ICMR
INDIAN COUNCIL OF MEDICAL RESEARCH
Serving the nation since 1911

NIV
NATIONAL INSTITUTE OF VIROLOGY

Annual Report

2019- 2020

ICMR-NATIONAL INSTITUTE OF VIROLOGY

CONTENTS

From the Director's desk

Greetings from ICMR-NIV!

It is indeed a privilege to present the research progress report of the Institute for the year 2019-20. The journey has been phenomenal. We have and are living through history as the epic challenge from SARS CoV-2 emerged in early 2020 like a tsunami. As an Institute we shouldered the national responsibility to meet this gargantuan challenge. And we did it as team NIV.

Every hand, every resource blended into one platform and we as NIV could meet this challenge with swiftness, accuracy and response as was needed in this unprecedented public health crisis of this decade. I am proud to present the work done by my colleagues on SARS CoV-2 as a special section in the present research report. Despite this challenge, straining every fabric of the Institute, my colleagues also carried out excellent research in their respective domains in the current reporting period. The Institute got approval from WHO for establishing the country's only Polio Essential Facility as compliant with the Polio Global Eradication Program. The NIV field units at Bengaluru, Kerala, and Mumbai continued with their essential services to assist the State Health authorities with diagnostic virology support, human resource development and provide testing facility for SARS CoV-2.

The ICMR One Health Program on zoonotic diseases initiated in collaboration with Maharashtra Animal and Fishery Sciences Univeristy (MAFSU), has progressed with boundry wall construction and detailed contruction plans to build this unique facility in Nagpur. ICMR-NIV continues to be a WHO collaborating center (CC) on emerging viruses and the Institute has provided a significant number of diagnostic kits for dengue, chikungunya and Japanese encephalitis to national and international programs. The National Influenza Center (NIC) proved its mettle by excelling in meeting the challenge of SARS CoV-2 testing, training and reagent supply as well as providing diagnosis for other respiratory viruses.

As an Institute committed to support the domain of public health, our scientists have been part of different central teams investigating outbreaks and also providing active support to many states as an Apex VDRL laboratory. A unique project was undertaken by the epidemiology group in collaboration with ICMR to develop mobile app applications.Needless to say, the quality of basic research in

dengue, chikungunya, influenza, hepatitis, entomological and veterinary studies and emerging viruses has been of a high standard. The Institute's computational biology & bioinformatics and electron microscopy work on SARS-CoV-2 has been at par with international standards as is evident from the list of publications. We even succeded in isolating SARS-CoV-2 paved the way for several other engagements with industry in the direction of vaccine manufacture and immunotherapy.

We also helped the country of Maldives in the early days of screening SARS-CoV-2 cases as well as sending out a team to screen Indian nationals stranded in Iran, prior to evacuation.We were also engaged in screening returnees from China, Italy and the Diamond Princess Cruise ship.

The pandemic however dulled the physical participation of colleagues in human resource development activities but needless to say this was compensated adequately through several virtual platform engagements. M.Sc Virology, PhD programs continued – a bit delayed on the timeline but not in content. Of course such phenomenal activities cannot be possible without the active support from the administration division and all technical support staff including the engineering core. I would like to congratulate each and every one of my NIV family who have stood by me, with me and for me to help me execute my responsibilities as Director.

My sincere gratitude to Professor (Dr.) Balram Bhargava, DG ICMR for his constant support, Chairman of the SAC, Lt. General (Dr.) Velu Nair for his continued guidance. The Epidemiology and Communicable Diseases (ECD) Head Dr. Raman Gangakhedkar, the present Head Dr. Samiran Panda, Dr. Nivedita Gupta, Scientist F and Head Virology Unit (ECD) and team ICMR are gratefully acknowledged for their timely help rendered to the institute.

I am confident that the Institute shall in every way meet the challenge posed and live upto the expectation of the nation, now and in the future

Prof. (Dr.) Priya Abraham
Director

Composition of committees

Scientific Advisory Committee

Sr. No.	Name
1	Lt.Gen.(Dr) Velu Nair (Retd.)
	Group Head- Medical Services &
	Chief Consultant – Haemato-Oncology & Bone Marrow Transplant
	Comprehensive Blood & Cancer Center (CBCC)
	Address : 632, C-1,Ansals Palam Vihar, Carterpuri,
	Gurgaon-122017, Haryana
2	Dr. Jayprakash Muliyil
	Former Principal & Head of the Community Health and Development (CHAD) Department of CMC CMC Vellore
3	Dr. Shekhar Mande
	Secretary, DSIR and Director General,
	Council of Scientific & Industrial Research,
	Anusandhan Bhawan, 2, Rafi Marg,
	New Delhi - 110001
4	Dr. D.A. Gadkari
	Former Director, NIV, Shilpayatan Apartment 2/13, Erandwane Pune 411 004
5	Dr. Lalit Dar, Professor,
	Dept. of Microbiology,
	All India Institute of Medical Sciences,
	Ansari Nagar, New Delhi-110029.
6	Dr. Neeraj Dhingra
	Director
	National Vector Borne Disease Control Program,
	Directorate General of Health Services,
	Ministry of Health and Family Welfare,
	22-Sham Nath Marg, Delhi-110054.
7	Dr.Sujeet Kumar Singh
	Director
	National Centre for Disease Control
	Directorate General of Health Services
	22, Sham Nath Marg, New Delhi-110 054.
8	Dr. Ashok Kumar, ADG (AH), ICAR
	Krishi Bhavan, Raisina Road, Opp Rail Bhavan, New Delhi, Delhi 110001
9	Director, Health Services, Directorate of Health Services, 8th Floor, Arogya Bhavan, St.George's Hospital Compound, P.D'Mello Road, Mumbai-400001.
10	Dr.R.Gangakhedkar
	Scientist G & Head, Division of ECD
	Indian Council of Medical Research,
	V. Ramalingaswami Bhawan,
	P.O. Box No. 4911 Ansari Nagar,
	New Delhi – 110029
11	Dr. Priya Abraham MD, FRCPath, PhD
	Director, ICMR-NIV, Pune
12	Dr.Swarup Sarkar, National Chair – C.G.Pandit (Medical), ICMR, New Delhi

Institutional Biosafety Committee

Chairman	Prof. Priya Abraham, Chairman, Pune, Maharashtra
DBT Nominee	Dr. Arvind Sahu, Scientist-G, National Centre for Cell Sciences,Pune, Maharashtra
Member Secretary	Dr. Kavita Lole, Member Secretary, Pune, Maharashtra
External Expert	Dr. Vikram Ghole, External Expert, Pune, Maharashtra
Biosafety Officer	Dr. Rima Sahay, Pune, Maharashtra
Internal Experts	# Dr. Anita Shete, Internal Expert, Pune, Maharashtra # Dr. Manohar Choudhary, Internal Expert, Pune, Maharashtra # Dr. Tejaswini Deshmukh, Internal Expert, Pune, Maharashtra

Institutional Human Ethics Committee

Sr. No.	Role	Name & Affiliation	Address	Mobile No	Email ID
1.	**Chairperson** (External)	Dr. Amitav Banerjee, MD Professor & Head Community Medicine D Y Patil Medical College, Pune 411 018	D Y Patil Medical College, Pune 411 018	9372434017	amitav.banerjee@dpu.edu.in amitavb@gmail.com
2	Clinician (External)	Dr. Rajesh Kulkarni, MD Associate Professor B J Medical College Pune 411 001	B J Medical College, Pune 411001	9224799523 8446221019	docrajesh75@ yahoo.com
3	Basic Medical Scientist (External)	Dr. Vikram Padbidri, MD Consultant Microbiology & Infection Control, Jehangir Hospital, Pune 411001	Dept. of Microbiology & Infection Control, Jehangir Hospital 32 Sassoon Road, Pune 411 001	020-66819958, 9822334543	vikram.padbidri@ gmail.com
4	Social Scientist (External)	Dr. Aarti Nagarkar, PhD Assistant Professor Interdisciplinary School of Health Sciences, Savitribai Phule University Ganeshkhind, Pune 411 007	Interdisciplinary School of Health Sciences, Savitribai Phule University Ganeshkhind, Pune 411 007	020-25691758 9822437743	amnagarkar@ gmail.com, aarati@unipune.ac.in
5	Legal Expert (External)	Dr. Jyoti S Bhakare, LLM, PhD Dept. of Law, Savitribai Phule University Ganeshkhind, Pune 411 007	Dept. of Law, Savitribai Phule University Ganeshkhind, Pune 411 007	9422008075, 7507779166	jyotibhakare@ gmail.com
6	Person from Community (External)	Mr. Joseph Cherian BA, Diploma in Health D.Y. Patil Medical College Pune-411018	D Y Patil Medical College Pune 411018	8379910021	jcherian160@ gmail.com
7	Basic Medical Scientist (Internal)	Dr. V.K. Saxena, M.Sc (Microbiology), Ph.D. Scientist E NIV Mumbai Unit, Mumbai 411012	National Institute of Virology Mumbai Unit, Haffkine Institute Campus, A D Marg, Parel Mumbai 400 012	9322070139	ercinternalvks@ gmail.com

Sr. No.	Role	Name & Affiliation	Address	Mobile No	Email ID
8	Basic Medical Scientist (Internal)	Dr. Anuradha Tripathy, PhD Scientist E, Hepatitis Group ICMR-National Institute of Virology, Pashan, Pune 411 021	ICMR-National Institute of Virology, Pashan, Pune 411 021	9822914708	anuradhastripathy@ hotmail.com
9	**Member Secretary** (Internal)	Dr. Rajlakshmi Viswanathan MD (Microbiology) Scientist D, Bacteriology Group ICMR-National Institute of Virology, Pashan, Pune 411 021	ICMR-National Institute of Virology, Pashan, Pune 411 021	8149716655	rupamicro11@ gmail.com

Institutional Animal Ethics Committee

Name, Designation & Address	Role
Dr. Mangesh Shamrao Kamble C-901, Aarohi Project, Sr. No. 123, Susgaon, Tal. Mulshi, Dist-PUNE - 411021	Main Nominee
Dr. Balasaheb Siraskar Principal, SVHNT's College of B. Pharmacy, Rahuri Factory, Pin: 413706, Dist. Ahmednagar	Link Nominee
Dr. Ramanamurthy Boppana Scientist G & In charge, Animal House, National Centre for Cell Science, Pune University Campus, Ganeshkhind, PUNE – 411007	Scientist from outside the Institute
Shri. Ravindra P. Kulkarni B-5, Building C, Anjira Sankul Vidyanagar, Karad-Masur Road, Karad Tal. , Dist. SATARA- 415124	Socially Aware Nominee
Dr. Jayati Mullick Scientist F & Group Leader, Polio Virus Group (former Avian Influenza), ICMR-National Institute of Virology, Microbial Containment Complex, 130/1, Sus Road, Pashan, PUNE- 411021	Biological Scientist, Chairperson
Dr. Dilip Rewa Patil Scientist D & Group Leader, Animal House Group, ICMR-National Institute of Virology, 20-A, Dr. Ambedkar Road, Post Box 11, PUNE-411001	Scientist In charge of Animal House Facility, Member Secretary
Dr Paresh Sumatilal Shah Scientist E & Group Leader, Diagnostic Reagent Facility, ICMR-National Institute of Virology, 20-A, Dr. Ambedkar Road, Post Box 11, PUNE-411001	Scientist from different biological discipline
Dr. Sreelekshmy Mohandas Scientist B, Maximum Containment Laboratory, ICMR-National Institute of Virology, Microbial Containment Complex, 130/1, Sus Road, Pashan, PUNE- 411021	Veterinarian
Mr. Virendra Kumar Meena Scientist B, Electron Microscopy Group, ICMR-National Institute of Virology, 20-A, Dr. Ambedkar Road, Post Box 11, PUNE- 411001	Scientist from different biological discipline

List of samples tested by different groups during 2019-20

List of samples tested for SARS-CoV-2 till March 2020

Facility	No. of samples tested	No. tested positive
NIV-NIC	3905	221
NIV-Bengaluru	1195	33
NIV-Kerala	4701	371
NIV-Mumbai	256	12
Total	**10,057**	**637**

List of samples tested for other viruses of public health importance during 2019-20

Dept	Samples tested for	No. of samples
MCL	KFD (Serum)	2993
	Tick (pools)	1568
	CCHF	876
	Nipah	456
	Zika	45
	DEN/CHIK	48
	Others	14
Influenza	Influ A, B, RSV and others	7655
DEN-CHIK	DEN	4384
	CHIK	768
Hepatitis	HAV, HBV, HCV. HEV	1675
Encephalitis	Encephalitis	735
	Chandipura	469
	HSV	364
Enteric	Enteric viruses	10
PVG	AI	14
DVG	ZIKV, DEN/CHIK	5437
Bacteriology	Bacteria	13
	Pertusis	204
BU	Polio	6491
	Measles/RU	1421
	DEN/CHIK	3580
	Scrub typhus	238
	Lepto	89
KU	All viruses	5673
MU	Polio Measles/RU + others	14206
TOTAL		**59,426**

Section 1:
Report on COVID activities

Section 1: Report on COVID activities

Introduction

An outbreak of pneumonia was reported from Wuhan, People's Republic of China, in December 2019 that was linked to a novel coronavirus designated as SARS-CoV-2. On January 31, 2020, the International Health Regulations Emergency Committee of the World Health Organization (WHO) declared the COVID-19 outbreak as a Public Health Emergency of International Concern (PHEIC).

Activities undertaken by different research groups

National Influenza Centre (NIC)

Dr Varsha Potdar, Dr Manohar Lal Choudhary, Dr Sumit Bharadwaj and NIC team

The NIC played the most important role by being the key testing laboratory. WHO has pandemic preparedness in place for public health important respiratory viruses. During the current pandemic, being the Apex laboratory of ICMR NIV–NIC has quickly developed and deployed accurate diagnostic method to detect SARS CoV-2 in mid-January.
During January 22 to February 29, 2020, 362 clinical samples collected at the airport screening centre and a few samples of the SARS-CoV-2 suspected overseas returnees were received at NIC for diagnosis. Of the 362 clinical specimens tested, 84 (23.2%) were positive for one or more respiratory viruses (10 with co-infections) and 278 were negative (Fig. 3) Viral positivity was highest in the 41-50 yr age group and SARS-CoV-2 was detected only among four (1.1%) cases, all of them had a travel history to China or to the countries affected with COVID-19 outbreak; the first three SARS-CoV-2-positive cases were from Kerala and the fourth one from Delhi.
A one-step single tube multiples RT-qPCR assay for the detection of SARS-CoV-2 *in vitro* was successfully developed. COVID-19 testing to the Indian nationals in Iran and Italy, as well as for the travellers quarantined at the Indo-Tibetan border police was provided.
Reagent support was provided to Japan for the cruise outbreak and further samples of the Indian Nationals' returnees were tested. NIV provided the diagnostic reagent support for sustenance of quality testing at government laboratories. NIC served as a Central and Regional depot for ICMR for COVID-19 reagent stock and supplies to the Government testing laboratories. Genetic sequencing of the first two positive cases and sequences were performed and shared globally.

Maximum containment facility

Dr Pragya Yadav, Dr Anita Shete, Dr Sreelakshmi and BSL4 team

(i) Virus isolation

Initial COVID-19 cases (n=3) were reported from Kerala state. Later the cases were also reported from a group of Italian tourists and their contacts along with other reports from Agra, Uttar Pradesh. SARS-CoV-2 was successfully isolated from the clinical samples and was further characterized. Cytopathic effects were observed in 9/12 cultures in the first passage in Vero CCL-81 cells. A $TCID_{50}$ of $10^{5.5}$/ml was observed at passage 2 for different clinical specimens passaged in Vero CCL-81 cells. qRT-PCR of the envelope (E) gene demonstrated an increase in the E gene copy number for the 2nd Vero CCL-81 passage

in comparison with the 1st passage. The virus particles showed a distinct morphology of SARS-CoV-2 with typical peplomeric projections of the glycoprotein spike under electron microscope.

(ii) Genomic characterization of SARS CoV 2 isolates

Throat swab (TS) samples collected from suspected cases (n=881) were tested using the WHO recommended protocol and three of them were found to be positive. These positive samples were further sequenced using the NGS to retrieve the complete genomic sequences. However, the genomic sequences could be retrieved only for two samples.

The sequences of the Indian SARS-CoV-2 strains were clustered in different positions (Figure 3). The analysis of these two sequences with multiple sequences in the GISAID (https://www.gisaid.org/) indicated a globally emerging heterogeneity. This study also helped design the *in-silico* model for the spike gene region and predicted the B-cell and cytotoxic T-cell epitopes in the spike protein. These epitopes can be considered suitable for the vaccine design and diagnostic target in the future.

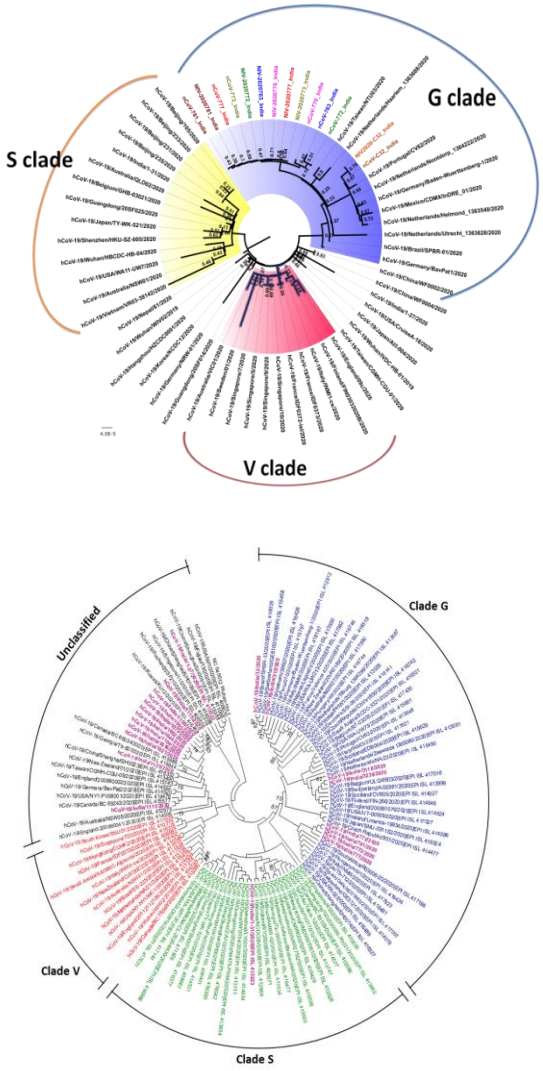
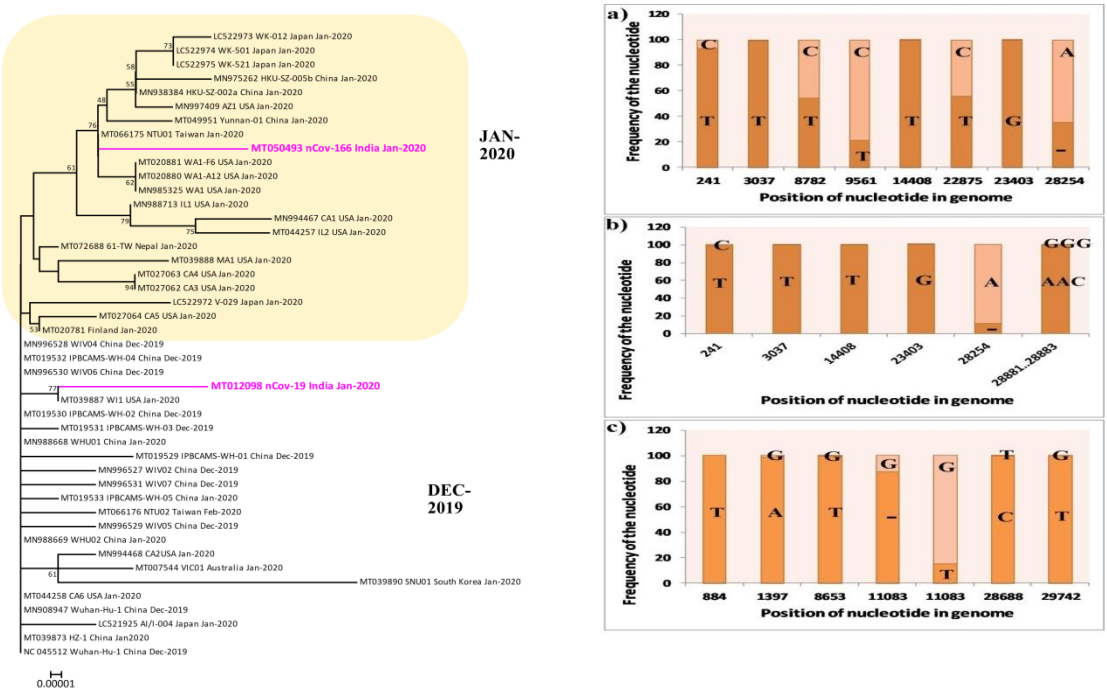


Figure: Neighbor joining tree based on complete Indian SARS-CoV-2 sequences using the Kimura 2-parameter. Nucleotide substitutions/site is indicated in the scale bars.

The sequences used in the study were retrieved from the throat/nasal swab samples of Italian tourist and their Indian contact cases, Indian nationals in the Iran and the first two SARS-Cov sequences from Wuhan, China. A total of 21 SARS-CoV-2 sequences were retrieved using the NGS approach and 1563 sequences were downloaded from GISAID. These sequences were aligned and a phylogenetic tree was generated (Figure 5). An overall divergence of 0.03% was observed in the sequences used in the study. Further the phylogenetic tree demonstrated the presence of 'G' and 'S' clade sequences in the retrieved set. Nucleotide mutations were compared using the Wuhan Hu-1 reference strain (Figure 6). The nucleotide variation observed in the sequences can be linked to the specific regions of their origin.



(iii) Developing a serologic assay for SARS-CoV-2 antibody detection

(Collaboration between MCL and VDRL)

With the rapid spread of SARS-CoV-2, the need to develop serological tests for antibody detection was undertaken. Such assay are important for both disease burden studies as well as spread of the virus. An attempt was made to develop and evaluate an IgG-based ELISA for COVID-19.

The indigenously developed ELISA was found to be 92.37 % sensitive, 97.9% specific, robust and reproducible. The positive and negative predictive values of the assay were 94.44 and 98.14 percent, respectively. The developed assay can be used for determining the seroprevalence of SARS-CoV-2 in a human population exposed to the virus.

(iv) Studies on virus quasi-species

The next-generation sequencing (NGS) methods to analyze the SARS CoV 2 sequences from Italian tourists, contact of the Italian tourist, and the Indian citizens sampled at Iran were used to analyze the quasispecies. The samples were analyzed by using the variant detection tool as implemented in the CLC genomics workbench v11.0. The reference sequence used for deriving variants from the sequenced reads was the SARS-CoV-2 Wuhan HU-1 strain (Accession number:

NC_045512).

The quasispecies identified in the three sets of clinical samples reveals the presence of variation in the nucleotide positions and virus evolution to adapt towards the new host. This study also indicates that a quasispecies is varied depending on the location and indicates the further exploration of larger data set to identify variability amongst different clinical samples.

(v) Animal studies with SARS-CoV2

SARS CoV-2 propagation was studied using animal models like infant mice CD1 (by intranasal, intracerebral and intraperitoneal route), AG129 (by intranasal and intraperitoneal route) and C57 BL/6 (intracerebral route). None of the animals supported virus propagation. Further, susceptibility of various adult rodent models like BALB/c mice, C57BL/6, AG129 mice and Syrian hamsters to SARS CoV-2 was studied in the BSL-4 laboratory. Syrian hamsters were found susceptible. The polyclonal sera required for the development of in-house IgM ELISA was raised in adult Balb/c mice against SARS CoV-2. Liver, formalin inactivated and Gamma irradiated SARS-CoV-2 were used as antigen for the study.

(v) SARS-CoV-2 antiviral testing study

Considering the need for effective antiviral for the treatment of COVID-19, antiviral testing study was planned with COVID antiviral team of ICMR-NIV. Candidate antiviral drugs from various institutes and companies for the evaluation of antiviral effect against SARS-CoV-2 were tested. Besides this, inactivated SARS-CoV-2 was provided to CSIR-CCMB for Molecular studies on SARS-CoV-2 infection and development of antiviral compounds and candidate vaccines.

Virus Research and Diagnostics laboratory (VDRL)

Dr Gajanan Sapkal, Dr Gururaj Deshpande, Dr Ulhas and team

(i) Building laboratory capacity for COVID-19 in the country

During the early stage of the COVID-19 pandemic in India, the Group actively contributed to build National preparedness to fight COVID-19 and enhanced laboratory capacity of 10 VRDLs for COVID-19 diagnosis by shipping necessary diagnostic reagents and kits. Scientists from the Group also visited state VRDLs at Bangalore Medical College & Research Institute and Gandhi Medical College, Secunderabad and provided technical and troubleshooting assistance in COVID-19 testing, during February 2020.

(ii) Assistance provided for COVID-19 testing of Indian expatriates in Iran (with support from NIV engineering core, Shri Ajay Khare and team)

As part of the special mission by the Govt. of India, Ministry of Health & Family Welfare and ICMR to assist testing and expatriation of the Indian nationals stranded in Iran during the COVID-19 pandemic, ICMR-NIV has deputed a team comprising six professionals. DVG group supported the move by deputing two Scientists and a Technical staff to organize collection and shipment of clinical samples from >2000 Indians citizens from various locations in Iran.

(iii) Development of anti-human SARS-COV-2 IgG ELISA kit

In collaboration with Maximum Containment Facility of NIV, developed anti-human IgG ELISA kit (COVID KAWACH Human IgG ELISA), and transferred the technology to Zydus Cadila, Ahmedabad and five other companies.

In addition, micro-neutralization test (MNT) and plaque reduction neutralization test (PRNT) for SARS-CoV-2 were also developed subsequently. Results of both the tests were found specific and had no cross reactivity. The intra-class correlation was calculated to assess the correlation between MNT and PRNT and found to be 0.520. The PRNT assay may find application to determine the antibody titer of samples collected from NAb in recovered/vaccinated or infected COVID-19 patients while MNT may be used for large scale serosurveys.

(iv) Validation of serological test kits for COVID-19

During the pandemic, a pressing need was felt by ICMR to have a validation mechanism for COVID-19 test kits in the country. In view of this, the Diagnostic Virology Group developed a well-characterized sample panel for validation of the serological test kits [including Rapid Diagnostic Tests, ELISA and Chemi-luminescent Immunoassay kits for detection of IgM, IgG and total antibodies against SARS-CoV-2 from RT-PCR-confirmed cases. Till March, 2020, 07 test kits were evaluated, of which 04 Rapid Diagnostic Test kits were found satisfactory. The reports were conveyed to ICMR and the manufacturers.

