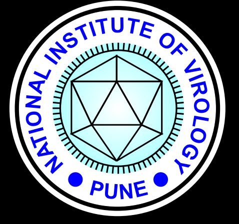


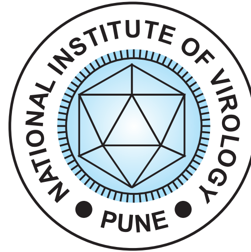
ANNUAL REPORT

2017 - 18

Bat trapping activity during Nipah outbreak in Kerala

ICMR - NATIONAL INSTITUTE OF VIROLOGY
PUNE, INDIA

आय सी एम आर – राष्ट्रीय विषाणु विज्ञान संस्थान, पुणे



ICMR-National Institute of Virology, Pune

Annual Report 2017-18

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LIST OF SCIENTISTS

DT Mourya
Scientist G & Director

Scientist G

MS Chadha	Human Influenza (Superannuated on 30th June 2017)

Scientist F

Atanu Basu	Electron Microscopy	Sarah Cherian	Bioinformatics
PS Sathe	Medical Entomology (Superannuated on 31st March 2018)		

Scientist E

V Gopalkrishna	Enteric virus	Kavita Lole	Hepatitis
BV Tandale	Epidemiology	Jayati Mullick	Avian Influenza
VP Bondre	NIV Gorakhpur Unit	Anuradha Tripathy	Hepatitis
Sunil Vaidya	Measles	PS Shah	DRF
VK Saxena	NIV Mumbai Unit	MC Mohanti	NIV Mumbai Unit
Pragya Yadav	MCL (BSL-4)	G N Sapkal	Diagnostic virology

Scientist D

YK Gurav	Epidemiology	SD Pawar	Avian Influenza
B Anukumar	NIV Kerala Unit	AB Sudeep	Medical Entomology
D Parashar	Dengue & Chikungunya	DR Patil	Animal House
Reethesh Babu	NIV Kerala Unit	Anita Aich	MCL (BSL-4)
Kanchan Patil	DRF		

Scientist C

MD Gokhale	Medical Entomology	Varsha Potdar	Human Influenza
Rekha Damle	Encephalitis	R Vishwanathan	Bacteriology
A Deosthatwar	Epidemiology	K Alagarasu	Dengue & Chikungunya
ML Chowdhary	Human Influenza	R Balasubramanian	NIV Kerala Unit
Pratip Shil	Bioinformatics	Hirawati Deval	NIV Gorakhpur Unit
Kamran Zaman	NIV Gorakhpur Unit	SS Nandi	NIV Mumbai Unit

Scientist B

T Deshmukh	M. Sc. Virology	PT Ullas	Diagnostic virology
Sreelekshmy Mohandas	MCL (BSL-4)	Sumeet Bharadwaj	Human Influenza
VK Meena	Electron Microscopy	A Munivenkatappa	NIV Bangalore Unit
Rajeev Singh	NIV Gorakhpur Unit	Anuj Kumar	Bacteriology
Himanshu Kausal	Encephalitis	Pradeep Sawant	Enteric virus
Rima Sahay	MCL (BSL-4)	Shilpa Tomar	Epidemiology

Administration

Dr R Lakshminarayan
Senior Administrative Officer

P Subramanian	AS Gaikwad	Vibha Shendye
Administrative Officer & DDO	Accounts Officer (stores)	Accounts Officer

FROM THE DIRECTOR'S DESK

Greetings from ICMR-NIV.

It is indeed my pleasure and privilege to present the annual report of ICMR-National Institute of Virology (NIV), Pune for the year 2017-18. NIV continued to contribute the mandated activities of the institute with more responsibility. I am proud to announce that NIV has created history by successfully transferring a number of technologies to private companies for production of diagnostic kits, fulfilling the vision of ICMR. Three technologies, *i.e.* Japanese encephalitis virus antigen capture ELISA for detection of antigen in mosquitoes, Crimean-Congo hemorrhagic fever (CCHF) IgG ELISA (Sheep/Goat) and CCHF IgG ELISA (Bovine) have been released by DG, ICMR for marketing in Indian and international markets. During the last few years, the institute has evolved from a diagnostic laboratory to a comprehensive research unit capable of meeting any eventuality as far as viral outbreaks are concerned. This has been convincingly demonstrated during the KFD and CCHF outbreaks as well as the Ebola scare. During the Nipah virus outbreak in Kerala recently, NIV has played a critical role in the containment by providing early diagnosis as well as guidelines for patient management. NIV continued to supply diagnostic reagents and positive controls to the virology networks as well as WHO SEAR countries to strengthen the diagnostic capacity.

The Enterovirus Research Centre, Mumbai has been merged with NIV with effect from 1st November 2017 as per ICMR's directive and has been renamed as NIV Mumbai Unit. This unit is involved in research on diseases caused by Enteroviruses, especially acute flaccid paralysis, acute hemorrhagic conjunctivitis, aseptic meningitis/encephalitis, Measles & Rubella surveillance, acute gastroenteritis caused by enteric viruses such as Rotavirus, Norovirus and other Enteroviruses. The merging has strengthened the capacity of NIV especially to deal with enteroviral infections in Mumbai and Western India.

Outbreak investigations and diagnosis of referred samples of vector-borne and other viral diseases of public health importance remained the mainstay of NIV's contribution to the Nation. NIV along with its four field units located at Mumbai, Gorakhpur, Bangalore and Alappuzha, have provided diagnosis and helped in the management of outbreaks. The field units are well established and have the basic infrastructure and trained manpower to provide diagnosis of their own to home state as well as neighboring states. During the year, **99,477** referred samples from across the country were provided with early diagnosis for better disease management and control.

NIV continued to develop serological and molecular diagnostics against emerging and re-emerging viruses to make the country competent enough to diagnose and contain viral pathogens at any level. In addition to enhancement in the diagnostic capabilities, NIV also played an important role in man power development by imparting training to staff of virology networks and other national laboratories.

The Institute is also involved in human resource development by organizing workshops and trainings to health professionals in India and Southeast Asian countries. During the year NIV provided hands on training to health care professionals of ICMR and other national laboratories in the diagnosis of Zika virus, Yellow fever virus etc. The RCVDL group (Apex laboratory for VRDL) of NIV continues to provide long term hands-on training in diverse areas *viz.,* serology, cell culture, molecular biology, epidemiology etc. routinely to scientists and staff of viral diagnostic network laboratories across the country to strengthen the diagnostic capacities. Training to work on highly pathogenic viruses in containment

facilities have been provided to scientific and technical staff in India and Southeast Asian countries. In addition, NIV scientists provided support to WHO-SEAR countries in establishing diagnostic capabilities.

Despite the busy schedule, NIV scientists have published 87 high quality publications in journals of national and international repute with an average impact factor of **3.9**. NIV has also contributed in the fields of academics and research by imparting their knowledge as lectures to budding scientists at universities and national institutes. M. Sc. Virology course conducted by NIV has remained a popular course under the Pune University and attracts high caliber students from different parts of the country. NIV believes in scientific progress of the country and helps researchers from other educational institutes by providing free access to its core facilities like animal house, library etc.

NVBDCP, the nodal agency that monitors vector borne diseases in India depends solely on NIV for the diagnostic kits against major vector borne diseases *viz.* Dengue, Chikungunya and Japanese encephalitis due to their high sensitivity and specificity. During the reporting period, NIV has supplied 11,106 diagnostic kits to different laboratories in India and WHO SEAR countries.

As the Director of NIV, I congratulate my fellow scientists for their commitment and hard work. I also appreciate the administrative, technical and engineering staff for their continuous support. I extend my sincere gratitude to Dr. Soumya Swaminathan, Secretary, DHR & Director General ICMR and the administrative staff of ICMR and DHR for their whole-hearted support and cooperation. I appreciate the same from Dr. Balram Bhargava, the new Secretary, DHR & Director General, ICMR for furthering the interest so that NIV could serve the nation much better.

D T Mourya
Director

INSTITUTIONAL COMMITTEES

Scientific Advisory Committee

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

Human Ethics Committee

Name, Designation & Address	Role
Dr. Sheila Bhave Consultant in Pediatric Research, Department of Pediatrics, KEM Hospital Research Centre, Pune-411011.	Chairperson
Dr. Devendra T. Mourya Director, National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune-411001.	Member Secretary
Dr. Babasaheb V. Tandale Scientist 'E', National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune-411001.	Member Coordinator & Basic Medical Scientist
Dr Shashikala Sangle, M.D.	Clinician
Dr Aarathi Nagarkar, Ph.D.	Social Scientist
Dr Jyoti Bhakare, LLM, Ph.D	Legal expert
Dr. Vikram Padbidri, Consultant, (Microbiology & Infection Control), Department of Microbiology & Infection Control	Basic Medical Scientist
Mr T Krishnamurty, B.Com., LL.B.	Legal Expert
Mr Malsidha B Shelke, B.A., MSW	Person From Community
Dr Anuradha Tripathy, Ph.D	Biological Scientist
Dr Yogesh Gurav, MD	Medical Scientist

Animal Ethics Committee

S.No.	Name, address & Designation of members	Role
1	**Dr. Mangesh Shamrao Kamble** C-901, Aarohi Project, Sr. No. 123, Susgoan, Tal. Mulshi, Dist **PUNE - 411 021** drmangesh.kamble@gmail.com	Main Nominee
2	**Dr. Balasaheb Siraskar** Principal, SVNHT's College of B. Pharmacy, Rahuri Factory, Ahmednagar, Pin: 413706, svnhtcop@gmail.com	Link Nominee
3	**Dr. Ramanamurthy Boppana** Scientist F & In charge, Animal House, National Centre for Cell Science, **PUNE-411 007** raman@nccs.res.in	Scientist from outside the Institute
4	**Shri R. P. Kulkarni** B-5, Building C, Anjira Sankul Vidyanagar, Karad-Masur Road, Karad Tal., Dist. **SATARA – 415124**	Socially Aware Nominee
5	**Dr Jayati Mullick,** Scientist E & Group Leader Avian Influenza Group, National Institute of Virology, 130/1, Sus Road, Pashan, **PUNE - 411 021** jayati_mullick@hotmail.com	Chairperson (Biological Scientist)
6	**Dr Dilip Rewa Patil** Scientist In charge of Animal House facility dipupatil@yahoo.com	Member Secretary
7	**Dr P. S. Shah** Scientist E & Group Leader, Diagnostic Reagent Facility, National Institute of Virology, **PUNE-411001** paresh17@hotmail.com	Scientist from biological discipline
8	**Dr. Sreelekshmy Mohandas** Scientist B, Translational Research Group, National Institute of Virology, Microbial Containment Complex, 130/1, Sus Road, Pashan, **PUNE-411021** sreelekshmy88@gmail.com	Veterinary Scientist
9	**Dr. Virendra Kumar Meena** Scientist B, Electron Microscopy Group, National Institute of Virology, 20-A, Dr Ambedkar Road, Post Box 11, **PUNE-411001** viren27091988@gmail.comdiscipline	Scientist from biological discipline

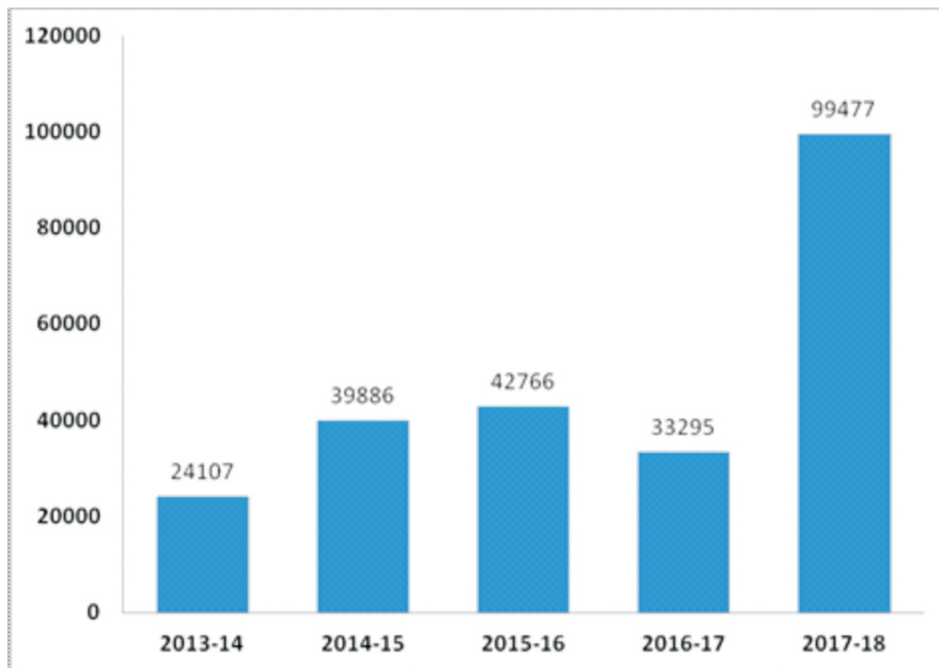
Institutional Biosafety Committee

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

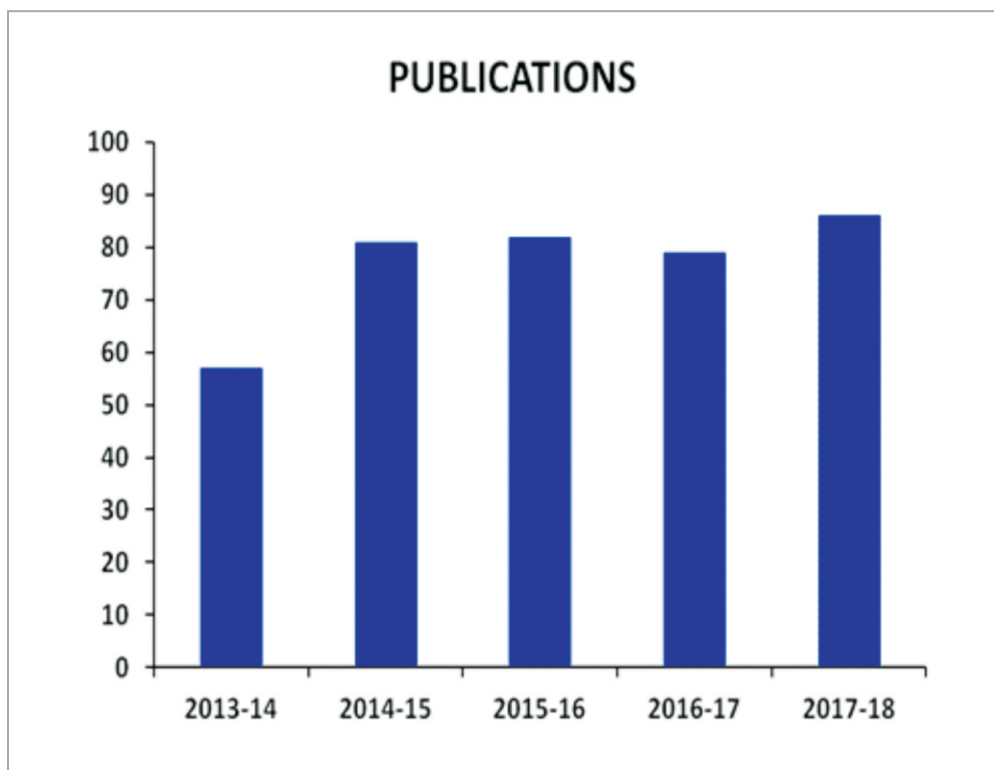
PERFORMANCE INDICATORS

1. Outbreaks investigated	Four
2. Number of samples tested for diagnosis	99,477
3. Number of diagnostic kits supplied	11, 106
4. Number of papers published	86
5. Number of technology developed and transferred	03
6. No. of Ph.Ds awarded	06

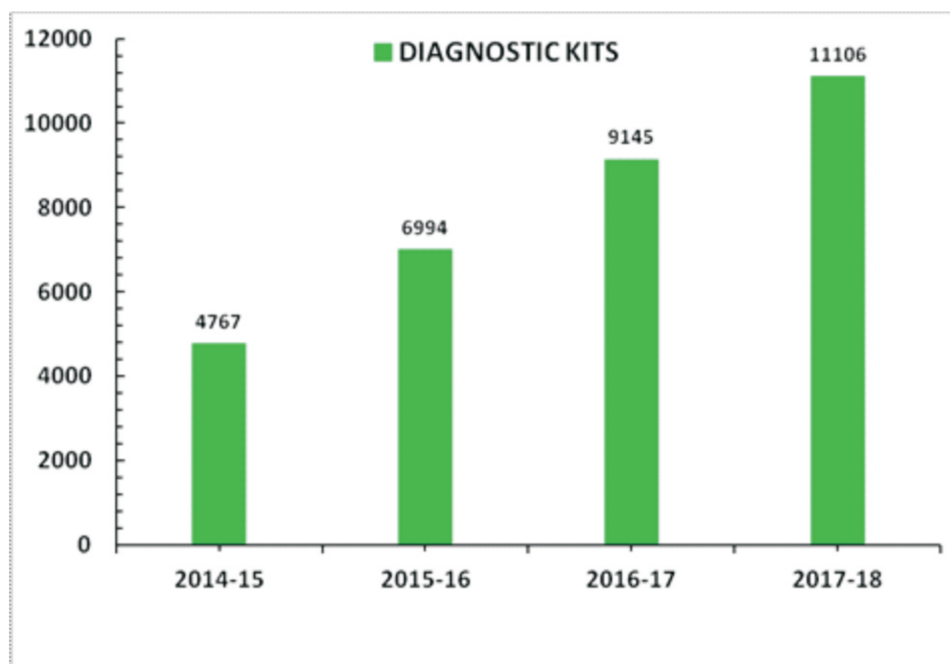
Samples tested during the last five years :



Research Papers published during the last five years



Diagnostic kits developed and supplied during the years



List of distinguished visitors during the year

1. **Jane Basile, Microbiologist,** Arbovirus Diseases Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, USA.
2. **Professor Joyce Wilson,** Dept of Microbiology, University of Saskatchewan, CANADA.
3. **Professor Vikram Mishra**, Dept of Microbiology, University of Saskatchewan, CANADA.
4. **Dr. Albarino Cesar,** Centers for Disease Control and Prevention, **Atlanta, USA**
5. **Dr. Harley M. Jenks,** Centers for Disease Control and Prevention, **Atlanta, USA**
6. **Dr. Lisa Guerrero,** Centers for Disease Control and Prevention, **Atlanta, USA**

List of Technologies developed and transferred to private companies:

The following technologies (ELISA) have been released by DG, ICMR for marketing in Indian and international markets.

1. Japanese encephalitis virus antigen capture ELISA for detection of antigen in mosquitoes
2. Crimean-Congo hemorrhagic fever (CCHF) IgG ELISA (Sheep/Goat)
3. CCHF IgG ELISA (Bovine)

RESEARCH GROUPS

MAXIMUM CONTAINMENT FACILITY

Scientific Staff

Dr. Yadav P	Scientist 'E' & Group leader (yadavpd@icmr.gov.in)
Dr. Shete A	Scientist 'D' (anitaaich2008@gmail.com)
Dr. Sahay R	Scientist 'B' (sahay.rr@icmr.gov.in)
Dr. Sreelekshmy M	Scientist 'B' (sreelekshmy88@gmail.com)

Technical Staff

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

Project Staff

Dr. Nyayanit D	Scientist 'C'
Dr. Pardeshi P	Scientist 'C'
Dr. Chaubal G	Scientist 'B'
Dr. Kumar S	Scientist 'B'
Dr. Jain S	Scientist 'B'
Mr. Srivastava A	Technical Officer-A
Mr. Patil S	Technical Officer-A
Mr. Gondane Y	Technical Officer-A (Engineering)
Mr. Patil P	Technical Officer-A (Engineering)
Mr. Soman V	Technician-C
Mr. Kore P	Technician-C
Mrs. Bargat A	Technician-C
Mrs. Melag S	Technician-C
Ms. Chopde Y	Technician-C
Mr. Kadam M	Project Technician III
Mr. Suryavanshi A	Project Technician I
Mrs. Amble S	DEO-CDC Project
Mr. Pacharne N	DEO-CDC Project

MCL 1504: Enhancing biorisk mitigation awareness in public health community and creating laboratory networks for enhanced diagnostic capabilities to deal with surveillance and outbreaks of viral hemorrhagic fever and respiratory illness diseases

Investigators: DT Mourya & PD Yadav

Funding: Extramural (CDC-GHSA)

Project Duration: 2015-2020

Objective 1: Enhancing awareness as well as conducting various levels of teaching and training programs on Biorisk management and engineering controls required for safely operating biomedical laboratories and infection control practices in the public health settings.

Work Done: Several on-site and off-site training programs for management, scientific, technical and engineering personnel were organized by NIV, Pune in various part of the country (Table 1). More than 50 participants were trained during these trainings during 2017-18 (Fig 1).

***Objective 2:* Creating laboratory network for enhancing diagnostic capabilities for surveillance, outbreaks and epidemic investigations of high-risk viral pathogens causing viral hemorrhagic Fevers**

Under the CDC-GHSA, network of laboratories for viral hemorrhagic fever (VHF) were formed across the country to enhance the surveillance of viral pathogens leading to hemorrhagic manifestations. The purpose was to enhance the laboratory's capacity by sharing best laboratory practices and expand the collective knowledge base to meet the objectives of Global Health Security.

- A total of 5839 samples of suspected VHF cases were screened for dengue (DEN), Chikungunya (CHIKV) and Zika virus (ZIKV) by 6 VHF network laboratories between April 2017 and March 2018, which yielded 811 positives for DEN, 429 for CHIKV and 166 samples showed dual positivity (Table 1). None of the samples tested positive for ZIKV.
- Age groups of 19 to 45 years (27.04 %) and 6 to 18 years (26.02 %) showed maximum positivity. Sero positivity was found more in males (27.43 %) than females (19.48 %).
- A total of 1612 samples of VHF suspected cases, but negative for DENV, CHIKV and ZIKV referred to NIV by six VHF laboratory networks were screened for CCHFV and KFDV. All the samples tested negative for both the viruses either by Real Time RT-PCR and/or IgM ELISA.
- A total of 74 CDC Trioplex Real Time PCR kits along with positive controls and reagents have been supplied to different national and medical laboratories. Also, Primer Probes and Positive controls for CCHFV and KFDV Real Time RT-PCR for 500 reactions were provided to NIRTH, Jabalpur.

Hands on training on NGS, Illumina MiniSeq for identifying emerging viral pathogens at BSL-4 facility [31 Jul -11 Aug, 2017]

On-site workshop "Biorisk Management in Laboratory, hospital and field settings for KFD", Sindhudurga Maharashtra [30 - 31 October 2017]

Fig 1: Glimpses of workshops/Seminars conducted during the year

Table 1: VHF samples screened at six centers from April 2017 to March 2018

VHF Center	Total patient Tested	Total Patient Positive	Only DENV		Only CHK		Only ZIKV		DENV+CHIKV	
			+ve	% Positivity	+ve	% Positivity	+ve	% Positivity	+ve	% Positivity
Bhubaneswar	915	183	154	16.83%	12	1.31%	0	0.00%	17	1.86%
Jabalpur	1744	219	122	7.00%	76	4.36%	0	0.00%	21	1.20%
Jaipur	1940	742	342	17.63%	305	15.72%	0	0.00%	95	4.90%
Lucknow	318	97	44	13.84%	21	6.60%	0	0.00%	32	10.06%
Port Blair	447	75	60	13.42%	15	3.36%	0	0.00%	0	0.00%
Srinagar	475	90	89	18.74%	0	0.00%	0	0.00%	1	0.21%
Grand Total	**5839**	**1406**	**811**	**13.89%**	**429**	**7.35%**	**0**	**0.00%**	**166**	**2.84%**

MCL 1503: Study on Kyasanur Forest Disease viremia and antibody kinetics in patients and disease progression in monkey model.

Investigators: PD Yadav, DR Patil, A Shete, R Vishwanathan, Sreelekshmy M & DT Mourya

Funding: Intramural

Project Duration: 2016-2018

Objective 1: Persistence of KFD viral RNA, NS1 antigen, IgM and IgG antibodies in human serum

Over the years, KFDV has shown geographic expansion within Karnataka and also to neighboring states of Kerala, Tamil Nadu, Goa and Maharashtra. A major outbreak was reported from Sindhudurg District of Maharashtra in 2016. With an intention to develop a diagnostic algorithm, a KFD disease progression study was planned in KFD patients in Sindhudurg District. Persistence of KFDV viremia, IgM and IgG antibody kinetics from 72 KFDV positive patients were studied over a period of two years. A total of 399 human serum samples were collected at different time-points for 740 days and screened for KFDV viral RNA, anti-KFDV IgM and IgG antibodies with the in-house developed real-time PCR and IgM and IgG ELISA.

Findings: KFDV was detected from 1st to 18th post onset day (POD). Anti-KFDV IgM and IgG antibodies were detected as early as 4th and 5th POD and persisted till 122 and 468 PODs respectively (Fig 2). KFDV was detected in human clinical specimens using Real-time RT-PCR [1-3 POD]; anti-KFDV IgM [24-145 POD] and IgG by ELISA [6th POD to at least 2-year post infection] (Fig 3). An algorithm was predicted from statistical analysis using GAM model in R software to support laboratory findings pertaining to viral kinetics. This study led to the development of an algorithm for conducting KFDV diagnostic tests as per post onset day.

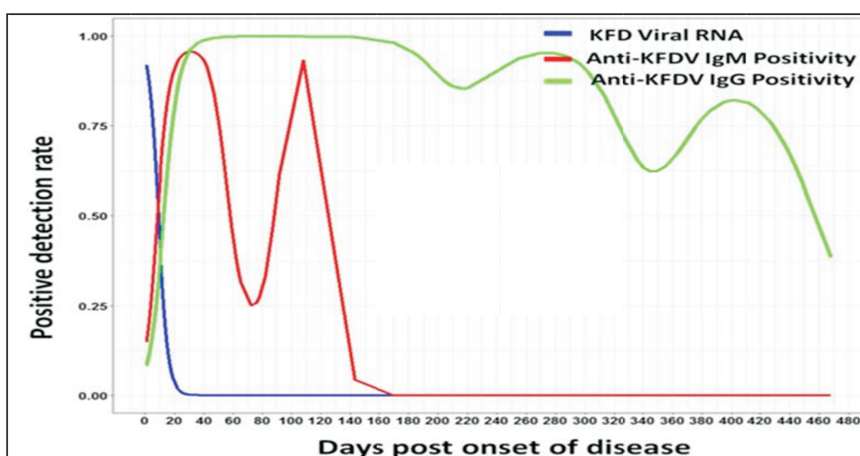


Fig 2: Response in terms of percent positivity for diagnostic markers at varying PODs: Blue line, red line and green line depicts percent positivity response for Real Time RT-PCR, anti-KFDV IgM antibody and anti-KFDV IgG antibody respectively. X-axis is increasing values of days post onset of illness and y-axis depicts the fitted values of the observed response.

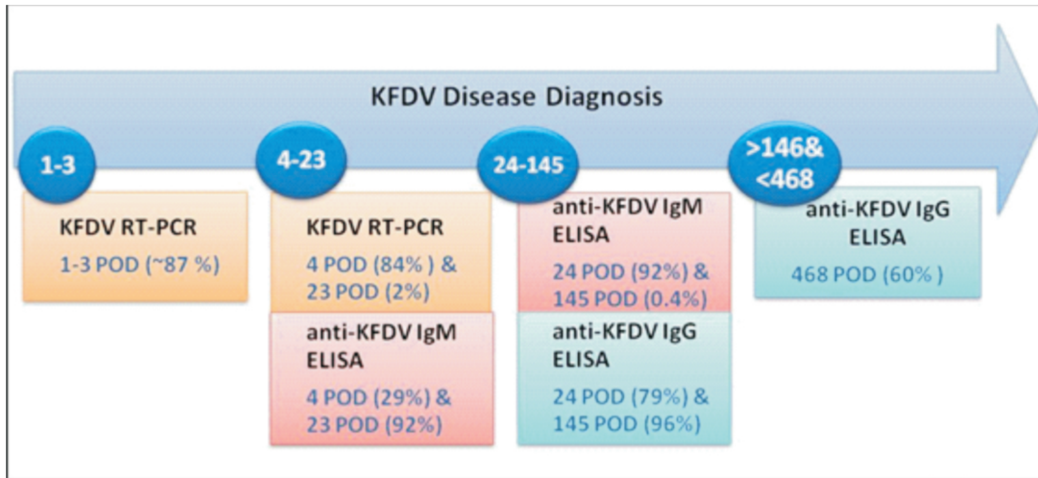


Fig 3: Diagnostic algorithm derived from KFDV for follow-up samples from Sindhudurg. Circle depicts the POD range and numbers in parenthesis indicate probability of percentage positivity.

***Objective 2:* Determining KFD disease progression in monkey model:**

Monkey is the only known natural host for KFDV apart from humans. In India, the black-faced langur (*Semnopithecus entellus*) and the red-faced bonnet monkey (*Macaca radiata*) are susceptible to KFDV. *M. radiata*, are local inhabitants in South India and are an excellent model to study disease progression of KFDV including persistence of viral RNA, anti-KFD IgM and IgG antibodies and cytokine profiles.

After obtaining necessary approvals from CPCSEA and Forest Department, 11 bonnet monkeys were procured through trapping agency authorized by Maharashtra government. Animals were quarantined in the monkey run (facility) at NIV, Pashan Campus and screened to rule out acquired infections if any. Hematological analysis, serum biochemistry, ultrasonography, chest X-ray, tuberculin test, deworming etc were also performed to confirm the fitness of the monkeys. Blood samples of the monkeys were screened to detect KFDV IgM and IgG antibodies before commencement of the study. Animals were weighed, randomly assigned into groups of three and infected with cell culture adapted KFD virus (Strain # NIV12839).

Findings: Daily monitoring of temperature, heart rate and weight is in progress. Blood samples are being collected on specific intervals to determine viremia, development of anti-KFDV IgM and IgG antibodies and also for hematology and biochemical parameters; stool samples to detect the presence of occult blood and urine for virus discharge. KFD progression study in monkey model is under progress.

MCL 1602: Studies on the vector competence of an Indian strain of Aedes aegypti to Zika virus

Investigators: DT Mourya, MD Gokhale, PD Yadav, GN Sapkal, Triparna Majumdar & Prasad Sarkale

Funding: Intramural

Duration: 2016-2018

Objectives:

- To determine the replication potential of an Indian strain of *Aedes aegypti* mosquitoes to Zika virus (ZIKV) and its growth kinetics in the mosquito.
- To determine vector competence of the mosquito to ZIKV
- Determination of transovarial transmission (TOT) of ZIKV in *Aedes aegypti* mosquitoes
- Interaction studies on the multiplication of ZIKV, dengue virus and chikungunya virus in concomitantly infected vector mosquitoes.

Word done: Cell culture grown (4 passages in Vero) ZIKV (African strain MR-766, procured through WHO) was used in the study. *Ae aegypti* mosquitoes procured from insectory maintained at NIV, Pune were experimentally infected with ZIKV and the growth kinetics was determined. Mosquitoes fed on viraemic mice were incubated for 10 days at experimental conditions and used for transmission studies in mice. The effect of co-infection/super-infection with dengue virus (serotype-2) and chikungunya virus (CHIKV) was carried out by experimentally infecting the

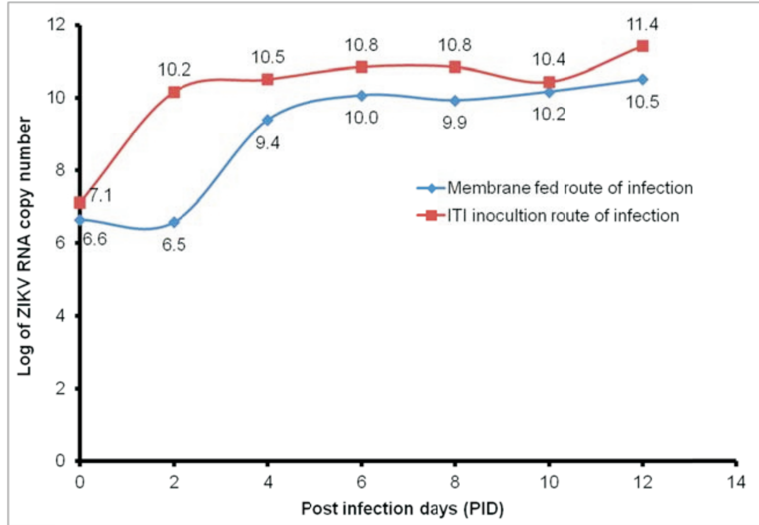


Fig 4: Replication kinetics of ZIKV in an Indian strain of *Aedes aegypti* infected via intrathoracic inoculation (ITI) and membrane-feeding

mosquitoes either alone or in combination. Replication of these viruses in mosquitoes was determined using real-time reverse transcription-polymerase chain reaction and immuno-fluorescence assay.

Findings: ZIKV replication in *Ae aegypti* (Fig 4) and transmission to infant mice was confirmed by clinical symptoms and presence of viral RNA in different organs of mice. Concomitant infection of *Ae aegypti* mosquitoes with DENV, CHIKV and ZIKV showed simultaneous propagation of all the three viruses. Infection of mosquitoes with CHIKV followed by ZIKV showed 7% dual positivity; 8.3% positivity for ZIKV followed by DENV; 8.3% positivity for DENV followed by ZIKV and 5% positivity for ZIKV followed by CHIKV in individual head squashes. TOT could not be demonstrated for ZIKV in the Indian strain of *Ae aegypti* mosquitoes. Super-infection experiments showed that ZIKV might have a relative advantage in replication dynamics over DENV.

Objective 2: Zika virus pathogenesis in mice after infection by the bite of infected mosquitoes

Work done: *Ae aegypti* mosquitoes were experimentally infected with ZIKV (MR-766 strain) by the oral route and allowed to feed on

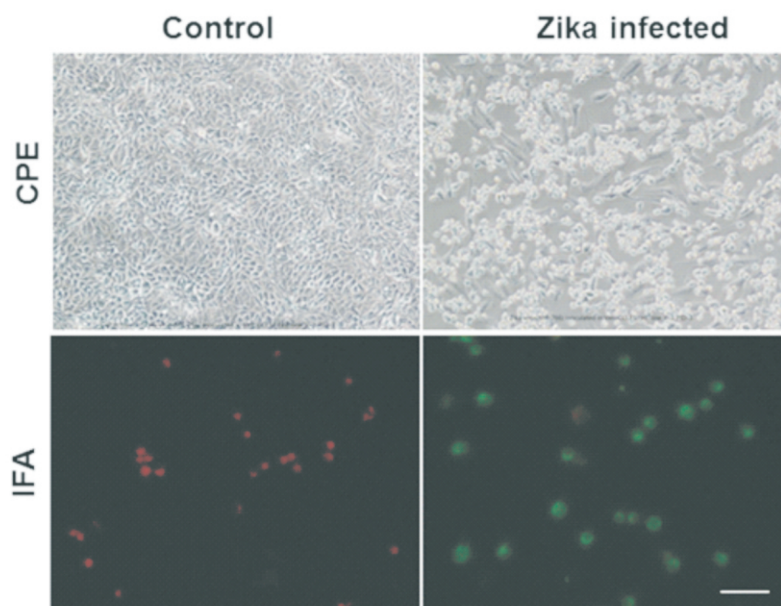


Fig 5: Cytopathic changes as observed by bright field as well as fluorescence microscopy in Zika virus infected Vero CCL 81 cell line. Scale bar = 100 μm.

infant mice (CD1) after an incubation of 8 days. Sick mice were euthanized; organs harvested and subjected to real-time RT-PCR, histopathology and immunohistochemistry studies.

Findings: Clinical symptoms commenced in infected mice after 4–5 days post exposure to mosquito bite. Real-time RT-PCR analysis showed the presence of viral RNA in different organs of mice, *viz*., brain, liver, kidney, spleen, lungs and intestines. Brain tissue showed higher viral loads as compared to other organs. Cytopathic changes were observed in *Vero CCL 81* cells when inoculated with ZIKV infected organ suspensions (Fig. 5). Histopathological and immunohistochemistry studies have revealed the presence of the virus and associated lesions in the brain indicating tissue tropism for neuronal cells (Fig. 6, 7).

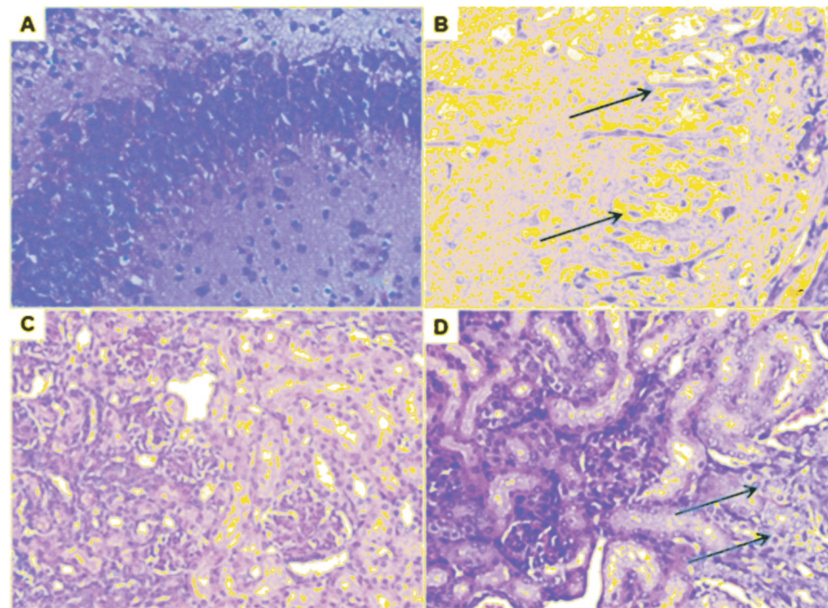


Fig 6: Histopathological changes in various organs of infant CD1 mice infected by ZIKV (mosquito bite) (x400) (shown by arrows). A: Control brain tissue showing normal neuronal tissue B: Infected mouse brain depicting degeneration and neuronal necrosis. C: Control kidney tissue D: Infected kidney depicting degenerative pathological changes. Scale bars are shown in individual figures (10 μm).

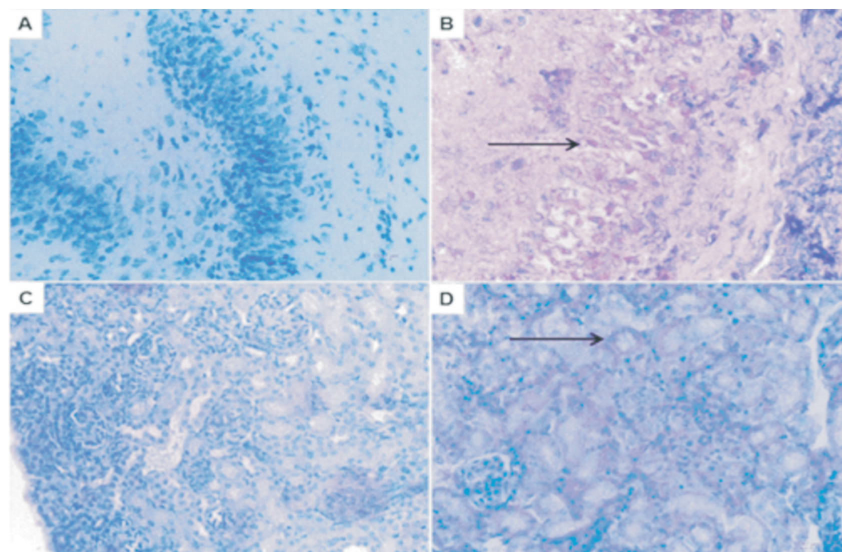


Fig 7: Immunohistochemical changes in organs of CD1 infant mice infected with ZIKV (x 400) (shown by arrows). A: Uninfected brain B: Infected mouse brain depicting presence of viral antigen in the neurons with degeneration and neuronal necrosis C: uninfected kidney D: Infected kidney depicting presence of viral antigens. Scale bars are shown in individual figures (10 μm).

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

Investigators: DT Mourya, PD Yadav, YK Gurav, PS Sathe, G Sharma (IVRI, Mukteshwar); D Raval (Commissionerate of Health, Gujarat), K Upadhyay (B J Medical College, Ahmadabad)

Funding: Intramural

Project Duration: 2015-2017

Objectives:

i) Development and standardization of CCHFV specific IgG ELISA for detection of IgG antibodies in domestic animals.

ii) Nationwide screening of domestic animal samples for CCHFV specific IgG antibodies.

iii) Epidemiology, risk factors and seroprevalence of Crimean-Congo Hemorrhagic Fever (CCHF) infection among humans in rural population in Gujarat

iv) Epidemiological profile of CCHF disease, its spread and risk factors in at- risk rural population in CCHF affected villages.

v) To estimate the extent of CCHF virus infection among high-risk population groups in CCHF affected villages in Gujarat.

vi) To estimate the extent of CCHF virus infection among high risk groups and general population in CCHF unaffected villages in Gujarat.

Objective I :

Work done: Developed and standardized an anti-CCHF Human IgG ELISA assay that has been found to be specific for detection of anti CCHF human IgG antibodies. The assay validation was performed in-house and at external laboratories. The technology has been transferred to Zydus Cadila through ICMR, New Delhi.

Objective III-V :

Word done & fingings:

A total of 4978 human serum samples from all the 33 districts of Gujarat were screened for anti-CCHFV Human IgG seroprevalence. Twenty-five samples tested positive for CCHF (0.5%, 95%CI 0.3%-0.74%) of which 17 belonged to category "A" (CCHF affected cases/ close contacts); 5 belonged to category "B" (Neighborhood) and one each from category "C" (Animal Handlers), Category "E" (Farmers) and Category "F" (Abattoir worker) (Figure 8).

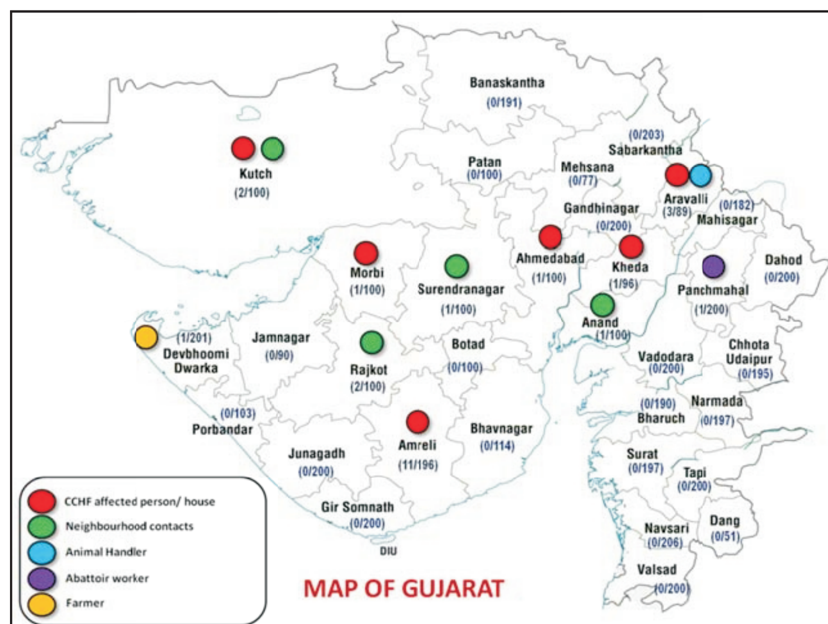


Fig 8: CCHF Seropositivity in districts of Gujarat State based on subject categories. The numbers in brackets indicate total number of positive samples/total number of samples tested

MCL 1803: Identification and characterization of novel viral isolates using Next-generation sequencing platform

Investigators: PD Yadav, AM Shete, TP Majumdar, DT Mourya

Funding: Intramural

Duration: 2018-2021

Background: Preparedness to diagnose known and unknown infectious disease agents is one of the mandates for NIV to help public health systems to initiate control measures. In such scenario, discovery and characterization of novel viruses are utmost important. As Next generation sequencing platforms can sequence a mixture of genetic materials from a heterogeneous mix, with high sensitivity making them ideal tools for detecting novel viruses. NIV, Pune has acquired an NGS platform and attempts are being made to establish the system with the help of CDC, USA for identifying viruses from samples of unknown etiology (Gorakhpur samples).

Objectives:

1. Establishment and standardization of Next Generation Sequencing platform at NIV
2. Identification of unknown virus isolates available with BSL-4 laboratory and virus repository.
3. Targeted sequencing of known positive samples to understand viral heterogeneity
4. Development of diagnostic assays for the newly identified viral pathogens

Work done: Next-generation sequencing machine was installed at the BSL-4 and a pool of scientific and technical staff was given training to work on the platform. Protocol was finalized and work has been initiated.

I. Isolation and identification of equine encephalosis virus, an arbovirus from horse samples using NGS platform.

Background: NIV received blood and necropsy samples (lung, liver, kidney, and spleen) from a horse suffering from fever, nasal discharge, loss of appetite and symptoms of weakness, for etiological investigation from a horse farm in Pune in July 2008. Nasal swab, blood, and serum samples of 13 other equines reported to have similar symptoms but recovered were also sent for investigation. Initial screening for equine influenza, Japanese encephalitis and West Nile viruses tested negative.

Work done & fingings: Blood specimens were processed for isolation in cell culture *(Vero CCL-81)* and the etiological agent was isolated. RNA and DNA extracted from the infected cells were analyzed on an Illumina Miniseq NGS platform, and the infectious virus was identified as equine encephalosis virus (EEV). The complete genome of the isolate was obtained and phylogenetic analysis of the VP2 gene sequences, which is used for the genotype classification of Reoviruses, revealed that the Indian isolate groups with Bryanston EEV strain belonging to serotype 4 (Fig. 9).

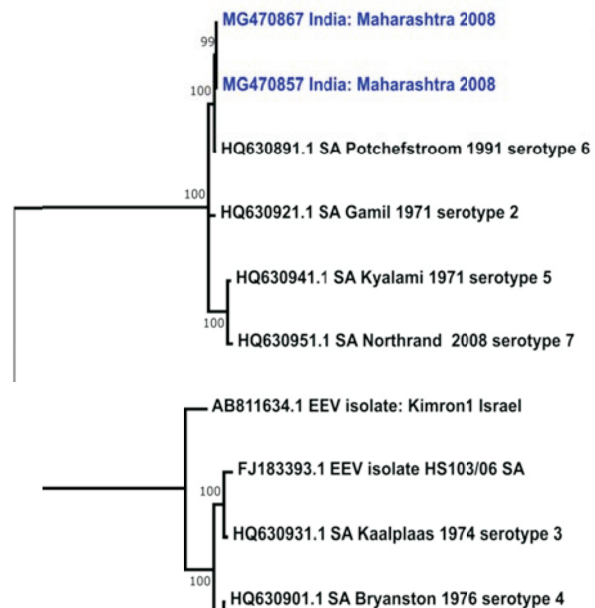


Fig 9: Phylogenetic tree of the VP2 segment with reference EEV strains.

The information about EEV detection has been intimated to Department of Animal Husbandry, Dairy and Fisheries of the Ministry of Agriculture and Farmers Welfare, New Delhi, India. A survey will be initiated to understand the EEV prevalence in equine populations in various parts of the country.

II. Identification and characterization of novel mosquito-borne (Kammavan-pettai virus) and tick-borne (Wad Medani) reoviruses isolated in India

Background, work done and findings: Wad Medani virus (WMV), isolated (1954) from *Hyalomma marginatum* ticks and Kammavanpettai virus (KVPTV) isolated (1963) from a Brahmini myna (*Sturnia pagodarum)* were characterized using the NGS approach. NGS led to the determination of their complete genomes, and identification of both the virus isolates as orbiviruses (family *Reoviridae*). Sequencing data showed that KVPTV has a genome of 12,710 nucleotides encoding proteins ranging 238–1290 amino acids (aa) in length. WMV genome contains 16,941 nucleotides encoding proteins ranging 214–1305 aa in length. Phylogenetic analysis of VP1 segment along with the capsid segments VP5 and VP7 revealed its phylogenetic relation with Reoviruses. Complete characterization of the newly identified orbiviruses will help in the development of diagnostic reagents as preparedness to combat outbreaks, in future.

III. Identification and confirmation of Quaranfil virus, an Orthomyxovirus in India.

Background, work done and findings: An unknown isolate obtained from soft ticks in Karnataka state was characterized using NGS platform and BLAST analysis of the RNA contigs led to the identification of the agent as Quaranfil virus (QRFV), a *Quarjavirus* belonging to family *Orthomyxoviridae.* QRFV has a wide distribution in Egypt, South Africa, Afghanistan, Nigeria, Kuwait etc and is reported to cause human infections. Data showed that genome (11,427 nucleotide base pair) consists of 6 viral RNA segments encoded for polymerase PB2, polymerase PB1, polymerase PA hypothetical protein (Nucleoprotein), Hemagglutinin (HA) and unknown (266 aa). Homology analysis showed this close to Afghanistan strain with 91% PB2 nucleotide identity.

IV. Optimization of Real Time RT-PCR for the detection of QRFV

Work done: A Real Time RT-PCR assay has been standardized for QRFV using varying concentrations of primers/probes. The specificity and sensitivity of the primers were checked with known viral RNAs of influenza A, influenza B, influenza C, avian influenza (Orthobunya viruses) and Tioman virus (Outlier virus). In addition, tick samples (Hemophysalis and Hyalomma ticks), human samples, control mouse brain suspension, control tissue culture fluid (from *Vero CCL81*, *Vero E6*, *BHK21*, *RD* and *PS* cell lines) were used and found that the assay is specific and did not cross-react with other viruses of the sub family Orthomyxoviridae, or other viruses. The assay was found sensitive up to 10^{-6} dilutions of viral RNA.

Complete genome sequencing of Kaisodi virus (Phlebovirus: *Phenuiviridae)*

Background: Kaisodi virus isolated from *Haemaphysalis spinigera* ticks was characterized on NGS platform and compared to other representatives from the Phlebovirus group. NGS based analysis of the sequence confirmed it as Kaisodi virus (KASDV). All the three segments of this virus (L, M, and S) could be retrieved using the *de-novo* approach. Phylogenetic analysis revealed its closeness to Silver Water virus (Fig 10).

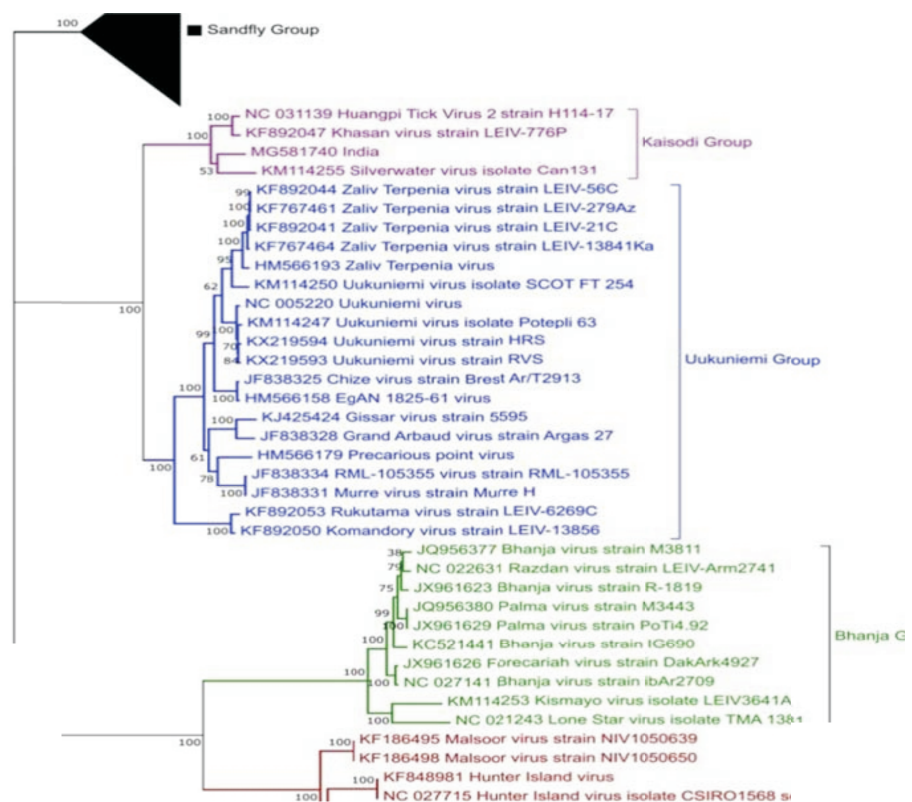


Fig 10: A phylogenetic tree for M segment of Kaisodi virus with other reference sequences.

MCL 1804: Development of multiplex antigen detection ELISA using group specificity approach for identification of viral agents

Investigators: AM Shete, PD Yadav, R Jain & PS Sathe

Funding: Intramural

Project Duration: 2018-2021

Background: Conventional viral detection methods rely upon symptoms and morphological identification of virus using electron microscopy, Real-time polymerase chain reaction (qRT-PCR) and RT-PCR/PCR, etc. However, slight mutation or change in antigen or viral sequences leads them undiagnosed by these methods due to their hypersensitivity. Known arboviruses *viz*., DENV, KFDV, JEV, Zika, Chikungunya, CCHFV, etc., are well characterized, however, there are more than 400 viruses that cause human infections and diagnostic assay for majority is not available.

In a country like India, a large number of viral fever cases go undiagnosed due to lack of diagnostic methods. Similarly, etiological agents for several outbreaks that caused significant morbidity and mortality were not identified. Identification of the agents responsible for causing high disease burden in the society will be useful from public health perspectives. Therefore attempts are being made to develop a detection method for initial screening to identify the agent at least to the genus level.

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

Investigators: PD Yadav, GN Sapkal, AB Sudeep, DT Mourya

Funding: Extramural (DHR)

Project Duration: 2016-2019

Background: Positive controls are essential for conducting molecular diagnosis and many of the Virology network laboratories (VDLs) lack the infra structure to produce positive controls especially of highly pathogenic viruses. NIV has taken up the call and initiated measures to supply reagents and positive controls to National laboratories, VDLs and WHO-SEAR countries.

Objective: **Preparation of lyophilized positive controls for ZIKV, KFDV, YFV (17D) and CCHFV**

Work done:

(i) ***In vitro*** **propagation of viruses:** Validated virus stocks were prepared in vertebrate/ invertebrate (mosquito) cell lines, titrated, aliquoted and stored at -80°C.

(ii) **Standardization of gamma irradiation dose for virus stocks, inactivation and supply to National Laboratories:** The gamma radiation dose and time for inactivation was standardized using the Gamma chamber installed at the BSL-4 Laboratory, NIV, Pune (with the help of BARC, Mumbai). The virus stocks were inactivated, confirmed inactivation by *In vitro* methods, concentrated using vacuum concentrator, aliquoted, lyophilized and stored at –80°C. The positive controls supplied to VDRL and other National laboratories are given in Table 6.The following stocks of gamma irradiated positive controls are available with BSL-4 group.

- Lyophilized positive control (2000 vials) for detection of Zika viral RNA by single step RT-PCR, Real Time RT-PCR that can detect both strains of Zika virus
- Lyophilized positive control (200 vials)of tissue culture derived KFD virus (Strain 12839)
- Lyophilized positive control of tissue culture derived CCHF virus (Strain 11704).

Table 6: List of viruses propagated and supplied to National Laboratories

Sr.No.	Antigen/other details	Virus stock supplied to VRDL centers/NIV Pune
1.	Japanese encephalitis virus (733913)	Bangalore Unit, NIV, Pune, PGIMER, Chandigarh, RMRC, Bhubaneswar, Entomology Dept. NIV, Pune
2.	West Nile virus (804994)	Bangalore Unit, NIV, Pune, PGIMER, Chandigarh, RMRC, Bhubaneswar
3.	Chikungunya virus (061573)	PGIMER, Chandigarh, RMRC, BhubaneswarEntomology Dept. NIV, Pune
4.	Mumps (0V-14-037)	KGMU, Lucknow
5.	Measles (1520640)	Stocked and stored
6.	Rubella virus (12195-m-09-060-2)	KGMU, Lucknow
7.	Cox. B3 virus	PGIMER, Chandigarh,RMRC, Bhubaneswar
8.	Rota virus	Stocked and stored
9.	Dengue-1	Stocked and stored
10.	Dengue-2	Animal House, (NIV), Entomology Dept. NIV, Pune.
11.	Dengue-3	Stock prepared and stored
12.	Dengue-4	Stock prepared and stored
13.	Zika (MR-766 strain) RT-PCR primers along with +ve controls	VRDL, NIV, Pune
14.	Zika virus (MR-766 strain) Lyophilized RNA	NCDC, New Delhi
15.	Kyasanur Forest Disease virus (KFDV strain 12839) Positive control and Real Time RT-PCR reagents	NIIRTH, Jabalpur
16.	CCHFV Positive control and Real Time RT-PCR reagents	● NIIRTH, Jabalpur
17.	Anti KFD Human IgM ELISA	● Manipal Centre for Virus research, Manipal
18.	Anti KFD Human IgM ELISA	● VDL, Shimoga
19.	KFD Real Time reagents	● VDL, Shimoga
20.	Yellow Fever (17D strain) Antigen	● VRDL, NIV, Pune
21.	Yellow fever	● Bangladesh through WHO office
22.	Yellow fever	● SEARO regional office through WHO office
23.	Nipah virus Real Time RT-PCR and positive controls	● BSL-4 Laboratory, NIV, Pune
24.	Chandipura virus	SMS Medical College, Jaipur, PGIMER, Chandigarh, RMRC, Bhubaneswar, Entomology Dept. NIV, Pune

MCL 1802 Preparation of reagents for highly infectious viruses

Investigators: PD Yadav, A Shete, P Sarkale

Funding agency: Intramural

Project Duration: 2018-2020

As per the Institute's mandate, the BSL-4 laboratory have developed diagnostic tests or many hemorrhagic fever viruses and other highly pathogenic viruses (KFD, CCHF, Nipah virus, Marburg, Ebola, Pox viruses etc) to diagnose the etiological agents during outbreaks/clusters of highly infectious diseases/ emerging or re-emerging viral threats.

MCL 1702: Susceptibility and vector competence study of Cat Que virus

Investigators: P D Yadav, M D Gokhale, AM Shete & TP Majumdar

Funding agency: Intramural

Duration: 2017-2019

Background: An agent isolated in 1961 from a Pond Heron (*A. grayii*) in Sagar district of Karnataka was identified as Cat Que virus (CQV), a member of Simbu group (Orthobunya: *Bunyaviridae*).

Work Done**:** Nucleocapsid gene-based RT-PCR and Real Time RT-PCR assay was developed and used for screening of CQV from retrospective febrile illness cases of human samples (n=1000) collected during 2014-2017. Simultaneously anti-CQV swine IgG and anti-CQV human IgG ELISA were also developed and screened 600 swine and 833 human samples of which two human serum samples tested positive.

Experiments were conducted to determine the susceptibility and vector competence of *Aedes aegypti*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* to CQV. Results indicated multiplication of the virus both by intrathoracic as well as artificial membrane/oral feeding routes. Vector competence studies are in progress.

NABL accreditation:

Authors: PD Yadav, AM Shete, DT Mourya

NABL accreditation system was implemented with the objective to provide the quality and technical competence of diagnostic testing done by the BSL-4 laboratory. Internal audits of the laboratory were regularly conducted as per the Quality Manual.

The scopes & services of the laboratory for NABL accreditation are as below

1. Anti-CCHF human IgM & IgG ELISA: By NIV kit and Commercial kit
2. CCHF Real Time RT-PCR: to detect CCHF Viral RNA from human, animal and ticks
3. KFD Real Time RT-PCR: to detect KFD Viral RNA from human, animal and ticks
4. Anti-KFD IgM ELISA: to detect IgM antibody in human sample

A surveillance audit for continuation of the NABL accreditation was conducted on 3rd and 4th February 2018. Lead Assessor, Dr. Sonia Nandre, General Manager, Laboratory Operations, TUV India (Pvt) Ltd Pune and Technical Assessor Dr. Pramod Kumar Chug, (OC DOG, New Delhi). Diagnostic tests included in NABL accreditation's scope were performed under the observation of the technical assessor. Records of the documents related to testing of the samples *viz.*, sample receiving, aliquoting, processing, storage and reporting as well as equipment calibration status were reviewed. Dr. Nandre conducted the audit of the Quality system of the laboratory and detected one major and one minor non-conformities. However, the auditors have considered continuation of our Quality System after correcting the nonconformities. Report of closure of the non-conformities and corrective action taken was submitted to NABL office.

Technologies developed and transferred to commercial partner through ICMR, HQ

Three technologies (Anti-CCHF sheep/ Goat IgG ELISA, Anti CCHF cattle IgG ELISA and Anti KFD Human IgM ELISA) developed by BSL-4 group were transferred to Zydus Cadila in 2017, of which two technologies (CCHF animal IgG ELISA) were released by ICMR in November 2017 (What about the third). In March 2018, three more technologies were transferred to Zydus Cadila. The details are as below:

1. **Anti CCHF human IgM ELISA**
2. **Anti CCHF human IgG ELISA**
3. **Anti KFD human IgG ELISA**

Two PCR based technologies were submitted to ICMR for transfer of technology (displayed on ICMR website for technology transfer)**.**

1. **KFD Real Time RT PCR and RT-PCR assay:**
2. **Yellow fever antigen capture ELISA:**

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

Investigators: DT Mourya, PD Yadav, Anita Shete

Funding: Intramural

Project duration: 2015-2017

Work done:

I. Re-validation of BSL-4 laboratory: The BSL-4 laboratory was shutdown during November-December 2017 for annual maintenance/re-validation. During the shutdown period, complete maintenance and servicing of the equipments inside the BSL-4 laboratory was carried out. The validation of complete laboratory, including bio-safety doors, inlet and exhaust plenums, biosafety cabinets, autoclaves, BLED Plant and tissue digester etc. was done. Insulation and fixing of false ceiling sheets of ground floor atrium area, BSL-2 laboratory area, and washrooms, which were damagd due to leakage were also completed during the period.

II. Re-commissioning of Gamma chamber: The existing Gamma chamber GC-5000 has been replaced with a replenished Cobalt-60 GC-5000 with guidance from BRIT and BARC on 12th January 2018 as the decay rate has affected its radiation activity and inactivation of viral agents.

III. Annual Inspection of IBR Boiler: The IBR boiler installed at the BSL-4 facility needs to be inspected every year as per the Indian Boiler Act. The boiler inspector from "Directorate of Steam Boiler Department" visited the site in October 2017, measured/examined the boiler and determined the maximum pressure in the prescribed manner. On receipt of the report from the boiler inspector, the Chief Inspector validated and assigned a "Certificate for the use of IBR boiler" for the next one year.

