period between the appearances of clinical symptoms to hospitalization was 7.8 days and the case fatality rate was 25.5%. There is no visible gender bias as male (918) to female (818) ratio. Based on the clinical and biochemical histories, cases were classified as confirmed JE (IgM positive), non-JE viral encephalitis and other causes of AES [respiratory failure, pneumonia (aspiration/ventilator associated), disseminated intravascular coagulation, protein energy malnutrition, tubercular meningitis, enteric fever, malaria, peripheral ventricular failure] etc. Routine follow up of >1500 line listed AES cases marked the presence of high-grade fever as the primary clinical symptom followed by neuronal symptoms in majority cases.

In all AES cases investigated during 2015, neurological symptoms included altered sesnorium (81%), seizures (45%), hypertonia (40%), general symptoms including vomiting (47.6%), headache (13.6%) abdominal pain (14%), laboratory findings increased (>13000 cells/mm3) total blood leukocyte count (45%) and thrombocytopenia (34%). An increase in CSF glucose level (>75mg/dl) in 27% and



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2013	26	40	31	47	41	28	117	413	412	534	330	91
2014	19	22	29	44	41	76	144	475	551	424	293	90
2015	29	36	57	45	33	61	78	331	514	319	166	91

Fig 2: Graph showing month wise seasonality pattern of AES cases from eastern Uttar Pradesh during the last three years (2013-2015).

CSF protein (>45mg/dl) in 52% of cases were observed . CSF pleocytosis was noted in 57% cases. In majority of JE positive cases, CSF protein (46.2 mg/dl mean) and glucose (67 mg/dl mean) were found under normal range. Elevated serum SGOT and SGPT indicate hepatic involvement in 49% cases Meningeal signs were recorded in 13% of JE positive cases and 15% of non-JE AES cases. Anemia was recorded in 50% JE positive cases while moderate to mild thrombocytopenia was recorded in 38% cases.

GKP1504: Isolation, identification and genetic characterization of viruses isolated from AES cases from eastern Uttar Pradesh.

***Investigators**: VP Bondre, D Hirawati, V Janardhan, K Sah, RS Singh, SP Behera, BR Misra, V Nagose, G Patil, K Sanjeev and K Neeraj*

Funding: Intramural

Duration: 2015-2017

Background

To improve upon the diagnosis of AES, 400 CSF specimens collected from suspected viral encephalitis cases classified on the basis of clinical and biochemical data analysis were processed for virus isolation in Porcine Stable kidney (PS) cells. While selection of the specimens, emphasis was given to the specimens collected during early phase of infection (hospitalized within 4-7 days of clinical symptom appearance) and meeting the AES case definition.

Objectives

- Isolation of viruses from AES cases from Gorakhpur

Work done

Three consecutive passages were made in PS cells, cultures showing CPE in P3 (n=11) were passed for 2 more passages and total nucleic acid was extracted for RT-PCR for detection of different encephalitis causing viruses.

Findings

The isolates tested negative for JEV, WNV, Dengue, Mumps, Measles, Rubella, Adeno, Influenza A / B, Parecho, Human Herpes virus 1, 2, 6, 7, 8, Epstein-Barr and Cytomegaloviruses by PCR. They were also tested negative for flavi, alpha, entero and bunya viruses by generic RT-PCR.

Summary

Since, these virus isolates are obtained directly from the CSF of AES cases accurate identification will contribute towards understanding the etiology of AES.

GKP1505 Laboratory conformation of dengue virus infection in referred cases from Gorakhpur region.

***Investigators**: D Hirawati, BR Mishra, V Janardhan, SP Behera, AK Agrawal, K Sah, RS Singh, V Nagose, K Sanjeev & VP Bondre*

Funding: Intramural

Duration: 2015-2017

Background

During September 2015, an increase in dengue fever (DF) cases was documented in Gorakhpur region. The laboratory confirmation of suspected viral infection was much needed.

Objective

- Testing of referred samples from suspected dengue and other febrile illness cases.

Work done

Blood samples were collected from 155 clinically suspected hospitalized cases (Gorakhnath Hospital), screened using NS1 detection kit (J. Mitra diagnostics) and found positive for DF. Further analysis is in progress.

Findings

Most of the DF cases were from Gorakhpur, Maharajganj, Kushinagar, Deoria, Basti and adjoining districts of Bihar. Adults of the age group 21-45 years were mostly affected with clinical symptoms such as fever, headache, chills, nausea/vomiting, abdominal pain, arthralgia and myalgia. Rash was documented only in 4.5% cases. Biochemical and haematological analysis of cases were indicative of thrombocytopenia and compromised liver functions. Dengue (DEN) IgM ELISA detected

antibodies in 81/155 (52.2%) specimens while diagnostic RT-PCR detected viral genome in 110/155 (70.9%) cases. Typing of dengue virus by RT-PCR amplification suggested prevalence of dengue 2-virus serotype. Amplification of a 1700 nucleotide product spanning complete envelope protein coding region from 44 blood specimens using type 2 specific RT-PCR further confirmed these findings.

Summary

Inoculation of available 150 sera in PS cells for 4 consecutive passages resulted in isolation of virus from 7 specimens. Typing of these isolates confirmed isolation of 6 DEN-2 virus strains. Further sequence analysis and isolation work is in progress.

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Summary of samples received for viral diagnosis in NIV, BU

During the period, 15694 samples received from 10289 cases for different viral diagnosis. Under the WHO programs viz. (i) surveillance of acute flaccid paralysis (AFP) cases from Karnataka, Kerala and southern parts of Bihar (10191 samples from 5271 cases) and (ii) surveillance of Measles and Rubella cases from Karnataka, Kerala and Andhra Pradesh (538 samples from 454 cases)

Under NVBDCP program, 4735 samples from 4485 cases were received from Bangalore urban and neighboring areas for diagnosis of dengue, chikungunya and JE viruses. In addition, 149 samples from 29 retinitis cases; 39 samples from 13 cases from Mumps; 42 samples from respiratory virus infections and 37 samples from CMV cases were received. All the samples were processed for virus isolation (47%), IgM detection by ELISA (45%) and virus detection by RT-PCR (8%).

BNU9702: Studies on Poliovirus (1): Surveillance of AFP cases from Karnataka, Kerala and southern parts of Bihar as part of WHO-SEAR Polio Lab Network in the WHO's global eradication of poliomyelitis program.

***Investigators**: CG Raut & H Hanumaiah*

Duration: Ongoing

Funding: WHO

No wild poliovirus was isolated during the period, which confirmed the country polio free (P1) for five years. The global commission for Certification of Poliomyelitis Eradication (GCC) has declared that P2WPV (wild poliovirus) has been eradicated on 20th September 2015 as the last P2 WPV in the world was reported from Aligarh, UP, India in 1999. Similarly, last P3WPV was reported from Pakur district Jharkhand in 2010. However, 44 cases of vaccine derived poliovirus (VDPV) and two cases of P2 VDPVs have been reported in India during 2015 and 2016 respectively.