Bioinformatics Group

Dr Sarah Cherian and team

(i) Repurposing of drugs for the coronavirus disease 2019 using drug docking studies

We analyzed the binding potential of HIV-1 protease inhibitors, lopinavir and ritanovir against the main protease (3C-like protease) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), using computational docking studies. The X-ray structure of SARS-CoV-1 3CLPro dimer bound with aza peptide epoxide (APE) as an inhibitor, (2A5K.pdb) was used for the modeling studies. We detached the APE from the crystal structure complex and re-docked it computationally using the same protocol as for the selected study inhibitors to obtain the docking score and it was found to be -8.27 Kcal/mol. The two inhibitors in this study, had better binding potential (docking score = −11.29 kcal/mol for ritonavir, and −9.6 kcal/mol for lopinavir) (**Fig. 1**) when compared to APE. Comparison of the docked poses revealed that lopinavir occupies the P1’ and P1 subsites with excellent complementarity while ritanovir occupies the P3 and P4 subsites with excellent complementarity. These structural features indicate the possible mechanism by which these inhibitors can block the function of the 3CLpro. Molecular dynamics simulation studies for the complexes obtained in this study would be essential to identify specific interactions between the enzyme and drug in the stable complexes.

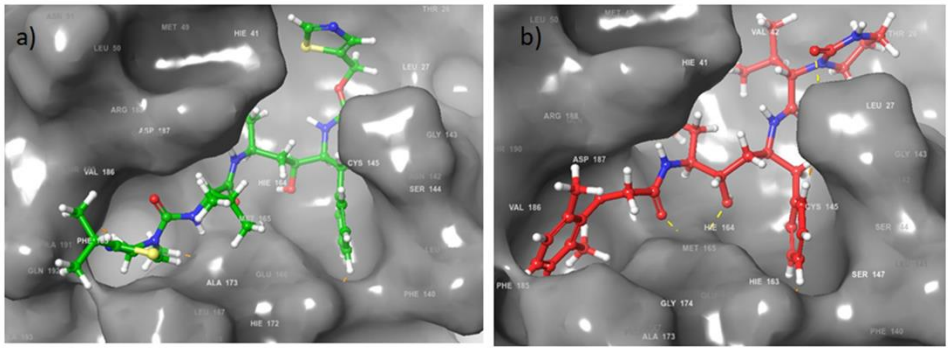


Fig. 1: Docking interaction analysis of HIV inhibitors in the substrate binding cavity of modeled SARS-CoV-2 main protease (3CLpro) (A) ritonavir (B) lopinavir. The P4–P1' side chains of the inhibitor are labelled.

Prediction of B- & T-cell epitopes on the spike protein of the Indian SARS-CoV-2 strain

Investigators: *Dr Sarah Cherian, Mrs Megha Agarwal, Dr Pragya Yadav, Dr Dimpal Nyayanit*

As the spike protein of SARS corona viruses has been found to elicit neutralizing antibodies, prediction of B- and T-cell epitopes for the Indian SARS-CoV-2 sequences were undertaken using bioinformatics tools. Predicted linear B-cell epitopes were found to be concentrated in the S1 domain of spike protein. Prediction of conformational B-cell epitopes (**Fig. 2**) revealed that one of these (residue positions 341-507) in the spike protein incorporates two of the predicted linear epitopes (327-342 and 404-419) having good antigenicity along with a favourable IFN-γ response that enables differentiation and proliferation of the B-cells. The epitope also lies within the receptor binding domain (RBD).

The spike protein of SARS-CoV has also been reported to be immunogenic and elicit high IFN-γ-specific T-cell response. The prediction of T-cell epitopes revealed that nine possible CTL epitopes possessing good antigenicity and inducing IFN-γ response were present in the S protein. Among the T-cell epitopes predicted, four epitopes 89-97 and 256-264 in the S1 domain and 825-833 and 1058-1066 in the S2 domain were found to have good CTL prediction scores with a broad human leucocyte antigen (HLA) allele coverage of A and B supertypes. These HLA supertypes being predominant in the Indian population, the predicted epitopes may be considered suitable for future experiments towards vaccine design.

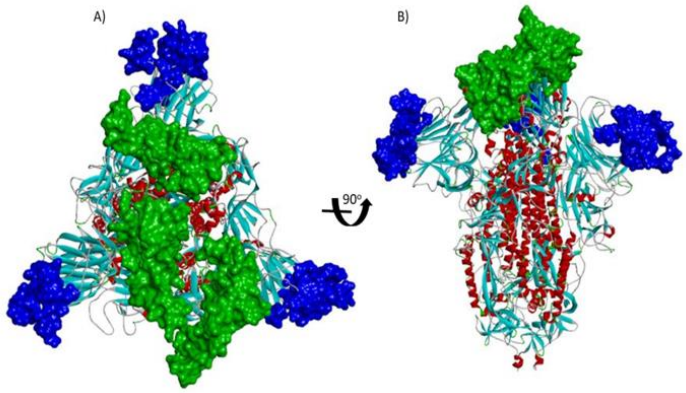


Fig. 2. Predicted conformational B-cell epitopes mapped on the pre-fusion structure of the modelled Indian SARS-CoV-2 spike protein using the pre-fusion structure of SARS-CoV-2 (6VSB.PDB) (colour key: blue - epitopes 67-261; green - epitopes 341-507 (A) Top view (B) Side view.

Electron Microscopy Group

Prof Atanu Basu and team

The EM group carried imaging studies on SARS-CoV-2. These included direct imaging the virus in clinical specimens, developing an immune electron microscopy assay and changes in the virus morphology at different temperatures.

Other Miscellaneous activities

Reagent supply and ICMR hub creation: Under directives from ICMR NIV acted as the central hub for diagnostic reagent supply to all SARS-CoV-2 testing labs under the VDRL network as well as to state health department testing laboratories. The activity was co-ordinated by close coordination of the logistic cell created at the NIV.

Section 2:
Outbreak investigations

Section 2: Outbreak investigations

1. Nipah virus outbreak in Ernakulam district, Kerala state, June 2019:

Investigators: Yadav PD, Sudeep AB, Gokhale MD, Anita Shete-Aich, Balasubramanian R, Ullas PT, Majumdar T & ICMR team.

On 4th June 2019, MoH&FW, Govt. of India, declared an outbreak of Nipah virus in Ernakulam district, Kerala state after confirmation of a case of a 21-year-old male student from Vadakekara. ICMR constituted a multidisciplinary team comprising experts from ICMR-National Institute of Epidemiology, Chennai; ICMR-National Institute of Virology, Pune; ICMR-National AIDS Research Institute, Pune; Government Medical College, Kozhikode and TD Medical College, Alappuzha to coordinate facilities for prompt laboratory diagnosis of suspected patients and to explore the association of fruits bats in virus transmission.

Two teams from ICMR-NIV, Pune were deputed to the outbreak area to support the state health authorities in outbreak management. The first team was to establish a temporary laboratory with all possible equipments to support diagnosis and patient management while the Field Biology team was to investigate the role of fruit bats (*Pteropus* and *Rousettus*) in virus maintenance and transmission.

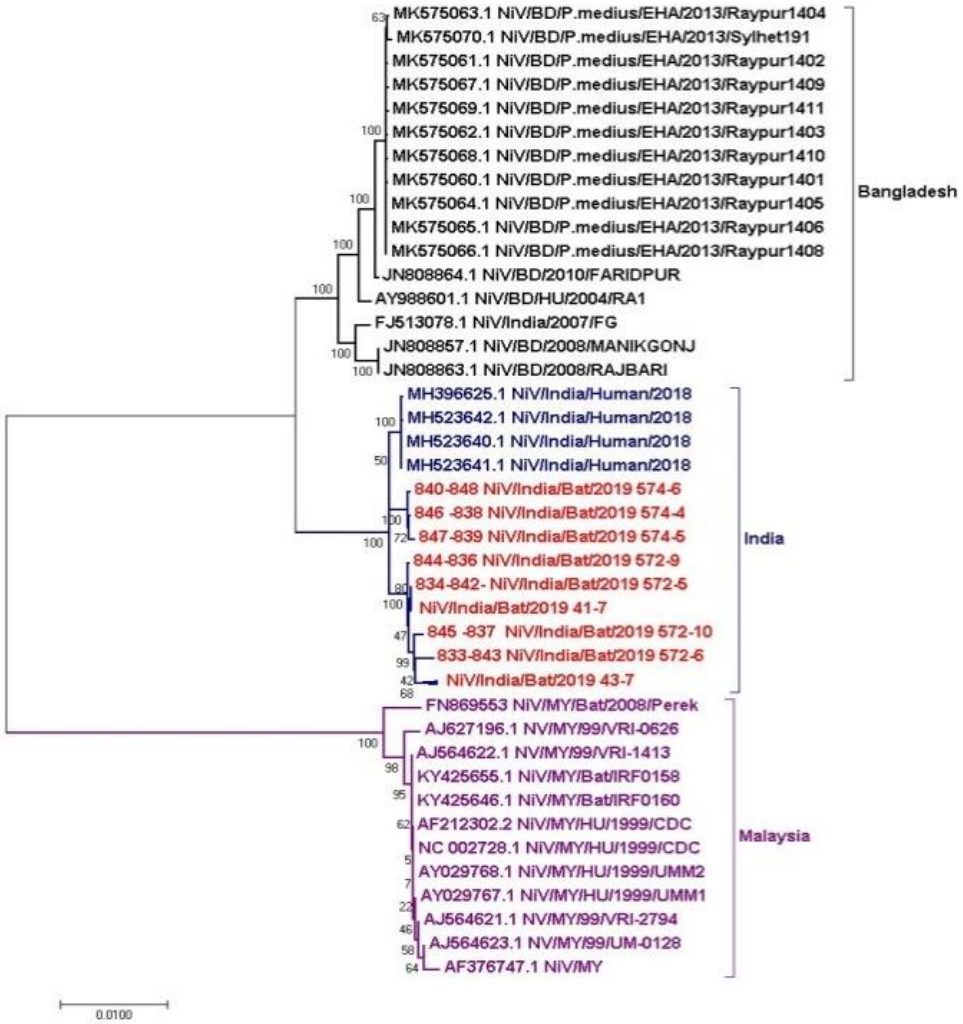


Fig 1: Phylogenetic tree of Nipah virus: NiV sequences from Kerala, along with reference sequences indicating the circulation of a new genotype in South India.

Work done:

(a) A temporary laboratory was established at Government Medical College, Kalamasseri, Ernakulam with installations necessary for conducting serological and molecular detection of NiV activity in human samples. NIV team conducted intensive training sessions on risk mitigation, donning and doffing of Personal Protective Equipment (PPE) to local medical practitioners, nursing staff, and state health team. About 30,000 HCWs of different cadres were trained within two weeks of the outbreak onset. The following are the key contributions made by NIV team.

(i) Confirmation of Nipah outbreak by detecting NiV in patient sample
(ii) Strengthened the diagnostic capacity of GMC, Ernakulam to diagnose NiV.
(iii) Helped in the containment of the outbreak in association with the state authorities
(iv) Validated the Point of care (PoC) real time RT-PCR assay for NiV
(v) Provided diagnostic support to referred samples during the outbreak.
(vi) Provided hands on training to Kerala state staff in donning and doffing of PPE
(vii) Provided training in handling PoC real time RT-PCR assay to microbiologists and technical staff
(viii) Nipah virus genomic ancestry was revealed by complete sequencing of the sample.
(ix) ICMR-NIV enhanced the capacity of VRDLs to deal with NiV like emergencies

(b) To determine the association of fruit bats in virus transmission, 141 bats (109 *Pteropus* and 32 *Rousettus*) were sampled from five sites in Ernakulam and Idukki districts of Kerala especially from the neighborhood of the residence and the college where the patient was studying. Nipah virus RNA was detected in one throat sample and 03 liver/spleen samples. Serological studies have revealed the presence of anti-NiV antibodies in 12 of 58 bat samples. NGS analysis of the sequences of the present study revealed a distinct cluster of NiV sequences indicating the circulation of a new genotype of NiV in South India (Figure 1).

2. Acute encephalitis syndrome outbreak among children, Muzaffarpur (Bihar)

Investigators: Tandale BV, Deoshatwar AR, Sapkal GN, Yadav P & ICMR team.

A mystery illness ccharacterized by sudden onset of generalized tonic-clonic seizures that caused unusual deaths in children was reported from Muzaffarpur. ICMR-NIV scientists as part of the Central Team investigated the outbreak to determine the etiological agent (s).

Findings: Almost 75% of the cases were reported from the Muzaffarpur district only. Clonic, occasional seizures continued in many patients even after heavy sedation and anti-epileptics. No significant illness in past/recent past was recorded. WBC counts were mostly normal, but MRI scans shown generalized edema of cerebrum without focal changes. EEG was suggestive of encephalopathy than encephalitis. Almost 10% patients reported 'hyperactivity/aggressive behavior or partial loss of power in an extremity [paresis]. Glucose level was found low in majority of patients (43mg/dl). CFR of 0.3% was seen as per government records. Time distribution of cases is given in Fig 2.

All serum/CSF samples collected from the patients and their contacts were tested for measles, mumps, rubella and Chandipura virus but with negative results. NGS analysis was also carried out at ICMR-NIV, but no novel/unknown viral agent (s) was associated with acute encephalopathy/ encephalitis could be detected.

The investigators attributed the etiology to malnutrition and starvation. Children going to sleep without a meal could be the probable reason for the deaths.

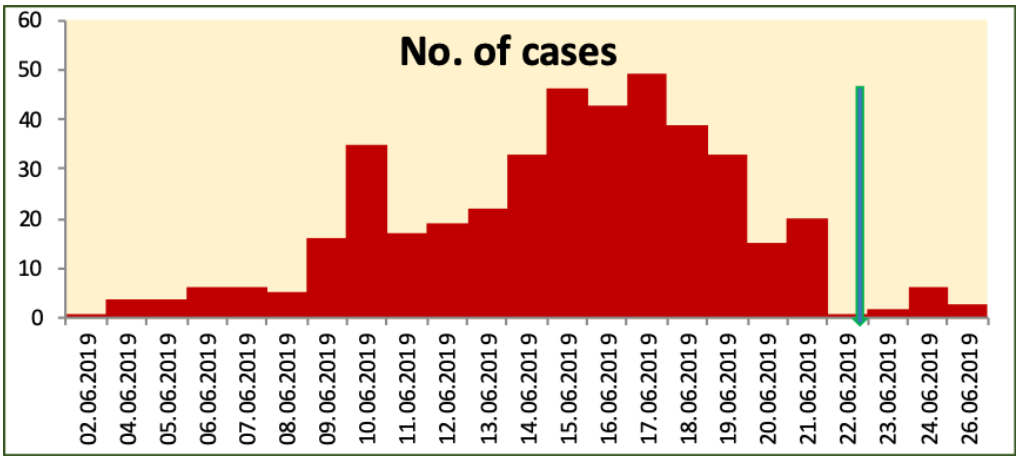


Fig 2: Time distribution of cases of Muzafarpur outbreak

3. Investigation of pediatric deaths due to unknown etiology at Udhampur, Jammu & Kashmir

Investigators: Deoshatwar AR, Tomar S. Potdar V, Yadav PD

Eighteen healthy children in the age group of 11 months to 5.5 years presented with unusual clinical manifestations, *i.e.*, vomiting, decreased urine output, anuria and high blood pressure in Udhampur district of Jammu. Ten deaths were reported at the time of investigation followed by one more death taking the tally to 11. ICMR-NIV investigated the outbreak as part of the central team.

Work done and findings: Screening of 13 samples collected from cases and contacts at ICMR-NIV detected the presence of adenovirus, RSV and parainfluenza viruses. NGS analysis has shown the presence of rhinovirus A, RSV-A, Human respirovirus 3, Human rhinovirus B35 and Human coronavirus OC43. However, no common etiology could be determined for the unusual deaths. Toxicological analysis of drug samples conducted by Drug Controller, Jammu revealed the presence of diethylene glycol and could be the cause of the illness and death. The timeline is presented in Fig 3.

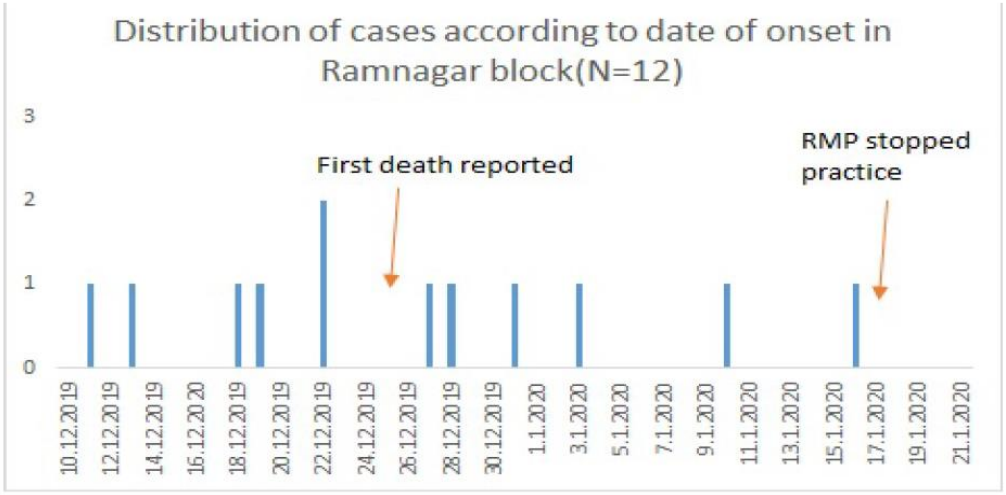


Fig 3: Distribution of cases from Ramnagar block by date of onset

4. Hepatitis outbreak in Shirole, Dumala, Kavri, Kolhapur (Maharashtra)

Investigators: Deoshatwar AR (Epidemiology group) & Lole KS (Hepatitis group).

An outbreak of hepatitis-like illness was reported from a small village in Kolhapur district during November - December 2019. On the request of state health department, ICMR-NIV team investigated the outbreak and collected samples from 25 affected people and tested. Hepatitis A virus IgM antibodies were detected in 11 serum samples and the samples tested negative for hepatitis E virus antibodies suggestive of a Hep A virus outbreak. The source of the contamination was determined as a well situated 4 km uphill from where water was supplied to the village. NIV team recommended regular monitoring of water quality and chlorination at regular intervals to avert such outbreaks.

5. Investigation of Cholera outbreak in western Maharshtra

Rajlakshmi Viswanathan (Bacteriology), Avinash Deoshatwar (Epidemiology), Gopalkrishna Varanasi, Madhuri S Joshi (Enteric Viruses)

An outbreak of acute watery diarrhea was reported in Sawarpada village of Kalwan taluka, Nashik district, in October 2019. Case-patients (n=288) were identified among 1074 residents. A team comprising members from Epidemiology, Enteric Viruses and Bacteriology groups investigated the outbreak with the help of local health authorities. *Vibrio cholerae* O1 Ogawa Biotype El Tor was isolated from four of 13 refereed faecal samples. No other viral or bacterial pathogen was detected. Leakagae in pipe supplying water from a well to the main water tank of the village, and subsequent sewage contamination was implicated as the likely source of the outbreak. Appropriate prevention and control measures were implemented by local health authorities.

Section 3:
Scientific work reports

ANIMAL HOUSE GROUP

Scientific Staff

Dr. D.R. Patil	Scientist D & Group Leader

Technical Staff

Mr. S.N. Fulari	Sr. Technical Officer-1
Mr. H.L. Chakankar	Sr.Technician-3
Mr. R.H. Chavan	Sr. Technician-2
Mr. R.J. Sarpatil	Sr. Technician-2
Mr. A.N. Shitole	Sr.Technician-1
Mr. S.M. Doke	Technician C
Mr. V.B. Kalangade	Technician B
Mr. M.B. Kamble	Technician B
Mr. A.V. Kondayya	Lab Assistant
Mr. A.D. Dalvi	Lab Assistant
Mr. A. Thimanapalli	Lab Assistant
Mr. V.P. Sasane	Lab Assistant
Mr. S.B. More	Lab Assistant
Mr.K.S.Nikalje	Technician-A
Mr.S.K.Athwal	MTS
Mr.N.V.Chavan	MTS

Project Staff

Mr.A.S.Shinde
Mr.M.S.Parsuram
Mr.S.Poharkar

- Core experimental animal facility
- Maintaining special mice like Jax, CRL and AG 129 mice
- State-of-art primate facility

Experimental Animal Facility

Authors: Dilip Patil & Sidharam Fulari
Funding Agency: Intramural
Project Duration: Ongoing Institutional service project

Background:

Animal House Group is a core service department catering to the needs of animal experimentation in the institute. Large and small animal facilities at the Institute are registered with CPCSEA under "Research for education and breeding for in house use" vide Registration No. 43/GO/ReBi/SL/99/CPCSEA having validity until March 2022. Ten strains of mice (Inbred: BALB/c (Jax and CRL), C57BL/6, DBA/2, C3H, Outbred: CD-1, Immuno-deficient: CD-1 *nu/nu,* BALB/c *nu/nu,* RAG-1 and AG129) and Golden Syrian hamsters are being maintained, bred in the individually ventilated caging (IVC) system (Figure) and supplied for in house research in filter top cages.

***Work Done*:**

During the report period, a total of 1436 mice and 85 hamsters (for Covid-19 research) were supplied to institutional scientists against 24 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 637 ml blood from different species of laboratory animals, as diagnostic reagent in various assays was supplied to institutional scientists. The animals are maintained under controlled environmental conditions (temperature (22±2ºC), relative humidity (50 ±10 %), 12:12 h light and dark cycle with 100 % of fresh air exchange in animal rooms) with uninterrupted power supply.

Husbandry and veterinary care was provided to rhesus monkeys under rehabilitation at the institute. Comprehensive health monitoring was conducted for individual animals. The tests were conducted through NABL accredited laboratory, which included, Complete Blood Count, Differential Count, Peripheral Blood Smear Examination, hepatic profile, kidney profile, lipid profile, Chest X-ray, intra-dermal tuberculin test. Beside institutional care programme for primates, services of consultant veterinarians were also sought.

As per the recent directive to animal facilities by CPCSEA vide letter. No 25/28/2017, annual health check-up of animal house staff was carried out.

IAEC activities: Institutional animal ethics committee reviews the research protocols and also ensures compliance with the CPCSEA norms. Accordingly, two meetings of Institutional Animal Ethics Committee (IAEC) for animal experimentation projects evaluation and one mandatory meeting for animal house inspection was conducted.

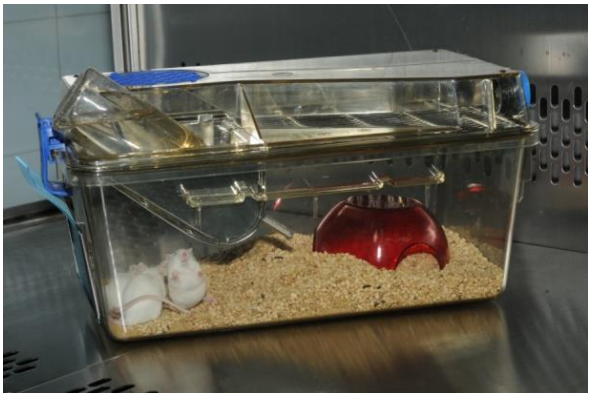


Figure: IVC with mice

BACTERIOLOGY

Scientific Staff

Dr Rajlakshmi Viswanathan	Scientist D (vishwanathan@gov.in)

Technical Staff

Mrs Savita Dhurandhare	Technician B

Support Staff

Mr Rajendra Khedkar	Multi Tasking Staff

Project Staff

Ms Shradha Maheshwari	Technician C (from January 2020)
Mr Gajanan Zhambre	Field Worker (from January 2020)

- Core facility for bacteriology.
- Initial study finding on seroepidemiology of pertussis suggests 20% seropositivity in unvaccinated pregnant women.

BACT1803: **Seroepidemiology, maternal immune status and missed diagnosis of pertussis among young infants in India - a multicentric study**

Rajlakshmi Viswanathan, Babasaheb V Tandale, in collaboration with clinical partners
Funding Agency: Extramural: DBT Wellcome India Alliance Intermediate Career Fellowship in Clinical and Public Health
Duration: 1st October 2019-30th September 2024 (5 years)
Budgte: 2.83 crore INR

Background:
Pertussis, caused by *Bordetella pertussis* is one of the most poorly controlled vaccine-preventable diseases in the world and remains a major cause of childhood morbidity and mortality. Clinical diagnosis of pertussis in infants is challenging due to non specific presentation and absence of classical symptoms. Young infants, who have not completed their primary immunization series, and are dependent on maternal immunity, are the most susceptible. No systematic information on pertussis is available from India.

Objectives:

1. To generate information on seroepidemiology of pertussis in India
2. To estimate seroprevalence of pertussis in infant-mother dyads & follow up for vaccine uptake and susceptibility to pertussis
3. To determine the role of pertussis in severe respiratory infection among young infants: strengthening laboratory capacity

Work done and Findings: Two hundred and four of 276 eligible infant-mother dyads were enrolled in the study, including women who had received acellular pertussis vaccine in pregnancy. Estimation of anti pertussis toxin IgG was performed using recommended commercial ELISA kit. More than half of the vaccinated mothers were seropositive. Among the unvaccinated subset, 20% showed seropositivity. The geometric mean titre of anti-PT IgG antibodies was significantly higher in the vaccinated subset in comparison to the unvaccinated subset (Table1). Median placental transfer ratio of anti-PT antibodies was 1 (IQR 0.8 – 1.3). Seroprevalence of pertussis antibodies was low (20%) in unvaccinated pregnant women and suggests exposure to natural infection. Further studies will be performed to strengthen these preliminary findings.

Table 1: Estimation of anti pertussis toxin antibodies in infant-mother dyads

Anti PT antibodies	**Vaccinated (*n*=90)**	**Unvaccinated (*n*=114)**
Maternal Seropositivity n (%)	49 (54)	23 (20)
GMC IU/ml (Range)	34* (24-47)	4 (3-7)
Infant Seropositivity n (%)	49 (54)	19 (17)
GMC IU/ml (Range)	34* (24-47)	4 (3-7)

**Significantly higher than unvaccinated group, p<0.01*

BIOINFORMATICS AND DATA MANAGEMENT GROUP

Scientific Staff

Dr Sarah Cherian,	Scientist F & Group leader
Dr Pratip Shil,	Scientist D

Technical Staff

Mr A.M. Walimbe,	Technical Officer B
Mr Santosh Jadhav,	Technical Officer A
Mr N V Gujar	Technician - B
Mr Avinash Patil	Technician - B

Ph.D. Scholars

Ms Dipali Bhoye	UGC's Rajiv Gandhi Senior Research Fellow
Ms Prachi Jagtap	ICMR Junior Research Fellow
Ms. D. Pavitrakar	ICMR-NIV Technical Officer

Project staff

Ms Bhagyashri Kasabe	SRF (ICMR extramural project)
Mrs Megha Agarwal	SRF (ICMR extramural project)
Ms Sucheta Patil	Research Associate (ICMR extramural project)
Dr Nitin M. Atre	Research Associate (ICMR extramural project)

- Core Facility services
- *In-silico* modeling of lopinavir and ritanovir binding to SARS-CoV-1 3CLPro dimer showed excellent binding to P1 and P1' sites.
- Phylogeography analysis of full genome of CCFH shows multiple introductions of the virus in different times.
- *In-silico* modeling suggests benzothiazol compounds to have affinity for Chikungunya virus oilymerase enzyme.
- A new database named ArVirInd was developed that contains information on all arboviruses isolated from India
- Mathematical modeling studies on climatologic factors and vector borne viruses initiated.