MCL 1805: Providing diagnostic support for referred samples of viral hemorrhagic fever and other unknown etiology and outbreak investigation

Investigators: PD Yadav, AM Shete, RR Sahay

Funding agency: Intramural

Duration: Ongoing Project

1. Investigation of Varicella Zoster outbreak in Mumbai, Maharashtra state

Varicella (chickenpox) is a highly contagious disease caused by Varicella Zoster virus (VZV). The disease is usually benign but can be life-threatening to immunocompromised individuals, with an attack rate of >85%. Public Health Department of Municipal Corporation of Greater Mumbai (MCGM), reported increase in the number of cases of Varicella in March & April months. Investigations were carried from 7-20th April 2017 and 50 acute cases of clinically suspected Varicella were enrolled for investigation. All the 50 cases were residing in slum areas of Mumbai and had vesicular rash. Forty-one cases (82%) showed positive contact history with known case of clinically diagnosed chickenpox (friends/ room-mates/ co-workers) signifying high secondary attack rate. None of the cases had history of immunization or exposed to infection. As per the feasibility of collection and attrition, 22 patients were followed up and detected VZV DNA by Real Time PCR in serum, urine, throat swab, vesicle fluids and crust (Fig.11). To understand the circulating genotype of the virus, a phylogenetic tree of the sequences was created for envelope protein gB and analysis has shown that thirteen sequences belonging to M1 genotype were clustered with clade 4/5 and three sequences belonging to M2 genotype were clustered with clade 1/3.

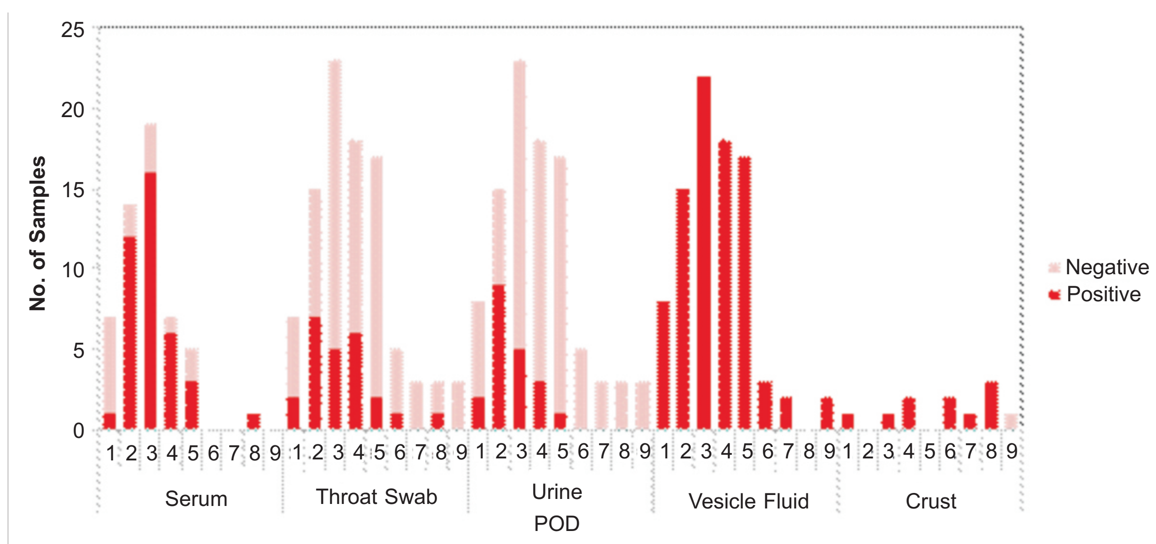


Fig11: Detection of VZV DNA in Clinical specimens (serum, throat swab, urine, vesicular fluid and crust) by Real Time PCR at different PODs

2. Immunization program for the staff during the year 2017-2018

As part of the routine immunization program, 25, and 21 NIV staff were immunized against KFDV and Hepatitis B virus respectively during 2017-2018 after collecting baseline serum samples from all the vaccinees before immunization. Twenty staff members were administered with bivalent oral Polio vaccine (bOPV) in May 2017 and fractional dose of Injectable Polio vaccine (f-IPV) in June 2017.

Samples tested : The total number of samples tested and the results are given in Table 7 & Fig. 12.

Table 7 : Details of high pathogenic virus samples tested at BSL-4 laboratory

Suspected etiology	Number tested	Sample tested for	No. positive	Place of origin of positive samples	Assays used
KFDV	374	KFDV	21	Karnataka	
CCHFV	54	CCHFV	5	Gujarat	
Yellow Fever	35	YFV	0	Andhra Pradesh	Real Time RT-PCR
Hantan	22	Hantan	0	—	Nested RT - PCR, IgM ELISA
VHF	13	YF, JE, DEN, CHIK, ZIKV, WNV, CHPV	1 (dengue)	Tamil Nadu	CDC Trioplex Real Time RT-PCR, Real Time RT-PCR
VZV	7	VZV, Measles, Rubella	0	—-	Real Time PCR, anti-Measles anti Rubella IgM ELISA
Nipah virus (Gujarat)	01	NiV	0	-	Real Time PCR, IgM ELISA
VHF (Oman)	01	CCHFV, KFDV, DEN, CHIV, ZIKV	1 (CHIKV)	Oman	CDC Trioplex, Real Time RT-PCR
Lassa Fever (NRI from Ghana and referred by Himalayan Hospital, Dehradun)	01	Lassa fever, CCHFV, YFV	0	0	RT-PCR

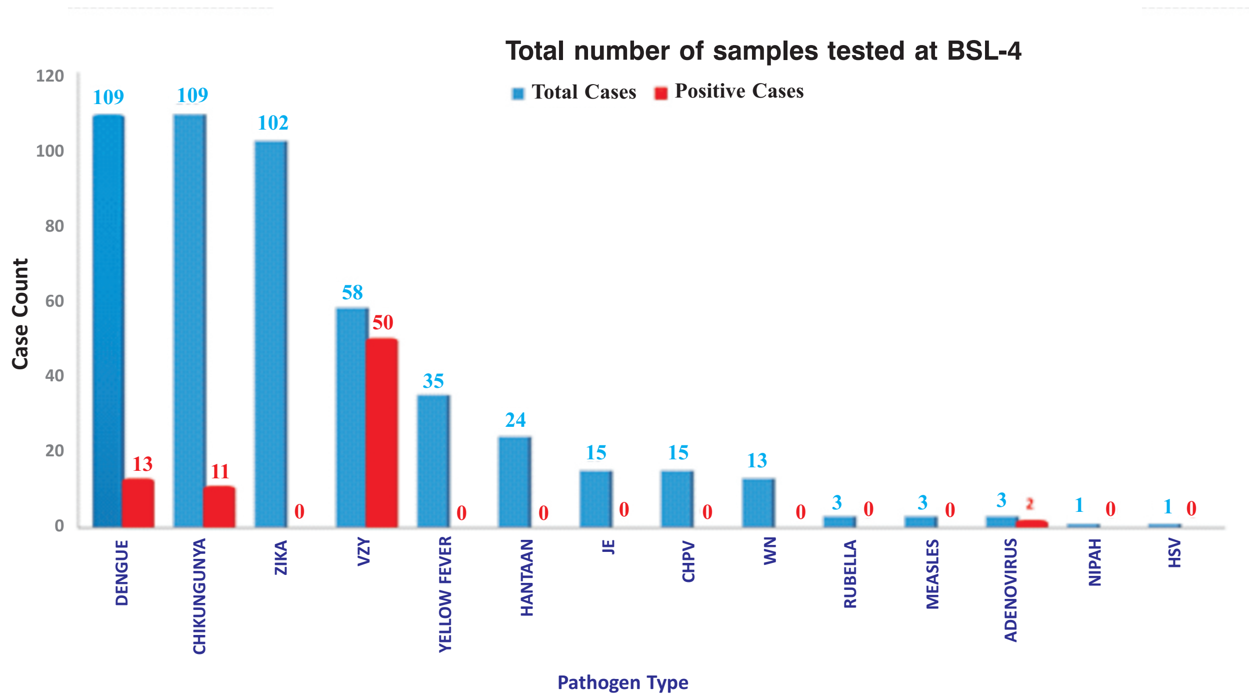


Figure 12: Details on clinical specimens tested for different viruses at BSL-4 Laboratory

INFLUENZA

Scientific Staff

Dr. MS Chadha	Scientist G & Group Leader (mscniv@gmail.com) (superannuated in June 2017)
Dr. VA Potdar	Scientist 'C' & Group Leader (From 22 Jan 2018) (varshapotdar9@yahoo.co.in)
Dr. ML Choudhary	Scientist C (mlchoudhary@gmail.com)
Dr. SD Bhardwaj	Scientist B (sumitduttbhardwaj@gmail.com)

Technical Staff

Mrs. SM Karambelkar	Technical Officer
Mrs. VC Vipat	Technical Assistant
Mrs. SY Jadhav	Technician C
Mr. HK Kengale	Technician C
Ms. A Vaidyanathan	Technician C
Mr. AS Awhale	Technician B
Mr. VN Autade	Technician B

Project Staff

Dr. Chandankumar Maurya	Sc. B, Non-Medical
Dr. Rohan Ghuge	Sc. B, Medical (From 17 July 2017)
Mr. K Mahendra Reddy	Programmer (From 19 Dec 2017)
Mr. DD Hinge	Technical Assistant
Mrs. TS Rale	Technical Assistant
Mrs. Urmila Choudhary	JRF (From 15 May 2017)
Miss Poonam Shinde	JRF (From16 Oct 2017)
Mr. Nandadeep Jadhav	SRF (From 16 Oct 2017)
Miss Spardha Doshi	Technical Assistant (From 13 Nov 2017)
Miss Prapti Deshmukh	Technical Officer (From 13 Nov 2017)
Mr. Paramvir	Staff Nurse (From 13 Nov 2017)
Mr. Pramodkumar Sunda	Staff Nurse (From 13 Nov 2017)
Mr. Meena Ramphool	Staff Nurse (From 13 Nov 2017)
Mr. Solanki Lalaram	Staff Nurse (From 13 Nov 2017 to 14 March 2017)
Mr. Shubham Joshi	Staff Nurse (From 4 Dec 2017)
Lab attendants (Three)	

INF1601: Diagnostic Services / Outbreak Investigation

Investigators: MS Chadha, VA Potdar, ML Choudhary & SD Bhardwaj

Funding Agency: Intramural

Duration: 2015-2020

Background: Clinical samples from Influenza infection suspected patients were referred for diagnosis of influenza A/H1N1pdm09 by different clinics/hospitals across Maharashtra.

Objective: To provide diagnosis for referred samples received from suspected Influenza patients.

Work done: 3374 clinical samples were tested using Real Time RT-PCR and 665 (19.7%) samples were found positive for influenza viruses. 579 (17.1%) were Influenza A/H1N1pdm09 positive, 42 (1.24%) influenza A/H3N2) positive and 44 (1.3%) were Influenza B positive (Fig 1).

Virus isolation and characterization:

Representative influenza positive samples received from Srinagar, Delhi, Jaipur, Ahmadabad, Rajkot, Maharashtra, Hyderabad, Bhubaneswar, Kolkata, Guwahati, Chennai and Kerala were attempted for isolation in MDCK cell line, which yielded 294 isolates (H1N1pdm09: 188/270; H3N2: 50/91; Influenza B: 56/100). Genome analysis of influenza A/H1N1pdm09 virus HA gene (n=80) showed that 2017-18 isolates grouped in clade 6B.1, which is similar to A/Michigan/45/2015 strain and is the 2018-19 northern hemisphere vaccine component with S84N, S162N and I216T signature amino acid change (Fig 2). Phylogenetic analysis showed HA gene (n=39) of influenza A/H3N2 virus grouped in clade 3C.2a1 and is characterized by signature amino acid change N171K, F159Y, N144S and similar to A/Singapore/INFIMH-16-0019/2016, which is the 2018-19 northern hemisphere vaccine component. Phylogenetic analysis of HA gene (n=10) of influenza B isolates showed that both the lineages Victoria and Yamagata are in circulation and are similar to 2018-19 northern hemisphere vaccine components Colorado/06/2017 and B/Phuket/3073/2013.

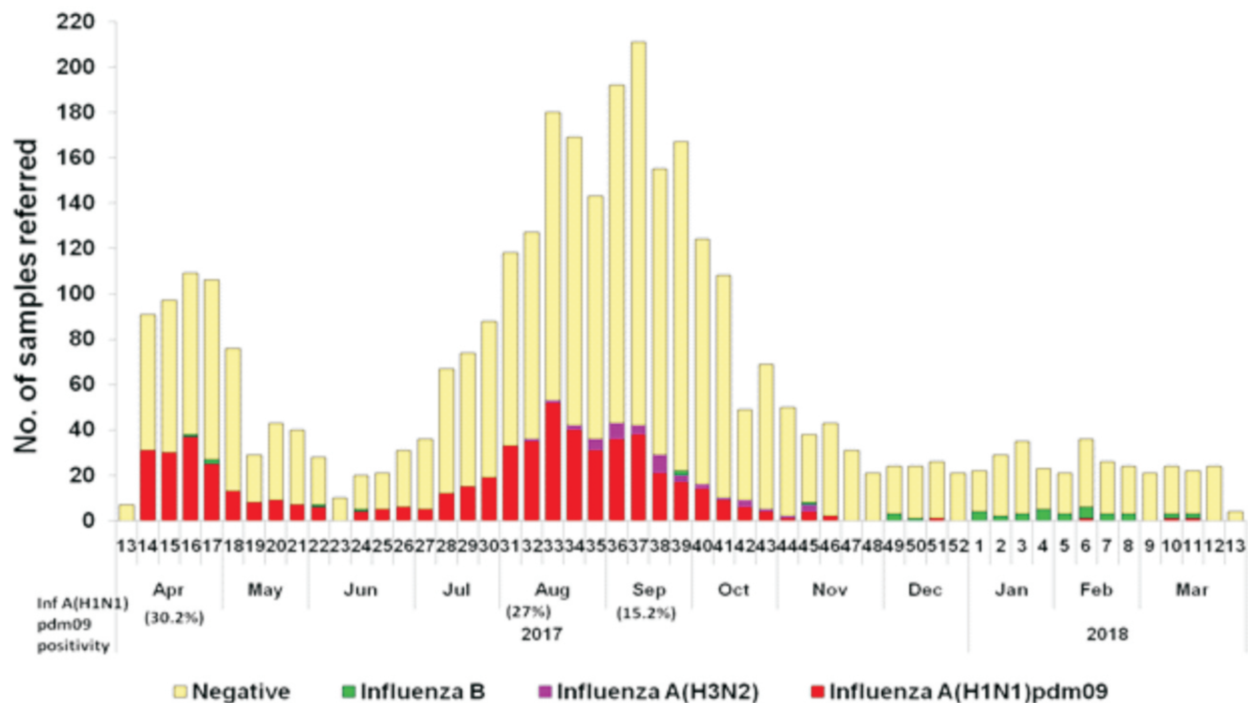


Fig 1: Influenza activity in referred samples

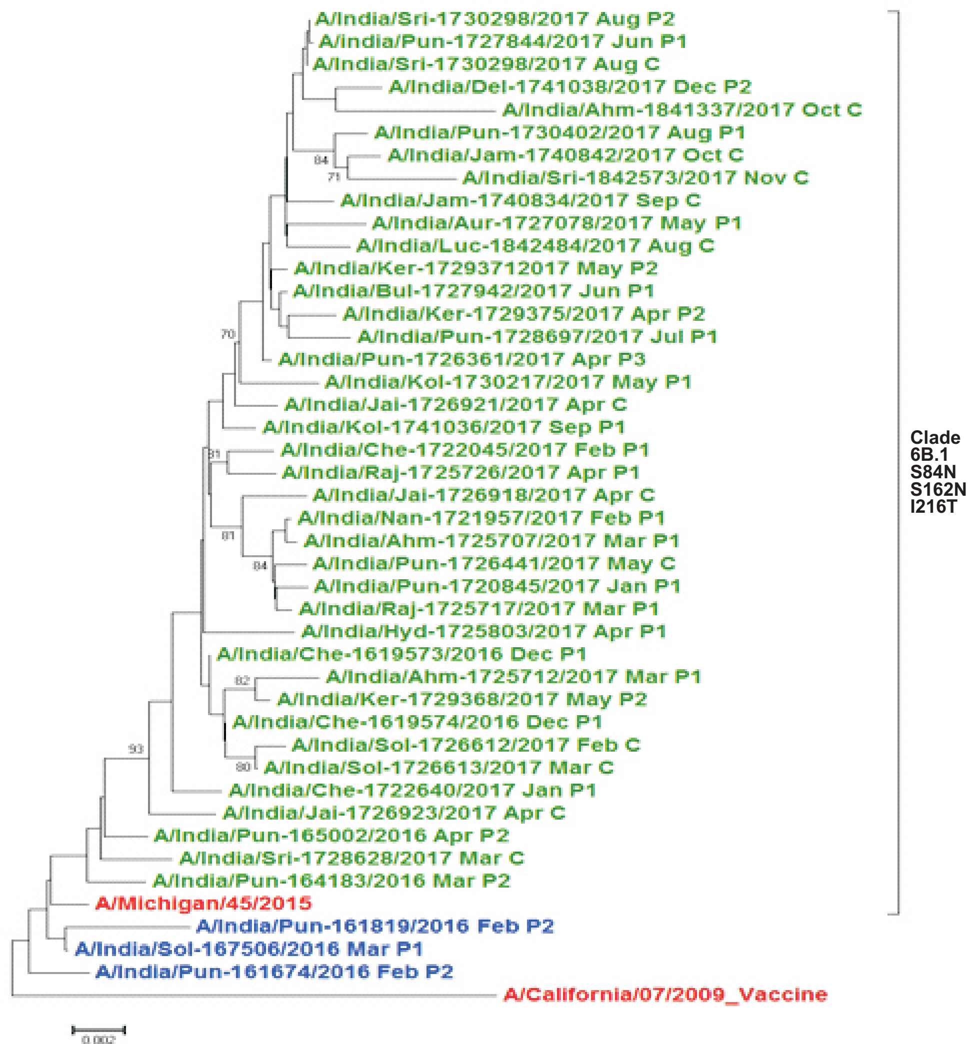


Fig 2: Phylogenetic analysis of influenza A/H1N1pdm09 HA gene (n=80) from 2017-18

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

PI: MS Chadha, VA Potdar and ML Choudhary

Funding Agency: GHSA-CDC

Duration: 2015-2020

Background: Acute respiratory tract infections are a leading cause of morbidity and mortality. A network of laboratories has been established in the country to carry out surveillance/ investigate outbreaks caused by respiratory viral pathogens.

Objectives: To conduct epidemiological and virological surveillance for influenza and non-influenza respiratory viruses, *viz.*, RSV, hMPV, PIV, adenoviruses, rhinoviruses etc. in India.

Work done: Of the 2188 patients identified for the study, 655 throat/nasal swabs collected from acute respiratory infections (ARI) and 1533 from SARI patients were tested for different respiratory viruses by duplex Real Time PCR. 15% and 36.2% positivity were detected in ARI and SARI cases respectively. Over all 29.8% (653/2188) were positive for different respiratory viruses (Influenza A/H1N1pdm09 (9%), RSV (6%), rhinovirus (4%), PIV (3%), hMPV (3%), adenovirus (3%), influenza B (2%) and H3N2 (1%). Weekly distribution of samples tested and positives were shown in figure 3.

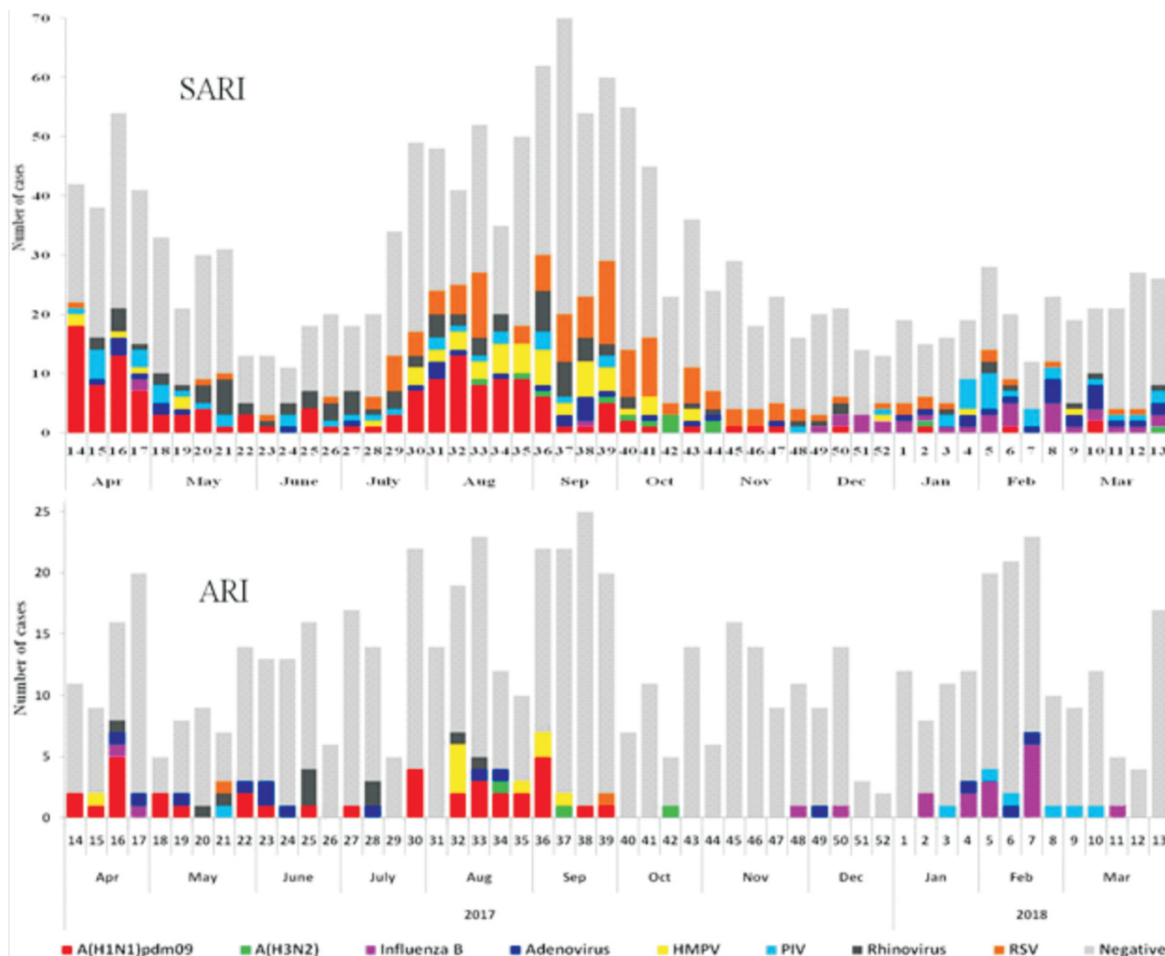


Figure 3: Weekly distribution of respiratory viruses in SARI and ARI cases

INF1602: Antiviral Susceptibility profiling of influenza virus in India

Investigators: VA Potdar, MS Chadha

Funding Agency: DHR

Project Duration: 2016-19

Background: Neuraminidase inhibitors (NAIs) are drugs of choice to treat influenza patients. Through binding in the conserved catalytic domain of the enzyme, these drugs inhibit all types/subtypes of influenza neuraminidase. All influenza RT-PCR positive samples were cultured on MDCK cells and subsequently sequenced for haemagglutinin [HA], NA or full genomes and tested phenotypically for NAI resistance.

Work done & findings: During the reporting period, 200 Pandemic A/H1N1pdm09 isolates were tested for oseltamivir by phenotypic assay (NAI). Eight isolates [Pune 3, Delhi 2, Solapur 1, Nanded 1 and Srinagar 1] had high IC50 values resulting in reduced susceptibility. The detection of resistance was significantly more frequent in the 1–5 year-old age group as compared to older age groups. All the patients had an uneventful recovery. All the eight companion isolates with clinical samples were confirmed by allelic discrimination Real Time PCR for H275Y mutation. Whole genome sequencing of five out of eight resistant viruses confirms that no mutation other than H275Y is known to be associated with reduced sensitivity. A total of 55 influenza A/H3N2 and 57 type B isolates were also tested for oseltamivir resistence by phenotypic assay (NAI) and were found susceptible.

INF1603: World Health Organization pilot study on RSV surveillance based on the Global Influenza Surveillance and Response System

Investigators: MS Chadha, VA Potdar, ML Choudhary and SD Bhardwaj

Funding Agency: WHO

Duration: 2016-2019

Background: Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and pneumonia in infants and young children. RSV surveillance will provide epidemiological information as well as the disease burden to support RSV vaccine development.

Objectives:

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

Work done: Screening of 1284 clinical samples from OPD (192) and hospitalized cases (1092) belonging to all age groups for RSV by Real Time PCR yeided 66 (5.14%) samples positive (RSV A=1 and RSV B= 65). The data has been uploaded on FluNet. Only one sample detected positive in the OPD samples.

INF1604: Feasibility Study: Tracking community mortality due to Respiratory Syncytial Virus in collaboration with University of Colorado and MAHAN Melghat.

Investigators: MS Chadha, VA Potdar

Funding Agency: Bill & Melinda Gates Foundation

Duration: 2016-2019

Introduction: RSV is a major cause of morbidity and mortality in children in developing countries. Paucity of data from India on RSV associated mortality in infants and children nececitated the study.

Objectives: To identify RSV associated mortality in infants/children below the age of 2 years in Melghat, a tribal area in Maharashtra, India.

Work done: Screening of 833 samples for RSV by Real Time PCR yielded 18 RSV B positives. The samples were also screened for Influenza and other respiratory viruses. Rhinovirus activity was observed throughout the year, whereas, influenza was detected in August and September 2017. An outbreak of human metapneumo virus was observed in September 2017 (Fig 4).

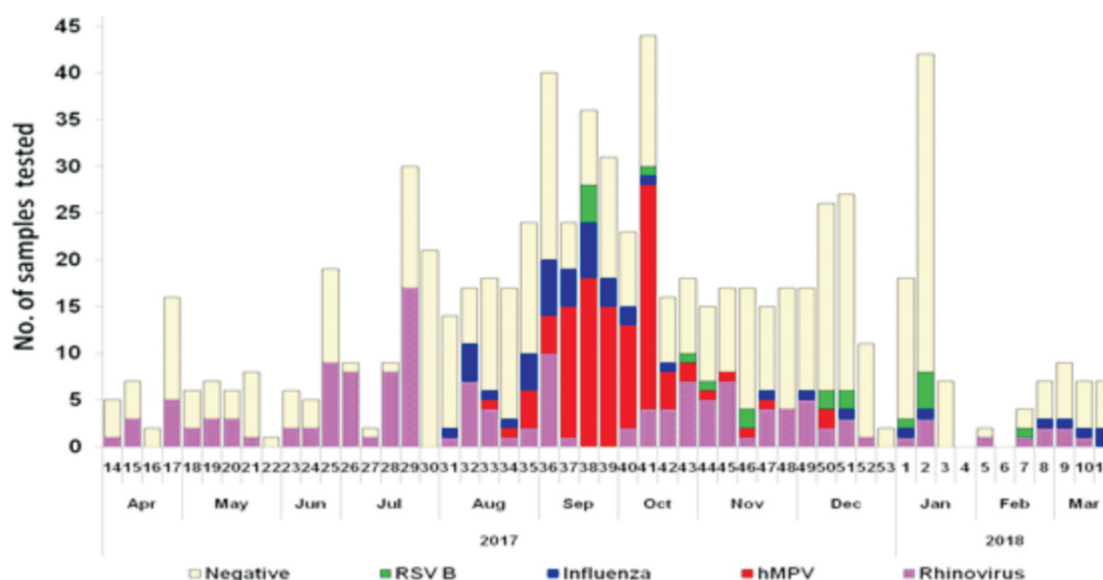


Fig 4: Weekly distribution of RSV and other respiratory virus positives from Melghat area

INF1701: Immunogenetics of severe pandemic H1N1pdm09 infections

Investigators: ML Choudhary, MS Chadha and K Alagarasu

Funding Agency: DBT

Duration: 2017-2020

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

Objectives: To determin 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 samples (n=250) from influenza A/H1N1pdm09 positive patients were collected and genotyping of SNPs for TLR3, IFITM3, MxA genes was done. TLR3 is a pattern recognition receptor involved in the sensing of viral RNA while IFITM3 and MxA mediate broad spectrum antiviral activity. These proteins have major role in the innate immune response against Influenza A/H1N1pdm09. The level and functions of TLR3, MxA and IFITM3 are affected by single nucleotide polymorphisms (SNPs) in the genes coding for these proteins. We investigated the association of *TLR3* -1377, *MxA* -123 & -88 and *IFITM3 rs12252* SNPs with severity of disease in Influenza A/H1N1pdm09 infected patients. SNPs were studied in 129 mild cases and 117 severe cases using PCR-RFLP based methods. The results revealed the genotype frequencies of these SNPs were not significantly different between mild cases and severe cases or between survived and fatal cases (Table 1). More samples are being studied.

Table 1: Percentage genotype frequencies of TLR3, MxA and IFITM3 gene polymorphisms in Influenza A/H1N1pdm09 infected patients with different grades of disease severity

Genotypes	Mild cases (n =129)	Severe cases (n = 117)	Mild vs. severe cases		Survived cases (n =219)	Fatal cases (n =27)*	Survived vs. Fatal cases	
			Odds ratio	P value			Odds ratio with 95% confidence intervals*	P value
TLR3 -1377								
G/G	62 (48.1%)	56 (47.9%)	1.00		108 (48.9%)	10 (40.0%)	1.00	
G/A	61 (47.3%)	49 (41.9%)	0.84 (0.49-1.42)	0.14	97 (43.9%)	13 (52.0%)	1.31 (0.55-3.17)	0.78
A/A	6 (4.7%)	12 (10.3%)	2.38 (0.83-6.81)		16 (7.2%)	2 (8.0%)	1.52 (0.30-7.71)	
MxA -123								
C/C	84 (65.1%)	70 (59.8%)	1.00		138 (63%)	16 (59.3%)	1.00	
C/A	41 (31.8%)	43 (36.8%)	1.19 (0.67-2.11)	0.81	74 (33.8%)	10 (37%)	1.11 (0.47-2.61)	0.97
A/A	4 (3.1%)	4 (3.4%)	0.90 (0.20-4.18)		7 (3.2%)	1 (3.7%)	1.15 (0.12-10.66)	
MxA -88								
G/G	63 (48.8%)	45 (38.5%)	1.00		99 (45.2%)	9 (33.3%)	1.00	
G/T	54 (41.9%)	59 (50.4%)	1.42 (0.80-2.52)	0.48	98 (44.8%)	15 (55.6%)	1.62 (0.67-3.94)	0.54
T/T	12 (9.3%)	13 (11.1%)	1.12 (0.43-2.90)		22 (10.1%)	3 (11.1%)	1.12 (0.27-4.64)	
IFITM3 rs12252								
T/T	99 (76.7%)	76 (65%)	1.00		159 (72.6%)	16 (59.3%)	1.00	
T/C	30 (23.3%)	40 (34.2%)	1.51 (0.83-2.75)	0.32	59 (26.9%)	11 (40.7%)	1.61 (0.69-3.76)	0.42
C/C	0 (0%)	1 (0.8%)	NA		1 (0.5%)	0 (0%)	NA	

INF1702: Development of recombinase polymerase amplification (RPA) based point of care molecular diagnostic assays for detection of respiratory viral and vector borne viral diseases".

Investigators: Dr. ML Choudhary, Dr. K Alagarasu

Funding Agency: SERB

Duration: 2017-2018

Introduction: Diagnosis of respiratory and vector borne viral diseases at the early stage of onset of disease is crucial for patient management and control.

Objectives: To develop RT-RPA based detection method for influenza A/H1N1pdm09, RSV, dengue and chikungunya virus.

Work done: Primer and probe were designed for influenza A/H1N1pdm09 and RSV. Primer-probe evaluated on viral isolates of influenza A/H1N1pdm09 is working well and specificity has been evaluated using other respiratory viruses like influenza A/H3N2, influenza B and RSV. No cross reactivity was observed. Further evaluation of clinical samples is in progress.

INF1703 Etiology of childhood pneumonia in India: an ICMR task force study (Multicentre study):

Investigators: MS Chadha, VA Potdar

Funding Agency: ICMR

Project Duration: 2017-19

Introduction: Considering the importance of childhood pneumonia, multisite taskforce study was initiated by the ICMR at 4 sites in India.

Objective: Determination of the **e**tiology of childhood pneumonia in Pune.

Work done: NIV was leading the virological aspects of the study and developed lab-testing algorithm (SOPs and training modules). Trainings were conducted for respiratory virus diagnosis at CMC Vellore. PT panel and positive controls were dispatched and results are awaited. Laboratory support is being provided to two sites *i.e.* KEM Hospital, Pune and PGI, Chandigarh. Enrollment of subjects has started since November 2017 and one tested positive for RSV B among 12 samples sent by KEM.

INF1704: Establishing a network of population based influenza surveillance platforms for elderly persons in India

Investigators: MS Chadha, Atanu Basu, SD Bharadwaj, V Potdar

Funding Agency: CDC

Duration: 2017-18

Site development and enrollment of study population: Janata Vasahat, an urban slum in Pune with a population of 31,489 has been selected for the study. Mapping and listing of the study site was done. A cohort of 1009 elderly was recruited and baseline data (Individual and household) of all the participants were recorded. Weekly surveillance of the cohort was started in January 2018. ARI cases were detected during the surveillance and a total of 28 ARI samples were collected by the nurses based on the random roster. None of the samples tested positive for Influenza virus.

Contribution to Global Influenza Network: Virological data for 13115 clinical samples including 1504 positive influenza A(H1N1)pdm09, 232 A(H3N2), 217 Influenza B, 138 PIV, 213 Rhinovirus, 118 hMPV, 125 Adenovirus and 236 RSV were submitted to Global Influenza Surveillance and Response System (FLUNETPLUS). Fifty influenza isolates (H1N1pdm09: 31, H3N2: 15, Influenza B: 4) in three lots (Apr-17, Aug-17, Jan- 18) were submitted to WHO CC, CDC, Atlanta.

WHO External Quality Assessment Programme (EQAP): Panel number 16 (2017) for influenza A Real Time PCR from WHO, CHP Hong Kong was received; this contained 10 samples of A(H3), A(H5), A(H1)pdm09, influenza B and other Influenza A, the results were 100% concordant.

NABL Accreditation: Real Time PCR test for influenza virus diagnosis has been assessed and accredited in accordance with the standard ISO/IEC17025:2005 in the discipline of biological testing by National Accreditation Board for Testing and Calibration Laboratories (NABL).

AVIAN INFLUENZA

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Ms. Rewati Kasbe	SRF (UGC)
Ms. Deeksha S. Tare	JRF

AVI1302: Operation and maintenance of High Containment Laboratory. (Service project)

Investigators: Jayati Mullick, Shailesh D. Pawar, Ajay B. Khare, Dinesh K. Singh & JPN Babu.

Funding Agency: Intramural

Project Duration: Ongoing

Abstract: The Biosafety Level 3 (BSL3) laboratory is a high containment facility to conduct experiments on highly infectious agents providing a clean and safe environment for the personnel and the community. During the reporting period, the BSL3 lab has provided infra structural support to NIV staff working on avian influenza H5N1, avian influenza H5N8, influenza A H1N1pdm09, vaccinia, Kyasanur Forest disease and Zika viruses. Specialized biosafety training for working in the BSL3 and animal BSL3 laboratories were imparted to NIV staff (n=9). A primate experiment inside the ABSL3 was initiated by the BSL4 group and is ongoing. As part of the M.Sc. Virology course, the students (n=21) were provided hands-on-training in biosafety and donning and doffing of personal protection equipment. The facility is being maintained for smooth functioning.

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

Investigators: Shailesh D. Pawar, Jayati Mullick, Sarah S. Cherian (Collaborator-Bioinformatics)

Funding Agency: Intramural

Duration: 2016-2019

***Objective 1:* Substitutions in the neuraminidase gene of avian influenza H9N2 virus that confer reduced susceptibility under selection pressure of Oseltamivir and Zanamivir.**

Amino acid substitutions in the viral neuraminidase (NA) that are associated with reduced susceptibility to NA inhibitors are both drug and virus sub-type specific. The NA gene sequence of an H9N2 virus isolate from Maharashtra, India was analyzed to detect the presence of markers conferring reduced susceptibility to NA inhibitors Oseltamivir and Zanamivir followed by *in ovo* susceptibility studies.

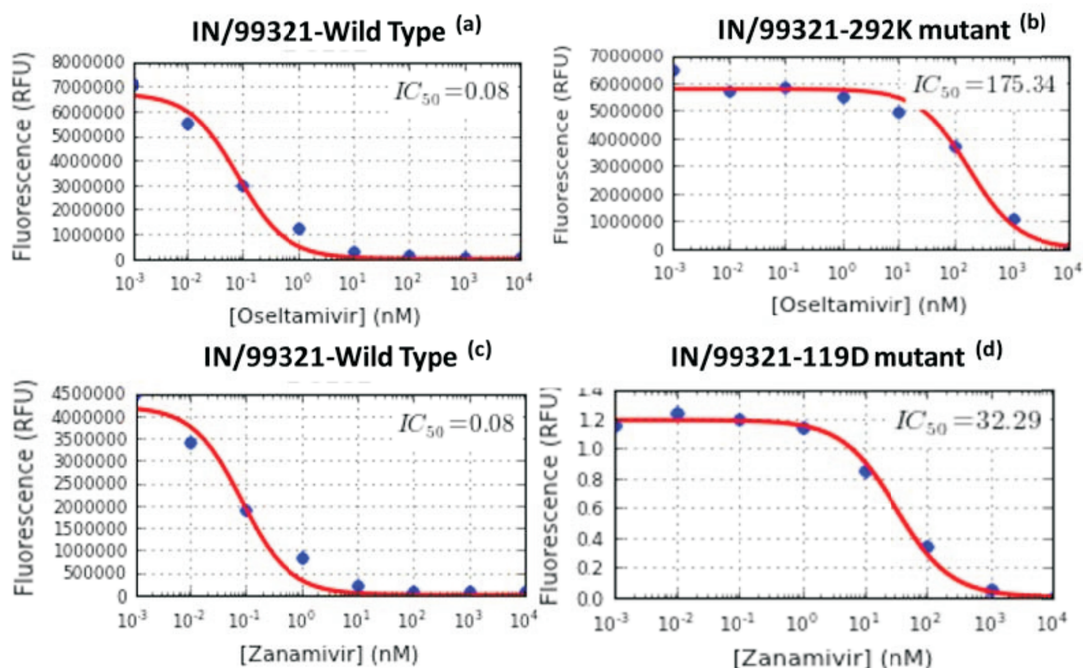


Fig 1: Half maximal inhibitory concentration (IC_{50}) of H9N2 viruses in neuraminidase inhibition assay. Figures 1a and 1c indicate IC_{50} values of In/99321-WT virus tested against oseltamivir and zanamivir, respectively. Figures 1b and 1d indicate IC_{50} values of the R292K and the E119D mutants tested against oseltamivir and zanamivir, respectively.

Findings: Susceptibility studies using embryonated chicken egg as a model revealed that the NA gene sequences of H9N2 viruses in presence of oseltamivir and zanamivir showed the occurrence of R292K and E119D substitutions. Neuraminidase inhibition using fluorescence-based MUNANA assay (Fig 1) showed that the marker mutations conferred highly reduced susceptibility to oseltamivir and zanamivir, respectively. Viral fitness studies by determining 50% egg infectious titers showed that both variant viruses were unfit, with wild type viruses outgrowing the variant viruses following three passages in the absence of NA inhibitor.

Further, molecular dynamics simulations of oseltamivir and zanamivir bound to H9N2 NA revealed that the R292K mutant disrupted oseltamivir drug binding due to loss of a salt bridge and hydrogen bond (H-bond) between R292 with the carboxylate group of the drug while the E119D substitution resulted in the loss of an H-bond with the guanidine group of zanamivir and an interaction with E276, affecting zanamivir drug binding. The study also elucidates the molecular basis of the identified marker substitutions in determining the drug binding potential of H9N2 NA.

(2) Susceptibility of highly pathogenic avian influenza viruses isolated from India to antiviral drugs: Oseltamivir and Zanamivir are currently the approved neuraminidase (NA) inhibitors for the control of influenza infections. Susceptibility of high pathogenic avian influenza (HPAI) H5N1 viruses to antiviral agents varies globally. Information on susceptibility of avian influenza viruses from India is lacking.

Susceptibility of 65 HPAI (H5N1) viruses (clades 2.2 and 2.3.2.1) isolated from various states of India during 2006-2012 were analyzed for known molecular markers in the NA gene by one step RT-PCR and sequencing. The sequences were analyzed for the mutation sites 97, 99, 116, 179, 255 and 275 which are associated with reduced/ highly reduced inhibition in group N1 neuraminidases. *In vitro* fluorometric neuraminidase inhibition (NAI) assays were performed to assess the susceptibility of these viruses to both the NA inhibitor drugs. The virus growth in the presence and absence of oseltamivir and zanamivir was assessed by determining the infectious virus titres (EID_{50}) in an egg model.

Findings: The NA gene sequencing and amino acid analysis of the isolates revealed that 9 out of 65 isolates exhibited amino acid substitutions which are known to confer reduced inhibition to NA inhibitors. Three isolates each showed E99A and N275S substitution, two isolates were having I97V and one was having I97T amino acid substitution. These isolates showed reduced inhibition to NA inhibitors in NAI assays. Susceptibility studies using embryonated chicken egg model results are in line with the NAI results. When the growth of the isolate with highly reduced inhibition to zanamivir (1000-fold) in egg in presence or absence of zanamivir is compared, it yielded to equivalent HA titres (no significant difference, P=0.52). The present study highlights the need of antiviral surveillance of avian influenza viruses.

(3) Avian influenza virus flocculation and detection from water samples using polyelectrolytes: Environmental samples such as water from sites where migratory and resident birds congregate, as well as poultry drinking water obtained from the cages where poultry are housed are important sources of avian influenza (AI) virus. There is a need to concentrate and detect the virus from environmental water specimens. Polyelectrolytes act as flocculating agents in waste water treatment plants. Flocculation of AI virus was thus studied using a cationic polyelectrolyte.

A reverse genetically modified AI H5N1 RG virus (Vaccine strain) was spiked in 2 liters of dechlorinated tap water in a separating funnel, followed by addition of milk powder (0.02%) to facilitate flocculation. A 100-ppm solution of the electrolyte was added to the funnel followed by thorough mixing. Funnels were incubated overnight at room temperature. The floccules containing the virus were collected, centrifuged and Real Time RT-PCR was performed to detect viral RNA from the pellet. Ten days old

embryonated chicken eggs were also inoculated with the resuspended pellet.

A spiked AI virus concentration of 1 and 0.001 infectious particle/ml could be detected by real-time RT-PCR and virus isolation in embryonated chicken eggs, respectively. It was also found that the virus viability was not hampered by the chemical as the virus could be isolated in embryonated chicken eggs. The polyelectrolyte could also flocculate spiked virus from sea water, fresh water from reservoirs, borewell, etc., independent of pH. Further work needs to be standardized to detect AI virus from field samples.

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

Jayati Mullick, Milind M Thube, Shailesh D. Pawar, Pratip Shil.

Funding Agency: Intramural

Duration: 2012-2018

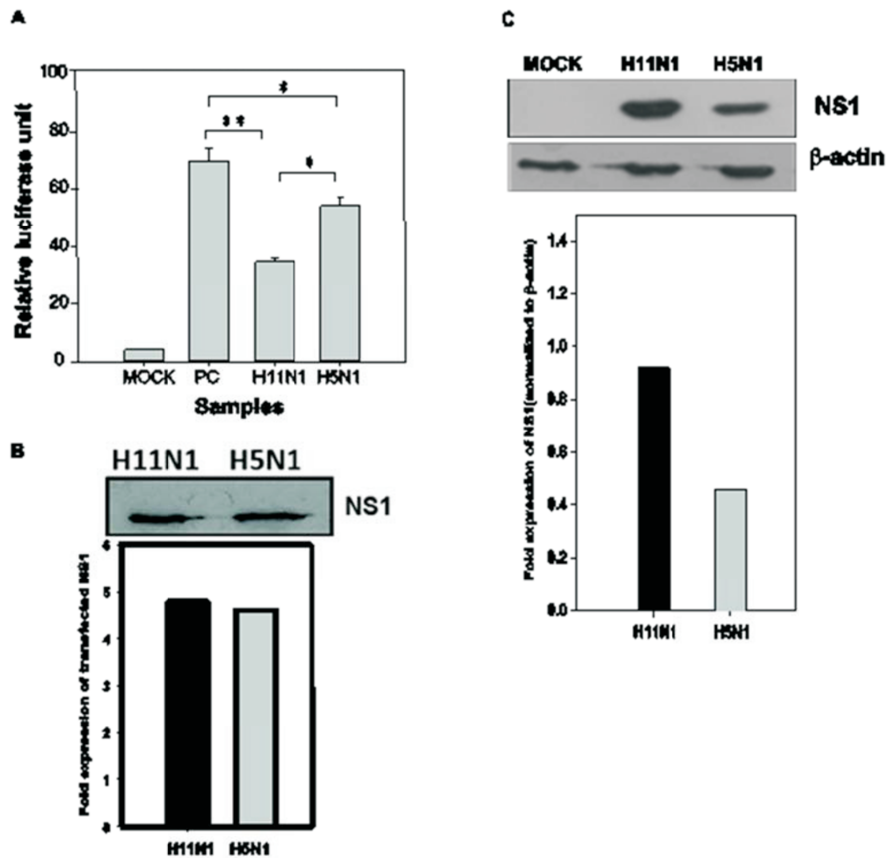
Abstract: Earlier we have shown comparison of highly pathogenic avian influenza (HPAI) H5N1 and low pathogenic avian influenza (LPAI) H11N1 viruses isolated from India, for their replication kinetics and ability to induce IFN-β and interferon-stimulating genes (ISGs). The H5N1 virus showed a higher replication rate and induced less IFN-β and ISGs compared to the H11N1 virus when grown in the human lung epithelial A549 cells, reflecting the generation of differential innate immune responses during infection by these viruses. Non-structural 1 (NS1) protein is a major IFN antagonist known to help the virus in evading host innate immune response. During this period, we have studied the role of non-structural 1 (NS1) protein, from both the strains using bioinformatics tools as well as by comparing the capacity of transiently expressed NS1 of the respective strains for inhibiting the IFN-β promoter in A549 cells using the Luciferase reporter assay system (Figure 2) and IFN β concentration by ELISA. Analyses revealed differences in the composition of the NS1 proteins from the two strains that may have an impact on modulation of the innate immune response. Intriguingly, H5N1 virus attenuated IFN-β response in a non-NS1 manner, suggesting the possible involvement of other viral proteins (PB2, PA, PB1/PB1-F2) of H5N1 in synergy with NS1. Preliminary analyses of the above proteins of the two strains by sequence comparison show differences in charged residues. The insight gained will be useful in designing experimental studies to elucidate a probable role of the polymerase protein(s) in association with NS1 in inhibiting the IFN signaling and understanding the molecular mechanism governing the difference.

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

Quality Manager: *Dr. Shailesh D Pawar*

NABL conducts annual Surveillance which is aimed at evaluating continued compliance with ISO/ IEC 17025: 2005 and relevant NABL specific criteria and Policies.

Surveillance Assessment of Human Influenza, Maximum Containment Laboratory and Avian Influenza laboratories was performed on 3rd and 4th February, 2018 by a team of NABL empanelled Assessors led by Ms. Sonia Nandre, General Manager, Laboratory Operations, TUV India (Pvt.) Ltd., Pune and Technical Assessor Dr. Pramod Kumar Chug (OC DOG, New Delhi). The team expressed satisfaction and recommended continuation of accreditation of NIV in the Biological field as per the scope (Figure 3).

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H5N1_NS1    MDSNTVSSFQ VDCFLWHVRK RFADQELGDA PFLDRLRRDQ KSLRGRGNTL GLDIETATRA GKQIVERILE EESDEALKM- [80]
H11N1_NS1   MDSNTVSSFQ VDCFLWHVRK RFADQELGDA PFLDRLRRDQ KSLRGRGSTL GLDIETATRA GKQIVERILE EESDEALKMT[80]

                          CPSF30                                                  CPSF30 -
H5N1_NS1    ----PASRYL TDMTLEEMSR DWFMLMPKQK MTGSLCIKMD QAIMDKTIIL KANFSVIYDR LETLILLRAF TEEGAIVGEI [160]
H11N1_NS1   IASVPASRYL TDMTLEEMSR DWFMLMPKQK VAGSLCIRMD QAIMDKNIIL KANFSVIFDR LETLILLRAF TEEGAIVGEI [160]

            - binding sits
H5N1_NS1    SPLPSLPGHT NEDVKNAIGV LIGGLEWNDN TVRVSEIIQR FAWRSIDEDG RPPLPPDQKR KMARTIESKV [230]
H11N1_NS1   SPLPSLPGHT DEDVKNAIGV LIGGLEWNDN TVRVSETLQR FAWRSSNEDG RPPLPPKQKR KMARTIESEV [230]
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Fig 2: Ability of NS1 to inhibit IFN-b, expression of NS1 in infected cells and pairwise alignment of NS1 proteins of H5N1 and H11N1 viruses. A. Luciferase activities of A549 cells co-transfected with luciferase reporter plasmid under the control of IFN-b promoter followed by induction with PolyI:C. B & C. Western blot of total cellular protein from H11N1 and H5N1 –NS1 transfected A549 cells (B) and cell lysate from A549 cells infected with H11N1 and H5N1 viruses (C) immunoblotted with anti-NS1 antibody and reprobed with anti-b-actin antibody. Graphs below the blots are densitometric analysis of the respective blots. D. Pairwise alignment of NS1 protein sequences of H11N1 and H5N1 viruses showing the CPSF30 binding sites.

Fig 3: NABL team during the final assessment of Avian Influenza, Human Influenza and BSL4 laboratories at ICMR-NIV, Pune.

HEPATITIS

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Dr. Preksha Jain	Research Associate
Ms. Sonam Lata	

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

Investigators: Kavita Lole & Supriya Hundekar

Funding: Intramural

Duration: 2014-2018

Background: HAV outbreaks in the recent past have indicated the need of hepatitis A vaccination of susceptible individuals. HAV grows slowly in cell culture and requires multiple passages for adaptation. Though commercial vaccines are available, they are expensive. Hence, alternative methods for obtaining HAV proteins for use as candidate vaccines or for diagnostic assays have been explored. We are trying to develop recombinant HAV VLPs and evaluate utility of these VLPs in developing diagnostic ELISA and candidate vaccine for HAV.

Objective: Exploration of bacterial, insect and mammalian cell expression systems for HAV VLP development.

Work done: VP2, VP3 and VP1-2A encoding regions were cloned in pET-28a separately and expressed. For insect cell expression, capsid (VP1-2A) and protease (3C) encoding regions were cloned in pFastbac dual vector (PH3Cp10P12A), recombinant baculovirus was developed and expressed protein in Sf9 cells. Cell lysate and supernatants were loaded on to 10-60% discontinuous sucrose gradient, collected fractions and analyzed by ELISA using anti-HAV IgM and IgG positive human sera. The fractions showing reactivity were processed for electron microscopy. Concurrently, attempts were made to adapt genotype IIIA hepatitis A virus to cell culture system (MRC-5) using purified virus by serial passaging at every 10 days. No CPE could be observed in the virus exposed cultures and presence of HAV viral RNA was monitored by quantitative Real Time PCR.

Findings: Overall reactivity of cell supernatant was more as compared to cell pellet indicating that expressed HAV proteins were secreted out. Though all the sucrose gradient fractions showed some extent of reactivity with anti-HAV IgG or IgM, fraction numbers 7-15 exhibited both IgG and IgM reactivity (Fig 1A, 1B). Analysis of these fractions on electron microscope revealed presence of ~30nm particles indicating possible formation of HAV VLPs. Viral RNA was observed in the infected cells from passage 3 onwards, though at a very low level (titer). Currently, the culture is at the 10th passage level. For the HAV VLPS, up scaling and purification using HPLC is ongoing.

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

Investigators: Kavita Lole & Swapnil Desai

Funding: Extramural (DST)

Duration: 2015-2018

Background: This study focuses on the viral papain-like cysteine protease (PCP) from genotypes 1 and 4 of Hepatitis E virus (HEV). A PCP domain encompasses 440–610 amino acid residues in ORF1 polyprotein of HEV. *In vitro* enzymatic analysis of HEV PCP had shown ability to hydrolyse and remove SUMO, NEDD and ISG15 and ubiquitin moieties from proteins. These findings prompted us to look for the potential HEV PCP target proteins with modifications like ubiquitination SUMOylation, NEDDylation and ISGylation in HEV replicating cells.

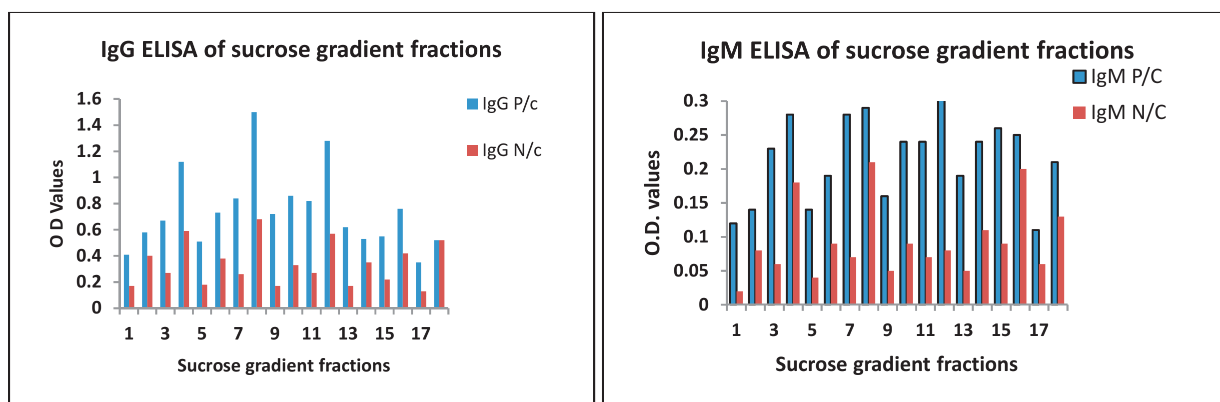

Fig 1: Sucrose gradient fractions showing IgG and IgM reactivity

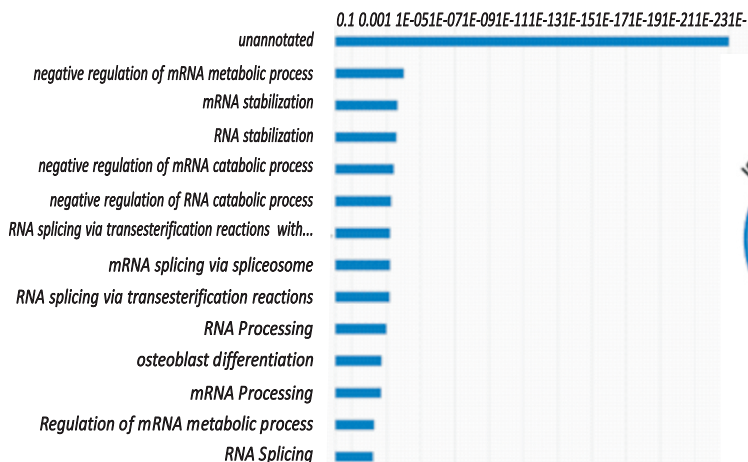

Fig 2A: *The GO analysis of the deubiquitinated proteins*

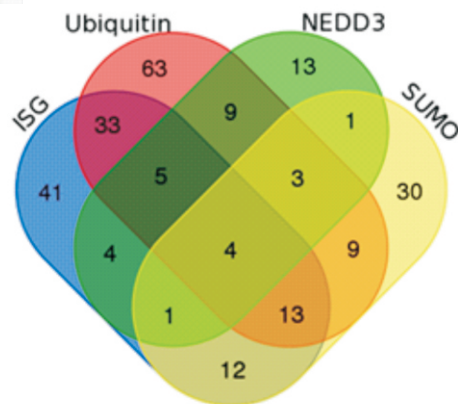

Fig 2B: Venn Diagram for the proteins which were processed by HEV PCP

Objectives:

- To assess the effect of catalytic mutations in PCP on HEV replication kinetics *in vitro*.
- To assess the role of HEV PCP/ cellular PCPs in ORF1 polyprotein processing.

Work done: Hepatoma cell lysates were incubated with HEV PCP and modified proteins were pulled down with respective antibody specific for the above-mentioned modifications and identified the proteins using quantitative LC-MS analysis. There were 139 putative target proteins that were deubiquitinated by HEV PCP. Functional annotations of these proteins showed their enrichment in the RNA metabolic and catabolic processes, mRNA/RNA stabilization, RNA splicing etc. (Fig 2A). Interestingly, it was seen that HEV PCP deubiquitinates components in RNA processing and maturation.

Findings: It was seen that HEV down regulates activities of proteins involved in mRNA transportation and translation. Venn diagram of the proteins with different modifications showed that several proteins had multiple modifications (Fig 2B). It would be interesting to see how these protein modifications help HEV during replication. Further analysis of host protein modifications in HEV replicating host cells is ongoing.

Hep1501: Antiviral agents against hepatitis E virus

Investigators: Kavita Lole & Neha Bhise

Funding: Intramural

Duration: 2015-2018

Background: It has been reported that genotype-3 HEV can establish chronic infections in immunocompromised/ immunosuppressed people leading to rapid cirrhosis. Hence there is a need to explore the possibility of developing antivirals against HEV to prevent liver damage. Such drugs may be also useful in fulminant hepatic failure cases associated with genotype-1 HEV infection.

Objectives:

- To evaluate inhibitors of hepatitis E virus helicase and RNA dependent RNA polymerase (RdRp) by *in vitro* enzymatic assays
- To evaluate the effect of helicase and polymerase inhibitors on HEV replication

Work done and findings: HEV RdRp was purified using ProBond Nickel chelating resin column (native conditions) followed by the gel filtration chromatography and a non-radioactive calorimetric replicase assay was optimized for detecting phosphate using malachite green molybdate reagent. Similarly, recombinant HEV helicase protein was purified and malachite green reagent-based ATPase assay was established to screen helicase inhibitors.

Twelve compounds (Table 1) were first screened for cytotoxicity using MTT assay and based on these values, concentrations were determined to study their inhibitory effect on hepatitis E virus. Testing of antiviral activity of additional compounds against HEV is ongoing.

Table 1: CC_{50} and 100% cell viability concentration by cytotoxicity assay

Sr. No.	Drug	CC_{50}	100% cell viability
1	Sofosbuvir	315 μM	7.8 μM
2	Dalatasvir	150 μM	7.8 μM
3	Ribavirin mono phosphate	48.23 μM	0.09 μM
4	Ribavirin di phosphate	37.84 μM	0.09 μM
5	Ribavirin tri phosphate	100 μM	10 μM
6	2'C methylcytidine	1000 μM	3.9 μM
7	Voglibose	50 μM	15.6 μM
8	Miglitol	1000 μM	250 μM
9	Benzimidazole	160 μM	7.8 μM
10	Psammaplin A	10 μM	3.12 μM
11	MG132	2.5 μM	0.31 μM
12	Artesunate	168.19 μM	23.43 μM

HEP1302: Immunological memory in Hepatitis E infection

Investigators: Anuradha Tripathy & Shruti Kulkarni

Funding: Intramural

Duration: 2013-2017 (completed)

Background: HEV pathogenesis is immune-mediated and several groups have assessed host immune response following HEV infection. However, no confirmatory data is available on the generation and persistence of the immunological memory responses. In addition, identification of additional correlates of protection (CoPs) against HEV infection, besides anti-HEV antibodies is needed to assess the efficacy of prospective HEV vaccine candidates. To fill these gaps, an attempt was made to envisage immunological memory responses in (a) individuals recovered from infection (1-30 years post HEV infection), and (b) mice immunized with liposome-encapsulated recombinant neutralizing epitope protein (rNEp), the indigenously developed HEV vaccine candidate.

Objective:

- To study immunological memory in individuals infected with hepatitis E virus 1-30 years age.
- To assess immunological memory in mice immunized with HEV vaccine candidate developed at NIV, Pune.

Work done and results: For evaluation of memory responses post HEV infection, anti-HEV antibody response, phenotypic analysis of HEV-specific immune cells and functionality of memory B and T cells were assessed in hepatitis E recovered individuals. Acute hepatitis E patients, exposed individuals (recovered from subclinical HEV infection), and healthy controls were selected for the study. Robust anti-HEV IgG antibody response was observed in recovered individuals 1-30 years post HEV infection with 91% sero positivity, showing the persistence of HEV-specific long-lived plasma cells (Fig 3). Memory B cells were functional in 95% of anti-HEV positive recovered individuals 1-16 years post HEV infection. Higher frequencies of $CD3^{+}$ $CD4^{low}$ $CD8^{high}$ cells (proposed as effector memory cells), $CD4^{+}$ and $CD8^{+}$ effector memory T cells in recovered individuals hold importance as effector memory cells are considered essential in controlling viral infections with long incubation periods.