The Polio eradication endgame strategic plan 2013-2018 was developed to end all poliovirus disease. In India OPV2 containing vaccine will be withdrawn on 29th April 2016 and switched over to bOPV containing P1 and P3. The laboratory needs to implement Biorisk Management System as per Global Action Plan III for diagnostic facilities of all the sixteen elements of Annexure- 6 of GAPIII.

Environmental Sample surveillance

The laboratory has been identified for testing environmental sample concentrate from Hyderabad by the program. Accordingly one personnel has been trained at ERC Mumbai and the facility has been kept ready for testing.

Procurement of cell lines and their characterization

Under the GPEI, two cell lines *i.e.* L20B (P#20+1)and RD (P#231+1) were received from GSL-ERC Mumbai and Master cell bank (MCB) and Working cell bank (WCB) were prepared. Cover slip cultures were prepared, fixed using Carnoy's fixative and screened for mycoplasma using DAPI staining. The coverslip cultures were also sent to ERC Mumbai for external quality control (DAPI & PCR) and found concordant. The cultures were maintained in 25cm^2 flasks by sub-culturing thrice a week.

Virus isolation: During 2015-16, 10191 stool specimens from 5159 AFP cases were processed for virus isolation. The state wise receipt of AFP cases are Karnataka (958), Kerala (351), Bihar (3808) and 42 cases from other states (Andhra Pradesh, Goa, Haryana, J & K, Rajasthan, Jharkhand, Maharashtra, Odessa, Punjab, Tamil Nadu, Uttar Pradesh, West Bengal).

All the L20B cell line-grown virus isolates (484 poliovirus isolates) were referred to ITD Laboratory. Real time RT-PCR intratypic differentiation (ITD) assay and vaccine virus serotype wise VDPV assay are performed for ITD of polioviruses following WHO protocols and CDC supplied kits. ITD Laboratory was accredited by the WHO for the year 2013-14 and 2015.

Poliovirus isolates for sequencing & banking to GSL, ERC Mumbai:

A total of 470 VDPV isolates were sent to ERC Mumbai for virus banking. Fourteen discordant ITD isolates were sent for sequence confirmation and found 10 isolates belonging to P2 vaccine with one nucleotide change for two isolates; two nucleotide changes for three isolates; three nucleotide change

for one isolate and four nucleotide changes for four isolates. P2 vaccine isolates were not sent for banking since January 2016 as per National Polio Meeting 2016 recommendation and are retained at NIV BU.

Polio isolate on FTA card (WHO-Pilot study):

Polio isolate spotted on FTA card and sent to ERC Mumbai for pilot study. All results were tallied. Intratypic differentiation of poliovirus isolates from AFP cases received from National Polio Laboratory as part of WHO-SEAR Polio Lab Network in the WHO's Global Eradication of Poliomyelitis Program.

A total of 522 poliovirus isolates received and tested for ITD by rRT-PCR and VDPV rRT-PCR for confirmation of vaccine isolates following WHO protocols and CDC supplied kits. Result s of the study is given in Table 1.

BNU0603: Surveillance of Measles cases from Karnataka and Kerala as part of WHO-SEAR Measles Laboratory Network in the WHO's Global Measles Elimination Program.

During 2015-16, 454 serum specimens from 89 outbreaks received from 15 districts of Karnataka, 13 districts of Kerala and 1 district of Andhra Pradesh were processed for measles and rubella IgM. State

Table 1: ITD results of stool sample isolates from AFP cases.

	rRT-PCR Results				
Total No. of Isolates Tested	P1 Vaccine	P2 Vaccine (P2 Discordant: 10)	P3 Vaccine	Vaccine Mixtures (P1+P2=15 Discordant:1), (P1+P3=73), (P2+P3=10, Discordant:3) & (P1+P2+ P3=15)	NPEV
522	149	57	153	117	46

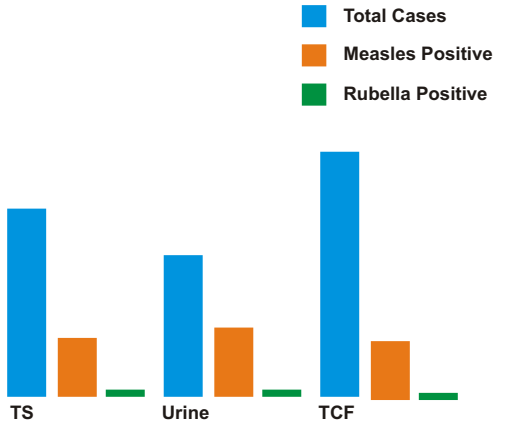
Table 2: State wise distribution of Measles/Rubella IgM positive cases.

Sample details		IgM results						Outbreaks				
State	No. of Samples	Me IgM POS	Me EQ	Ru total tested	Ru +ve	Ru EQ	Me/ Ru Both -ve	Total Out break	Me	Ru	Me + Ru	Both Neg
Karnataka	178	49	1	129	80	2	45	31	8	14	4	5
Kerala	271	117	3	157	103	2	51	57	23	18	14	3
AP	5	1	1	4	2	0	2	1	0	0	1	0
Total	454	167 (36.7%)	5	287	184 (64.1)	4	94 (20.7)	89	31	32	19	8

wise distribution of cases are given in Table 2.

Molecular detection of Measles / Rubella virus by RT-PCR:

Sixty-three specimens (27 urine and 36 throat swabs) received from different states of India were screened for measles and rubella by RT-PCR and sent to NIV for genotyping. The details regarding specimen and results for Measles/Rubella summarized in Fig 1. The samples were also processed for virus isolation and yielded 11 measles and 2 rubella isolates in Vero h/SLAM cell line (Fig 1). Genotyping of 33 measles positives demonstrated predominance of D8 (n=31) and 1 each for D4 and B3. Genotype distribution is given in Fig 2. Confirmed measles genotypes were deposited in NCBI database.



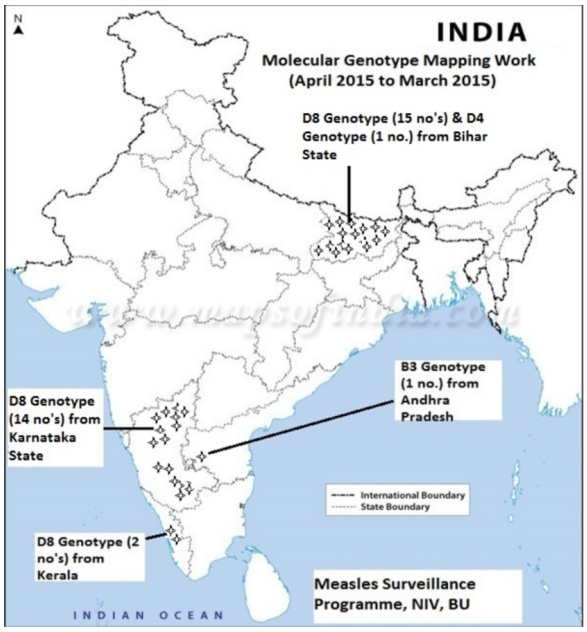


Fig 2: Measles genotype distribution in India (April 2015 to March 2016)

BNU8801: Etiological and epidemiological investigations on arbovirus infections and laboratory diagnosis in Bangalore, Karnataka and neighboring areas with particular reference to Dengue, Chikungunya and Japanese Encephalitis viruses.