BDM1001: Core facility services
(Sarah Cherian, Pratip Shil, A.M. Walimbe, S.M.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 was done on regular basis. Regular maintenance of connectivity between NIV, MCC and three field units is being done for data transfer, AIMS, LIMS Software and intercom services. NIV website is being monitored on a regular basis. Audio and video conferencing units are maintained on a regular basis. Technical support has been provided for conferences, workshops, M.Sc (Virology) classes, dissertation presentations, meetings held by NIV. The indigenously developed softwares like payroll, pension, supplementary bills, arrears calculator, computer complaint register, etc. were maintained.

Phylogeography analyses of Crimean Congo Hemorrhagic Fever Virus
Investigators: *Dr Sarah Cherian, Mr S.M. Jadhav, Mr A.M.Walimbe, Dr Pragya Yadav*

The phylogeography analyses of CCHFV based on whole genomes (n=110, including 23 Indian isolates) 1956-2017, was undertaken. Analysis based on the S-gene revealed multiple introductions of the virus into India. The far-east Asia-2 genotype was most likely introduced from Tajikistan ~90 years ago while the middle-east Asia-1 genotype was introduced from Pakistan in the more recent entries ~10-30 years back (**Fig. 1**). The earliest entry of M2 genotype (based on the M gene) may have been ~350 years ago most likely from South Africa while, analysis of the L-gene, revealed entry of the virus via Afghanistan. Many of the Indian isolates having different origins in the S and L segments could be reassortents. Though the early entry of the virus into India was noted from South Africa either directly or via Tajikistan, Pakistan and Afghanistan, it was noted that India has also transmitted CCHFV to countries such as UAE, Iran and Afghanistan, Kazakhstan and Turkmenistan.

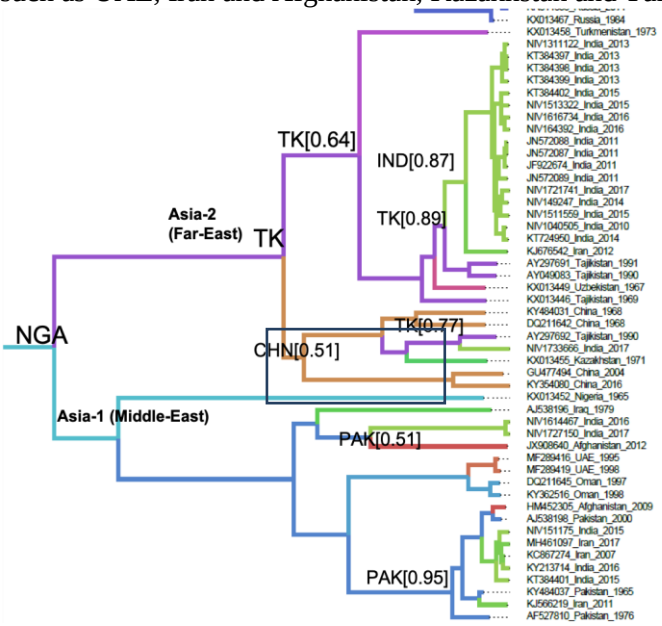


Fig. 1: Maximum clade credibility (MCC) tree based on the CCHFV S gene sequences (1956–2017) showing the G-IV (Asia genotype) strains clustering into the far-east Asia-2 and the middle-east Asia-1 sub-genotypes. The labels at the nodes correspond to the ancestral states with their probabilities wherever >0.5. The branches are colored according to the respective ancestral geographical region.

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

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

Collaborators: *Dr Prathama Mainkar & Dr Naveen Kumar (CSIR-IICT, Hyderabad)*

Funding: *Extramural (ICMR)* ***Duration****:* 2017-2020

Background**:** Natural polyphenolic compounds like flavonoids and xanthonoids possess wide ranging bioactivities such as anti-inflammatory, anti-oxidative, anti-bacterial, anti-fungal as well as antiviral activities. Such compounds and other groups of compounds which are known for their wide spectrum antiviral activity against multiple viral protein targets of different viruses, *viz*., dengue virus, HCV virus, herpes virus, etc. can also be investigated for possible chikungunya virus (CHIKV) antiviral activity.

Objectives: (i) To identify and shortlist drug compounds with antiviral properties by screening of available compound libraries (ii) Evaluation of the antiviral activity of selected compounds against CHIKV (iii) Identification of viral drug targets of the effective compounds.

Findings: Docking studies were undertaken to understand the molecular mechanism of action of few natural compounds and a few synthetic compounds bearing the benzo-thioazol scaffold that were found to be effective against CHIKV by *in vitro* studies. Among several benzo-thioazol derivatives, the *in-vitro* antiviral effect of EM-GU-BTT-1 and EM-GU-BTT-17 showed post-treatment inhibition of CHIKV replication. The molecular docking analysis revealed that these derivatives interacted significantly with CHIKV RNA dependent RNA Polymerase (RdRP) enzyme at the nucleotide triphosphate (NTP) binding site having the GDD and two highly conserved motifs (Fig. 2 a,b). This may be attributed to their scaffold structure which mimics the NTP structure of the RNA molecule. *In vitro* studies revealed that among tested flavonoids, OCL-101, was effective under the posttreatment condition while OCL-113 was effective both under pre and posttreatment conditions. OCL-113 bound to an allosteric site in the thumb domain of the RdRp structure (Fig. 2 c,d). OCL-101 showed highest binding affinity with CHIKV nSP3 macrodomain though its role as a potential inhibitor needs to be elucidated as an interaction with the crucial residue Asp10 which is required for stabilization of the ligand-receptor complex is not observed (Fig. 2e,f). The *in vivo* effect of these compounds would however, needed to be undertaken.

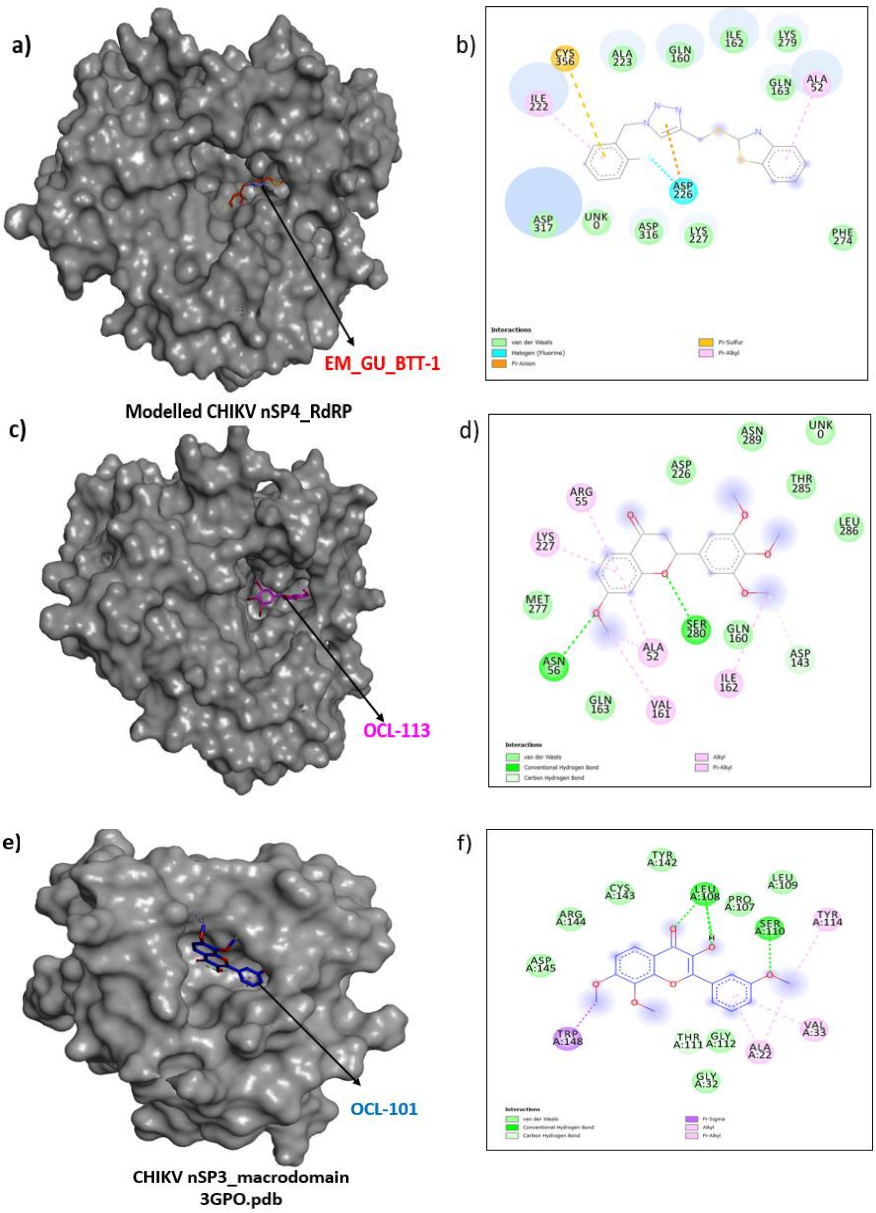


Fig. 2: Molecular docking of compounds that showed *in vitro* antiviral activity with putative targets of CHIKV. Figures to the left show surface overview of the docked poses (a) EM-GU-BTT-1 with nsP4 RdRP domain (c) OCL-113 with nsP4 RdRP domain and (e) OCL-101 with nsP3 macrodomain. Figures (b), (d) and (f) show the corresponding 2D interaction diagrams.

Repurposing of drugs towards anti-Dengue and Chikungunya viruses using the systems biology approach

Investigators: *Dr Sarah Cherian, Ms Bhagyashri Kasabe, Dr Deepti Parashar & Dr K. Alagarasu*

Funding: *Extramural (ICMR)* ***Duration:*** 2019-2022

Background: Repurposing of drugs has emerged as a novel concept to combat pathogens. Though, several drugs have been tested in general for repurposing as anti-DENV and anti-CHIKV agents, none are yet recommended for specific use. The studies based on computational approaches using systems biology

data for shortlisting potential FDA-approved drugs have only recently been initiated, and experimental testing has not yet been undertaken for the predicted drugs. Further multitarget drug repurposing to develop drugs that are able to interfere with multiple pathways involved in pathogenesis of co-infections has not been undertaken. Thus, there is need to use a systems biology approach for analysing "multi-omics" data to repurpose FDA approved and investigational drugs against these viruses to identify effective novel drug candidates.

Objectives: (i) Identification of the differentially expressed signature gene/ protein profiles for dengue and chikungunya viruses (DENV/ CHIKV) and also common signatures based on available literature and appropriate databases (ii) Identification of the specific metabolic pathways involved and shortlisting of FDA-approved drugs to be repurposed for DENV/ CHIKV using computational systems biology approach (iii) Evaluate the mechanism of drug action of shortlisted drugs against DENV and CHIKV, which have potential viral targets

Findings: Based on the analysis of three publicly-available DENV transcriptomic datasets, we identified 1585 differentially expressed signature genes. In addition, we obtained 390 differentially expressed proteins from available proteome data and 489 human-DENV interacting proteins from interactomics data. These signature profiles were further combined and used for identification of significant pathways and gene ontology. We identified 875 significant functional hits for signature genes and 2378 hits for specific biological processes. Further, based on the inverse gene signature analysis of the transcriptome data using Connectivity Map 'CMap', drug candidates, staurosporine, vorinostat, wortmannin, doxorubicin and triptolide were identified for DENV.

ArVirInd: A database of Arboviral proteins from the Indian subcontinent

Contributors: Dr. Pratip Shil, Dr Nitin Atre & Dr. K Alagarasu

Funding: ICMR Extramural; Duration: 2019 - 2022

Background*:* Climate change brought resurgence of arbo-viruses worldwide, necessitating scientific research towards diagnostics, prevention and viral evolution. Keeping this in view and the role of bioinformatics in virology, we proposed to develop a knowledgebase of arbo-viral antigenic proteins from strains isolated in Indian subcontinent. Current databases do not enlist genome/proteome information by country or year of outbreak.

Objectives*:* to design and develop a bioinformatics database with protein information about arbo-viruses isolated from Indian subcontinent.

Key Findings*:* The ArVirInd database has been developed in HTML, CSS, MySQL and PHP. While the webpage was designed in HTML and CSS, backend database was built in MySQL and for the server side preprocessing PHP was used (Fig. 3).

To begin with, we have curated a total of 200 records of antigenic proteins for dengue, chikungunya, Japanese encephalitis and West Nile viruses from India, Bhutan, Bangladesh, Maldives, Sri Lanka, Pakistan and Nepal. For the flaviviruses, the envelope proteins and NS1 were considered. For Chikungunya, envelope proteins (E1 and E2) were analyzed. Record content involves: amino acid sequence, predicted epitopes, N-glycosylation sites, functional sites, year of isolation, etc. The ArVirInd knowledgebase is available at: http://www.arvirind.co.in.

It is available for free usage. User can search for sequences by "Virus Name", "Country of Origin" and "Year of Origin" (year of outbreak or sample collection) OR as combinations. This is a unique feature of the database. Work on expanding the database is undergoing.

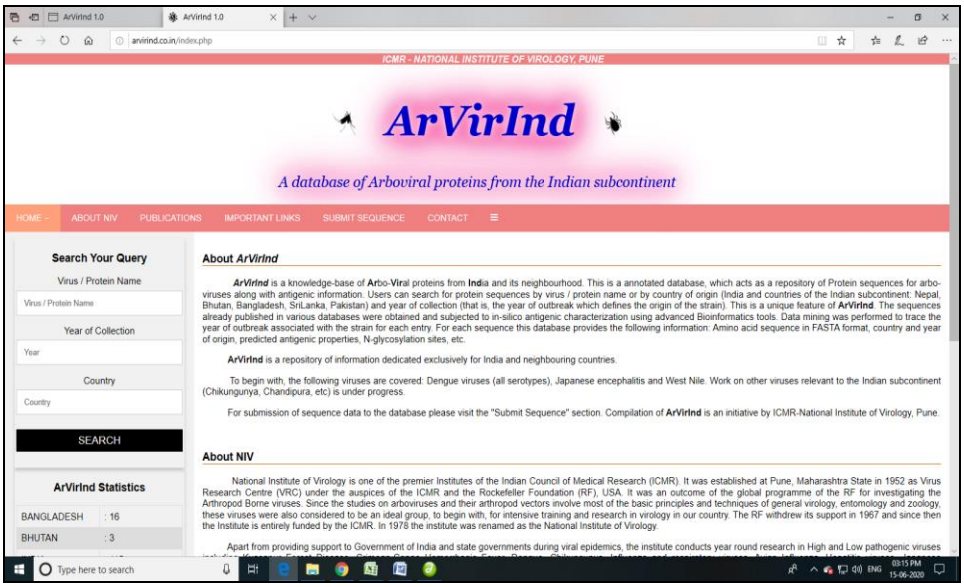


Fig. 3: The webpage for ArVirInd - a database for arbo-viral proteins from the Indian subcontinent.

Bioinformatics characterization of Chandipura virus proteins
PI: P Shil
CI: AS Tripathy
PhD student: D Pavitrakar.

Background: Chandipura virus (CHPV) is an emerging pathogen responsible for acute encephalitic syndrome (AES) in pediatric population in India. Several outbreaks of CHPV have been reported from different states of India since the year 2003. At present there is no vaccine or therapeutic measures available to curtail the disease. Thus, theoretical studies on the characterization of the proteins using Bioinformatics tools are necessitated. Earlier we had identified a neutralizing epitope (Pavitrakar *et al*, Arch Virol 2018) on the surface protein (G).
Objectives: Bioinformatics characterization of Chandipura virus proteins *i.e*., N, P and M proteins
Findings: In this study, we have identified both T-cell and B-cell epitopes of different antigenic proteins of CHPV like Nucleoprotein (N), Phosphoprotein (P) and Matrix protein (M) along with the immuno-dominant glycoprotein (G) and conducted *in silico* characterization for the same. For the B-cell epitope predictions standard protocols were used.
For MHC Class-I epitope predictions, we have used alleles predominant in the population of the Indian states affected by Chandipura virus. The following alleles were considered: HLA-A*03, HLA-A*69:01 and HLA-A*26. The predicted epitopes were primarily shortlisted with percentile rank of ≤5 (high binding affinity). The peptides detected by all the three alleles were considered as potential CTL epitope. We identified 2, 1 and 3 such peptides from N, P and M proteins respectively.
The most prevalent HLA class II alleles were: HLA-DRB1-04, HLA-DRB1-07 and HLA-DRB1-15, HLA- DRB1-03 HLA-DRB1-11, HLA DRB1-13 and HLA-DRB1-15. The peptides detected by ≥6 alleles were considered as potential HTL epitope. This led to the identification of 4 potential HTL epitopes from the N protein. Further, *in silico* characterization of the peptides is going on.

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

a) Meteorological parameters modulate Dengue occurrences in India (2010- 2016)
Contributions: *P Shil*

Background: Climate change is a reality and along with came the global surge in mosquito-borne viral diseases like Zika, dengue, chikungunya, etc. India has a huge burden of dengue and chikungunya with more than 10 million individuals affected between 2010 and 2016. This necessitated research to understand the role of environmental parameters in the spread of these diseases.
***Objectives*:** In the present project, we have compared all India occurrences (confirmed cases) of dengue and chikungunya with meteorological factors like Maximum and minimum temperatures, rainfall, etc.
Findings: Disease data was obtained from National Vector Borne Disease Control Programme (NVBDCP) and Infectious Diseases Surveillance Programme (IDSP) of the Government of India.
The Area weighted average annual rainfall (ARF) was calculated by Thiessen method:

$$ARF = \frac{\sum_{i=1}^{n} X(i) \times A(i)}{\sum_{i=1}^{n} A(i)}$$

where *X(i)* is the average annual rainfall for any (i th) meteorological sub-division and
A(i) is the area in sqKm for the (*i* th) sub-division and *n* is the number of sub-divisions covering any particular region of India.
Spatio-temporal analyses revealed the distributions of rural outbreaks of dengue across India (**Fig. 4**). Meteorological data were analyzed and possible correlations with confirmed cases of dengue were obtained. It was observed that annual rainfall (ARF) modulates dengue incidences differently in the northern and southern (peninsular) states of India. While the northern states experience more cases due to high annual rainfall, the southern states, on the other hand, experience increased occurrences with lesser rainfall. Both this phenomenon can be attributed to geography, land use, and water management practices. Hence, we conclude that rainfall affects dengue occurrences differently between northern and southern states of India (BMRJ 2019).

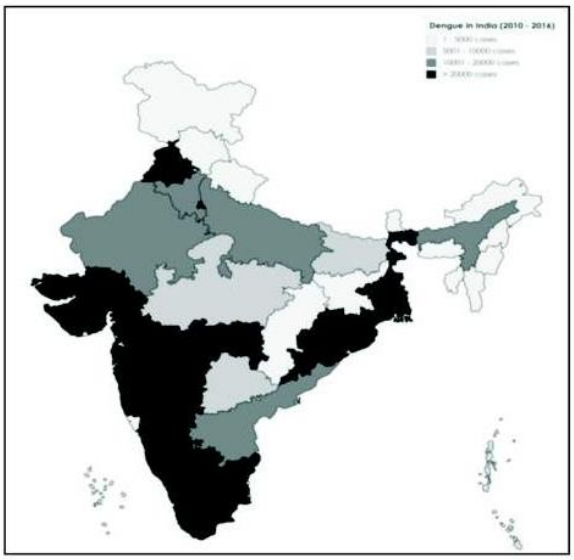


Fig.4: All India dengue scenerio 2010 - 2016.

DENGUE - CHIKUNGUNYA GROUP

Scientific staff

Dr. Paresh Shah	Scientist 'E'& Group Leader
Dr. D. Parashar	Scientist 'E'
Dr. K. Alagarasu	Scientist 'D'

Technical staff

Dr (Mrs) Jayashri A Patil	Senior Technical Officer I
Dr (Mrs) Rupali V Bachal	Senior Technical Officer I
Mr. Mahadeo Kakade	Senior Technician (1)
Mrs. Ashwini More	Senior Technician (1)
Mrs. PoonamPatil	Senior Technician (1)
Mrs. Minal Bote	Technician 'C'
Mr. H B Supe	Technician 'B'
Mr. SK Pandey	Technician 'A'
Mr. P B Gore	Multitasking staff
Mr. D M Jadhav	Multitasking staff

Project staff

Dr. Manish Kumar Jeengar	Research Associate (DST Nano Mission)
Mr. Priyanka K Newase	Project Technical Assistant
Mr. YogeshBalaKarthik	Project Technical Assistant

- Apex referral laboratory for National Vector Borne Disease Control Programme
- Studies using molecular genotyping suggest prevalence of Dengue 2 virus in eastern and southern states, Dengue 3 in western and some northern states, Dengue 4 in Andamans and North Maharashtra and Dengue-1 in Maharashtra.
- Phylogeographic studies suggest that India is an endemic reservoir for Chikungunya virus and global transmission could have originated from here.
- Lipid nanoparticles were seen to have higher efficacy in delivery anti siRNA against Chikungunya virus in mice model.

Apex referral laboratory for National vector borne disease control programme
Investigators: K Alagarasu, D Parashar, JA Patil, Kakade MB, A More, PS Shah
Funding agency: NVBDCP/Intramural **Duration:** 2019-2020

(i) Molecular characterization of dengue viruses circulating in India

Background: A large number of dengue outbreaks were reported from Maharashtra as well as other states of India during 2019-20. Information on circulating serotypes is an essential component of surveillance of dengue.
Objective: To find out the distribution of serotypes of dengue virus and genotypes within serotypes in Maharashtra and other states during the 2019.
***Findings*:** During the year, 1138 samples from different districts of Maharashtra (mainly Pune district) were tested by real-time RT-PCR for dengue (DENV) and chikungunya and detected 227 cases positive for DENV and 39 for chikungunya. Serotyping of the 192 DENV positive samples from Pune district revealed 77 as DENV-1, 83 as DENV-2, 29 as DENV-3, 02 as DENV-4 while one sample had mixed infection. DENV-1 and DENV-2 emerged as the pre-dominant serotypes during 2019 in Pune district, Maharashtra (Figure 1). Screening of 217 samples received from Goa showed DENV positivity in 119 samples with DENV-2 and DENV-3 as predominant serotypes (18 DENV-1, 55 DENV-2, 43 DENV-3 and 03 DENV-4).

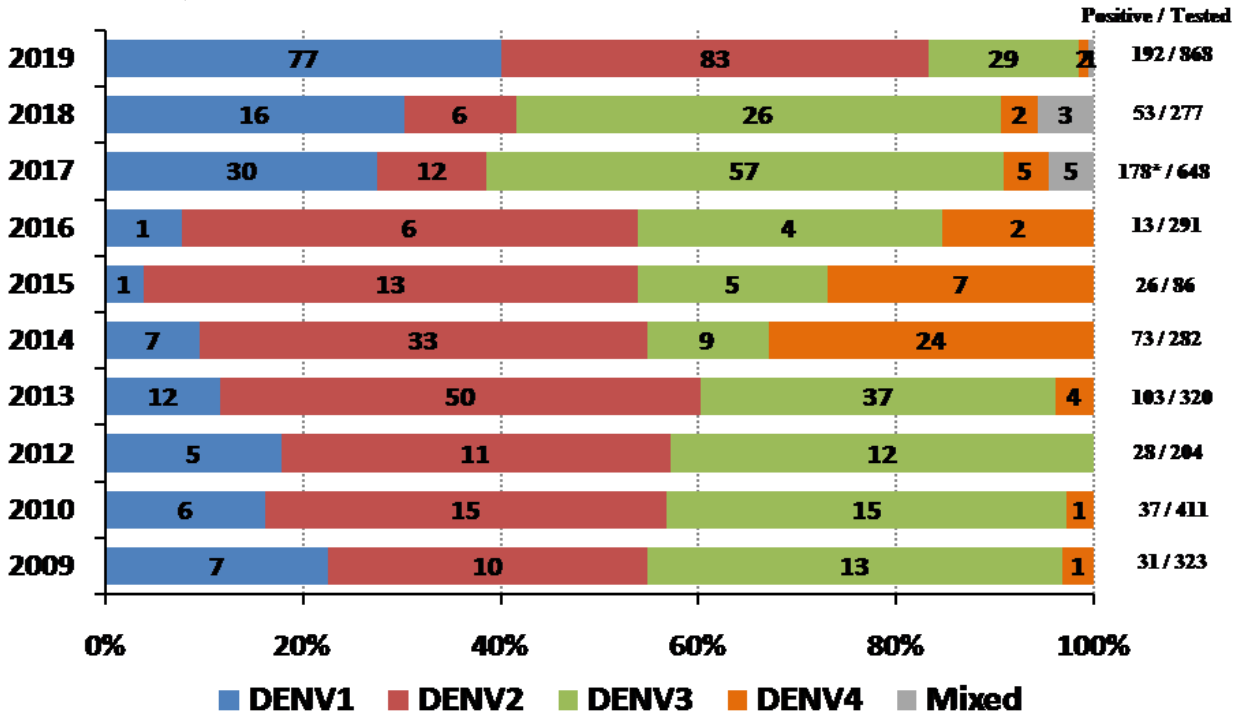


Figure 1: Prevalence of circulating DENV serotypes in Pune district from 2009-2019

(ii) Clinical evaluation of an in-house developed real-time RT-PCR assay used in Apex referral laboratory for serotyping of dengue virus

***Background*:** For serotyping of dengue virus strains, a real-time reverse transcription based polymerase chain reaction assay (rRT-PCR) was developed which has been evaluated for best performance.

Objective: To evaluate the performance of an in-house developed rRT-PCR for serotyping of DENV in clinical samples.
Findings: Five hundred sixteen clinical samples representing confirmed DENV-1 to DENV-4 serotypes and 96 DENV negative samples were used to evaluate the assay. In comparison to the composite reference standard, the in-house developed rRT-PCR had an overall sensitivity with 95% confidence interval (CI) of 97.5% (95.7-98.6) and a specificity of 100% (96.2-100.0). The assay had 100% sensitivity for detection of DENV-1 and DENV-4 while it was 95.6% and 96.9% for DENV-2 and DENV-3 respectively. The study reports that the in-house developed rRT-PCR can efficiently detect DENV-1 and DENV-4 while it suggests modification of primers and probes used for serotyping of DENV-2 and DENV-3. Periodical evaluation of real-time RT-PCR assays for detecting DENV serotypes with large number of samples and the usage of at least two assays which targets different regions of DENV genomes is recommended.