In immunized mice, anti-HEV antibody responses, immunophenotyping of splenocytes, and functionality of memory B cells were assessed at different time points post immunization (PI). Age and sex matched mice administered with adjuvant alone and unimmunized mice were used as controls. Strong anti-HEV IgG response was elicited in vaccine candidate immunized mice that

persisted up to 420 days PI, and was statistically estimated to persist for the lifetime of mouse. Upon boosting, 4-6 fold rise in titers established the anamnestic response by B cells against rNEp. Higher percentage of memory B cells in vaccine candidate immunized mice compared to adjuvant only mice were observed. All mice immunized with rNEp+ liposome exhibited HEV rNEp-specific antibody secreting plasma cells establishing long term immunological memory following vaccination. Higher frequencies of $CD4^+$ T_{CM} and T_{EM} cells post booster dose in vaccine candidate immunized mice suggested the participation of $CD4^+$ memory T cells in recall response

Findings: Evaluation of immunological memory responses in both hepatitis E recovered individuals and in vaccine candidate immunized mice established salient roles of anti-HEV antibodies and memory B cells. Our study has provided a rational approach to assess the immune status of recovered/ vaccinated individuals. We propose that besides anti-HEV antibodies, memory B cell responses could also be considered as co correlates of protection against HEV infection

Hep1405: Identification, characterization and validation of neutralizing monoclonal antibodies (MAbs) against Hepatitis E Virus

Investigators: Anuradha Tripathy & Preksha Jain

Funding: DST SERB (grant no: EMR/2016/004977)

Duration: 2017-2020

Background: Infection with HEV is mostly self-limiting, but in a subpopulation of high risk individuals including pregnant women, the mortality rate can reach up to 30% and the disease course may lead to chronicity in immunocompromised individuals, requiring antiviral treatment options. Current therapeutics used to treat HEV infection *viz*., ribavirin and Interferon-β have severe side effects and are contraindicated in pregnant women. Monoclonal antibodies are the largest and fastest growing group of 'biologicals' (i.e. therapeutic proteins) and could be used to generate immune responses when administered without much side effects. The advent of phage display technology permits the facile isolation of fully human antibodies from large combinatorial repertoires and has been applied for development of therapeutic monoclonals against important viral pathogens. The current study was undertaken to identify, characterize and finally validate HEV specific neutralizing monoclonal antibodies in rhesus monkeys, the animal model for HEV. The two of the objectives of study include

- Construction of antibody phage library from the lymphocytes of HEV recovered individuals
- Isolation of monoclonal antibodies from the cDNA library

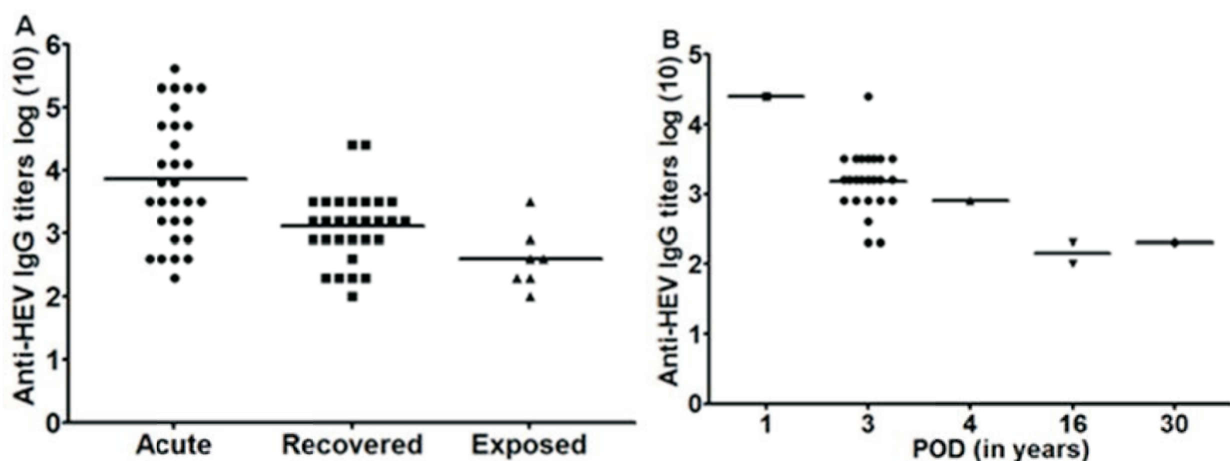

Fig 3: Anti-HEV IgG antibody response. (A) Anti-HEV IgG titers, presented as log transformed values, in acute hepatitis E patients, hepatitis E recovered individuals and HEV-exposed individuals, (B) Anti-HEV IgG titers of hepatitis E recovered individuals, 1-30 years post HEV infection. POD: Post onset days of illness

Work done and results: For construction of recombinant monoclonal HEV antibody phage library, two previously identified HEV recovered individuals were selected based on their high IgG anti HEV antibody titres. RNA was isolated from the lymphocytes of the recovered individuals and cDNA synthesis was done using random hexamer and Superscript II reverse transcriptase enzyme.

To prepare the Fab gene cassette, human light chain and heavy chain were amplified by PCR from cDNA. To increase the diversity of the combinatorial library, the kappa light chain and Heavy chain amplification was done using all possible combinations of each family specific and constant region-specific primer including the synthesis of control Fab antibody also. Five variable and two different constant region primers were used for the amplification of kappa light chain. Touchdown PCR and seven variables and constant domain primers were used for the amplification of specific heavy chain PCR products. All the primers were designed with restriction enzyme sites compatible for direct cloning into the phagemid vector. The PCR products were sequenced using the gene specific primers. Analysis of the sequences was done using IgBlast from NCBI.

Screening of monoclonal antibodies from cDNA phagemid library will be done by panning and ELISA using HEV ORF2 protein. The HEV ORF2 protein has previously been cloned in Bac to Bac baculovirus system and expressed in SF9 insect cells. This HEV rORF2 protein was purified from the stored high viral titre stock using anion exchange chromatography and HiPrep 16/10 DEAE column. The immunoreactivity of the purified HEV rORF2 protein was checked in ELISA and western blot using human HEV IgG/IgM positive sera as primary antibody.

Findings: Sequence analysis of nine PCR products for human kappa light chain including the controls using IgBlast revealed sequence similarity with human kappa light chain variable germline gene segments. Similarly, the sequence search for human IgG heavy chain products revealed sequence identity up to 99% with human heavy chain genes. The products of both light chain and heavy chain will be cloned into phagemid vector for the construction of antibody phage library. The HEV ORF2 protein purified using anion exchange chromatography was immunoreactive both in ELISA and western blot done using human IgG/ IgM positive sera as primary antibody.

Hep1313: Study of B regulatory cells in Hepatitis E virus infection

Investigators: Anuradha Tripathy & Meenal Sharma

Funding: Intramural

Duration: 2014-2018

Background: Reports from the Indian subcontinent indicating high mortality in patients with hepatitis E infection has resulted in attempts to understand hepatitis E virus (HEV) pathogenesis. Understanding the immune correlates that contribute to the host immune response towards recovery may help in designing an efficacious vaccine/immune based treatment strategy. The presence of robust antibody response to the open reading frame 2 in the recovered individuals from hepatitis E infection has formed the basis for the development of a vaccine against Hepatitis E. B cells have been identified as potent regulators of T cell immune responses and their functions are not just confined to antibody production. B regulatory cells (Bregs), a subset of B cells has been identified to play an important role in viral pathogenesis and they modulate other immune cells primarily via IL-10 cytokine production. We have reported elevated IL-10 levels in acute hepatitis E patients and its association with immunosuppressive responses in hepatitis E. This information has prompted us to undertake this study to identify/assess the role of B regulatory cells and IL-10 in HEV pathogenesis.

Work done and findings: Forty-five acute hepatitis E patients, 32 recovered individuals and 54 healthy controls were our study population. B cells were comparable in acute hepatitis E patients, healthy controls and recovered individuals whereas immature B cells (Bregs, $CD19^{+}CD24^{hi}CD38^{hi}$) were high in acute hepatitis E patients compared to healthy controls as well as recovered individuals (Fig 4). HEV rORF2p stimulated Bregs were assessed by flowcytometry at 72h post antigen (affinity purified HEV rORF2p) stimulation in 12 HEV acute hepatitis E patients, 15 recovered individuals and 15 healthy controls. B cells, mature B cells and memory B cells were comparable among all the three categories

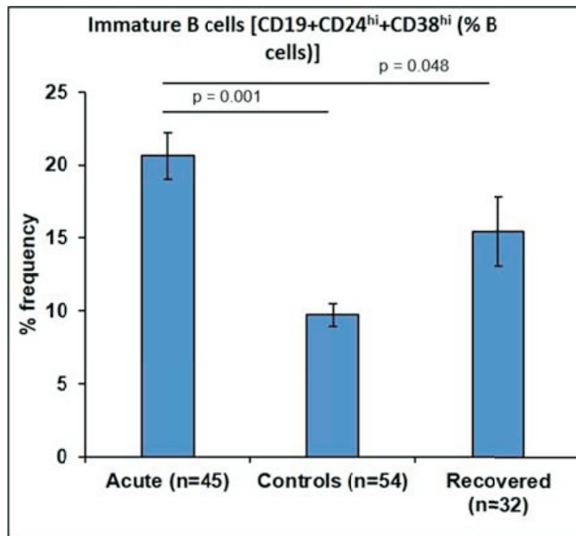

Figure 4: Percentage frequencies of immature B cells [%B cells] in HEV acute patients (n=45), recovered individuals (n=32) and healthy controls (n=54).

whereas immature B cells (Bregs) were high in rORF2p stimulated PBMCs of acute hepatitis E patients compared to healthy controls as well as recovered individuals (Fig 5). To determine the functionality of the regulatory B cells, intracellular cytokine staining (ICS) was done for the expression of IL-10 cytokine. The ICS assay following CpG/PMA/Ionomycin and rORF2p stimulation was performed in 10 acute hepatitis E patients, 15 recovered individuals and 13 healthy controls. Results showed that percentage IL-10 production by B cells was higher in acute hepatitis E patients compared to controls as well as recovered individuals (p= 0.04 in both).

The current study done so far indicated that peripheral Bregs frequencies as well as HEV rORF2p stimulated Bregs frequencies are significantly higher in hepatitis E acute patients compared to healthy controls and recovered individuals. Moreover, IL-10 production by the B cells of acute hepatitis E patients compared to controls as analyzed by intracellular cytokine staining suggested that the regulatory B cells of hepatitis E acute patients are functional. Our preliminary analysis indicated that B regulatory cells do have a role in the self-limiting of HEV infection.

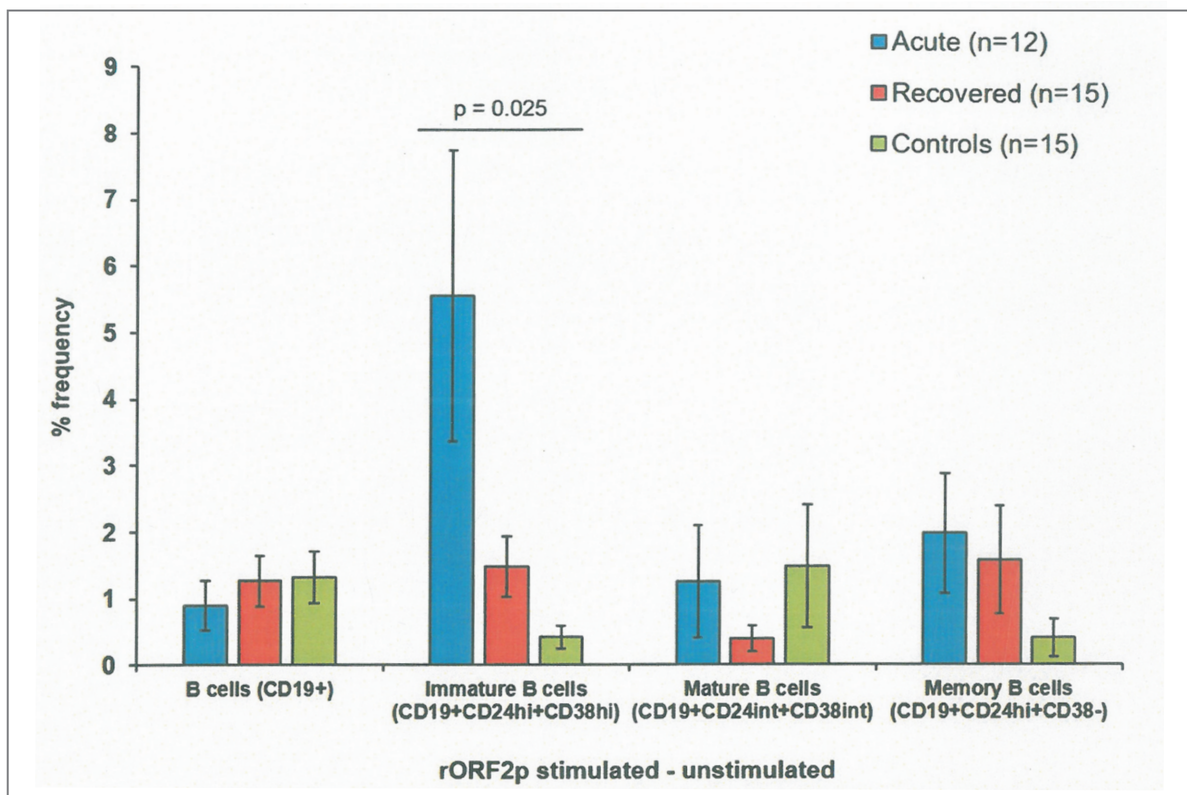

Figure 5: Percentage frequencies of immature B cells [%B cells] in HEV acute patients (n=45), recovered individuals (n=32) and healthy controls (n=54).

HEP1602: Cellular antiviral responses against hepatitis B virus

Investigators: Kavita Lole & Bhukya Prudhvi Lal

Funding: Intramural

Duration: 2016-2019

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

Objective: Analysis of cellular innate immune pathways that are activated in response to infection of hepatoma cells with wild type and mutant 1.3 mer HBV clones.

Work done: After the development of both Wild type and mutant 1.3 mer HBV clones, we carried out replication kinetics of the two viruses and noted that mutant virus induces apoptosis in the infected cells. To understand the mechanism of induction of apoptosis, we carried out whole transcriptome analysis of hepatoma cells (HepG2C3A), infected with the wild type and mutant 1.3 mer HBV clones.

Findings: Global transcriptome analysis of hepatoma cell infected with wild type and mutant strains revealed the significant differential expression of the genes belonging to host apoptotic anti-viral pathways.

Hep1604: Screening of blood donors from Pune city for Occult Hepatitis B infection

Investigators: Anuradha Tripathy & S Puranik

Funding: Intramural

Duration: 2016-2018

Background: HBV infection is an important global public health concern. Despite mandatory screening for HBsAg by ELISA for over 20 years, transfusion-associated HBV (TAHBV) continues to be a concern in India. It has been demonstrated that in some HBsAg-negative individuals, HBV continue to replicate. Thus the absence of HBsAg in the blood of apparently healthy individuals does not ensure lack of

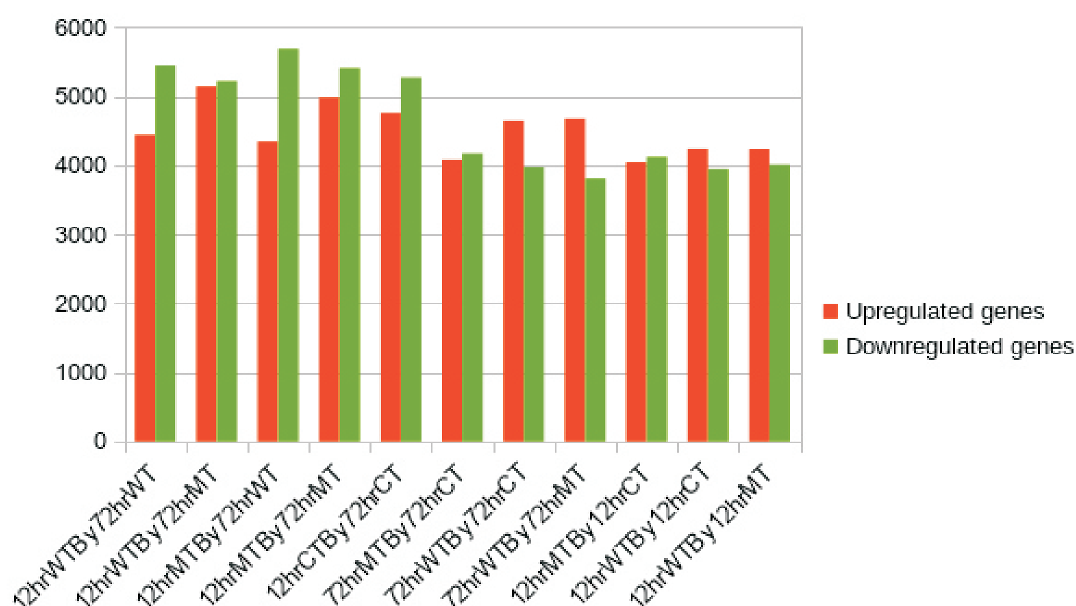

Fig 6: Global transcriptome analysis of hepatoma cell infected with wild type and mutant strains revealed the significant differential expression of the genes belonging to host apoptotic anti-viral pathways.

circulating HBV. Persistent low levels of HBV DNA in serum and liver tissues after HBsAg clearance observed during acute self-limited or chronic HBV infection has brought about the concept of “occult” HBV infection, indicating the presence of HBV DNA in the absence of detectable HBsAg. Occult hepatitis B virus (HBV) infection (OBI) refers to the presence of HBV DNA in the absence of detectable hepatitis B surface antigen. No such data is available from western India. Detection of HBsAg in blood is a diagnostic marker for infection with HBV. In the blood banks screening for HBsAg is carried out routinely to detect HBV infection. It is possible that, donors with occult HBV infection, who lack detectable HBsAg might have exposure to HBV infection and are a potential source of HBV infection. Hence, the aim of this study is to determine the presence of HBV DNA among HBsAg negative blood donors from Pune city and to assess the magnitude of occult HBV infection in these subjects.

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

Work done and findings: Screening of blood samples from volunteer blood donors (n=2230) from Pune city who are negative for HBsAg yielded five samples positive for HBV DNA indicating a 0.22% prevalence of OBI. (0.22%; 95% CI: 0.09-0.5). One of the OBI positive samples was IgG anti-HBc positive, while the rest four were IgG anti HBc negative. The sole IgG anti HBc positive sample was also positive for anti-HBs. HBV DNA quantitation was carried out in all 5 OBI positive samples. Three OBI positive samples had HBV DNA viral load in a range of 10^2-10^3 copies/ml, while the rest 2 had less than 250 copies/ml of .HBV DNA. Upon sequencing, all the samples belonged to HBV genotype D. Current data of an OBI prevalence suggested that further studies are needed for better understanding of the epidemiology of OBI among the blood donors in western India. OBI is transmissible by blood transfusion, though the clinical outcome of occult HBV transmission primarily depends on recipient immune status and the number of HBV DNA copies present in the blood products.

Hep1409: Study of the involvement of host factors in chikungunya infection

Investigators: Anuradha Tripathy, Mohini Ganu & Sonam Lata

Funding: Extramural ICMR (Grant no: VIR/75/2013/ECD-1)

Duration: 2017-2020

Background: Outcomes in chikungunya infection have been attributed to host/viral factors. There has been an increasing interest in the understanding of the role of host genetic factors in the pathogenesis of chikungunya. Variable cytokine production by different individuals is attributed to polymorphisms within the regulatory regions or signal sequences of cytokine genes. Though, substantial evidence indicates that IgG antibodies to CHIKV play a role in protection from chikungunya, the involvement of biologically different functional differences among the IgG subclasses and their association with the chikungunya disease status has not been studied. Therefore, this study was undertaken to understand the pathogenesis of CHIKV infection to elucidate the specific host responses in acute, recovered and in long-lasting chronic conditions that may help to distinguish patterns between patients who either “recovered” or suffer from “chronic” chikungunya infection.

Objective: Assess the polymorphisms of cytokine genes with susceptibility/resistance to chikungunya infection in Maharashtra.

***Work done and results*:** Chikungunya virus specific cytokine assay was done in 25 acute chikungunya patients, 55 chronic chikungunya arthritis patients, 30 recovered individuals and 25 healthy controls. The frequency distribution of IL1RN, NKG2D (rs7980470) and NKG2A (rs 2734440) alleles was carried out in 166 chronic chikungunya arthritis patients and in 355 healthy controls. Polymorphism study has the following data. Frequency of IL 1RN genotype 1/1 was significantly higher in chronic chikungunya arthritis patients compared to controls (p=0.0181) (Table 2) indicating it as a susceptible genotype. Frequency of IL 1RN genotype 2/2 was significantly higher in the control group compared to the chronic chikungunya arthritis patient population indicating it as a resistant allele that needs further validation in a larger sample size with stringent statistical analysis. Genotype frequency distribution of three SNP rs2734440 in NKG2A *i.e.* AA, AG and GG were carried out and the data is summarized in Table 3. GG genotype was more frequent in patients with chronic chikungunya than the healthy controls (28.43% vs. 11.76%)(p=0.002) indicating it as a susceptible allele, whereas, AA genotype was more frequent in healthy controls than the patients with chronic chikungunya (52.94% vs. 31.37%) indicating it as a protective allele.

Table 2 Frequency distribution of IL-1RN alleles:

Genotypes	Controls (n=355)	chronic chikungunya patients (n=195)	p value
1/1	129(36.33%)	91(46.66%)	**0.0181**
2/2	150(42.25%)	62(31.79%)	**0.0160**
1/2	68(19.15%)	36(18.46%)	0.8434
1/3	3(0.84%)	3(1.53%)	0.4553
3/3	1(0.28%)	0(0%)	-
2/3	3(0.84%)	3(1.53%)	0.4553
2/4	1(0.28%)	0(0%)	-

Table 3. Frequency distribution of NKG2A (rs2734440) alleles:

Genotypes	Healthy Controls (%) (N=102)	chikungunya chronic cases n (%) (N=102)	P value
AA	54 (52.94)	32(31.37)	**0.0019**
AG	36(35.29)	41(40.19)	-
GG	12(11.76)	29(28.43)	**0.0030**

HEP1601: Outbreak Investigation and providing diagnosis

Investigators: Kavita Lole & Anuradha Tripathy

Funding: Intramural

Duration: Ongoing

This project is part of the collaborative study with Regional Medical Research Centre (ICMR), Port Blair. During the year, 241 samples collected from the tribal population of Andaman and Nicobar were processed for detecting HBsAg and HBsAg positive samples for hepatitis B virus (HBV) DNA detection. All HBV DNA positive samples were further processed for genotype analysis using 'S' gene sequence based phylogenetic analysis. DNA positive samples were also processed for viral load determination using real-time PCR based assay. Of the 63 HBsAg positive samples, 44 samples were HBV DNA positive. Viral loads in these samples were comparatively low (<10^4 copies/ml) and all belonged to the genotype D, which is prevalent in the mainland India.

Technical support / consultancy provided:

1. Screeing products: A total of **647** products from the Drug Controller of India were tested for HBsAg and HCV RNA and reports were submitted (Commercial basis).
2. Chronic hepatitis B and C patients: A total of **4** and **01** sample were tested for the presence of HCV RNA and HBV DNA respectively in PCR & 113 samples were processed for HBV quantitative PCR.
3. Sporadic hepatitis patients: Referred serum samples were tested for anti-HAV-IgM (198), anti-HEV-IgM antibodies (199) and HBsAg (44) and provided diagnosis.
4. Serosurvey samples: **751** sera collected from Pune, Bhor & Shikrapur (District Pune) during serosurvey of viral hepatitis were tested for anti-HAV IgG, anti-HEV IgG, HBsAg and anti-HCV
5. **Testing of Water Samples:** Water samples [109] from Shimla (Himachal Pradesh), Khed, Aurangabad, Nashik (Maharashtra) and Dadara Nagar Haveli were tested for Hepatitis A & E viral RNA and reports were provided.
6. **Providing sequencing facility for other departments in NIV:** A total of 9663 samples given by other departments of NIV were sequenced on the 3130XL Genetic Analyzer and chromatograms were provided.

DENGUE - CHIKUNGUNYA

Scientific staff

Dr. Cecilia Dayaraj	Scientist 'F'& Group Leader (Retired on 30.04.2017)
Dr. Paresh Shah	Scientist 'E'& Group Leader (From 01.05.2017)
Dr. D. Parashar	Scientist 'D'
Dr. K.Alagarasu	Scientist 'C'

Technical staff

Mr. Anand Singh	Technical Officer
Dr. (Mrs.) Jayashri A Patil	Technical Officer
Dr. (Mrs.) Rupali V Bachal	Technical Officer
Mr. Mahadeo Kakade	Technician C
Mrs. Ashwini More	Technician C
Mrs. Poonam Patil	Technician C
Mrs. Minal Bote	Technician 'C'
Mr. HB Supe	Technician 'B'
Mr. SK Pandey	Technician 'A'
Mr. PB Gore	Multi tasking staff
Mr. DM Jadhav	Multi-tasking staff

Project staff

Dr. Shahdab Almelkar	Research Associate (DST Nano Mission)
Mr. Nandadeep Jadhav	Junior Research Fellow (upto 13.10.2017).
Monika Seervi	Research Assistant

Molecular characterization of dengue and chikungunya viruses circulating in India

Investigators: K Alagarasu, D Parashar, JA Patil, Kakade MB, A More, PS Shah

Funding agency: NVBDCP/Intramural

Duration: 2017-18

Background: Dengue is highly prevalent in India with >100000 cases annually and is caused by four serotypes, *i.e.,* DEN 1-4. Recent years have seen a spurt in dengue cases with complications and death across the country. Hence there is a need to characterize the viruses at molecular level to detect serotypes and genotypes involved in outbreaks.

Objective: To find out the distribution of serotypes of dengue virus (DENV) and genotypes within serotypes in India during the 2017 dengue outbreak.

Findings: Screening of samples from Maharashtra (1510), Tamil Nadu (92) and Jammu & Kashmir (85) were for the presence of DENV or chikungunya virus (CHIKV) by CDC Trioplex Real Time RT-PCR, 895 tested positive for dengue and 234 for CHIKV. Distribution of dengue serotypes detected from the three states is given in Table 1.

Table 1: Distribution of dengue serotypes in Maharashtra, Tamil Nadu and Kashmir during 2017

States & positives	Maha-rashtra (769)	Tamil Nadu (70)	Jammu & Kashmir (56)
Dengue serotypes			
DEN-1	27.2%	38.2%	64.3%
DEN-2	10.4%	23.6%	12.5%
DEN-3	30.6%	8.5%	17.8%
DEN-4	6.2%	30.2	1.7%
DEN & CHIK Co-infections	7.4%	00	00

E gene sequencing was carried out for 17 DENV-1, four DENV-2, 15 DENV-3 and one DENV-4 samples obtained from Maharashtra. Phylogenetic analysis revealed co-circulation of V and I genotypes of DENV-1 in Maharashtra while, it was GIV genotype for DEN2, GIII for DENV-3 and GIII and GI for DENV-4 (Fig 1 a & b). DENV-3 and DENV-1 serotypes dominated in Maharashtra, DENV-1 dominated in Kashmir while DENV-1 and DENV-4 dominated in Tamil Nadu. Circulation of GI genotype of DEN1 is reported for the first time in Maharashtra. Other samples are being sequenced.

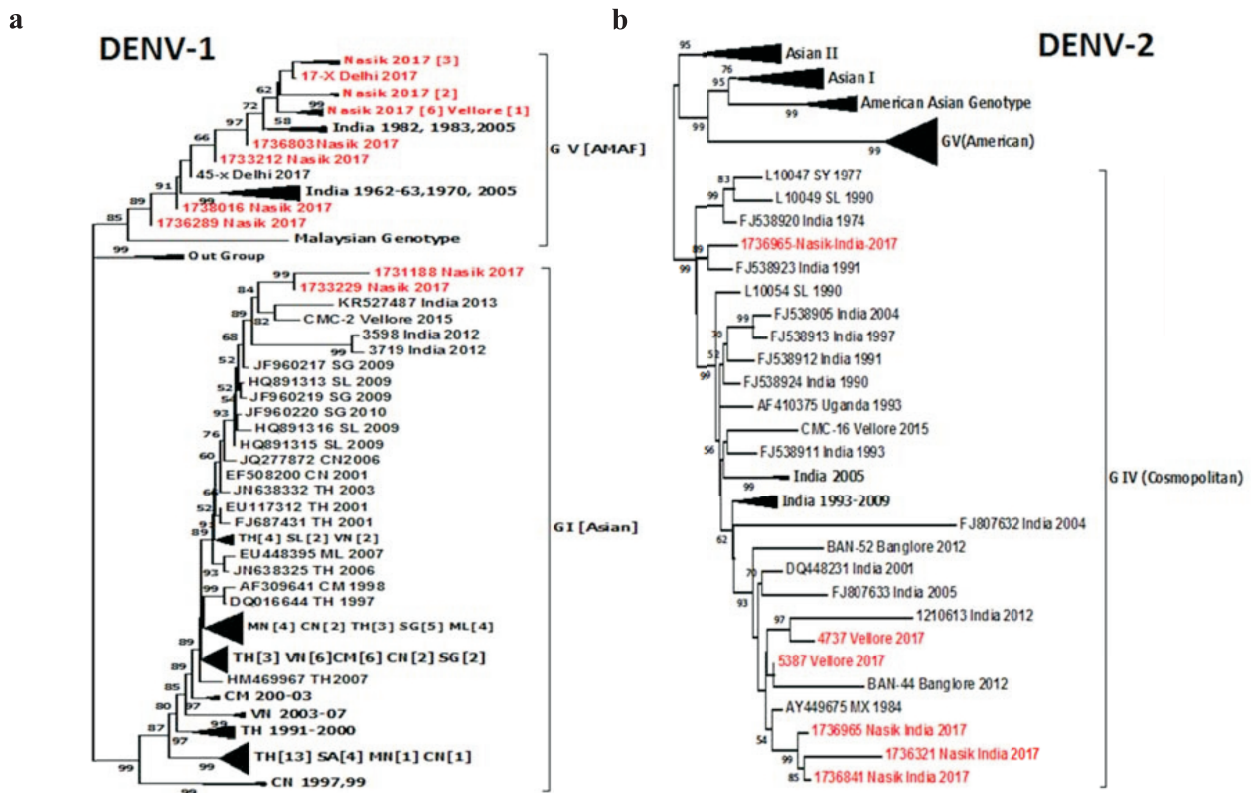

Fig 1. Phylogenetic tree of DENV-1 &DENV-2 from 2017 based on E gene sequences

Genetic characterization and molecular clock studies of chikungunya viruses circulating in India (2005-2016) using complete genome sequences

D Parashar, S Cherian, P Newase (Bioinformatics Group)

Funding agency: Intramural

Duration: 2017-18

Background: During the Chikungunya virus (CHIKV) outbreak in India in 2005-06, the Indian Ocean Lineage (IOL) has emerged within the East, Central and South African (ECSA) genotype. During the outbreak which persists till today, enhanced CHIKV activity with case severities in Karnataka, Maharashtra, New Delhi etc. Genetic characterization of CHIKV isolates obtained from these outbreaks is therefore important to understand the overall evolution of the virus in the context of the global phylodynamics and epidemiology of the IOL.

Objective: To carry out time-scaled molecular clock analysis of the IOL genomes

Findings: Molecular clock analysis was carried out with 200 whole genome sequences of the IOL that included six Indian isolates obtained from Maharashtra (Pune 2015, 2016, 2017; Nasik 2015), Delhi (2015) and Karnataka (Bangalore 2015). Beast analysis showed the Uncorrelated Lognormal relaxed molecular clock with bayesian skyline population demography as the best fit model. The mean rate of evolution was found to be 5.686 x 10^{-4} substitutions/site/year. The maximum clade credibility (MCC) tree (Figure 2) showed that the IOL root dated back 14 years with the Indian Subcontinent group showing time to the most recent common ancestor (tMRCA) around 12.062 years back, dating to ~2005. A common ancestor of the newly sequenced strains of 2015-17 except a single strain from Bangalore of 2015, was noted about 5.6 years ago dating to ~2011. The group was found to include the strains from the southern and northern regions of India along with 2016 strains from Hong Kong, Australia, Italy and Pakistan, indicating that the current strains have a wide-spread circulation.

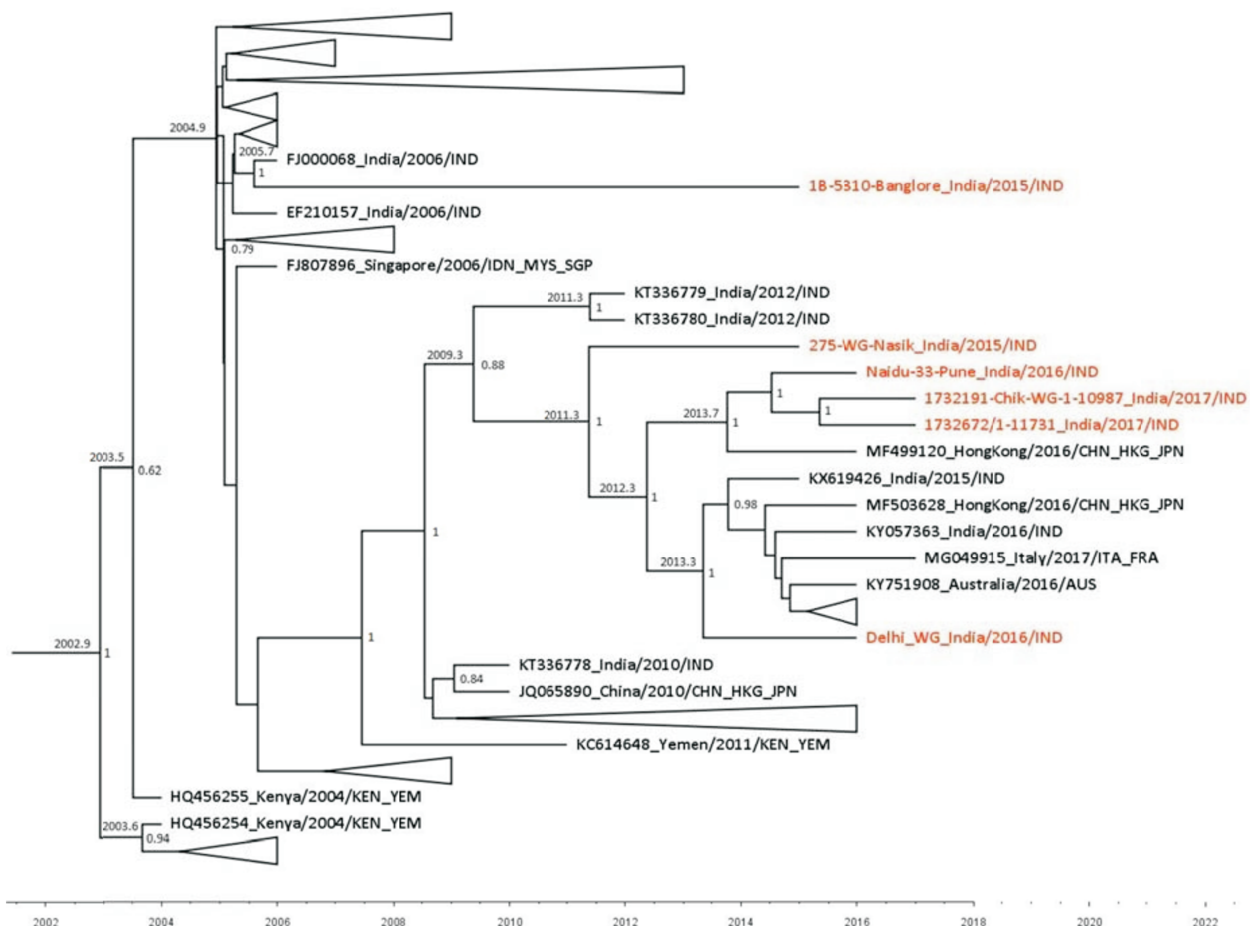

Fig 2. Maximum clade credibility (MCC) tree of the Indian Ocean Lineage of CHIKV. The time to the most recent common ancestor (tMRCA) at major nodes are indicated in years. Indian isolates sequenced are given in brown font color.

DEN 1304: Dengue virus infection in India: Molecular characterization of dengue viruses circulating in India

Investigators: JA Patil, M Kakade, K Alagarasu K, PS Shah

(In collaboration with ICGEB Delhi, AIIMS Delhi, CMC Vellore and Emory University, USA)

Funding Agency: NIH (ICIDR) and Intramural

Duration: 2015-2020

Background: Molecular epidemiological studies have established that dengue viruses evolve phylogenetically, move geographically and certain genotypes associate with severe disease outcome.

Objective: To perform molecular characterization of DENV circulating in different regions of India

Work done: During 2017, 20 samples were received from New Delhi, of which 15 detected positive for Dengue. DENV-3 was the predominant serotype and was found in 80% of the samples. Thirteen samples tested positive from the 25 samples received from Vellore, Tamil Nadu. Molecular typing of the positives has shown DENV-3 (5); DENV-1 (3), DENV-2 (2) and DENV-4 (1). E gene sequencing of two DENV-1 isolates of Vellore, two DENV-2 isolates from Delhi, three DENV-3 isolates (Vellore and Delhi) and one DENV-4 isolate (Delhi) was carried out. Whole genome sequencing has been completed for four isolates and further sequencing is in progress.

Findings: DENV-3 was the predominant serotype found circulating in Delhi while all four serotypes were observed in Vellore, Tamil Nadu. DENV-3 isolates belonged to GIII genotype. Larger number of samples needs to be sequenced to understand the association of genotypes with immune response and disease severity (Fig 3).

DEN1308 Influence of Vitamin D on innate immune response to dengue virus

Investigators: K Alagarasu, NJ Jadhav, PS Patil, M Seervi

Funding Agency: Extramural (SERB-DST)

Duration: 2015-2017 (completed)

Background: Vitamin D, an immunomodulator, is considered as a therapeutic agent against DENV.

Objective: To study the effect of vitamin D on the expression of genes coding for RNA sensing pattern recognition receptors, downstream signaling components including oligoadenylatesynthetases (OAS) and interferon stimulated gene 15 (ISG15) and T helper (Th)1, Th2 and Th17 cytokine response in DENV infected monocytic cell lines.

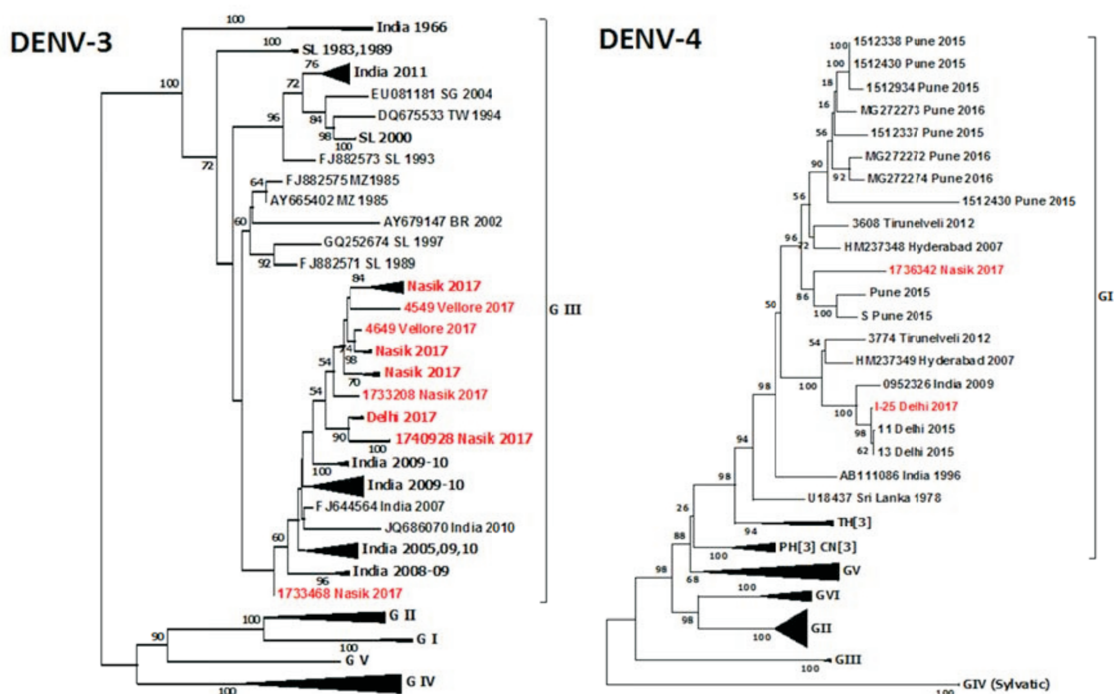

Fig 3. Phylogenetic tree of DENV-3 &DENV-4 from 2017 based on E gene sequences

Work done & Findings: U937-DC-SIGN cells treated with 0.1 μM and 1 μM 1,25(OH)$_2$D$_3$for 24 hrs. were infected with DENV-2 and 1,25(OH)$_2$D$_3$ treatment was continued for 72 hrs. THP-1 macrophages infected with DENV-2 virus were treated with1, 25(OH)$_2$D$_3$ for 72 hrs. U937-DC-SIGN RNA was investigated for the expression of *TLR3*, *DDX58*, *IFIH1*, *OAS1*, *OAS2*, *OAS3*, *CAMP* and *ISG15* genes using gene expression assays. Interleukin (IL)-12p70, IL-10, IL-4 and IL-17A levels were assessed in the THP-1 macrophage culture supernatants and the results revealed that 1,25 (OH)$_2$D$_3$enhanced the expression of *DDX58, OAS1, OAS2* and *OAS3* at 0.1 μM while higher concentration had diminishing effect. 1,25(OH)$_2$D$_3$increased the expression of *ISG15*and *CAMP* genes. 1,25(OH)$_2$D$_3$ suppressed the levels of IL-4 and IL-17A. IL-12p70 and IL-10 levels were suppressed by 0.1 μM 1,25 (OH)$_2$D$_3$ while a higher concentration increased the levels (Fig 4). The results suggest that 1,25 (OH)$_2$D$_3$may have concentration dependent immunomodulatory effects and higher dose of 1,25(OH)$_2$D$_3$ might have an immunoregulatory role in ameliorating inflammation during dengue infections. Further studies are needed to evaluate the efficacy of different doses of 1, 25 (OH)$_2$D$_3$ in preventing severe dengue.

CHK1501: Use of lipid nanoparticles for effective delivery of siRNA in chikungunya virus

D Parashar, S Almelkar, S Ramakrishna, V Rajendran (IICT, Hyderabad)

Funding agency: Extramural (DST Nano Mission)

Duration: 2017- 2020

Background: RNA interference (RNAi) is a natural cellular process that can be exploited to silence specific genes by intracellular delivery of small interfering RNAs (siRNAs). We have recently shown that siRNAs against the ns1 and E2 genes of CHIKV are effective in controlling CHIKV replication *in vitro* and *in vivo*. However, their real therapeutic potential is limited.

Objectives:

(i) To develop and characterize CHIKV siRNA encapsulated solid lipid nanoparticles (siRSLN).

(ii) To evaluate the efficacy of siRSLN in inhibiting CHIKV replication in *in vitro* and *in-vivo* systems.

Findings: Cationic based SLNs were successfully prepared by emulsification-solvent evaporation method at different mass ratio. Glycerylmonostearate as key component of

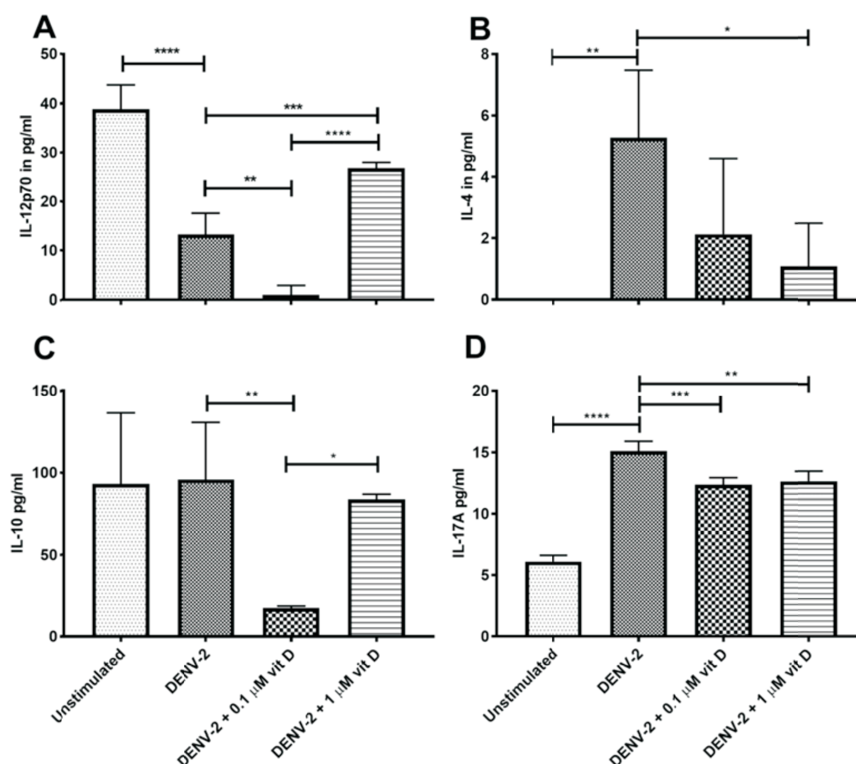

Fig 4: Effect of 1,25(OH)$_2$D$_3$ on cytokine response in DENV infected THP-1 macrophage cultures.

A. IL-12p70, B. IL-4, C. IL-10, D. IL-17A.Cytokines levels are given as pg/ml. Cytokine levels are given as mean±standard error. ****P<0.0001; ***P<0.0005; **P<0.005; *P<0.05.

solid lipid and soya lecithin acts as stabilizer or co-lipid which facilitates in packing density of lipids.

We determined the binding potential of siRNA with SA-SLN cationic complexes using gel retardation assay. The results showed retardation of siRNA movement indicating the strength of complexes and exhibiting stable complexation. We have also evaluated efficiency of naked siRNAs in reducing CHIKV replication in Vero cells transfected with Lipofectamine. Plaque assay results showed complete reduction in viral titers for 100-200 pmol Comb-siRNA in comparison to control (only virus) ($P < 0.001$), suggestive of the potential of higher concentration of Comb-siRNA transfection in gene silencing activity and may play a pivotal role in controlling CHIKV replication in cells (Fig 5).

BDM1401: Structure-based design and evaluation of lead compounds targeting Chikungunya virus

S Cherian, D Parashar & S Almelkar

Funding agency: Extramural (ICMR)

Duration: 2017- 2020

Background: Currently no approved drugs or effective vaccine is available for CHIKV. Previous studies have shown certain compounds (chloroquine, furin inhibitors, arbidol, mycophenolic acid, combination of ribavirin and alpha interferon, harringtonine, suramin, baicalin etc.) with anti-CHIKV activity *in vitro*. But none of them has been approved as a therapeutic agent against CHIKV. Since the virus is spreading, there is an urgent need to discover novel antivirals to combat CHIKV infection.

Objectives: Evaluate the antiviral activity of selected lead compounds

Findings: Lead compounds with antiviral activity against CHIKV were screened at the National MolBank repository of compounds at IICT, Hyderabad and 13 compounds were shortlisted for *in vitro* antiviral testing. Initial screening has shown that, a compound, OCL-105 has the potential as an anti-viral against CHIKV as effective reduction was observed in viral titres when used in pre and post and co-treatments (Fig 6). Dose dependent reduction in RNA copy number by Real Time PCR was observed during pretreatment.

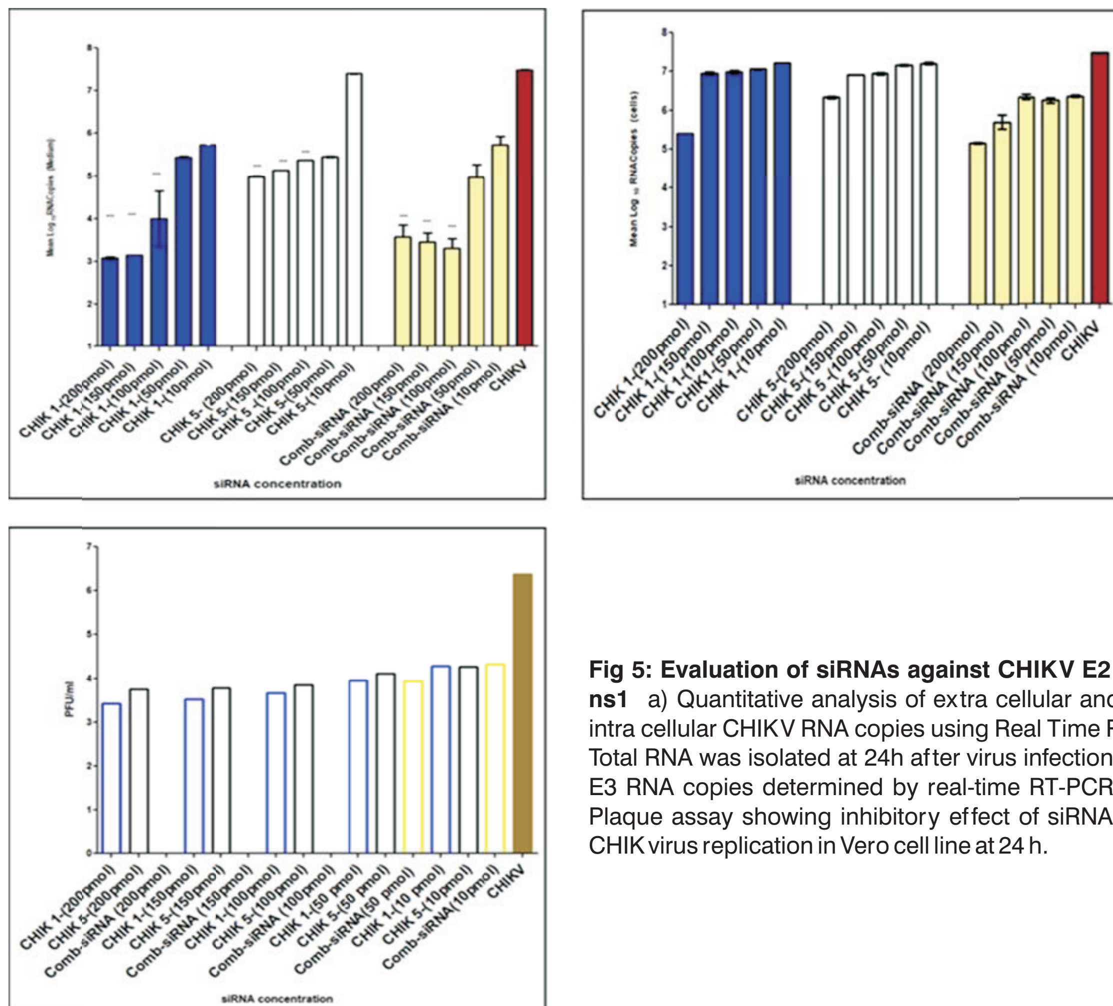

Fig 5: Evaluation of siRNAs against CHIKV E2 and ns1 a) Quantitative analysis of extra cellular and b) intra cellular CHIKV RNA copies using Real Time PCR: Total RNA was isolated at 24h after virus infection and E3 RNA copies determined by real-time RT-PCR, c) Plaque assay showing inhibitory effect of siRNAs on CHIK virus replication in Vero cell line at 24 h.

Fig 6: Focus forming unit assay for pre and post-treatment of virus at 24hr (a & b) and 48hr (c & d) and co-treatment of virus at 48 hr (e)

Samples tested during the year (Jan-Dec 2017)

Dengue IgM	No. Tested: 3729	No. Positive: 2131 (57.1%)
Chikungunya IgM	No. Tested: 1583	No. Positive: 854 (53.9%)

Kit Validation

DEN: 50 batches CHIKV: 10 batches

ENCEPHALITIS

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

Investigators: Sapkal GN, Damle RG, Kaushal H, Pavitrakar DV, Mahamuni SA, Sankararaman V

Funding Agency: Intramural

Duration: Ongoing (service project)

Background: Encephalitis group is involved in providing diagnosis to referred clinical specimens as well as sample collected during viral encephalitis outbreaks.

Objective 1: Diagnosis of referred human clinical specimens

Work done & findings: During 2017-18, 480 cases were referred to encephalitis group, 370 from Government hospitals and 110 from private hospitals. State wise distribution, samples and tested results are given in Table 1.

Objective 2: Diagnostic support provided for AES outbreak in Sangli, Maharashtra, August 2017

Work done & findings: Clinical samples were collected from index case, siblings of index case admitted with encephalitis and family contacts and health care providers (n = 65). The samples were tested by RT-PCR for JE, WNV, CHPV, EV, and PCR for HSV and VZV. None of the samples tested positive for any of the viruses. Serum and CSF samples from contacts were tested by ELISA for JEV and CHPV IgM but tested negative for both the viruses. The samples were further tested for DENV-CHIKV-Zika and Measles-Rubella (by DVG) and found to be positive for Rubella Genotype 2B by NGS (CSF), RT-PCR (urine) and IgM antibody (blood).

***Objective 3:* Virus isolation from clinical specimens:**

Work done: To have the conclusive evidence of the etiological agent, attempts were made to isolate virus from 38 positive samples (26 from Maharashtra (CSF 23, serum 03), 14 from Gujarat, 01 from Tamil Nadu and 12 from Sangli AES outbreak (CSF 01, Serum 03, throat swab 03, rectal swab 02, and nasopharyngeal aspirate 01) in Vero, RD, BHK 21 and MRC 5 cells lines.

Findings: No virus could be isolated.

***Objective 4:* Neutralization test** for **JEV and CHPV antibodies**

Work done & findings: Ninety-nine serum Samples (Aurangabad 01/05, Jalna 05/18, Mumbai 06/08, Solapur 01/03 and Dahod 02/18) were subjected to *in vitro* neutralization test and antibodies to JEV was detected in 15samples. Antibodies to CHPV were observed in 16 samples (Bhandara 01/02, Dahod 11/15). Seven samples received from endemic areas with death cases *viz*., Chennai 01/01, Gujarat 03/03, Maharashtra 01/01, Telangana 02/05 tested positive for neutralizing antibodies to JEV while two samples received from Maharashtra showed positivity for CHPV.

Objective 5: Testing of animal sera for JEV

Work done & findings: Eighteen animal sera referred by District Malaria Officer *viz*. Aurangabad (5 Pigs, 1 Buffalo, 1 Cow), Jalna (6 Pigs), and Beed (1 Horse, 1 Ox, 1 Cow, 2 Buffalo), were screened for neutralizing antibodies to JEV. Pig sera from Jalna and all the animal sera from Beed and one buffalo serum from Aurangabad showed Nabs to JEV.

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

Investigators: Damle RG, Pavitrakar D, Tandale BV, Aiyer S.

Funding Agency: Extramural (DST)

Duration: Two Years

Background: Development and characterization of anti CHPV MAb clones was done. The MAbs having IgG isotype were considered for the development of Ag Cap ELISA.

Objective: To develop Ag Cap ELISA for detection of CHPV from hospitalized encephalitis patients.

Work done: IgG of seven MAbs representing five hybrids was purified on protein G column and the protein yield was found to be in the range of 3-5 mg/ml. Purified IgG of all seven MAbs was biotinylated (MAb-B) and the dilution giving OD value 1.5 ± .01 with background OD less than 0.1 was considered optimum. MAb IgGs were screened for their ability to capture and the MAb-B for their ability to detect captured TC derived CHPV antigen. A combination of capturing (CAb) and detector antibodies (DAb) giving higher OD value to known CHPV antigen and OD value < 0.10 to NC in turn giving higher P/N ratio was considered as suitable pair. All individual IgGs captured CHPV antigen and 03/07 MAb-B

satisfactorily detected captured antigen (P/N range 5 to 11). In order to increase assay sensitivity, we screened 21 combinations of mixture of two MAbs for capturing. Increase in P/N was observed when a mixture of MAbs was used in place of singular MAb (P/N range 18-20).

In further refinement, overnight incubation of Ag yielded higher P/N ratio 30.1 Vs 21.7 (3 hrs.) and 18.3 (1 hr.). ELISA detected 6250 PFU and 100 ng of purified viral proteins respectively. In cross-reactivity testing, the ELISA did not react with other encephalitic viruses *viz*., JEV, WNV, HSV & or dengue and CHIKV.

Findings: Combination of MAb CHP-I2E13 and CHP-IV4D14 was determined as the best CAb and CHP-II8B13-B as suitable DAb.

ENC 1802: Development of IgG detection ELISA to diagnose past exposure to Chandipura virus

Investigators: Damle RG, Butte DK, Sapkal GN

Funding Agency: Institutional Funding

Duration: Two years

Background: An indirect antibody detection ELISA was developed to detect anti CHPV IgG antibodies from human serum samples.

Objective: Detection of anti CHPV IgG from archived human blood

Work done & findings: CHPV was purified using discontinuous sucrose density gradient and coated on to ELISA wells (1 ug/well). A small panel of sera which included 3 sera each of CHPV Nab positive and negative was employed for the assay development. P/N ratio of positive samples was in the range of 3.0 to 11.0. Further studies with 114 clinical samples showed 95% positivity in comparison to neutralization test. OD value of 0.56 was considered as cut off for positivity. The assay was standardized with 95% sensitivity and specificity. Further refinement with more samples is in progress.

ENC1503: Role of Histidine residues in membrane fusion of Japanese encephalitis virus.

Investigators: Bondre VP, Mali DN.

Funding agency: Intramural

Duration: 2015-2018

Background: The work is undertaken to define the role of Histidine (His) residues conserved in envelope E protein of the JEV and members of flavivirus genus in sensing alterations in pH during virus – host cell interaction. The interaction and movement of virus along with cellular compartments against different time intervals after infection will be detected through fluorescent signals emerging from lipophilic dye (DiD) labelled virus and antibodies specific to different cellular compartments. This standard time kinetics of virus tracking will serve as standard control in experiments that will be carried out with expressed virus like particles (VLPs) and genetically mutated VLPs at conserved His residues to define their role in pH sensing. His residues involved in pH sensing and internalization are going to be important targets for development of therapeutics.

Objective: Labelling of JEV with lipophilic dye and confirmation of expression of virus like particles (VLPs) by western blotting.

Work done & findings: Labelling of virus with lipophilic dye was done as per the manufacturer's instructions. The unbound dye was removed by size exclusion filtration through Sephadex 25 coarse column and fractions were analyzed by standard ELISA for viral particles and confirmed under fluorescent microscope. The labeled virus will be used for tracking experiments.

Previously Confirmed clone having partial C-prM and complete E protein genome insert was transfected in BHK-21 cells. Transfected cells soup harvested at different time intervals was analyzed for expression of VLPs. Constitutive expression of VLPs was confirmed in all fractions through western blotting (Fig. 1). The formation

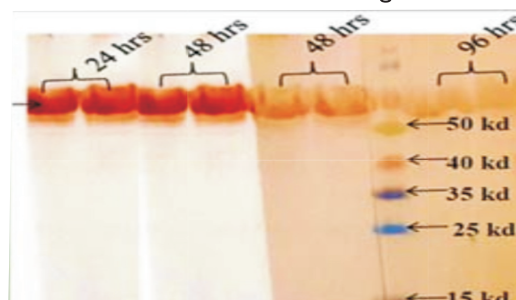

Fig 1: Western blotting analysis of expressed JEV E protein post transfection at 24, 48 and 96 hrs.

ENC 1803: Detection of CHIKV antigen from infected mosquito in C MAb based antigen cap ELISA

Investigators: Damle RG, Sudeep AB, Parashar D

Funding Agency: Intramural

Duration: Two years

Background: An antigen capture ELISA was developed using a panel of MAbs developed earlier (Damle *et al.,* 2016). A mixture of antibodies (C1/C4) for capture gave better detection and the cut off for positivity was determined as P/N e ⩾ 2.0.

Objective:To detect CHIKV from oral fed mosquitoes

Work done & findings: CHIKV exposed *Aedes aegypti* mosquitoes via oral route were processed for virus detection in the newly developed Ag Cap ELISA and Real Time PCR. CHIKV was detected in the former on 2nd to 6th PID giving P/N ratio e" 2.0, which was found comparable to the latter. In order to mimic field conditions, infected and uninfected mosquitoes in different combinations were also screened by the two assays and the results were found comparable (Table 2). More studies are needed to standardize the assay for screening of field collected mosquitoes.

Delineation of an epitope on the Chikungunya virus (CHIKV) capsid protein using monoclonal antibody and immuno-informatics approaches (M. Sc Project)

Investigators: Damle RG, Thite A, Pavitrakar DV, Agarwal M, Cherian SS

Funding: Intramural

Duration: six months

Background: The Capsid protein (CP) of CHIKV plays a crucial role in the assembly of nucleocapsid and packaging. The un-conserved N-terminal domain of CP is involved in non-specific RNA binding, while the highly-conserved C-terminal region harbors a globular chemotrypsin-like serine protease and contains the binding site for the spike protein. A recent study (Goh et al., 2015) has revealed that the CHIKV CP contains linear B-Cell epitopes in the N-and C-terminal regions that are dependent on an intact C-terminus for antibody recognition.

Objective: To study the epitope specific to the MAb (CI VE4/B4) on the CHIKV capsid protein and to understand the binding by antibody modeling and antigen-antibody (Ag-Ab) interaction studies.

Findings: PRNT performed to test the neutralizing activity of the antibody CI VE4/B4 (IgG1 type with Kappa light chain), showed that it was found to be non–neutralizing. PCR products of heavy and light chains were sequenced, translated and the amino acid sequences were

Table 2:ELISA results of CHIKV oral fed mosquitoes

Capture MAb **Detector MAb**	Mixture MAbC1/C4 Pools of Infected and Uninfected Mosquitoes	OD 490	P/N ratio
	1+4	**0.245**	**2.22**
	1+9	**0.449**	**4.08**
	1+19	0.178	1.61
	2+3	0.133	1.20
	2+8	**0.280**	**2.54**
	2+18	**0.317**	**2.88**
MAb C1-B	3+2	0.136	1.23
	3+7	0.162	1.47
	3+17	0.156	1.41
	TC Pos Ctrl	**0.800**	**8.98**
	Inf Mosquito Pos Ctrl	**0.220**	**2.0**
	Un infect Mosquito Ctrl	0.11	

used for modeling of the antibody Fv region using an online server Kotai (http://kotaiab.org/). Docking of the antibody model with the available crystal structure of the C-terminus of the CHIKV CP using 'ZDOCK' server, revealed that the antibody can interact with residues crucial in the dimerization of CP (Fig 2). The region is also predicted as a B-cell epitope. Further mutational studies are needed for confirmation of the binding specificity. Dimerization of CP being a very important step during assembly of the nucleocapsid and in turn packaging and budding of the alpha viruses, these MAbs may have application in therapeutics or prophylactic use in CHIKV infection.

Other activities

I. Development of a panel of samples for JE IgM Kit validation: A panel of known positive and negative serum and CSF samples for anti JEV IgM antibodies was developed for validation of JE MAC ELISA Kits. Eighty-one samples were screened for the presence of IgM antibodies to JEV by three different kits *viz*. JE MAC ELISA kit, NIV, batch no (16-011), In Bios (In Bios International Inc Seattle USA) and DRG (DRG International Inc Springfield USA). Samples showing reactivity in all the three kits were selected for inclusion in validation panel. Forty out of eighty-one samples comprising ten samples each of serum and CSF, showing positive and negative reaction for JEV IgM was included in final validation panel.

II. Validation of JE MAC ELISA Kits: *Validation of 12 batches of JEV IgM ELISA kit (Batch No 17-011, 17-013, 17-017, 17-028, 17-031, 17-041, 17-053, 17-059, 17-070, 18-05, 18-006, 18-011) was done using a panel of 10 sera/CSF samples for D*iagnostic Reagent Facility, NIV.

III. Preparation of Positive control for conventional PCR

(i) Positive control was prepared for Chandipura virus using 034627 strain in BHK-21 cell line.

(ii) Cell culture-based antigen was also prepared for HSV using BHK-21 cell line.

After confirmation on Agarose gel electrophoresis of first and Nested PCR products the positive controls were aliquoted and stored at –70°C till further use

IV. Revival, screening and cryo-preservation of MAb clones: As part of routine activity of the group, two MAbs specific to JEV, seven to CHPV and two MAbs to CHIKV were revived, tested for their ability to secrete virus specific antibodies. They were cryopreserved again in liquid nitrogen.

V. Characterization of JEV received from HLL: Plaque titration of JEV 014178, Vero P2 (Virus Bank-1 26-12-2016) received from HLL was performed which yielded a titer of 3×10^7 PFU/ml. Additionally complete genome sequencing of this virus along with the original virus stock adapted to 56°C (JEV 014178, Exm. DVG 138,139 10% M Br. Suspension 0.5ml 19-12-2015) was carried out and the results were communicated to DVG.