***Investigator**: CG Raut, H Hanumaiah & MJ Manjunath*

Funding agency: NVBDCP & NIV

During 2015-16, 4495 specimens from 4474 were screened for dengue and chikungunya IgM antibodies and found 1935 (43.0%) and 1079 (24.0%) positive for dengue and CHIKV respectively. Majority of the cases were recorded during June-October 2015. Though dengue cases were reported from 13 districts, maximum number of cases was from Bangalore City (821/3570) and Bangalore Urban (161/468) areas. JEV anti IgM antibodies were detected in 35 samples and has a wide distribution with maximum cases from Bangalore City and Bangalore Urban (n=19), Chikkaballapura (n=4) Hassan (n=1) Kolar (n=1), Ramnagar (n=4) and Tumkur (n=4).

Respiratory virus diagnosis:

Screening of 64 throat swab specimens received from city hospitals using Multiplex RT-PCR detected 14 Influenza A, 4 Adenovirus, 2 PIV-1, 6 PIV-2,4 Rhino, 4 hMPV A1A2, 4 hMPPV B1B2 and 2 RSV A viruses. Though attempts were made to isolate the viruses in cell culture, it was not successful.

Investigation of Mumps outbreak in Ramanagara district, Karnataka:

Thirty-two clinical specimens (blood 10, throat swabs 11, urine 11) collected from suspected mumps patients in Ramanagara district in March 2016 were screened for mumps virus. Serological investigation showed antibody positivity in 10 samples while nested PCR targeting SH gene detected 6 out of 11 samples. Genetic analysis of the PCR products have shown to be matching with genotype 'C'.

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KLU 1107: Development of NS1 protein capture ELISA for detection of West Nile virus infection

***Investigator**: Anukumar Balakrishnan*

Funding Agency: Intramural

Project Duration: 2013-2016

Background

West Nile virus (WNV) activity has been detected in humans and mosquitoes in Kerala during 2011-12. Since diagnosis of WNV using existing serological techniques are difficult due to cross reaction with other flaviviruses, the new project is proposed.

Objective

- The aim of the study is to develop NS1 capture ELISA for WNV to detect early stages of infection.

Work done

Along with NS1, envelop (E) protein was also expressed in baculovirus vector system, purified and used as a coating antigen to detect the WN specific IgG from serum sample. The ELISA was standardized with samples specific for WN IgG, JEV IgG and negatives for WNV and JEV IgG. The samples were selected based on the presence of specific neutralizing antibodies. Comparative screening of samples were made using WNV IgG ELISA (InBios international, USA). The test results revealed that the recombinant E protein cross-react with both JEV and WNV specific IgG. Polyclonal and monoclonal antibodies were raised against the recombinant NS1 protein (In collaboration with Dr. GN Sapkal, NIV, Pune). The monoclonal antibodies recognized the native NS1 protein expressed in the WNV infected cells. The antibodies will be used for developing NS1 capture ELISA.

Conclusion

Further optimization is necessary to develop the WNV specific IgG ELISA.

KLU 1301: Virus-host protein-protein interaction: a preliminary study to identify putative host protein (s) interacting with Chandipura virus

***Investigator**: A Balakrishnan & VK Kavathekar*

Funding Agency: Intramural

Duration: 2013-2016

Intra-peritoneal inoculation of CHPV led to neurologic dysfunction in mice within 48h PI. Recent studies in mice have demonstrated that a right footpad injection of CHPV causes progressive viral replication in all nervous tissues. So far, there has been no significant effort to understand CHPV interactions with neurons and its proteins. This study aims to profile the interaction of whole virus and individual protein components of CHPV with the neuronal proteins. During the reporting year, CHPV Matrix (M) protein gene was cloned in pcDNA3.1 (mammalian expression vector) and expression was checked in Neuro2a cell line using indirect immune-fluorescence assay (IFA) using anti-Chandipura polyclonal antibody raised in mice. The expression of M protein was confirmed. Isoelectric-focusing protocol for Vero cell lysate using IPG strip (pH range 3-10) (BioRad) was standardized. The technique will be used for protein profiling in future.

KLU 1302: Effect of mutations in chikungunya virus E1 and E2 proteins in virus entry: a virus like particle based approach

Investigator: A Balakrishnan & AM Mathew

Funding Agency: Intramural

Duration: 2014-2017

The entire structural polyprotein region of CHIKV genome was cloned into a baculovirus vector earlier for studying the mutations observed during the recent outbreak. The recombinant baculovirus was transfected into Sf-9 cell line where they self assemble to form Virus Like Particle (VLP) of CHIKV. Analytical assays like SDS-PAGE, Western Blot, IFA and ELISA were done to confirm the production and the immunoreactivity of VLPs. Purification of VLPs was done using PEG precipitation followed by discontinuous density gradient ultracentrifugation. The characterization of VLP product was done by transmission electron microscopy (TEM) and the particles were found to be morphologically similar to the native CHIKV (Fig.1). The structural polyprotein region of CHIKV was further cloned to the N-terminus of the AcGFP protein (pAcGFP-N1 vector) to produce a VLP that expresses GFP and transfected into Vero cell line. However, the protein could not be expressed. For studying mutations, the mutations observed in the envelope regions and some other important mutations have to be introduced to the CHIK VLPs produced. Primers are designed

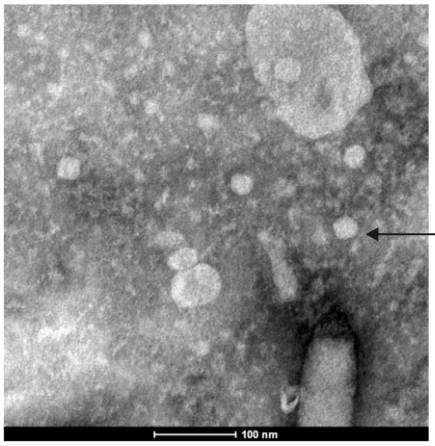

Fig 1: Chikungunya virus like particles (VLP) observed under transmission electron microscopy

accordingly and by site directed mutagenesis method the mutations are introduced to the envelope region of structural cassette.

KLU 1102.Grade I virology Laboratory network

***Investigator**: A Balakrishnan, BV Tandale & R Balasubramanian*

Funding Agency: ICMR (Extramural)

Project Duration: 2011-2016

The unit provides diagnosis for >40 viruses using serological and molecular based diagnostic tests. During the year, 1290 samples collected and/or received from 800 patients were processed against 31 viruses. 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).