(iii) Comparison of whole blood and plasma in detecting of dengue virus RNA

Background*:* Early diagnosis of dengue, a disease caused by dengue virus (DENV), depends either on the detection of viral RNA or detection of non structural protein 1 antigen (NS1 Ag). The sensitivity for detection of viral RNA and NS1 Ag detection decreases with an increase in day of illness after onset. Therefore there is a need to look for alternative sample types which can enhance the detection of viral RNA.
***Objective*:** To compare the advantage of whole blood over plasma for detection of dengue virus
Findings: The utility of viral RNA isolated from whole blood over plasma for detection of DENV was investigated in 80 pair of whole blood and plasma samples by DENV serotype specific one step real-time RT-PCR. Dengue virus RNA was detected in 71.25% of the whole blood samples in comparison to 46.25% of plasma samples. In secondary dengue infections, DENV RNA was detected in 83.3% of whole blood samples against 40.5% in plasma samples (P=0.0001). Compared to viral RNA detection in whole blood, NS1 ELISA was positive in 54.8% of the secondary dengue infections (P= 0.0015).The detection rate of DENV RNA in whole blood is found higher as compared to plasma. One step real-time RT-PCR using RNA from whole blood combined with NS1 ELISA should be the choice for dengue diagnosis in dengue vaccine trials.

Monitoring of dengue and chikungunya viruses circulating in India for changes in the serotypes, genotype and lineages utilizing Viral Research & Diagnostic Laboratories Network

Investigators: K Alagarasu, JA Patil, Kakade MB, A More, D Parashar, PS Shah
Funding: DHR/ICMR **Duration:** 2018-2020

***Background*:** Dengue and chikungunya have emerged as the major health concerns for India. Recent studies have shown an association of DENV genotype/lineage with change in magnitude of outbreaks and disease severity. Similarly, recent studies have shown that the 2015-17 CHIKV outbreaks in different states of India was due to indigenous evolution rather than importations. Data on circulating serotypes/genotypes are available from limited regions; mostly from places where National institutions are located, that rarely reflect the exact scenario of the entire nation.
Objective: Molecular monitoring of dengue & chikungunya viruses circulating across the country for changes in serotypes, genotypes and lineages involving DHR/ICMR VRDL network
***Work done and findings*:** **A** total of 4543 DENV suspected samples were tested by ICMR-NIV, NIV field units and different VRDLS that revealed 473 positive for DENV-1, 575 for DENV-2, 421 for DENV-3, 127 for DENV-4. Multiple serotype infections were detected in 90 samples. CHIKV RNA was positive in 141 samples, while 32 samples had co-infections DENV/CHIKV. DENV-1 was more prevalent in Kerala, Karnataka, Maharashtra, Himachal Pradesh, Punjab and Assam. DENV-2 was more prevalent in Kerala, Tamil Nadu, Andhra Pradesh, Telungana, Odisha, West Bengal, Jharkand, Uttar

Pradesh, Rajasthan, Gujarat and Punjab. DENV-3 was mainly found in Maharashtra, Madhya Pradesh, Delhi, Haryana and Punjab. DENV-4 was detected only Port Blair (Andaman & Nicobar) and Nagpur region of Maharashtra (Figure 2). Sequence analysis of DEN envelope gene was done for 120 samples (33 DENV-1, 53 DENV-2, 24 DENV-3 and 10 DENV-4). Phylogenetic analyses revealed circulation of Asian and AM/AF genotypes of DENV-1; Asian genotype in South India (Kerala and TN), Maharashtra and Himachal Pradesh while AM/AF in other states. Phylogenetic analyses of DENV-2 revealed circulation of multiple lineages of cosmopolitan genotype in different regions of India. For DENV-3, Genotype III was the circulating genotype detected. For DENV-4, genotype I was the circulating genotype in India.

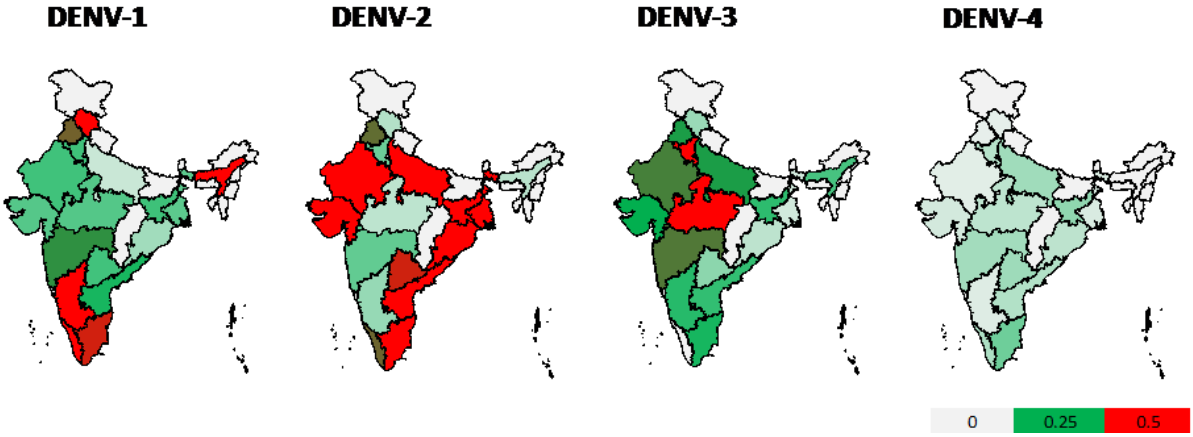


Figure 2: Prevalence of circulating DENV serotypes in different states of India during 2018

Phylodynamics of the Indian Ocean lineage of chikungunya viruses (2005-2018) using complete genome sequences

Investigators: D Parashar, P Newase, More A, JA Patil, P. Patil, K Alagarasu, PS Shah
Collaborators: S Cherian, S Jadhav (Bioinformatics Group)
Funding agency: Intramural Duration: 2018-19

***Background*:** Since the re-emergence of CHIKV in India in 2005-06, the Indian sublineage of the (IOL) has continued endemic/epidemic circulation in India. Phylogeography analysis is important to understand the transmission pattern of CHIKV in the global context.

Objective: Phylogeography analysis of IOL CHIKV strains for estimation of geographical ancestry for Indian subgroups by whole genome sequencing of 2014-18 Indian isolates

Findings: Whole genome sequencing followed by phylogeography analysis of Indian isolates representing CHIKV outbreaks (2014–2018) from selected states of India was carried out using Bayesian Markov chain Monte Carlo method and selection pressure analysis. Phylogeography analysis revealed indigenous evolution in India at least at three time points, with specific mutations that conferred viral fitness in *Aedes* vector species. Further dispersal of the strains from India was noted to neighbouring and distant countries with multiple exportations to Sri Lanka, Bangladesh and China (Figure 3). The study reveals India as an endemic reservoir for CHIKV and persistent global transmissions. Though natural selection does not appear to play a major role in the establishment of the IOL, sustainable efforts towards vector control may help address the issue.

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

Investigators: D Parashar, MK Jeengar, P Patil, More A
Collaborators: S Ramakrishna, M Kurakula (CSIR-IICT, Hyderabad)

Funding agency: Extramural (DST Nano Mission) **Duration:** 2017-2020

Background: The efficacy of siRNAs against ns1 and E2 genes of CHIKV has been demonstrated by both the *in vitro* and *in vivo* studies by this group. The main challenge for siRNA therapy is its inability to reach the intended targets in the cytoplasm to exert gene silencing activity. Hene, there is a need for novel siRNA delivery systems to overcome these challenges.
Objectives: To explore solid-lipid nanoparticles for their applicability as siRNA delivery system (s).
Findings: Stearylamine (SA) was used as a cationic lipid and charge inducing agent in the solid-lipid nanoparticle (SLN) formulation. The antiviral activity of SA against CHIKV was demonstrated in the *in vitro* system. *In vivo* studies in C57BL/6 mice model revealed that SA at 10 mg/kg and 20 mg/kg dose per day up to 3, 5 and 7 days, showed inhibition of CHIKV (Figure 4A). Histopathology studies of muscle tissue (Figure 4B) showed inhibited inflammatory cell migration and muscle tissue necrosis (Figure4C).

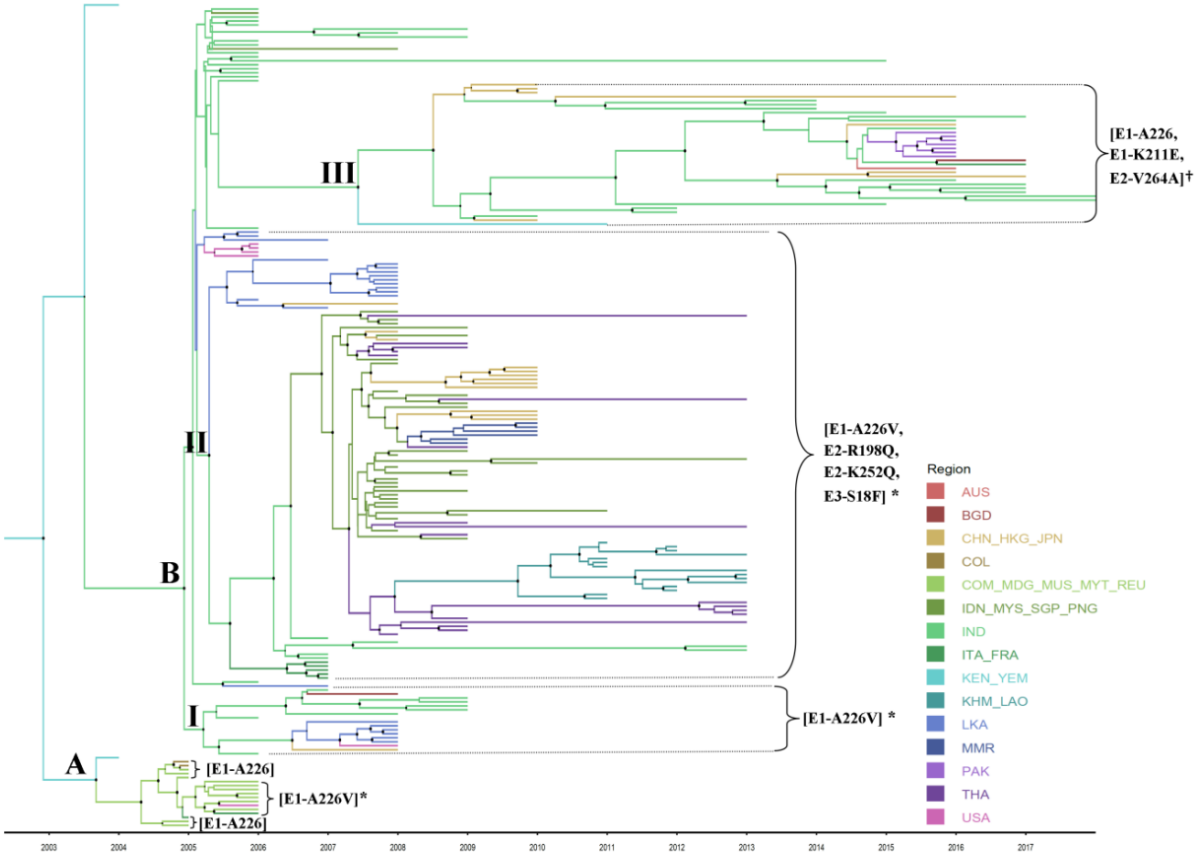


Figure 3: Maximum clade credibility tree based on CHIKV whole genome sequences (2005–2018) of the IOL under the uncorrelated relaxed clock (lognormal). The branches are colored according to respective geographical region. Circles at the nodes indicate posterior probabilities with size reflecting the confidence. Key nodes are labeled as A, B, I, II and III. The sequences containing *Ae. aegypti* adaptive mutations are labelled with symbol† and those containing *Ae. albopictus* adaptive mutations are labeled with*****.

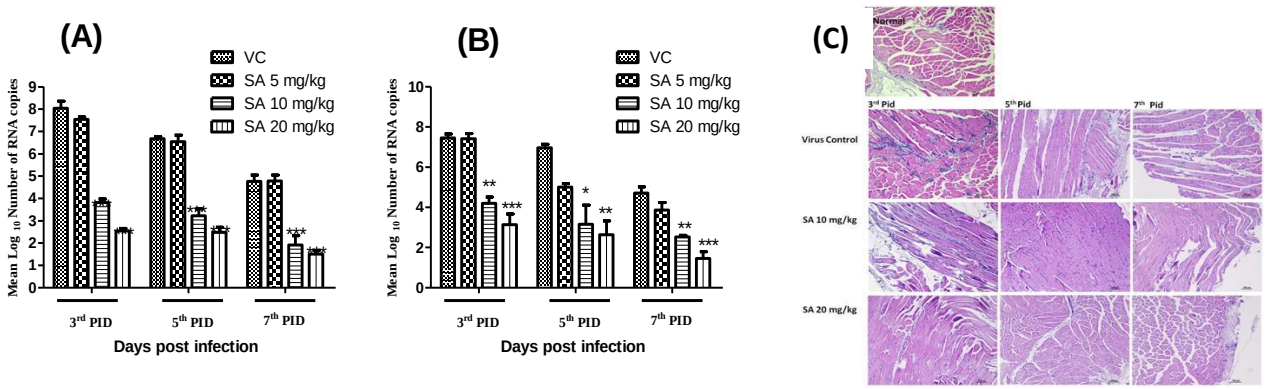


Figure 4: The reduction in CHIKV copies/ml in (A) serum (B) muscle tissue. C) Histopathology changes in skeletal muscle at different time points of different group of C57BL6 mice

BDM1401: Structure-based design and evaluation of lead compounds targeting Chikungunya virus
Investigators: S Cherian, M Agarwal, D Parashar, P Patil, A More, K Alagarasu
Collaborators: PS Mainkar & NV Kumar (CSIR-IICT, Hyderabad)
Funding agency**:** Extramural (ICMR) Duration: 2017- 2020

Background: Chikungunya virus (CHIKV) is the causative agent of one of the most perplexing human arboviral infections having global significance. Approved antiviral therapies or vaccines for the treatment or prevention of CHIKV infections are not available. Natural polyphenolic compounds like xanthonoids and flavonoids possess wide ranging bioactivities such as anti-inflammatory, anti-oxidative, anti-bacterial, anti-fungal as well as antiviral activities.

Objective: to evaluate the anti-viral efficacy of certain natural and synthetic compounds against chikungunya by *in vitro* and *in vivo* studies.

Findings: *In vitro* studies have revealed that OCL-101, EM-GU-BTT-1, EM-GU-BTT-7 and EM-GU-BTT-16 are effective as therapeutics while compound EM-GU-BTT-15 was found effective as prophylactic. Two compounds, *i.e.*, OCL-108 and OCL-113 were found effective as both prophylactic and therapeutic while OCL-105 and EM-GU-BTT-17 showed effectiveness under all the treatment conditions (pl clarify).The effective benzothiozole derivatives among several, EM-GU-BTT-1, EM-GU-BTT-17, showed inhibition of CHIKV replication under post-treatment conditions also, but need to be investigated in the *in vivo* model. α-Mangosteen (OCL-105) treatment exhibited significant reduction in the viral RNA load in mice. At 3rd dpi, OCL-105 high dose group showed 2.1 $\log_{10}$ reduction (99.2% reduction) of viral RNA compared to untreated animals (VC) ($p<0.05$). However, on 5th dpi, reduction in viral RNA copy number with low dose and high dose was 1.8 $\log_{10}$ ($p<0.01$) and 2.23 $\log_{10}$ (99.4% reduction) ($p<0.001$) respectively. At 7th dpi, reduction in viral RNA was found at par with 5th dpi (2.22 $\log_{10}$) with high dose treatment, compared to VC ($p<0.01$) (Figure 5a).

Reduction in CHIKV RNA copies in muscle tissue of mice was observed from day 3 in OCL-105 both with low and high dose treatment (Figure 5b). Reduction of 1.41 $\log_{10}$ and 2.36 $\log_{10}$ of CHIKV RNA copies respectively was found on 3rd dpi while it was 2.44 $\log_{10}$ and 3.1 $\log_{10}$ (99.91% reduction) on 5th dpi when treated with low and high doses. However, no significant reduction in virus load was detected on 7th dpi with low dose regimen but reduction of 3.4 $\log_{10}$ (99.96% reduction) was observed with high dose regimen.

Histopathological studies have shown marked degeneration, atrophy, MNC infiltration and edema (at day 3, 5 and 7) in CHIKV infected muscles in comparison to control. While treatment with low dose of OCL-105 showed improvement in inflammatory signs, high dose treated mice showed regeneration of muscle tissues from 5 dpi onwards (Magnification X100) (Figure5c).

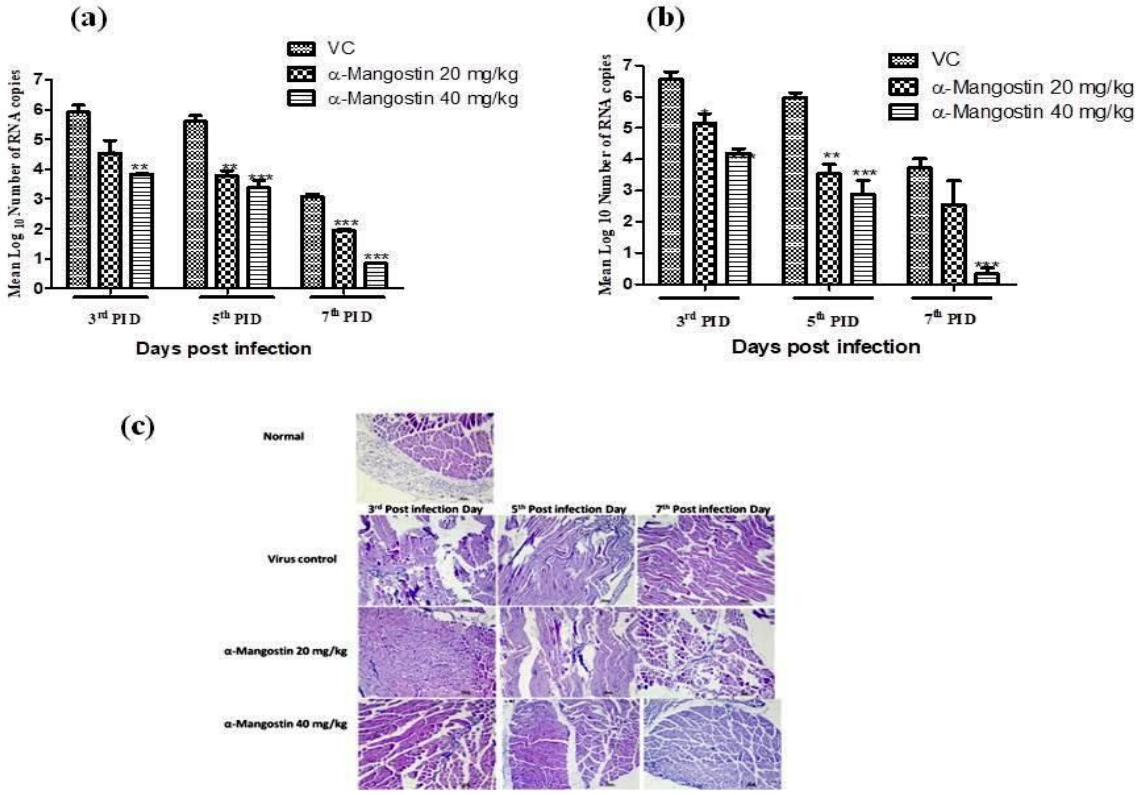


Figure 5: *In vivo* inhibition of CHIKV using OCL-105 in (a) serum and (b) muscle tissue of C57/BL6 mice. Values are given as $\log_{10}$ RNA copies/ml. serum. Data shown are mean ± SEM. ***P < 0.001, **P < 0.01 and *P < 0.05 vs. VC; Histopathological changes in mouse muscle tissues after CHIKV infection and OCL-105 treatment (c)**.**

DIAGNOSTIC REAGENT FACILITY

Scientific Staff:

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

Technical Staff:

Dr. Sujeetkumar Belani	Technical Officer-A (Up to Jan-2020)
Mrs. Deepika Chowdhary	Technical Officer-A (Up to Nov-2019)
Mr. Kishor Kshirsagar	Technical Assistant
Mr. Anant Deshpande	Technical Assistant
Mrs. Reva More	Technician-C
Ms. Snehal Veer	Technician-C
Mrs. Arti Waghmare	Technician-C
Mrs. Anjana Ugale	Technician-C
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. Anuja Satvekar	Technical Assistant
Mr. Tushar Raut	Technical Assistant
Mr. Tanmay Dharmadhikari	Technical Assistant
Ms. Madhura Punekar	Technical Assistant
Ms. Trupti Rale	Technical Assistant
Ms. Archana Verma	Technical Assistant
Ms. Daisy Saini	Technical Assistant
Ms. Vaishnavi Salvi	Technical Assistant
Mr. Vishal Tate	Technician-A
Mr. Sushant Sable	Technician-A
Mr. Rahul Tiwade	Technician-A
Mr. Zubair Attar	Technical Officer-A (Engineering)
Mr. Suraj Pandey	Technical Assistant (Engineering)
Mrs. ShwetaChondhe	Technician-C (DEO)
Mr. Prashant Gore	Technician-A (DEO)

A total of 12823 indigenously developed diagnostic reagent kits were supplied to various National Programs and other organizations including 700 sentinel Centers around the country.

DRF 0601: Scaling up facilities for production of Diagnostic kits/ Reagents for detection of Japanese encephalitis (JE), dengue (DEN) & chikungunya (CHIK) viruses
Investigators: Director, ICMR-NIV & Dr. Paresh Shah
Funding agency: NVBDCP **Duration:** Ongoing

***Background*:** India has become endemic to dengue, chikungunya and Japanese encephalitis virus (JE) and large scale outbreaks are reported at regular intervals from different parts of the country. Since no vaccines or antivirals have been commercialized yet, early diagnosis is recognized as the back bone of disease management and control. The Indian Council of Medical Research and NVBDCP together conceptualized the idea of developing a GFP facility at ICMR-NIV to manufacture highly specific and sensitive MAC-ELISA kits, which are at par with world standards, to cater the need of the country to diagnose anti-IgM antibodies of dengue, chikungunya and JE in a short time period. The facility has been approved by the DCGI and FDA.

Work done and findings

During the reporting year, 12,823 diagnostic kits of dengue, chikungunya and JE were prepared in the facility, carried out in-house quality checking and supplied to different sentinel centers, ICMR institutes, government hospitals and laboratories across the country under the national program (Table 1). Approx. 700 Sentinel Centers have been identified all over the country and the kits were provided to the Centers based on their requirements. Month-wise distribution of kits is given in Fig 1. Kits were also supplied to CDC-VHF project facilities, acute encephalitic syndrome centres and even to Southeast Asian countries (through WHO).

Table 1: Supply of MAC ELISA kits in 2019-2020

	JE	DEN	CHIK	TOTAL
National Program	850	8066	2426	11342
VRDL (DHR)	238	686	361	1285
Others*	93	60	43	196
Total	1181	8812	2830	12823

*CDC-VHF project centers, AES project centers, NIMHANS (Bangalore), RMRC-Bhubaneswar, RMRC-Gorakhpur

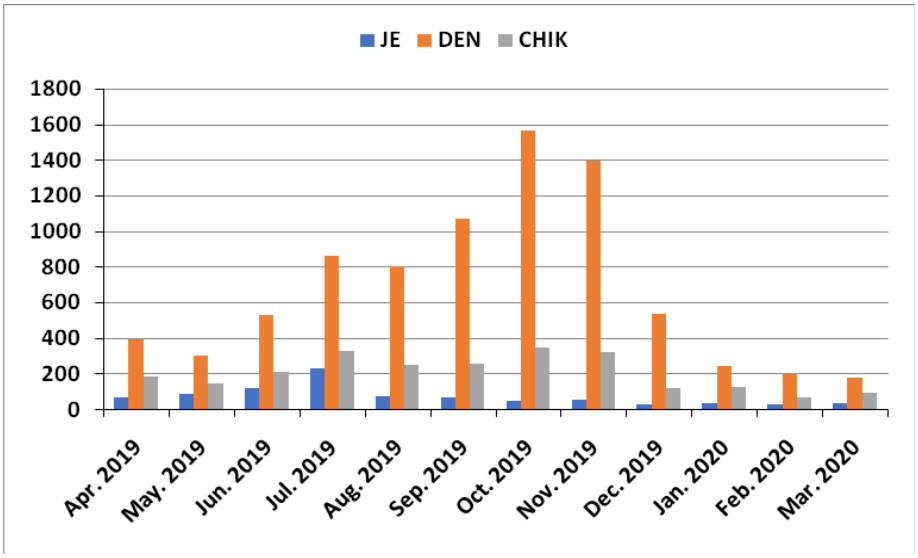


Figure 1: Month-wise supply of MAC ELISA kits under National Program

Electron Microscopy & Histopathology Group

Scientific staff

Dr. Atanu Basu	Scientist G & Group Leader
Dr. Virendra Kumar Meena	Scientist B

Technical staff

Ms. Sharda Prasad	Technician A
Dr. Preksha Jain	DST WOSA Fellow
Shri. Satish Mate	Multitasking staff (EM)
Shri. Ulhas Gadekar	Multitasking staff (Pathology)

- Core Electron microscopy and pathology services
- Inhibition of endothelial cell myristoylation may inhibit dengue 2 virus replication suggesting post translation lipid modifications to play a possible role in virus-host interplay.
- Dengue virus NS1 protein shows differential affinity to cellular membrane lipids.
- Imaging of SARS-CoV-2 virus particles in clinical sample by transmission electron microscopy

Core facility activities

The EM is a central facility and high resolution TEM application support was provided in virus imaging and thin section analysis to in-house researchers as well as researchers from other national laboratories. An important activity was extending support to industry for EM requirement for quality control purposes.

Research projects

Studies on the effect of dengue virus and viral NS1 protein on endothelial cell dysfunction

Principal investigator: Atanu Basu
Co-investigator(s): Virendra K Meena, Sharda Prasad
Funding: Intramural 2018-2021

This is an umbrella project that has multiple components addressing different aspects of dengue-vascular endothelial cells interactions at cellular and molecular levels. During the reporting year we focused in-depth on profiling the binding affinity of the NS1 protein of all four serotypes of dengue virus (DENV) with different membrane phospholipids *in-vitro*. The cellular lipids 2-oleoyl-1-palmitoyl-sn-glycer-3-phosphocholine, 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphoethanolamine, 2-oleoyl-1-palmitoyl-*sn*-glycer-3-phospho-L-serine and phosphatidylcholine were studied. The NS1 protein of DENV was commercially purchased and *in-vitro* interactions assays designed. Previous studies showed that NS1 protein of all four serotypes of DENV may have affinity for anionic membranes. Studies are ongoing to determine the specificity of the same using correlative computation biology, in-vitro biochemical assays and electron microscopy tools.