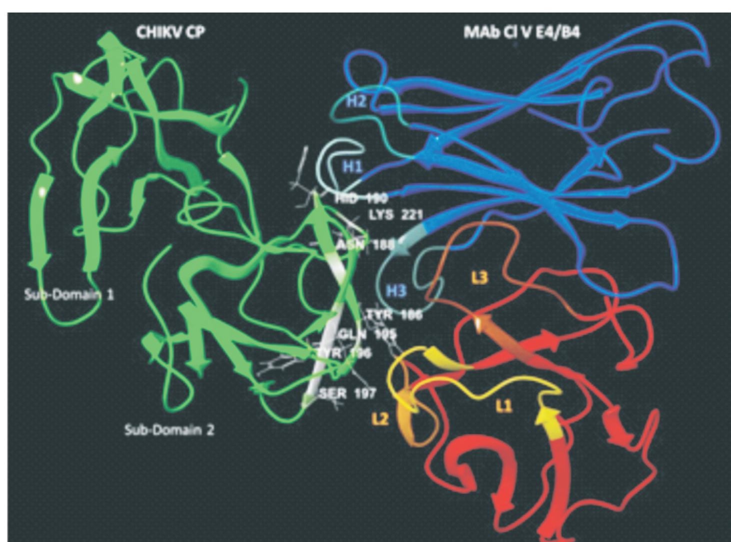

Fig. 2. The docked complex of monoclonal antibody Cl VE4/B4 with CHIKV capsid protein (CP). The heavy chain (VH) and the light (VL) of the MAb and the CHIK CP are shown in blue, red and green ribbon form respectively. The interacting residues of the CHIKV CP with the Complementarity determining regions (CDRs) L1, L2 and H1, H3 of the antibody are labeled.

Table 1: Details of samples (referred) processed by Encephalitis Group

	Total samples tested		Positive		Indeterminate	
	Serum	CSF	Serum	CSF	Serum	CSF
Maharashtra (Serum 182, CSF 183)						
JE MAC ELISA	182	183	32	08	**62**	**03**
JE RT- PCR	38	20	00	00	NA	NA
CHPV MAC ELISA	19	21	00	00	NA	NA
CHPV RT- PCR	07	09	00	00	NA	NA
WN RT-PCR	17	05	00	00	NA	NA
Enterovirus RT-PCR	03	02	00	00	NA	NA
HSV PCR	16	25	00	00	NA	NA
CMV, EBV, Measles (each)	03	00	00	00	NA	NA
VZV PCR	04	03	00	00	NA	NA
Total			32	08	62	03
Gujarat (Serum 118, CSF 91)						
JE MAC ELISA	118	91	18	02	**08**	**07**
JE RT- PCR	44	46	00	00	NA	NA
CHPV MAC ELISA	106	82	00	00	NA	NA
CHPV RT- PCR	50	60	03	00	NA	NA
HSV PCR	06	06	00	00	NA	NA
Total	324	285	18	02	08	07
Other states (Serum 60, CSF 27) A.P, Chhattisgarh, M.P, Tamil Nadu, Kerala, Bihar, Rajasthan, Telangana, Manipur, Puducherry						
	Total samples tested		**Positive**		**Indeterminate**	
	Serum	**CSF**	**Serum**	**CSF**	**serum**	**CSF**
JE MAC ELISA	60	27	21	02	04	15
JE RT- PCR	18	05	00	00	NA	NA
CHPV MAC ELISA	04	07	00	00	NA	NA
CHPV RT- PCR	02	03	00	00	NA	NA
WN RT-PCR	14	13	00	00	NA	NA
HSV PCR	03	07	00	01	NA	NA
Total	101	62	21	03	04	15

EPIDEMIOLOGY

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Multi-centric study to estimate the seroprevalence of dengue virus infection in India

Investigators: Tandale BV, Sapkal GN, Murhekar M (ICMR-NIE)

Funding agency: Extramural (ICMR-NIE)

Duration: 2017–2018

Background:

As a multi-state study, Maharashtra state survey was undertaken during August to December 2017 to determine the seroprevalence of dengue virus in rural and urban areas.

Objectives:

- To estimate the age-specific sero-prevalence of dengue virus infection in India
- To identify risk factors for dengue infection

Findings: Four districts in Maharashtra were selected; two urban and two rural enumeration blocks randomly. Enumeration of households and members was done using tablet computers and the data were uploaded onto the servers at NIE. The subjects selected for the study were contacted for consent for data collection and sampling of venous blood specimens.

In all, 774 blood samples were collected; sera separated and aliquoted into two parts. One aliquot was tested for anti-dengue IgG antibody by indirect MAC ELISA at NIE while the other was used for quality control to be undertaken along with PRNT at NIV Pune.

The prevalence of anti-dengue IgG antibodies was 71.32% (95% CI 68.03%-74.39%). The highest seroprevalence of 90.43% was observed in 18-45 year age group, followed by 73.76% in 9–17 years and the lowest of 51.91% in 5 – 8 year age group (Fig 1). Seroprevalence was significantly higher in females (74.8%) than males (67.8%). Urban population (78.00%) showed higher seroprevalence in comparison to rural areas (65.80%). Highest seroprevalence was observed in Osmanabad district (82.63%) while the lowest was in Pune district (47.45%).

Seroprevalence of hepatitis viruses in Pune district, Western India

Investigators: Avinash Deoshatwar, Yogesh Gurav, Kavita Lole

Funding agency: Generated fund (Hepatitis Group)

Duration: March 2016 – December 2017

Background:

Viral hepatitis poses a significant challenge to the public health in India. Epidemiology of viral hepatitis changes according to the socio-economic transitions along with other factors. We have been conducting periodic serosurveys for hepatitis viruses in the Pune district since 1978. This study is aimed at assessing transitions over the last two decades in the same area.

Objectives:

- To estimate sero-prevalence of hepatitis viruses in children and adults in Pune district.
- To compare the sero-prevalence across age-groups and between urban and rural settings.
- To compare sero-prevalence between high and low socio-economic strata.

Findings: A total of 767 blood samples were collected from rural areas and 671 from the urban

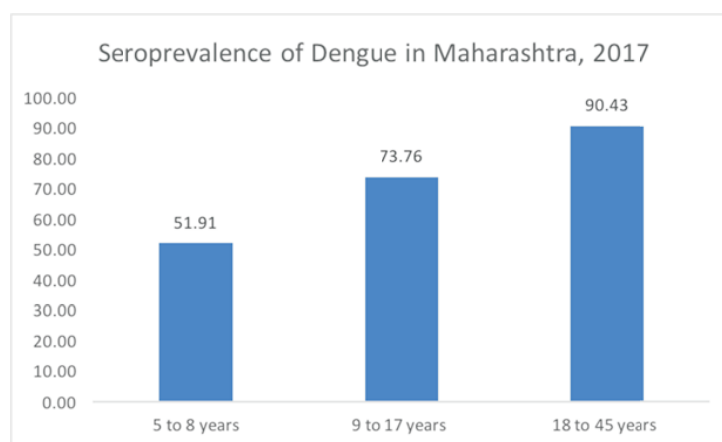

Fig1. Seroprevalence of dengue in Maharashtra state in 2017

areas and screened for hepatitis viruses. Preliminary analyses suggested that hepatitis A virus [HAV] seroprevalence was highest among the hepatitis viruses followed by hepatitis E virus [HEV], HBV, and HCV. Among those who reported history of jaundice, most were positive for HAV. HCV and HBV infection were found to be very low (<0.2% and <1% respectively).

Outbreak Investigation: Acute febrile illness (AFI) incidence in Dervan, Ratnagiri district, Maharashtra

Investigators: Tandale BV, Rima Sahay, Pragya Yadav

Funding agency: Intramural

Duration: Ongoing

Background: An alarming rise in cases of acute respiratory distress syndrome (ARDS) and multi-organ dysfunction syndrome (MODS) was reported from Dervan, Ratnagiri district (Maharashtra). NIV investigated 4 acute AFI cases, 2 convalescent cases and reviewed records of 4 death cases. Acute fever of 2-5 days with vomiting, diarrhea, myalgia, bodyache & breathlessness were reported. Moderate leukocytosis and raised neutrophil counts were reported with raised bilirubin, liver enzymes & creatinine. X-rays reported consolidation of lower and middle lobe zones. ARDS and MODS were reported as the cause of death. Death cases were adults (>45 years) with co-morbidity. Local hospital laboratories reported positivity for Malaria (*Plasmodium vivax*) and Leptospirosis in a few cases. Additionally, dengue NS1 positives were reported in some patients.

Objectives:

- To understand the magnitude of acute febrile illness cases with unusual clinical manifestations
- To detect etiologies of acute febrile illness, especially association of viral agents, if any.

Findings: Clinical specimens such as serum, urine and throat swab were screened for viral hemorrhagic fever etiologies *viz*., CCHF, KFD, dengue, Chikungunya, Zika, Hantaan, respiratory viruses either by Real Time RT-PCR or by IgM or IgG ELISA assays. All the samples tested negative.

MEDICAL ENTOMOLOGY & ZOOLOGY

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

Investigators: Sudeep AB & Gokhale MD

Funding: Extramural (ICMR)

Project Duration: 2017-2019

Background: Vidarbha region of Maharashtra is endemic to Chandipura virus and sporadic cases are reported annually with case fatalities. Sand flies belonging to Phlebotomine group are indicted as the vector of the virus as the virus has been isolated from the sandflies repeatedly from the outbreak areas. It has also been experimentally shown to transmit the virus both vertically and horizontally. Recently, the virus has also been isolated from sand flies belonging to genus *Sergentomyia*. It was therefore decided to study the bionomics of sand flies in Vidarbha region to determine the vectors involved in virus transmission.

Work done: Field surveys were carried out in 16 villages in Nagpur and Bhandara districts of Vidarbha division of Maharashtra for sand fly surveillance as part of ICMR project (Table 1). Collection sites were selected based on two criteria *viz*; 1. Past incidence of Chandipura virus activity either in human or vector specimens. 2. Records of high vector density in the area. The general ecological set up of the households is of village type intermixed with Katchha and Pucca houses. The walls of the houses are either mud plastered or brick work and most had modern toilets. The houses are surrounded by shrubs and bushes. Agriculture and small level merchandize seems to be the main occupation of the population. Many houses have cattle and other bovines in the close vicinity.

Adult sand fly collections were done between 12 hrs. to 19 Hrs. every day mainly from indoors (houses, toilets, cattle sheds and associated premises). A total of 2837 sand flies were collected, identified and pooled according to locality, gender and species. Per Man Hour Density ranged between 2.6 (Wadi) to 56.8 (Chinchtola). *Sergentomyia* species constituted almost 99 percent of the catch (Fig 1). *Phlebotomus* species were found low. All the pools were brought to NIV, Pune maintaining cold chain and processed for virus isolation in cell culture. **But no virus could be isolated**.

Table 1: Sand fly collection sites in Nagpur and Bhandara districts

Sr. No.	Name of place	District	Sand fly species encountered
1	Vaddhamna	Nagpur	*Phlebotomus argentipes* *Sergentomyia babu* *Sergentomyia baily* *Sergentomyia punjabensis*
2	Chacher	Nagpur	
3	Tarsa	Nagpur	
4	Navegaon	Nagpur	
5	Wadi	Nagpur	
6	ADHS Campus	Nagpur	
7	Police Lane (Primary school campuses)	Nagpur	
8	Nagardhan	Nagpur	
9	Dudhada	Nagpur	
10	Zhonad	Bhandara	
11	Gopipada	Bhandara	
12	Varathi	Bhandara	
13	Karbhi	Bhandara	
14	Chinchtola (Pipalgaon)	Bhandara	
15	Nurva	Bhandara	
16	Mathani	Bhandara	

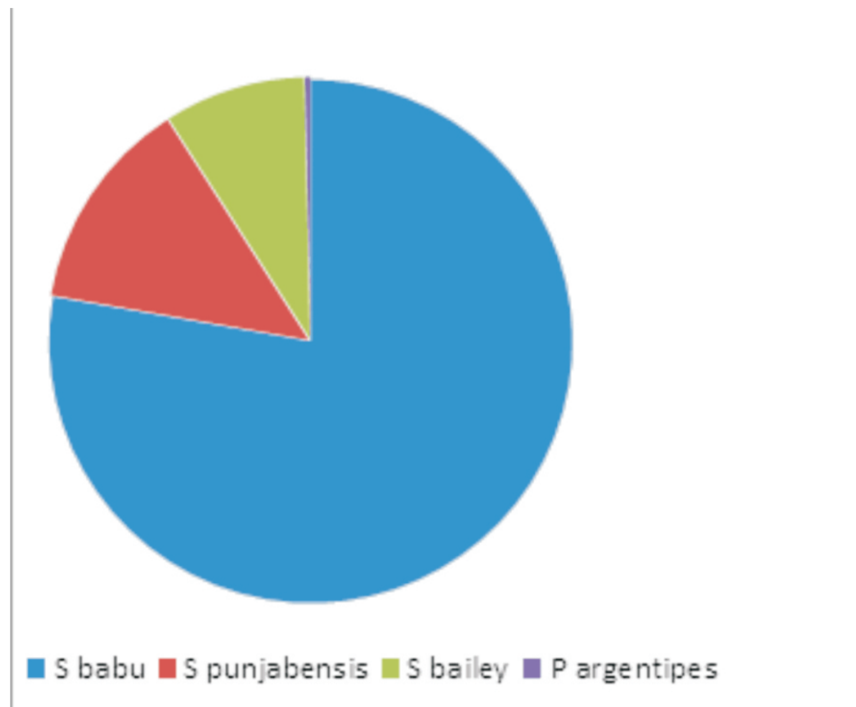

Fig 2: Distribution of *Phlebotomine* sand flies in Nagpur and Bhandara districts during 2017-18

Dengue outbreak investigation in Nagpur: Investigation of a dengue like illness in Wadi area, Nagpur:

Investigators: Dr AB Sudeep & MD Gokhale

During our survey for sand flies, we have investigated a dengue like outbreak in Vyahad PHC of Wadi area within the Nagpur Municipal Corporation during September-October 2017. Major symptoms reported were high fever (103-106°F), acute joint pains, myalgia, drowsiness, breathlessness, etc. Five patients succumbed to the infection of which two had symptoms of dengue hemorrhagic fever. An investigation was conducted to confirm the etiological agent, its characterization and the vectors involved in the outbreak. Serological analysis revealed presence of dengue IgM antibodies in 44 out of 158 sera samples. Molecular typing revealed involvement of DEN-2 and DEN-3 serotypes. Very high *Aedes aegypti* breeding was observed in the affected areas with Breteau index and house index ranging from 23 to 70 and 17 to 56 respectively (Table 2). Major breeding habitats encountered were cement tanks used tyres, and refrigerator trays among others (Table 3). Cement tanks used for water storage contributed to the high breeding of mosquitoes. Attempts were made to isolate virus from the mosquitoes in cell culture, but failed. Clinical manifestations, presence of IgM antibodies to dengue virus in 28% (44/158) of patients, absence of chikungunya virus antibodies and the extensive breeding of Aedes aegypti mosquitoes, the principal vector, clearly confirmed a dengue outbreak in Wadi.

Miscellaneous studies: (M.Sc. projects)

1. **Susceptibility and vector competence of Aedes vittatus mosquitoes to certain encephalitis causing arboviruses.**

   PI: AB Sudeep

   Preliminary studies have shown that Japanese encephalitis (JEV), West Nile (WNV) and Chandipura (CHPV) viruses replicate in *Aedes vittatus* mosquito species when infected via intra thoracic inoculation (Annual Report 2016-17). Attempts were therefore made to study the vector competence of the mosquito to the above viruses after infection through the natural route (oral feeding). Replication of JEV and WNV could be observed in the mosquito while no replication of CHPV was observed. Vector competence studies with JEV and WNV are in progress.

Table 2: Details of Aedes larval collection and entomological indices

Name of ward	No. of houses searched	No of Houses +ve for *Aedes* larvae	No. of container searched	No. of container tested positive	House index for *Aedes*	Container index	Breteau index
Indrayani Nagar	13	06	42	08	46.15	19.05	61.54
Bakshi Layout	30	05	82	07	16.67	8.54	23.33
Suraksha Nagar	27	15	65	19	55.56	29.23	70.37
Shivaji Nagar	36	02	49	02	5.56	4.08	5.56
Dhammakirthi Nagar	26	01	37	02	3.85	5.41	7.69
Samarth Nagar	25	01	42	01	04.00	2.38	04.00
Total	**157**	**30**	**317**	**39**	**19.11**	**12.30**	**24.84**

Table 3: Breeding habitats of Aedes mosquitoes in different wards

Type of container	Indoor	Outdoor	Total No. +ve /No searched & %	% positivity (to total)
	No. +ve/ No. searched	No. +ve / No searched		
Cement tank	00	13/133	13/133(9.77)	4.1
Buried mud pots	6/15	00	06/15 (40)	1.89
Metalware/plastic-ware	00	5/88	05/108 (5.68)	1.58
Rubber tyres	00	06/09	06/09 (66.7)	1.89
Refrigerator trays	09/72	00	09/72 (12.5)	2.84
	15/87	24/230	39/317 (12.3)	12.3

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2. Studies on the bionomics of Culex sitiens and its competence to replicate and transmit Japanese encephalitis virus

PI : Dr. MD Gokhale

Field studies were conducted at Aleppey for the collection of *Culex sitiens* species. Eggs and larval stages were collected from the brackish (saline) waters and brought to NIV, Pune and a cyclic colony was established by gradually adapting the species to laboratory conditions. Base line data with reference to life cycle parameters, morphometric parameters and the insecticide susceptibility status has been generated. Susceptibility studies of *Culex sitiens* to JEV are in progress.

3. Susceptibility of Aedes mosquitoes collected from different parts of Pune District with reference to dengue and chikungunya virus (es).

Aedes aegypti and Aedes albopictus populations were collected from 10 different transport hubs in and around Pune. Temporary colonies of field strains were established. Baseline data with reference to morphometric parameters of the IVth stage larvae and the insecticide susceptibility bioassay has been generated. The virus susceptibility studies on these strains are in the progress.

Processing of referred Arthropod specimens:

The Entomology Group receives arthropod specimens from different Public Health Centers (PHC), Municipal Corporations and the State Entomology units for identification as well as detection of arboviral agents. These referred arthropod specimens were identified and pooled according to species, gender and locality. Details of the arthropod specimens processed are as below.

Mosquitoes:

From Pune Municipal Corporation: *Aedes aegypti* mosquito: Males (19), Females (56) Larvae (116) were received. The male and female mosquitoes were screened for detection of dengue and chikungunya viruses by indirect immunofluorescent antibody technique. All the specimens tested negative for both the viruses. The larvae were allowed to pupate and the F_1 generation adults were also tested for dengue and/ chikungunya viruses, but found negative (No trans ovarial transmission could be detected).

From Aurangabad District: *Aedes aegypti* mosquito: Two male specimens were tested for dengue/chikungunya viruses. The mosquitoes tested negative for both the viruses.

Ticks: Tick specimens were received as part of the KFD monitoring program in ticks. The specimens were identified and handed over to Dr. Pragya Yadav for processing in BSL-4 lab.

1. Sindhudurg District
   - i. PHC Bhedshi: *Haemaphysalis spinigera* (nymphs-5); *H. turturis* (nymphs-5); *Amblyomma* spp. (larvae-41).
   - ii. PHC Pandur: *H. spinigera (*nymph-1); *H turturis* (nymphs-2)
   - iii. PHC Walawal: *Amblyomma* spp. (larvae-13); *H spinigera* (nymphs-11)
   - iv. PHC Kanadi: *H. spinigera* (nymph-1); *H. turturis* (nymphs-2)
   - v. PHC-Banda: : *H. spinigera* (nymphs-2), *H. turturis* (nymphs-6)
2. Shimoga District (Karnataka):

   H. spinigera (nymphs-292); *H. turturis (*nymphs-20, larvae-12); *Amblyomma* spp. (nymphs-4, larvae-143); *H.* spp (larvae-22); *H. bispinosa* (nymphs-3), *H. intermedia (*adult female-1, nymphs-5); *Boophilus micrpoplus* (adult male-5, adult female-5)
3. Uttar Kannada District (Karnataka):

   Amblyomma spp (nymphs-8); *H. bispinosa* (nymphs-19); *H. spinigera* (nymphs-31)

ENTERIC VIRUSES

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ENV1301: Investigation of outbreaks and diagnostic services

Investigators: Gopalkrishna V, Joshi MS, Vishvanathan R.

Funding Agency: Intramural

Duration: Ongoing

(i) Acute gastroenteritis outbreak in Cantonment area of Aurangabad (Maharashtra)

Background: An outbreak of acute gastroenteritis was reported in the Cantonment area of Aurangabad (Maharashtra), during November 2017 with 3690 cases, majority above the age of 14 years. Most of the patients presented with severe abdominal pain, vomiting and fever with chills alongwith profuse watery stools with foul smell. Patients were treated with IV Fluids, IV Metrogyl, Antimetics, antibiotics etc. No cases were reported in rest of the corporation area. Drinking water supply to the area is from a single storage tank and contamination of water through the pipeline was suspected as the source of infection.

Objective: To investigate the gastroenteritis outbreak for viral and bacterial etiology.

Findings: Screening of 46 stool samples collected from patients revealed absence or negligible role of known viral etiological agents *i.e*. Group A rotavirus, Group B rotavirus, Group C rotavirus, Norovirus, Adenovirus and Enterovirus. Electron Microscopic studies also showed absence of any viral agents.The bacterial culture studies have showed presence of *Vibrio cholerae* O1 Ogawa in six fecal samples while *Salmonella, Shigella* and *E .coli* O157:H7 were absent. Stored water samples collected from houses of 4 patients were concentrated in the Hepatitis group of NIV and Nucleic acid was extracted. Further studies are in progress.

ii) Acute gastroenteritis outbreak in Devalikarad, Nashik district of Maharashtra

Investigators: Gopalkrishna V, Joshi MS, Lole KS

Background: An outbreak of acute gastroenteritis with 251 cases was reported at Devalikarad village in Nashik district of Maharashtra, western India during November 2017. The total population of the village was recorded to be 1350.

Objective: To investigate the acute gastroenteritis outbreak for viral etiology.

Work done and findings: Ten stool specimens collected from the gastroenteritis patients were tested for different enteric viruses using conventional methods. Reverse transcription polymerase chain RT-PCR and nucleotide sequencing confirmed the presence of Group B Rotavirus (GBR) in all the samples. The water samples collected from the well and boar well from the outbreak area showed positivity for GBR by RT-PCR and nucleotide sequencing. None of the other agents *i.e*. Group A and C rotavirus, Norovirus, Adenovirus and Enterovirus could be detected in the samples. Both well and borewell water supply were subjected to chlorination and absence of GBR was detected subsequently.

iii) Investigation of Hand, Foot and Mouth disease cases for viral etiology

Investigators: Gopalkrishna V, Ganorkar NN

Funding agency: Intramural

Duration: Ongoing

Background: Hand, Foot and Mouth disease (HFMD) is a common childhood disease generally caused by Coxsackie virus A-16 (CVA-16) and Enterovirus 71 (EV71). Cases were reported in Pune (Maharashtra) during December 2017 to January 2018.

Objective: To investigate the cases of HFMD for viral etiology.

Findings: Seventeen clinical samples from 12 HFMD cases collected from three local hospitals in Pune were tested to detect EV-RNA. Sixteen samples (94.11%) were found positive for enterovirus by RT-PCR using 5'NCR gene. All the EV positive specimens were subjected to molecular typing using VP1 gene and sequencing and phylogenetic analysis of the partial VP1 gene revealed the presence of CVA6 [12, (75%)] and CVA16 [4 (25%)].

ENV1310: Hospital based surveillance of rotaviruses and strains in children with acute gastroenteritis

Investigators: Gopalkrishna V, Joshi MS

Funding Agency: Intramural

Duration: Ongoing

Background: Rotavirus infections are the major cause of severe diarrhea in children. Among different group A rotavirus genotypes, G1P[8], G2P[4], G3P[8], G9P[8] genotypes are predominantly detected across the world. In addition, uncommon and untypeable rotavirus strains co-circulate and mixed infections with different rotavirus genotypes are reported to occur in developing countries. Currently two live oral vaccines, *i.e*., Rota Teq and Rotarix have been licensed globally. In India, Rotavac vaccine

has been included under the National Immunization programme since 2016. Surveillance studies would be helpful to define the need and utility of rotavirus vaccines to estimate the proportion of rotavirus diarrhea and circulation of different strains in India.

Objective: Hospital based surveillance of rota virus disease and virus strains among children <5 years age for determination of prevalent RV G-P genotypes, seasonal distribution and diseases burden.

Work done and findings: Screening of 177 fecal specimens collected from children hospitalized with acute gastroenteritis in Pune (Maharashtra) showed 28.2% rotavirus positivity by ELISA. All positive specimens were subjected to multiplex PCR for RV VP7 (G) and VP4 (P) genotyping. Nearly 96% and 94% of the strains were typed for G and P genotypes respectively while 92% were typed for both. The study showed circulation of G3P[8] (70%), G1P[8] (10%), G1P[6] (2%), G9P[4] (2%), G12P[11] (4 %), G10P[8] (2%), GNTP[8] (2%), G3P[NT] (2%), G12P[NT] (2%) and GNTP[NT] (2%) rotavirus strains (Fig 1). Mixed infection of rotavirus genotype G3P[4]P[8] was detected in a single specimen. The present rotavirus surveillance will be continued further to monitor the common, uncommon and emerging rotavirus genotypes causing acute gastroenteritis among children.

The present study highlights predominance of RV G3P [8] in 70.0% of the strains followed by RVG1P[8] in 10% and unusual RV strains at low level in Pune clinical recruitment site.

ENV1302: Assessment of genetic diversity in group A rotaviruses in patients with acute gastroenteritis.

Investigators: Tatte VS & Gopalkrishna V

Funding Agency: Intramural

Duration: 2013-2018

Background: The diversity within group A rotaviruses remains a challenge to the efficacy of currently available group A rotavirus (GARV) vaccines. In view of the circulation of unusual rotavirus genotypes/strains and their diversity reported over the years, it is mandatory to study and to understand the genetic diversity of the predominant unusual RV strains for better vaccine coverage.

Objective: to characterize representative unusual group A rotavirus strains and cell culture isolates to determine their genogroups and genetic diversity.

a. In the study, three representative unusual Group A rotavirus strains *viz*., G9P [4],G9P[6] and G1P[6] were selected, classified them as RV G9 and RV G1 genotypes, P[4] and P[6] genotypes, I1 and I2 genotypes, A1 and A2 genotypes, N1and N2 genotypes and T1 and T2 genotypes for the VP7, VP4, VP6, NSP1, NSP2 and NSP3 genes respectively. All the study strains showed genotype constellation of G9-P [4]-I2-A2-N2-T2, G9-P[6]-I1-A1-N1-T1 and G1-P[6]-I1-A1-N1-T1. Nucleotide and amino acid identity of 74.3-100% and 59.7-100% for all the 6 genes with reference strains was noted. Nucleotide / amino acid divergence of 4.5-12.2% / 0.3-14.1% (identity of 87.8-95.5% / 85.9-99.7%) for all the 6 genes within the three strains was noted. (Fig 2a & b)

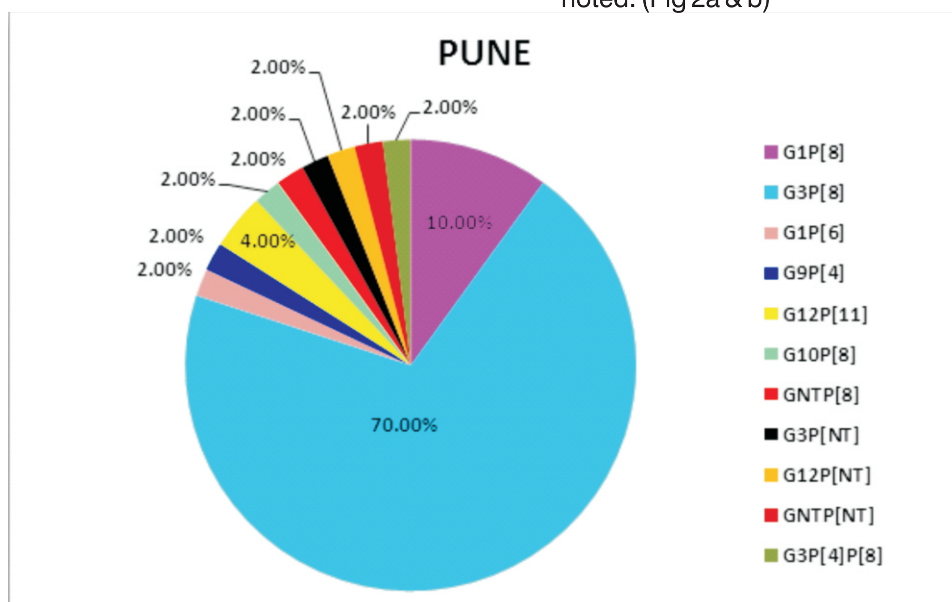

Fig. 1 : Distribution of different rotavirus strains in Pune

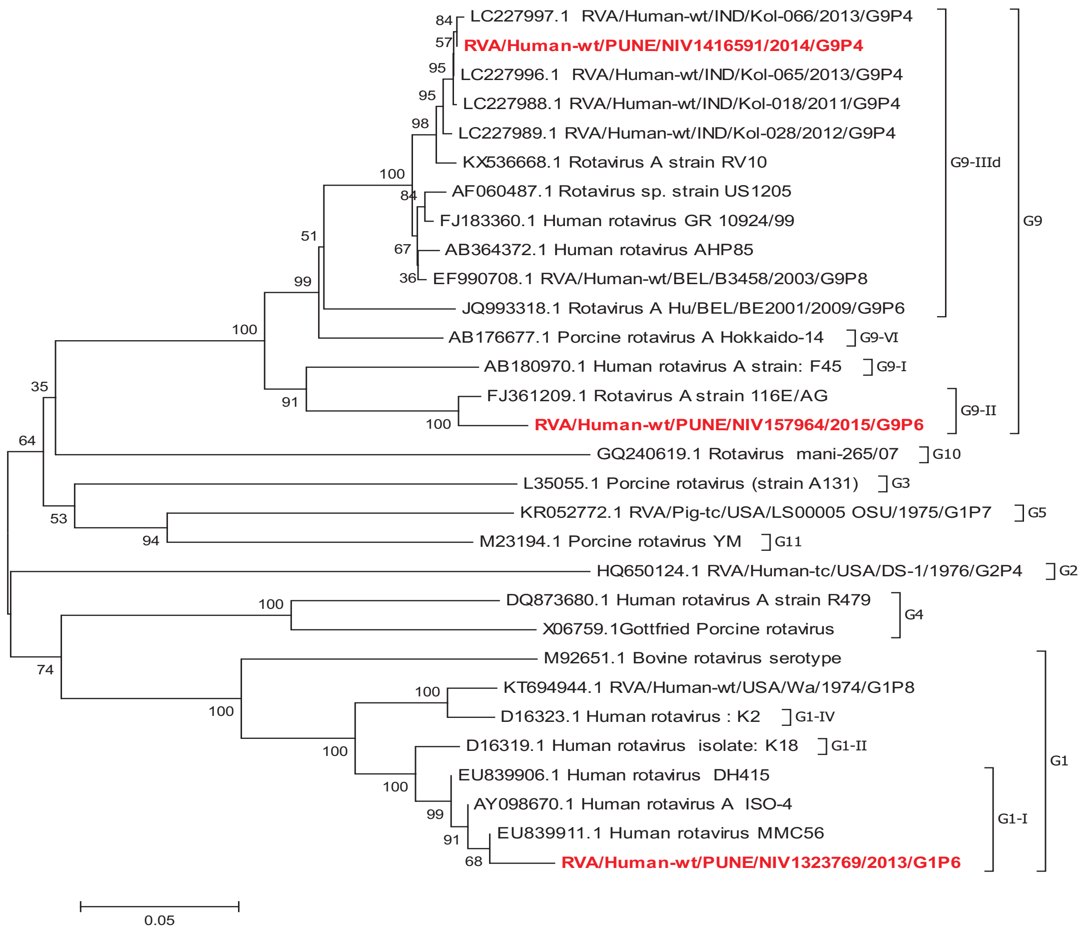

Fig 2a: Phylogenetic dendrogram based on nucleotide sequence of VP7 gene (nt 50-1026) of rotavirus study strains. Reference sequences used in the analysis were obtained from GenBank database. Scale indicates genetic distance. The study is highlighted in red color.

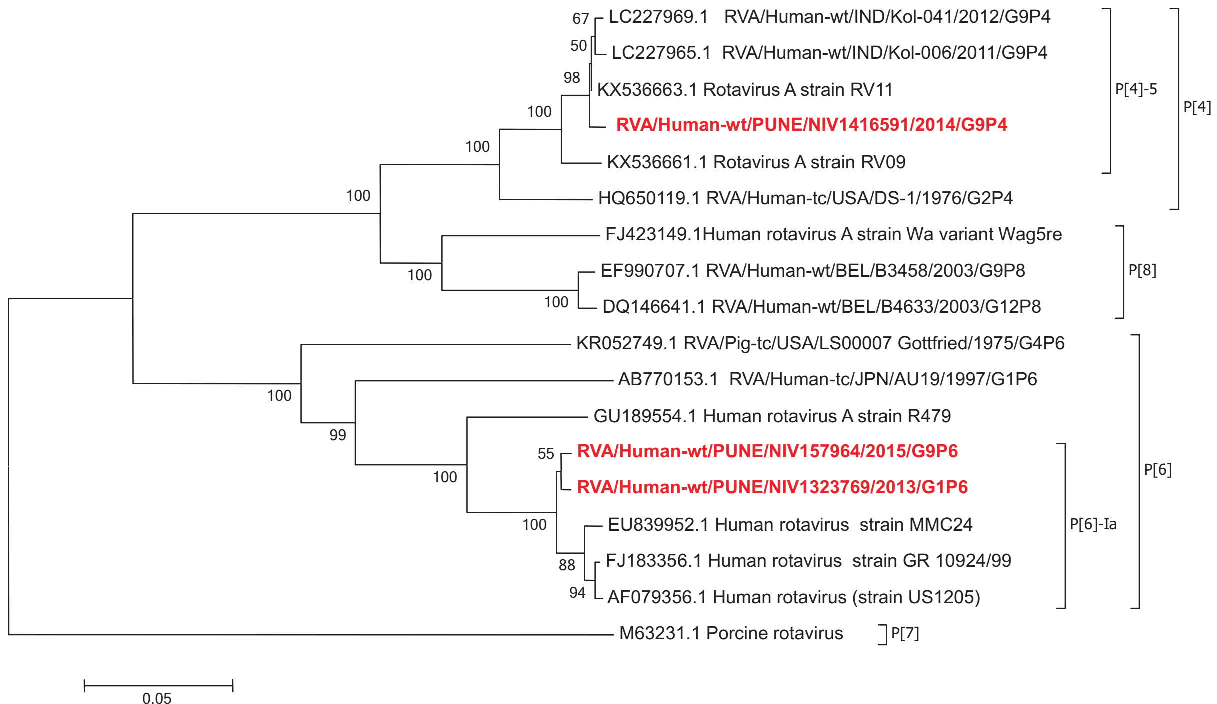

Fig 2b: Phylogenetic dendrogram based on nucleotide sequence of VP4 gene (nt 1441-2328) of rotavirus study strains. Reference sequences used in the analysis were obtained from GenBank database. Scale indicates genetic distance. The study strains are highlighted in red color.

b. Sequencing of structural and non-structural genes of tissue culture isolates of rotavirus strains:

In continuation to the earlier work reported (Annual Report 2016-2017), sequencing and phylogenetic analysis of the structural gene VP1 and nonstructural gene *i.e.* NSP2 of the three G9P[4] isolates was carried out and results showed 95-98% nucleotide identity with Japanese strain G2P[4] for the VP1 genes and 95-99% nucleotide identity with Australian strain (G1P[8]) for the NSP2 genes. Sequencing and phylogenetic analysis conducted on the nonstructural gene *i.e.* NSP1 of the RV G9P[4] strains showed 98-99% identity with Kenya strain (G2P[4]), China TB-Chen strain (G2P[4] and Japan strain (G8P[4]) for the NSP1 genes. Sequencing of the RV strains isolated in MA104 culture is in progress.

ENV1601: Identification and Molecular Characterization of Rota virus and Noro viruses in Neonates admitted at Neonatal Intensive Care Units (NICU).

Investigators: Ranshing SS & Gopalkrishna V

Funding Agency: Intramural

Duration: 2016-2019

Background: Nosocomial infections are among the important causes of morbidity and mortality in neonates, particularly those born preterm. In India, rotavirus (RV) infections and their genotype distribution is well documented in children < 5 years of age, however, very limited data is available on RVs in neonates admitted at Neonatal Intensive Care Unit (NICU).

Objective: To determine the prevalence of RV infections and characterization of strains to determine genotype distribution among neonates.

Findings: A total of 324 stool specimens were collected from 180 & 112 neonatal cases admitted at KEM Hospital NICU and SKNMC&GH respectively. All the stool specimens were tested for the presence of Group A rotavirus by commercially available ELISA Kit. Rotavirus positivity was detected in 24.4% (44/180) and 8.92% (10/112) of the neonates of KEM and SKNMC&GH neonatal units respectively. Majority (~98%) of the neonates examined were asymptomatic. All RV positive samples (n=54) were subjected to multiplex RT-PCR for VP7 (G) and VP4 (P) genotyping. Rotavirus strains non-typeable in multiplex PCR were confirmed by sequencing. Nearly 88.8% and 70.3% of the strains were typed for G and P genotypes respectively, 70.3 % were typed for both while 11.11% strains remained non-typable for both the genes. The study highlights predominance of unusual RV strain (Human–Bovine Reassortant) G12P[11] (89.47%) in neonates. Only four samples showed presence of G12[6] genotype specificity.

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

Investigators: Joshi MS & Gopalkrishna V.

Funding Agency: Intramural

Duration: 2015-2018

Background: Group C rotavirus (GCR) infections associated with sporadic or large outbreaks of acute diarrhea in all age groups have been reported worldwide. GCR, an emerging zoonotic infection with evidences of cross species transfer needs continuous monitoring for elucidation of genetic diversity and evolution studies. The scanty data on full genome sequencing is the main hurdle during such studies. The occurrence of GCR in outbreak cases of gastroenteritis was reported recently from India (Joshi et.al 2016). However, the molecular characteristics of the GCR strains circulating in the region are unknown.

Objectives:

- To detect and characterize GCR in patients with acute gastroenteritis, bovine and porcine species.
- To elucidate evolutionary relationship, time scale stasis or dynamics existing in GCR.

Findings: Full genome sequencing of structural genes of three strains isolated from three different gastroenteritis outbreaks occurred in western India during 2010-2014 was reported in Annual report 2016-17. In the current year the nonstructural genes of these strains were analyzed by nucleotide sequencing. Nearly complete nucleotide sequences of all 11 gene segments of 3 GCR strains showed percent nucleotide and amino acid identity values for all genes closer to human compared to porcine, bovine and canine strains. Phylogenetic analysis classified study strains into G4-P[2]-I2-M2-R2-

C2-A2-N2-T2-E2 and H2 genotype of VP7, VP4, VP6, VP3, VP1, VP2, NSP1, NSP2, NSP3, NSP4 and NSP5 genes respectively. This is the first report on the complete genome characterization of the GCR strains circulating in India. The genes suitable for lineage classification as well as codon sites under positive selection pressure in globally circulating human GCR strains were identified in the study. The results are pointing towards monitoring of these genes in future to ascertain their role in genetic variability. Retrospective stool specimens of pigs collected from Nagpur and Shirval (Maharashtra), are being tested for GCR using VP6 based RT-PCR. To determine the genetic evolution of GCRs, phylogenetic trees were constructed using sequences from the present study and GenBank strains and the evolutionary rates and the time of most recent common ancestors were being determined using Bayesian Markov chain Monte Carlo (MCMC) method implemented in BEAST v1.8.4.

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

Investigators: Gopalkrishna V, Joshi MS

Funding Agency: Intramural

Duration: Ongoing

Background: Acute gastroenteritis continues to be a significant cause of morbidity and mortality in humans. Among enteric viruses, rotavirus is the leading viral agent associated with severe diarrhea while non-rotavirus infections and bacterial pathogens also contribute. Association of other enteric viruses such as Calici, Astro, Adeno, Picorna, Parvo viruses have been reported in sporadic cases and outbreaks of gastroenteritis globally. The causative agents in such cases are rendered unidentified in the absence of concerted efforts in most of the episodes of gastroenteritis. In India, limited studies are reported on non-rota enteric viral pathogens associated with acute gastroenteritis. Keeping in view of the lacunae, a study was undertaken to understand the spectrum of enteric viruses and yet unknown etiological agents in sporadic cases and outbreaks.

Objective: Identification and molecular characterization of Norovirus and Adenoviruses in patients with acute gastroenteritis.

Findings: Fecal specimens (n=108) collected from children hospitalized for diarrhea in Pune (western India) during May - December 2017 were tested for Group A rotavirus by ELISA and 21.6% were found positive. A total 102 specimens were tested for Norovirus (Genogroup I and II) and Adenovirus using RT-PCR and PCR respectively followed by nucleotide sequencing. Norovirus GII was confirmed in 6% and GI in 4% of the specimens. Adenovirus was detected in 13.7% of specimens by using hexon gene specific primers. Genotyping of Adenovirus using Pentone gene specific primers showed presence of Adeno 41 (5%), Adeno 7 (6%), Adeno 2 (1%) and Adeno 12 (1%). Among these specimens, three showed mixed infections, one with GAR and norovirus GII, second with GAR and adenovirus while third with adenovirus and norovirus GII. Genotyping is in progress.

ENV1701: Detection and molecular characterization of potentially zoonotic enteric viruses in animals

Investigators: Pradeep Sawant &
Gopalkrishna V.

Funding Agency: Intramural

Duration: 2017-2020

Background: A wide range of enteric viruses cause diseases in animals. Some of these viruses are considered to have zoonotic potential, thus constituting a risk for humans by direct transmission through milk, milk products and meat. Zoonotic potential has been reported for RV, AstV, AdV, NoV and SaV, because of close genetic relationship of strains detected in humans and animals. However, no such studies have been reported from India.

Objectives:

1. To detect enteric viruses in bovine, porcine, canine and feline species.
2. Molecular characterization of detected enteric viruses to understand the genetic diversity.

Findings: In the present study, Bovine (30) and porcine (10) diarrheic faecal samples collected from private cattle and pig farms around Pune (Maharashtra) were tested for viruses. In the VP6 gene-based RT-PCR analysis, 14 bovine and 4 porcine faecal samples were found positive for Group A rotaviruses (GAR). Molecular typing of bovine GAR positive samples using VP7 and VP4 genes indicated presence of RV G10P[11] genotypes, while porcine samples showed presence of G4P[6] and G5P[6] genotypes. Further studies are in progress.

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

Investigators: Tikute SS & Gopalkrishna.V

Funding agency: Intramural

Duration: 2013-2018

Background: HFMD is a common childhood viral infection, characterized by ulcerating vesicles and lesions in mouth, hands and feet. Among the enteroviruses, Coxsackie virus A-16 (CVA-16) and Enterovirus-71 (EV-71) are the main viruses associated with outbreaks. Clinical studies reported from Asian countries indicated that CVA-16 infections do cause neurological complications leading to fatalities. Therefore, a study was initiated to understand the pathogenicity of CVA-16 strains associated with HFMD in mice.

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

Findings: Coxsackie virus A-16 (CVA16/311) infected neonatal mice showed marked changes in hind limb muscles, brain and heart muscles on day 5. Brain, heart, lung, liver, intestine, spleen, kidney and skeletal muscles of the CVA-16 infected mice were stored in 10% neutral buffered formalin as per scheduled time-points and processed for histopathology. Hind limb muscles, brain and heart muscles showed marked changes on day 5 as compared to day 3. Hind limb muscles revealed severe muscle necrosis, dissolution of muscle fibre cells, swelling and infiltration of inflammatory cells as compared to control mice. Histopathological changes of brain indicated marked dilated ventricle, hemorrhages and neuronal degeneration upon infection with CVA-16. Degeneration in cardiomyocytes and infiltration of inflammatory cells was observed in sections taken form heart as compared to controls. Further, localization of the CVA-16 specific viral antigens in organ tissue localization was performed by immunohistochemistry (IHC). Tissue sections of 3-5μm were fixed on glass slides coated using albumin / poly –L-Lysine. The assay was carried out by Novolink Polymer Detection System as per manufactures instructions. The CVA-16 specific viral antigens were detected and showed gradual increase from day 3 to day 7 showing concordance between the histopathological findings and IHC.

SRF Project: Development of immunodiagnostic assay for identification of Coxsackievirus A16 (CVA16) in patients with Hand Foot and Mouth disease.

Investigators: Ganorkar NN, Gopalkrishna V.

Funding agency: Extramural

Duration: 2016-2019

Background: In India, coxsackievirus A16 (CVA16) has been reported as the major causative agent in both sporadic infections as well as outbreaks of HFMD. Diagnosis of HFMD is being carried out based on clinical characteristics alone but identification of the etiological agent mainly relies on laboratory approach. So far, no attempts have been made to develop a rapid immunodiagnostic assay to detect viral pathogens associated with HFMD. Sporadic cases of HFMD were reported earlier from Western, Southern and Eastern regions of India. Eighty nine clinical specimens collected from 61 HFMD cases reported during 2009-10 and 158 clinical specimens of 64 cases reported in 2012-14 were included in the study.

Objectives:

1) Genomic characterization of circulating strains of coxsackievirus A-16 (CVA16) associated with HFMD in India.
2) To generate laboratory reagents for development of immunodiagnostic assay (ELISA), for identification of CVA16 in HFMD patients.

Findings: EV RNA was detected in 47.1% (42/89) of the clinical specimens from 57.4% (35/61) of the HFMD cases reported during 2009-10 and in 63.92% (101/158) of the specimens of 2012-14. A total of 51 CVA16 strains *i.e.*, thirty-seven strains of 2012–14 and 14 strains of 2009–10 were genotyped and phylogenetic analysis showed that these strains exhibited highest nucleotide (97.53–99.33%) and amino acid (99.70–100%) identity with Chinese and Japanese strains (KM235916 and AB771998) on the basis of full VP1 region. All the CVA16 study strains clustered with rarely reported B1c sub genotype and shared 96.07–100% nucleotide and 99.33–100% amino acid identity within the strains. Seven representative CVA16 strains recovered from different regions of India were subjected for amplification of P1 region (2586 bp) using specially designed primers. Sequencing and phylogenetic analysis of P1 region showed that

the present CVA16 Indian strains showed 96.60-99.70% nucleotide identity and 98.80-100% amino acid identity within them.

ENV1502: Development of multiplex PCR assay for the detection of viral Pathogens associated with diarrheal diseases

Investigators: Tatte VS & Gopalkrishna V

Funding Agency: Intramural

Duration: 2015-2018

Background: Diarrhea is a major public health problem in developing countries. Among the viral pathogens, Rotaviruses, Noro viruses, enteric adenoviruses, Astro viruses, Sapo viruses etc. are the main contributors for the disease. Development of a simple, rapid and cost effective multiplex PCR for the simultaneous detection of majority of the enteric pathogens will be useful for hospital-based surveillance studies and outbreak investigations

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

Findings: Stool samples (n=185) collected from infants and children admitted with acute gastroenteritis in local hospitals of Pune, Maharashtra were tested for different enteric viruses by multiplex PCR. All nine enteric viruses (Rota, Noro, Sapo, enteric Adeno, Astro, Entero, Parecho, Aichi, and Boca virus) were identified by this approach. A total of 42.1% of the samples analyzed by the multiplex-PCR showed high frequency of mixed-infection between 2 and 3 viruses in contrast to the conventional RT-PCR. Using this approach, Group A rotavirus (79.5%) was detected as predominant followed by enterovirus (10.0%,), Astrovirus (4.1%,), Adenovirus (2.3%,) and norovirus GII (1.2%). The specificity and sensitivity for each pathogen was examined using positive and negative controls. This is the first report on the development of Multiplex RT-PCR assay for the detection of multiple enteric viruses.

ENV1401: Development of NIV Rota virus antigen capture ELISA Kit (novel invention).

Investigators: Gopalkrishna V. Ranshing SS, Sathe PS.

Funding Agency: Intramural

Duration: 2016-2018

Background: Studies conducted worldwide on pediatric nosocomial diarrhea have identified rotavirus A (RVA) as the major etiological agent. Isolation of rotaviruses in cell culture directly from human faecal samples is time consuming and difficult, and hence, rotavirus infections are generally identified by the direct detection of antigen(s) in diarrheal samples by immunoassay methods.

Objective: To develop Simple, Rapid and cost-effective kit.

Findings: An antigen capture Rota virus ELISA has been developed indigenously for the first time. The sensitivity and specificity of the kit was found 97% and 100% respectively. The kit showed stability for 18 months when maintained at 2-8°C. It is cost effective. Technical Dossier has been submitted to ICMR forTechnology transfer.

Number of clinical samples tested: Four conjunctivital samples were tested and three were found positive for Adeno virus by PCR.

MEASLES

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Mr. Roben P. George	Technician-A (Till 19th March 2018)

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

Investigator: Vaidya SR

Objective 1: Measles virus diagnosis of referred samples: During the year, 57 serum samples (male-32; female-25) were referred for measles diagnosis from 13 hospitals and institutions of which 49 were<15yrs and the rest were >15 years. History of measles vaccination (by documentation or parent's recall) during childhood was not available. Tests revealed 34 cases (59.64%) positive for measles IgM antibody. Of the 8 adult cases (>15 yrs., 4 male & 4 female), 2 (males) had lab confirmed measles. Of the 23 measles negatives, two samples tested positive for rubella IgM antibody and 16 were negative, fourwere equivocal (single sample was not tested for rubella). Laboratory reports were submitted to the concerned authorities for case management and control measures. Weekly/monthly reports were sent to WHO.

Virus isolation: Twenty-five clinical specimens (TS-2, Urine- 23) collected from different outbreaks or sporadic cases from Maharashtra (n=24) and Gujarat (n=1) were subjected to virus isolation in Vero hSLAM cells. These clinical specimens were collected from 14-male and 11-female cases. Amongst these, 23 cases were <15 yr old and two were >15yrs old. No virus could be isolated despite RT-PCR positivity of 23 specimens for measles virus.

1.1. Outbreak based measles surveillance in the State of Maharashtra

Investigator: Vaidya SR

Funding: Extramural (WHO)

Duration: 2015-20

As part of Maharashtra government's outbreak-based measles surveillance since January 2013, measles laboratory of NIV receives 3-5 representative serum samples from each suspected outbreak for confirmation. During the year, the State health agencies investigated 38 suspected measles outbreaks in 18 districts and 191 serum samples (male-92; female-99) were referred for diagnosis of measles/ rubella. Majority of the suspected measles cases were under 15 years (180, 94.24%) and 33 had history of measles vaccination. Overall, 125 (65.44%) clinically suspected measles cases were serologically confirmed. Measles was also confirmed in 11 vaccinated cases. Rubella IgM positivity was confirmed in 23 (34.84%) of 66 measles negative and equivocal samples. Results of the outbreak-based surveillance in the Maharashtra are summarized in Table 1. Of the 11 suspected adults cases (>15 yr, 2 male & 9 female), three were laboratory confirmed measles (one male & two female). Among the 38 suspected measles outbreaks, 28 were confirmed as measles outbreaks; 4 as rubella outbreak, 5 as mixed outbreaks of measles and rubella and 1 outbreak could not be confirmed.

Table 1: Laboratory diagnosis performed on the referred serum samples

District & No. of Outbreaks	Serum samples	MeV IgM Positive	MeV IgM Negative	MeV IgM Equivocal	RuV IgM Positive	RuV IgM Negative	RuV IgM Equivocal
Akola (1)	6	6	0	0	0	0	0
Aurangabad (4)	20	15	5	0	3	2	0
Beed (2)	7	7	0	0	0	0	0
Buldhana (1)	5	4	1	0	0	1	0
Gondia (2)	8	8	0	0	0	0	0
Hingoli (3)	13	1	12	0	11	1	0
Jalgaon (1)	5	4	1	0	0	0	1
Jalna (1)	5	3	2	0	0	2	0
Latur (1)	5	4	1	0	0	1	0
Nagpur (1)	5	3	2	0	0	2	0
Nandurbar (1)	6	2	4	0	0	3	1
Nasik (6)	32	23	8	1	2	7	0
Osmanabad (2)	10	6	3	1	1	2	1
Parbhani (3)	22	15	7	0	0	7	0
Pune (3)	11	9	1	1	0	2	0
Wardha (2)	10	5	5	0	5	0	0
Washim (3)	15	8	7	0	0	7	0
Yavatmal (1)	6	2	4	0	1	3	0
Total (38)	191	125	63	3	23	40	3

1.2. Measles virus genotyping in India

Six throat swabs (3 males & 3 females) and 97 urine specimens (55 males & 42 females) were referred to NIV from Gujarat, Maharashtra and Goa for virus detection and genotyping. Of the 103 samples, 51 were positive by measles RT-PCR. Sequencing and phylogenetic analysis of 39 PCR products, revealed circulation of measles genotypes D4 (n=2) and D8 (n=37). These measles virus sequences were deposited in the WHO global measles sequence database *i.e.* MeaNS/GenBank (Figure 1 & Table 2). In addition, 234-PCR products (measles N gene/ rubella E1-E2 gene) were referred from four national measles laboratories *i.e.* NIV Unit Bangalore (n=15), SGPGIMS Lucknow (n=66), KIPM&R Chennai (n=81) and Government Medical College Guwahati (n=72) for virus sequencing and genotyping. Circulation of measles genotypes D4 (n=5) and D8 (n=207) has been evidenced. Twenty-two PCR products could not be sequenced due to poor quality. Seven PCR products received from NIV Bangalore unit and one from KIPM&R Chennai were positive for rubella and sequencing of PCR products revealed rubella genotype 2B strain. All sequences were deposited in the WHO global measles sequence database (MeaNS & RubeNS) and GenBank by the respective laboratories.

1.3. Utility of matrix-fusion region for the genetic characterization of measles viruses circulating in India

Circulation of measles genotype D4 and D8 were detected in India. Sequence analysis of a larger fragment will enhance the resolution of the genotypes. Therefore, a study was designed to determine the genotyping potential of matrix-fusion (M/F) gene for measles virus (MeV) circulating in India. Genetic characterization of circulating MeV is essential to measure the impact of immunization. Currently, MeV genotyping is based on sequencing of nucleoprotein (N) gene and further confirmation of new genotype using hemagglutinin (H) gene. This study reports the utility of M/F region for genotyping of 67 Indian MeV isolates

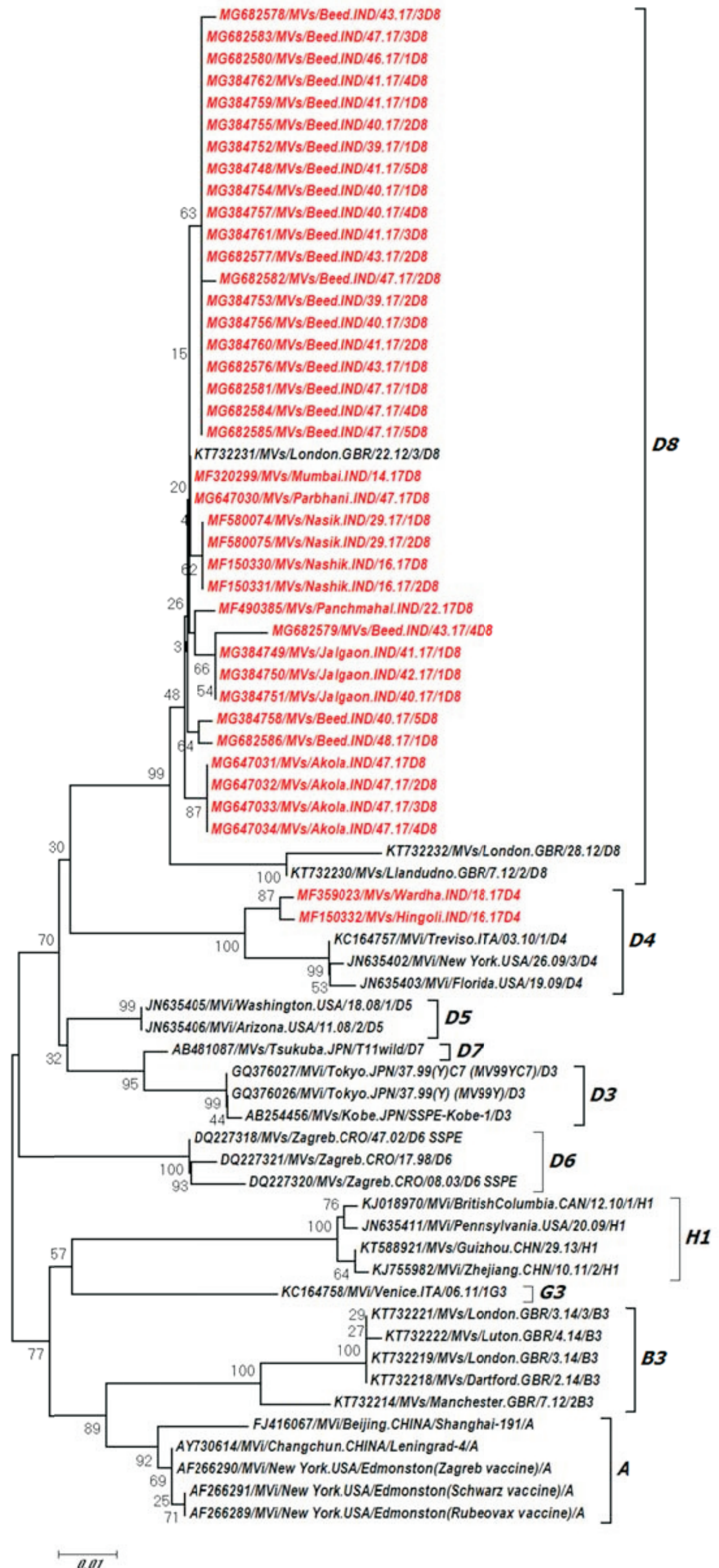

Fig. 1: Phylogenetic tree of measles viruses detected from the Maharashtra state, 2017-18.

obtained during 2012-17. These MeV isolates were obtained from Maharashtra (n=23), Gujarat (n=19), West Bengal (n=7), Madhya Pradesh (n=5), Karnataka (n=4), Kerala (n=2), Uttarakhand (n=2), Chhattisgarh (n=2), Delhi (n=1), Haryana (n=1) and Uttar Pradesh (n=1). Results revealed, 7.47% and 5.40% mean percent divergence for M/F and N gene, respectively. Notably, the most heterogeneous genotype for M/F and N are genotype D4 and genotype D8 respectively. Study concludes that M/F may be useful for MeV genotyping along with the routine N gene sequencing and genotyping.

MMR1202 & MMR1402: Genetic and antigenic characterization of measles, mumps and rubella virus isolates

Investigator: Vaidya SR

Ten mumps virus isolates obtained from Maharashtra, Karnataka, Uttar Pradesh, and Dadra & Nagar Haveli were sequenced and complete genomes were deposited in the GenBank (reported earlier). In addition, 20 measles virus isolates and 5 rubella virus isolates obtained from Gujarat, Karnataka, Kerala, Maharashtra and West Bengal were sequenced. All these complete genomes (seven MeV D4, thirteen MeV D8 & five RuV 2B) were studied at nucleotide and amino acid level (Fig 2 & 3). The genome diversity and mutations in different epitopes were studied. For the first time, the complete genomes of Indian measles (genotypes D4 & D8), mumps (genotypes C & G) and rubella (genotype 2B) viruses were sequenced. Preliminary BLAST analysis revealed 98-99% sequence identity with the recent measles virus genomes published from France, Germany, Italy and USA. Comparison with measles Edmonston vaccine strain revealed 97% and 96-97% sequence identity with D8 and D4 genomes, respectively. Similarly, preliminary BLAST analysis revealed 98-99% sequence identity with the rubella virus genomes published from China, Korea and USA. Comparison with rubella vaccine strain (Wistar RA 27/3) revealed 92% sequence identity. Additional bioinformatics analysis is in progress.

MMR1701: Measurement of virus specific IgM antibody, IgG antibody and neutralizing antibody levels in suspected Measles & Rubella cases.

Investigator: Vaidya SR

As part of the World Health Organization Southeast Asia Region's elimination program of measles and control of rubella by 2020, measles outbreak-based surveillance has been initiated. The role of laboratories is crucial to support measles elimination and rubella control goal in India. It has been recognized that the commercial EIAs may provide false positive or negative results and difficult to interpret equivocal results in the clinically suspected cases. Moreover, collection of second sample (after 3-4 weeks) is not easy in the field. Hence, a study was designed to understand the qualitative and quantitative correlation of IgM, IgG and neutralizing antibody in the suspected measles/ rubella cases. A panel of 162 serum samples referred during 2015-2016 was used in the study. These samples were collected from the suspected measles patients aged between 5 months to 35 yrs. Majority of the suspected cases (88.8%) were under 15 yrs. The history of measles vaccination was available for 65 individuals. A subset of serum samples (n=156) were tested in rubella FRNT. Overall positivity by rubella FRNT, rubella IgG EIA and rubella IgM EIA were 76.2%, 60.8% and 26.2%, respectively. Altogether, 19.2% samples (n=30) were found positive in the three assays. Comparison of measles specific IgM, IgG and neutralizing antibodies in the suspected measles cases is in progress.

5. Other Activities

5.1. Support provided for ELISA kit development

Technology dossier "anti-measles IgM ELISA" has been transferred to the DG ICMR on 24th May 2017. In connection with this technology, laboratory support was provided for the development and validation of anti-measles IgM ELISA kit in collaboration with Virus Diagnostic Laboratory (VDL), NIV Pune. Now this technology is available on the ICMR portal for industrial transfer.

5.2. WHO IQA & EQA program

1) As a part of the WHO's internal quality assurance, a panel of serological samples (n=50) was sent to KIPM&R Chennai for reconfirmation of measles/ rubella IgM. Results indicated 100% concordance for measles (50/50) and rubella (35/35) IgM antibody detection.

2) As a part of the WHO's external quality assurance, a panel of serum samples (n=20) was received from the Victorian Infectious Diseases Reference Laboratory (VIDRL), Australia. These samples were tested using the WHO approved standard kit for measles/ rubella IgM antibody detection. Results indicated 100% for measles/ rubella testing.

Table 2: Measles virus genotypes detected during 2017-18

State referred	Cases processed	Specimens genotype	Measles	GenBank ID
Maharashtra	100	100 (50POS[#]) 2D4	36D8,	MG647031, MG647032, MG647033, MG647034, MG384752, MG384753, MG384754, MG384755, MG384756, MG384757, MG384758, MG384759, MG384760, MG384761, MG384762, MG384748, MG682576, MG682577, MG682578, MG682579, MG682580, MG682581, MG682582, MG682583, MG682584, MG682585, MG682586, MF320299, MF150332, MG384749, MG384750, MG384751, MF150330, MF150331, MF580074, MF580075, MG647030, MF359023
Gujarat	2	2 (1POS)	1 D8	MF490385
Goa	1	1 (1NEG)	—-	—-
Total	**103**	**103**	**D4=2 D8=37**	

[#]The quality of PCR products (n=12) was weak and not sufficient for the sequencing reaction.