Sl. No.	Virus	IgM ELISA	IgG	Convent PCR	Real time PCR	Virus Neut.	HAI	Ag. Detect	Virus Isolate
1	JE	308/18	–	–	54/0	1/0	–	–	–
2	Dengue	65/17	–	139/51	12/3	–	–	5/1	15/14
3	West Nile	–	32/26	1/0	68/0	1/0	–	–	–
4	Chikungunya	25/8	–	–	6/0	–	–	–	–
5	HSV 1	–	–	131/4	–	–	–	–	–
6	HSV 2	–	–	131/4	–	–	–	–	–
7	HSV 1 & 2	24/0	–	–	–	–	–	–	–
8	Rota	–	–	7/5	–	–	–	–	3/2
9	Rubella	15/1	–	18/2	–	–	–	–	–
10	Measles	5/0	–	7/0	–	–	–	–	–
11	HAV	–	–	2/0	–	–	–	–	–
12	HBV	–	–	5/0	–	–	–	11/0	–
13	HCV	11/0	–	4/1	–	–	–	–	–
14	Entero virus	–	–	5/0	279/12	–	–	–	–
15	Adeno	–	–	–	25/1	–	–	–	–
16	CMV	24/0	–	70/24	126/15	–	–	–	–
17	Varicella Zoster	–	–	–	10/2	–	–	–	–
18	Influenza A	–	–	2/0	9/0	–	–	–	–
19	Influenza B	–	–	2/0	9/0	–	–	–	–
20	H3N2	–	–	–	–	–	1/-	–	–
21	PandemicA (H1N1)	–	–	2/0	136/9	–	1/0	–	11/10
22	RSV - A	–	–	38/4	–	–	–	–	–
23	RSV - B	–	–	38/0	–	–	–	–	–
24	Parvo V	–	–	1/0	–	–	–	–	–
25	EBV	33/0	–	2/0	94/19	–	–	–	–
26	Mumps	10/0	–	6/0	–	–	–	–	–
27	Rhino	–	–	2/0	–	–	–	–	–
28	HHV 6	–	–	7/1	–	–	–	–	–
29	HHV 7	–	–	4/1	–	–	–	–	–
30	Mers	–	–	–	2/0	–	–	–	–
31	KFD		–	–	1/0	–	–	–	–
TOTAL / POSITIVE		520/44	32/26	624/97	831/61	2/0	2/0	16/1	29/26

KLU 1602: Impact of climate change on mosquito abundance in coastal wetlands of Alappuzha district, Kerala

***Investigator**: R. Balasubramanian, B. Anukumar*

Funding agency: Intramural

Project duration: 2016- 2019

The aim of this study was to evaluate mosquito abundance, species diversity, larval population dynamics in abandoned wells and wetlands integrated in coastal system. Mosquito larvae were collected from water bodies within one km proximity to seashore and brackish water bodies at different sites in Alappuzha district from January to March, 2016. Collections were made bi-monthly using standard dip count method. Initial studies have shown the predominance of *Ae. albopictus*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus*, *Cx. bitaeniorhynchus* and *Anopheles subpictus*. Mosquito larvae were absent in wells where Copepods and zooplankton were present. Further studies are in progress.

KLU1603.Ixodid (Acari: Ixodidae) ticks diversity in Southern Kerala

***Investigator**: R Balasubramanian*

Funding agency: Intramural

Project duration: 2016-19

Ticks are important vectors of pathogens of humans and animal. The aim of the present study was to find out species diversity of ticks, which infested the domestic animals in Alappuzha district, Kerala. Ticks were collected randomly from infested goats and cattle and stored in 70% ethanol and transported to the laboratory for identification. In this study, a total number of 189 adult ticks (104 males and 85 females) were collected during summer season, identified and found that *Haemaphysalis* species are dominant in the surveyed area. The major species encountered are *Rhipicephalus microplus* (6.34%), *R sanguineus* (0.5%), *H turturis* (62%) and *H bispinosa* (31%). Further studies are in progress.

INFORMATION CENTRE AND LIBRARY

Scientific Staff

Dr. Alok K Chakrabarti	Scientist C &Library in charge

Technical staff

Ms. Vandana Chandere	Asstt. Library & Information Officer
Mr. Vishal R. Mali	Library & Information Assistant
Ms. Ekta Jain	Library & Information Assistant
Mr. GR Gorayya	Attendant

Project staff (Apprentices)

Mr. Girish Moghe	(Till 16.11.2015)
Ms. Jayshree Walhe	(From 16.11.2015)
Ms. Sharvari Chavan	(From 20.11.15)

(1) Major activities

During this year, Library continued to provide its academic support to Scientists, Staff, M.Sc. & PhD students, Project staff of NIV and other institutions in Pune by providing references, citation analysis, Document Delivery Services(DDS), reprints, literature search, etc. Library continued to participate in exchange of documents through inter library loan services from NCCS, ARI and BJMC. Assistance provided to scientists for sending papers for publication and database of the published papers is made accessible through LIBSYS software. In addition, the following assignments were also carried out

- Weeding out of library documents *viz.*, loose issues, annual reports, un-accessioned books, catalogs, etc.
- Installed the Turn it in plagiarism detection software for PhD students.
- Newspaper clippings on scientific aspects were scanned and sent to NIV scientists by email on daily basis.
- Approval for implementing Barcode System.
- Renewal of Newspapers and magazines.
- Subscription to JCCC@ICMR and J-Gate has been renewed for the period Jan-Dec 2016 by Indian Council of Medical Research, which provides 2717 journals access.
- On-line subscription of four Journals such as NATURE, SCIENCE, LANCET and NEJM has been renewed by ICMR is accessible to all.
- National Medical Library (NML), New Delhi has provided on-line 243 e journals for full text access, based on IP address.
- Settled the long pending issues with M/S Panima Educational Centre, New Delhi.
- Physical Stock Verification of books and journals.
- Updated and maintained in-house NIV Scientific Publications from 1953-2015.
- Updated list of NIV Holdings from 1953-2015 for ready reference.

(2) Information and Library Services Continued, Added/Started

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

S. No.	Description		Quantity
1.	**Books: Purchased /Gifts/Gratis**		35
	Bound Volumes		114
	Annual Report Received		34
	Reprints		NIL
2.	**Journals: Current titles**		
		Print	14
		Online	04
		J Gate	2717
		ERMED Consortia	243
		e- Journals Gratis/Free	56
	Loose Issues		513
	Bound Volumes		115
3.	PhD thesis		8
	MSc dissertation		17
4.	Others; CDs, Microforms, floppies etc.		28

(3) SDI/CAS, Reference Services, Inter Library Loan (ILL) and Document Delivery Services (DDS) services provided:

Table-2: Services Provided -Circulation, Reference, SDI/CAS, ILL, DDS, etc.