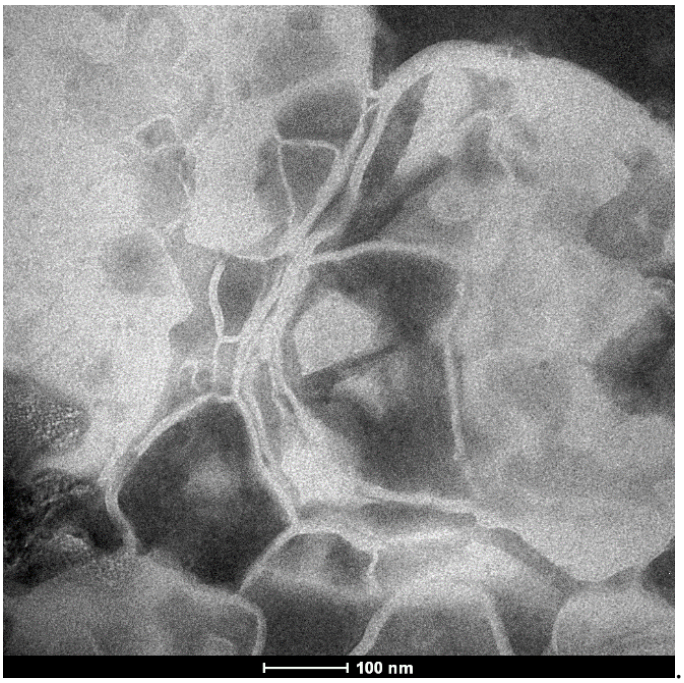


Figure 1: TEM imaging of choline membrane structure forming multifaceted membrane interfaces *in-vitro*

Investigating the role of dengue virus non-structural protein NS1 on pathophysiological basis of endothelial cell dysfunction
Principal investigator: Atanu Basu
Co-investigators: Nitali Tadkalkar, PhD thesis student.
Funding*: Intramural (2018-2020)

Funding*: Core funding was received as task force mode from DBT 2014-18. Project was completed in 2018. Leads obtained were studied further and the component during 2019-20 as continuation of the PhD thesis work is presented.

During the reporting year, *in-vitro* studies were carried out to examine the possible role of myristoylation of DENV2 NS1 protein (as predicted from *in-silico* studies) in the biology of the virus and on host-virus interactions. The effect of inhibition of cellular myristoylation with hydroxymyristoic acid treatment showed reduction in DENV virus negative strand copy numbers. In addition, transfection studies using a deletion mutant of NS1 also showed significant difference in the cellular expression and localization profiles. At this phase of the study we could establish the proof-of-principle that myristoylation of DENV NS1 may have an important role in both virus biology and host interaction events. Further studies are ongoing using putative motif specific mutation targeting and its effect.

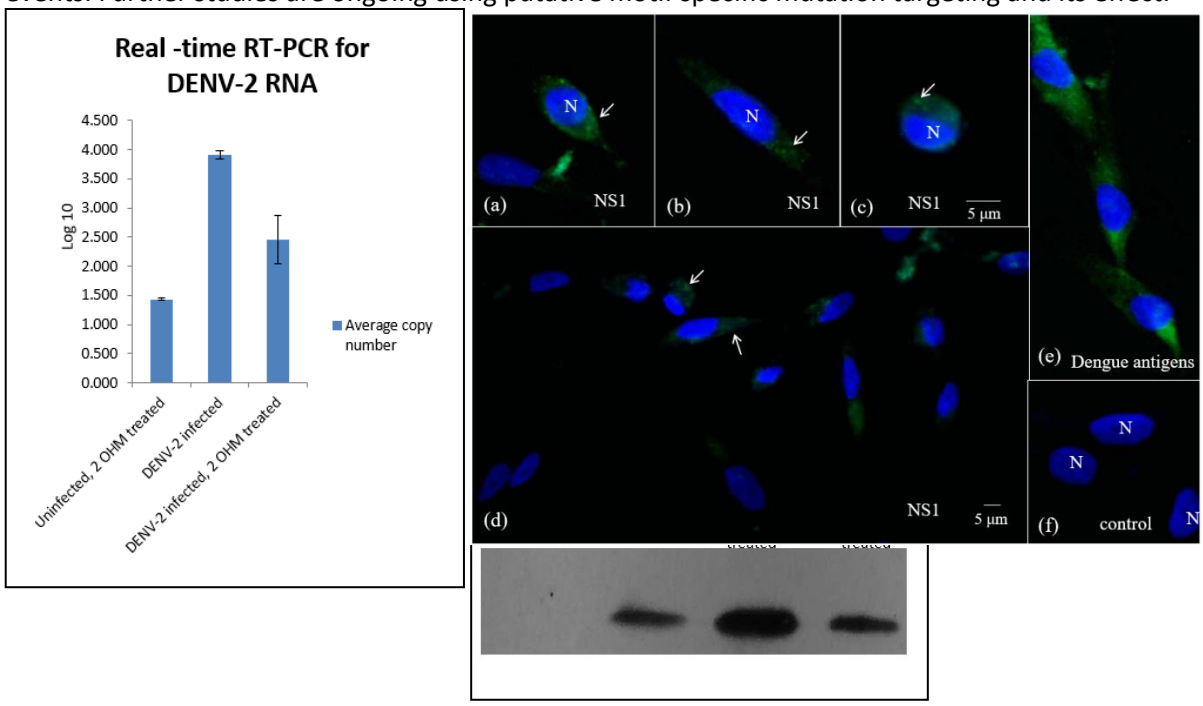


Figure 2: Effect of inhibition of myristoylation on DENV replication. Representative IFA images of NS1 localization pattern in DENV2 infected SK Hep1 cells treated with 2-OHM. (a, c) Single cells showing perinuclear punctate NS1 expression in infected cells not treated with 2-OHM and probed with anti-NS1 monoclonal and anti-DENV polyclonal primary antibody, respectively. (b,e) Distribution of DENV2 antigens in infected cells treated with 2-OHM and labeled with anti-NS1 monoclonal and anti-DENV polyclonal antibody, respectively. (d) A wide-angle large field showing overall staining pattern in infected, untreated cells. (f) Uninfected control. All magnification bars are built into the micrographs and correspond to 5µm.

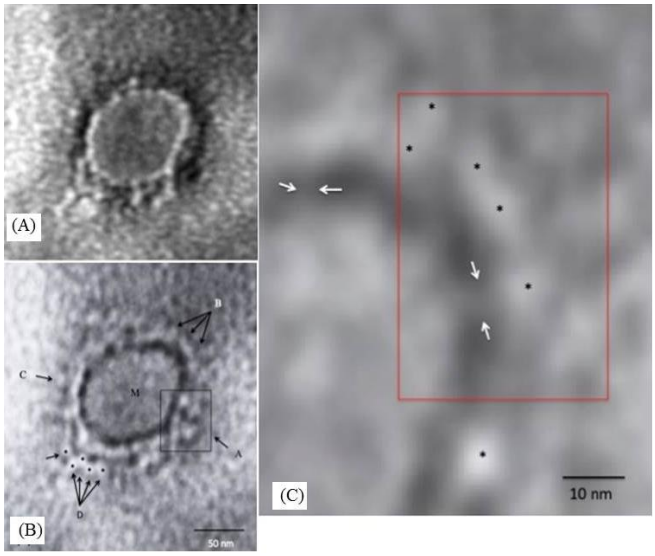


Figure 3: Transmission Electron Microscopy imaging of COVID 19. (a) A representative negative stained COVID 19 particle showing morphodiagnostic features of family Coronaviridae (b) defocussed image of the same particle resolving the virus envelope glycoprotein morphology in finer details. The boxed area A shows a tetramer-like aggregate of four distinct peplomers, arrows shown by B shows a more orthodox morphology of Coronavirus surface projections. C shows a distinct "peplomer head" with negative stain silhouette. The area D is interesting as a possible linear of projections could be imaged. Five distinct peplomers could be imaged as shown by the arrows. (c) A highly magnified processed image for pixel corrections show a distinct evidence of direct "stalk" connecting the peplomer to the viron surface. The peplomers are shown with asterix and the stalk with an arrow. Magnification bars are built into the micrographs.

ENTERIC VIRUS GROUP

Scientific Staff

Dr. V. Gopalkrishna	Scientist-F&Group Leader **(gopalvk58@hotmail.com)**
Dr. Mallika Lavania	Scientist-D **(mallikalavania@gmail.com)** (w.e.f. 27.01.2020)
Dr. Pradeep Sawant	Scientist-B **(pradeep.immuno@gmail.com)**

Technical Staff

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

Project Staff

Mrs. NitalGanorkar	ICMR-SRF(up to 22.12.2019)
Ms. Shweta Bhosale	Data Entry Operator

Sr. No	Title	Duration
1	ENV1301: Investigation of outbreaks and diagnostic services**.**	Ongoing
2	ENV1310: Hospital based surveillance of rotaviruses and strains in children with acute gastroenteritis	2017-20
3	ENV1302: Assessment of genetic diversity in group A rotaviruses in patients with acute gastroenteritis.	2013-20
4	ENV1601: Identification and Molecular Characterization of Rota virus and Noroviruses in Neonates admitted at Neonatal Intensive Care Units	2016-20
5	ENV1501: Identification and molecular characterization of group C rotaviruses in patients with acute gastroenteritis and animals from western India: A retrospective study	2015-20
6	ENV1306: Diversity of non-rota enteric viruses associated in patients with acute gastroenteritis (AGE)	2017-20
7	ENV1701: Detection and molecular characterization of potentially zoonotic enteric viruses in animals	2017-20
8	ENV1307: Pathogenesis of Coxsackie virus A-16 (CVA-16) associated with Hand, Foot and Mouth Disease in Neonatal Mice	2014-20
9	SRF Project: Development of immunodiagnostic assay for Identification of Coxsackie virus A-16 in patients with Hand Foot and Mouth disease.	2016-19

ENV1301: Laboratory diagnostic services

The Enteric virus group provides laboratory diagnostic referral services for various enteroviruses.

ENV1310: Hospital based surveillance of rotavirus strains in children with acute gastroenteritis
Investigators: Goplkrishna V, Joshi MS
Staff: Shinde MS, Chavan NA
Funding Agency: Intramural **Duration:** 2017-20

***Background*:** Rotavirus infections are the major cause of severe dehydrating diarrhea in children <5 years old despite the availability of two oral vaccines, *viz.*, RotaTeq and Rotarix. In India, the RotaVac vaccine has been introduced and implemented in the National Immunization program since 2016. Epidemiological and molecular studies would be helpful to define the effect of rotavirus vaccines in diarrhea control and to understand the different genotypes and their changing trends in India.
***Objective*:** To determine the disease burden, seasonal distribution and circulation of the prevalent and unusual rotavirus A (RVA) G-P genotypes.
Work done and findings:

Investigation of fecal specimens collected from 72 children hospitalized with acute gastroenteritis at Pune city, showed 30.6% (n=22) positivity to RVA antibodies by ELISA. RVA positive cases were mainly seen in children <2 yr of age (86%). Among the RVA positive patients, 28.5% cases showed severe disease while 66% were moderate. Seven different G-P types of RVA were identified with predominance of G3P[8] (62%) genotype followed by G9P[4], G2P[4], G1P[6],G12P[6], G12P[11] and G1P[8] (Fig1). Mixed infection of RVAG1G3P[8] genotypes was also detected in a single specimen.

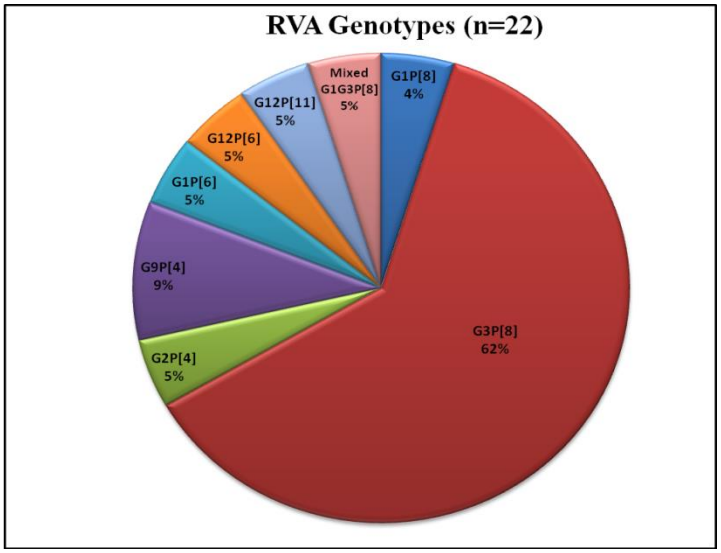


Fig 1: Distribution of G (VP7 gene) and P (VP4 gene) types among rotavirus A strains isolated from children with acute Gastroenteritis.

ENV1302: Assessment of genetic diversity in Group A rotaviruses (RVA) in patients with acute gastroenteritis.

Investigators: Tatte VS and Goplkrishna V.

Funding Agency: Intramural Duration: 2013-2020

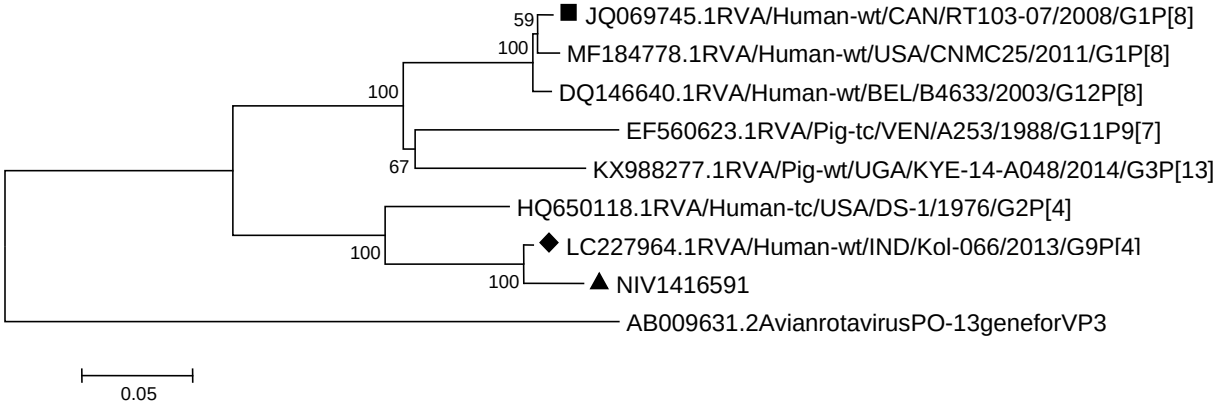
***Background*:** Group A Rotaviruses (RVA) are genetically diverse pathogens causing acute gastroenteritis in children. These are the most evolutionarily adaptable organisms having multitude of mechanisms for evolutionary change. To date, full genome classification of RVAs has been proved to be an excellent tool for studying the evolution of unusual rotavirus strains. As limited data is available from Western India, a study was undertaken to understand the genetic changes and their diversity in three unusual RVA stains, *i.e.*, (G9P[4], G9P[6] and G1P[6]) strains circulating in Pune city, during 2013-2015.

***Objective*:** Full genome characterization of three unusual RVA (G9P[4], G9P[6] and G1P[6]) strains circulating in Pune city, Western India.

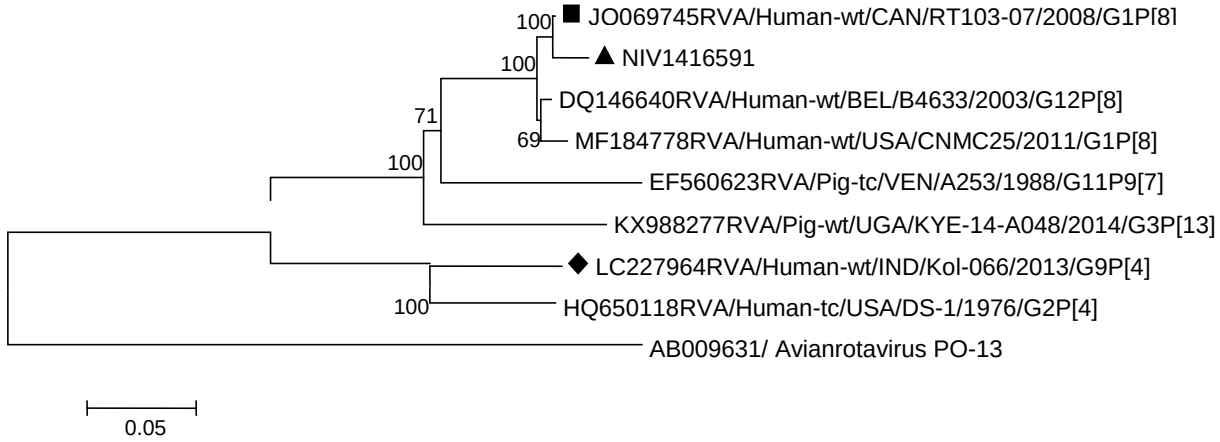
Findings: Full genome analysis of these strains classified them as G1-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1, G9-P[4]-I2-R2-C2-[M1-M2_R]-[A1-A2_R]-N2-T2-E6-H2 and G9-[P4-P6_R]-I1-R1-C1-M1-A1-N1-T1-E1-H1. Sequencing and phylogenetic analysis of structural and non structural genes of these unusual RVA strains showed nucleotide / amino acid identities of 82.3-98.5% / 77.3-99.8% and 86.6-97.6% / 89.6-97.8% between the strains. Evidence of recombination events was found within the genes encoding VP3, VP4 and NSP1 that showed re-combination of genetic information of genogroup 1 [M1/P[6]/A1] and genogroup 2 [M2/P[4]/A2] strains (Figure 2a,2b).

Observations of the current study will facilitate for future investigations into the molecular pathogenesis of such RVAs as exchange of whole or partial genetic material between rotaviruses by recombination events contributing directly to their diversification, adaptation and evolution.

Nucleotide 1-1620



Nucleotide 1621-2597



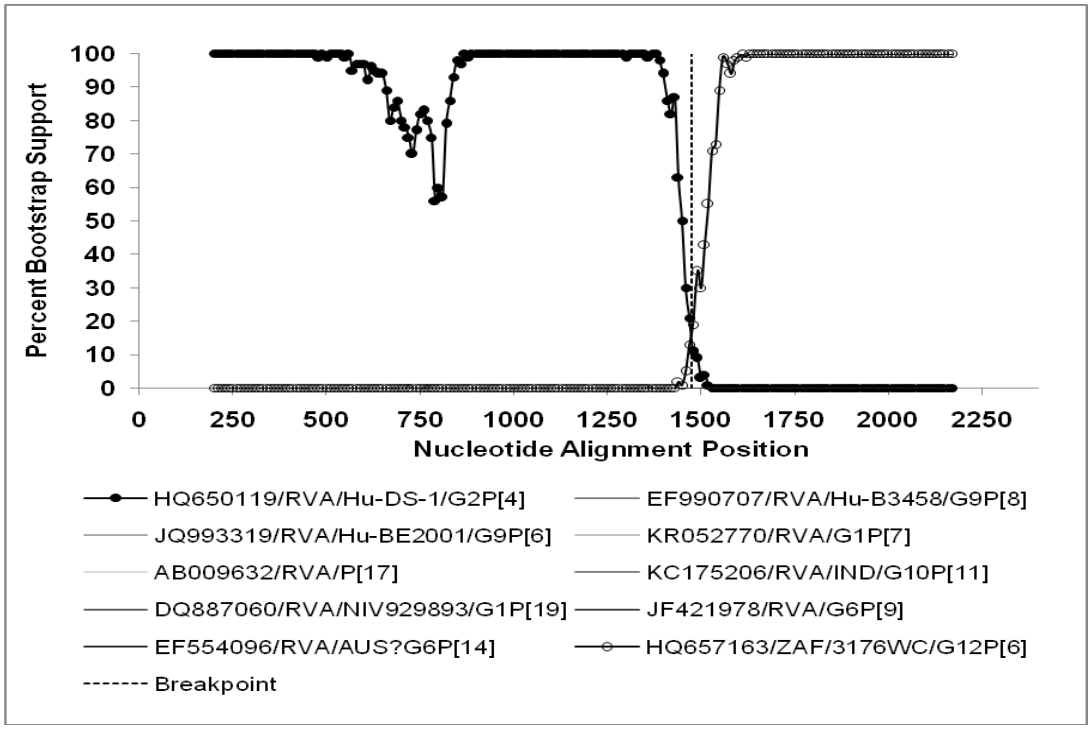
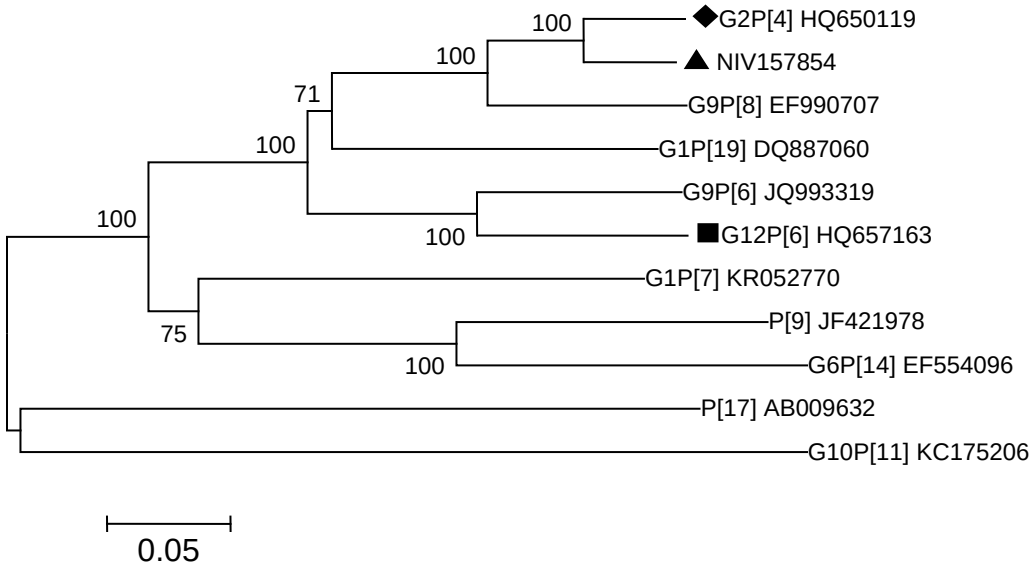
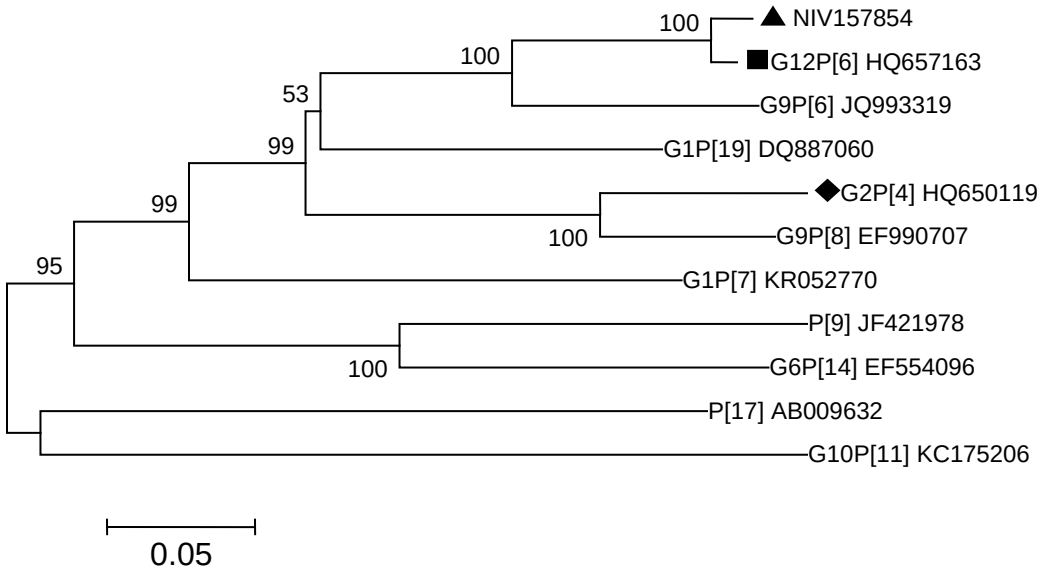


Figure 2a**:** Boot Scan plot for RVA/ Human / NIV 157984/G9P[4-6_R] VP4 gene against VP4 derived from strains RVA/Human-tc/USA/DS-1/1976/G2P[4] (major parent) and RVA/Human-wt/ZAF/3176WC/G12P[6] (minor parent) is shown. Plot is modeledon a pairwise alignment with a window size 400, step size of 10 and 100 bootstrap replicates. The vertical dotted line indicates break point. ML trees showing phylogenetic relationship of the recombinant strain NIV157984/G9P[6-4_R] with reference strains prior to (nt 1-1474) and after the break point (nt 1475- 2379).