Table 3: Number of samples tested in the reporting year 2017-18

Sr No	Test	Number of samples tested
1	Measles IgM ELISA	248
3	Mumps IgM ELISA	23
4	Mumps IgG ELISA	2
5	Rubella IgM ELISA	88
7	VZV IgM ELISA	23
8	VZV IgG ELISA	1
9	Measles RT-PCR	344*[#$]
11	Rubella RT-PCR	152
12	Measles PCR product sequencing	234
13	Rubella PCR product sequencing	13
14	VZV DNA PCR	9

*103 throat swabs/ urine specimens from suspected measles cases were directly tested by RT-PCR.

[#]Altogether, 125 throat swabs/ urine were tested for Polio GAP-III program.

[$]116 tissue culture fluids obtained by passaging specimens (suspected measles) in Vero hSLAM cells.

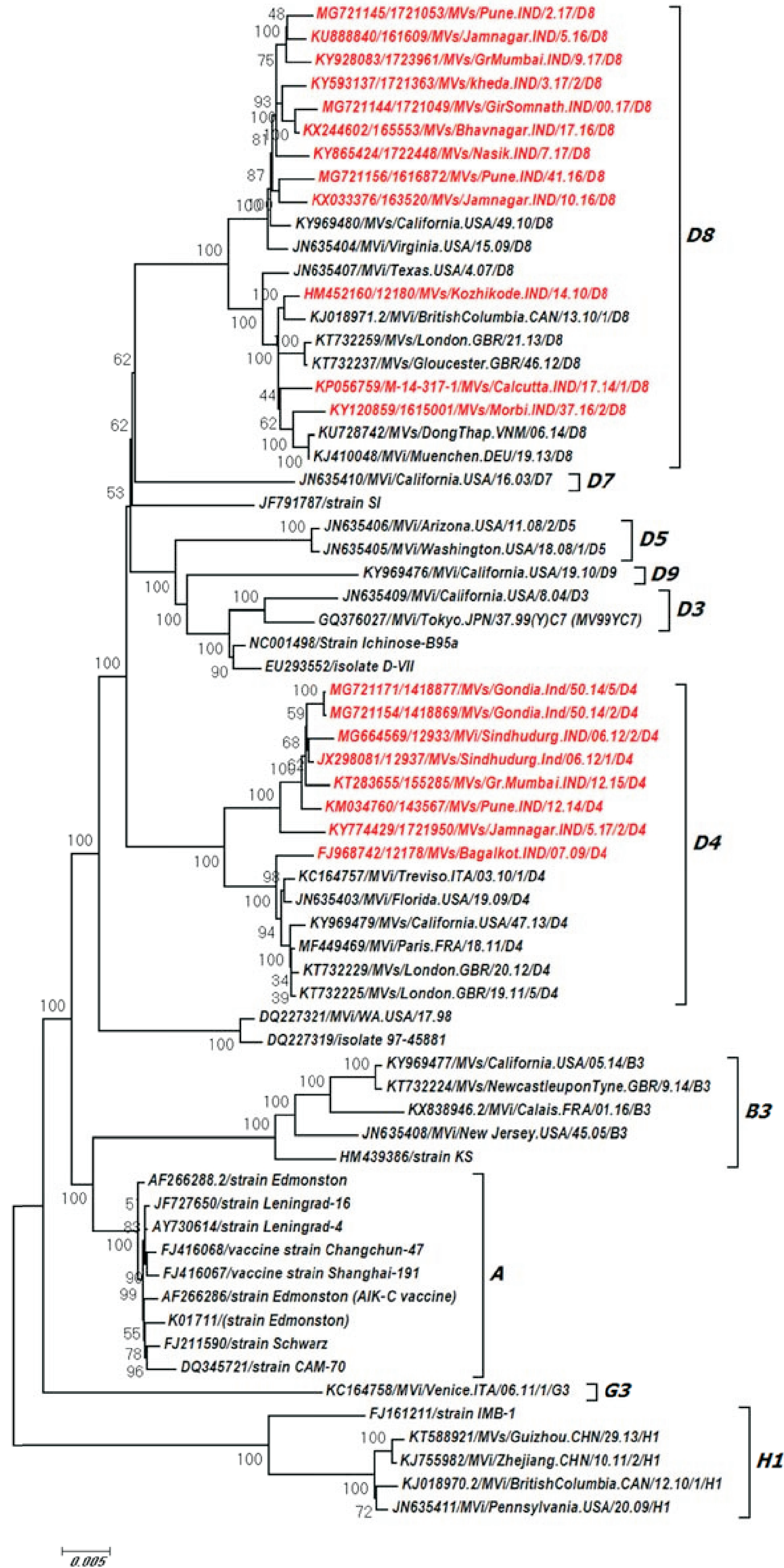

Fig. 2: Phylogenetic tree of measles virus full genomes (n=20) detected from India.

RVi Tokyo.JPN 0.90 1D
RVi Saitama.JPN 0.94 1D
1D
RVi Shandong.CHN 0.02 1E
RVi KualaLumpur.MYS 0.01 1E
1E
RVi Kagoshima.JPN 22.04 1J
RVi Miyazaki.JPN 10.01 1J
1J
RVi Shandong.CHN 0.00 1F
RVi Anhui.CHN 0.00 1F
1F
RVi Pennsylvania.USA 0.64 VAC 1a
RVi Toyama.JPN 0.67 1a
1A
RVi Tiberius.ISR 0.88 1B
RVi Jerusalem.ISR 0.75 1B
RVi BeneBerak.ISR 0.79 1B
1B
RVi Milan.ITA 46.92 1I
RVi London.GBR 0.86 1I
1I
RVi Minsk.BLR 28.05/2 1H
RVi Ryazan.RUS 09.08 1H
1H
RVi Kampala.UGA 20.01 1G
RVi Ontario.CAN 27.05 1G
RVi Minsk.BLR 29.04 1G
1G
RVi PanamaCity.PAN 0.99 1C
RVi SanSalvador.SLV 0.02 1C
RVi California.USA 0.91 1C
1C
RVi NewJersey.USA 0.61 VAC 1a
RVi Brussels.BEL 0.63 VAC 1a
1A
RVi Moscow.RUS 0.67 2C
RVi Moscow.RUS 0.97 2C
2C
RVi Beijing.CHN 0.80 VAC 2A
RVi Beijing.CHN 0.79 2A
2A
RVi TelAviv.ISR 0.68 2B
RVi Anhui.CHN 0.00/2 2B
924773 RuVi/Pune.IND/12.92
12195 RVi/Kannur.IND/08.09/2B
RVi Washington.USA 16.00 2B
12162 RVi/Chitradurga.IND/11.07
12163 RVi/Kolar.IND/15.07
12164 RVi/Bangalore-Urban.IND/14.08
2B

Fig. 3: Phylogenetic tree of rubella virus full genomes (n=5) detected from India.

BACTERIOLOGY

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BAC1601: Diagnostic Services

Rajlakshmi Viswanathan, Anuj Kumar, Savita Dhurandhare

Funding: Intramural

Duration: Ongoing

Background: Laboratory renovation is completed and microbiological diagnostic services functionalized for conventional and molecular tests.

Work done & findings: Standard strains have been procured from authentic sources. Species specific PCR for detection of three diarrheagenic bacteria, *viz*., *Escherichia coli, Shigella* spp. and *Vibrio cholerae* have been standardized apart from broad range PCR using 16S rRNA detection.

Diagnostic support was provided for detection of bacterial pathogens, in samples referred from an outbreak of acute diarrheal disease in Aurangabad. *Vibrio cholerae* O1 Ogawa was confirmed in 6 of 46 fecal samples.

BAC1801: Evaluation of a panel of biomarkers as a tool to differentiate acute viral and bacterial respiratory infections: pilot study

Anuj Kumar, Rajlakshmi Viswanathan, Manohar Lal Chaudhary, Sumit Dutt Bhardwaj

Funding: Proposed Extramural

Duration: 2 years

Background: Acute respiratory infections (ARI) are estimated to have caused 6.5 million deaths in India and 38 million deaths worldwide in 2015. ARI has a diverse etiology of viral and bacterial pathogens, which are difficult to differentiate clinically. Aetiological diagnosis of ARI can be a lengthy process susceptible to errors and delays, leading to inappropriate use of antibiotics. A panel of three serum biomarkers will be evaluated - Procalcitonin (PCT), Myxo virus antigen protein (MxA) and Lactate dehydrogenase (LDH), which can enhance the sensitivity and specificity of diagnosis. Determination of levels of biomarkers in case of bacterial/viral infection, co-infection and microbiologically unconfirmed respiratory illness in comparison to healthy controls will provide information to differentiate these conditions.

Objectives:

1. Estimation of selected biomarkers - Procalcitonin (PCT), Myxovirus resistance protein A (MxA) and Lactate dehydrogenase (LDH) levels in adult patients with bacterial respiratory infection, viral respiratory infection and co-infection infection.
2. Evaluation of diagnostic performance of these biomarkers for differentiation of acute bacterial and /or viral respiratory infections.
3. Development of scoring system using the studied markers to differentiate acute viral and bacterial respiratory infections in adults

Work done: Project has been approved by institutional project review committee, scientific advisory committee and institutional biosafety committee. Patient information sheet, patient informed consent form in English and local language and case report form are prepared. Panel of proposed biomarkers identified and standardization of laboratory tests is ongoing. Project is presently under consideration of institutional human ethics committee.

BACT1803: Missed diagnosis of Pertussis in young infants and role of maternally derived immunity

Investigators: Rajlakshmi Viswanathan, Anuj Kumar in collaboration with clinical partners

Funding: Intramural

Duration: 3 years

Background: Pertussis (whooping cough) caused by *Bordetella pertussis* is one of the most poorly controlled vaccine-preventable diseases in the world. Pertussis remains a major cause of childhood morbidity and mortality, with WHO estimating worldwide occurrence of 1,39,535 cases in 2016 of which, India alone have contributed 37,274 cases. Non-immunized / partially immunized children depend on maternal antibodies for protection and are at greater risk of developing pertussis. Clinical diagnosis of pertussis in infants is challenging due to nonspecific presentation and absence of classical symptoms. Almost no systematic information on pertussis is available from India including seroepidemiology among pregnant women or laboratory confirmed cases in infants.

Objectives:

1. To estimate seroprevalence to pertussis and to determine the antibody concentrations in cord and maternal blood in both preterm and term infant–mother pairs and to evaluate the efficacy of transplacental antibody transfer.
2. To study missed diagnosis in young infants (<6 months) for pertussis presenting with clinical features of respiratory infection.

Work done: Following approval by project review committee and ratification by scientific advisory committee, approval of institutional bio-safety committee and human ethics committee has been obtained. Case report forms have been developed (EpiInfo). Participant information sheets and consent forms have also been developed and validated (English and vernacular language). Linkages have been established with four study sites for collaboration. Positive control is obtained and standardization of laboratory tests has been initiated.

DIAGNOSTIC VIROLOGY

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DVG 1301: Resource Centre for Virus Diagnostic Laboratories (RCVDL) at NIV, Pune

Investigators: GN Sapkal, Ullas PT, GN Deshpande, R Shejwalkar, V Vidwans, VA Potdar

Funding Agency: ICMR

Duration: 03 Years

Background: RCVRDL was constituted in response to the recommendation of the Virology Task Force monitoring the VRDL network, with an objective to provide training for the categories of staff in the VRDL network, conducting quality assurance (QA) and quality control (QC) programs as well as extending scientific and technical expertise for establishing new and existing VRDLs in the country.

Work done: During 2017-18, the RCVRDL conducted 06 trainings and trained 97 scientific and technical staff from different VRDLs. During the consolidated VRDL training program, 4 PIs, 2 Co-PIs, 11 Scientists, 3 Research Assistants and 18 other staff were trained on different aspects of bio-safety, outbreak investigation, biomedical waste management, ELISA, PCR and Real Time PCR-based viral diagnostic methods.

i) Response to National emergency

Need-based training programs, *viz.,* hands on training for Zika virus diagnosis, on-site hands-on training program for detection of Zika virus by real-time RT-PCR and hands-on training program on real-time RT-PCR-based allelic discrimination for Influenza virus was conducted during the year and 59 participants from 21 centers were trained **(Table 1)**. The training modules included lectures, case studies, demonstrations and hands-on practicals. Pre and post assessment tests were conducted. Feedback was invited from participants and taken into account while preparing subsequent training modules.

ii) Quality control program for VRDLs

a) The Quality control program for the VRDL network is ongoing. A total of 2649 samples were tested (Dengue IgM-1079, Dengue NS1-492, CHIK IgM-394, JE IgM-155, Measles IgM-73, and HAV IgM-144, HBsAg-57, HCV IgG-85, HEV IgM-118 and other viruses-52 (CMV IgM/IgG, Rubella IgG, Parvovirus B19). The overall concordance rate of VRDL results with the RCVRDL test results was 93%.

b) **NIV-External Quality Assurance Program for virus diagnosis by ELISA**

An external Quality Assurance program was designed to retrospectively monitor the laboratory performance using 'blind'/coded samples. Serological tests remain the mainstay for diagnosis of most viruses of public health importance at the VRDLs. Quality in testing and reporting assumes critical importance in ensuring the reliability of the results and maintaining consistency across the laboratory network. The RCVRDL has initiated the EQA program with the following objectives:

a. To maintain and improve analytical quality of the VRDLs

b. To evaluate inter-laboratory comparability of test results

c. To identify the causes of potential discordance and to implement corrective action

In the first phase of the EQA program, sample panels were provided to 21 VRDLs for dengue IgM, chikungunya IgM and JE IgM ELISA. The decoding and analysis of the test results from the VRDLs is in progress.

iii) Influenza reagent supply:

From March 2017 till date, test reagents for 1500 reactions (for 07 targets and positive controls) were provided to 07 VRDLs. In addition, positive control for HSV tests was provided to the VRDL at SVIMS, Tirupati.

DVG1606: Surveillance for Zika virus infection in India

Investigators: DT Mourya, GN Sapkal, PD Yadav, Ullas PT, GN Deshpande, RS Gunjikar, Heena Shaman

Background: As a continued effort for enhancing the laboratory capacity for Zika virus (ZIKV) surveillance in India, on-site hands on training programs were conducted for VRDLs of Gujarat, Rajasthan and Punjab **(Table 1)**. The topics covered included the global scenario of ZIKV infections and the preparedness of India for ZIKV diagnosis, basic concepts in ZIKV diagnosis, specimen management, packing and transport, biosafety, ZIKV real time RT-PCR, CDC Trioplex real time RT-PCR for simultaneous detection of dengue, chikungunya and Zika viruses, troubleshooting and quality control.

Table-1: List of trainings conducted during April 2017 to March 2018

Sl. No.	Name of Training	Date	Number of participants attended the training
1	Hands on training for ZIKV detection by real time PCR	June 7-9, 2017	6
2	Training program for VRDLs 2017/03	August 3-11, 2017	16
3	Training program for VRDLs 2018/01	February 5-12, 2018	22
4	Hands-on Training Program on Real-time RTPCR-based Allelic Discrimination for Influenza virus	February 14-15, 2018	9
5	On-site Hands-on Training Program on Detection of Zika Virus by Real-time RTPCR (for laboratories in Gujarat)	February 14-16, 2018	27
6	On-site Hands-on Training Program on Detection of Zika Virus by Real-time RTPCR (for laboratories in Rajasthan)	March 26-27, 2018	17
	Total number of participants trained		**97**

Work done: The RCVRDL and the VRDL network have screened 46235 serum samples, 18491 urine samples and 64 other samples. The test samples included those from cases of acute febrile illnesses, ante-natal women, babies with microcephaly and retrospective specimens (fever samples negative for dengue and chikungunya). All the samples tested negative for ZIKV and the results were communicated to respective VRDLs and ICMR.

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

Investigators: DT Mourya, GN Sapkal, PD Yadav, GN Deshpande

Funding Agency: Intramural

Duration: 03 years

Work done and findings: Generation of monoclonal antibodies (MAbs) reactive against Kyasanur Forest disease virus (KFDV) was reported earlier. During the reporting year, MAbs of IgG isotypes were scaled up, purified, biotin labeled and optimized for their reactivity. Work on assay sensitivity and specificity is ongoing.

DVG1602: Development of sero-diagnostic assays for detection of anti-Zika virus antibodies

Investigators: GN Sapkal, PD Yadav, GN Deshpande

Funding: Intramural

Duration: 03 years

ZIKV strain was procured from WHO and monoclonal antibodies (MAbs) reactive against the virus were generated. MAbs of IgG isotypes were characterized and purified. Biotinylation of Zika specific MAbs and optimization of Enzyme Linked Immunosorbent Assay (ELISA) are in progress.

Simultaneously, three commercially available Zika IgM kits were procured and evaluated with samples from patients with acute febrile Illness, antenatal cases and retrospective serum samples tested negative for dengue and chikungunya viruses. The following studies are in progress:

- Comparison of the performance of these kits in detection of Zika-specific IgM antibodies.
- Preparation of a Serum Panel for the evaluation of a Zika IgM ELISA being developed by NIV
- Standardization of PRNT for Zika virus

DVG 1604: Congenital Rubella Syndrome (CRS) Surveillance in India

Investigators: GN Sapkal, R Viswanathan, GN Deshpande, S George, V Jagtap, O Kaduskar

Funding Agency: ICMR

Duration: 05 years

Background: In India, reliable estimates of CRS burden are not available. In view of the proposed introduction of rubella vaccine in the country and immediate need to set up CRS surveillance, the Ministry of Health and Family Welfare has requested the Indian Council of Medical Research (ICMR) to establish surveillance for CRS, which will provide a baseline estimate of disease burden and monitor the impact and progress made by rubella vaccination over a period of time. This multicentric project has been initiated in six centers across India and is coordinated by National Institute of Epidemiology, Chennai. RCVRDL, NIV, Pune is the laboratory partner for laboratory SOP development, training, trouble shooting and QA/QC. RCVRDL will also perform PCR of throat swabs from all the centers as well as genotyping of positive samples.

Work done & findings: During the reporting period, NIV has received 285 throat swab samples, screened 225 samples by Rubella diagnostic PCR of which 33 tested positive. Tests for the remaining samples are in progress. Genotyping of 12 samples obtained from different geographical areas revealed the circulation of 2B genotype. An ELISA proficiency panel was sent to all the participating laboratories in the CRS surveillance. Based on the result of the proficiency test, the performance of the laboratory was assessed by a scoring system. All the centers performed well in the proficiency test.

Translational Research Activities

During the current year, NIV has transferred the following technologies to M/s. Zydus Cadila, Ahmadabad, Gujarat.

1) ELISA kit for detection of IgM antibody against HEV
2) CCHFV bovine, sheep & goat IgG antibody detection ELISA
3) CCHFV Human IgG antibody detection ELISA
4) KFD Human IgM antibody detection ELISA
5) Chandipura virus IgM ELISA
6) ELISA for JE virus detection in mosquito [For epidemic prediction/surveillance]

Japanese encephalitis virus Vaccine technology

The technology for production of tissue culture-derived vaccine against Japanese encephalitis virus (JEV) has been transferred to HBL [HLL], a Govt. of India undertaking.

TRG 1601: Evaluation of certain compounds for inactivation of viruses of public health importance

Investigators: DT Mourya, GN Sapkal, GN Deshpande, R Gunjikar, BN Tilekar, S Vidhate, K Khutwad, SA Gaikwad

Clinical samples associated with high risk necessitates: a) handling under strict safety controls b) processing in containment laboratories c) safe means of sample transport. To address these issues and fulfill the requirements in resource limited settings, NIV Pune, has developed a formulation for virus inactivation. The technology has been standardized and evaluated at NIV, Pune and three external laboratories *viz*., SMS Medical College, Jaipur, Rajasthan, Sri Venkateswara Institute of Medical Sciences and allied hospitals, Tirupati, Andra Pradesh and King George's Medical University Lucknow, Uttar Pradesh. The field evaluation report has been submitted to ICMR, New Delhi.

New Project Initiatives

DVG1801: Impact of measles/rubella (MR) vaccination campaign on population immunity in India [IMRVI study]

Investigators: GN Sapkal, Ullas PT, GN Deshpande, BV Tandale

Funding Agency: intramural

Duration: 02 years

Background: Community-based, cross-sectional serosurveys in different age groups to estimate the population immunity to measles and rubella viruses in districts with MRHRUs and other priority areas has been proposed and will be compared with a facility-based serosurvey from participants in a similar population (e.g., children at the outpatient department).

Objectives:

1. To estimate age-specific population immunity to measles and rubella viruses within a specified precision of ±10% within three age strata (children 9 months to 4 years and 5 to 14 years of age and women 15 to 49 years of age) in India using serological surveys.
2. Compare the accuracy, precision and cost of estimating age-specific measles and rubella population immunity using convenience samples from health care facilities versus community-based serosurveys.

Work done: Initiated training of staff at study sites in Punjab and Assam. Diagnostic Virology Group, NIV being the apex laboratory for testing of all study samples, devised a kit for collection of serum samples and shipped to MRHUs of both the states.

ELECTRON MICROSCOPY & HISTOPATHOLOGY

Scientific Staff

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Mr VS Gadekar	Multi Tasking Staff
Mrs SS Mohol	Multi Tasking Staff

Project Staff

Dr Preksha Jain	Post doc

Project 1: Studies on the effect of dengue virus (DENV) NS1 protein on vascular endothelial cell biology

Investigator: Dr. Atanu Basu

Funding: Extramural (DBT)

Duration: 2017-18 (Completed)

Previous studies had shown that exposure of DENV NS1 protein can alter physiology of cultured endothelial cells in conventional and 3D systems. Further analysis of the gene expression profile of these cells using limited arrays showed evidence for both universal and serotype specific regulation. Important among these being genes for ACE and TGFâ.

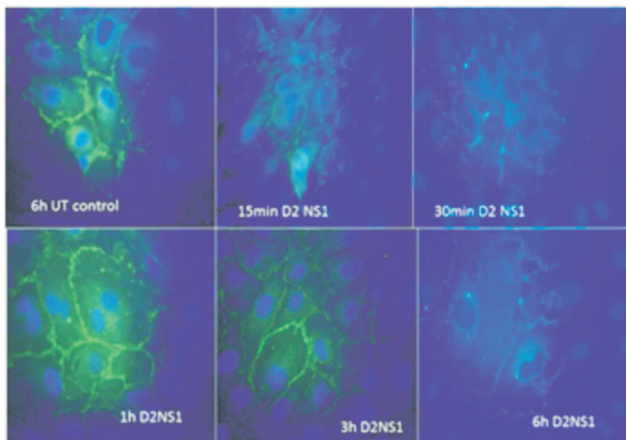
Dengue virus NS1 protein can affect VE-cadherin and catenin expression in-vitro

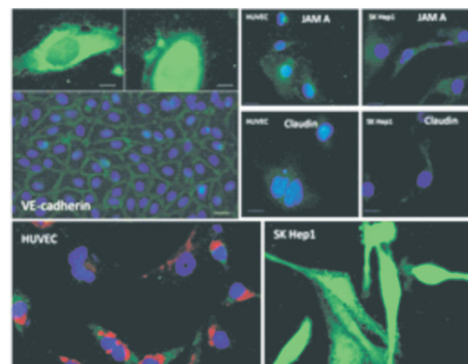
VE cadherin is an essential adhesion molecule that is important for physiological regulation of endothelial cell junctions thereby affecting permeability properties and rheological dynamics of capillary beds. Pathophysiological processes affecting VE cadherin expression have been shown to be key features of multi-system organ failure during sepsis. In severe dengue disease the role of VE cadherin remains incompletely understood. Here we investigated the role of exogenous DENV NS1 on VE cadherin expression in HUVECs.

Findings:

We profiled the normal expression of all physiologically important adhesion molecules in both SK Hep1 and HUVEC cells and found that VE cadherin expression was most prominent. In HUVECs the VE cadherin was typically a marginally localized protein outline cell contacts but in SK Hep1 it was associated with mitochondrial clusters with a relatively higher expression level as imaged by fluorescence microscopy compared with HUVEC. Both infection with DENV 2 virus and exposure to exogenous NS1 protein (5ìg) showed an effect on VE cadherin expression when examined over a timeline data. In early post-exposure time (15 min- 1 hr.) there was a transient increase followed by a decrease.

These findings are very important in the context of vascular physiology. The capillary dysfunction that is seen as dramatic increase in vascular





Exogenous DENV 2 Ns1 affects HUVEC junctional integrity

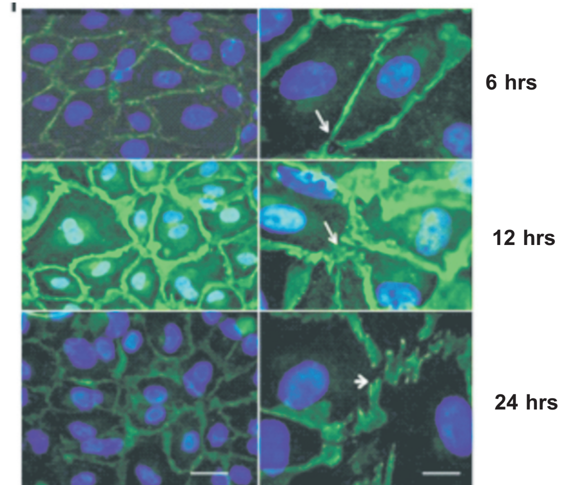


Fig 1: The expression of adherence molecules in SK Hep1 and HUVEC. VE is most prominent in both.

permeability during severe dengue disease is fully reversible. The transient increase in VE cadherin and beta catenin that is associated with cellular signaling could constitute early triggers for this event. A more detailed dissection of the pathway is in advanced stage of completion using a proteome approach.

Core facility and academic activities

High resolution electron microscopy applications like virus morphodiagnosis, thin section analysis, electron tomography volume imaging and cryo-sectioning were provided to both in-house researchers and to other National facilities. Application support to industry was also provided. Successful completion of EQA on virus morphodiagnosis was done.

Teaching and training of postgraduate students on application aspects of electron microscopy was carried out as part of human resource development program. This included assistance to Indian Institute of Technology (Mandi) on operational aspects of the TEM cluster.

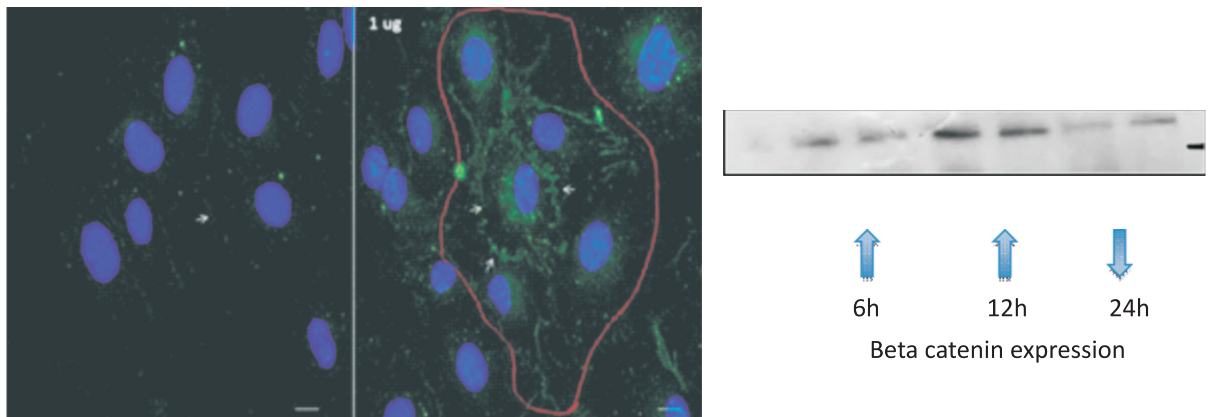


Fig 2: Expression of VE cadherin in HUVEC in short time post exposure with DENV 2 NS1 protein. In Panel A the short time intervals are shown and Panel B shows a longer expression timeline. (Bar =1 micron). Arrows show inter-endothelial junctions. Panel C shows the changes.

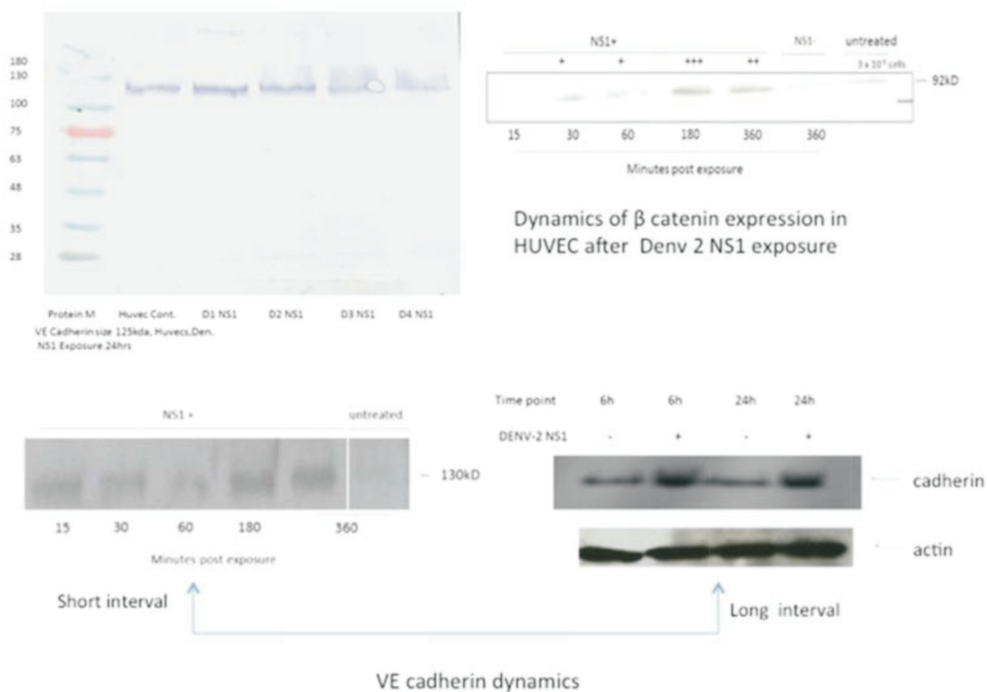


Fig 3: Changes in VE-cadherin expression in endothelial cells exposed to NS1 and infected with dengue virus.

BIOINFORMATICS AND DATA MANAGEMENT

Scientific Staff

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Mr. Avinash Patil	Technician - B

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Mr. Abhisek Behera	ICMR SRF
Ms. Dipali Bhoye	UGC's Rajiv Gandhi Senior Research Fellow

Project Staff

Mrs. Meghan Agarwal	ICMR extramural project

BDM1001: Core facility services

(Sarah Cherian, Pratip Shil, A.M. Walimbe, Mr. Vijayasimha, Mr. 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, mathematical modeling etc. were provided. Management and maintenance of computers, servers, laptops, printers, computer peripherals, network and internet services were done on regular basis. Regular maintenance of connectivity between NIV, MCC and three field units is being done for data transfer, AIMS, LIMS Software and intercom services. NIV website is being 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 indigenously developed softwares like payroll, pension, supplementary bills, arrears calculator, computer complaint register etc. are being maintained.

Mathematical biology principles (with computational techniques) applied to analyze viral disease epidemiology, vector propagation, etc. are described below:

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: Sarah Cherian & Mr. Abhisek Behera

Funding: ICMR (Grant #2014-20880)

Duration: 2014-17

Background**:** This database aims at providing information on co-evolutionary mutations obtained from the implementation of tools based on a statistical information theory approach and mutual information (MI). Best MI scores, good entropy values and frequency/proportion (in percentage) of the residues at the predicted co-evolutionary sites are evaluated for short-listing the co-evolutions.

Objective: To determine inter- and intra-segment MIs from the alignment of the HA and NA sequences of 2009 pandemic H1N1 (pH1N1) viruses using the MISTIC server.

Findings: Within HA of the pH1N1 viruses, the predicted co-evolved mutations associated with the antigenic regions were 179-180 (Sa), 202 (Sb)-391, 202-204 (Sb), 180 (Sa)-391, 180-202 (Sa-Sb), 202 (Sb)-214 and 204(Sb)-207 (Fig. 1). The residue, 391 lies within a conserved epitope in the HA2 domain. Within NA, the major antigenic sites (reported escape mutants) such as Asp199Asn, Asn200Ser, Lys221Asn, Asn248Asp, Lys249Glu, Asn336Gly, Asp341Asn, Asn365Iso, Arg366Ser and Lys369Asn/Ala showed evidence of co-evolutions. Co-evolution analysis of the HA-NA segments revealed that amino acid position 200 in the antigenic site of NA was predicted to co-evolve with antigenic sites 202 (Sb), 180 (Sa) of HA.

Phylogenetic analysis of pH1N1 viruses show that the emergence of the new sub-clade 6B, involved mutations K163Q (~pos.180-Sa) and S185T (~pos. 202-Sb) while the emergence of sub-clade 6B1 involved an antigenic drift due to S162N (~pos. 179) mutation in the Sa antigenic site. Thus, the co-evolution of the pair 179-180, both in the 'Sa' antigenic site relates to the fitness

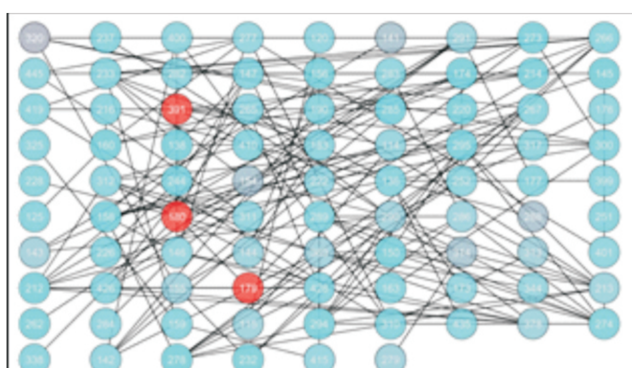

Fig. 1: Top 50 highly co-evolved sites in HA gene of pH1N1 viruses (2009-2017) shown using the grid layout mode of MISTIC

advantage of the newer strains, which also reflected in the change in the vaccine strain from A/California/7/2009 to A/Michigan/45/2015.

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

Investigators: Sarah Cherian & Ms. D. Bhoye

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

Duration: 2014-2018

Background: The 2009 pandemic influenza H1N1 (pH1N1) virus was known for rapid global spread and successive outbreak waves with varying intensities at a global level. The heterogeneity in the epidemiological patterns of the pH1N1 worldwide is well evident. Reconstruction of viral phylogeny that reflects the rates of transmission, time scales of diversification and effective population sizes, can provide information related to host susceptibility in a population

Objective: We investigated rates of evolution and genetic diversity of the viral population based on polymerase genes of pH1N1 viruses (2009-2016) and compared with that of HA gene.

Findings: The mean substitution rate of HA gene was relatively higher than the mean rate observed for PB2 gene. The Bayesian skyline plot revealed three peaks around Oct. 2010, Oct. 2011 and Aug. 2012 (Fig. 2) most likely corresponding to emergence of co-evolved mutations at positions 344 and 354 during 2010/2011 and establishment during 2012-13 in different regions globally. The Bayesian skyline plot based on HA gene sequences showed 2 major peaks corresponding to emergence and initial spread of the pandemic (~Dec. 2009) and corresponding to change in vaccine strain (~Dec. 2016), with smaller secondary peaks around Oct. 2010, March 2013 and March 2015 corresponding to spurts in influenza activity.

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

Investigators: *Sarah Cherian, Mr. A.M. Walimbe & S. Vaidya*

Funding: *Intramural*

Duration: 2015-18

Background: Measles virus (MeV) 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 transmission pathways of MeV and understand the dispersal trends.

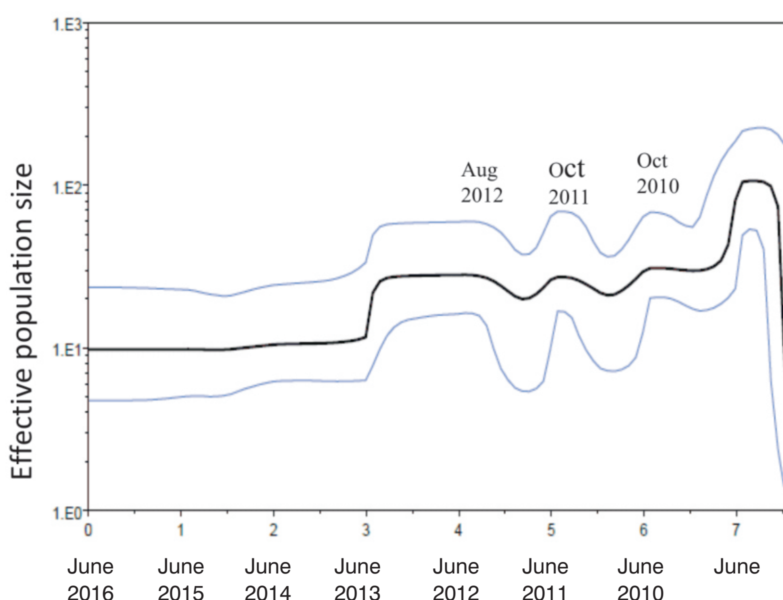

Fig.2: Bayesian skyline plot for PB2 gene sequences of pandemic H1N1 viruses (2009-2016)

Objective: To understand the spatiotemporal global transmission dynamics of clade D genotypes circulating in India.

Findings: We used nucleoprotein gene sequences (n=756) from GenBank, representing 86 countries (1973-2016), to study the spatiotemporal transmission dynamics of clade D (Fig. 3). Genotype D4 was introduced into India around 1991 and genotype D8 around 1994. Recent transmissions of the D4 genotype of MeV were noted from India to Australia, North America and Western Europe. In genotype D8, subgroups associated with past introductions showed higher frequencies of transmission from India as compared to those associated with the more recent introductions. D8 genotype importations to India from North America were noted in recent years. The bayesian skyline plot reflecting the genetic diversity of clade D of MeV revealed a growth phase from around 1985 to around 2000, followed by a marginal drop upto the year 2010. This trend is reflective of the vaccination campaigns in several countries since the last two decades resulting in MeV elimination in several WHO regions. Beyond 2010, a nearly stable phase was noted, which can be explained as a consequence of the recent transmissions from both measles endemic countries and from the countries that have eliminated or are nearing elimination. Sustained MeV surveillance and active immunization policies are thus essential to achieve the measles elimination target.

BDM 1401 Structure-based design and evaluation of the antiviral activity of potential lead compounds against the Chikungunya virus

Investigators: Sarah Cherian, Mrs. Megha Agarwal; Deepti Parashar (Dengue-Chikungunya group)

Funding: Extramural (ICMR)

Duration: 2017-2020

Background: The availability of the crystal structures of several proteins of the CHIKV RNA genome and other related alphaviruses is encouraging the drug discovery process through target structure-based pharmacophore modeling, virtual library screening and drug docking approaches.

Objectives: To identify and shortlist drug compounds with antiviral properties by screening of available compound libraries. Evaluation of the antiviral activity of selected compounds and identification of viral drug targets of the effective compounds.

Findings: The possible molecular mechanism of antiviral action of one of the compounds (OCL-105) obtained from IICT Hyderabad that showed anti-CHIKV efficacy in pre-treatment studies was analyzed by molecular docking against the envelope protein (E2-E1) complex. Results showed good binding of OCL-105 to domain A of E2 protein, interacting with residues crucial for acidic pH-based de-stabilization of the E2-E1 complex which may vitiate the fusion process (Fig. 4). The moderate inhibitory effect of compound OCL-105 in post-infection treatment against CHIKV encouraged us to look into the molecular mechanism of interaction between OCL-105 and targets of viral replicase machinery. The docking interaction analysis of CHIKV nsP3 macrodomain with OCL-105 and its natural substrate ADP-ribose showed that both the ligands bind to the same binding site with almost equivalent binding affinity. The receptor-ligand interaction analysis of the docked complex revealed that OCL-105 binds with comparatively stronger intermolecular interactions than ADP-ribose. Overall, these results indicate that OCL-105 can interact with multiple CHIKV target proteins.

BDM1601: ArVirInd - a database for Arboviral proteins from Indian subcontinent

Investigators: Pratip Shil and Kalichamy Alagarasu

Funding: *Intramural*

Duration: 2017-2020

Background: Finding protein sequences specific to strains of arboviruses from the internal databases is difficult and time consuming. Also, no existing database provides sequences corresponding to the year of outbreak. To overcome these difficulties for researchers, we have designed a database that would be exclusively for antigenic proteins from arboviral strains isolated from the Indian subcontinent.

Objectives: Establish a knowledge database of antigenic proteins for Arboviruses of public health importance from the Indian subcontinent

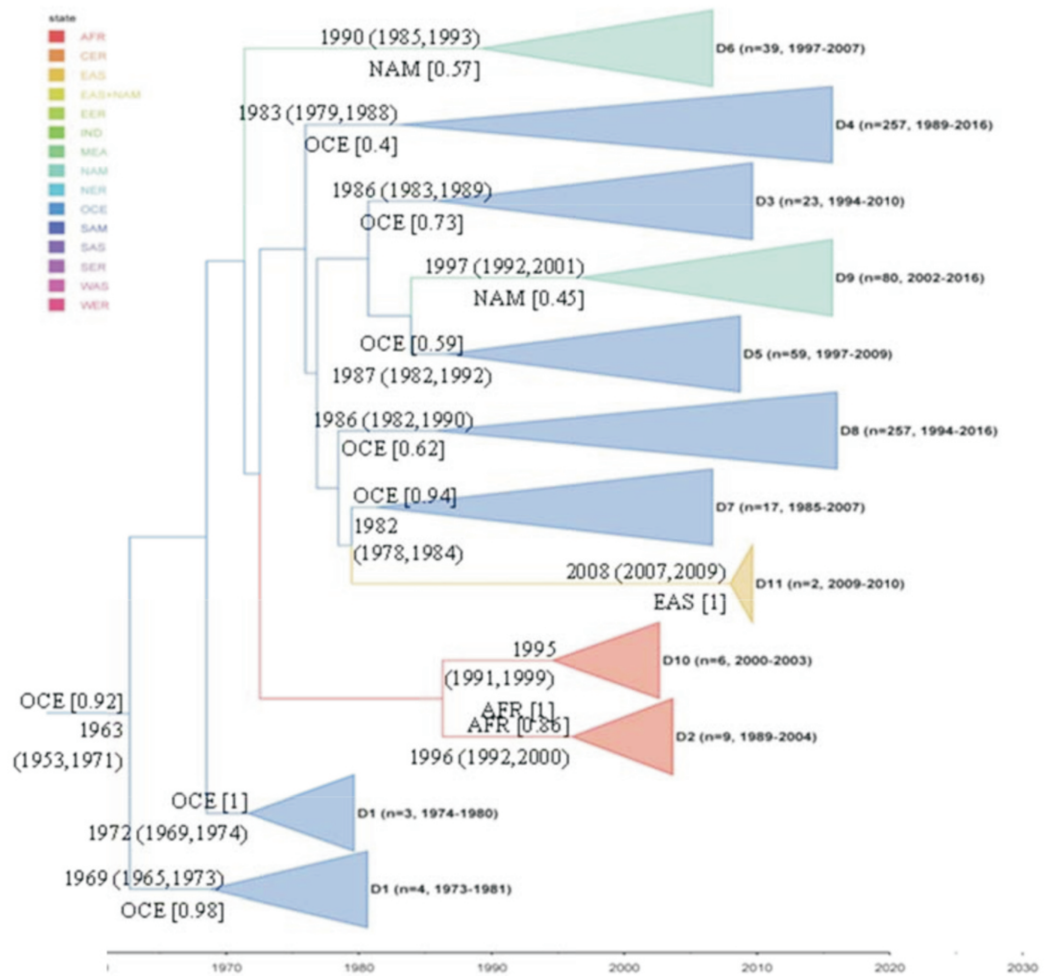

Fig. 3: Maximum Clade Credibility tree for MeV clade D based on 756 nucleoprotein gene sequences

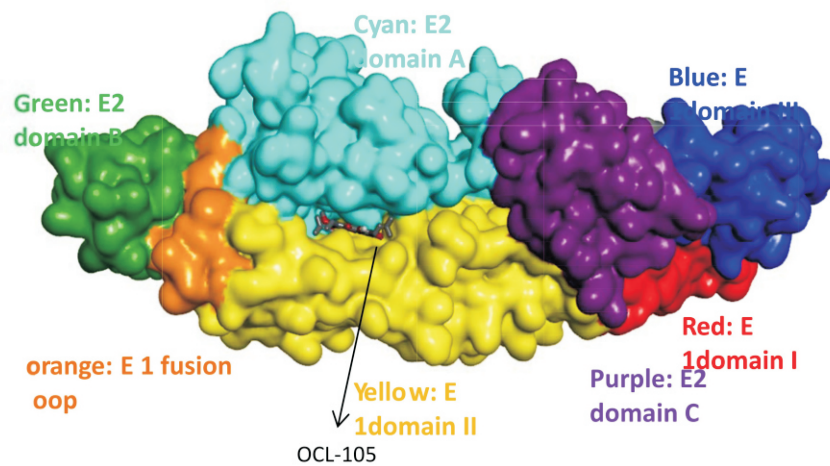

Fig. 4. Molecular docking of OCL-105 with CHIKV envelope glycoprotein complex in mature (3N42.pdb) form. (different colors represent the different domains of E1, E2 and E3 protein).

Findings: A total of 181 amino-acid sequences were collected from various databases with details of the strain and the actual outbreak. The 181 sequences for dengue, West Nile, Japanese encephalitis, Chikungunya, etc. have been corresponded to India, Bangladesh, Nepal Bhutan and Sri Lanka. For flaviviruses, we concentrated on the two antigenic proteins *viz*., envelope glycoprotein (E protein) and non-structural protein (NS1). For each sequence, B-cell epitopes were predicted and antigenicity determined using bioinformatics tools. Results of these bioinformatics analyses were entered along with sequence information in the database entry. This **Ar**bo **Vi**ral database for antigenic proteins from **Ind**ian subcontinent (**ArVirInd**) is designed as a knowledge-base which provides primary as well as derived information on arboviral proteins to users. The demo version of the database is made operational in the laboratory. Work on launching of the database on web is in progress.

BDM 1602: BIOINFORMATICS CHARACTERIZATION OF CHANDIPURA VIRUS PROTEINS

Investigators: Pratip Shil, Daya Pavitrakar, AS Tripathy and RG Damle

Funding: Intramural

Duration: 2017-2020

Background: Chandipura virus (CHPV), a member of the family, *Rhabdoviridae*, is associated with an encephalitic illness in humans. First discovered by NIV from Nagpur area of Maharashtra state, India in 1965, it caused multiple outbreaks in central and western India in the recent years. The present study was undertaken to compare surface glycoprotein (G-protein) from prototype and recent outbreak strains using bioinformatics tools along with laboratory experiments.

Objectives: Sequence analyses and predicted 3D structures; B-cell, T-cell epitopes of CHPV proteins with comparative analysis between prototype and currently circulating strains.

Findings: Analysis of G-protein of CHPV strains circulating in India from 1965-2014 was carried out using bioinformatics tools. 3D structure prediction and comparison for the G-proteins strains: I653514 (Year 1965), CIN0327 (Year 2003) and 148974 (Year 2014) revealed that the CHPV G-protein is stable and major antigenic determinants are conserved. *In silico* epitope predictions (B-cell and T-helper) were performed for these representative strains. In order to find neutralizing epitopes, a monoclonal antibody (MAb) was developed against strain CIN0327. The antibody named NAbC was found experimentally to neutralize prototype I653514 as well as recently circulating strain 148974. *In silico* antigen-antibody interaction studies were conducted using molecular docking of predicted structures of NAbC and G-proteins of various strains. Results lead to the identification of a conserved neutralizing epitope (in the fusion domain of G-protein), which also contains a T-helper peptide. The finding is a significant outcome which may help in diagnostics developments.

Mathematical Modelling Applications in Public Health: Effects of environmental factors on Chikungunya incidences in India between 2010 -2014

Investigators: Pratip Shil and AB Sudeep (In collaboration with DR Kothawale, IITM, Pune*)*

Changing climate scenario has resulted in emergence and re-emergence of various arboviral diseases. Re-emergence of chikungunya virus (CHIKV) has been observed since 2004 in Africa and spread across the globe including the Americas. The virus re-emerged in India in 2005, after a gap of 32 years, causing massive outbreaks in south Indian states initially and in other states subsequently with huge impact on human health and economy. In the present work we analyzed CHIKV incidence data from India (2010–2014) with a view to understand its association with environmental parameters. Data on country-wide occurrence of CHIKV cases were procured from the National Vector Borne Disease Control Board, India. Meteorological data for different climatic subdivisions of India were obtained from IITM and processed mathematically using computational tools. Relevant statistical analyses were performed to evaluate the effects of various meteorological parameters like rainfall, temperature, etc. We observed that prevailing temperature range was favorable for CHIKV propagation and the occurrences were modulated by average rainfall (strong positive association). Most affected state was West

Bengal, followed by Maharashtra and Karnataka. Overall for India, favorable climatic conditions have contributed to incidences of CHIKV during the study period with majority of the rural outbreaks occurring in Peninsular India.

Effects of climatic factors on mosquito abundance and seasonal diversity in Pune urban zone

Investigators: Pratip Shil and AB Sudeep

The present study was undertaken to investigate the composition and seasonal abundance of mosquito population in Pashan area of urban Pune, which has shown rapid urbanization since 2001. Mosquitoes were trapped and identified to determine species composition and abundance. Association of meteorological parameters like temperature, humidity and rainfall with mosquito abundance was analyzed from June to November 2016. Raw meteorological data was obtained and analyzed mathematically to determine derived parameters like diurnal temperatures and fortnightly averages of all parameters. A total of 21 mosquito species were observed across four genera *viz. Aedes*, *Anopheles*, *Culex* and *Armigerous*. Mosquito abundance peaked during South West (SW) Monsoon and correlated positively with maximum and minimum relative humidity and rainfall. In post-monsoon season mosquito abundance decreased along with relative humidity. Interestingly, the mosquito abundance is modulated by diurnal temperature range (DTR). During SW monsoon, low DTR corresponded to high mosquito abundance. The trend was reversed in the post-monsoon season as DTR increased by ~4 folds in comparison to SW monsoon with corresponding decrease in mosquito abundance. Mosquito population in the study area showed diversity and seasonal variability influenced by meteorological parameters. DTR seemed to be the major factor affecting seasonal variability in mosquito abundance.

C) Investigation of a Dengue outbreak in Wadi, Nagpur: Mathematical analysis (In collaboration with Dr. AB Sudeep)

Investigators: AB Sudeep, Pratip Shil and State Health authorities Nagpur, Government of Maharashtra

Entomological, virological and epidemiological investigations of a dengue-like-illness in Wadi, Nagpur district, Maharashtra 2017 was conducted by NIV, Pune (Entomology Group) and State Health authorities, Nagpur Division, Government of Maharashtra. Mathematical epidemiology protocols were applied to estimate the growth rate of the epidemic, basic reproduction number and extrinsic incubation period for dengue. The intrinsic exponential growth rate was estimated as 0.12 per day. Assuming standard disease parameters: the intrinsic incubation period of dengue in humans to be 5.9 days, the average infectious period in humans to be $1/\tilde{a}_H$ = 5 days, mean lifespan of *Aedes* mosquito (vector) to be 15 days and using the computed values of EIP and growth rate r = 0.12, the R_0 was estimated as 29.12, which is consistent with spread for moderately high transmissions in other tropical countries.

ANIMAL HOUSE

Scientific Staff

Dr. D.R. Patil	Scientist D & Group Leader patil.dr@niv.co.in,

Technical Staff

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Mr. A.N. Shitole	Technician B
Mr. A.B. Sunsuna	Multi-Tasking Staff (MTS)
Mr. A.V. Kondayya	MTS
Mr. A.D. Dalvi	MTS
Mr. V.P. Sasane	MTS
Mr. S.B. More	MTS
Mr. D.L. Nagayya	MTS
Mr. T.S. Ramayya	MTS
Mr. R. Venkatayya	MTS
Mr. A. Thimanapalli	MTS

AHG 1501: Breeding, Supply and Maintenance of Laboratory Animals.

Investigators: Dilip Patil & Satish Nipunage

Funding Agency: Intramural

Duration: service project

The institute is having well equipped infrastructure to cater to the needs of *in vivo* research in the field of viral infectious diseases. This includes mouse breeding, small animal experimentation, primate experimentation and rehabilitation facilities. Animal facility at the Institute is registered with CPCSEA under "Research for Education Purpose and Breeding for in house use on Small and Large Animals" vide Registration No. 43/GO/ReBi/SL/99/ CPCSEA having validity until March 2022. All the animal species are housed in species appropriate cages, which are designed as per the CPCSEA guidelines. Eight strains of mice (Inbred: BALB/c, C57BL/6, DBA/2, C3H, outbred: CD-1, Immuno-deficient: CD-1 *nu/nu* and BALB/c *nu/nu*, Double knock out: AG129) are currently being bred and maintained in the individually ventilated caging (IVC) system. Mouse breeding is carried out in three tier system of foundation, nucleus and production colonies. Pedigree records are maintained at foundation and nucleus colonies. Controlled environmental conditions are strictly ensured with animal room temperature maintained between 22 ± 2°C, relative humidity between 45-55 %, 12:12 hr. light dark cycle and 15-16 air changes per hour with 100% fresh air. Health monitoring programme at the mouse colony includes genetic, microbial and parasitological examinations. Genetic quality analysis of inbred strains of mice is regularly done to rule out genetic contamination. Microbial quality analysis of inbred/outbred strains of mice is carried out for seven rodent pathogens (MP, MHV, MVM, LDHV, Sendai virus, Tyzzer's organism, *pneumocystis carinii)*. Biannual testing of mouse fecal samples is carried out for physical, microscopic, parasites and occult blood examination. During the report period, a total of 2392 mice belonging to different strains were supplied to institutional scientists against 26 IAEC approved research projects.

Other species of laboratory animals *viz.,* guinea pig, rabbit, fowl, turkey, goose are procured from CPCSEA authorized sources as per requirement. A total of 741 ml blood from different species of laboratory animals, as diagnostic reagent in various assays, was supplied to institutional scientists.

Rhesus monkeys are currently under rehabilitation at the eco-friendly group housing enclosures. Apart from routine husbandry and veterinary care, annual health monitoring is conducted and individual records are maintained. Primate facility inspection and verification of records is done regularly by IAEC. Besides institutional care programme for primates, services of consultant veterinarian are also sought.

Records are maintained at the facility as per CPCSEA requirements, which include livestock register, breeding records, pedigree, microbial and genetic monitoring of animals, staff health records, consumables testing records, individual project files and form D (animal utilization), AMC of equipment's.

During the reporting period, two meetings of IAEC were organized for evaluation of animal experimental protocols. One mandatory annual inspection meeting of IAEC was organized. Inspection of the facility and verification of various records pertaining to animal breeding and experiments at the institute was conducted by the committee and the report submitted to CPCSEA, New Delhi.

As part of mandate, the facility also conducts training/course work for the research fellows and staff of the institute as per requirement in the area of 'Laboratory Animal Experimentation and Ethics'.

DIAGNOSTIC REAGENT FACILITY

Scientific Staff:

Dr. Paresh Shah	Scientist-E& Group Leader
Dr. Kanchankumar Patil	Scientist-D

Technical Staff

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

Project Staff

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

Title: Scaling up of facilities for production of diagnostic kits/ reagents for detection of JE, DEN & CHIK virus antibodies.

Investigators: DT Mourya & PS Shah

Funding agency: NVBDCP Pro

Duration: Ongoing

Work done:

During the year 2017-2018, 11,106 MAC ELISA kits (JE, Dengue and Chikungunya) were produced and supplied to SSH and 15Apex laboratories under the national program and also to WHO SEAR for onward transmission to neighboring countries (Table 1). Dengue kits were in high demand due to dengue outbreaks across the country and 7941 kits were supplied. Monthwise distribution of kits is given in Fig 1. Maximum number of kits was supplied to Karnataka followed by Delhi, West Bengal, Gujarat and Andhra Pradesh. Nagaland, Arunachal Pradesh and Himachal Pradesh received the least number of kits during the year (Fig 2).

Table 1: Supply of MAC ELISA kits in 2017-2018

Supplied to	JE	DEN	CHIK	TOTAL
National Program	652	7694	2290	10636
WHO-SEAR	2	2	5	9
VRDL (DHR)	27	140	73	240
Others*	9	9	5	23
Total	749	7941	2416	11106

*ICMR-NARI, Pune for research project and to NIB, Noida for panel preparations

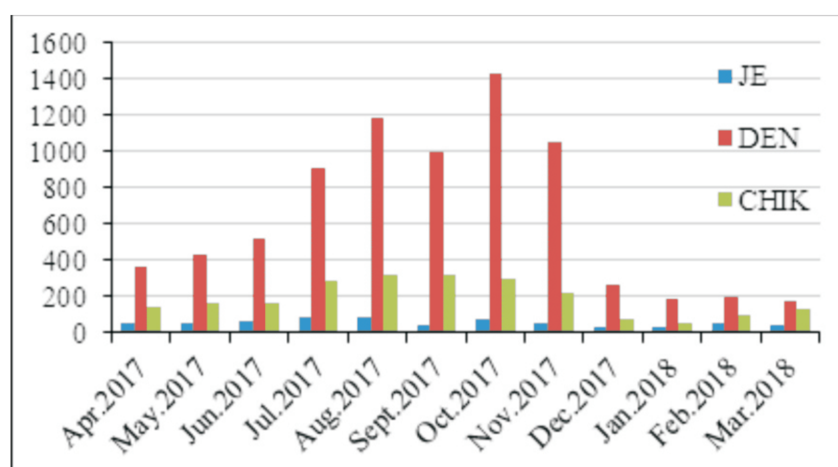

Fig 1: Month-wise supply of MAC ELISA kits under National Program

Fig 2: State-wise supply of MAC ELISA kits under National Program

ACADEMICS

Scientific Staff

Dr. Kavita S. Lole	Scientist E and Academic Cell In-Charge
Dr. Tejaswini M. Deshmukh	Scientist B

Technical Staff

Mr. Shrinivas S. Bedekar	Technical Officer
Mr. Hemant R. Band	Technician C

Project Staff

Dr. Vikram S. Ghole	Co-ordinator Academic Cell (Retired Professor, SPPU, Pune)
Mrs. Neethi Jayaram	Technical Officer
Ms. Sanmati P. Dilpak	Technical Assistant

M. Sc. Virology

NIV conducts a two year M. Sc Virology course under the IBB Program of the Savitribai Phule Pune University since 2005. During the year, eighteen students from a total strength 19, have passed out. Two students got 'O' Grade while eight students received A+ Grade.

Ph.D Cell

Staff

Mrs. Swati	Technical Officer

Twenty-one students are pursuing Ph. D degree in different disciplines. The following students were awarded Ph.D. degree by Savitribai Phule Pune University during the year.

1. Ms. Neha Lasure
2. Ms. Pradhan G
3. Mr Ashsh Kumar Tiwari
4. Mr Himanshu Tillu
5. Mr Subrat Thanapati
6. Ms Shakuntala Mahilkar

Characterization of hepatitis E virus specific antigen-antibody interactions *in vitro*

Investigator: Tejaswini Deshmukh, Kavita Lole

Funding: Exra mural (ICMR)

Duration: 2017 to 2018

Background: Hepatitis E virus (HEV), the etiological agent of Hepatitis E infection, causes immune associated pathogenesis. Antibody based cross protection is well documented in HE, but a complete understanding of the associated humoral immune response is lacking. Unavailability of a robust cell culture system makes characterization of specific antibodies induced post natural/experimental infection or immunization using conventional methods difficult. It becomes imperative to characterize anti-HEV humoral immune response in context of recurrent epidemics, large number of subclinical infections and for development of better diagnostic/vaccine targets.

Objectives: To characterize anti-HEV humoral immune response in terms of quality (affinity maturation/avidity) and quantity, simulate/validate HEV binding assay using chaotrope based avidity assays and kinetic binding assays employing Surface Plasmon Resonance technique (SPR).

Findings: ORF2 and T1NE protein based ELISAs were comparable in their performance in detecting anti-HEV IgM and IgG antibodies and each of these ELISAs were modified to the corresponding avidity ELISAs. Avidity index (AI) percentages determined in ORF2 avidity ELISA for the anti-HEV IgG antibodies from patients' serum samples collected during HEV outbreaks distinctly defined the phases of HEV infection. Approximately de30 percent AI values defined the cut-off between acute (Mean AI 21.7%) and convalescent/prior exposure phases. Mean AI percentage values determined for convalescent and prior exposure phases were similar, 71% and 71.9%, respectively. Kinetic interaction analyses (SPR) of T1NEp e His tag with anti-HEV IgG antibodies indicated high affinity specific IgG antibody response in early phase. These results are promising and merit further investigation.

Fig 1: M. Sc Virology topper of 2017 Ms. Janice Chithelen being conferred gold medal by Dr Atanu Basu, Scientist 'F', NIV, Pune

LIBRARY & INFORMATION SERVICES

Scientific Staff

Dr. M.D. Gokhale	Scientist "C" & Library In charge

Technical Staff

Ms. Vandana Chandere	Senior Library & Information Officer
Mr. V.R. Mali	Library & Information Assistant
Ms. Ekta Jain	Library & Information Assistant

Library Apprentices

Mr. Dinesh Tribhan	(Till 20/11/2017)
Mr. Suresh Manekar	(From 01/11/2017 to till date)
Mr. Mukesh Sonawane	(From 01/11/2017 to till date)

Major Activities:

Library continued to cater its services to scientists, technical staff, project staff, M.Sc. and PhD students of NIV as well as scientists and students of other research institutions in Pune. Services provided included citation analysis, document delivery service, reference service, procurement of reprints, support in publication of papers, literature search etc. In addition, books/journals and other related documents were procured through inter library loan from other research institutes in Pune, *viz.*, ARI, NCCS, and Serum Institute of India Pvt. Ltd etc.

The following consortiums for access to full text of journals were also managed by NIV library.

- ICMR Consortium for full text online access for four journals *viz*., Science, Nature, Lancet and NEJM.
- J-Gate Plus database for access for 3497 journal titles with access to full text, abstract and on Document Delivery Request (DDR) from other ICMR libraries.
- ERMED Consortium through National Medical Library, an IP based consortium for access to 237 e-journals from five publishers i.e., BMJ Publishing Group, Cambridge University Press, Lippincott Williams & Wilkins, Oxford University Press, Wiley Blackwell.
- Web of Science databases service started for access for Journal Citation Report, Abstracts of various journals on trial basis.

During the year, the following activities were conducted:

- Renewal of Magazines and Newspapers.
- Implementation of Barcode system for books and journals (completed).
- Xeroxing, Lamination & Binding service to scientists/staff.
- Retrieving of medically important articles daily from English and Marathi Newspapers and sending to NIV Scientists through email.
- Preparation and maintenance bibliographic details of NIV research articles, hyperlinked with full text to upload on NIV website.
- Updating and maintenance of in-house NIV Scientific Publications from 1953-2017.
- Updated list of NIV holdings from 1953-2017 for ready reference.
- Library Stock Verification 2016-17.
- Weeding Out of library documents (Old Books, Loose Issues, Maps, Publication Files, Annual Reports, catalogues, and other miscellaneous) carried out (3588 documents).