1.	Books & bound volumes Issued	Staff: 159 Students: 847
	Books & bound volumes returned	Staff: 242 Students: 806
2.	**Newspaper Clippings**: Reports, articles of medical importance published in local newspapers were clipped, scanned and sent to Director and other scientists of NIV for ready reference.	2128 Clippings
3.	Inter Library Loan (ILL) - received and sent	30
4.	Photocopy Services	4144
5.	Binding	25 documents
6.	Lamination	03 documents
7.	Reference services + Document Delivery Services (DDS-Print + Electronic)	537
8.	NIV Annual Reports	88
9.	SDI/CAS (Routinely)	On demand
10.	Citation Analysis for NIV Scientists	12 Scientists
11.	Plagiarism detection using software	05 Ph. D. students

Publication of NIV (April 2015 to March 2016): **82**

VIRUS REGISTRY

Scientific Staff

Dr. Basu A	Scientist 'F' & Group leader

Technical staff

Mr. Umrani U	Technical Officer (A)
Mr. SK Deshpande	Technical Assistant
Mr Raju Rahurkar	Technician B
Mrs SS Mohol	MTS

Maintencance of Virus Repository and Registry (VRR)

U Umrani, & A Basu

The main function of VRR is to provide an interface for receiving and cataloging samples from various organizations that are sent for diagnostic testing at NIV and co-ordinate subsequent test logistics till reporting. This is a central facility. During the period of 2015-16. 20,160 referred samples were handled. In situations of public health emergencies, like the recent episodes of Ebola, KFD, CCHF and H1N1, VRR directly co-ordinated the logistic flow with the maximum containment BSL4 laboratory.

Virus Repository Unit caters to the need of providing reference virus strains to both academic and industrial laboratories as per international/national biosecurity regulatory directives. During 2015-16 a total of 09 reference virus strains were provided to 14 such institutions in the country.

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

MAINTENANCE (ENGINEERING SUPPORTS) DIVISION

Name of staff	*Designation*
Mr. ST Perumal	Technical Officer-C
Mr. AB Khare	Technical Officer B
Mr DR Kumbhar	Technical Officer-A
Mr. JD Pacharane	Technical Officer-A
Mr. AK Kasar	Technical Officer-A
Mr. VJ Bhosale	Technical Assistant
Mr. Gawate CB	Technical Assistant
Mr. Surbhaiya SN	Technician 'C'
Mr. Pote SD	Technician 'C'
Mr. Bathe SD	Technician 'A'
Mr. Ishte VT	Technician 'A'
Mr. Dedunda IR	Technician 'A'
Mr. NV Bhongale	Technical Assistant
Mr. AJ Suresh	Technical Assistant
Mr. GK Bagul	Technical Assistant
Mr. RM Shukla	Technician-C
Mr. NS Dhawale	Technician-C
Mr. VD Jagtap	Technician-C
Mr. YM Taru	Technician-C
Mr. BS Shelar	Technician-B
Mr. SS Utale	Technician-A
Mr. AB Kelkar	Technician-A
Mr. SS Holkar	Technician-A
Mr. RS Gadhave	Technician-A
Mr. AM Pawar	Technician-A
Mr. DK Jagtap	Technician-A
Mr. MV Gadhave	Technician-A
Mr. AN Kale	Technician-A
Mr. SR Jagtap	Technician-A

The staff-members of the Division along with the ITI Apprentice of various trades have worked efficiently throughout the year keeping all equipments in good working condition by carrying out routine / preventive / breakdown maintenance works. Regular servicing, overhauling and repairs of conventional machineries, LT / HT electrical installations and other laboratory equipments were undertaken and satisfactorily completed to extend the life of equipment. During the year 669 jobs of different nature have been completed.

NIV ADMINISTRATION

Foreign visits

S.N.	Name & Designation	Place	Purpose	From	To
1	Dr. R. Laxminarayanan, Senior Administrative Officer	CDC, Atlanta, Georgia	Grantees Meeting	10.02.2016	12.02.2016

Training courses attended by Administrative Staff at ISTM, New Delhi

S.N.	Name & Designation of the Official	Course Title	Duration	From	To
1	Mr. A.S. Gaikwad, SO	Purchase Management in Govt.	3 days	02.11.15	04.11.15

Training courses attended by administrative staff at Regional Training Centre, Mumbai

Sr. No.	Name & Designation of the Official	Course Title	From	To
1	Mrs. R.S. Moghe, Asst.	Budget Process	22.07.15	23.07.15
2	Ms. D.S. Pisal, LDC			
3	Ms. M.J. Shaikh, LDC	Pension and Retirement Benefits	27.07.15	29.07.15
4	Mrs. M.L. Rupnar, LDC			
5	Mrs. D.D. Marathe, UDC	Leave Travel Concession	03.08.15	03.08.15
6	Mr. P.N. Chabukswar, LDC	Maintenance of cash book	05.08.15	05.08.15
7	Mrs. S.S. Dube, Stenographer	Long term and short term advances	07.08.15	07.08.15
8	Ms. Shakila I Choudhari, PA	Fundamentals of computers and internet	10.08.15	14.08.15
9	Mrs. S.M. Bhave, PA			
10	Mrs. A.S. Deshpande, Asst.	APAR	31.08.15	31.08.15
11	Mrs. Aleyamma Mathai, Asst.	Medical / CGHS / CSMA	01.09.15	02.09.15
12	Mr. K.S. Galange, UDC			
13	Mrs. Amruta S Bakare, Asst.	Roster Maintenance and Reservations & Concessions (including practical)	07.09.15	09.09.15
14	Mrs. Shibi Jacob, PA			
15	Mrs. P.B. Aher, UDC			
16	Mrs. M.R. Kannalu, LDC	Noting and drafting	14.09.15	15.09.15

Sr. No.	Name & Designation of the Official	Course Title	From	To
17	Mrs. S.H. Khamkar, Asst.	Pay fixation: ACP / MACP	28.09.15	30.09.15
18	Mrs. S.B. Chakole, LDC			
19	Mr. P. Subramanian, DDO	Drawing & Disbursing Officers (DDO) Functions	26.10.15	27.10.2015
20	Mr. Y.K. Mazire, ACO	General Financial Rules & Budget	28.10.2015	29.10.2015
21	Mrs. J.S. Gadre, UDC	New Pension Scheme and Uploading of Data	02.11.2015	03.11.2015
22	Ms. M.J.A Shaikh, LDC			
23	Mrs. S.H. Khamkar, Asst.	Leave Travel Concession	04.11.2015	04.11.2015
24	Mrs. S.P. Mulay, Asst.	Long Term & Short Term Advances	19.11.2015	19.11.2015
25	Mr. P.N. Jadhav, UDC	Government Accounting	23.11.2015	24.11.2015
26	Mrs. D.S. Pisal, LDC			
27	Mrs. P.S. Joshi, Asst.	Office Procedure/ File Management & Record Management	30.11.2015	02.12.2015
28	Mr. S.R. Vasam, UDC			
29	Mrs. V.V. Shendye, SO	Medical (CGHS/CSMA) Rules	03.12.2015	04.12.2015
30	Mr. B.K. Wadke, SO	Seniority Promotion & DPC	07.12.2015	08.12.2015
31	Mrs. Shibi Jacob, PA	Fundamental Rules & Supplementary Rules	09.12.2015	10.12.2015
32	Mrs. R.K. Amale, PS	Conduct Rules & Disciplinary Proceedings	14.12.2015	16.12.2015
33	Mrs. S.B. Chakole, LDC			
34	Mrs. A.S. Deshpande, Asst.	Stress Management	15.12.2015	15.12.2015
35	Mrs. R.S. Moghe, Asst.			
36	Mr. J.R. Kumbhare, PA	Public Procurement (GFR)	17.12.2015	18.12.2015
37	Mrs. S.S. Pathak, Asst.			
38	Mr. V.C. Chavan, Asst.			
39	Ms. Prajakta Bapat, LDC	Basics of MS Access	13.01.2016	15.01.2016
40	Ms. Sadhana Ubhe, LDC			
41	Ms. Madhuri Tandan, LDC			