Nucleotide 1-1474



Nucleotide 1475-2379



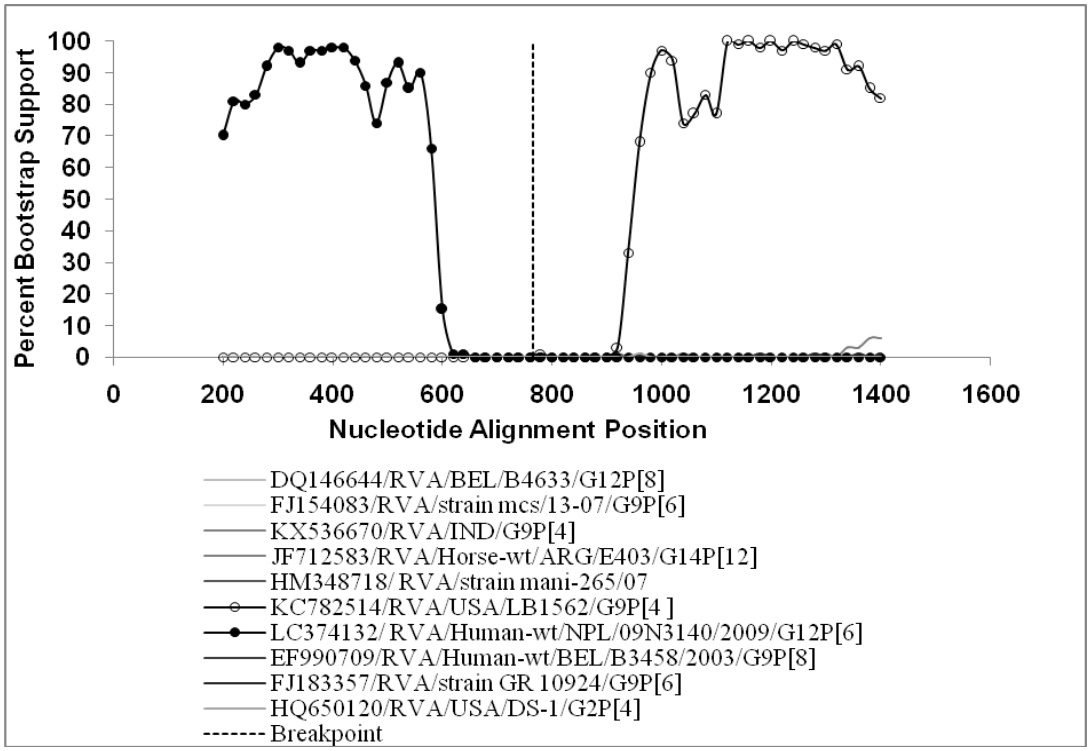
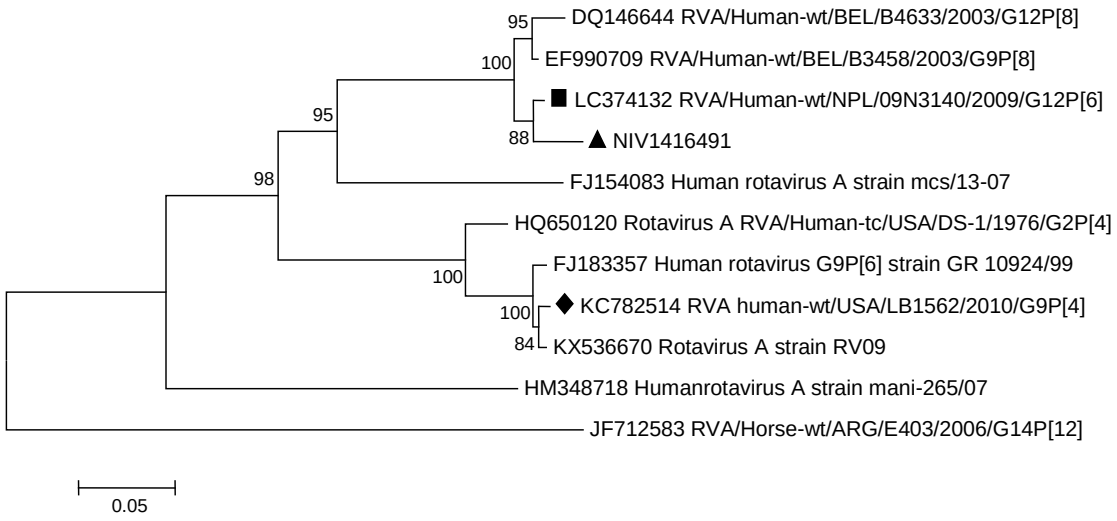
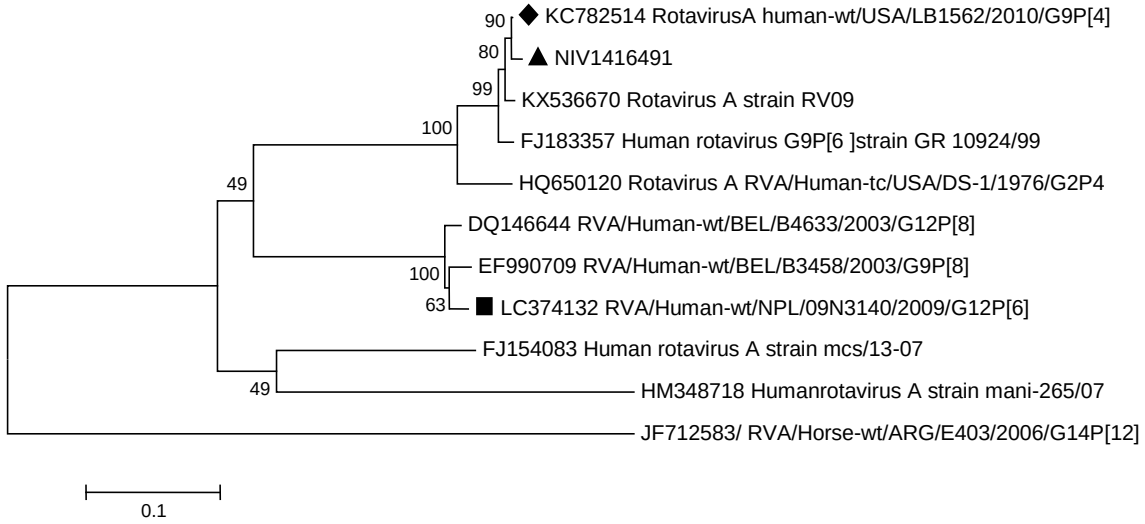


Figure 2b: BootScan plot for RVA/ Human/NIV1416591/G9P[4]–A[1-2_R] NSP1 gene against NSP1 derived from strains RVA/Human-IND/Kol-066/ G9P[4] (major parent) and RVA/Human-wt/NPL/N3140/G12P[6] (minor parent) is shown. Plot is modeledon a pairwise alignment with a window size 200, step size of 20 and 100 bootstrap replicates. The vertical dotted line indicates break point. ML trees showing phylogenetic relationship of the recombinant strain NIV1416591/G9P[4]–A[1-2_R] with reference strains prior to (nt 1-766) and after the break point (nt 767-1494).

Nucleotide 1-758



Nucleotide 759-1494



ENV1601: Identification and molecular characterization of rota and Noroviruses in neonates admitted at Neonatal Intensive Care Unit (NICU).

Investigators: Ranshing SS and Goplkrishna V

Funding Agency: Intramural Duration: 2016-2021

(i) ***Complete genome sequence analyses of human-bovine-like rotavirus strain, G12P[11] isolated in neonates: Evidence of multiple reassortment events***

***Background*:** Investigations conducted during 2016-18 in neonates in Pune, have shown early exposure to rotavirus infections and predominance of unusual human-bovine-like G12P[11] strains. Moreover, of the two P[11] neonatal strains reported earlier in India, *viz*., I321 G10P[11], and 116E, G9P[11], the former had a bovine RV backbone while the latter had only VP4 of bovine origin. Thus Complete genome sequence analyses of bovine-like strain, G12P[11] reported in the current study is important to evaluate whether these strains have originated due to interspecies transmission or reassortment events.

Objectives: To determine complete genome analyses of wild type as well as culture adapted G12P[11] strain to understand their genome constellations.

- To investigate genetic relatedness of neonatal vaccine isolate, 116E and other G/P type- matched strains.

***Work done and findings*:**

A new set of primers specific for P[11] were designed for amplification of VP4 gene. PCR amplicons were sequenced and sequence identity was determined through RotaC, (http:// www.regatools.be/rota20/). For each gene segment, a maximum-likelihood tree was constructed with the best-fit model of nucleotide/amino acid substitution. The nucleotide sequences were submitted to GenBank database with accession numbers MN529944 to MN529965.

Analysis of complete genome sequences of 11 gene segments of the RV strain, NIV-1740121 (both wild type and culture adapted) showed 100% homology at nucleotide and amino acid level at the 8th passage level. Phylogenetic analysis of outer capsid VP7 protein of the study strains showed clustering in G12 lineage III, With reference to VP4 gene, G12P[11] strains clustered within lineage I comprising of 116E, G9P[11] and shared the highest 94.39% (nucleotide) and 96.37% (amino acid) identities defining maximum evolutionary relatedness between them. The VP1 and VP2 genes of NIV-1740121 showed the maximum nucleotide and amino acid identities (>98%) with that of G12P[8]/[6] and other common genotypes. In contrast, the VP6 gene exhibited 96.73% nucleotide and 98.74% amino acid identities with

116E and clustered in a single branch of the tree. Most surprisingly, the VP3 gene exhibited highest homology with porcine rotaviruses within M1. Among the non-structural genes, the NIV-1740121 NSP1, NSP2 and NSP4 genes clustered closely with the common G12P[8]/P[6] and G1/G9P[8] strains showing >97% nucleotide and amino acid identities distinct from 116E. In contrast, NSP3 and NSP5 clustered very close to 116E strain in a single branch of the phylogenetic tree with >96% nucleotide and amino acid identities. Moreover, all the genes (except VP3) were genetically distinct from the porcine strains.

(ii) Structural characterization of outer capsid proteins of an unusual neonatal rotavirus G12P[11] strain

***Background*:**
Rotavirus outer-layer proteins, VP7 and VP4 are the principal targets of neutralizing and protective antibodies. Differences in amino acids at the antigenic epitopes of these proteins can affect the ability of antibodies to neutralize the virus. To date, G12 is not a component of any RV vaccine and 3D structures of its proteins are not available. Also, human rotavirus histo-blood group antigens (HBGAs) have host attachment receptors in a strain-specific manner. The expression of HBGAs is genetically determined and developmentally regulated. Crystallographic studies showed that the VP8* of P[11] forms a novel binding site that exclusively recognize either type I or type II precursor glycons.

Objectives:

- To carry out 3D structure modeling of VP4 (VP8*) and VP7 proteins from strains; NIV-1740121 G12P[11] and 116E, G9P[11] (Rotavac Vaccine strain).
- To understand the basis for the glycan specificity in neonatal G12P[11] strain.

Findings:
The 3D structures of VP7 and VP4 (globular head VP8*) proteins of NIV-1740121 and 116E were predicted using SWISSMODEL, a protein structure homology-modeling server in the fully automated mode. Homologous templates 3GZT.PDB and 4YFZ.PDB were used to model VP7 and VP8* respectively. Visualization of the structures was carried out using Discovery studio (BIOVIA). The surface electrostatic charge distribution analysis was performed using Maestro 11.5 (Schrodinger Inc., USA).

(iii) Comparison of VP7 antigenic epitopes of NIV-1740121 with 116E and G12P[8]/[6] strains

Comparative analysis of antigenic epitopes of VP7 protein of NIV-1740121 and the virus formulating ROTAVAC revealed 17 amino acid differences distributed in all the three epitopes. Within 7-1a antigenic epitope, seven non-conservative substitutions were identified and in most of these substitutions hydrophobic, non-polar residues were noted to have changed to polar residues (87Ile → Ser; 94Gly → Thr; 100Gly → Asn; 125Ala → Ser). However, the overall structure superimposed well with a root mean square deviation (RMSD) of 0.267 Å for the matching Cα atoms (main chain 0.370 Å). The least RMSD difference was noted within 7-1b region (C-α- 0.212 Å, main chain 0.304 Å) with four non-conservative substitutions. An additional N-linked glycosylation site was noted in the G12 strains, as a result of a change from Asp → Asn at amino acid position 238 of 7-1b epitope. The maximum RMSD difference has been observed in case of 7–2 epitope region (C-α-0.479 Å, main chain 0.491 Å) despite only two non-conservative substitutions. The surface charge distribution analysis of the modelled VP7 protein of NIV-1740121 revealed that the changes in the amino acid composition contributed to changes in charge density over the protein in comparison to 116E.

(iv) Comparison of VP4 (VP8*) antigenic epitopes and glycan binding site of NIV-1740121 strain with the neonatal P[11] strains

Sequence analysis of NIV-1740121 G12P[11] VP8* protein showed two substitutions at amino acid positions 148Ala → Thr and 188Val → Ile in 8–1 antigenic epitope and one substitution 182Ala → Thr in the immediate vicinity of the 8–2 epitope region compared to 116E VP8* and revealed negligible differences in the structural RMSD (C-α 0.017 Å, main chain 0.024 Å). Visualization of the receptor-glycan interaction structures of NIV-1740121 and 116E VP8* revealed that the type I glycan binds with a similar conformation at the same active site as represented in the crystal structure of N155 VP8* (PDB 4YFZ). The glycan was stabilized by several hydrogen-bonding and hydrophobic interactions. The residues Asn155, Phe156, Asp185, Arg187 and Ile158, Trp178, Gly179 of the P[11] VP8* were involved in hydrogen bond and hydrophobic interactions with the reducing end of LNT respectively, whereas Try183 and Ser 180 formed hydrogen bond and hydrophobic interactions with the non-reducing terminal Galb1,3-GlcNAc moieties of LNT respectively.

(iii). ***Determination of neutralizing antibody response against neonatal G12P[11] strain using hyper immune antisera against G1-G4 and G9 RV genotypes***

Background:

Of 36 G and 51 P RV genotypes that have been identified to date, only five major genotypes, G1- G4 and G9 in combination with P[8] and P[4] cause most of the disease in humans. More recently, the G12 genotype, has emerged as an increasingly common type globally.

Objectives:

- To determine neutralizing antibody response against neonatal G12P[11] strain in hyper immune antisera raised in rabbits against G1-G4 and G9 RV genotypes

Findings:

Neutralization assays based on a combined tissue culture and ELISA were employed using RV strains and hyper-immune antiserum against reference strains raised in rabbits. Significantly higher homotypic NAb responses to all five RV vaccine strains (G1, G2, G3, G4 and G9) were observed as compared to fully heterotypic strain, G12P[11]. Assays performed indicated that the rabbit antiserum neutralized homotypic strains with 16-128 fold higher titers as compared to G12P[11] RV strain.

IV. ***Prevalence of Noroviruses (NoV) in neonates admitted at NICUs in Pune, Western India***

Background: Neonatal NoV infections have rarely been surveyed in India. Also the **c**linical manifestations of the disease in neonates are not clearly understood.

Objective: To study the prevalence Noroviruses among neonates admitted at NICUs in Pune, Western India

Findings: A total of 701 stool specimens collected from 621 neonates (including 2 to 4 follow-up samples from 55 neonates) admitted at NICUs of two hospitals in Pune from April 2016 to March 2018 were analyzed during the study using single step RT-PCR targeting the conserved region of RNA-dependent RNA polymerase (RdRp) gene and sequencing. None of the neonate fecal samples tested positive during the present study.

ENV1501: Identification and molecular characterization of group C rotaviruses in infected humans and animals from Western India: A retrospective study

Investigators: Joshi MS, Walimbe AM and Goplkrishna V.

Funding Agency: Intramural **Duration:** 2015-2020

Background:

Group C rotavirus (GCR), is a known causative agent of sporadic and outbreak gastroenteritis cases globally. The zoonotic potential of GCR has been documented by studies indicating occurrence of porcine GCR in bovine species; bovine GCR in porcine species, porcine GCR in human and human GCR in porcine species. In this context, studies on all eleven genes of GCR strains were undertaken to identify cross species transmission and re-assortment events.

Objectives:

- To detect and characterize GCRs from humans, bovines and porcines with acute gastroenteritis and elucidate evolutionary relationship and time scale stasis or dynamics

Work done &Findings:

Full/nearly-full length nucleotide sequences of the GCR strains available with GenBank up to July 2018 representing different countries and hosts (Human, Porcine, Bovine, Canine, Ferret and Sloth Bear) were analysed. Final data set consisted of 81, 77, 74, 111, 166, 235, 83, 86, 81, 87, 88 sequences for VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4, NSP5 genes respectively isolated between 1970 and 2017. Multiple sequence alignment was performed using the MAFFT web server (https:// mafft.cbrc.jp/ alignment/server/index.html) while the best-suited nucleotide substitution model was identified by using MEGA6. Using these models phylogeographic analysis was carried out with country and host as traits for each gene by using the Bayesian Markov Chain Monte Carlo (MCMC) algorithm as implemented in BEAST 1.8.4. Two models such as molecular clock, Strict and Uncorrelated Relaxed Lognormal, were fitted to the datasets with Bayesian skyline tree prior. The marginal likelihood values, estimated by path-sampling and stepping-stone methods, were compared to choose the best-fitted molecular clock model. The strict clock model fitted best for the NSP4 and NSP5 genes while Lognormal clock model was found suitable for the remaining genes. Along with the evolutionary time estimates (tMRCA: time to Most Recent Common Ancestor), the most probable ancestral country and the most probable source host were inferred for nodes of MCC tree by fitting the standard continuous-time Markov chain (CTMC) model with the Bayesian stochastic search variable selection (BSSVS) to the data. The SPREAD 1.0.3 software was used to identify significant transmission links between different countries/host.

The human strains were monophyletic for all the genes except for M3 genotype (consisted of both human and porcine strains) of the VP3 gene. All human strains further grouped into two lineages, lineage I (mainly from China and Japan) and lineage II (mainly from India) for all the genes except the VP7 gene and M3 genotype of the VP3 gene. In case of the NSP4, VP4 and VP6 genes, few human strains remained ungrouped in any of the two lineages. The porcine strains were monophyletic for the NSP5 and VP1 genes and the remaining genes were classified into 2 to 4 groups on the basis of high posterior support. The MCC trees of all 11 genes showed monophyletic grouping for all bovine strains. The root mean age for all the genes indicated circulation of the GCR for over 800 years. Overall, the tMRCA of human strains dated back to the beginning of 19th century. The VP6, VP7 and NSP2 genes had lowest rates of evolution as compared to other genes. Most of the human strains originated from Japan or China with the exception of VP3 M2 genotype and NSP1 human strains from India. The most probable ancestral host for human strains was porcine for majority of the genes.

ENV1306: Diversity of non-rota enteric viruses in patients with acute gastroenteritis.
Investigators: Gopalkrishna V, Joshi MS
Staff: Chavan NA, Shinde MS
Funding Agency: Intramural **Duration:** 2017-2020

Background:

In India, disease burden and molecular epidemiology of Rotavirus A (RVA) is well studied and documented. However, the role of non-RVA viral agents among acute gastroenteritis patients were reported in limited studies.

Objective: Identification and molecular characterization of non-RVA viral agents *viz.*, Norovirus (NoV), Adenovirus (AdV), Astrovirus (AstV) in patients with acute gastroenteritis.

Findings:Fecal specimens (n=72) of acute gastroenteritis patients hospitalized in Pune city during Jan 2018- March 2019 were screened for non-RVA enteroviruses. RT-PCR studies for NoV-genogroup I and II detected NoV genogroup II six specimens (8.3%). Hexon gene specific PCR showed AdV in eight specimens (6.9%) while ORF-1 region based RT-PCR yielded two specimen positive for astrovirus. Genotyping analysis of the ADV confirmed presence of four different subgroups of AdV with predominance of subgroup F strains. Mixed infection of RVA and AdV was seen in two patients and single specimen each with RVA-AstV and AdV - AstV. Overall, the study highlights circulation of NoV, AdV and AstVs in acute gastroenteritis cases in addition to RVA. However, in 47.2% of the cases, the etiology remained unknown (Figure 3).

i) Screening of fecal specimens (n=302) of children hospitalized in Pune city for acute gastroenteritis during May 2017 to March 2019 yielded 4.9% positive for AstV. Genotyping confirmed presence of five different genotypes of AstV with predominance of HAstV-1 genotype.
ii) Retrospective studies of fecal specimens collected from children hospitalized for acute gastroenteritis in different hospitals of Surat (n=300) and Ahmadabad (n=300) in Gujarat during 2013-2016 using NoV and AdV specific RT-PCR/PCR have shown 7% and 6% NoV (Genogroup II) positivity in Surat and Ahmadabad respectively. Circulation of twelve different NoV genotypes with predominance of GII.4 along with its recombinant genotype strains was observed. Detection rate of AdV in patients was 5.6% (n=17) in both Surat and Ahmadabad and circulation of multiple genotypes of the subgroups A, B, C and D was noted with predominance of AdV-40 and AdV-41 genotypes of subgroup-F.

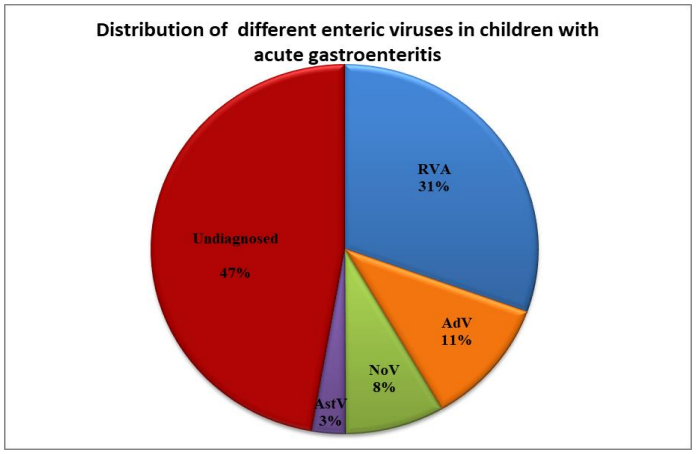


Fig 3: Distribution of different enteric viruses in children hospitalized with acute gastroenteritis during Jan 2018-March 2019 in Pune city.

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

Investigators: Sawant PM and Gopalkrishna V

Funding Agency: Intramural Duration**:** 2017-2020

***Background*:**

A wide range of enteric viruses have zoonotic potential, thus pose a risk for humans either by direct transmission from animals or through diary or meat products. The zoonotic potential has been reported for RV, AstV, AdV, NoV and SaV. However, limited data is available from Western India.

***Objectives*:**

Molecular characterization of enteric viruses in bovine, porcine, canine and feline species to understand their genetic diversity.

Findings:

Bovine (n=153) and porcine (n=29) diarrheic fecal samples collected from private cattle and pig farms in and around Pune city (Maharashtra) were screened. RVAs were detected in 27 (17.64%) samples bovine samples; showed predominance of G10P[11] (51.85%), followed by previously unreported genomic constellations, G6P[14] (14.81%), and, G6P[4] (7.40%) and G10P[33] (3.70%). The G10P[33] is bovine-simian (SA11-like) reassortant strain, G6P[4] was bovine-human reassortant strain while G6P[14] strain is human. In this study, we also sequenced nearly full genome of porcine EVG-15 besides detection of PTV-4 and putative PTV-17 genotypes. The detected EV-G strain showed highest identity in VP1 gene at nucleotide (78.61%) and amino acid (88.65%) levels with the prototype EV-G15 strain. However, its complete genome analysis was found homologous with nucleotide (78.38% identity) and amino acid (91.24% identity) level to Ishi-Ka2 strain (LC316832), an unassigned EV-G genotype detected from Japan. The diversity of enteric viruses detected in the present study highlights the need for exploring their genetic diversity and zoonotic potential from other geographical regions of India.

Genetic characterization of enteroviruses in Hand, Foot and Mouth disease (HFMD).

Investigators: Gopalkrishna V, Ganorkar N.

Funding agency: Intramural

Background:

Hand, Foot and Mouth disease (HFMD) is a mild exanthematous and febrile childhood viral disease that affects children. Coxsackie virus A-16, Enterovirus-71 and Coxsackie virus A-6 (CVA-16, EV-71, CVA-6) types were found associated in sporadic and outbreaks cases. The study was conducted to understand the EV types associated and their genotypic distribution in HFMD cases reported.

Objective: To determine the genotype and sub-genotype distribution of EVs associated with HFMD.

Findings:Molecular typing targeting VP1/2A junction or VP1region of 71 EV positive HFMD specimens revealed CVA-16 as the predominant type (57.7%) followed by CVA-6 (40.8%) and Echo-1 (1.4%). All the CVA-16 strains (n=41) exhibited highest nucleotide (96.86-98.32%) and amino acid (99.33-100%) identity with Indian strains and clustered with rarely reported B1c sub genotype. Within the strains, nucleotide and amino acid identity of 95.29-100% and 98.65-100% respectively was observed. CVA-6 strains (n=29) showed identities of 96.6-97.8% and 99-99.70% at nucleotide and amino acid levels respectively with Indian strains and clustered with E2 sub lineage. Within the strains, 95.1-100% nucleotide and 98-100% of amino acid identities were noted. The study highlights the circulation of B1c sub-genotype strains of CVA-16 and E2 sub-lineage strains of CVA-6 in Western India.

ENV1307: Pathogenesis of Coxsackie virus A-16 (CVA-16) associated with Hand, Foot and Mouth Disease (HFMD) in Neonatal Mice.
Investigators: Tikute SS and Gopalkrishna V
Funding agency: Intramural **Duration:** 2014-2020

***Background*:**
Clinical data suggests that CVA-16 infections can cause neurological complications leading to fatalities. The objective of the study is to determine the pathogenicity of CVA-16 strains in mice.
***Objective*:** to understand the pathogenicity of CVA-16 infection in HFMD using neonatal mouse model.
Finding: ICR neonatal mice infected with CVA-16 strain revealed progressive pathological changes from 3rd day post infection (PI). Minimal neuronal degeneration of brain and enlargement of ventricles were observed from 3 to 7 days PI. Lung tissues showed hemorrhages on day 3 PI and progressed to day 7PI. Microscopic observations revealed vacuolations in the enterocytes on day 3 PI in intestinal tissue. Cardiac tissues showed vacualations in cardiomyocytes; degeneration in cardiomyocytes and infiltration of inflammatory cells were observed on day 5 PI. Skeletal muscles showed degeneration of myocytes on day 3 which progressed to severe infiltration of inflammatory cells on 7 day PI. Immuno-histochemical analysis revealed high level expression of CVA-16 specific viral antigens in hind limb muscles, brain and cardiac tissues suggestive of the target organs of CVA-16 infection. CVA-16 specific viral antigen were also detected in kidney, lung, liver and intestine on day 5 PI, suggestive of infection in vital organs. Estimation of viral RNA in organ tissues using real time PCR using ITD kit (WHO) also showed gradual increase in virus titer in skeletal muscles and brain tissues.

SRF-project: Development of immunodiagnostic assay for Identification of Coxsackie virus A-16 (CVA-16) in patients with Hand Foot and Mouth disease.
Investigators: Ganorkar N, Hundekar S, Lole K and Gopalkrishna V
Funding agency: ICMR (SRF) **Project duration:** 2016-2019
Background:
Diagnosis of HFMD is usually carried out based on clinical characteristics and confirmation relies mainly on laboratory approach. So far, no attempts have been made to develop a rapid immunodiagnostic assay to detect viral pathogens associated with HFMD. The study involved development of CVA16 rVLPs and the corresponding hyper immune sera will be used to generate reagents for detection of CVA-16. This is a rapid, sensitive and cost effective immunodiagnostic assay using cloning and expression of CVA16, full capsid/VP1 gene using Bac-to-Bac baculovirus expression system. The study will help to develop diagnostics for rapid detection of CVA-16 in HFMD cases.
Objectives
- To generate laboratory reagents for development of immunodiagnostic assay (ELISA), for identification/detection of CVA16 in HFMD patients.

Findings:
Genomic characterization of CVA-16 strains identified from HFMD cases from India has been carried out by amplification of complete P1 region of EV (capsid 2586 bp)/VP1 (891 bp) genome. Phylogenetic analysis using complete VP1 and P1 sequences has been carried out to determine the genogroup distribution of circulating CVA-16 strains in HFMD patients. Cloning and co-expression of complete P1 and 3CD gene of CVA-16 strain into baculovirus donor vector (pFastBac Dual vector) resulting the CVA16 P1-pFdu recombinant plasmid for the purpose of generation of CVA-16 virus-like particles (VLPs) to develop immunodiagnostic assay (ELISA). CVA-16 P1-3CD was expressed in Sf-9 insect cell line (Invitrogen, USA) and CVA-16 VLPs were generated. The recombinant CVA-16 VLPs was confirmed against anti-CVA-16 polyclonal antibody raised in rabbit using ELISA, SDS-PAGE and Immunoblotting.