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

Table- 1: Details of books/journals added to NIV Library during the year

Description		Quantity
Books	Purchased/Gifts/Gratis	25
	Bound Volumes	107
	Annual Reports Received	23
Journals	Print (Subscribed)	14
	Print (Gratis)	56
	ICMR Consortium	4
	J Gate	3497
	ERMED Consortium	239
	Loose Issues	591
	Bound Volumes	107
Ph.D. These		3
M.Sc. Dissertations		22
Others; CDs		6
Papers sent for Publication		27

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

Table 2: Service Provided- Circulation, Reference, CAS, ILL, DDS, etc.

1.	Book & Bound Volumes Issued	Staff : 190 Students : 672
	Book & Bound Volumes Returned	Staff : 155 Students : 649
2.	Newspaper Clippings sent to scientists	2547
3.	Inter Library Loan Received and Sent	124
4.	Photocopy Service	1686
5	Binding	Nil
6.	Lamination	30
7.	Reference Service	284
8.	NIV Annual Reports Distributed	17
9.	Citation Analysis	17 Scientists
10.	Plagiarism Detection using Software conducted· ● Ph.D. Theses ● Manuscripts	 3 7
11.	CAS/SDI	On Demand

FIELD UNITS

MUMBAI UNIT

Scientific Staff

Dr. S D Pawar	Scientist 'D'& Officer-in-Charge
Dr. V K Saxena	Scientist 'E'
Dr. (Mrs.) M C Mohanty	Scientist 'E'
Dr. S S Nandi	Scientist 'C'

Technical Staff

Mr. R L More	Technical Officer
Dr. (Mrs.) D K Sharma	Technical Officer
Mrs. S V Rane	Technical Officer
Mrs. R H Bhavasar	Technical Officer
Mrs. N R Shanbhag	Technical Officer
Mrs. U P Nalavade	Technical Officer
Ms. S A Sawant	Technical Assistant
Mr. S Y Varose	Technical Assistant
Mr. V S Dhuri	Technician – C
Mr. D S Jagtap	Sr. Technician
Mr. C B Sawant	Laboratory Assistant
Mr. G J Bane	Laboratory Assistant
Mr. P P Gurav	Laboratory Assistant
Mr. V V Kadu	Laboratory Assistant
Mr. P A Ahire	Laboratory Assistant
Mr. K N Rathod	Laboratory Assistant
Mr. S R Saware	Laboratory Assistant
Mr. N G Koktare	Laboratory Assistant
Mr. N H Sawant	Laboratory Assistant
Mr. S M Parab	Laboratory Assistant
Mr. B M Ghadigaonkar	Laboratory Assistant
Mr. M S Solanki	Laboratory Attendant -2
Mr. P M Modak	Laboratory Attendant -2
Mr. R S Vallakati	Laboratory Attendant -2
Mr. N K Salunkhe	Driver
Mr. T Y Jadhav	Driver

Project Staff

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Ms. S M Bobade	Res. Asst. (NPSP Project)
Ms. N A Karkare	Sr. Lab. Tech (NPSP Project)
Ms. S S Kawale	Sr. Lab. Tech (NPSP Project)
Mr. M G Narkar	Lab. Tech (NPSP Project)
Ms. P A Bandivdekar	Lab. Tech (NPSP Project)
Ms. D N Jatekar	Lab. Tech (NPSP Project)
Ms. J Khan	Lab. Tech (NPSP Project)
Mr. K Kalgutkar	Lab. Tech (NPSP Project)

Mr. V S Dhanawade	Lab. Asst. (NPSP Project)
Ms. S T Padelkar	Asst. Data Manager (NPSP Project)
Ms. A Rani	Res. Asst (Atlas Project)
Mr. T H Qureshi	Res. Asst (Atlas Project)
Mrs. R Sheshware	DEO (NTF Project)

Administration

Mrs. A B Palkar	Section Officer
Mr. A R Magade	Assistant
Mr. D V Muneshwar	Assistant
Mr. M S Malvankar	Upper Division Clerk
Mr. R R Jaiswal	Lower Division Clerk
Ms. Y C Bhandare	Lower Division Clerk
Ms. P A Kamble	Clerical Asst (NPSP Project)
Mr. R G Bane	MTS (NPSP Project)

NIV - Mumbai Unit

As per the recommendations of the Indian Council of Medical Research (ICMR), the Enterovirus Research Centre (ERC) was merged with National Institute of Virology (NIV), Pune with effect from 1st November 2017 and has been renamed as NIV Mumbai Unit (NIV MU).This unit has been involved in research on diseases caused by Enteroviruses, especially acute flaccid paralysis (AFP), acute hemorrhagic conjunctivitis, aseptic meningitis/encephalitis, Measles-Rubella surveillance, acute gastroenteritis caused by enteric viruses such as Rotavirus, Norovirus, and Enterovirus.

1. NIV MU 9701: National Polio Surveillance Project, India

Shailesh Pawar, Mrs. Uma Nalavade, Deepa Sharma & Vinay Saxena

Funding agency: Extramural (National Polio Surveillance Project, WHO, India)

Duration: Ongoing (since 1997)

Abstract

ICMR-National Institute of Virology, Mumbai is one of the seven Global Specialized Laboratories (GSL) of Polio Laboratory Network in South East Asia Region (SEAR), World Health Organization. Wild poliovirus has been eradicated from SEAR with the last case detected in India in January 2011. As SEAR region has been certified free of wild poliovirus in 2014, the surveillance has now become more stringent as there is always a risk of importation of wild poliovirus from neighboring polio-endemic countries such as Pakistan and Afghanistan. The objective of this programme is to eradicate wild poliovirus from India and carry out stringent surveillance with continuous monitoring until global eradication of poliovirus is achieved.

During April 2017 to February 2018, the unit tested 5230 stool specimens from AFP cases reported in Maharashtra, Madhya Pradesh, and Goa. A total of 167 isolates Sabin-like (SL) polioviruses (P1SL=49; P3SL=56; P1+P3SL=36 and NPEV growing in L20B = 26) were isolated. Under environmental surveillance, the unit has tested 297 sewage specimens/ concentrates and 208 isolations were made. All the isolates were found to be Sabin-like poliovirus type 1 and type 3 (P1SL-26, P3SL-99, P1SL+P3SL-83, and NPEV-88). Wild poliovirus/VDPV/Sabin2 has not been detected in any of the AFP cases/sewage specimens.

2. NIV MU 1601: Measles and Rubella Surveillance

Shailesh Pawar, Deepa Sharma, Mrs. Uma Nalavade

Funding agency: Extramural (National Polio Surveillance Project, WHO, India)

Duration: Ongoing

Background: In India, there are a total of 13 Measles and Rubella WHO network laboratories of which NIV Mumbai Unit is the reference laboratory for sequencing. Under this surveillance, the center receives serum specimens for confirmation of suspected cases from outbreaks detected by NPSP as well as those from the hospitals. The measles and rubella surveillance programme have high importance in relation to national and global measles and rubella elimination by 2020. The objective of this surveillance programme is the elimination of measles and control of rubella.

Work done: NIV MU receives serum specimens from suspected cases of measles and rubella from Maharashtra, Madhya Pradesh, Chhattisgarh, and Goa. The serum specimens are tested using the Measles and Rubella IgM ELISA. During the period April 2017 to Feb 2018, the unit has tested 335 serum samples, of which, 220 (65.67%) tested positive for Measles and 28 (8.35%) for Rubella. The remaining specimens were equivocal for Measles (2.98%) or Rubella (2.38%) or negative for both (20.5%).

3. NIV MU 1804: Strengthening laboratory quality management systems including EQA of DHR/ICMR VRDL laboratories for laboratory confirmation of outbreaks including measles and rubella.

Investigators: Shailesh Pawar, Deepa Sharma, Mrs. Uma Nalavade

Funding: Extramural (DHR)

Duration: 2018-2020

Background: The NIV Mumbai Unit, WHO reference laboratory for measles and rubella sequencing, was identified as a nodal laboratory for capacity building of VRDLs by providing training and continued technical support for testing Measles and rubella surveillance specimens in coordination with KIPM, Chennai.

Objective: to develop an External Quality Assurance System (EQAS) for the VRDLs along with on-site assessment to make them WHO proficient laboratory for measles and rubella diagnosis.

Work done: A workshop on "Strengthening of Measles and Rubella case confirmation by serology IgM detection and conventional RT-PCR" was conducted at NIV Mumbai unit for the 6 selected VRDLs from 19-23 February 2018. In order to develop WHO proficient laboratories for measles and rubella surveillance, plan was developed for the establishment of EQAS and on-

site assessment which will be executed in four phases. The Phase I of this plan which included the provision of practice panels for both serological assay and molecular methods to the six VRDLs has been completed successfully. The Phase II plan is currently under process.For establishing a team for an on-site assessment, a team of selected members from NIV MU and ICMR visited seven WHO laboratories. Dr. SD Pawar and Dr. DK Sharma visited the labs at Guwahati, Kolkata, Patna and Dr. DA Gadkari and Mrs.UP Nalavade visited the labs at Lucknow and Delhi during their accreditation review along with Dr. David Featherstone from WHO. By the end of 2018, the four phases of the plan are expected to be completed.

4. NIV MU 1602: Comparative evaluation of immunogenicity of bivalent oral poliovirus vaccine (bOPV) and monovalent oral poliovirus vaccine type 1 (mOPV1) when administered in the EPI schedule with a dose of inactivated polio vaccine (IPV) at week 14 and assessment of immunogenicity of IPV only schedule in the EPI: A multicentric open label randomized controlled trial.

Investigators: Jacob John, SD Pawar, Mrs. U Nalavade, D Sharma, DT Mourya, Jayati Mullick

Funding agency: World Health Organization (WHO)

Duration: 10 months

Background: Although wild poliovirus has been eradicated, globally, the Sabin type 2 component of trivalent OPV (tOPV) was found to be responsible for the majority of circulating VDPV (cVDPV) outbreaks accounting to more than 95% of cVDPV cases due to poliovirus type 2 in the last few years. To eliminate this risk due to VDPV2, the program decided to make a global switchover from tOPV to bivalent OPV (bOPV) in April 2016. Considering eradication of type 3 poliovirus in upcoming years and to further reduce the burden of cVDPVs due to type3 poliovirus, a switchover from bOPV to monovalent OPV type1 (mOPV1) is expected in the near future. Finally, all live OPV forms will be stopped and replaced with IPV. The goal of this study is to compare immunogenicity against poliovirus type1 by bOPV and mOPV1 when given as a part of routine immunization schedule at birth, 6, 10 and 14 weeks along with one dose of IPV at week 14. Also, this study will assess the immunogenicity of IPV only.

Work done & findings: The unit has completed testing of all sera (n=2254) received under this study against poliovirus type 1 and type 3. Results of these specimens have been submitted to NPSP, WHO for analysis. As Sabin 2 is under containment, the testing against poliovirus type 2 has to be performed in a BSL-3 facility. Further work is in progress.

5. NIV MU 1707: Comparative evaluation of immunogenicity of various schedules, dosages and delivery options to provide fractional Inactivated Poliovirus Vaccine (IPV) in routine immunization in the post tOPV-bOPV period: A multi-centric open-label randomized controlled trial"(India IPV fractional dose study)

Jagadish Deshpande, Shailesh Pawar, Mrs. Uma Nalavade, Deepa Sharma, DT Mourya, Jayati Mullick

Funding agency: World Health Organization

Duration: 2017-2018

Background: The Polio Eradication and Endgame Strategic Plan 2013–2018 includes the introduction of at least one dose of inactivated polio vaccine (IPV) into routine immunization schedule as a strategy to mitigate the potential risk of re-emergence of type 2 polio following the withdrawal of Sabin type 2 strains from oral polio vaccine (OPV). All OPV-using countries have switched from tOPV to bOPV in April 2016. OPV2 withdrawal requires the integration of $\geq$1 dose of IPV into routine immunization schedules for risk mitigation purposes. In October 2015, the Strategic Advisory Group of Experts (SAGE) reaffirmed that the switch should proceed in April 2016, irrespective of further IPV supply constraints. Intradermal (ID) IPV administration of a fractional dose of IPV (0.1mL or 1/5 of a full dose) enables to improve immunogenicity while stretching the limited IPV supply. Though various options to ease IPV supplies are under consideration it seems that switching to fractional-dose IPV (fIPV), instead of full doses may be the most rapid antigen sparing option available. This could be implemented countrywide or in states within a country.

Objectives:

1. To compare the immunogenicity against types 1 and 3 polioviruses in all the three vaccination schedules:
    a) One full dose IPV at 14 weeks
    b) Two dose f-IPV given at weeks 6 and 14 delivered by BCG needle and syringe
    c) Two f-IPV doses at weeks 10 and 14 delivered by BCG needle and syringe
2. To compare the effect on immunogenicity and operational convenience in administering by f-IPV either through BCG needle and syringe or intradermal (ID) adapter (West/Helm).

Work done & findings: The NIV Mumbai unit has received 2203 sera under this study which have to be tested against all three serotypes of poliovirus using microneutralization assay as per WHO guidelines. The testing against poliovirus type 2 is ongoing.

6. Contract Research Project: NIV MU 1703: A Phase IV, interventional, open label, multicentric, single arm clinical trial to assess the safety, tolerability & immunogenicity of bivalent oral polio vaccine (bOPV) in healthy Indian infants.

Principal Investigators from BIBCOL: Ravi MD, Rao Y K, Bora C, Awasthi S, Ramanan P

The team at NIV, Mumbai: Shailesh Pawar, Mrs. Uma Nalavade, Deepa Sharma

Funding agency: Bharat Immunologicals and Biologicals Corporation Limited (*BIBCOL)*

Duration: 2017-2018

Background: Data from previous studies suggest that type2 vaccine viruses often interfere with responses to vaccine virus type1 and type3. It is also suggested that viral interferences may be overcome by modifying the absolute and relative dosage of the three Sabin serotypes. Wild poliovirus type 2 was eliminated in 1999 but the continued use of tOPV contributes to ongoing type 2 vaccine-associated paralytic poliomyelitis and vaccine-derived poliovirus outbreaks (cVDPV2). The SAGE working group on polio has recommended a switch from tOPV to bOPV to remove the threat of cVDPV2. Therefore, there is a need to accelerate the elimination of wild polio types 1 and 3 as bOPVis a more immunogenic vaccine. The study aims to explore the safety and efficacy of BIBCOL bivalent oral polio vaccine in newborn/infants. Also, this study will be helpful to assess the safety, tolerability, and immunogenicity of Bivalent Oral Polio Vaccine (bOPV, Type 1 & 3) in healthy Indian infants.

Work done & Findings: The unit has received and tested 161 sera till date against 200 sera to be delivered from five sites across India *viz*. Mysore, Lucknow, Kanpur, Assam, and Chennai to test the neutralizing antibody titers against poliovirus types 1 and 3 using micro-neutralization assay as per WHO protocol. Results of these sera have been submitted to the collaborating agency. The remaining 39 sera are expected to be delivered in May/June 2018 for testing.

7. NIV MU 1603: Variant analysis of vaccine-derived poliovirus RNA by next-generation sequencing

Shailesh Pawar, Deepa Sharma, Mrs. Uma Nalavade

Funding agency: Intramural (ICMR)

Duration: January 2016 to December 2019

Background: Poliovirus isolates from AFP cases are reported in India (by 7 WHO polio network laboratories) and other countries like Bangladesh, Myanmar and Sri Lanka. Vaccine-derived polioviruses (VDPVs) are polioviruses in which genetic properties indicate prolonged replication or transmission. Three categories of VDPVs are recognized as circulating VDPVs (cVDPVs), immunodeficiency-associated VDPVs (iVDPVs), and ambiguous VDPVs (aVDPVs). In molecular definition, vaccine-derived polioviruses (VDPVs) are isolates of Sabin OPV (oral poliovirus vaccine) origin that have incorporated >6 nucleotide substitutions (Sabin2) or e"10 nucleotide substitutions (Sabin1 and Sabin3) in the VP1 region. VDPVs are neurovirulent strains of polioviruses mutated from the oral polio vaccine and pose a challenge to global polio eradication program. Genome analyses of VDPVs are very important to understand their spread, virulence, evasion of immune response and viral evolution. Differentiation between cVDPV and iVDPV is necessary for their classification. The reversion analysis provides information on the risk of transmission of the virus in the community. There are no reports of analysis of iVDPV and cVDPV for India.

Objective: to perform complete genome variant analysis using Next Generation Sequencing (NGS) to enable differentiation of Vaccine-derived polioviruses (VDPVs) into subclasses especially aVDPV to cVDPV or iVDPV.

Work done & Findings: Under this study, 9 iVDPV1 isolates were tested using NGS. The data analysis is in progress.

8. NIV MU 1701: Complete genome sequencing of Rubella and Measles virus using Next Generation Sequencing

Shailesh Pawar, Deepa Sharma, Mrs. Uma Nalavade

Funding agency: Intramural

Duration: 2017 - 2018

Background: NIV, Mumbai Unit is WHO reference laboratory for sequencing for measles and rubella for India, since December 2017. Genotyping of rubella and measles is necessary for outbreak investigations. However currently used genotyping strategies are insufficient to distinguish repeated importations of strains. Therefore, higher resolution genotyping methods are urgently required for outbreak investigation. The present study proposes complete genome sequencing of rubella and

measles viruses using next-generation sequencing (ION-torrent).

Objective: complete genome sequencing of rubella and measles viruses to study genetic diversity using NGS.

Work done & Findings: NIVMU has recently started testing and isolating viruses from urine/throat swabs received under measles and rubella surveillance. Processing of isolates is in progress.

9. NIVMU 1702: Molecular detection of Enteroviruses from Acute Flaccid Paralysis (AFP) cases in Maharashtra not detected by routine cell culture

Vinay Saxena, Tarique Quereshi, Arokia Rani, Priyanka Surve

Funding Agency: ICMR Intramural

Duration: April 2017-January 2018

Background: AFP surveillance in India includes systematic virus isolation in cell cultures from stool samples of AFP cases detecting both polio and non-polio enteroviruses (NPEV). Many Enteroviruses (EV) mainly coxsackievirus A (CVA) serotypes fail to grow on cell cultures used routinely requiring animals for isolation. Molecular methods using sequencing of the partial VP1 capsid protein gene have been used extensively for direct identification of EV from clinical samples i.e., stool samples, cerebrospinal fluid (CSF) etc.

Objective: to identify and characterize EVs from the stool samples of AFP cases in 2017 from Maharashtra, by molecular methods of RT-PCR and sequencing.

Work done & Findings: EV RNA was found in 40 (5.5%) out of 720 cell culture negative AFP cases with EV serotypes of 21 (52.5%) being identified as EV species C (CA1, CA11, CA13, CA19, CA20, CA22, CA24, EV99 and EV116) followed by EV species A (CA2, CA6, and EV71) and EV species B (EV80 and EV84) (**Table 1**). EV species C strains CA1, CA19, CA22 and EV116 requiring suckling mice were also detected. Attempts were also made to grow all 720 stool samples in RD and Hep2 cell cultures yielding 4 virus isolates in RD and Hep2 cells. One virus isolate grown in RD cells was identified as EV120 which was not grown earlier. The study using molecular methods improved identification of EV serotypes which were missed out by routine cell culture methods during AFP surveillance. Also, several CVA were identified which require suckling mice

Table1: EV detection from culture-negative stool samples of AFP cases from Maharashtra.

Total No. of AFP cases (n=1191)			Total No of EV positive culture negative AFP cases						Total No of AFP cases with EV Serotypes identified
			Molecular Methods					Cell Culture positive (RD cells)	
Cell Culture Positive cases	Cell Culture Negative cases		EV +ve (panEV RT-PCR)	EV Serotypes identified by partial					
	Total	Tested		EV Species	EV Species	EV Species	EV Species		
235 (19.7%)	956 (80.2%)	720 (75.3%)	40 (5.5%)	3 (CA2,	2 (EV80, EV84)	15 (CA11, CA20, CA24, EV99, EV116 (1 EACH), CA13, CA1, CA22 (2 EACH), CA19 (4)	0	1 (EV120)	21 (52.5%) (CA2,CA6, EV120*, EV71* *, EV80, EV84* *, CA11, CA20***, CA24, EV99, EV116 (1 Each), CA13, Ca1, CA22 (2 Each) and CA19 (4)

Note: * EV120 was identified by cell culture only from one case. * * EV71 and EV84 were identified by the molecular method and RD cell culture from 2 cases. * * * CA20 was identified by the molecular method and cell cultures (RD and Hep2 both) from one case.

10. NIVMU 1501: Genetic diversity of Enterovirus 71 from non-polio-Acute Flaccid Paralysis (nonpolio-AFP) cases in India (2014-2015).

Vinay Saxena, Tarique Quereshi, Arokia Rani, Priyanka Surve

Funding Agency: ICMR Extramural

Duration: April 2017-January 2018

Background: Since its isolation from a case of encephalitis in 1969 in California, USA, EV71 has been considered a serious health problem due to its association with aseptic meningitis, encephalitis and acute flaccid paralysis (AFP) with severe and sometimes fatal neurologic complications. In Southeast Asia region, EV71 has shown increased epidemic activities since 1997 causing large HFMD outbreaks. However, in India, the epidemiology of EV71 has been unexplored as it is not considered a serious public health problem. Several studies from around the globe on the genetic diversity of EV71 by phylogenetic analysis of the capsid protein gene VP1, suggested genogroups B and C being circulating most (Genogroup A included prototype strain BrCr). However, over the years, EV71 genome evolved with several new genogroups being identified. Limited studies on the molecular epidemiology of EV71 are available suggesting that EV71 genogroups C, D, and G have been circulating in India during 2001-2012.

Objectives: to provide insights into the circulation and genetic diversity of EV-A71 in India.

Work done & Findings: We retrospectively analyzed 4870 nonpolio enteroviruses (NPEV) isolated during routine AFP surveillance in 2014–2015. Only 33 isolates were identified as EV71 from AFP cases mostly from Madhya

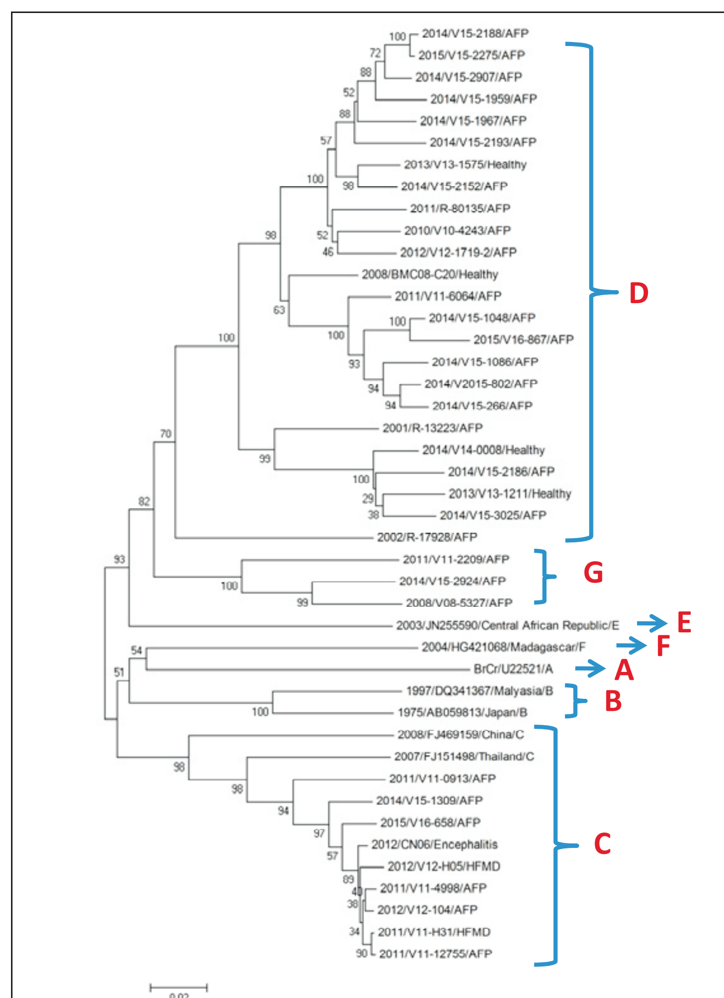

Figure 1: Phylogenetic relationships between EV71 isolates using complete VP1 nucleotide sequences (891 nt).

Pradesh, Maharashtra, Bihar, UP, Jharkhand, West Bengal, Orissa, and Assam. The complete VP1 (891 *not*) gene of 17 EV71 isolates was amplified using RT-PCR and the sequences were compared with the sequences of EV71 VP1 sequence database available from NCBI. The phylogenetic tree was constructed using a Neighbor-joining method which suggested that genogroup D has been the most predominant and widely circulating genogroup since 2001 (Fig 1). The genogroup D EV71 strains were isolated from both healthy children and AFP cases. The genogroup C was also found circulating during 2011 – 2015 which were mostly isolated from AFP, encephalitis and HFMD cases. Interestingly, the newly identified genogroup G circulating since 2008 was also found circulating in 2014. The study clearly revealed that multiple genogroups of EV71 with high sequence divergence have been circulating in the country. With the only limited number of EV71 isolates being studied, the real circulation of different genogroups is probably underestimated. Although EV71 has not caused any serious HFMD outbreaks in India in recent years, systematic efforts should be made to understand the molecular epidemiology of Ev71.

11. NIVMU 1502: Studies on poliovirus infections in children with immune-deficiency

Investigators: Madhu Mohanty, Manisha Madkaikar, Mukesh Desai

Funding Agency: Intramural

Duration: 2015-2018

Work done: Immunodeficient children, when exposed to poliovirus, may harbor poliovirus infection for several months or even longer posing risk to community and the polio eradication programme.

Objective: This project is being carried out to screen the immunodeficient patients for polio and non-polio enterovirus infections, to characterize the isolates and identify the long-term excretors.

Work done & Findings: A total number of 175 stool samples of 68 patients (4 SCIDs, 3 CVIDs, 7 XLAs and 28 other PIDs) with humoral, combined and other PIDs were assessed and followed up for enterovirus excretion. Stool specimens of 10 patients (14%) were tested positive for enteroviruses of which 6 (9 %) patients tested positive for non-polio Enteroviruses and 4 (6%) were tested positive for polioviruses. A 6 years old male child, a case of leaky SCID was excreting poliovirus for two years and then stopped excretion. Genetic sequencing of virus isolates identified type 3 VDPV with up to 41 (4%) nucleotide changes at the age of 4 years which subsequently showed up to 93 (10%) nucleotide divergence at 6 years of age (Table 2A). The comparison of immunological parameters during excretion and after the child stopped poliovirus excretion, revealed rising cytotoxic T lymphocytes with exceptionally high NK cells (Table 2B). However, the B cell count of the child was still low with no change in immunoglobulin status (Table 2B). The result indicated that the T cells and not the B cells, which are important to stop virus replication. Further studies to analyze the immune responses of the SCID patient, who cleared poliovirus infection, are being carried out.

Table - 2A: Detailed follow up of the P3VDPV case with nucleotide changes

Case	Sample No.	ID Type	Culture Result		ITD Result	Sequencing	No. of nt changes	Final Result	Current Status
			L20B	RD					
1	1	SCID	+ve	+ve	P3SL	P3 VDPV	41	P3 VDPV	
	2		+ve	+ve	P3SL	P3 VDPV	46	P3 VDPV	
	3		+ve	+ve	P3SL	P3 VDPV	49	P3 VDPV	
	4		+ve	+ve	P3SL	P3 VDPV	48	P3 VDPV	
	5		+ve	+ve	P3SL	P3VDPV	55	P3 VDPV	
	6		+ve	+ve	P3SL	P3VDPV	61	P3 VDPV	
	7		+ve	+ve	P3SL	P3VDPV	64	P3 VDPV	
	8		+ve	+ve	P3SL	P3VDPV	73	P3 VDPV	
	9		+ve	+ve	P3SL	P3VDPV	66	P3 VDPV	
	10		+ve	+ve	P3SL	P3VDPV	93	P3 VDPV	
	11		+ve	+ve	P3SL	P3VDPV	68	P3 VDPV	
	12		+ve	+ve	P3SL	P3VDPV	67	P3 VDPV	
	13		+ve	+ve	P3SL	P3VDPV	76	P3 VDPV	
	14		-ve	-ve	–	–	–	-ve	Negative for poliovirus
	15		-ve	-ve	–	–	–	-ve	
	16		-ve	-ve	–	–	–	-ve	

Table 2B): Comparison of Immunological parameters of the SCID child excreting Poliovirus with that of his parameters when he stopped excreting poliovirus (Poliovirus +ve Vs Poliovirus –ve status)

Polio virus Positive		Polio virus Negative			
Lymphocyte Subpopulation	Result % Lymphocyte	Absolute Lymphocytes Count/mm3	Result % Lymphocyte	Absolute Lymphocyte Count / mm3	Biological reference intervals
ALC		3355		9255	2300 – 5400
$CD19^{+/}$ B lymphocytes	3	101	2	185	390-1400
$CD3^{+}$ T lymphocytes	55	1845	72	6664	1400-3700
$CD3^{+}$ / $CD4^{+}$ Th lymphocytes	10	336	12	1111	700-2200
$CD3^{+}$ / $CD8^{+}$ Tc lymphocytes	30	1007	39	3610	490-1300
$CD3^{-}$/ $CD16^{+}56^{+}$ NK Cells	38	1275	25	2314	130-720
Immunoglobulin	**Values**		**Values**		**Normal ranges**
IgG	<1.41 g/L		0.778 g/L		3.5 - 16.2
IgA	< 0.244 g/L		<0.239 g/L		0.17 – 3.18
IgM	0.203 g/L		<0.168 g/L		0.30 – 2.65
IgE	< 15.3 g/L		<4.45 IU/ml		3 – 423

NIVMU 1401: Host-virus interaction and the significance of apoptosis in infections by viruses causing Hand, Foot and Mouth disease.

Madhu Mohanty, Mr. Swapnil Varose

Funding Agency: Intramural

Duration: Jan 2015- Dec 2018

Background: The most common causes of Hand, Foot and Mouth disease (HFMD) are Enterovirus 71 (EV71) and Coxsackie virus A16 (CVA16). Coxsackie virus A6 (CVA6) infections are emerging as a new cause of HFMD. CVA6 has been identified as an emerging pathogen for a series of HFMD outbreaks in Europe, North America, and Asia.

Objectives: to investigate whether CVA6 Indian isolates can infect human neuronal cells *in-vitro* and induce apoptosis.

Work done: Our earlier study have confirmed that CVA6 infects human neuronal cell lines and induce TLRs, cytokines as well as apoptosis. To confirm the mechanism of apoptosis human neuronal cells (SK-N-SH) were infected with two CVA6 strains from patients with different clinical features (**CVA6 isolated from a case of HFMD and a case of AFP).** The mechanism of apoptosis was studied by flow cytometry which showed a marginal increase in expression of cytochrome C and caspases in infected cells suggesting apoptosis. CVA6 infection increased the expression of caspase 7 and 8 genes as studied by real-time PCR. TUNEL and Annexin assay by flow cytometry confirmed that CVA6 induces apoptosis in neuronal cells. Further study by a luminescent assay that measures caspase-3 and -7 activities (Caspase Glow assay) showed a significant increase in caspase 3/7 activity as compared to the uninfected cells (Figure 2A,B). Our study has shown a response in both mitochondrial (Intrinsic) and TNF associated pathway (extrinsic) in neuronal cells infected with CVA6.

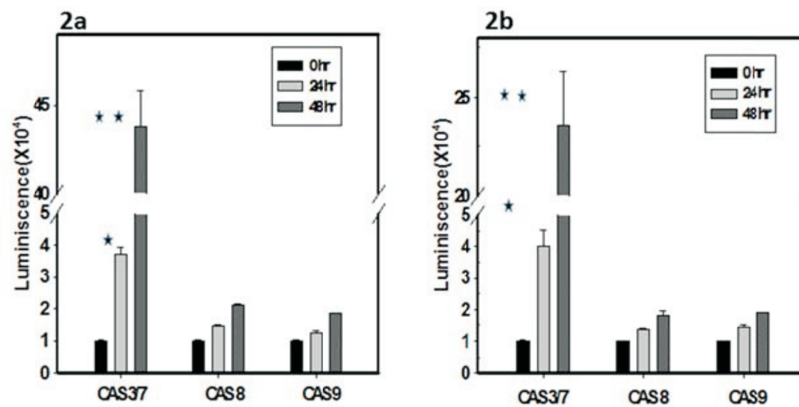

Fig 2: Caspase glow assay to measure caspase activity in human neuronal cells infected with two clinically different CVA6 viruses; 2A) CVA6 S8, 2B) CVA6-2997 at 24 and 48 HPI. (P< 0.01, P< 0.001).

NIVMU 1604: Cytokine responses to EV71 genotypes in cultured human cells

Dr. Madhu Mohanty, Dr. Vinay Saxena, Mr. Swapnil Varose

Funding agency: Intramural

Duration: 2017- 2020

Background: Enterovirus 71 (EV71) is the second most important enterovirus of public health after poliovirus. EV71 causes HFMD, herpangina, neurological diseases with potentially serious complications and its epidemics are on the rise in South East Asia including India. Excessive pro-inflammatory cytokines and chemokine responses were thought to contribute to the severity of the infection. EV71 isolates have been reported at NIVMU from cases of AFP, HFMD and encephalitis in Mumbai, India which has been classified as genotype D, genotype C and a new genotype G (Saxena et al, 2015). Although EV71 outbreaks have been reported in India there has been no study to investigate the infectivity and cytokine/chemokine pattern of the Indian strains/genotypes. Therefore, studies on cytokine/chemokine responses are needed to understand the interplay between host factors, immunopathology, and severity of EV71 (Indian isolates) infection.

Work done & Findings: Our earlier study has shown that human muscle cells do not upregulate inflammatory cytokines in response to most of the EV71 genotypes. In this study, the pattern of cytokine release by human neuronal cells (SK-N-SH cell line) infected with EV71 indigenous genotypes were analyzed *in-vitro* in order to gain information on clinical severity and pathogenesis of these strains.

SK-N-SH cells were infected with C1, C2, G and D genotypes of EV71 isolated at NIVMU and the culture supernatants were tested by Multiplex Luminex bead ELISA. From the two indigenous genotypes (D and G), infection with genotype D did not release any of the inflammatory cytokines as compared to that of the other genotypes whereas infection with genotype G showed significantly higher TNF, IL-6, IL-8, IP-10 production in infected neuronal cells (Fig 3) Results of the study indicated that the Indian isolates of EV71 have tendency to cause neuronal complications.

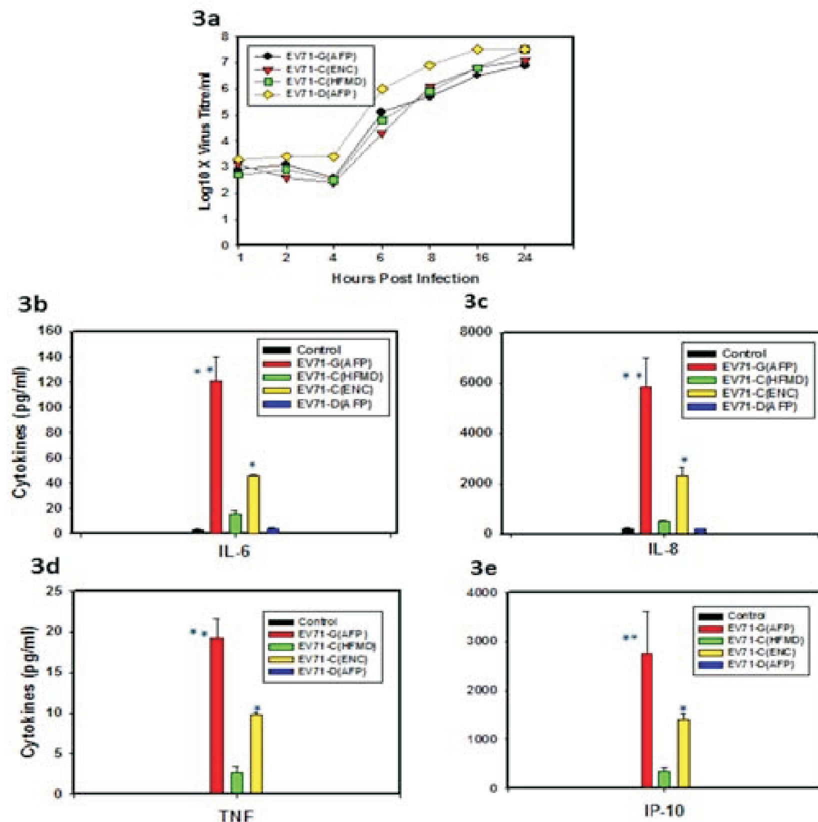

Fig 3: a) Single-step growth curve of EV71 genotypes C, D, G up to 24 HPI, b) IL-6, c) IL-8, d) TNF-? and e) IP10 secretion of human neuronal cells infected with different genotypes of EV71 (Black control uninfected cells, Red - G (AFP), green -C (HFMD), yellow- C (Encephalitis), Blue- D (AFP), ** p<0.01).

14. NIV MU 1705: Investigation of host genetic susceptibility markers to Enterovirus A71 infection in Indian population

Shyam Nandi & Jagadish Deshpande

Funding: Intramural

Duration: 2017-2020

Background: In India, EV-A71 has been isolated sporadically from AFP and encephalitis patients as well as apparently healthy children. However, no major EV-A71 caused outbreaks of HFMD or AFP have been reported from India. The reason for this has not been explored. Recent research reports have identified point mutations (SNPs) in a small number of host genes leading to susceptibility to severe EV-A71 infections. The aim of the present study is to determine the prevalence of markers of genetic susceptibility to EV-A71 in Indian population and to analyze the data in the light of published information to predict public health impact of EV-A71 in the country. The objectives are to study EV-A71 genetic susceptibility markers are located on a number of different genes. Therefore, multiple region sequencing or deep sequencing (NGS) methods are used to identify the SNPs at the specific sites.

Objective 1: To design a rapid and simple method to detect EV-A71 genetic susceptibility markers.

Objective 2: To determine the prevalence of the EV-A71 genetic susceptibility markers in Indian population.

Work done & Findings: Multiplexed single nucleotide polymorphism assays (two) were designed and developed to probe 15 SNPs in 12 different genes. All the SNP's primers position/mobility were checked by Primer Focus reaction. Variation in genetic markers of EV71 susceptibility was checked using samples of healthy persons. The information is used for sample size determination for large-scale countrywide screening. Assay profile is shown in figure 4. The assay is ready for population-based screening for genetic susceptibility to EV-A71 in Indian population. The assay was submitted for patent filing via IPR Cell, ICMR HQ on 31/10/2017.

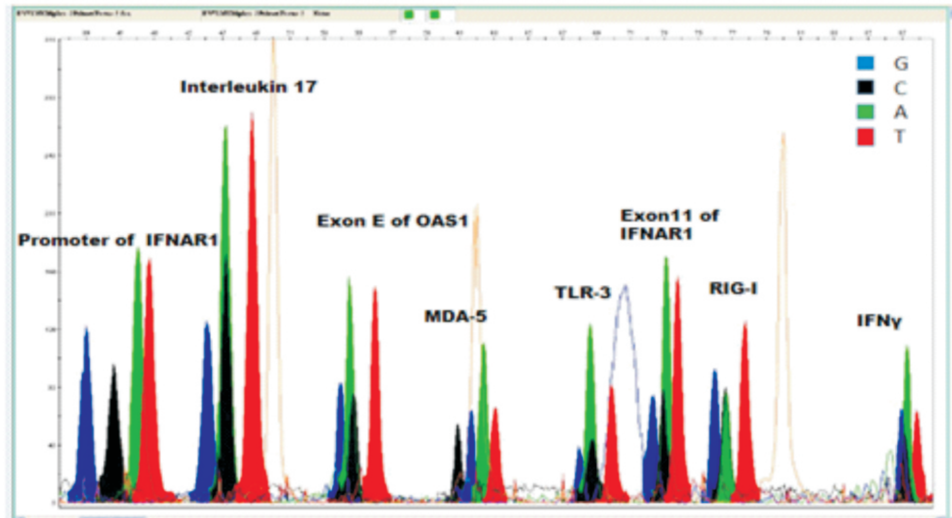

Fig 4: Multiplexed SNP assay for EV71 susceptibility markers. Clear separations of eight different SNPs are shown using primer focus reaction. Gene names are indicated.

Major Achievements:

1. NIV Mumbai Unit has been designated as the Reference Sequence Laboratory by WHO for Measles and Rubella network laboratories in India.

2. The NIV-MU has achieved following scores in WHO Proficiency Test panels for polio, measles, and rubella.

Polio:

- Virus Isolation PT panel - 100%
- ITD PT panel - 100%
- Sequencing PT panel - 100%

Measles and Rubella:

- Serology PT panel - 100%
- Molecular PT panel - 100%

3. The NIVMU has been fully accredited for 2018 and has scored 89% for the WHO accreditation review visit during 12-15 September 2017.

4. The first ICMR-DHR-WHO workshop entitled "Strengthening of Measles and Rubella case confirmation by serology IgM detection and conventional RT-PCR for ICMR VRDLs" with regard to the elimination of Measles and control of Rubella. This was conducted for the six VRDL laboratories from India at NIVMU from19-23 February 2018. A total of 19 participants from VRDL laboratories and DHR along with the faculties from the National Institute of Health, Thailand and WHO participated in this workshop. (Fig. 5)

5. As a follow-up of the above training and as part of the Phase I activity, practice panels for serology and molecular diagnosis were prepared by NIVMU and sent to the six VRDLs.

   They were guided to perform assays, to analyze results and to send reports to WHO, SEARO, DHR, and ICMR. All the labs performed well which will help them to become WHO proficient.

6. Participated in pilot testing of WHO environmental surveillance proficiency test panel received from RIVM, Netherlands. This will be used in future for yearly assessment of WHO polio laboratory network handling environmental surveillance project for polio detection.

Table3: No. of samples tested (virus-wise details)

Virus	Specimens	No. of cases	No. of samples	Test	Output
Polio	Stool	2642	5230	Virus isolation	167 poliovirus
Polio	Sewage	-	297	Virus isolation	208 poliovirus
Measles / Rubella	Serum	335	335	IgM ELISA	Measles positive 220, Equivocal-10 Rubella positive-28, Equivocal-8

Fig 5: Workshop on "Strengthening of Measles and Rubella case confirmation by serology IgM detection and conventional RT-PCR for ICMR VRDLs" at National Institute of Virology, Mumbai Unit, during 19-23 February 2018.

GORAKHPUR UNIT

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Administrative staff	
Mr. Amol Lohbande	Lower Division Clerk
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GKP1001: Diagnostic services for suspected Japanese encephalitis (JE) cases from eastern Uttar Pradesh.

Investigators: VP Bondre, Deval H, Zaman K, Patil G, Sah K, Niraj Kumar & Mittal M.

Funding: Intramural

Duration: Ongoing

Background: NIV Gorakhpur unit undertake routine investigation of clinically suspected acute encephalitis syndrome (AES) cases admitted to BRD Medical College (BRDMC), Gorakhpur and provides diagnosis that guide the management of cases. Although JE is the known cause of AES in the region, our investigations during 2016 confirmed higher positivity for anti- *Orientia tsutsugamushi* (OTs - cause of scrub typhus) IgM antibodies in AES cases. In addition to it, to rule out the antigenic cross reactivity between JE and Dengue (DEN), all the AES cases hospitalized during 2017 were investigated for detection of anti - JE IgM, anti - OTs IgM and Dengue NS-1 antigen by ELISA assays as per the ICMR recommendations. The findings were communicated within 24-36 hrs to BRDMC and concerned State Health authorities. A total of 3662 clinical specimens (CSF and serum) from 2131 AES cases were screened and anti-JE IgM, anti-OTs IgM and dengue NS-1 antigen positivity was detected in 299 (14.0%), 992 (47%) and 97 (5.5%) cases respectively. Among the OT positive cases, anti-OTs IgM antibodies were also detected in 33.5% (108/322) CSF specimens from cases in which either the serum was not available or inadequate for testing.

Maximum AES cases were reported from Gorakhpur district (576) followed by Kushinagar (450), Deoria (321) and a few cases from other 4 districts (Fig. 1). The AES cases started from July, peaked during August to October and declined. Incidence of cases was also noted in the month of November 2017 (Fig. 2). The most affected population was in the age group of 1-5 years (877) followed by 5-10 years (n=655) (Fig. 2). Of the cases, JE was reported maximum from Kushinagar (57) followed by Gorakhpur (47). Children of 5-10 years of age were found maximum affected during September and

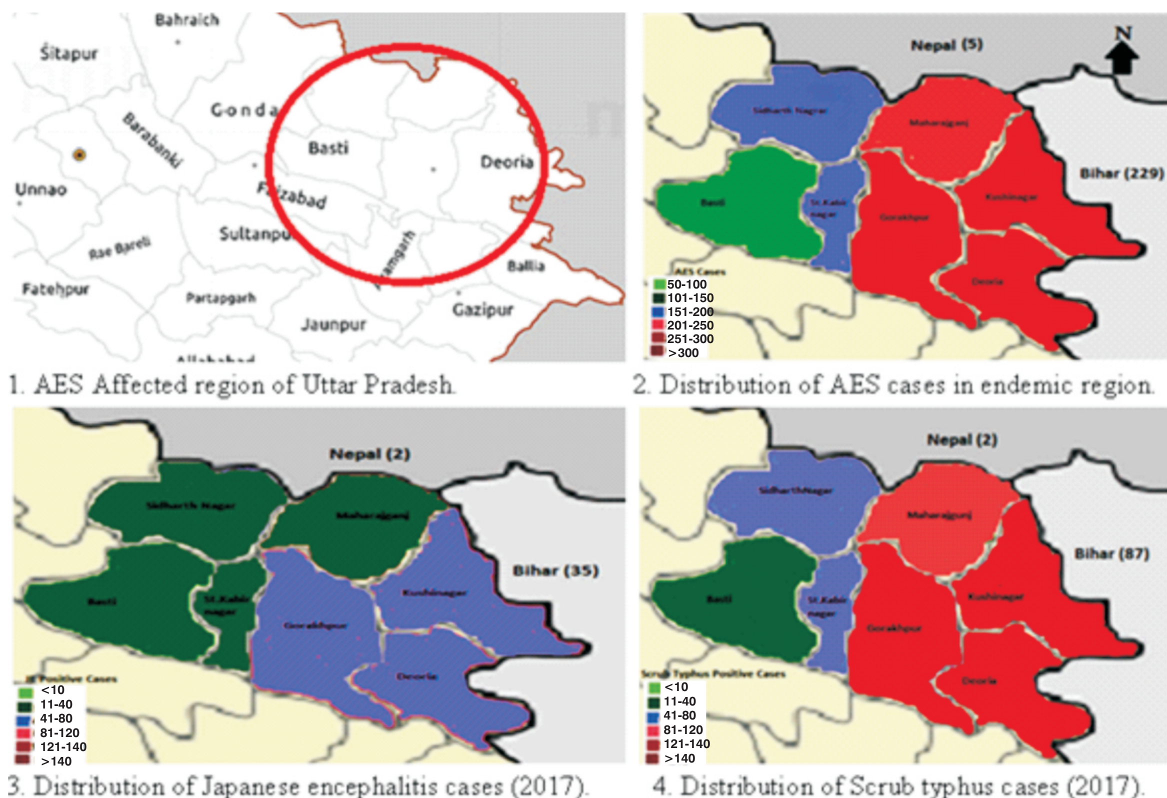

Fig. 1. Grogaphic distribution of AES, JE and Scrub typhus cases in affected areas of UP state.

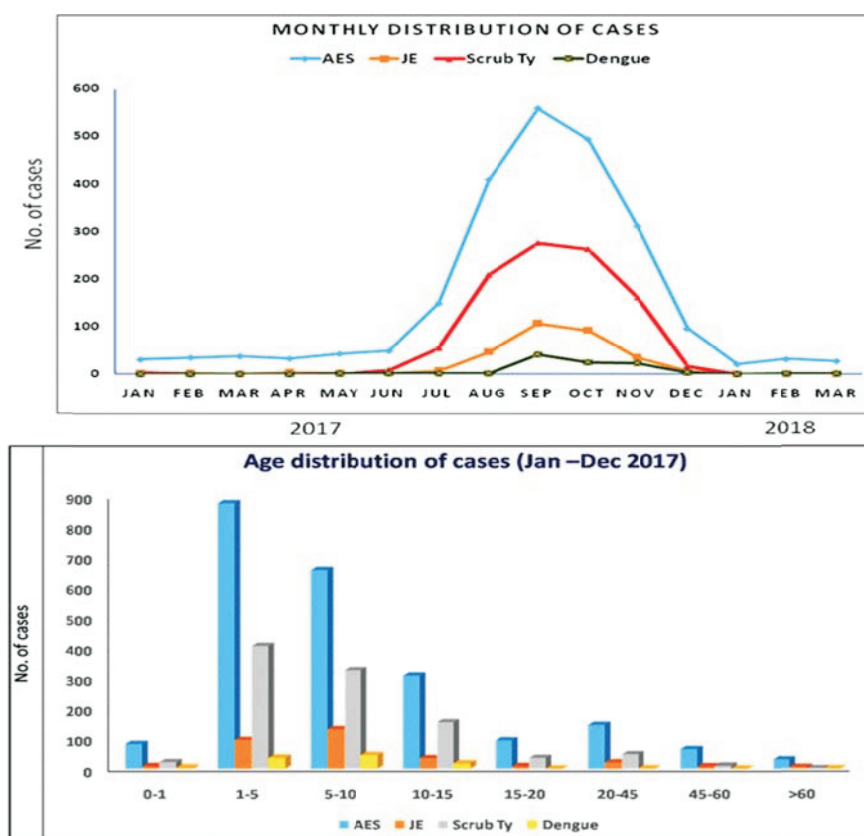

Fig. 2: Seasonal and age group wise distribution of AES cases during 2017.

October. Similarly Scrub typhus (ST) was reported maximum from Gorakhpur (283) followed by Kushinagar and Deoria (172 in each district). Children of age group 1-5 year (405) were affected maximum and cases peaked during August to November. In AES cases, dengue positivity was mostly documented during the months of September (41) and October (25) while a few cases were documented during August and November (Fig. 2). These findings suggest that scrub typhus is associated as one of the important etiological agents amongst AES cases in this region. However, increase in the incidence of JE cases is also alarming, despite good vaccination coverage in the endemic area. Appropriate intervention to control mites and mosquitoes with the focus on increasing the coverage of JE vaccination is the need of the hour.

GKP1501: Etiologic investigations in clinical specimens collected from AES cases from eastern Uttar Pradesh.

Investigators: VP Bondre, Hirawati Deval, Kamran Zaman, Rajeev Singh, AK Pandey, SP Behera, BR Misra, NM Rao, Niraj Kumar & Sanjeev Kumar.

Funding: Intramural

Duration: Ongoing

Globally etiological identification of AES is ascertained to the maximum of 50% cases. JE is historically a known cause of AES in this region. In addition to it, anti-OTs IgM antibodies and genome was detected in about 50% cases investigated during 2016. Hence, to streamline the utility of minimum amounts of available clinical specimens and identification of the cause associated with AES, comprehensive efforts were made. Investigation of cases through the best use of epidemiological, clinical and biochemical parameters collected from each case, a diagnostic algorithm for investigation of JE negative cases hospitalized in BRDMC was developed to investigate the viral as well as bacterial infections (Fig. 3). As per clinical presentation, virological diagnosis in CSF samples were done by PCR assay for Herpes simplex virus (HSV 1/2/7), Cytomegalovirus (CMV), Varicella Zoster virus (VZV), Epstein Bar

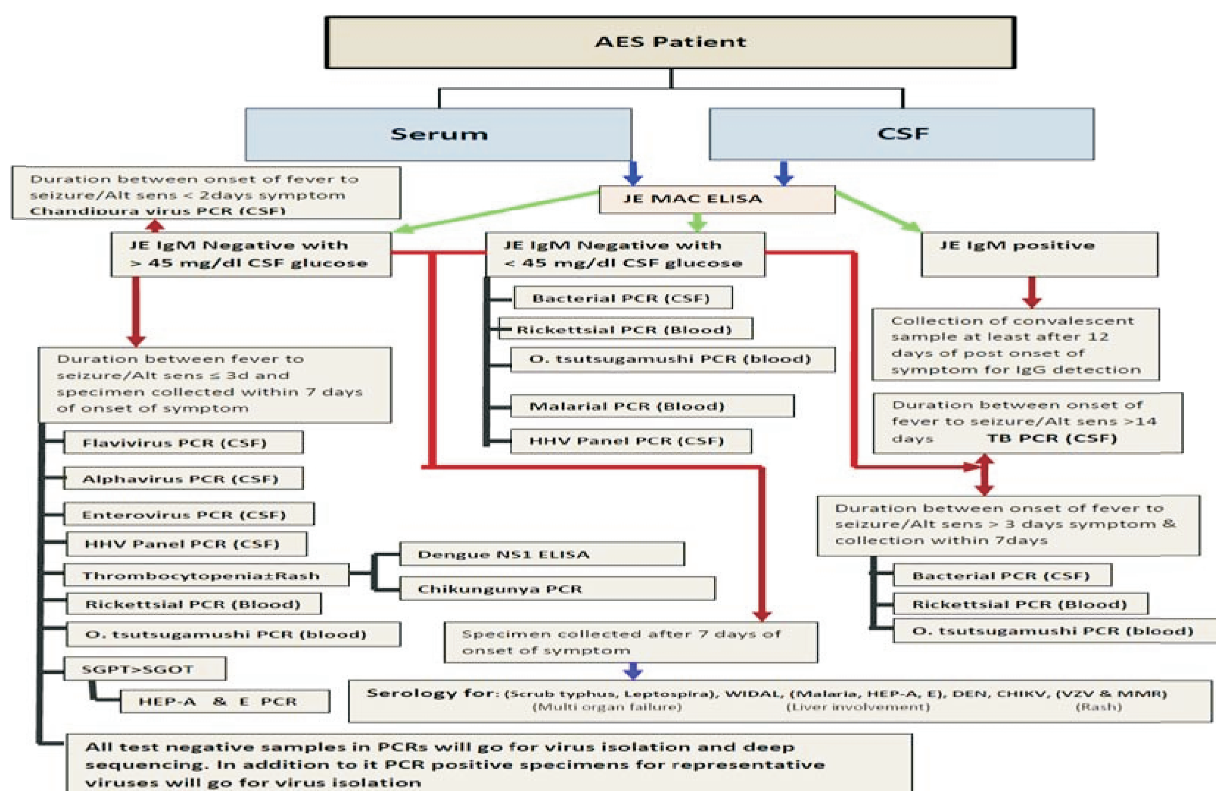

Fig 3. Laboratory diagnostic algorithm developed for investigation of AES cases hospitalized in BRDMC.

virus (EBV), Enterovirus (human, bovine, porcine), Parvovirus P4, Parvovirus B19 and Flaviviruses (including majority of the human infectious viruses *viz.,* JEV, WNV, DENV, tick borne encephalitis (TBE) virus, ZIKA virus, etc.). Depending on the CSF and serum biochemical characteristics, the JE negative CSF samples were also tested to detect bacterial infections including *Streptococcus pneumoniae, Neisseria meningitidis* & *Haemophilus influenzae* by multiplex PCR.

Clinically suspected viral encephalitis cases with abnormal brain functions (EEG) and brain pathology (MRI) were found positive by PCR for HSV-1 (4/314: 1.2%), VZV (3/294: 1%), EBV (1/294, 0.3%) while HSV-2, HSV-7, CMV and flaviviruses were not detected in any of the cases (Table 1). Cases selected on the basis of rash and anemia tested positive for enteroviruses (6/241: 2.4%) by RT-PCR, Parvovirus P4 (2/39: 5.1%) while Parvovirus B19 was not detected in any of the cases investigated. CSF samples from the selected JE negative cases suspected of bacterial infection (meningeal symptoms) also were investigated for bacterial infection including *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae* by multiplex PCR, but none of them were detected. In 7.5% (41/542) of the whole blood specimens from JE and OTs negative AES cases with rash and thrombocytopenia, infection with rickettsia of spotted fever group was detected by PCR. Genome amplification of OTs in 21.2% (504/1170) cases positive by anti-OTs IgM ELISA further strengthen the finding on high OTs positivity by sero-diagnosis. Even in CSF where whole blood was not available, 30.4% (14/66) was positive for OTs by PCR. These findings suggest major contribution of OTs and other rickettsia in the non-JE AES occurring in the region (Table 1).

Table 1: Summary of diagnostic finding of AES cases during 2017-18

S. No.	Etiologies	Specimens	Assay(s)	Outcome	% Positivity	Positive in tested	Cumulative Positivity (%)
1.	**JEV** Serum	CSF IgM ELISA	IgM ELISA 241/1810	179/1849 13.3	9.7	299/2131	14
2.	**DENGUE** Serum	Serum IgM ELISA	NS1 ELISA 4/602	93/1169 0.6	8	97/1771	5.5
3.	O. tsutsugamushi Serum	CSF IgM ELISA	IgM ELISA 884/1780	108/322 49.6	33.5	992/2102	47.2
4.	**VZV**	CSF	PCR	3/294	1.02	8/314	2.54
5.	**HSV-1**	CSF	PCR	4/314	1.27		
6.	**HSV-2**	CSF	PCR	0/314	0		
7.	**HSV-7**	CSF	PCR	0/294	0		
8.	**EBV**	CSF	PCR	1/294	0.34		
9.	**CMV**	CSF	PCR	0/294	0		
10.	**Parvovirus P4**	CSF	PCR	Feb-39	5.12	2/39	5.12
11.	**Parvovirus B19**	CSF	PCR	0/39	0		
12.	**Flavivirus Genus (JE/DEN/WNV/Zika)**	CSF	RT-PCR Generic	0/148	0	-	-
13.	**Enterovirus Generic**	CSF	RT-PCR Generic	6/241	2.49	6/241	2.49
14.	**H. influenzae**	CSF	PCR	0/32	0	-	-
15.	N. meningitides	CSF	PCR	0/32	0	-	-
16.	S. pneumonia	CSF	PCR	0/32	0	-	-
17.	O. tsutsugamushi	Blood	PCR	504/1170	21.2	511/1178	43.4
18.		CSF	PCR	14/66	43		
19.	**Other Rickettsia**	Blood	PCR	41/542	7.56	41/542	7.56

GKP 1502: Epidemiological and clinical correlation of acute encephalitis syndrome cases with JE, non-JE viral and other AES associated etiologies from eastern Uttar Pradesh.

Investigators: VP Bondre, Kamran Zaman, Avinash Deoshatwar, Hirawati Deval, Rajeev Singh, Niraj Kumar, Asif Kavathekar & Vishal Nagose

Funding: Intramural

Duration: 2015-2018

Background: JE, OTs, other rickettsia and dengue have emerged as the associated causes with about 70% AES cases investigated during 2017 as these infections were documented in 14, 47.2, 7.56 and 5.5% cases respectively. However, clinical outcome in lab confirmed JE, OTs, Rickettsia and dengue cases was largely similar independent of the associated etiology. Thus, to define any clinical, biochemical and / or pathologic features that might be helpful in differentiating these infections, clinical, biochemical treatment and physiological parameters were collected from all the cases hospitalized during 2017 from the day of hospitalization to recovery / death. A total of 2247 AES cases were hospitalized in BRDMC during the year. Mean age of JE cases was higher (10.49 years) than the OTs infected cases (8.39 years) indicating relatively younger group is affected by JE. During 2017, overall AES case mortality was 22.8%, which is 4.2% lesser than the previous season (26.4%). In all the AES cases, fever appeared to be the first clinical symptom followed by headache (96.7%), vomiting (99.2%) and abdominal pain (96.8%). Up rolling of the eyeball and frothing from mouth was recorded as the predominant neurological features in almost all AES cases (>99%) (Table 2). Neurological examination marked severe brain injury (GCS=3-8) in a large number of patients (AES 46.8%; JE-AES 54.6% and OTs-AES 43.4%). The SGOT, SGPT and CSF-protein levels were significantly higher in OTs-AES patients as compared to JE-AES patients (for SGOT mean±SEM 177±5.6 vs.131.8±9.4, $p<0.001$; for SGPT mean±SEM 116.53±4.05 vs. 89.3±10.4, $p<0.01$; for CSF-protein mean±SEM

116.02±2.6 vs. 99.6±4.49, p<0.01). However, the CSF-glucose level was significantly lower in OTs-AES patients as compared to JE-AES patients (mean±SEM 61.4±0.94 vs. 72.4±2.44, p<0.001) as shown in Fig. 4. In addition to it, the JE, dengue and OTs negative 541 AES cases were investigated for infection with other rickettsial (Rick) Spp. Rick DNA was detected in whole blood collected from 41 AES cases. The comparison of clinical and biochemical parameters between Rick positive and negative cases showed that duration of onset of fever, total leukocyte counts (TLC) and serum urea were significantly higher in Rick positive cases than Rick negative cases (for onset of fever median 6 day [range 3-15day] vs median 5day [range 1-61day], p<0.05; for TLC median 19500 cells/mm3, range 6200-37800 vs median 12900cells/mm3, range 600-49900, p<0.01; for serum urea median 55.3 mg/dl, range 19.2-424.6 vs median 35.3mg/dl, range 13.6-316.4, p<0.01). However, the platelet count (PC) was significantly low in Rick positive cases than the negative cases

Table 2: Comparison between JE-AES and OTs-AES patients with respect to the demographic, clinical and biochemical features.