Sr. No.	Name & Designation of the Official	Course Title	From	To
42	Mrs. Amruta S Bakare, Asst.	Advanced Course on Roster Maintenance, Reservations & Concessions	18.01.2016	19.01.2016
43	Mrs. Shibi Jacob, PA			
44	Mrs. P.B. Aher, UDC			
45	Mr. P.S. Jadhav, Asst	Budget Process	21.01.2016	22.01.2016
46	Mr. V.C. Chavan, Asst			
47	Mr. Y.C. Pote, LDC			
48	Ms. M.J.A. Shaikh, LDC	Travelling Allowance	27.01.2016	27.01.2016
49	Mrs. S.P. Mulay, Asst			
50	Mr. P.Subramanian, AO	Annual Performance Appraisal Report	28.01.2016	28.01.2016
51	Mrs. R.K.Amale, SO			
52	Mrs. S.P.Chakole, LDC	Noting Drafting & File Management	01.02.2016	03.02.2016
53	Mr. A.E.Matkar, LDC			
54	Mrs. D.D. Marathe, Asst.	Leave Rules & Maintenance of Service Book	04.02.2016	05.02.2016
55	Ms. M.R.Kannalu, LDC			
56	Mr. P.N.Chabukswar, LDC			
57	Mr. Akram Khan, LDC	Hardware/ Software Troubleshooting	08.02.2016	12.02.2016
58	Ms. Prajakta Bapat, LDC			
59	Mr. S.E.Matkar, Assistant	Change Management	10.02.2016	10.02.2016
60	Mrs. T.S.Yadav, LDC			
61	Mr. B. K.Wadke, SO	Seminar on RTI	15.02.2016	16.02.2016
62	Mr. H.S. Pasalkar, Asst			
63	Ms. Madhuri Tandan, LDC	Advanced MS Excel Training	15.02.2016	16.02.2016
64	Ms. Sadhana Ubhe, LDC			
65	Ms. Sadhana Ubhe, LDC	Basics of Computer & Internet	22.02.2016	26.02.2016
66	Ms. Madhuri Tandan, LDC			

Sr. No.	Name & Designation of the Official	Course Title	From	To
67	Mrs. Amruta S Bakare, Asst.	Seniority Promotion & DPC	25.02.2016	26.02.2016
68	Mrs. Shibi Jacob, PA			
69	Mr. A.S.Gaikwad SO	Drawing & Disbursing Officers (DDO) Functions	07.03.2016	08.03.2016
70	Mr. J.S.Rangan, SO			
71	Mrs. V.V.Shendye, SO	Pension & Retirement Benefits	09.03.2016	11.03.2016
72	Mrs. J.V. Gadre, Asst			

List of Admin Staff as on 31st March 2016

Sr#	Name	Designation
1	Dr. R. Lakshminarayanan	Senior Administrative Officer
2	Mr. P Subramanian	Administrative Officer
3	Mr. Y K Mazire	Accounts Officer
4	Mr. A S Gaikwad	Section Officer
5	MS. S N Ponkshe	
6	Mr. J S Rangan	
7	Mrs. V V Shendye	
8	Mrs. P K Ratnaparkhi	
9	Mrs. A S Deshpande	
10	Mr. B K Wadke	Private Secretary
11	Mrs. R K Amale	
12	Mrs. A V Shendrikar	Technical Officer 'A'
13	Mrs. A S Palshikar	Assistant
14	Mr. H S Pasalkar	
15	Mrs. A S Bakare	
16	Mrs. A Mathai	
17	Mrs. S. Srinivasan	
18	Mr. S E Matkar (MCC Budget)	
19	Mrs. A A Bapat	
20	Mrs. A G Ghorpade	
21	Mr. V C Chavan	
22	Mrs. S S Pathak	
23	Mrs. S P Mulay	
24	Mrs. A R Nair	
25	Mrs. P S Joshi	
26	Mrs. R S Moghe	
27	Mrs. S H Khamkar (MCC Budget)	
28	Mr A.D. Pardeshi	
29	Mrs. J V Gadre	
30	Mrs. D D Marathe	
31	Mr. P N Jadhav	
32	Mr. J R Kumbhare	Personal Assistant
33	Mrs. Shibi Jacob (MCC Budget)	
34	Ms Shakila Imam Choudhari	
35	Mrs S.M. Bhave	

Sr#	Name	Designation
36	Mrs. S S Dube	Stenographer
37	Mrs. S P Bohodkar	Technical Assistant
38	Mr. B T Chandane	Technician 'C'
39	Mr. S R Vasam	Upper Division Clerk
40	Mrs. P B Aher	
41	Mr. K S Galange	
42	Ms. MJA Shaikh	Lower Division Clerk
43	Mr. Y C Pote	
44	Mrs. S B Chakole	
45	Mrs. T.T. Yadav	
46	Mr. P N Chabukswar	
47	Mrs. M L Rupnar	
48	Mr. A E Matkar	
49	Mr. H D Raut	
50	Mr. V A Bisht	
51	Ms. D S Pisal	
52	Mrs. M R Kannalu	
53	Ms. Madhuri S Tandan	
54	Mr. Akram Khan	
55	Ms. Prajakta Bapat	
55	Ms. Sadhana Ubhe	

राजभाषा रिपोर्ट

संस्थान भारत सरकार के राजभाषा विभाग द्वारा जारी नियमोंका एवं भारतीय आयुर्विज्ञान अनुसंधान परिषद, नई दिल्ली के आदेशोंका अनुपालन करने के लिए सदैव कार्यरत हैं।

राजभाषा अधिनियम 1973 की धारा 3 की उपधारा(3) का अनुपालन करते हुवे संस्थान के अधिकतम दस्तावेज हिंदी और अंग्रेजी दोनों मे तैयार किये जा रह हैं।

राजभाषा नियम 1976 के नियम 10(4) के अनुसार कर्मचारी हिंदी का कार्यसाधक ज्ञान प्राप्त कर रहे हैं। इस साल में हिंदी टंकन-1, प्राज्ञ-13, पारंगत-4, कर्मचारी परिक्षा उत्तीर्ण हुए हैं और टंकन-2, प्राज्ञ-15, पारंगत-21 कक्षा के अंतर्गत कर्मचारी ज्ञान प्राप्त कर रहे हैं।