EPIDEMIOLOGY GROUP

Scientific Staff

Dr. Babasaheb Tandale	Scientist F & Group Leader	
Dr. Yogesh Gurav	Scientist E	(30th August 2019 onwards)
Dr. Avinash Deoshatwar	Scientist D	(10th May 2019 onwards)
Dr. Shilpa Tomar	Scientist B	(Up to 12th December 2019)

Technical Staff

Mrs. Vasanthy Venkatesh	Sr. Technical Officer 2
Mr. Kailas Gadekar	Technical Assistant
Mr. Machindra Karanjawane	Senior Technician
Mr. Rahul Jagtap	Technician B
Mr. Avanish Pandey	Technician A

Support Staff

Mr. Vishal Khond	Multi-Tasking Staff

Project Staff

Dr. Susmit Sambhare	Scientist C (8th January 2020 onwards)
Dr. Pravin Deshmukh	Scientist B
Mrs. Poornima Khude	Technical Officer
Mr. Yovhan Landge	Technical Officer

- Investigation of suspected viral outbreaks and assistance to central teams
- Seroprevalence studies on dengue and chikungunya viruses in Western India shows higher endemicity in Pune city in 2019
- Hospitalizations due to acute encephalitis in children and adults were found similar during 2018-19. However, high number of acute encephalitis related hospitalizations was recorded in children during 2019-20. JE was reported year-round among children in both the years.
- Continued development of mobile apps for immunization data in India
- A Health Technology Resource Hub of Dept of Health Research set up at ICMR-NIV

EPD1605: Seroprevalence of dengue and chikungunya virus infections in Western India: A retrospective and prospective cross-sectional study

Investigators: Tandale BV, Tomar S, Parashar D, Alagarasu K, Shah PS

Funding agency: Intramural Duration: 1 year (2017-19)

Background: Dengue (DEN) and Chikungunya (CHIK) virus transmission in Western India needs planned seroepidemiological surveys to adequately quantify and characterize the extent of infection in the population.

Objectives:

(i) To compare the changes in overall, age-specific and rural-urban seroprevalences over the baseline seroprevalences in Maharashtra and Goa states

(ii) To explore the factors associated with seropositivity for DEN and CHIK virus infections

Findings:

In continuation to the findings reported in previous annual reports, a prospective population-based serosurvey in the age group of 05-60 years was undertaken in Pune city from 18th March to 10th April 2019. Multistage cluster random sampling of 30 clusters (n=1654) was done using probability proportional to size (PPS) sampling with 50-60 participants per cluster. The overall CHIK and DEN IgG seroprevalence was 53.2% and 87.8% respectively. Dengue seroprevalence in Pune city has increased from 59.5% in 2009 to 87.8% in 2019 suggestive of high endemicity. The age-specific seroprevalence to dengue in Pune City is presented in Fig. 1.

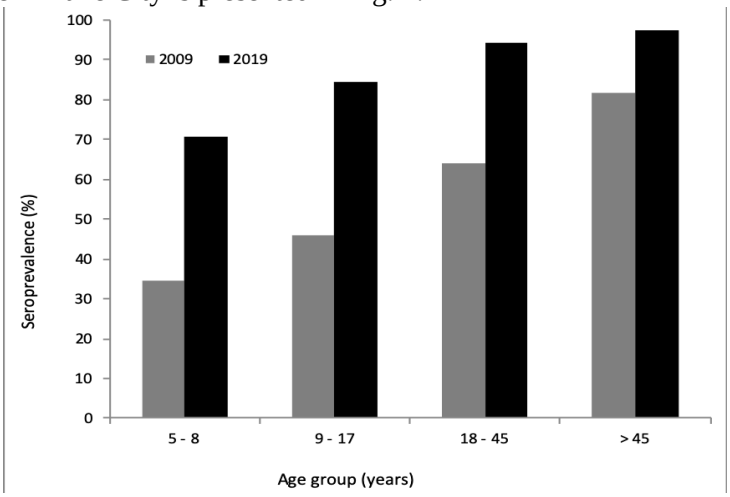


Fig 1: Age-specific seroprevalence of dengue in Pune City during the years 2009 and 2019

EPD1803: Japanese encephalitis (JE) epidemiology in central part of India: enhanced sentinel surveillance for etiological contribution and burden following vaccination in Maharashtra and Telangana

Investigators: Tandale BV, Sapkal GN, Damle R, Bondre VP, Tomar S, Narang R (MGIMS Sewagram), Mohiuddin Qazi (GMC Nagpur), G Padmaja (KMC Warangal)

Funding agency: ICMR (Extramural) Duration: 2.5 years (July 2018-Dec 2020)

Background:

Hospitalizations due to acute neurological illness among children and adults at Nagpur, Wardha and Warangal were tracked to detect the incidence of JE and other viral/bacterial etiologies.

Objectives:

- To estimate the incidence of acute encephalitis syndrome during hospitalizations
- To detect contribution of Japanese encephalitis and other etiologies of AES

- To estimate JE vaccination coverage among vaccine-eligible age groups
- To assess the effectiveness of JE vaccination in preventing JE disease

Findings:

An average 75-100 hospitalization with acute neurological illness was recorded at the three sites every month. The proportion of AES among acute neurological illness patients was found to be 9.1 % (127/1382) and 1.3 % (24/1816) in children and adults respectively.

Clinical specimens (n=463) were collected from 151 AES cases during a 12 month period. JE was confirmed in 18.8% (24/127) children and 12.5% (3/24) adults. The trend of AES hospitalizations along with JE confirmation among children and adults during the two year study period is presented in Fig. 2. The other viral/non viral etiological agents detected during the study are Herpes simplex (8), dengue (8), Chandipura, leptospirosis (3), typhoid (2) and scrub typhus (1). High number of acute encephalitis related child hospitalizations was recorded during 2019-20 in comparison to 2018-19. JE was reported year-round among children in both the years.

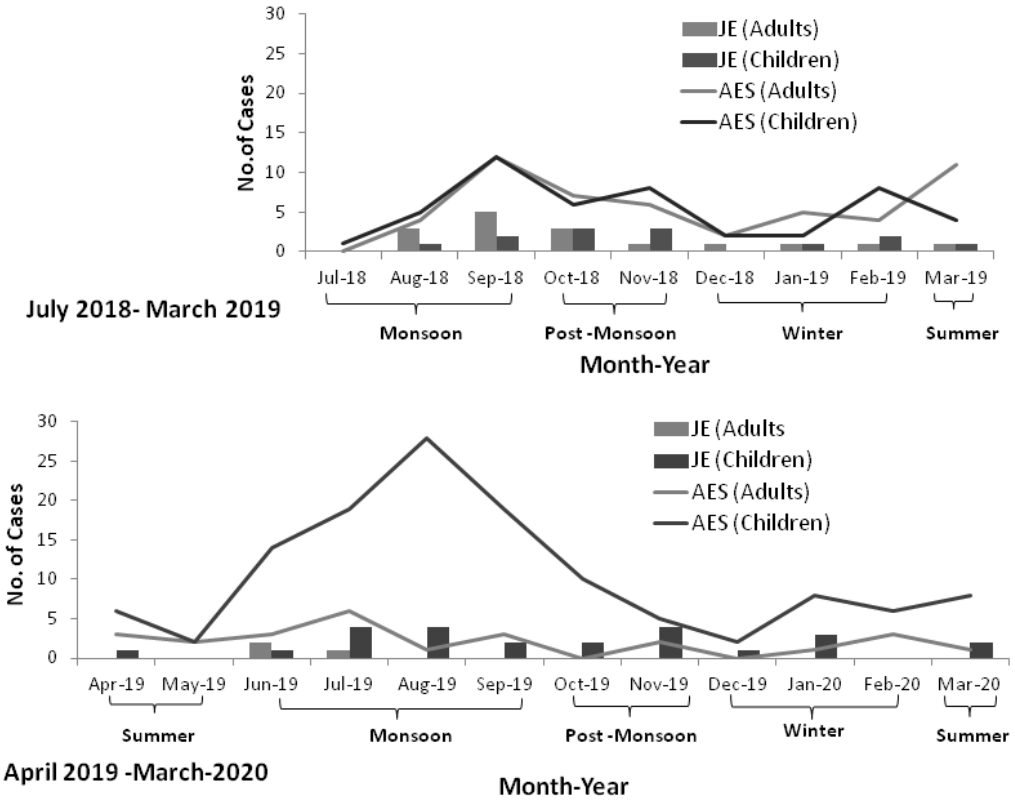


Fig. 2: Acute encephalitis hospitalizations along with JE confirmation, July 2018-March 2020

EPD2002: Mobile Application for Immunization Data in India [MAIDI]

Investigators: Tandale BV (Site PI), Gupta N (PI, ICMR)

Funding: ICMR [BMGF through DBT-BIRAC] Duration: 1.5 years (Feb 2019-August 2020)

Background:

The project involves the collaboration of ICMR institutes in six states located at different geographical regions of India. For undertaking pilot testing and operational feasibility of MAIDI mobile application, a Primary Health Center in Khadakwasla, Pune, has been identified.

Objectives:

(i) To develop an integrated mobile application for beneficiaries, health care providers and health system along with basic m-course on vaccine and immunization.

(ii) To validate the pilot mobile application in selected facilities at the community level.

(iii) To study the operational feasibility of the mobile application.

Findings:

Baseline situation analysis of mobile applications for immunization like ANMOL, eVIN and RISE was done. The features of MAIDI mobile application were compared with existing applications.

Workflow chart of immunization relevant to beneficiaries, *i.e.*, ASHAs and ANMs were prepared. Community beneficiaries approach ASHAs, who are acting as the link workers for immunization services. ANMs undertake immunization services with the help of ASHAs. The activities carried out during routine immunization are included in MAIDI mobile application development (Fig. 3). However, the feasibility and use of application needs to be evaluated.

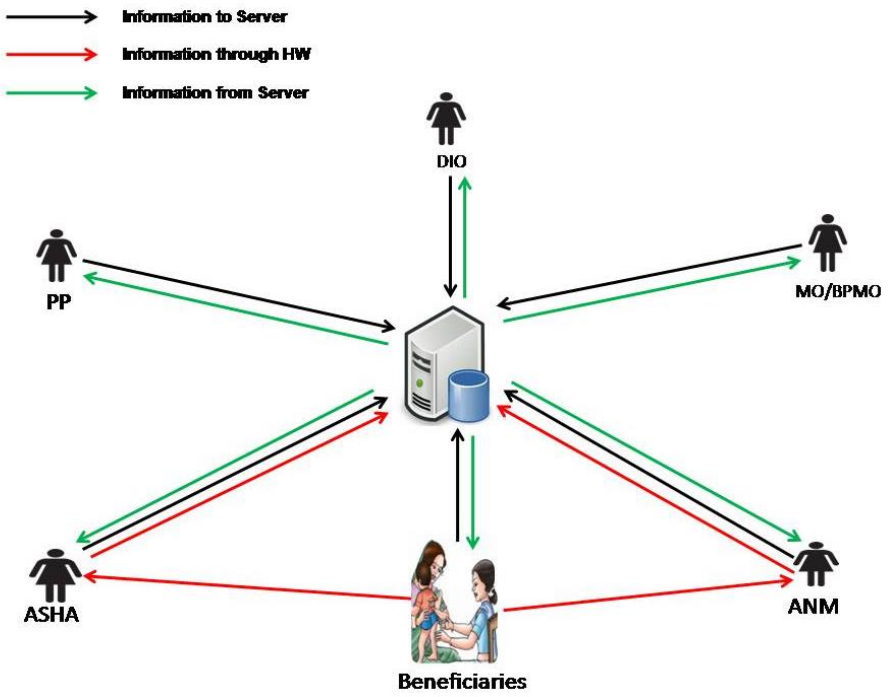


Fig. 3: Work flow of ASHAs and ANMs before, during and after immunization sessions

HTA3103: Health Technology Resource Center/Hub at ICMR-National Institute of Virology, Pune

Investigator: Gurav YK

Funding agency: D H R (Extramural) Duration: 2019 – 2022

Background: Health Technology Assessment (HTA) is a multidisciplinary process that uses explicit methods to determine the value of a health technology to promote an equitable, efficient and high-quality health system. To facilitate the process of transparent and evidence based decision making in the field of health, an institutional arrangement called the Health Technology Assessment in India (HTAIn) was initiated by the Department of Health Research (DHR). Health Technology Resource center (HTARC) at ICMR-NIV Pune has got sanctioned in October 2019 with a mandate to work closely with the state health services on HTA topics submitted by them to the DHR. Two HTA topics *viz*., syphilis-HIV combined point of care testing and Rubella vaccine for routine immunization in adolescent girls were allotted to HTARC at ICMR-NIV, Pune in March 2020.

Objectives:

- To identify the gap of knowledge for the allotted HTA topics and to finalize the appropriate research question, objectives for the allotted HTA topics with state health authorities.
- To prepare a research proposal on HTA topics and get the approval from the Technical appraisal committee of HTAIn at DHR
- To produce the evidence for the allotted HTA topic so that the transparent and evidence informed decision making in the field of health can be taken by the policy maker.

Work done*:* Project scientists were recruited and are being trained to carry out HTA research. Meetings were held with the health officials to finalize the research question and research objectives. Research proposals on the allotted HTA topics will be presented to Technical Appraisal Committee at DHR, New Delhi.

ENTOMOLOGY GROUP

Scientific staff

Dr. Sudeep AB	Scientist 'E' (sudeepmcc@yahoo.co.in)
Dr. Gokhale MD	Scientist 'D' (gokhale40@gmail.com)

Technical staff

Mr. Ghodke YS	Sr Technical Officer
Mr. Khude PD	Technical Assistant
Mr. Sonawane PA	Technical Assistant
Mr. Ingale VS	Technician-'C'
Mr. Lekhraj KN	Technician-'B'
Mr. Dhaigude SD	Technician-'B'
Mr. Meliya MZ	Multi Tasking Staff (MTS)
Mr. Raju Mewati	Multi Tasking Staff (MTS)

- Metagenomic analysis of *Aedes* mosquitoes from India are in progress
- Bionomics studies of Phlebotomine sandflies shows significant reduction in population.
- Field work on bat capture for studies on Nipah virus prevalence showed presence of antibodies in 12/58 samples tested.

ENT 1901: **Metagenomics analysis of viromes of *Aedes* mosquitoes in India**

Dr AB Sudeep, Dr SC Cherian & Dr Kavita Lole

Funding: Extramural (ICMR) NEW: Oct. 2019- Sept 2022)

Background*:* *Aedes* mosquito-borne virus diseases *viz*., dengue, chikungunya and Zika viruses have drawn special attention in India due to recurring outbreaks with high morbidity and mortality. Recent studies have shown high abundance and diversity of arthropod-associated RNA viruses, demonstrating the role of arthropods in viral evolution, potentially serving as a hotspot where insect-specific viruses have evolved into dual-host viruses.

Objectives: To study *Aedes* mosquito viromes from different parts of the country to determine the region-wise prevalence of arboviruses and differential vectorial capacities of the *Aedes* mosquitoes using metagenomics approach.

Findings: *Aedes aegypti* (n=803) and *Ae. albopictus* (n=586) mosquitoes were collected from 11 locations in Pune district. In the pilot study, two pools of *Ae aegypti* mosquitoes comprising 50 male and 50 female mosquitoes were processed to standardize the cDNA preparation protocol. RNA was extracted from the mosquitoes using QIAAMP viral RNA mini kit. The cDNA synthesis for the mosquito pools was carried out using random anchored primers. The cDNA library preparation using ligation Sequencing Kit (SQK-LSK109) from ONT is being optimized. A nanopore sequencer, MinION Mk1B (ONT, UK), was installed and a control lambda phage sequencing was carried out to evaluate the instrument. The tools required for metagenomic analysis such as Nanopolish, Minimap2, Canu, Medaka, Samtools and Bcftools were installed on the Linux server. Further work is in progress.

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 (in continuation to last year's Annual report).**

Sudeep AB & Gokhale MD

Funding: Extramural (ICMR) Duration: 2017- 2019

Background: Sand flies are the incriminated vector of Chandipura virus (CHPV) (Vesiculovirus*: Rhabodoviridae*), an encephalitis causing virus that inflicts high fatality among children and cause death within 24-48 hr of exhibition of symptoms.

Objective: to study the abundance and species distribution of sand flies in Vidarbha region of Maharashtra, an endemic region for Chandipura virus.

Finding: An year round survey of sand flies was carried out in 25 sites in Vidarbha region of Maharashtra and collected 5987 sand flies (Table 1). The study has shown drastic decline in genus *phlebotomus* population in the area (Data given in AR 2018-19). District wise species distribution has showed predominance of *Sergentomyia babu* in the three districts. Average per man hour density (PMHD) for the entire collection was determined as 35 (Fig 1). The PMHD for the urban areas were found comparatively low (≥1) while in villages it ranged from 1.5 to 101. Density of sand flies in remote villages that still retained the rural culture was very high and PMHD ranged from 32 to 101. Navegaon and Nagardhan in Nagpur district and Chinchtola, Varity and Kharbi in Bhandara district had the highest sand fly density. Influence of meteorological parameters, *i.e*., relative humidity and temperature on seasonal variability and abundance of sand flies was observed in the study.

Table 1: Species distribution of sand fly in different study areas (% to total collection) in three districts of Vidarbha region, Maharashtra

Name of village	District	Sand fly species distribution in different study sites (%)				
		Ph. papatasi	*Ph. argentipes*	*Ser. babu*	*Ser bailyi*	*Ser punjabensis*
Wad Dhamna	Nagpur	0	0.67	3.3	0.62	1.81
Wadi		0	0	1.48	0.05	0.58
Navegaon		0	0	12.67	1.36	2.83
Nagardhan		0	0	8.68	0.9	3.78
Dudhada		0	0.12	2.85	0.43	2.1
Chachar		0	0	0.69	0.14	0.05
Tarsa		0	0	1.51	0.37	0.23
Police line (U)		0	0	0.21	0.2	0.05
ADHS Campus U)		0	0	0.05	0	0
Mauda		0	0	0.33	0	0.32
Rangpeth	Bhandara	0	0	0.32	0	0
Chinchtola		0	0.02	13.6	0.88	1.78
Varithi		0	0	5.66	0.17	0.41
Kharbi		0	0	6.79	0.6	1.36
Zonad		0	0	0.72	0	0
Gopipada		0	0	1.96	00	0.15
Mathani		0	0	0.97	0	0.18
Narva		0	0	0.37	0.15	0.08
Tumsar		0	0	0.08	0	0
Chulhad		0.32	0.02	0.84	0	0.32
Kharadi		0	0	0.49	0.11	0.3
Marudi		0	0	0.32	0	0.32
Patampur	Gondiya	0	0	0.91	0	0.27
Wagh tola		0	0	1.61	0.12	0.11
Nandora tola		0	0	0.67	0	0.67

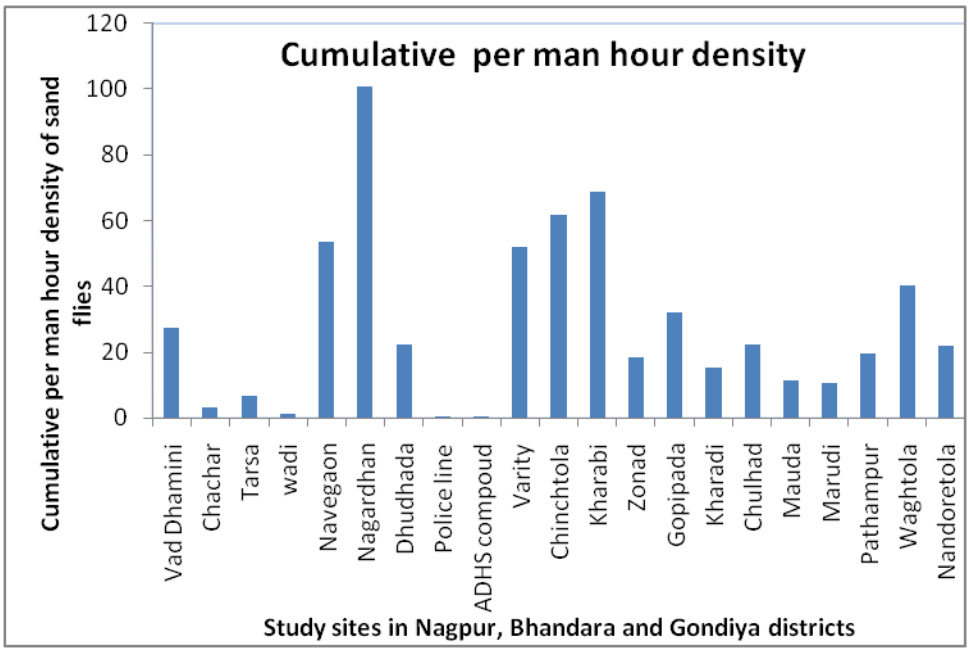


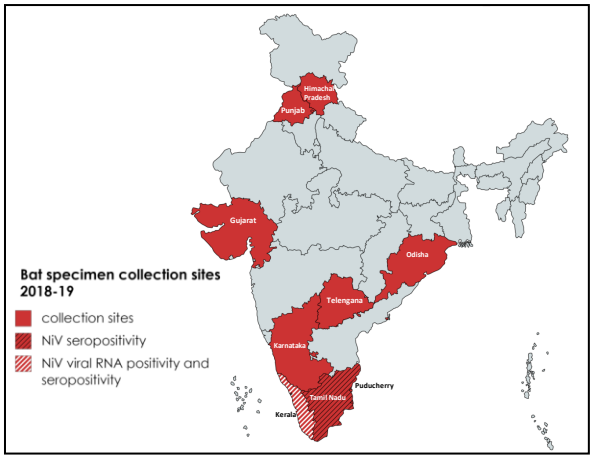
Fig 1: Cumulative PMHD of sand flies in selected study areas (sites) in Vidarbha region

ENT1701: Country-wide survey of Nipah virus in *Pteropus* bats
Dr AB Sudeep, Dr MD Gokhale, Dr PT Ullas, Dr Balasubramanium (Kerala unit)

Background: Kerala experienced an outbreak of Nipah virus during May-June 2018 which killed 17 people of 18 laboratory confirmed cases. This has warranted the need for a countrywide survey of fruit bats to determine the prevalence of Nipah virus in India. During the reporting year, bat sampling was done in two states (Table 1)
Objective: to determine the prevalence of Nipah virus in fruit bats across the country

Findings:

(i) Kerala: A follow up study was conducted during April 2019 to determine the persistence of Nipah virus and anti-NiV antibodies in fruit bats from the same rousting sites. Sampled 74 fruit bats (*Pteropus medius*) but none of the samples tested positive for NiV RNA suggestive of waning of NiV antibodies in bats after a period of one year. During December 2019, 36 *Pteropus* bats were also sampled from Malappuram and Kozhikode districts, but none tested positive.



(ii) Karnataka: During January 2020, sampling of 76 *Pteropus* bats from Kanakpura and Ramnagara districts of Karnataka was done. Nipah virus activity could not be detected in any of the bats.

(iii) Maharashtra: sampling of bats was carried out at Mahabaleshwar, Satara district, Maharashtra during 16th -18th March 2020 and 79 bats (62 *Rousettus leishnaulti* and 17 *Pipistrellus pipistrellus* species) were sampled during the study. Screening of samples showed presence of NiV RNA in two bats each of *Rousettus* and *Pipistrellus* bats as well as antibody positivity in 34 serum samples suggestive of the role of these two bat genera for the first time.

ENT 1902: Vector competence studies on *Aedes aegypti* with reference to Zika virus
M D Gokhale, G K Sakpal, P D Yadav (Intramural 2019-2021)

Background: Recent introduction of Zika virus has posed an additional challenge to India over the already existing heavy burden of vector borne diseases. So far the disease has been reported from Jaipur, Ahmadabad, Chennai and Bhopal. Despite its presence as a clinical entity the virus interaction with the vector in the present scenario needs to be explored in the context of its potential to spread and establish in the mosquito population.
Objectives: Vector competence studies on *Aedes aegypti* with reference to two Zika virus strains
***Work done and Findings*:** Laboratory grown *Aedes aegypti* mosquitoes were infected with two strains of Zika virus namely Zika (MR) strain and Zika (Indian) at low and high (mouse adapted) pathogenic doses. The mosquitoes were infected by three routes, *viz*., parenteral, membrane feeding and feeding on the infected host and were periodically harvested and tested by real time RT-PCR to determine the virus load. Horizontal and vertical (trans-ovarial) transmission of virus was also attempted using the standard protocol. High virus quantity (titre) was observed in the harvested organs of the infected mouse as well as in the mosquitoes. Mouse adapted Zika virus has exhibited the highest infection and transmission rates including the phenomenon of TOT which is a very significant observation. Presence of Zika virus (MR strain) in the infected eggs has epidemiological significance.

Miscellaneous Work:

a. **Vector competence of Anopheles mosquitoes to Chittoor virus, an Indian variant of Batai virus** (M Sc Project)

Background: Chittoor virus, an Orthobunyavirus of the Peribunyaviridae family has been isolated repeatedly from mosquitoes in India since 1957. Though no major outbreaks involving humans has been reported yet, the close antigentic relationship to Batai which is known to cause infections in humans in Europe and Africa.

Objective: To study the vector competence of *Anopheles stephensi* mosquitoes to Chittoor virus alongwith its potential to transmit the virus both horizontally and vertically (TOT).

Work done and findings: Mosquitoes were fed on viremic mouse, sampled and tested for virus growth on pre-scheduled time intervals. Horizontal and vertical (trans-ovarial) transmission of virus was also attempted using the standard protocol. The mosquitoes replicated and maintained the virus till 15th day post infection. The mosquitoes were able to transmit the virus to infant mouse demonstrating horizontal transmission. TOT could not be demonstrated, may be due to the low number of mosquitoes processed.

Studies on Dengue and Chikungunya Virus (M Sc Project):

Dual infection studies were conducted on *Aedes aegypti* with reference to dengue and Chikungunya virus in single infection, co-infection and super infection status. The infected mosquitoes were screened by immunofluorescence and real time PCR assay. It was of interest to note that both the viruses multiplied efficiently in the vector despite the co-infection or super infection status.

Detection of dengue virus antigen in infected mosquitoes using a NS1 antigen detection kit (commercial): *Aedes aegypti* mosquitoes were infected with Dengue-1, Dengue-2, Dengue-3 and Dengue-4 serotypes. The infected mosquitoes were tested for virus detection using the NS-1 detection kit and Real Time PCR assay. It was observed that the kit could detect the dengue virus antigen from the mosquito specifically.