Parameters	Findings in AES cases [n=2247]	Findings in JE cases [n= 299]	Findings in OTs cases [n=992]
Sex Ratio [M/F]	1173/1074	151/148	495/505
Age Mean [Years]	10.49	10.54	8.39
Mortality [%]	22.9 [516/2247]	25.4 [76/299]	13 [129/992]
High Grade Fever [%]	75.8 [1401/1846]	71.4 [185/259]	77.4 [664/857]
Fever [in days] before hospitalization	7.55 [n=2106]	7 [n=284]	8.28 [n=924]
Headache	96.7 [381/394]	95.3 [61/64]	96.3 [187/194]
Vomiting	99.2 [1277/1287]	99.3 [160/161]	99.1 [639/645]
Abdominal Pain	96.9 [279/288]	89.6 [26/29]	97.2 [177/182]
Altered level of consciousness	67.2 [1511/2247]	71.9 [215/299]	63.7 [632/992]
Up rolling of Eye Boll	99.6 [1397/1402]	100 [201/201]	99.5 [648/651]
Frothing from mouth	97.9 [573/585]	98.7 [78/79]	97.1 [204/210]
Glasgow COMA scale = 7	92.2 [1602/1737]	91.8 [225/245]	95 [781/822]
Neck rigidity	9.95 [184/1848]	8.3 [22/265]	10.4 [86/825]
Kerning's sign	8.13 [150/1843]	6.8 [18/265]	8.86 [73/823]
Hepatomegaly	15.8 [328/2073]	13.6 [38/279]	23 [209/907]
Splenomegaly	2.6 [53/2047]	2.17 [6/276]	4.05 [36/888]
Hemoglobin >10gm/dl	40.4 [774/1913]	45.9 [118/257]	28.2 [238/844]
Total leukocyte count >13000 cells/mm^3	49.3 [946/1915]	48.4 [124/256]	51.4 [435/845]
Total leukocyte count in CSF >5 cells/mm^3	80.7 [1376/1704]	88.2 [209/237]	92.2 [710/770]
Platelet count <$1x10^5$ cells/mm^3	62.8 [1198/1907]	69.1 [177/256]	47.1 [397/842]
SGOT >45 IU/L	77.8 [1430/1838]	76.9 [187/243]	88.3 [713/807]
SGPT >45 IU/L	58.5 [1080/1835]	47.4 [116/243]	77.4 [624/806]
Urea >40gm/100ml	41.9 [766/1827]	41.1 [99/241]	42.2 [346/819]
Creatinine>1gm/100ml	16.2 [303/1869]	13.8 [34/246]	16.2 [133/822]
CSF protein [>45mg/dl]	73.7 [1278/1733]	83.6 [205/245]	91.3 [707/774]
CSF glucose [>75 mg/dl]	30.2 [523/1730]	36.7 [90/245]	23.1 [179/776]

(median 41000 cells/mm3, range 11000-413000vs median 245000 cells/mm3, range 10000-891000, p<0.001). Further, comparison between Rick positive and OTs positive cases showed that TLC, polymorphonuclear leukocytes (PML) and serum urea were significantly higher in Rick positive cases (for TLC median 19500 cells/mm3, range 6200-37800 vs median 13350cells/mm3, range 1400-81000, p<0.001; for PML median 72.7%, range 36.2%-89% vs median 56%, range 3.7%-92.6%, p<0.001, for serum urea median 55.3 mg/dl, range 19.2-424.6 vs median 36.5mg/dl, range 2.4-282.5, p<0.05). However, the duration of fever onset, lymphocytes count (LC) and PC were significantly lesser in Rick positive cases than OTs positive cases (for onset of fever median 6-day, range 3-15 days vs median 7 days, range 1-31 days, p<0.05; for LC median 20.8%, range 7.9%-56.7% vs median 35.6%, range 4.7%-80.3%, p<0.001; for PC median 41000 cells/mm3, range 11000-413000 vs median 95000cells/mm3, range 4000-975000, p<0.001). These findings clearly indicate that the critical demography, age group, clinical and physiological analysis of the cases can be helpful in differentiating the AES associated with different etiologies. The age group, mortality, duration of illness, thrombocytopenia, CSF protein and glucose levels along with serum urea levels emerged as differentiating factors in different infections.

GKP 1503: Isolation, identification and genetic characterization of viruses isolated from acute encephalitis syndrome cases from eastern Uttar Pradesh.

Investigators: VP Bondre, Deval H, Singh R, V Janardhan, Ravishankar Singh & Sanjeev Kumar

Funding: Intramural

Duration: ongoing

Virus isolation is regarded as the gold standard in the investigation of viral etiologies. To improve upon the diagnosis of AES, 71 CSF specimens collected from suspected viral encephalitis cases and 10 dengue RT-PCR positive serum specimens were processed for virus isolation in different cell lines *viz*., PS, BHK 21, and Vero-E6 cells. None of the cultures showed any cytopathic effect (CPE) till the 4th passage. In addition to the above, 6 enterovirus PCR positive samples were attempted for cell culture isolation in RD cell line, but only one sample showed CPE in the 3rd passage after 48hr of incubation. Confirmatory studies by RT-PCR and sequencing is in progress.

GKP 1504: Etiological investigations of non-AES referred cases from Gorakhpur region.

Investigators: Hirawati Deval, Niraj Kumar, SP Behera, Kamlesh Sah, Gajanan Patil, Sanjeev Kumar, AK Agrawal & VP Bondre

Funding: Intramural

Duration: 2015-2018

Apart from diagnostic services to referred AES cases, NIV Gorakhpur unit also provided diagnosis to non-AES cases referred from

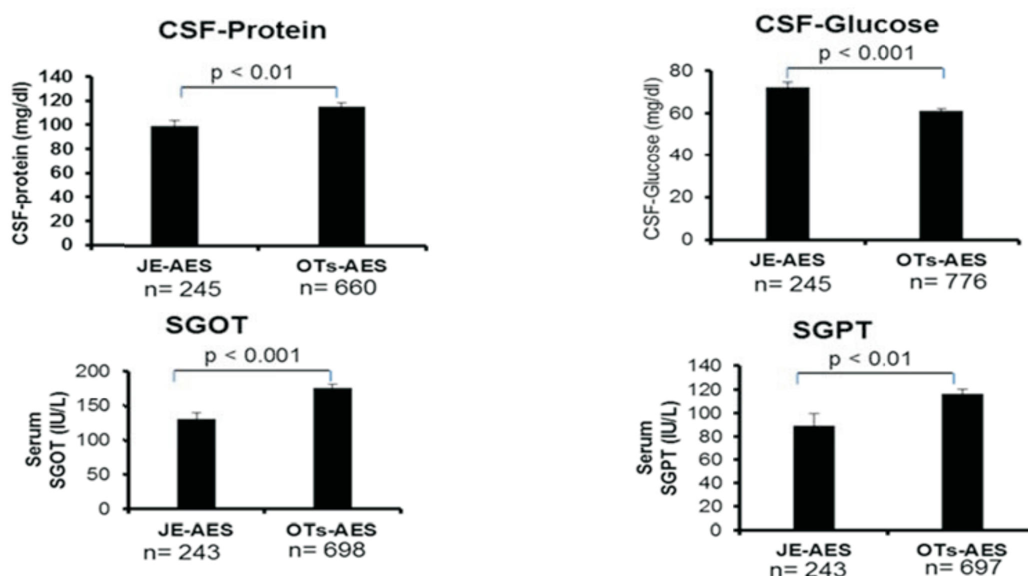

Fig. 4. Comparison of biochemical features between JE and OTs positive AES cases.

BRDMC and other tertiary care centers in the region. Depending on the clinical diagnosis, 147 clinical specimens (97 CSF and 50 blood / serum) were referred for investigations for JE, ST, DEN, HSV, VZV, EBV and Measles infections. JE was detected in 5.15% (5/97) of CSF and 20% of serum (10/50); ST in 39% of sera (14/36) while anti-dengue IgM tested negative in 21 sera. Anti-Measles IgM antibodies detected in 1/21 sera. Molecular diagnosis of these specimens for HSV-1/2, VZV, CMV and EBV in CSF specimens detected HSV-2 in 2/92 (2.17%) while VZV and EBV were detected in one each of the 92 (1.08%) CSFs tested. In addition to it, sera from clinically suspected dengue cases admitted in Gorakhnath Hospital were referred for identification of dengue serotypes associated with outbreaks occurring in the region. Phylogenetic analysis of complete envelope gene sequence directly amplified from sera suggests circulation of three genetically distinct strains belonging to the cosmopolitan genotype of dengue virus serotype 2 (Fig. 6). Further sequence analysis clearly suggests their close genetic relationship with DENV-2 strain recently isolated in Singapore and Delhi, probably introduced through febrile travelers. In addition to it, studies on 33/48 (68.7%) RT-PCR positive dengue fever cases are in progress.

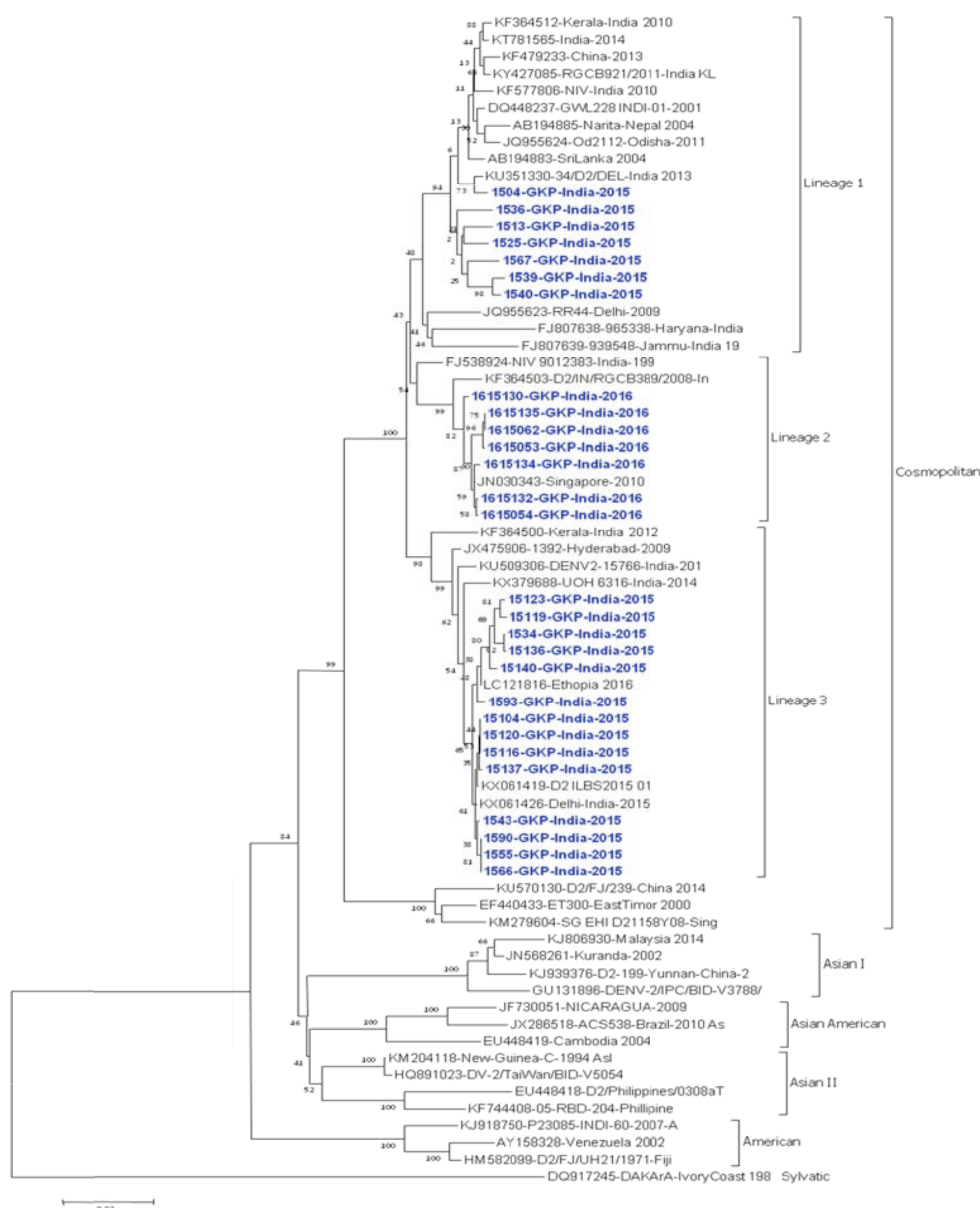

Fig. 6. Phylogenetic analysis of Dengue serotype 2 complete envelope gene sequences directly amplified from patient's sera and isolates.

GKP1702: Detection of anti-JEV IgM in urine samples of Japanese encephalitis-acute encephalitis syndrome (JE-AES) patients.

Investigators: Rajeev Singh, Niraj Kumar, Kamran Zaman, Deval H, Nagose V & VP Bondre

Funding: Intramural

Duration: Ongoing

JE diagnosis is mainly based on detection of virus or IgM antibody primarily in CSF and/or serum collected during acute phase of illness. However, lumbar puncture is a difficult task and expertise is not available in peripheral health settings. In addition to it, mainly pediatric age group is affected by JE and blood collection encounters a number of difficulties. Therefore, there is a need for non-invasive specimens which can be explored in disease diagnosis. Number of laboratories worldwide explored use of non-invasive body fluids *viz.*, urine has been successfully explored in molecular diagnosis of many viral infections including Zika, DENV, CHIKV, TBE, WNV, BK, JC, Mayaro, CMV, Hepatitis B and HEV. To determine the utility of urine specimens collected during the acute phase of illness, a study was carried out on urine specimens collected from hospitalized, lab diagnosed JE IgM positive cases from BRDMC.

Urine samples from 136 JE cases were simultaneously processed by RT-PCR / nested PCR (140μl) and IgM ELISA (10μl). Anti-JE IgM antibodies were detected in 22/136 (16.17%) cases while viral RNA was not detected in any of the cases. To further explore the utility of urine in diagnosis, it was concentrated (10 and 20 – fold) by ultra-filtration using vivaspin2 (Sartorius, Inc.) with 10kDa cutoff. Use of different concentrations for detection of IgM antibodies by ELISA yielded positive results in 34 JE cases (18 at 10X and additional 16 at 20X concentration). Out of 136 JE patients, 56 (40%) tested positive for anti-JEV IgM in urine samples after concentration. These experiments were repeated twice which confirmed IgM positivity in 22, 18 and 16 cases at neat, 10X and 20X concentrations respectively. The level of anti-JEV IgM antibodies in serum didn't correlate with its presence in urine. However, the concentration of anti-JEV IgM in urine showed significant correlation with its level in CSF samples ($r = 0.345$, $p<0.05$). Among the 136 patients investigated, anti-JEV IgM was detected in CSF of 82 cases and in serum of 119 cases. Further clinical data analysis of these cases did not correlate the present of IgM antibodies in urine with disease severity. However, the cases tested positive for IgM antibodies in urine showed higher concentration of IgM antibodies in both CSF and sera than the cases tested negative for IgM antibodies in urine (for serum mean P/N ratio± SEM12.55±0.85 vs 8.27±0.61, $p<0.001$; for CSF mean P/N ratio± SEM 15.49±1.28 vs 8.82±1.09, $p<0.001$). Further studies on duration of IgM detection post infection, any correlation with CNS pathology or damage to non-CNS organs is in progress. Standardization of this assay based on use of non-invasive body fluids needs more cases to be incorporated and investigated for different parameters.

GKP1703: Case based entomological investigation in AES affected area of Gorakhpur region.

Investigators: Brij Ranjan Misra, Vijay Kumar, S P Behera &Vijay Bondre

Funding: Intramural

Duration: 2016-2017

Our investigations during 2016 AES season established association of OT and Rickettsia infection with AES cases occurring in the endemic region. Considering the primary role of different arthropods ectoparasites (ticks, mites, fleas and louse) in transmission of these bacterial infections along with a variety of human infectious viruses and protozoa, case based entomological survey was carried out to substantiate their role in the natural cycle. Upon diagnosis, mites were collected from rats in the vicinity along with ticks from different domestic animals during September and October, 2017. A pilot study was performed on 50 AES cases from villages located in the Chargawan, Bhathat and Pipraich blocks of Gorakhpur district. Engorged arthropods were separated from the body of animals. Arthropods specimens were identified according to their morphological keys and the identified pools were subjected to molecular diagnosis for Flaviviruses, *Orientia tsutsuga-mushi*, Rickettsia genus and *Ehrlichia*/*Anaplasma* genus using standard reagents. Out of 307 pools tested, 4 pools tested positive for *O. tsutsugamushi*, 18 pools for rickettsia and 39 pools for Anaplasma/Ehrlichia genus (Table 3). The positivity of *O. tsutsugamushi* in chigger mite was 16.6% as this region is endemic for scrub typhus. One pool of *H. suis* and *X. cheopis* tested positive for OTs. Among the 18 rickettsia positive pools, 3 pools were identified as *Rickettsia felis* by sequencing.

Table 3: Table showing arthropods and the assosciated pathogens detected by PCR assays.

Arthropod Species (no of pools tested)	Flavivirus	O. tsutsugamushi	Rickettsia genus	Ehrlichia/ Anaplasma
Rhipicephalus (Boophilus) microplus **(84)**	0	0	3	4
Rhipicephalus sanguineus **(1)**	0	0	0	1
Dermacentor auratus **(1)**	0	0	0	0
Hyalomma Kumari **(5)**	0	0	0	0
Haematopinus suis **(26)**	0	1	3	6
Pediculus humanus capitis **(70)**	0	0	1	5
Polyplax spinulosa (6)	0	0	0	1
Echinolaelaps echidninus **(1)**	0	0	0	0
Leptotrombidium deliense **(12)**	0	2	0	1
Ornithonyssus bocoti (49)	0	0	5	13
Xenopsylla cheopis (52)	0	1	6	8
Total (307)	0	4	18	39

GKP1601: Setting up of AES cell at Baba Raghav Das Medical College, Gorakhpur.

Investigators: Mittal M, VP Bondre, Deval H, Murhekar M, Kamran Zaman & AES cell group

Funding: Extramural (ICMR)

Duration: 2016 - 2018

'AES cell' was established on the recommendations of ICMR to streamline the process of clinical specimen collection, distribution for different investigations and storage for future research on AES cases. The clinical and epidemiological data set along with different lab investigations and findings was created for each case. Apart from investigating all the hospitalized AES cases primarily for JE, ST, and dengue infections, the negative cases were also investigated for other known encephalitic etiologies including rickettsia and neurotropic viruses by different serological and molecular techniques. Genetic characterization of ST and rickettsia was carried out to define prevalence and circulation of different strains and to define their genetic relationship with worldwide identified strains. All the OTs-IgM positive whole blood specimens and CSF (if whole blood was unavailable) were processed by standard universal PCR followed by nested PCR to detect 456 bp product from 56kDa protein (outer membrane) coding gene of all OTs serotypes. The percentage positivity was 21.2% (14/66) in CSF (tested in cases where adequate sera were not available for diagnosis) and 43% (504/1170) in whole blood specimens. The phylogenetic study was carried out using reference sequences (GenBank) and 19 representative sequences from OTs positive AES patients. The genetic analysis suggests that most of the Gorakhpur sequences clustered in JG related serotype and 3 in Karp serotype while 2 sequences grouped with the Kato serotype of OTs (Fig. 6). In addition to it, the OTs IgM / PCR negative 541 cases with signs of rash and multi-organ involvement were also investigated for infection with Spotted Fever Group (SFG) of Rickettsia. Genetic analysis of SFG specific genome sequence amplified from 41 cases suggests prevalence of multiple strains of Rickettsia *viz.*, *R. conorii*, *R. felis* and *R. parkeri* associated with AES cases (Fig. 7).

GKP 1701: Genetics of susceptibility of infected children from Uttar Pradesh to Japanese encephalitis virus.

Investigators: Hirawati Deval, Alagarasu K, VP Bondre & Mittal M.

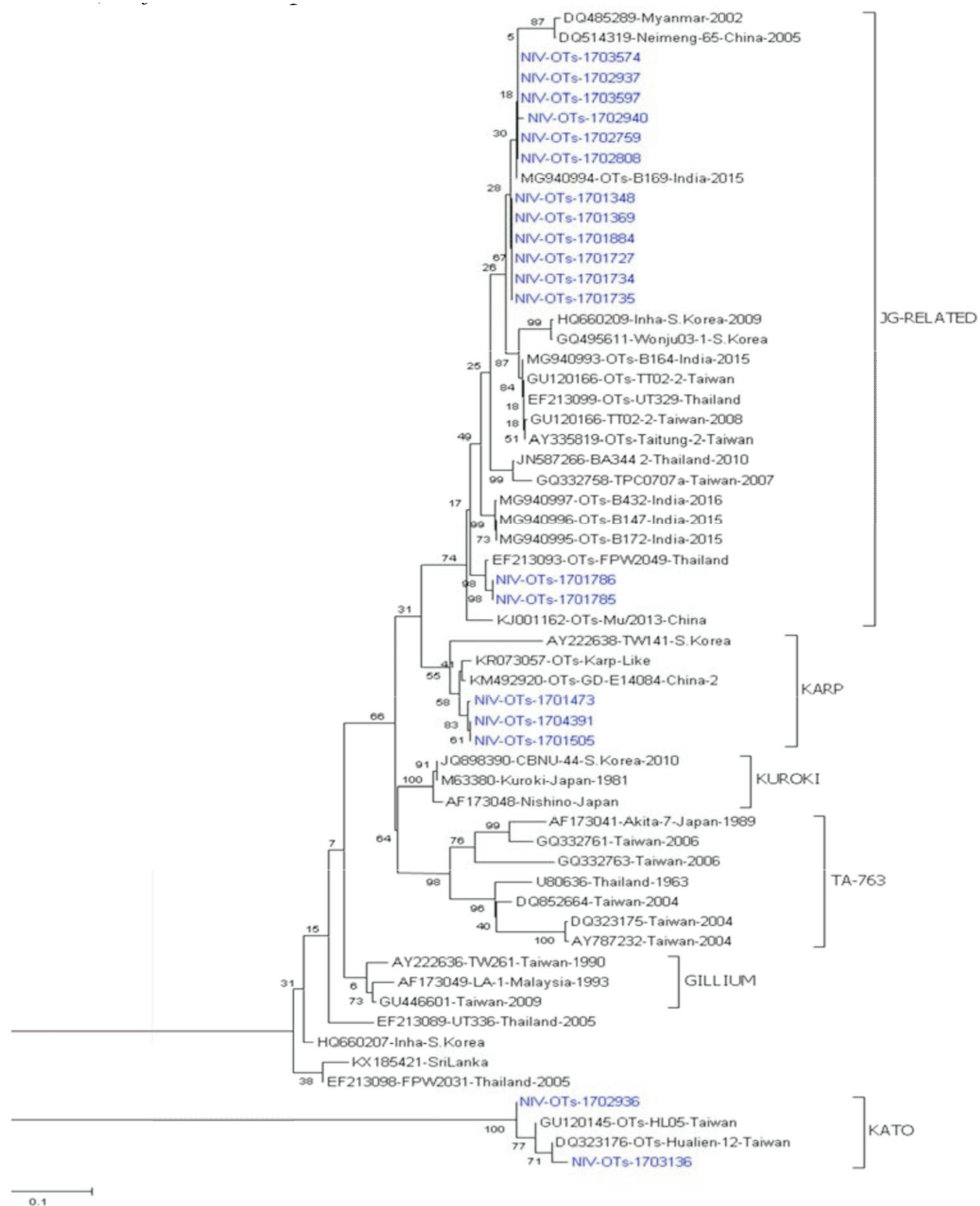

Fig. 7. Phylogenetic tree of *Orientia tsutsugamushi* based on the nucleotide sequences of 56-kDa cell surface antigen gene.

Funding: Extramural-ICMR Neuroscience Task Force

Project Duration: 2017-2020

JEV contributes to approx. 8-10% of AES cases in UP. The asymptomatic to symptomatic disease ratio is reported to be in the range of 25-1000:1. About 70% of the symptomatic infections manifest as encephalitis of which 30% are fatal. Clinical outcome of JE is influenced by host factors, virus and environment. There are only a few reports on the role of host genetic factors in the development of encephalitis in JEV infected children. Present work was carried out to study single nucleotide polymorphisms in genes coding for pattern recognition receptors, inflammatory mediators and receptors and matrix metalloproteinase and its association with AES caused by JEV in children from UP. During the study period, 197 apparently healthy controls without any history of encephalitis and 97 JE cases were recruited and genomic DNA was extracted from blood.

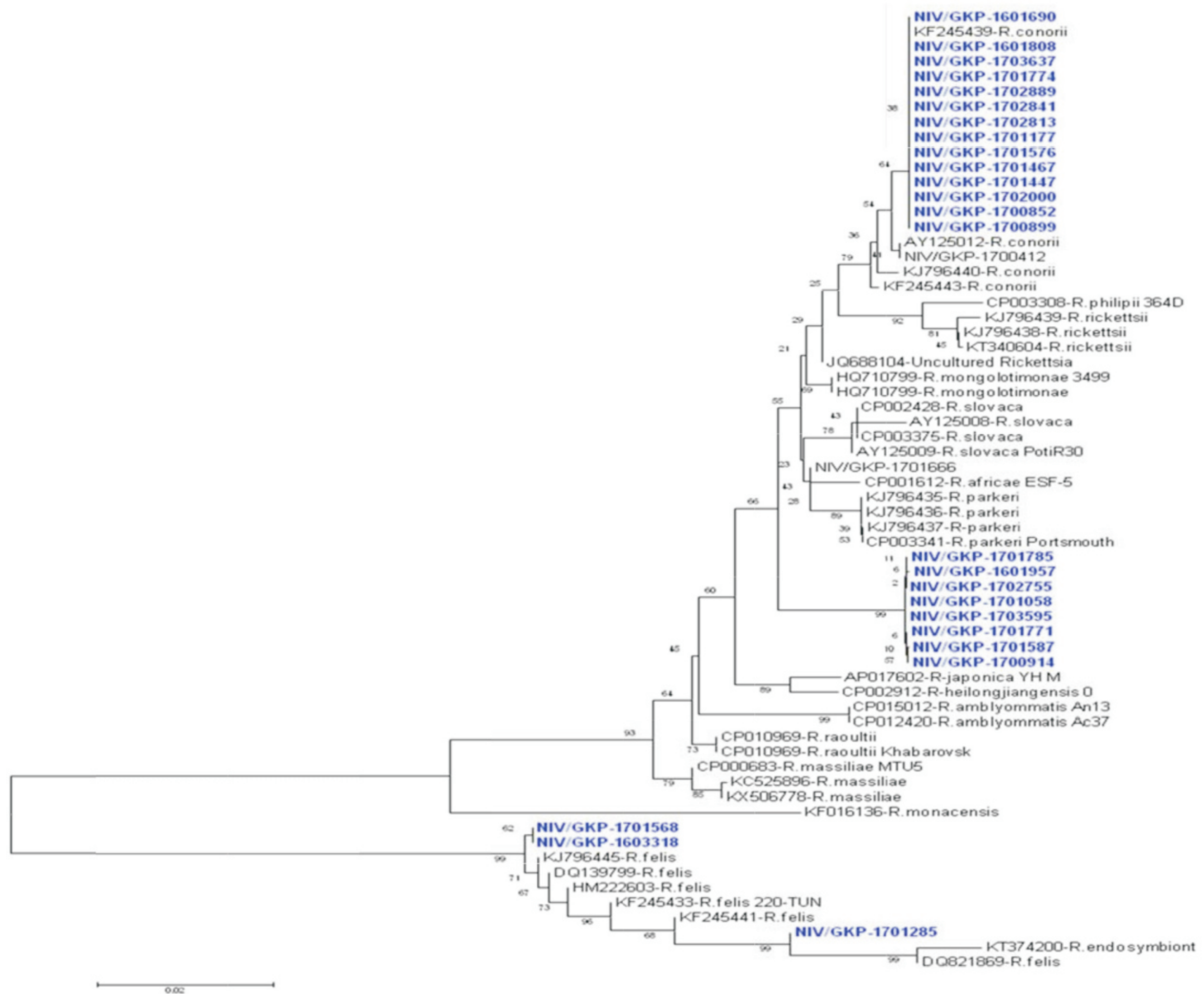

Fig. 8. Phylogenetic tree of SFG Rickettsia directly amplified from whole blood of AES cases.

Genotyping of *TNFA*-308 was performed by allele specific PCR while genotyping of *IL10* -592, *IFNG* +874, *CCL2* -2518, *TLR3* rs3775290 and *OAS1* rs10776471 was performed by PCR-RFLP based methods in 87 encephalitis and 37 healthy control DNA samples. Genotyping of *IL10* -1082 has been performed by allele specific PCR in 38 samples and *OAS1* rs1131454 genotyping was done by PCR-RFLP in 95 samples.

Genotype frequencies of *TNFA* -308, *IL10* -592, *IFNG* +874, *CCL2* -2518, *TLR3* rs3775290, *OAS1* rs10776471 and *OAS1* rs1131454 in encephalitis cases and healthy controls were provided in Table 3. The frequencies of *TNF*α-308 G/A genotype, *IL10* -592 C/C genotype, *CCL2* -2518 A/A and G/G genotype, *TLR3* rs3775290 G/G genotype, *OAS1* rs10776471 G/G genotype and *OAS1* rs1131454 G/G genotype were higher in encephalitis cases while the frequencies of *IL10* -592 A/A genotype, *CCL2* -2518 A/G genotype, *TLR3* rs3775290 G/A genotype and *OAS1* rs10776471 A/G genotypes were higher among healthy controls. The frequency of OAS1 rs10776471 G/G genotype was significantly higher in encephalitis cases and was not observed in healthy controls (P = 0.022). Further sample collection and studies are in progress.

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BNU8801: Surveillance of dengue, chikungunya and Japanese encephalitis cases from Bangalore city, urban, rural and neighboring areas under NVBDCP.

Investigators: Ashok M, Manjunath MJ, Hanumaiah

Funding Agency: Extramural (NVBDCP)

Duration: Ongoing

Background: The project is an umbrella program for detection of vector borne diseases. The transmission of vector borne diseases depends on the prevalence of infective vectors and human vector contact, which is further influenced by climatic factors, sleeping habits of humans, density of vectors etc. The program covers six diseases of which JE, dengue, & chikungunya are performed in the Bangalore unit. In Karnataka, Bengaluru unit is one among the 32 sentinel surveillance centers for dengue & chikungunya.

Objectives: Serological investigation of dengue & chikungunya infected cases from urban and rural areas of Bangalore district.

Work done & Findings: During the reporting period, Bangalore unit has investigated 2320 serum and 102 CSF samples received from rural and urban areas of Bangalore district. Tests revealed 33.75%, 10.5%, 24.91% cases positive for anti-IgM antibodies for dengue, chikungunya and mixed positivity. Among dengue positive cases, 55% were males and 47% of cases belonged to <15year and the rest >15 years of age group. Among Chikungunya positive cases, 54% were males and 70% of cases belonged to >15 years of age group. Increase in infection was observed from June, peaked in August and declined (Fig.1). Of the 102 CSF samples, five cases tested positive for anti-JE IgM antibodies.

BNU1604: Congenital Rubella Syndrome (CRS) Surveillance.

Investigators: Ashok M, Hanumaiah, Manjunath MJ

Funding Agency: ICMR

Duration: Ongoing project

Background: In India, reliable estimates of CRS burden are not available. WHO recommended options for assessing the disease burden are (A) establishing nationwide CRS surveillance to estimate the disease burden (B) investigating rubella outbreaks to describe rubella cases by time, place and person and (C) conducting sero-surveys to document the population immunity. The CRS surveillance focuses on identifying infants of 0-11 months of age attending health facilities and testing them for rubella infection.

Objective: To establish a facility-based surveillance for CRS in selected medical colleges/hospitals in different parts of country to monitor the time trends of the disease. Continued surveillance in these facilities for 7-10 years will generate data about the impact of rubella vaccination.

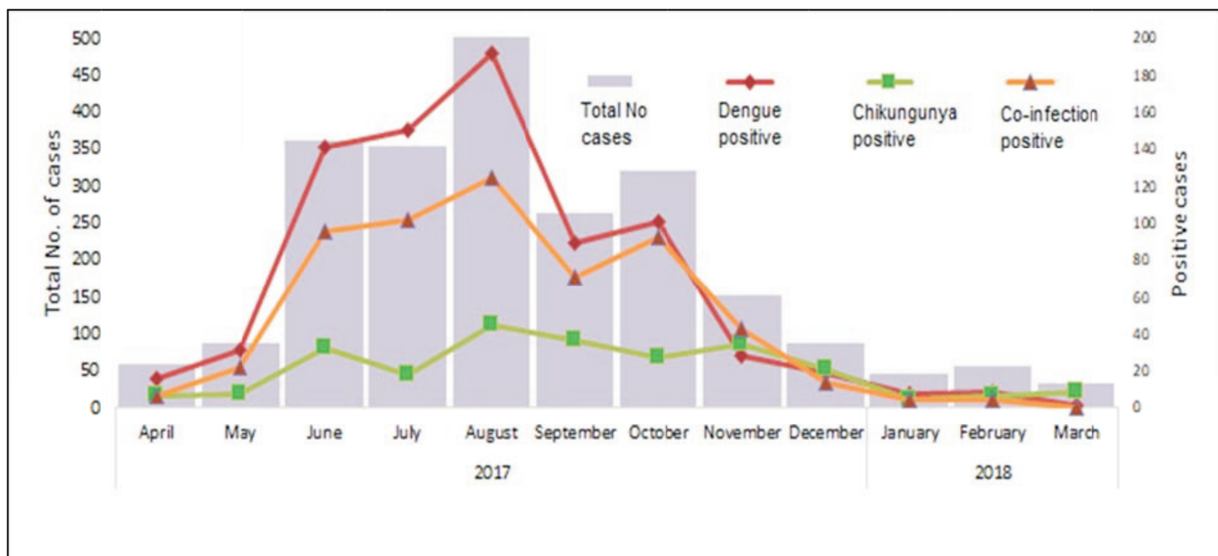


Fig1: Month wise distribution suspected Dengue/Chikungunya cases and their laboratory results

Table1. Laboratory investigation of CRS cases as per study algorithm

Age in Days	First Sample	IgM Positive	Second Sample received	Final result – positive for	
				IgM	Both IgM & IgG
<30	9	4	3	4	0
31-150	20	0	2	0	0
>151	20	8	5	3	5
Total	49	12	10	12	

Findings: During the reporting period, Bangalore unit has investigated 49 cases fulfilling all clinical criteria for CRS. Mean age was 136.34 days in the age group of 8 to 330 days. Male/female distribution was similar with 26 males and 23 females. As per the study protocol 9 cases were within 30days, 20 cases within 31-150 days and 20 cases above 151 days. Second samples after a one-month interval were available only for 10 cases. Tests indicated 7 cases positive for IgM antibodies while 5 cases had both IgM and IgG antibodies (Table 1).

Evaluation of Point of Care test device under field conditions for measles diagnosis and genotype determination

Investigators: Ashok M, Vikas Singhal, D P Sinha

Funding Agency: Extramural (WHO)

Project duration: Ongoing project

Background: Point-of-care tests (POCTs) based on a lateral flow technology are increasingly used for rapid diagnosis of infections by detection of antibody or microbial antigen. They can be performed in a single incubation step at ambient temperature under field conditions without the use of complex electrical equipment and the results can be read visually. POCT for detecting measles specific IgM antibodies in both serum and oral fluid specimens has recently been described from hospital samples. But data from field level are limited. The POCT by its virtue of providing real time results under field conditions could be a path breaking development for measles surveillance.

Objectives: To standardize IgM EIA and N gene PCR on oral fluid samples collected in fields.

Findings: A total of 668 cases from 82 outbreaks from 14 districts of Utter Pradesh was investigated between August 2017 and March 2018 (Fig.2). Of the 200 oral fluid samples tested by IgM EIA revealed 73.5% cases positive for Measles IgM antibodies and 68.5% positive for Measles virus (confirmed by N gene PCR test).

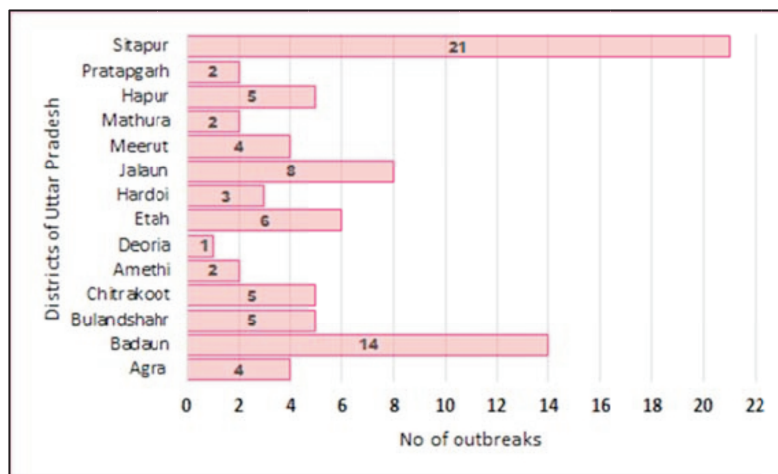


Fig 2. Total number of outbreaks received from various districts of Uttar Pradesh

BNU1701: Laboratory investigation of Severe Acute Respiratory Infection (SARI) cases.

Investigators: Ashok M, Manjunath MJ, Jaya Prakash

Funding Agency: Intramural

Project duration: Ongoing project (Since September 2017)

Background: SARI is defined primarily by clinical, radiological and/or histopathological evidence of pulmonary parenchymal disease (e.g., pneumonia, pneumonitis, or Acute Respiratory Distress Syndrome), typically associated with the need for hospitalization, intensive care unit management and/or other severity marker (such as death). There are numerous pathogens that may cause SARI, including but not limited to novel influenza viruses and other respiratory viruses. For laboratory diagnosis in patients with no epidemiological risk factors for unusual or emerging pathogens, common pathogens should be ruled out first.

Objective: To investigate SARI cases using Real Time RT-PCR for 13 viruses

Findings: During September 2017 to March 2018, Bangalore unit has tested 80 throat swabs and found 33 positive for various respiratory viruses. Majority of the cases were positive for RSV-B (n=18), followed by hMPV (n=3), Influenza-B virus (n=3) and one each for Rhino, Adeno and Influenza-A (H1N1) virus. Combination of two respiratory viruses was detected in six cases (Fig.3)

Investigation of serum samples for Zika virus

Investigators: Ashok M, Manjunath MJ, Hanumaiah

Funding Agency: Intramural

Project duration: Ongoing project (Since January 2018)

Background: On May 15, 2017, the Ministry of Health and Family Welfare, Government of India, reported three laboratory-confirmed cases of ZIKV from Bapunagar area, Ahmedabad, Gujarat. Since then health agencies have been on alert and kept a watch on the Zika situation in India. There was anticipation that a ZIKV outbreak might be possible due to the ubiquitous presence of the vector, *Aedes aegypti* mosquitoes. With the recent confirmation of one more Zika case from Chennai in India attempts are being made to screen more DEN/CHIKV negative samples.

Objective: Laboratory screening for ZIKV in acute febrile illness samples

Findings: Bangalore unit has investigated 187 acute febrile illness samples, which were tested negative for DENV/CHIKV. None of them tested

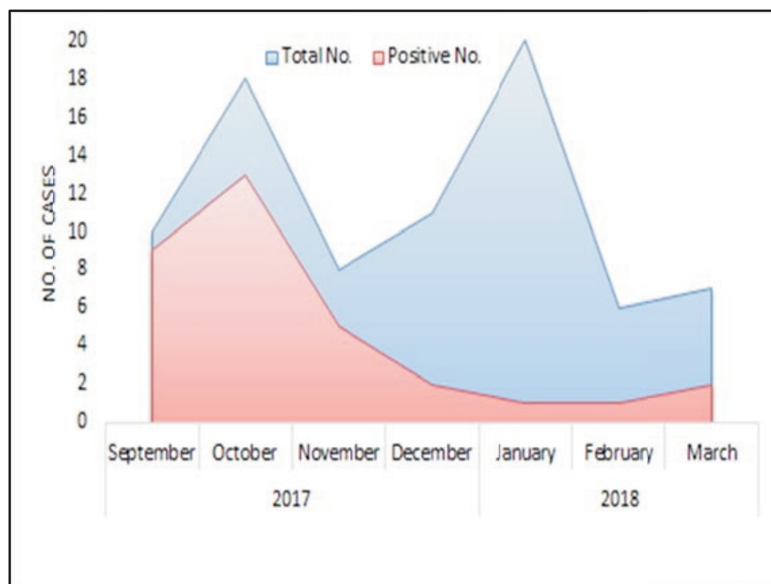


Fig 3: Month wise distribution of suspected SARI cases and virus positivity

KERALA UNIT

Scientific Staff

Dr. B. Anukumar	Scientist D
Dr.Retheesh Babu G	Scientist D
Dr. R.Balasubramanian	Scientist C

Technical Staff

Mr.Bestin Payyapilly	Tech.Asst
Mrs.Kunjila Konikkara	Tech C
Mrs.Sreelekha, K.P	Tech B
Mr.Amol B. Mun	Tech B
Mrs.Amalmol Peter	Tech B
Mr.Nikil T.L	Tech B
Mr. Jijo Koshy	Tech B
Mr.Mayur Mohite	Tech Asst (Engg)
Mr.Dipak Sutar	Tech B (Engg)
Mrs.Mangala Gangatharan	UDC
Mr.Satish Maind	LDC
Mr.Kiran S.Pharande	LDC
Mr.Harshal Kumthekar	Staff car driver
Mr.Govindsing Patil	Staff car driver
Mr.Vikrant Talape	MTS

Project Staff

Ms.Anju Murali	TO
Mr.Mohammad Shafi	TO
Ms. Aswathy P	DEO
Ms.Sahina S	Tech C
Ms.Arathy Nadh V	MTS

Students

Ms.Asha Maria Mathew	SRF
Mr.Vishal Kavathekar	SRF

KLU1301: Virus-host protein-protein interaction: a preliminary study to identify putative host protein(s) interacting with Chandipura virus

Investigators: B.Anukumar & VK Kavathekar

Funding Agency: Intramural

Project Duration: 2013-2018

Background: Chandipura virus has been associated with acute encephalitis in children infecting the central nervous system and replicate in neuronal cells. Yet no efforts have been made to understand the virus interaction with neuronal protein(s) and neuronal transmission. The interacting proteins may be the target for antiviral therapy. Structure based computational approach was used by researchers to predict the host-viral protein interaction, but lacks comprehensive data.

Objective: This study aims to profile the neuronal proteins that interact with the whole virus and individual protein components of Chandipura virus.

Findings: During the reporting year, differentially expressed proteins were examined in CHPV infected Neuro 2a cells by two-dimensional electrophoresis assay (2DE). At two time points (6 & 12h PI) the protein expression was compared with mock-infected cultures. Twenty-three (ratio>4, $p<0.05$) and twelve proteins (ratio>4, $p<0.05$) were differentially regulated at 6 and 12 h PI respectively (Fig.1). At six hours PI, 11 proteins were up-regulated and 12 were down-regulated. Similarly, five proteins were up-regulated and 7 down-regulated at 12 h PI. Additional studies are necessary to identify the differentially expressed proteins. CHPV N gene was cloned in pET28a plasmid vector and the expression of N protein was confirmed. The recombinant N protein was purified using Ni-NTA column. The protein will be used for future protein-protein interaction studies.

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

Investigators: Anukumar & AM Mathew

Funding Agency: Intramural

Project Duration: 2014-2017

Background: Chikungunya virus is an emerging arthropod borne virus, which caused large scale outbreaks in the past decade. Several mutations were reported in the structural genes of Chikungunya isolates obtained from different outbreaks. Some mutations in the structural protein region played a crucial role in the previous outbreaks. A few approaches are available to study the mutations in the structural

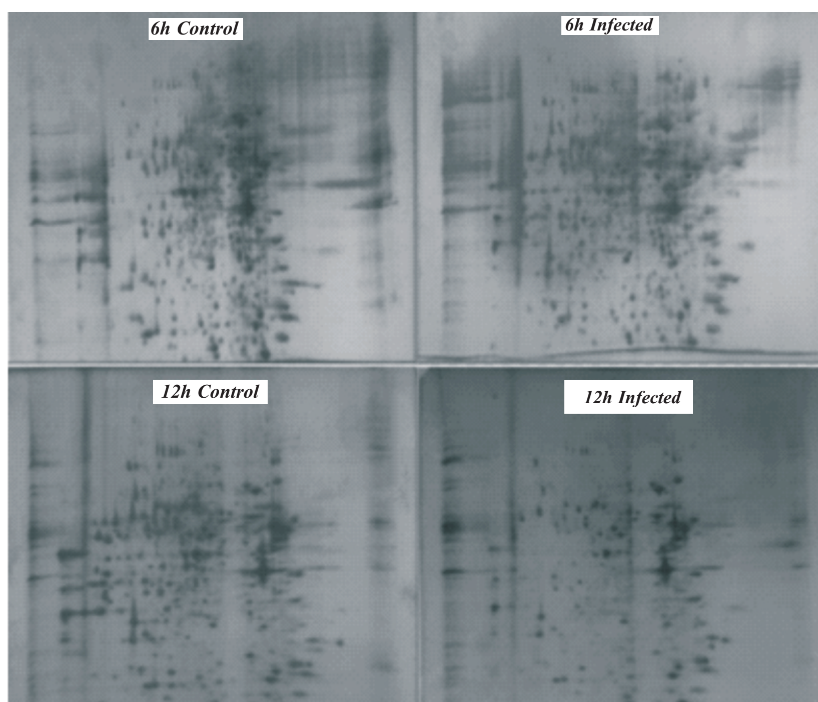


Fig.1: Two-dimensional electrophoresis analysis of Chandipura virus infected Neuro 2a cells at different time points

protein region and virus like particle (VLP) based approach is one among them.

Objective: To study the role of selected mutations on virus entry using virus like particles

Findings: A VLP of CHIKV has been produced during the earlier phase of the study by cloning and expressing the structural polyprotein region of CHIKV in baculovirus expression system which was validated by different techniques. The VLP produced with some previously reported mutations has been employed to see the effect of these mutations on virus entry to susceptible cell lines. Attachment, binding and entry of VLPs/mutated VLPs at different time points were also analyzed. Native CHIKV/UV inactivated CHIKV were used as control in these experiments. Immunofluorescence assay results suggested that VLPs enter into the cells within a 5 min time. After 24 hr post treatment, the VLPs were seen localized in different cytoplasmic compartments suggesting that the VLPs were following similar cellular localization pattern like the native or UV inactivated CHIKV (Fig.2).

KLU1401: Potential species distribution and detection of Kyasanur Forest Disease (KFD) virus in tick population in forest area of Kerala

Investigators: R. Balasubramanian, PD Yadav & Prathiush (Chief Disease Investigation Office, Palode, Kerala)

Funding Agency: DHR (Extramural)

Project Duration: 2016-2019

Background: KFDV activity was detected in Wayanad and Malappuram districts of Kerala. Tick collections were made in the two districts from March 2017 to April 2018.

Objective: to determine the species diversity, distribution and relative abundance of tick species in the study area as well as detection of KFD virus in collected ticks by Real Time RT-PCR.

Findings: A total of 13171 ticks were collected belonging to five genera and nine species. *Haemaphysalis spinigera* (60.4%) was the predominant species followed by *H turturis* (33.3%). *Amblyomma integrum* (3.76%), *H.bispinosa* (2.2%), *Rhipicephalus sanguinius* (0.12%), *H. kyasanurensis* (0.06), *Hyalomma species* (0.05%), *R. microplus* (0.02%) and *Dermacentor Spp*. (0.007%). Twenty-two tick pools collected from Wayanad and Malapuram districts tested positive for KFD viral RNA. The distribution of ticks collected from Malapuram and Wayanad districts and the KFD virus positivity is given in Table 1.

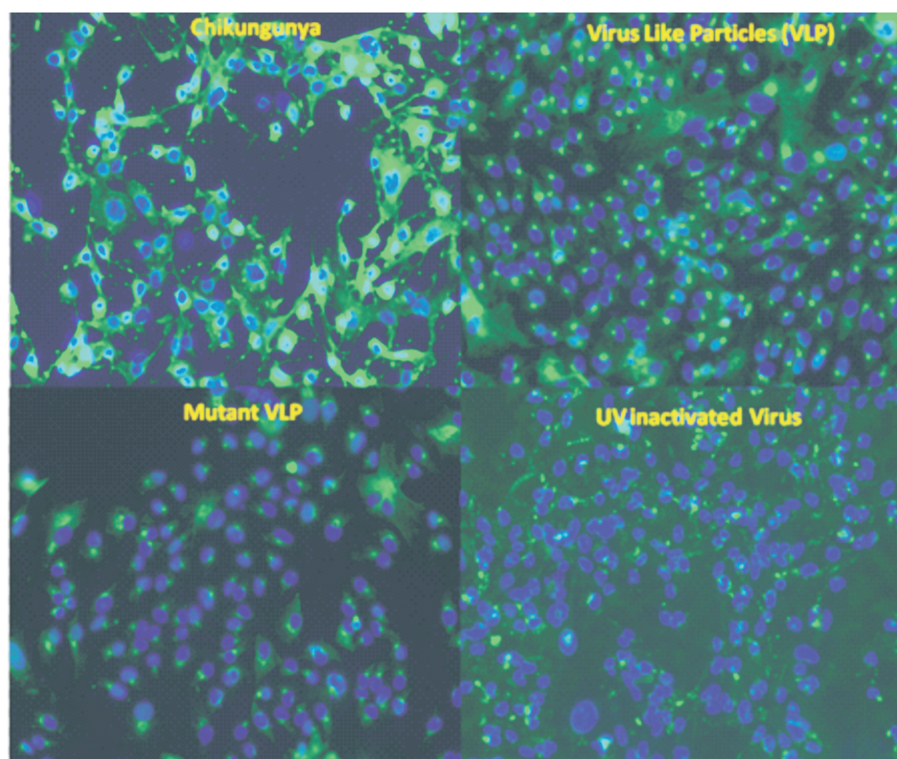


Fig.2 Vero cells exposed with Chikungunya virus, VLPs and inactivated virus were analyzed after 24h by indirect immune-fluorescence assay (20ximage, green fluorescence indicates Chikungunya positive cells).

Table 1: Species distribution and KFDV prevalence in tick samples collected from Malappuram and Wayanad districts from March 2017 to April 2018.

Sl.No	Tick Species	Wayanad			Malappuram		
		Total	%	+ve/pools tested	Total	%	+ve/pools tested
1	*Amblyomma integrum*	496	3.76	0/9	233	11.77	1/3
2	*Dermacentor sp.*	1	0.007	0	0	0	0
3	*Haemaphysalis bispinosa*	291	2.20	0/1	0	0	0
4	*H. cuspidate*	3	0.02	0	0	0	0
5	*H. kyasanurensis*	9	0.06	0	0	0	0
6	*H. spinigera*	7955	60.39	12/154	938	47.42	2/19
7	*H. turturis*	4390	33.33	6/74	796	40.24	1/15
8	*Hyalomma sp.*	7	0.05	0	0	0	0
9	*Rhipicephalus sangunius*	16	0.12	0	11	0.55	0
10	*R. microplus*	3	0.02	0	0	0	0
	Total	13171		18/238	1978		4/37

KLU1501; Community engagement in control of some vector borne diseases: a community-based intervention in Alappuzha Municipality

Investigators: Retheesh Babu G, R.Balasubramanian & Sairu Philip (Prof. & Head, Community Medicine, TDMCH, Alappuzha)

Funding Agency: ICMR (Extramural)

Project Duration: 2016-2018

Background: The second phase (intervention) of the study is based on the formative phase. The intervention plan emphasizes environmental management (along with other measures) as an important measure to control vector borne diseases.

Objective & Findings:

1. To develop a feasible community intervention model to reduce the vector breeding and vector borne diseases.
2. To understand the way the community intervention contributed to eco-bio-social factors to control vectors.
3. To understand the strength, weakness, opportunity and threat of these community-based intervention regarding sustainability and scaling up.

Work done: Selected wards were divided into six clusters and each cluster into two groups. Three types of committees (Steering committees, Cluster committees and Group level committees) were formed. These committees initiated continuous house to house monitoring of vector source activity along with poster campaigns, source reduction activity, plastic waste disposal and chemical spraying based on intervention plan. All the households and institutions in the selected wards were listed (Table 2) and vector control monitoring cards were distributed to register the engagement/ monitoring activities, by the committee members. The community has several reasons to engage and/or not to engage for the disease prevention and control activities. They were most commonly personal, political, cultural, social, religious, natural calamities, etc. which caused delay/lag in relation to coordination and implementation of the activities/engagements during the intervention. Evaluation meetings were conducted in which strength, weakness, opportunity and threats regarding intervention were discussed. However, the ongoing study could make progressive developments with the help of the community.

Table 2: Intervention program and participation status

Name of Municipal Wards	Total No of Household/ Institutions listed	No. of Participants (from different committees) Actively engaged in the house listing	Total No of family members listed
Pazhaveedu	**852**	**35**	**3134**
Vadackal	**771**	**45**	**3050**
Total	**1623**	**80**	**6184**

KLU 1601: Effect of antiviral compounds against Chandipura virus infection in mice

Investigators: B.Anukumar & Dilip Patil

Funding agency: Intramural Project

Duration: 2016-2018

Background: CHPV infection usually occurs in children with acute onset of fever and altered sensorium including coma or seizures. Death usually ensues within a few hours to 48 hours of hospitalization. There is no therapeutics or prophylactics available to date against CHPV. Emergency treatment is aimed at protecting the neurons against further ischemia to minimize neurologic sequel. Symptomatic treatment involves use of decongestants such as mannitol and furosemide to reduce cerebral edema and raised intracranial pressure.

Objective: to study the usefulness of antiviral drugs used to treat viral infection in human, against Chandipura virus

Findings: Seven common antivirals *viz* ganciclovir, acycloguanosine, adefovir dipivoxil, ribavirin, docosanol, inosine and lamivudine were included in this study. The effect of these drugs on CHPV replication was studied in Vero cells and the results indicated inhibitory action of ribavirin. In CHPV infected Vero cells at 150μg concentration, ribavirin inhibited 83% of plaques compared to controls. The inhibitory effect was noticeable in the cells after 4 hPI. The study concludes that ribavirin may be considered for treatment of Chandipura virus infection.

KLU1602: Impact of climate change on mosquito abundance in coastal brackish water and wetlands of Alappuzha district Kerala

Investigator: R.Balasubramanian

Funding Agency: DST (Extramural)

Project duration: 2017-2020

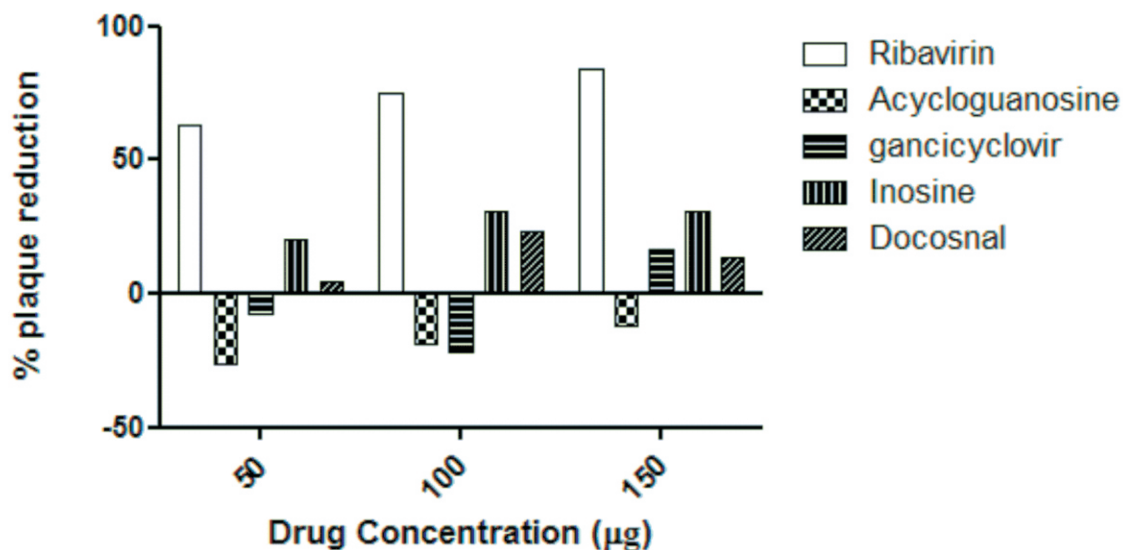


Fig.3: Percentage reduction of plaques in Chandipura virus infected Vero cells treated with different antiviral drug candidates

Background: An upsurge in the vector population of Japanese encephalitis, West Nile virus etc. has been observed in the coastal area. Hence a study was proposed to determine the vector population and their competence to transmit these viruses.

Objective: to evaluate the temporal distribution, spatial pattern and screening of mosquitoes for JE and WN virus in coastal and Paddy belts of Alappuzha district.

Findings: A total of 41503 immature larvae were collected from 92 larval habitats during the study period (Table 3). The overall distribution was Culex (95.74%), Anopheles and Aedes (0.93%). In paddy fields, *Cx. tritaeniorhynchus* was the predominant species while *Cx. sitiens* was dominant in the sea shore (brackish water) which had a salinity of 28 ppt. Adult mosquitoes were collected at dusk using light traps at monthly intervals. A total of 18659 adult mosquitoes belonging to six genera and 23 species were collected which comprised *Cx. tritaeniorhynchus* (54.10%), *Cx. gelidus* (12%), *Cx. quinquefasciatus* (8.96%), *Mansonia annulifera* (5.16%), *Ma. indiana* (4.57%) *Ma. uniformis* (3.82%), *An. peditaeniatus* (4.64%) etc. (Table 3). Attempts were made to isolate JEV and WNV from 197 pools (9119 mosquitoes), but no virus could be isolated. Following the onset of summer rains *Cx. sitiens* disappeared from the brackish water bodies. As the salinity of the pond was decreased to zero ppt, Cx *gelidus* and *Cx. tritaeniorhynchus* were seen breeding in the sites.

Table 3: Mosquito species collected for JE and WN virus detection from April 2017 – March 2018

Sl.No	Mosquito Species	Paddy field		Sea shore		Percentage		+ve/pools tested
		Larvae	Adult	Larvae	Adult	Larvae	Adult	
1	*Ae. albopictus*	0	0	452	8	1.08	0.04	0
2	*Ae. vittatus*	0	0	560	2	1.34	0.01	0
3	*Ae. vexans*	0	12	0	29	0	0.21	0
4	*An. barbirostris*	0	6	32	11	0.07	0.09	0
5	*An. peditaniatus*	61	850	15	16	0.18	4.64	0
6	*An. philipiniensis*	0	6	0	5	0	0.05	0
7	*An. subpictus*	0	88	284	0	0.68	0.47	0
8	*An. vagus*	0	0	1	1	0.002	0.005	0
9	*Ar. Subalbatus*	460	336	16	312	1.14	3.47	0/0
10	*Cx. bitaeniorhynchus*	22	1	2	0	0.05	0.005	0
11	*Cx. crossipes*	0	7	0	35	0	0.22	0
12	*Cx. fuscanus*	178	1	29	0	0.49	0.005	0
13	*Cx. gelidus*	1008	1486	2554	756	8.58	12.01	0/59
14	*Cx. infula*	0	18	0	1	0	0.1	0
15	*Cx. psuedovishnui*	0	0	31	32	0.07	0.17	0
16	*Cx. quinquefasciatus*	0	341	231	1332	0.55	8.96	0/16
17	*Cx. sinensis*	0	0	0	47	0	0.25	0
18	*Cx. sitiens*	0	22	28836	256	69.47	1.48	0/18
19	*Cx. tritaeniorhynchus*	5122	7285	1580	2811	16.14	54.1	0/102
20	*Cx. vishnui*	0	0	29	16	0.06	0.08	0
21	*Ma. annulifera*	0	487	0	476	0	5.16	0/1
22	*Ma. indiana*	0	530	0	323	0	4.57	0
23	*Ma. uniformis*	0	442	0	272	0	3.82	0/1
	Total	6851	11918	34652	6741			0/197

Number of Samples Tested (virus-wise details)

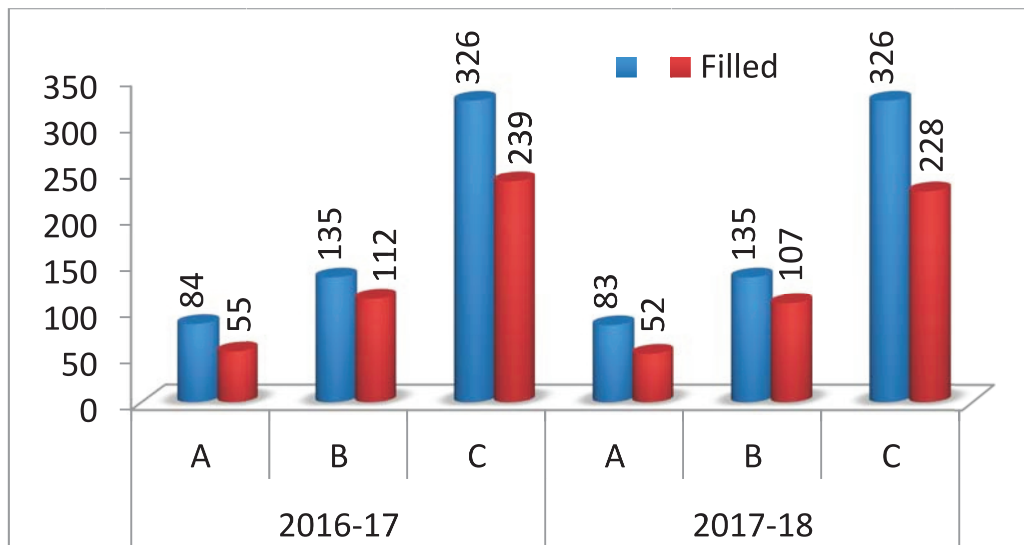
The unit provides diagnosis for different viruses using serological and molecular based diagnostic tests. Details of samples and tests are given in Table.4. During the year 916 samples were processed using 3024 testes yielding 41 isolates.