नगर राजभाषा कार्यान्वयन समिती की बैठकों में संस्थान के अधिकारी उपस्थित रहते हैं।

संस्थान में हिंदी सप्ताह के अंतर्गत दिनांक 21-22 सितंबर 2015 को ''जीव चिकित्सा उपशिष्ट (बायो मेडीकल वेस्ट) जोखिम, प्रबंध एवं पर्यावरण संबंधी समस्यांए'' इस विषय पर कार्यशाला संपन्न हुई। परिषद के सभी संस्थानों को आमंत्रित किया गया था। विभिन्न संस्थानों से कुल 55 प्रतिनिधि ने कार्यशाला में भाग लिया। 1) महाराष्ट्र प्रदूषण नियंत्रण बोर्ड, मुंबई के कनिष्ठ वैज्ञानिक डॉ. दयानंद तरे 2) पुणे महानगर पालिका के स्वास्थ अधिकारी डा. केतकी घाटगे 3) राष्ट्रीय रासायनिक प्रयोगशाला, पुणे के मुख्य वैज्ञानिक (सेवानिवृत) एवं सुरक्षा प्रबंध के प्रमुख डा. जी. एस. ग्रोवर ने उपरोक्त विषय में व्याख्यान द्वारा प्रतिभागियों कों महत्त्वपूर्ण जानकारी दी। सभी व्याख्यानों के सार की पुस्तिका का प्रकाशन किया गया।

संस्थान में दिनांक 27.10.2015 को सतर्कता जागरुकता सप्ताह में ''व्यक्तिगत-मौखिक साक्षात्कार श्रेणी ब, क और ड सिधी भर्ती प्रक्रियासे हटाने से भ्रष्टाचार के रोकथाम पर क्या असर पडेगा ?'' इस विषय पर हिंदी में वाद-विवाद प्रतियोगिता का आयोजन किया गया था।

संस्थान में आतंकवाद विरोध दिन, सद्भावना दिन, सतर्कता जागरुकता सप्ताह, कौमी एकता सप्ताह हिंदी में शपथ लेकर, व्याख्यान, संगोष्ठी, प्रतियोगिता का आयोजन करके मनाया जाते हैं।

LIST OF DISTINGUISHED VISITORS TO NIV [2015-16]

Name of the guest	Affiliation	Period
Dr. Kayla Laserson, Dr. Daves Sheron	CDC India Country Director, Division of Global Health Protection Centers.	18th May 2015
Dr. Meena, Joint Secretary,	DHR, Government of India	18th August 2015
Dr. Soumya Swaminathan,	Secretoy, DHR &DG, ICMR	29th August 2015 & 11th March 2016
Dr. Stuart Nichol	CDC Special Pathogens Branch, CDC USA	18th-21st November, 2016
Dr. Narshinha P. Argade	Chief Scientist, Division of Organic Chemistry, NCL, Pune	29th January 2016

MEETINGS AND CONFERENCES ATTENDED BY NIV SCIENTISTS (2015-16)

1. Dr. MS Chadha as a committee member attended and provided technical expertise to the Maharashtra communicable disease prevention and control technical committee (MCDPCTC) on 7th April, 2015.

2. Dr. (Mrs) K.S. Lole attended the 4th National conference on Molecular Virology organized at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, during 16-17 April, 2015 and delivered an invited talk entitled, "Use of hepatitis E virus genotype 1/ 4 chimeric cDNA clones to understand species specificity of the virus".

3. Dr. Gajanan Sapkal and Dr. YK Gurav attended the 31st Annual Clinical Virology Symposium at Daytona Beach, Florida, USA, 25-29 April 2015.

4. Dr. CG Raut attended the 8th Indo Global summit and Expo on Vaccines, Therapeutics & Healthcare (VTH-2015), held at HICC, Hyderabad, India on Nov 02-04, 2015

5. Dr. Hirawati Deval attended the meeting on Interdepartmental work on prevention and control of JE/AES in the affected area of eastern Uttar Pradesh held at Lucknow on June 03, 2015 at Secretariat, Lucknow (UP).

6. Dr. Shailesh D Pawar and SS Kode attended training on "Screening antiviral susceptibility of influenza viruses by sequencing and antiviral resistance testing" WHO CC for Reference & Research on Influenza, Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia, July 11-26, 2015.

7. Dr. J Mullick, Dr. Shailesh D Pawar attended Meeting of Expert Committee to review the ban on import of various pork and poultry products on account of Avian Influenza with respect to Dispute Settlement Body Ruling on India's notification SO 1663 (E) dated 19th July 2011 at Krishi Bhavan, Ministry of Agriculture & Farmers' welfare, Department of Animal Husbandry, Dairying & Fisheries, Government of India, New Delhi.

8. Dr. VP Bondre attended the Inter-departmental State Review Meeting – AES/JE under the chairmanship of Principal Secretary, Ministry of Health and Family Welfare, Govt. of UP at National Health Mission, Lucknow on July 10, 2015.

9. Dr. CG Raut and Mr H Hanumaiah attended the "Emerging issues in Environmental, Occupational Health & Safety, its National Scenario & regional needs at ROHC, ICMR Complex, Poojanhalli, Bangalore" during 22-24 July 2015.

10. Sunil R Vaidya participated in the WHO National Consultation Meeting of Virologist of Measles Laboratory Network held on 14th and 15th October 2015 at The Courtyard Marriott & Convention Centre Hyderabad, Telangana and delivered a lecture entitled, "Genotype characterization of measles & rubella viruses: challenges and way forward" and "Measles & rubella update: Jan 2014 - August 2015".

11. Dr. PD Yadav attended the Regional Workshop on poliovirus laboratory containment to be held from 23-24 November 2015 at Bangkok, Thailand.

12. Dr. MS Chadha provided technical expertise for Evaluation of laboratory capacity of the National Health Laboratory, Yangoon, Myanmar, during 07 to 11/09/2015

13. Dr. Shailesh D Pawar attended Training Sequencing and Phylogenetic Analysis of Influenza Viruses, at Centers for Disease Control & Prevention, Atlanta, GA, USA, October 26–Nov 6, 2015.

14. Dr. Potdar provided five day training on the molecular characterization of influenza viruses at Department of Virology, Institute of Epidemiology, Disease control and research (IEDCR) and national Influenza Centre in Bangladesh during 5th October to 9th October 2015

15. Dr. Potdar attended WHO Regional Office for South-East Asia (SEARO) and the WHO Regional Office for the Eastern Mediterranean (EMRO) cordially conducted the 2015 Sequencing Training Course CDC Atlanta 26th October -6November 2015

16. Dr. V Gopalkrishna attended the 3rd Annual review Meeting of National Rotasurveillance Net work (NRSN) at Goa on 9th October 2015 followed by Satellite symposium on Rotavirus Vaccines on 10th.Oct 2015.

17. Dr. V Gopalkrishna and Ms Preeti Jain attended the 12th International Double Stranded RNA Virus Symposium" held at Goa, India from 6-10 October 2015 and presented papers entitled, "Burden of rotavirus gastroenteritis in children below 5 years of age in western India" and "Effect of Rotavirus Vaccines on Rotavirus Disease".

18. Dr. AB Sudeep attended the National Conference on Entomology, held at Punjabi University, Patiala during 29-30 October 2015 and presented a paper entitled, "Vector competence of certain mosquitoes to Ingwavuma virus, a new bunyavirus recently found circulating in India".

19. Dr. DT Mourya and Dr. PD Yadav attended the WHO Consultation as Technical Advisor for validation / certification of BSL-3 Laboratory, Public Health Laboratory, Department of Public Health, Ministry of Health, Thimpu, Bhutan during 16-27th October 2015

20. Dr. MS Chadha attended the "International Severe Acute Respiratory and emerging Infection Consortium" at London, UK, during 30/11/2015 to 01/12/2015 ".