Table 4: The number of samples tested for different viruses using various diagnostics

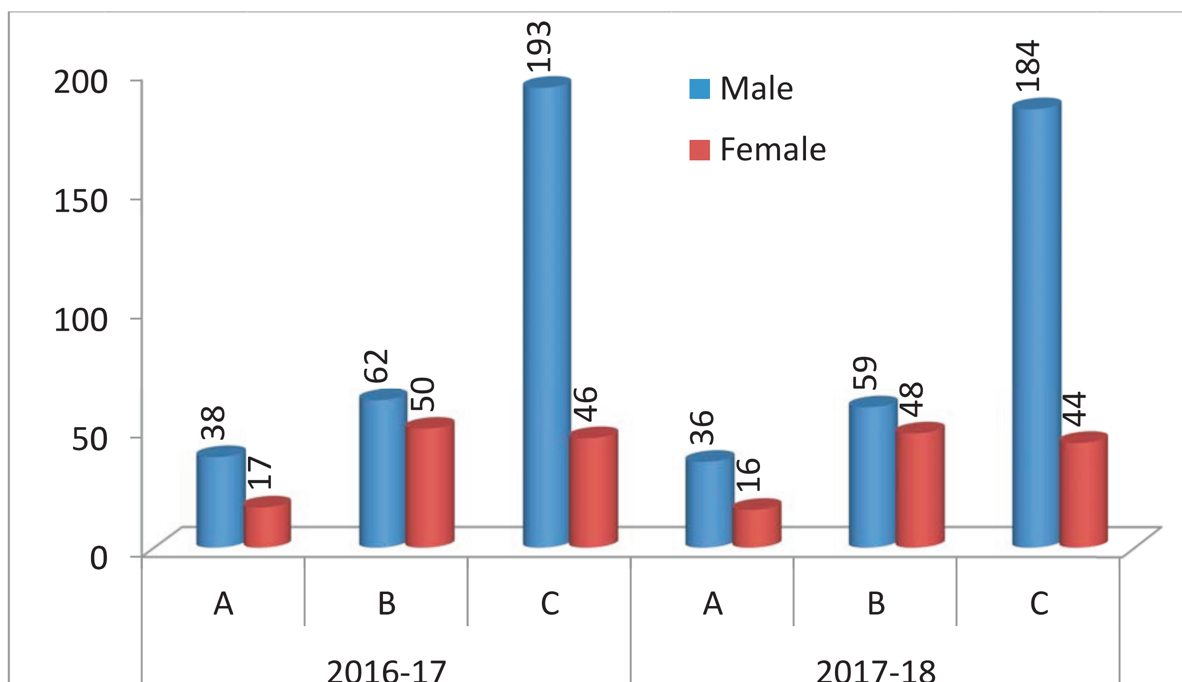
Sl. No	Virus	IgM ELISA	IgG	Conv. PCR	Real Time PCR	Virus Neut.	HAI	Ag. Detect	Virus Isolate	Seque-ncing
1	**JE**	112/0	-	52/2	22/0	-	-	-	-	-
2	**Dengue**	66/20	-	59/19	137/7	-	-	-	-	2/2
3	**West Nile**	-	-	14/0	21/0	-	-	-	-	-
4	**Chikungunya**	22/3	-	24/0	112/0	-	-	-	-	-
5	**HSV 1**	-	-	30/0	-	-	-	-	-	-
6	**HSV 2**	-	-	30/1	-	-	-	-	-	-
7	**HSV 1 & 2**	9/0	-	-	-	-	-	-	-	-
8	**Rota**	-	-	-	-	-	-	-	-	-
9	**Rubella**	8/0	-	7/0	-	-	-	-	1/1	1/1
10	**Measles**	4/0	-	2/1	-	-	-	-	-	-
11	**HAV**	-	-	-	-	-	-	-	-	-
12	**HBV**	-	-	6/0	-	-	-	-	-	-
13	**HCV**	-	-	7/3	-	-	-	-	-	-
14	**Entero virus**	-	-	53/4	35/2	-	-	-	2/2	1/1
15	**Adeno**	-	-	-	6/0	-	-	-	-	-
16	**CMV**	-	-	19/4	2/0	-	-	-	-	-
17	**Varicella Zoster**	-	-	3/2	-	-	-	-	-	-
18	**Influenza A**	-	-	43/16	630/214	-	-	-	-	-
19	**Influenza B**	-	-	43/0	630/14	-	-	-	-	-
20	**H3N2**	-	-	41/13	211/58	-	-	-	-	-
21	**PandemicA(H1N1)**	-	-	41/3	253/156	-	-	-	52/38	-
22	**RSV - A**	-	-	1/0	-	-	-	-	-	-
23	**RSV - B**	-	-	1/0	-	-	-	-	-	-
24	**Parvo V**	-	-	-	-	-	-	-	-	-
25	**EBV**	-	-	8/0	2/0	-	-	-	-	-
26	**Mumps**	-	-	-	-	-	-	-	-	-
27	**Rhino**	-	-	-	-	-	-	-	-	-
28	**HHV 6**	-	-	2/0	-	-	-	-	-	-
29	**HHV 7**	-	-	-	-	-	-	-	-	-
30	**Mers**	-	-	-	-	-	-	-	-	-
31	**KFD**	-	-	-	-	-	-	-	-	-
33	**ZIKA**	-	-	-	197/0	-	-	-	-	-
	TOTAL / POSITIVE	23/221	-	68/486	451/2258	-	-	-	41/55	4/4

ADMINISTRATION : STAFF PARAMETERS

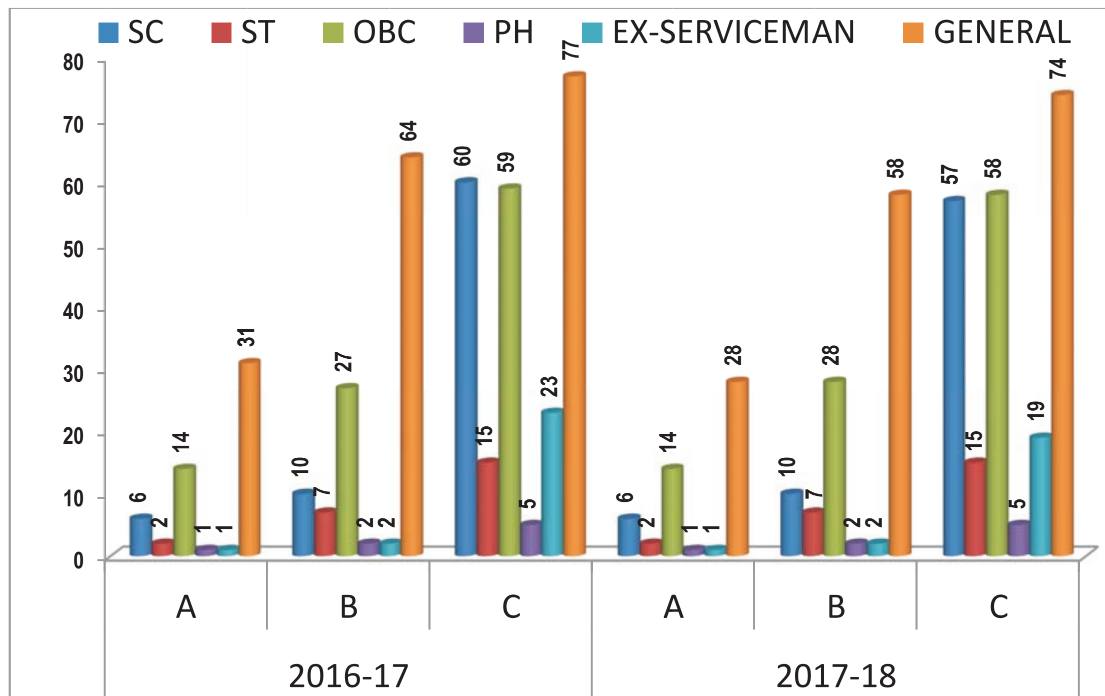
Group-wise staff strength



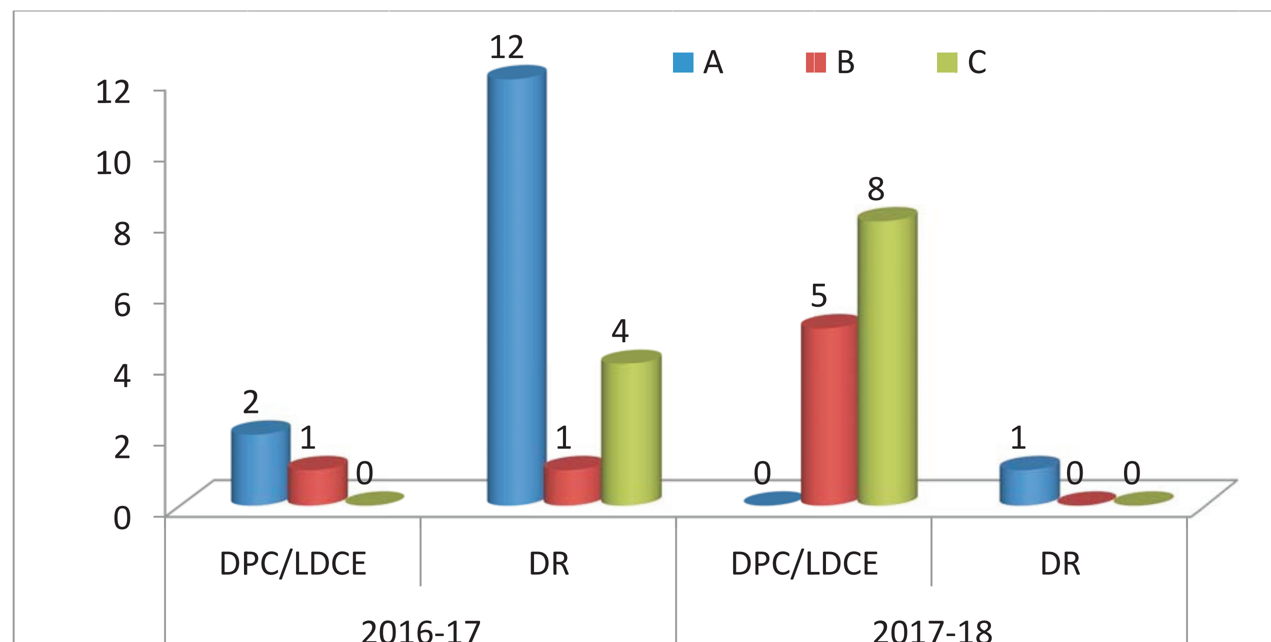
Group-wise gender distribution



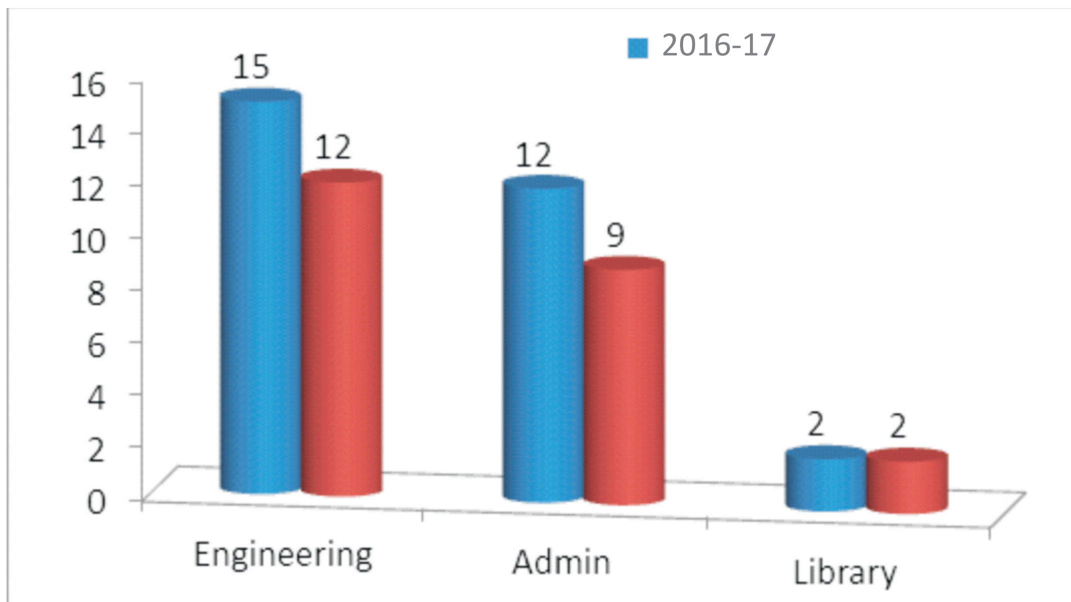
Group-wise category distribution



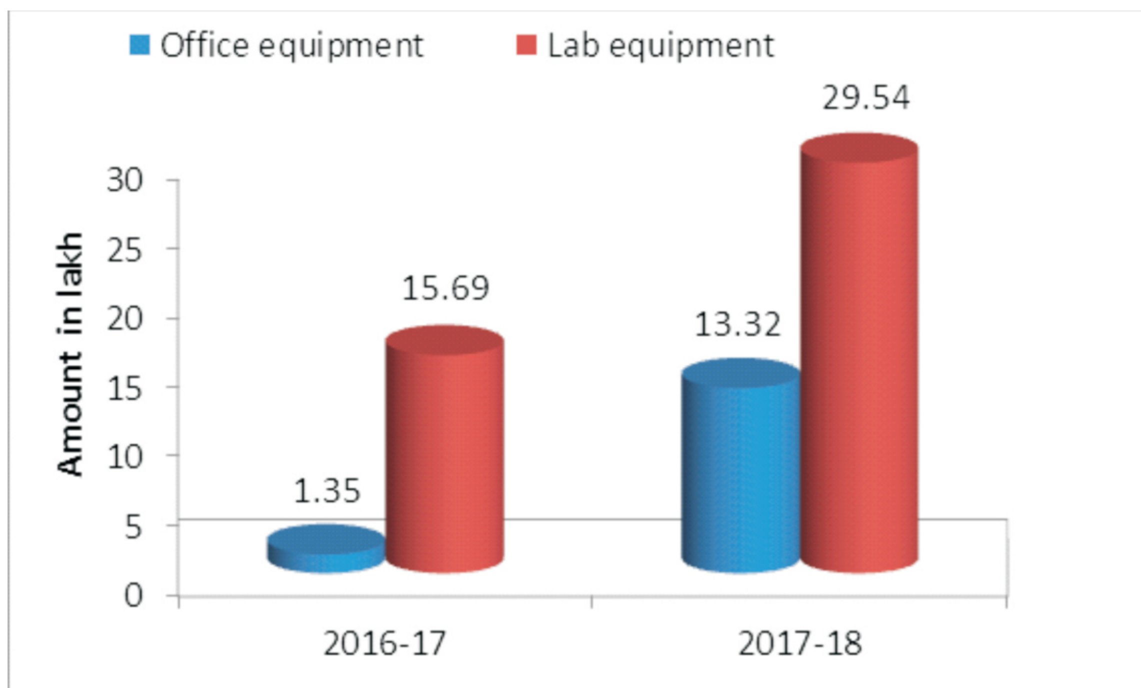
Group-wise vacancies filled



Apprentices engaged



Details of equipments supplied



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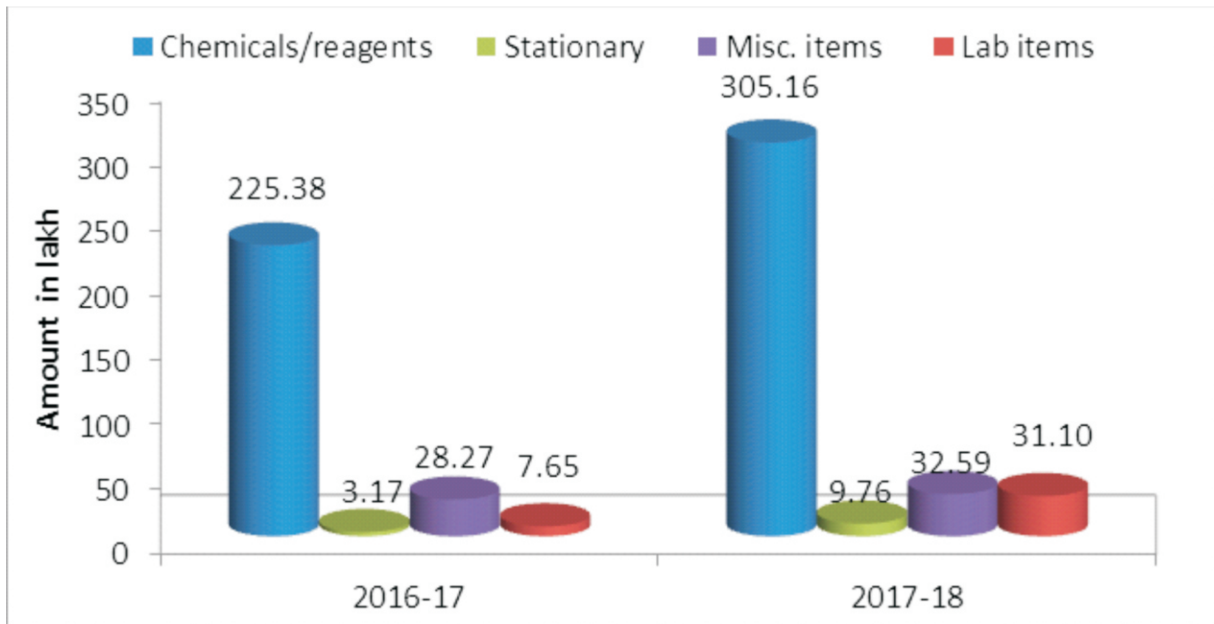
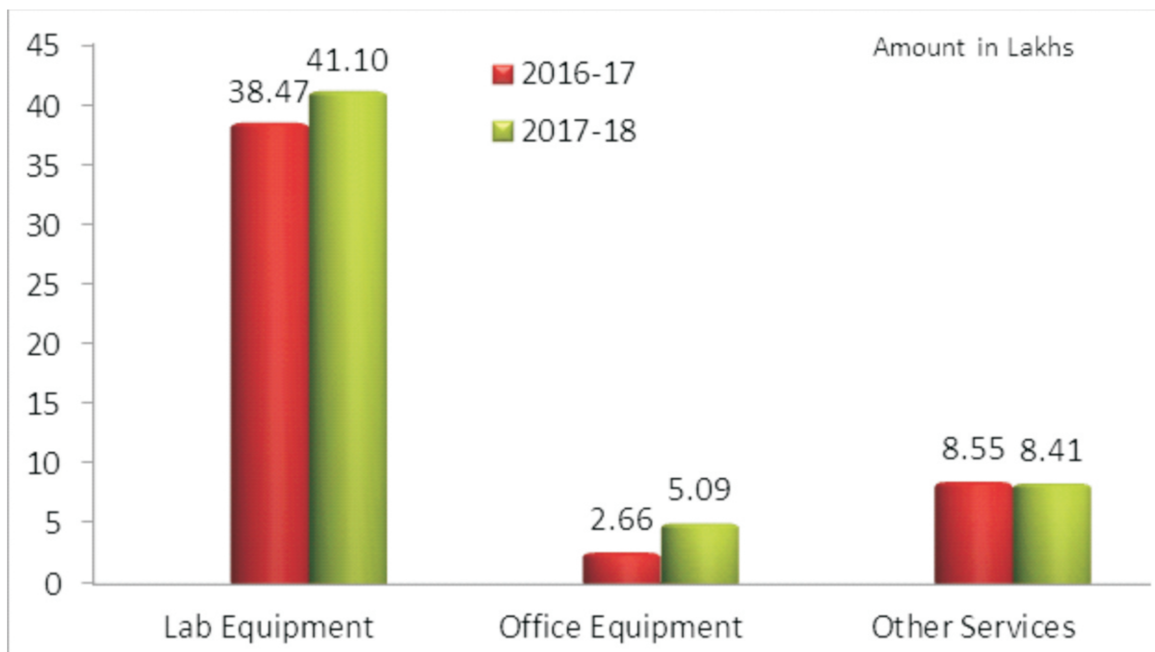
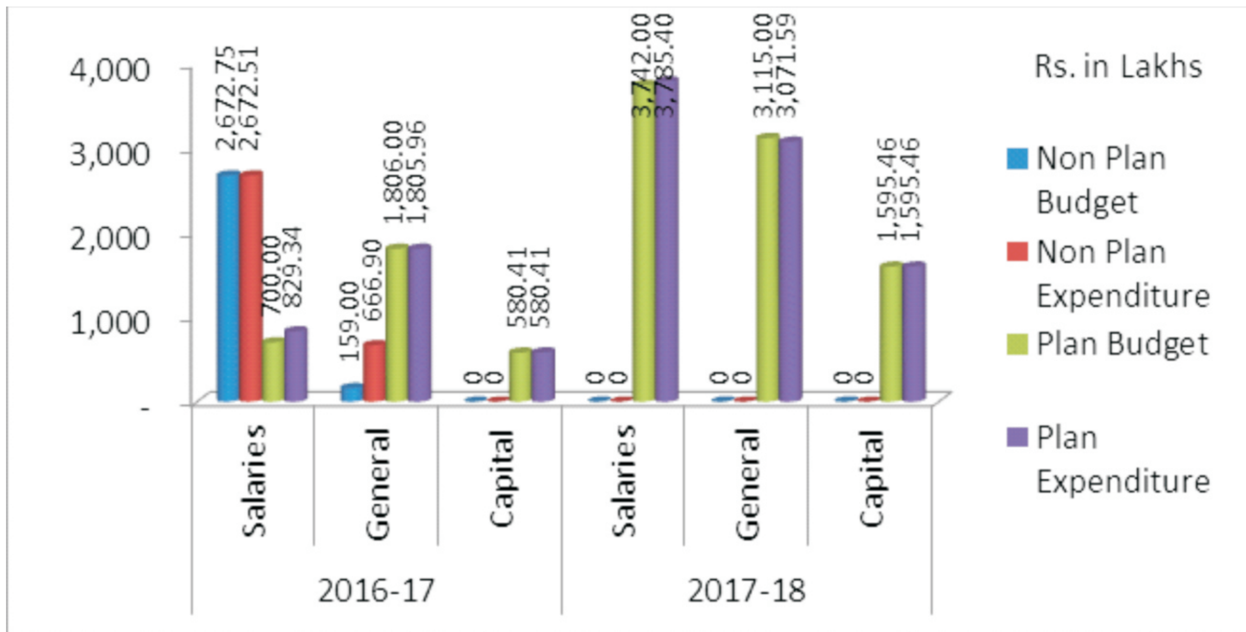


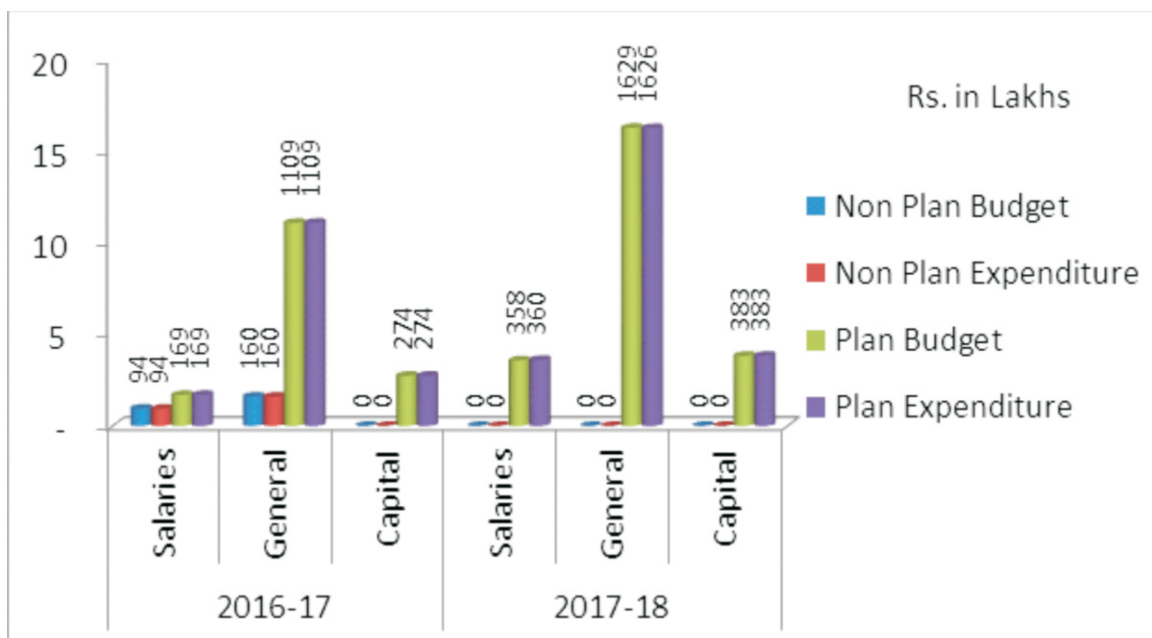
Details of annual maintenance contract



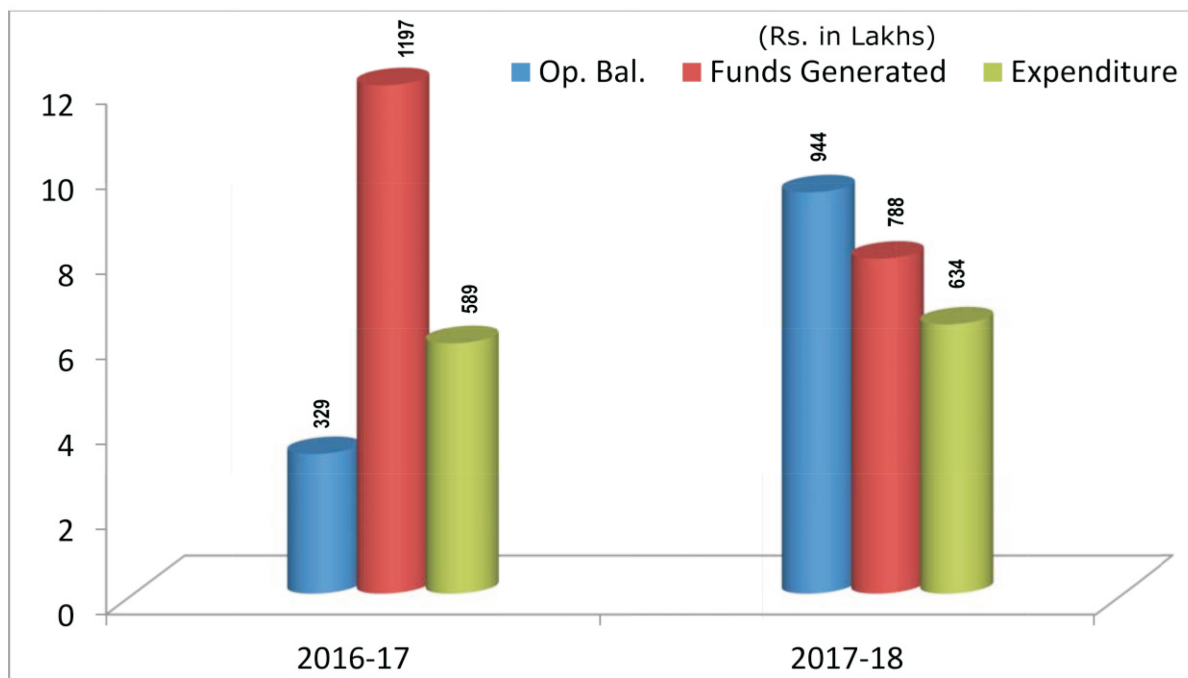
Budget : NIV, Pune



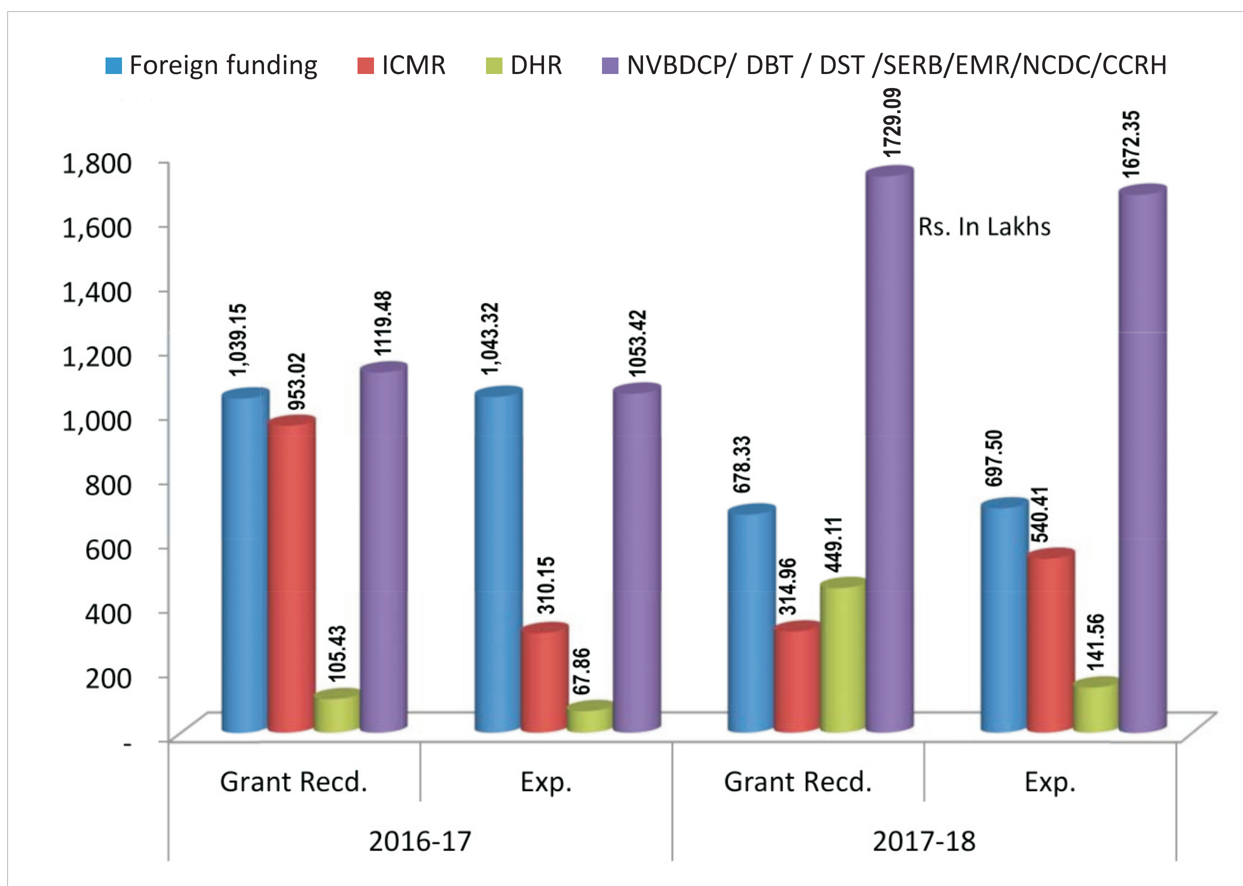
Budget : MCC



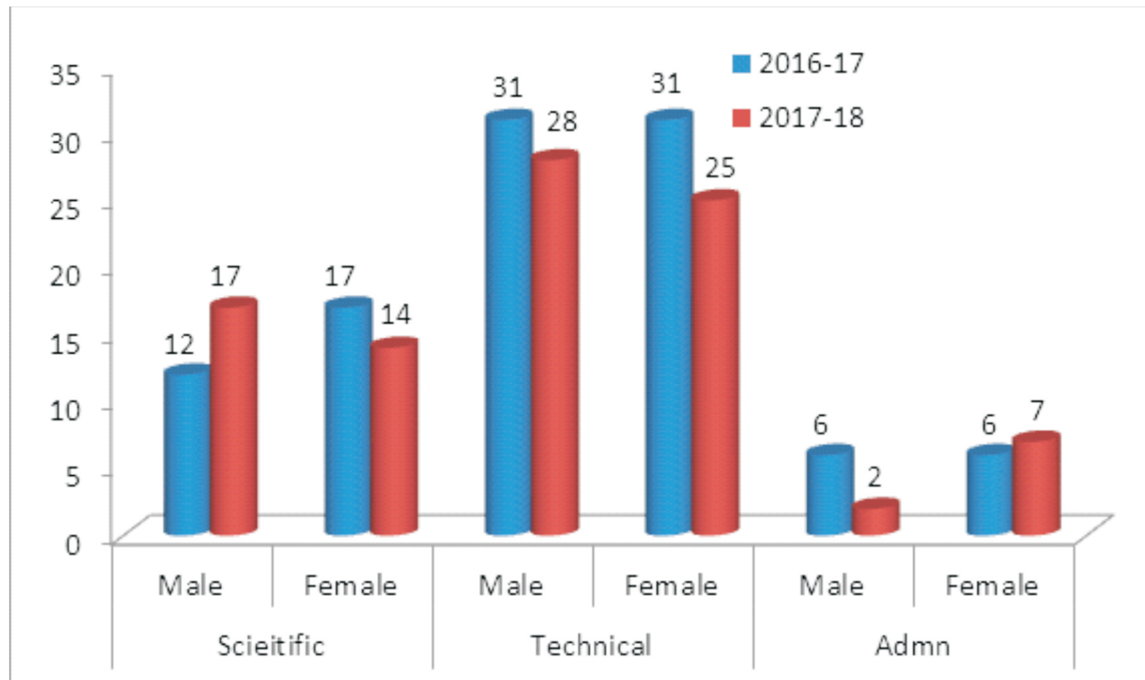
Budget : generated funds



Budget : project account



Project staff - cadre wise gender distribution



Training courses attended by Administrative Staff at Regional Training Centre, Mumbai

Sr. No.	Name & Designation of the Official	Course Title	Duration	From	To
1	Mr. P. Subramanian, AO	Retirement Benefits & Post Retirement Financial Planning	1 day	18.08.2017	18.08.2017
2 3	Mrs. A. S. Deshpande, SO Mrs. Prajakta Joshi, Assistant	Vigilance Matters, Disciplinary Proceedings, Prevention of Corruption Act, CVC Act & Guidelines and other related laws	3 days	18.09.2017	20.09.2017
4 5	Mrs. R.K. Amle, SO Ms. MJA Shaikh, UDC	Income Tax Rules, Calculation of E-Filing	2 days	09.11.2017	10.11.2017
6	Ms. Madhuri Tandan, LDC	Noting, Drafting, File & Record Management	2 days	16.11.2017	17.11.2017
7	Mrs. S.S. Dube, Stenographer	Leave Rules and Maintenance of Service Book	2 days	27.11.2017	28.11.2017
8 9	Mrs. R.S. Moghe, Assistant Ms. Madhuri Tandan, LDC	Pension & Other Retirement Benefits	2 Days	14.12.2017	15.12.2017
10	Mr. R. R. Jaiswal, LDC	Public Procurement Process & GeM	2 Days	16.01.2018	17.01.2018
11 12 13	Mrs. S.B. Chakole, UDC Mr. S.R. Vasam, UDC Ms. M.S. Tandan, LDC	NPS & Data Uploading	2 Days	18.01.2018	19.01.2018
14 15	Mrs. A.B. Palkar, SO Mr. M.S. Mavlankar, UDC	Right to Information Act-2005	2 Days	24.01.2018	25.01.2018
16	Mr. B. K. Wadke, SO	Stress Management	1 Day	08.02.2018	08.02.2018
17	Mr. A. E. Matkar, UDC	APAR	1 Day	19.02.2018	19.02.2018
18	Ms. J. Jayajyoti, Assistant	TA & LTC	2 Days	05.03.2018	06.03.2018
19	Mr. H. S. Pasalkar, SO	Loans & Advances	2 Days	07.03.2018	08.03.2018

Training courses attended by Administrative Staff at ISTM, New Delhi (2017-18)

Sr. No.	Name & Designation of the Official	Course Title	Duration	From	To
1	Mr.A S Gaikwad, AO (stores)	Workshop on Public Procurement under GFR-2017	2 Days	11.05.2017	12.05.2017
2	Mr.V C Chavan, Asst.		2 Days	08.06.2017	09.06.2017
3	Mrs.Tejaswini Yadav, LDC		2 Days	06.07.2017	07.07.2017
4 5	Mrs.Anita Nair, Asst. Mrs.Savita Chakole, LDC	Workshop on Pay Fixation	3 Days	12.07.2017	14.07.2017
6 7	Mr.P N Jadhav, Asst. Ms.M S Tandan, LDC	Workshop on Outcome Budget	2 Days	13.07.2017	14.07.2017
8	Mrs.V V Shendye, SO	Gender Budgeting	3 Days	26.07.2017	28.07.2017
9	Mr. P Subramanian, AO	Workshop on Income Tax	2 Days	27.07.2017	28.07.2017
10	Mr.B K Wadke, SO	Public Private Partnership	2 Days	03.08.2017	04.08.2017
11	Mr.A S Gaikwad, AO (stores)	Workshop on Emotional Intelligence	3 Days	02.08.2017	04.08.2017
12	Mr.Anil Matkar, LDC	Record Management - Right to Information	3 Days	16.08.2017	18.08.2017
13	Mr. Satish Matkar, Assistant	Workshop for Internal Finance Officers	2 Days	07.12.2017	08.12.2017
14	Mrs.V V Shendye, Accounts Officer	Workshop on Analysis of Financial Statements	2 Days	08.01.2018	09.01.2018

Training Courses attended by Administrative staff at Other Institutions

Sr. No.	Name & Designation of the Official	Course Title	Name of Institution	Duration	From	To
1	Mrs. Amruta Bakare, SO	Workshop on Reservation in Services and CCS (Conduct) Rules	National Institute for Research in Reproductive Health	2 Days	20.04.2017	21.04.2017
2	Mrs. Shibi Jacob, PA					
3	Mrs. Priyanka Aher, UDC					
4	Ms.Prajakta Bapat, LDC					
5	Mr. S.E. Matkar, Assistant	Public Financial Management System	Indian Council of Medical Research	1 day	20.06.2017	20.06.2017
6	Ms. Madhuri Tandan, LDC					
7	Mr. Akram Khan, LDC	E-Office	National Institute for Research in Reproductive Health	1 day	18.08.2017	18.08.2017
8	Mr. Vinod Chavan, Assistant					
9	Mr. Prashant Patil, LDC					
10	Mr. Prem Khandagale, LDC					
11	Mrs. M Gangadharan, UDC					
12	Mr. Kiran Pharande, LDC					
13	Mr. S.E. Matkar, Assistant					
14	Ms.Prajakta Bapat, LDC					
15	Mr. A.E. Matkar, UDC	Initial Translation Training	Central Translation Bureau	30 Days	01.01.2018	12.02.2018

ENGINEERING SUPPORT DIVISION

List of staff

Sr. No.	Name of Staff	Designation
Staff at NIV, Pune		
1.	Mr. A.B.Khare	HOD & Tech Officer B
2.	Mr. D.R. Kumbhar	Technical Officer-A
3.	Mr. A.K.Kasar	TO- A
4.	Mr. N.V. Bhongale	Sr. Technician-3 (ES)
5.	Mr. G.K. Bagul	Sr. Technician-2 (ES)
6.	Mr. V.J. Bhosale	Sr. Technician-2 (ES)
7.	Mr. A.J. Suresh	TA (ES)
8.	Mr. N.S. Dhawale	Sr.Technician-1 (ES)
9.	Mr. V.D. Jagtap	Sr.Technician-1 (ES)
10.	Mr. Y.M. Taru	Sr.Technician-1 (ES)
11.	Mr. B.S. Shelar	Laboratory Asst.- (ES)
12.	Mr. A.B. Kelkar	Sr.Technician-1 (ES)
13.	Mr. S.S. Utale	Laboratory Asst.- (ES)
14.	Mr. S.S. Holkar	Sr. Technician-1 (ES)
15.	Mr. R. S. Gadhave	Sr. Technician-1 (ES)
16.	Mr. D.K. Jagtap	Laboratory Asst. (ES)
17.	Mr. M.V. Gadhave	Technician-A (ES)
18.	Mr. A.M. Pawar	Technician-3 (ES)
19.	Mr. A.N.Kale	Technician-A (ES)
20.	Mr. S.R.Jagtap	Technician-A (ES)
Apprentice staff NIV, Pune		
1.	Mr. A.R. Meshram	Electrician
2.	Mr. G.K. Thorat	Electrician
3.	Mr. S.D. Sonawane	Electrician
4.	Mr. M.D. Jagtap	Electrician
5.	Mr. O.S. Dhamale	Electrician
6.	Mr. K.C. Bathe	Ref. & A.C. Mechanic
7.	Mr. S.M. Pawar	Plumber
Staff at Pashan unit		
21.	Mr. S.D.Pote	Technician-C (ES)
22.	Mr. S. N. Surbhaiya	Technician-C (ES)
23.	Mr. S.D.Bathe	Sr.Technician(1)
24.	Mr. G.R.Ghogare	Technician-B (ES)
25.	Mr. V.T.Ishte	Technician (2)
26.	Mr. I.R.Dedunda	Technician-A (ES)

Project Staff Pashan Unit		
27.	Mr. Y. Gondane	Technical Officer A
28.	Mr. P. Patil	Technical Officer A
29.	Mrs.P.H. Chakankar	Technical Officer
Apprentice Staff Pashan Unit		
30.	Mr.A.B. Javir	Carpenter
31.	Mr. A.K.Kare	Plumber
32.	Mr. V. S. Surbhaiya	Electrician
Staff at Field Units		
Kerala Unit		
33.	Mr. Mayur S. Mohite	Technical Assistant.
34.	Mr. Dipak V. Sutar	Technician 'B'
35.	Mr. Ramesh D. Mane	MTS
Gorakhpur unit		
36.	Mr.Ashish Choudhary	Technical Assistant
37.	Mr. Jitendra P Kumar	Technician-A
Bangalore unit		
38.	Mr.A.R. Sable	Sr.Technical Officer-2
39.	Mr. Arjun Jogangiri	MTS

MAJOR WORK CARRIED OUT BY MAINTENANCE STAFF

Pune Unit

- **Major works :**
  - Installation of new digital Ammeter and Voltmeter to LT panel and 500 KVA DG set essential panel at NIV Substation
  - Modification of control gear box with provision of MCB, wiring and cable laying with excavation work of staff quarter security light at Yamunanagar, Nigadi.
  - Replacement of old 36 watt PL street light fitting with 24 watt LED fittings at NIV Pune
  - Overhauling and 'B' Check and 'C' Check servicing work of 400 KVA Kirloskar DG set.
  - Servicing and spares replacement of AMF panel C&S 800 Amp. Breaker of substation.
- **Works Carried out at Mumbai Unit**
  - Servicing and Maintenance work of Electrical panels, Fire Alarm System and Intercom system of Mumbai Field Unit and physical verification of Electrical wiring at Nestle flat.

Pashan unit:-

- **ISO/IEC-17025:**
  - Assisted in preparation of documents and calibration of equipments of NABL accreditated labs. Also carried out internal audit of Avian Influenza Group.
- **Trainings and workshops:**
  - a. Attended training on Clean room Technology, Theory & Practice" at Hyderabad
  - b. Delivered Lecture on Bio safety cabinet, HVAC, Fire and electrical safety, autoclave, Incinerator, engineering control, installation management etc to MSC students
  - c. Delivered lecture on autoclave, incinerator etc. during Hindi workshop on 22.08.2017.
  - d. Lecture on Emergency Evacuation and demonstration of fire extinguisher
  - e. Attended meeting with CPWD, Lucknow, regarding RMRC, Gorakhpur
  - f. Visited NIRT Chennai for meeting with CPWD regarding containment lab.
  - g. Visited National Institute of Immunohaematology, Chandrapur regarding construction of **"National Satellite Center"**
- **Laboratory Set up:** Provided logistic support to set up new Bacteriology laboratory at Pashan.
- **Major works :**
  - Replacement of BMS system of BSL3 lab
  - Provided support in removing, Shifting and refixing of Gama Chamber of BSL4
- **Capital works:**
  - Preparation of SFC, Drawings, attended the meetings with CPWD, Mumbai for construction of NIV Mumbai Unit at Kharghar.
- **Emergency Attended:**
  - Heavy sparking was observed at Potential Transformer (PT) of HT panel at substation No 2 at late night (1:00 AM). Immediately attended the issue and restored the electric supply of institute within one hour by isolating the faulty PT.

Important meetings attended by Dr DT Mourya, The Director
(April 17 to March 18)

Date (s)	Meetings	Venue
09-10 April 2017	Meeting with principal investigators of RI network laboratories under the CDC GHSA project.	Jaipur, Rajasthan
8-9 April 2017	17th Indian Veterinary congress XXIV Annual Conference IAAVR & National Symposium at ICAR-IVRI Izatnagar	Bareilly
25-27 April 17	WHO Regional workshop on poliovirus Laboratory containment	New Delhi
17- 21 April 17	Visit to Special Pathogens Branch at CDC, Atlanta	USA
18 May 2017	Meeting with Health Minister regarding establishment of communicable research disease laboratory at sindhudurg	Sindhudurg
31 May 2017	'Measles ELISA assay', 'Anti-CCHF Human IgM Elisa assay' and 'Technology for testing water for presence of Water-borne Viruses'.	ICMR, New Delhi
19-22 June 2017	To Participate in Exhibit India Pavilion at the BIO International Convention at San Diego.	USA
29 June- 1st July 2017	Visit to Royal Centre for Disease Control, Serbithang, Bhutan	Thimphu
19-20 July 2017	SAG Meeting at ICMR	New Delhi
24 July 2017	HBL/HLL Meeting at ICMR	New Delhi
01-9 Aug 2017	National Health Laboratory, Yangon	Myanmar
17 Aug. 2017	Meeting with Dr. Kayla, Dr. Siddharth Saha and Dr. Walter	NIV, Pune
6 Sept. 2017	Meeting with CDC Officials regarding Influenza priorities	ICMR, Delhi
19 Sept. 2017	PI of VHF/RI Centers under CDC Project Annual Review Mtg.	Hotel O, Pune
20 Sept. 2017	Meeting for BSL-III Lab plan & budget sanction for PMC (UPRNN) at RMRC, Bhubaneswar	Bhubaneswar
06-16 Nov. 2017	Visit to Royal Center for Disease control, Bhutan	Thimphu,
23 Nov. 2017	Review Meeting at Centre for Stem Cell Research & Regenerative Medicine	Chennai
24 Nov. 2017	Meeting with Sri Lankan (WHO) visitors at MCC, Pashan	Pune
13-15 Dec. 2017	WHO BSL-4 high containment network meeting at International Agency for Research on Cancer	Lyon, France
20 Dec. 2017	Meeting on Measles kit development by NIV through zoom	NIV Pune
03-04 Jan 2018	1) Meeting on virus inactivation technology 2) Meeting with Shri V. K. Gauba. Jt. Sec. DHR	Delhi
17 Jan 2018	Global Health Security Agenda (GHSA) in India, Annual Review Meeting 2016-2017	Delhi
05-07 Feb. 2018	Annual Review of the WHO R&D Blueprint Priority List of Diseases	Geneva
19 Feb. 2018	Strengthening of Measles and Rubella case confirmation by serology IgM detection and conventional RT-PCR for ICMR VRDLs.	Mumbai NIV Unit
1-2 March 2018	Consultation for Nipah virus, as part of the WHO's R&D roadmaps process for priority diseases"	UK, London
16 March 2018	Meeting with Dr. Lipkin and Dr. Nishchay Mishra at NIV	Pune
20 March 2018	6th CBRN workshop at Delhi DRDO Bhawan	Delhi

Major Meetings/workshops/conferences conducted

Conference : Organized National conference on Virology during 26-27 October 2017 under the auspices of Department of Health Research, New Delhi. All the VDL networks were participated in the program.

Other meetings & workshops:

Date	Details of trainings	Venue	Resource person
3 and 4 August 2017	Training on Bio-safety and Bio-security and outbreak investigation to VDLN PIs and 16 technical Staff	Seminar Hall, MCC, Pashan	GN Sapkal
17-Aug-2017	CDC Project- PIs Meeting	NIV, Pune	DT Mourya, PD Yadav
29-Aug-2017	CDC Project administrative meeting with M/s Grant Auditor and VHF / RI network administrative staff	NIV, Pune	DT Mourya, PD Yadav
10-13 Sept 2017	CDC INSPIRE study. Molecular detection of respiratory viruses using real time RT-PCR	AIIMS Delhi	V A Potdar, Mr D Hinge
19-Sep-2017	Annual Review Meeting of CDC Project (For all the PIs of VHF and RI network)	NIV, Pune	DT Mourya, MS Chadha and P Yadav
10-12 Oct 2017	National workshop on Influenza surveillance & data management	Manipal Centre for Virus Research	V A Potdar S Bhardwaj
5-8 Dec 2017	ICMR Task force project Real time PCR training for virology	CMC Vellore	VA Potdar, D Hinge
11-Dec-2017	GHSA Annual Review meeting	NIV, Pune	D. T. Mourya, MS Chadha Pragya Yadav & G sapkal
17-Jan 2018	GHSA Annual Review meeting, under the chairmanship of DG, ICMR	Viceroy Hall of Hotel Claridges, New Delhi.	DT Mourya, Pragya Yadav
30 January 2018	CDC-GHSA Quarterly Review meeting (All PIs of CDC-GHSA project)	NIV, Pune campus	D. T. Mourya, MS Chadha Pragya Yadav & Dr.G sapkal
January 29 – February 2, 2018	Program on Integrated Scientific Project Management for Women Scientists/ Technologists	Centre for Organization Development, Hyderabad.	Pragya D Yadav
5 February 2018	Training on outbreak investigation and outbreak case discussion to VDLN PIs and 16 Technical Staff	Seminar Hall, MCC	DT Mourya, GN Sapkal
14 Feb 2018	VDRL training for Allelic discrimination real time PCR for H275Y	MCC Pune	V A Potdar
5-9 Feb 2018	Molecular detection of respiratory viruses using real time RT-PCR and antiviral testing	NIV Pune	VA Potdar, ML Choudhary, SD Bhardwaj

List of Papers Published including 'In Press'

(Period April 2017-March 2018)

Total Papers Published - 87
Journal Articles - 80
Book Chapters - 7
Average Impact Factors - 3.906

S.No.	List of Publications	Impact Factor
1.	Alagarasu K, Patil PS, Shil P, Seervi M, Kakade MB, Tillu H, Salunke A. In-vitro effect of human cathelicidin antimicrobial peptide LL-37 on dengue virus type 2. Peptides. 2017 Jun;92:23-30.	**2.778**
2.	Amdekar S, Parashar D, Alagarasu K. Chikungunya Virus-Induced Arthritis: Role of Host and Viral Factors in the Pathogenesis. Viral Immunol. 2017 Dec; 30(10):691-702.	**1.432**
3.	Arankalle VA, Ramdasi AY. Immune responses in pregnant women presenting with subclinical or clinical HEV infection are different and trimester-dependent: A whole transcription analysis of Peripheral Blood mononuclear cells. Plos Pathogens. 2017. (In Press)	**6.608**
4.	Balakrishnan A, Thekkekare RJ, Sapkal G, Tandale BV. Seroprevalence of Japanese encephalitis virus & West Nile virus in Alappuzha district, Kerala. Indian J Med Res. 2017 Jul;146(Suppl.):S70-S75.	**1.532**
5.	Bhardwaj S, Gokhale MD, Mourya DT. Zika virus: Current concerns in India. Indian J Med Res. 2017 Nov; 146 (5):572-5.	**1.532**
6.	Cecilia D, Patil JA, Kakade MB, Walimbe A, Alagarasu K, Anukumar B, Abraham A. Emergence of the Asian genotype of DENV-1 in South India. Virology. 2017 Oct;510:40-5	**3.353**
7.	Chaubal G, Sarkale P, Kore P, Yadav P. Development of single step RT-PCR for detection of Kyasanur forest disease virus from clinical samples. Heliyon 2018 Feb; 4(2):e00549.	**NA**
8.	Cherian SS, Walimbe AM, Moolpani K, Shirode A, Vaidya SR. Global spationtemporal transmission dynamics of measles virus clade D genotypes in the context of the measles elimination goal 2020 in India. Emerg. Infect. Dis. 2017 Dec. (In Press)	**8.222**
9.	Damle RG, Sankararaman V, Bhide VS, Mahamuni SA, Walimbe AM, Cherian SS. Genetic characterization of the glycoprotein G of Chandipura viruses in India with emphasis on an outbreak of 2015. Infect Genet Evol. 2017 Nov;55:112-6.	**2.885**
10.	Deoshatwar AR, Bondre VP, Tandale BV. Chickenpox and measles clusters among college students in Pune, Maharashtra. Virus Dis 2017 Sept;28(3):337-40.	**0.4**
11.	Deoshatwar AR, Parashar D, Gokhale MD, More A. Low intensity Chikungunya outbreak in rural Western India indicates potential for similar outbreaks in other regions. Asian Pacific J Trop Dis 2017 June; 7(7): 401-4.	**0.8**
12.	Ganorkar NN, Patil PR, Tikute SS, Gopalkrishna V. Genetic characterization of enterovirus strains identified in Hand, Foot and Mouth Disease (HFMD): Emergence of B1c, C1 subgenotypes, E2 sublineage of CVA16, EV71 and CVA6 strains in India. Infect Genet Evol. 2017 Oct; 54:192-9.	**2.885**

13.	Gore MM. View point- Diagnosis of seasonal viral outbreaks: Preference of antibody assays in contrast to gene detection. Indian J Med Res. 2017. (In Press)	**1.532**
14.	Gurav YK, Chadha MS, Tandale BV, Potdar VA, Pawar SD, Shil P, Deoshatwar AR, Aarthy R, Bhushan A. Influenza A(H1N1)pdm09 outbreak detected in inter-seasonal months during the surveillance of influenza-like illness in Pune, India, 2012-2015. Epidemiol Infect. 2017 Jul;145(9):1898-909.	**2.075**
15.	Gurav YK, Yadav PD, Gokhale MD, Chiplunkar TR, Vishwanathan R, Patil DY, Jain R, Shete AM, Patil SL, Sarang GD, Sapkal GN, Andhare MD, Sale YR, Awate PS, Mourya DT. Kyasanur Forest Disease Prevalence in Western Ghats Proven and Confirmed by Recent Outbreak in Maharashtra, India, 2016. Vector Borne Zoonotic Dis. 2018 Mar;18(3):164-72.	**2.045**
16.	Haldipur B, Bhukya PL, Arankalle V, Lole K. Positive regulation of hepatitis E virus replication by microRNA-122. J Virol. 2018 Mar. pii: JVI.01999-17. doi: 10.1128/JVI.01999-17. (E-Pub)	**4.663**
17.	Hegde NR, Gore MM. Japanese encephalitis vaccines: Immunogenicity, protective efficacy, effectiveness, and impact on the burden of disease. Hum Vaccine Immunother. 2017 Jun 3;13(6):1-18.	**2.157**
18.	Jain S, Mittal A, Sharma SK, Upadhyay AD, Pandey RM, Sinha S, Soneja M, Biswas A, Jadon RS, Kakade MB, Dayaraj C. Predictors of Dengue-Related Mortality and Disease Severity in a Tertiary Care Center in North India. Open Forum Infect Dis. 2017 May 5;4(2):ofx056.	**NA**
19.	Joshi MS, Ganorkar NN, Ranshing SS, Basu A, Chavan NA, Gopalkrishna V. Identification of Group B rotavirus as an etiological agent in the gastroenteritis outbreak in Maharashtra, India. J Med Virol. 2017 Dec;89(12):2244-8. 1.935	**1.935**
20.	Kulkarni R, Walimbe AM, Chitambar SD. Spatiotemporal evolutionary dynamics of norovirus GII.4 variants. Adv Pediatr Res 2017 May; 4:5.	**NA**
21.	Kulkarni SP, Ganu M, Jayawant P, Thanapati S , Ganu A, Tripathy AS. Regulatory T Cells and IL-10 as Modulators of Chikungunya Disease Outcome: A Preliminary Study. Eur J Clin Microbiol Infect Dis. 2017 Dec;36(12):2475-81.	**2.727**
22.	Kumar P, Kshirsagar A, Shil P. Estimation of epidemiological parameters for historical ship outbreaks of Influenza. Biomed Res J. 2017. (In Press)	**1.412**
23.	Lole K, Thorat N, Akolkar D, Pingle S. Detection of newly discovered KIs virus in India. Virus Res. 2017 Oct; 242:146-8.	**2.628**
24.	Mittal M, Thangaraj J, Rose W, Verghese V, Kumar C, Mittal M, Sabarinathan R, Bondre V, Gupta N, Murhekar MV. Scrub Typhus as a Cause of Acute Encephalitis Syndrome, Gorakhpur, Uttar Pradesh, India. Emerg Infect Dis. 2017 Aug ;23(8):1414-6.	**8.222**
25.	Mourya DT, Gokhale MD, Majumdar TD, Yadav PD, Kumar V, Mavale MS. Experimental Zika virus infection in Aedes aegypti: Susceptibility, transmission & co-infection with dengue & chikungunya viruses. Indian J Med Res. 2018 Jan; 147(1): 88-96.	**1.532**
26.	Mourya DT, Viswanathan R, Jadhav SK, Yadav PD, Basu A, Chadha MS. Retrospective analysis of clinical information in Crimean-Congo haemorrhagic fever patients: 2014-2015, India. Indian J Med Res. 2017 May;145(5):673-8.	**1.532**
27.	Mourya DT, Yadav PD, Gurav YK, Pardeshi PG, Shete AM, Jain R, Raval DD, Upadhyay KJ, Patil DY. Serosurvey for Crimean Congo Hemorrhagic Fever in humans for identifying high risk populations and high-risk areas in the endemic state of Gujarat, India. Parasites Vectors 2018 Feb. (In Press)	**3.035**

28.	Mourya DT, Yadav PD, Gurav YK, Shete AM, Jain R, Nyayanit D, Pardeshi P, Vishwanathan R, Chiplunkar T, Awate P, Majumdar T, Sahay R. Kyasanur Forest Disease patient's follow-up revealed the kinetics of viral RNA and IgM and IgG and IgG antibody in Maharashtra state, India- A prospective cohort study. Virology J. 2017 Oct. (In Press)	**2.139**
29.	Mourya DT, Yadav PD, Khare A, Khan AH. Certification & validation of biosafety level-2 & biosafety level-3 laboratories in Indian settings & common issues. Indian J Med Res. 2017 Oct ;146(4): 459-67.	**1.532**
30.	Mourya DT, Yadav PD, Majumdar TP, Nyayanit DA, Jain S, Sarkale P. Quaranfil virus an Orthomyxovirus originated in Egypt, confirmed in India, warrant an urgent need to know its relevance for human and animal. BMC Genomics 2018 Jan. (In Press)	**3.729**
31.	Mourya DT. Identifying equine encephalosis virus in horse samples from India using a next-generation sequencing approach. Emerg Infect Dis. 2018 Jan. (In Press)	**8.222**
32.	Nakamura K, Shirakura M, Fujisaki S, Kishida N, Burke DF, Smith DJ, KuwaharaT, Takashita E, Takayama I, Nakauchi M, Chadha M, Potdar V, Bhushan A, UpadhyayBP, Shakya G, Odagiri T, Kageyama T, Watanabe S. Characterization of influenzaA(H1N1)pdm09 viruses isolated from Nepalese and Indian outbreak patients in early 2015. Influenza Other Respir Viruses 2017 Sep;11:399–403.	**2.677**
33.	Nehul S, Kulkarni A, Pawar S, Godbole S, Ghate M, Thakar M. Cross-reactive influenza-specific antibody-dependent cellular cytotoxicity-mediating antibodies in HIV-infected Indian individuals. Infect Dis (Lond). 2018 Jan;50(1):35-43.	**1.119**
34.	Nyayanit D, Shete A, Yadav PD. Complete genome sequence of Tioman virus isolated from Pteropus giganteus bat in India. Genome Announc Feb 2018 (In Press)	**NA**
35.	Parashar D, Sudeep AB, More Ashwini, Patil P, Walimbe A, Mavale M, Amdekar S. Persistence of chikungunya virus in samples stored at different temperature. J Med Microbiol. 2017 May. (In Press)	**2.159**
36.	Patil DY, Yadav PD, Shete AM, Nuchinab J, Meti R, Bhattada D, Someshwar S, Mourya DT. Occupational exposure of cashew nut workers to Kyasanur forest disease at Goa, India. Int J Infect Dis. 2017 Aug; 61: 67-9.	**2.532**
37.	Patil PR, Ganorkar NN, Gopalkrishna V. Epidemiology and genetic diversity of human parechoviruses circulating among children hospitalised with acute gastroenteritis in Pune, Western India: a 5-years study. Epidemiol Infect. 2018 Jan;146(1):11-8.	**2.075**
38.	Patil SL, Balakrishnan A. Genetic characterization Respiratory Syncytial Virus in Kerala, the southern part of India. J Med Virol. 2017 Dec;89(12):2092-2097. 1.935	**1.935**
39.	Pawar SD, Pande SA, Tare DS, Keng SS, Kode SS, Singh DK, Mullick J. Morphological and Biochemical Characteristics of Avian Faecal Droppings and their Impact on Survival of Avian Influenza Virus. Food Environ Virol. 2018 Mar;10(1):99-106.	**1.847**
40.	Penumarthi A, Parashar D, Abraham AN, Dekiwadia C, Macreadie I, Shukla R, Smooker PM. Solid lipid nanoparticles mediate non-viral delivery of plasmid DNA to dendritic cells. J Nanopart Res. 2017 June; 19:210.	**2.020**
41.	Potdar VA, , Hinge DD, Dakhave MR, Manchanda A, Jadhav N, Kulkarni PB, Chadha MS. Molecular detection and characterization of Influenza 'C' viruses from western India. Infect Genet Evol 2017 Oct.; 54: 466-77.	**2.885**

42.	Sahu M, Singh N, Shukla MK, Potdar VA, Sharma RK, Sahare LK, Ukey MJ, Barde PV. Molecular and Epidemiological analysis of Pandemic and Post-pandemic Influenza A(H1N1)pdm09 virus from central India. J Med Virol. 2018 Mar; 90(3):447-55.	**1.935**
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राजभाषा रिपोर्ट

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

राजभाषा अधिनियम 1973 की धारा 3 की उपधारा (3) का अनुपालन करते हुए संस्थान के अधिकतम दस्तावेज़ हिन्दी और अंग्रेजी दोनों मे तैयार किए जा रहे हैं ।

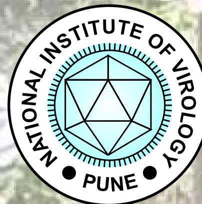
राजभाषा नियम 1976 के नियम 10(4) के अनुसार संस्थान के कर्मचारी हिंदी का कार्यसाधक ज्ञान प्राप्त कर रहें हैं । इस साल हिंदी टंकण - 2, आशुलिपि - 1, प्राज्ञ - 6, पारंगत - 9 कर्मचारी परीक्षा उत्तीर्ण हो चुके है तथा टंकण - 2, प्रबोध - 1, प्राज्ञ - 2, पारंगत - 13; कक्षा के अंतर्गत कर्मचारी ज्ञान प्राप्त कर रहे हैं ।

नगर राजभाषा कार्यान्वयन समिति द्वारा आयोजित की जा रही बैठकों में संस्थान के अधिकारी उपस्थित रहते हैं ।

संस्थान में हिंदी सप्ताह के अंतर्गत दिनांक 22-23 अगस्त 2017 को अनुसंधान प्रयोगशालाओं मे इंजिनिअरिंग एवं प्रशासनिक सहायता की महत्वपूर्ण भूमिका इस विषय पर कार्यशाला संपन्न हुई । परिषद के सभी संस्थानों को आमंत्रित किया गया था । विभिन्ना संस्थानों से कुल 22 प्रतिनिधियों ने कार्यशाला में भाग लिया । कार्यशाला में बँक ऑफ महाराष्ट्र के सहायक महाप्रबंधक श्री राजेंद्र श्रीवास्तवजी बतौर प्रमुख अतिथि उपस्थित रहें । कार्यशाला में श्री अजय खरे, वरिष्ठ तकनीकी अधिकारी(3), डा. शैलेश पवार, वैज्ञानिक डी, श्री डी. आर. कुंभार, वरिष्ठ तकनीकी अधिकारी(1), श्री अमित कासार, तकनिकी अधिकारी-ए, डा. आर. लक्ष्मीनारायणन, वरिष्ठ प्रशासनिक अधिकारी, श्री ए. एस. गायकवाड, प्रशासनिक अधिकारी(भांडार) ने व्याख्यान द्वारा प्रतिभागियों को महत्वपूर्ण जानकारी दी । संस्थान में दिनांक 14 सितम्बर से 22 सितम्बर 2017 तक हिंदी सप्ताह का आयोजन किया गया था । हिंदी सप्ताह के अंतर्गत दिनांक 20 सितम्बर 2017 को हिंदी अंताक्षरी का आयोजन किया गया साथ ही विमुद्रीकरण (ऊशोपशींळूरींळेप) इस विषय पर वाद विवाद प्रतियोगिता का आयोजन किया गया था ।

संस्थान में सतर्कता जागरूकता सप्ताह के उपलक्ष्य में भ्रष्टाचार मुक्त भारत : संभव या असंभव इस विषय पर वाद-विवाद प्रतियोगिता का आयोजन किया गया था ।

संस्थान में आतंकवाद विरोध दिन, सद्भावना दिन, सतर्कता जागरूकता सप्ताह, कौमी एकता सप्ताह के उपलक्ष्य में हिंदी में शपथ ले कर व्याख्यान, संगोष्ठी, प्रतियोगिता का आयोजन करके मनाया गया ।



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