21. Dr. M L Choudhary attended national training program on entrepreneurship development & management of scientist & technologist working in government sector from 7-11 Dec 2015 held organized by Entrepreneurship Development Institute of India, Ahmadabad.

22. Dr. MS Chadha provided onsite assessment and training at National Heath Laboratory, Male, Maldives during 24- 29/01/2016

23. Dr. Potdar, Dr. Manohar Lal Choudhary and Sheetal Jadhav attended laboratory management and internal audit as per ISO/IEC 17025:2005 conducted by STQC directorate, Govt. of India from 11th to 14th January 2016

24. Dr. Paresh Shah attended BIRAC CDSA Regulatory Workshop on "Current regulation on medical devices & *in vitro* diagnostic kits" at CDSCO, Shastri Bhavan, Chennai on 24 Feb 2016

25. Dr. CG Raut, Mr H Hanumaiah and Jayaprakash H attended the MICROCON KC- 16" held at BMCRI, Bangalore on 20.02.2016.

26. Dr. R. Lakshminarayanan, Senior Administrative Officer, NIV attended the Grantees meeting at CDC, Atlanta, USA during 10-2-2016 to 12.02.2016.

27. Dr. MS Chadha Co-chaired the session on "Ebola and beyond: Preparing for the next Pandemic" in the 17th International Congress on Infectious Diseases and the International Society for Infectious Diseases, held at Hyderabad during 2-5 March, 2016.

28. Dr. VP Bondre attended the meeting of AES/JE Program of UP under the Chairmanship of Principal Secretary (MH & FW) UP, held at the National Health Mission on March 11, 2016.

29. Dr. CG Raut attended the CME on World TB Day held at RGICD, Bangalore on 24/03/2016.

LIST OF PUBLICATIONS

(April 2015 - March 2016)

1) Alagarasu K, Bachal RV, Damle I, Shah PS, Cecilia D. Association of FCGR2A p.R131H and CCL2 c.-2518 A>G gene variants with thrombocytopenia in patients with dengue virus infection. *Hum Immunol*. 2015; 76(11):819-22.

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3) Alagarasu K, Bachal RV, Tillu H, Mulay AP, Kakade MB, Shah PS, Cecilia D. Association of combinations of interleukin-10 and pro-inflammatory cytokine gene polymorphisms with dengue hemorrhagic fever. *Cytokine*. 2015; 74(1):130-6.

4) Alagarasu K, Memane RS, Shah PS. Polymorphisms in the retinoic acid-1 like-receptor family of genes and their association with clinical outcome of dengue virus infection. *Arch Virol*. 2015; 160(6):1555-60.

5) Alagarasu K, Walimbe AM, Jadhav SM, Deoshatwar AR. A meta-analysis of the diagnostic accuracy of dengue virus-specific IgA antibody-based tests for Detection of dengue infection. *Epidemol Infect*. 2016; 144(4):876-86.

6) Ampofo WK, Azziz-Baumgartner E, Bashir U, Cox NJ, Fasce R, Giovanni M, Grohmann G, Huang S, Katz J, Mironenko A, Mokhtari-Azad T, Sasono PM, Rahman M, Sawanpanyalert P, Siqueira M, Waddell AL, Waiboci L, Wood J, Zhang W, Ziegler T, Chadha MS; WHO Writing Group. Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. *Vaccine.* 2015; 33(36):4368-82.

7) Awate P, Yadav P, 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; pii:S0163-4453(16)00088-8.

8) Bachal R, Alagarasu K, Singh A, Salunke A, Shah P, Cecilia D. Higher levels of dengue-virus-specific IgG and IgA during pre-defervescence associated with Primary dengue hemorrhagic fever. *Arch Virol*. 2015; 160(10):2435-43.

9) Balasubramanian R, Anukumar B, Nikhil TL. Stegomyia Indices of Aedes Mosquito Infestation and Container productivity in Alappuzha district Kerala. *Int J of Mosq Res*. 2015; 2(2):14-18.

10) Balasubramanian R, Nikhil TL. Effects of rainfall and salinity increase on the production of local vector mosquitoes (Diptera: Culicidae) in coastal areas of Alappuzha district, Kerala. *J Env Biol*. 2015; 36:1325-8.

11) Banskar S, Mourya DT, Shouche YS. Bacterial diversity indicates dietary overlap among bats of different feeding habits. *Microbiol Res*. 2016; 182:99-108.

12) Basu A, Yadav P, Badole S, Mourya DT. An early passage human isolate of Kyasanur Forest Disease virus shows acuteneuropathology in experimentally infected CD-1 mice. *Vector Borne Zoonotic Dis*. 2016 (In Press).

13) Bedford T, Riley S, Barr IG, Broor S, Chadha M, Cox NJ, Daniels RS, Gunasekaran CP, Hurt AC, Kelso A, Klimov A, Lewis NS, Li X, McCauley JW, Odagiri T, Potdar V, Rambaut A, Shu Y, Skepner E, Smith DJ, Suchard MA, Tashiro M, Wang D, Xu X, Lemey P, Russell CA. Global circulation patterns of seasonal influenza viruses vary with antigenic drift. *Nature.* 2015; 523(7559):217-20.

14) 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*. 2015; 12:1-31.

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19) Chaaithanya IK, Muruganandam N, Surya P, Anwesh M, Alagarasu K, Vijayachari P. Association of Oligoadenylate Synthetase Gene Cluster and DC-SIGN (CD209) Gene Polymorphisms with Clinical Symptoms in Chikungunya Virus Infection. *DNA Cell Biol*. 2016; 35(1):44-50.

20) Choudhary ML, Anand SP, Tikhe SA, Walimbe AM, Potdar VA, Chadha MS, Mishra AC. Comparison of the conventional multiplex RT-PCR, real time RT-PCR and Luminex xTAG® RVP fast assay for the detection of respiratory viruses. *J Med Virol*. 2016; 88(1):51-7.

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24) Gokhale MD, Paingankar MS, Sudeep AB, Parashar D. Chikungunya virus susceptibility & variation in populations of *Aedes aegypti* (Diptera: Culicidae) mosquito from India. *Indian J Med Res*. 2015 Dec; 142 Suppl: S33-43.

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27) Gurav Y, Hundekar S, Deoshatwar A, Lole K. Outbreak of Hepatitis A in an orphanage in Pune city, India. *Int J Trop Med Public Health*.2015; 5(2):1-4.

28) Hanumaiah H, Raut CG, Sinha DP, Yergolkar PN. Non-polio Enteroviruses in Karnataka, India: Virological surveillance of acute flaccid paralysis cases (July 1997-2013). *Indian J Med Microbiol*. 2016; 34(1):22-6.

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41) Makwana D, Yadav PD, Kelaiya A, Mourya DT. First confirmed case of Crimean-Congo haemorrhagic fever from Sirohi district in Rajasthan State, India. *Indian J Med Res*. 2015 142(4):489-91.

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