Comparative studies on *Aedes albopictus* and *Aedes aegypti* populations from Kerala (M Sc Project): Kerala state has exhibited repeated dengue and Chikungunya incidences during the monsoon and post monsoon period. With a view to study the vector biology of resident vector strains, both the *Aedes* species were collected at Kerala and brought to laboratory and a permanent colony was established. Comparative studies on the morphometric parameters, life table characteristics and Chikungunya virus susceptibility have been completed.

MAXIMUM CONTAINMENT FACILITY GROUP

Scientific staff

Dr. Yadav PD	Scientist 'E'& Head
Dr. Shete AM	Scientist 'D'
Dr. Sreelekshmy M	Scientist 'B'
Dr. Sahay RR	Scientist 'B'

Technical Staff

Mr. Upadhyay C	Senior Technical Officer (1)
Dr. Jain R	Technical Officer
Mrs. Patil S	Technical Officer
Mr. Lakra R	Technical Assistant
Mr. Sarkale P	Technical Assistant
Mrs. Majumdar T	Technical Assistant
Mr. S Baradar	Technical Assistant
Mr.DigheH	Technical Assistant
Mr. Mali D	Technician – C
Ms. GawandeP	Technician (1)
Mr. Kumar A	Technician (1)
Mr. Shende U	Technician-'B'
Mr. Gopale S	Technician-'A'
Mr. Aacharya M	Technician-'A'
Mr. Thorat S	Laboratory Attendant (2)
Mr. Chopade G	Laboratory Attendant (2)
Mr. Holepanavar M	Laboratory Attendant (2)

Project Staff

Dr. Nyayanit D	Scientist 'C'
Dr. Pardeshi P	Scientist 'II'
Mr. KadamM	Project Technician III
Mr.Suryavanshi A	Project Technician I
Ms. Kalele K	Technician- III
Ms. WaghmareA	Technician- III
Mr. Joshi Y	Technician- III
Ms. Kharde R	Technician- III
Mr. DespandeN	Technician- III
Ms.Salve M	Technical Assistant

Ms.BodakeP	Technical Assistant

Engineering Staff (Project)

Mr. Mohite M	Technical Assistant
Mr. Gaikwad V	Technician (1)
Mr. Sharma NK	Technician (1)

- National Apex Reference laboratory for laboratory diagnosis of high risk group 4 pathogens.
- Phylogeogrpahy studies revealed evolution and spread of Kyasanur Forest disease virus in the Western Ghats
- Using Next Generation Sequencing platforms, a Mason-Pfizer monkey virus was detected in a Nilgiri langur.
- Facility for production of reference reagents for laboratory of viruses of public health importance continued its mandated activities.
- An animal model of Nipah virus in golden Syrian hamsters and a rapid point-of-care assay developed.
- Countrywide surveillance of Nipah virus in bats showed activity in several states.
- Isolation and genomic characterization ofSARS-CoV-2 virus from India.

MCL 1801: Prospective investigation of transmission of Crimean Congo Haemorrhagic Fever amongst close contacts

Sahay RR, Yadav PD, Shete AM, Upadhayay K, Jain R, Patil S, Majumdar T

Funding agency**:** Extramural (ICMR**)** Duration: 2019-21

Background: Crimean-Congo haemorrhagic fever (CCHF) is a tick-borne viral disease with an average mortality rate of 30-50%. Gujarat and Rajasthan are endemic to the virus since 2011 and several outbreaks and sporadic cases of CCHF were reported during 2012–2020. During 2019, 40 confirmed cases with 20 deaths were reported from these states and ICMR-NIV, Pune provided diagnosis to suspected (referred) samples.

Objectives: To study transmission of CCHF virus among close contacts of positive patients and their clinico-epidemiological profile.

Findings: Laboratory investigations were carried out in 353 close contacts (symptomatic as well as asymptomatic) of 34 confirmed CCHF cases from Gujarat. Six symptomatic and one asymptomatic contact tested positive for CCHF either by qRT-PCR or anti-CCHF IgM ELISA. One asymptomatic contact tested positive for both anti-CCHF IgM and anti-IgG antibodies. This suggests human to human transmission of CCHF among close contacts with low sero-positivity (sub-clinical infection). On the contrary, none of the 291 close contacts of the six CCHF confirmed patients in Rajasthan tested positive for the virus or antibody.

MCL 1803: Identification and characterization of novel viral isolates using Next-Generation Sequencing platform

Yadav PD, Shete AM, MajumdarTD, Nyayanit D, Patil S, Mohandas S

Funding agency: Intramural Duration: 2018-2021

***Background*:** Next generation sequencing (NGS) is presently the most advanced approach for identification of unknown/untyped viruses independent of prior sequence information. Detection of novel pathogenic viruses in the clinical specimens has special significance in the public health as many recent disease outbreaks have been caused by novel pathogens.

***Objective*:** Identification of unknown virus isolates using targeted sequencing to identify viral heterogenicity and development of diagnostic assays for newly identified viral pathogens.

a. Next-generation analysis on acute encephalopathy samples from Muzaffarpur, Bihar

Work done & interpretation: Twenty-three clinical samples (5 CSF; 15 serum; 3 urine) received from acute encephalopathy patients from Muzaffarpur, Bihar were processed using NGS platform and data was analysed on the CLC genomics workbench version 11. *De novo* based assembly was performed to generate contigs of length greater than 1000bp and identified the contigs as per standard protocol. No specific virus or pathogenic bacterial sequences could be detected in the analysed samples. No correlation with the pathogenesis/aetiology of encephalitis could be established.

b. Screening of primate samples from Nandan Kanan Zoo, Bhubaneswar, Odisha

Tissues of a deceased Orangutan and a Nilgiri langur from the Nandan Kanan Zoo, Odisha were investigated to determine the etiological agent (s). Herpes virus specific real-time RT-PCR studies yielded negative results while RNA NGS analysis of langur samples suggested presence of Mason-Pfizer monkey virus in addition to a significant number of reads of rRNA specific sequences of *Chromobacterium violocecum*. Though tissue samples (heart, spleen, and intestine loop, liver, brain, Lungs, kidney, blood and serum) were processed for virus isolation in Vero CCL81 cells, virus could not be isolated. Samples from the orangutan provided no conclusive evidence of any pathogenic bacterial/viral sequences in the NGS analysis. Complete genome of Mason-Pfizer monkey virus was reported for the first time in Nilgiri langur.

c. Laboratory support to Ramnagar mystery outbreak, Udhampur district in Jammu and Kashmir and samples from PGIMER, Chandigarh in February 2020

ICMR-NIV received samples from children died during a mysterious outbreak in Udhampur district of Jammu as well as kidney biopsy samples from four patients admitted to PGIMER-Chandigarh for viral investigations. ICMR-NIV team also collected 17 serum samples, 04 nasal/oropharyngeal swabs, and peritoneal dialysis fluid from one of the patients of which 13 samples [2 nasopharyngeal aspirates, 02 nasal swabs, 04 renal biopsy sample, 02 endotracheal aspirates, 2 oropharyngeal swabs, and 01 peritoneal dialysis fluid were subjected to next generation sequencing. Viral contigs were identified as human coronavirus OC43 virus, human rhinovirus B35, human respirovirus 3, rhinovirus A and human respiratory syncytial virus A..The analysis did not give any conclusive evidence of a particular etiological agent responsible for the deaths.

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

Yadav PD, Sapkal GN, Sudeep AB, Mourya DT, Jain R, Majumdar T, Shrivastava R
Funding agency: Extramural [DHR] Duration: 2017-20

Background: Positive controls are the essential component of molecular diagnostic testing. However, availability of positive controls of high pathogenic viruses is not accessible to majority of diagnostic laboratories as these viruses are permitted to be handled only in high containment laboratories. Since ICMR-NIV has the containment facilities to handle these viruses, the responsibility was taken up.
Objective: to prepare positive controls (inactivated) of viruses of public health importance in India and to supply to National laboratory networks for research/diagnosis

Work done: During the year, lyophilized positive controls of Zika virus, KFDV, Yellow Fever (17D) virus, Nipah virus, HSV, CCHFV etc., were supplied to various VRDLs across India and SEAR countries. In addition, several diagnostic reagents were also supplied to different government/private organizations for development of indigenous assays/kits (Table 1).

Table 1: List of viruses, antigen/ diagnostic kits supplied by ICMR-NIV during 2019

Sr. No.	Antigen/kits details	Total volume of virus stock prepared/supplied	Supplied to
1.	CCHFV human IgG	CCHF Positive antigen – 05 ml	Zydus Cadilla, Ahmedabad [For Technology Transfer]
2.	CCHFV human IgM	CCHFV Positive antigen – 06 ml	
3.	CCHFV cattle IgG	CCHFV Positive antigen – 05 ml	
4.	CCHFV sheep/goat IgG	CCHFV Positive antigen – 05 ml	
5.	Nipah virus (NiV)	Anti NiV human IgM ELISA kit - 5 kits	GMC, Ernakulam
6.	Equine encephalosis virus (EEV)	Anti EEV horse IgG ELISA kit - 5 kits	FMD typing Scheme, Ahmedabad
7.	MERS-CoV	Real-time RT-PCR primers/probes-100 Rnx RT PCR primers-100 Rxn Positive controls – 100 Rxn	NCDC, New Delhi
8.	Ebola	Real-time RT-PCR primers/probes-100 Rnx RT-PCR primers-100 Rxn Positive controls – 100 Rxn	

9.	Kyasanur Forest disease virus (KFDV)	NIV Anti KFD Human IgM ELISA kit- 5 kits	NIV Bangalore Unit
10.	Dengue, Chikungunya and Zika virus	Trioplex Kit-3	
11.	Nipah virus diagnosis	Real-time RT-PCR primes/probes-500 Rnx Positive controls – 500 ul	NIV, Kerala Unit

A study of pathogenicity of Nipah virus in Syrian hamster model

Mohandas S, Yadav PD, Patil D, Sahay RR, Kaushal H
Jain R, Patil S, Majumdar T, Sarkale S, Kadam M
Funding agency: Intramural Duration: 2018-2020

***Background*:** Nipah virus (NiV), a highly pathogenic bat-borne paramyxovirus, has caused devastating outbreaks with high fatality in India since 2001. The 2018 outbreak of NiV was massive which killed 18 people with a case fatality rate of approx. 90%. No studies on the pathogenicity of the Indian strain of NiV have been conducted so far. Syrian hamsters closely mimic multiple aspects of human NiV disease and hence, a study has been initiated to determine the pathogenicity of the Indian strain.
***Objective*:** to determine the susceptibility, tissue tropism and viral kinetics of Indian isolate of Nipah virus in Syrian hamster model

Findings: Lethal dose 50 (LD_{50}) of the Indian strain was determined in Syrian hamsters by intraperitoneal (i.p.) route as $10^{2.5}$/ml. Intranasal route of infection could produce disease in only a few hamsters and hence intraperitoneal route was employed for the pathogenicity experiments. Thirty-five Syrian hamsters were inoculated though the i.p. route and a sequential disease progression study was carried out. The inoculated hamsters exhibited laboured breathing, profuse nasal discharge and neurological signs like head tilting and hind-limb paralysis. Viremia was observed from day 1 post inoculation (PI) to day 5 PI. Nasal washings were also found positive during the viremic period. In one of the hamsters, rectal and oral swab samples showed positivity. Viral RNA positivity was detected in the lung, brain, spleen, trachea, intestine, heart, kidney and urinary bladder of the infected hamsters. On histopathological examination, lungs showed severe congestion and hemorrhagic foci throughout. Kidney also showed severe changes.

MCL 1806: Development of sero-diagnostic assays for Nipah virus
Yadav P, Shete AM, Mohandas S, Jain R, Melag S, Chopade Y, Sarkale P, Lakra R, Majumdar *T*
Funding agency: Extramural (ICMR) Duration: 2018-2020

Background: Re-emergence of Nipah virus in an explosive manner in Kerala in 2018 killing 17of the twenty-two infected people warranted the need to develop serodiagnostic assays for diagnosis of the infection. In the present study attempts are made to develop Immunoglobin M and G based Enzyme-linked immunosorbent assay for the diagnosis of acute infection and for also surveillance activities.

Objective: (i) to develop immunoglobin M and G based Enzyme-linked immunosorbent assays

Findings: Anti-Nipah human IgG capture ELISA was developed and optimized. Sensitivity and specificity of the assay were evaluated with CDC anti-Nipah human IgG ELISA and found 93% and 99.28% respectively. Nipah virus negative samples of acute encephalitis (n=100) cases collected from different geographical areas were used to determine the cut off. Measles, mumps, rubella, CCHF and KFD IgG positive and negative human serum samples were used to test cross reactivity. No cross

reactivity could be detected. The assay was found to be highly specific for detection of anti-Nipah virus human IgG antibodies.Using the indigenously developed ELISA, antibody kinetics in serum of survivors of the NiV outbreak of 2018 and their close contacts (total 04 samples) was studied. Neutralizing antibodies were assessed usin g PRNT. Anti-NiV IgM antibodies were detectable from 5th day post infection (dpi) to 63 dpi while anti-NiV IgG antibodies could be detected from 5 to 438 days in symptomatic patients and 49-385 day in asymptomatic contacts.

Objective (ii) Development of anti-NiV bat IgG ELISA:

Findings: This assay was developed as a part of preparedness to detect the circulation of NiV in the reservoir bat populations. Sensitivity and specificity of the indigenous anti-NiV bat IgG ELISA was determined as described above and found to be100% and 83.3%, respectively. Screening of serum samples of *Pteropus medius* bats from Kerala state using the newly developed ELISA showed NiV seropositivity in 20.68% of bats during the 2019 outbreak.

MCL1901: Countrywide survey of Nipah and Corona viruses in different species of bats
Yadav PD, Sudeep AB, Gokhlae MD, Shete AM, Mohandas S,Patil DR, Balasubramanian, Ullas PT, P Sawant, Mathapati B
Contributing staff: Jain R, Patil S, Majumdar T, Gopale S, Suryawanshi S, Chopade G, Acharya M, Holeppanavar M, Daigude S.
Funding agency: Intramural Duration: 2019-2021

Detection of coronaviruses in *Pteropus* &*Rousettus* species of bats from different States of India
Background: Bats are known to harbour a number of viruses, most of which are potential human pathogens. In India, the association of *Pteropus medius* bats in the transmission of Nipah virus is well duering the NiV outbreak in Kerala in 2018 and 2019. SARS-CoV-2 virus which has emerged as a pandemic is also suspected to have its origin from bats. A survey of widely prevalent bat species in India as well as screening of retrospective bat samples was therefore initiated.
Objective (i): To sample widely prevalent Indian bats for Nipah and coronaviruses.
Work done and findings: To detect the presence of coronaviruses in bats, retrospective NiV negative bat samples collected during outbreaks and surveillance studies were screened. The study revealed the presence of bat CoV (BtCoV) in rectal swab specimens of eight *Rousettus* and 21 *Pteropus* bats. Partial RdRp sequences could be retrieved from three *Rousettus* and eight *Pteropus* bat specimens by Sanger sequencing. Phylogenetic analysis of the partial RdRp region demonstrated distinct subclustering of the BtCoV sequences. NGS analysis led to the recovery of four sequences covering approximately 94.3% of the whole genome of the BtCoVs from *Rousettus* bats. Three BtCoV sequences had 93.69 per cent identity to CoV BtRt-BetaCoV/GX2018 while the fourth BtCoV sequence had 96.8% identity to BtCoV HKU9-1.
Objective (ii): to determine the presence of Nipah and coronaviruses in the bat population of Maharashtra state
Work done and findings**:** Sampling of 65 *Rousettus* and 15 *Pipistrellus* collected from Mahabaleshwar area in Satara district of Maharashtra was carried out as part of the study. The bats tested negative for bat coronaviruses, but Nipah virus activity was detected in representative samples of both the species (Table 1). Detection of anti-NiV antibody in 33 out of 56 *Rousettus* bats is suggestive of active circulation of the virus in Maharashtra and needs immediate attention of the health authorities.

Table 1: Detection of anti-NiV activity in *Pipistrellus* and *Rousettus* bats from Maharashtra

Bat species	NiV RNA detection using rRT-PCR (Positive samples/total samples)				Anti-NiV IgG antibody positivity (Positive samples/total samples)
	Liver/ Spleen	Kidney	Throat Swab	Rectal Swab	
Pipistrellus sp.	2/10	1/10	0/15	0/15	01/04
Rousettus sp.	1/10	2/10	1/65	1/65	33/56
Total	3/10	3/10	1/80	1/80	34/60

Phylogeography of Kyasanur Forest Disease virus in India (1957-2017) reveals evolution and its spread in the Western Ghats region[1]

Kyasanur Forest Disease virus (KFDV), first isolated from Shimoga district of Karnataka, has shown geographical expansion to neighbouring states, *i.e*., Tamil Nadu, Maharashtra, Kerala, and Goa. Spread of KFDV across a large geographical region highlighted the need to understand the evolution and transmission dynamics. Genome analysis (E-Gene & complete genome) revealed two KFDV subgroups, *viz*., 1957–1972 strains and 2006–2017 strains that differed by ~2.76%. A probable route of KFDV transmission from Karnataka-Goa-Maharashtra is indicated in Figure 1. Selection pressure analysis indicated an adaptive evolution at the site near to the vicinity of the envelope protein dimer. The study highligts the need for more intense surveillance of KFDV in India.

MCL 1303: **Testing and rectification of different components of the BSL-4 facility & preparation of basic documents**
Yadav PD, Shete AM, Sahay RR, Mohandas S, GondaneY, MajumdarTP, Sarkale P
Funding: Intramural Duration: 2018-2020

MCL 1802: Preparation of reagents for highly infectious diseases
Yadav PD, Shete AM, Sarkale P, Jain R, Patil S, Majumdar T, Lakra R
Funding agency: Intramural Duration: 2018-2020

Background*:* Maximum containment laboratory has undertaken investigations of many outbreaks/clusters and cases of highly infectious diseases of public health importance. ***Objectives*****:** to prepare reagents for developing diagnostic assays against highly infectious viruses
Findings**:** Bulk propagation of Nipah and KFD viruses were made, aliquoted and stored. Polyclonal immune sera against Nipah, CCHF and KFD viruses were also raised in CD1 and Balb/c mice models.

Indigenous anti-monkey KFD IgM and IgG ELISA assays were developed to determine the presence of anti-KFDV antibodies in monkeys. Both the assays were validated at three internal laboratories. This assay was successfully used in animal experiments conducted to understand the persistence of anti KFDV IgM and IgG antibodies in monkey serum samples.

Standardization and Validation of KFDV Point of Care assay:
Background: In the background of geographic expansion of KFDV, a need to have a technical devise to provide correct diagnosis at the field level was felt and this has led to the development of a new assay.
Objectives**:** Standardization and validation of KFDV Point of Care (PoC) assay
Findings: The sensitivity of Trueprep AUTO RNA extraction system was compared with Magmax RNA extraction method and tested simultaneously by KFDV real-time RT-PCR assay [TaqMan based] and the Truelab UnoDx PCR system. Results by both the methods of RNA extraction were found at par when compared with UnoDx PCR and TaqMan real-time RT-PCR assays. Both the systems could detect equal number i.e. 10 copies of KFDV RNA. Specificity of KFDV PoC assay using UnoDx Real Time PCR system was found concordant with that of TaqMan real-time RT-PCR for a total of 53 positive KFD specimens that included human serum (n=20), monkey necropsy specimens (23) (kidney, heart, liver, brain and lungs specimens) and tick pools (10). Cross-reactivity of the KFDV PoC assay was checked by testing 25 flavivirus positive specimens and specimens positive for Chikungunya (3), Rubella (n=5) , Measles (n=5) and Influenza (n=5)viruses. No cross reactivity was observed which was concordant with the results by TaqMan Real-Time RT-PCR.

DRDE and ICMR-NIV team jointly evaluated KFD RT-LAMP, CCHF RT-LAMP, CCHF IgM and CCHF Antigen ELISA

DRDE and ICMR-NIV team jointly evaluated the following diagnostic assays during 10-12 April 2019 and 22-23 October 2019 with additional samples/commercial kit. In addition, immuno-reactivity of DRDE produced rNS1 protein of KFD virus was also carried out.
CCHF RT–LAMP: The assay was evaluated with 26 human serum samples (5 positive and 21 negative) collected from a recent outbreak of CCHF during August-September 2019 in Gujarat and Rajasthan and the results were compared with RT-PCR
Result: All the 26 human samples were found negative. The result also revealed 100% concordance with NIV qRT-PCR.
CCHF Human lgM indirect ELISA: This assay was standardized using rNP and was tested with CCHF negative samples at DRDE, Gwalior. Due to non-availability of CCHF positive human sample at DRDE, the system could not be evaluated earlier. Out of the 26 human samples, 04 samples tested positive while

22 samples were found negative for CCHF IgM antibodies. The comparison of results with NIV MAC ELISA revealed 96% concordance.

CCHF Human lgG indirect ELISA: Out of the 26 human samples, 5 samples were found positive for IgG and 21 were found negative at a dilutions of 1:100 and 1:10,000 of primary (Patient sera) and secondary antibody (Anti-human IgG HRP conjugate) respectively. Comparative evaluation with NIV IgG ELISA revealed 100% concordance.

CCHF Antigen ELISA: The comparative result revealed 100% concordance with Vector-Best commercial kit. Further evaluation with sample may be carried out depending on availability of antigen positive sample in future

Immuno-reactivity of DRDE produced recombinant NS1 protein of KFD virus.

Reactivity at 45KDa with patient sera was observed that confirms the immunoreactivity of rNS1 protein. The rNS1 protein needs to be further used for development of immunological assay.

Potential species distribution and detection of Kyasanur Forest disease virus in tick population in the forest area of Kerala

Author: Yadav PD, Bala S, Jain R, Patil S, Majumdar T, Lakra R

Funding agency: Intramural **Duration: 2018-2020 [ongoing]**

Objectives:

Findings: Ticks (804 pools comprising 32783 ticks belonging to five species) collected from four forest ranges of Wayanad and two forest ranges of Malappuram districts of Kerala state were screened for KFDV using KFD specific real-time RT-PCR. KFD viral RNA was detected in 16 pools of *Hemophysalis spinigera*, 7 pools of *H. turturis* and one pool of *Ambliomma integrum*. The study provides baseline information to build public awareness about the potential exposure to ticks and tick-borne diseases like KFD.

India-Bangladesh collaborative study on Nipah virus

Yadav PD, Shete AM, Sahay R, Jain R, Majumdar T

Funding agency: Intramural Duration: 2018-2020 [ongoing]

***Background*:** An MoU for research collaboration has been signed between Institute of Epidemiology, Disease Control and Research (IEDCR) and ICMR-NIV Pune in November 2019. Consultation meeting for the implementation of Nipah clinical trial was attended by ICMR team in Dhaka, Bangladesh from 24th to 26th September 2019

Onsite validation of Point of care [POC] real time RT-PCR system and Nipah human IgM and IgG ELISA for the detection of Nipah virus in the field settings

Under the MoU between Molbio Diagnostics and ICMR-NIV, Pune, a POC system for the detection of NiV has been validated. It is a battery operated, handy system/equipment that can be used efficiently in field settings. The testing and clinical protocol was also approved by Drug Controller General of India (DCGI) in August 2019. This is the first make in India product which was approved by DCGI and first of its kind of approved PoC for high risk pathogen testing. External onsite validation of Nipah PoC and indigenously developed anti-Nipah human IgM and IgG ELISA assays was conducted at the IEDCR, Dhaka, Bangladesh by trained scientific and technical staff of BSL-4 facility during 3-7 November 2019.

***Findings*:** Anti-Nipah human IgM antibody positive and negative [N=10 each] and anti -Nipah human IgG antibody positive and negative serum samples [N=10 each] and Nipah viral RNA positive [N=10]

and negative throat swab samples [N=10] were provided to IEDCR. RNA was extracted from these stored samples and was used for performing both Real Time RT-PCR (TaqMan) and Point of Care test simultaneously. All positive and negative samples were checked by Nipah Real time RT-PCR. Nine of the ten samples were positive by Nipah Real Time RT-PCR (TaqMan)**.** Results of the both assays anti-Nipah Human IgG and IgM ELISA were concordant with the results of IEDCR.

NABL accreditation ISO/IEC 17025:2005

Yadav PD, Shete AM, Sahay RR,Jain R, Patil S, Majumdar T, Gondane Y, Melag S, Chopade Y, Kore P

Funding agency: Intramural **Duration: 2016-Till date**

Background: ICMR-NIV, Pune is the Apex laboratory for testing and providing diagnosis of high pathogenic viruses for the country. BSL-4 facility routinely provides diagnosis for VHF causing pathogens, *viz.*, KFDV, CCHFV etc. NABL accreditation of the facilities including different diagnostic testing is the highest standard in the country.

Objective: to provide the quality and technical competence of diagnostic testing as per international standards in compliance with ISO/IEC 17025:2017.

Findings**:** Desktop surveillance audit for continuation of NABL accreditation was conducted on 16th September 2019. Documents for closure of non conformity records, training and evaluation records, QC rechecks etc., were submitted to the quality cell for submission to NABL office. Internal audits were conducted as per schedule. Various trainings were conducted for NABL awareness test and biosafety aspects. After successful completion of the four years of accreditation a desktop surveillance audit was organized by NABL.NCs raised during the re-assessment for their corrective actions and documentary evidence of continued compliance was provided for the year 2019.

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

Yadav PD, Shete AM, Sahay RR, Mohandas S

Contributing Staff**:** Jain R, Patil S, Maujumdar T, Sarkale P, Lakra R, Melag S, Chopade Y, Kadam M

Funding agency: Intramural **Duration:** Service Project

Background**:** BSL-4 facility is the only facility in India to provide diagnosis of VHF causing high risk viral pathogens like KFD, CCHF, Ebola, Marburg, Rift valley fever, Nipah, etc.

Objectives (i): to provide diagnostic support referred samples of suspected NiV cases.

Work done:

(i) Clinical samples (CSF and serum) of 118 Acute Encephalitis Syndrome cases reported in April and May 2019 were referred to ICMR-NIV, Kerala. These samples were also tested for NiV at BSL-4 laboratory. All the samples were found negative for Nipah virus either by qRT-PCR and/or anti-Nipah IgM and IgG ELISA.

(ii) Seroprevalence of NiV specific antibodies among close contacts Kerala, 2019

Serum samples from close contacts as well as hospital staff who attended the NiV patient during the NiV outbreak in Ernakulam in 2019 were screened for anti-NiV IgM antibodies. None of the samples tested positive for NiV antibodies. The absence of respiratory symptoms in the index case and the proper training and use of PPEs by the hospital staff could be the probable reasons for absence of antibodies.

(iii) Efforts for Nipah Clinical trial using monoclonal (m102.4)

The m102.4 monoclonal antibodies to NiV, developed by Australian scientists is the only approved therapeutic available against NiV infection. NIH/NIAID, USA in collaboration with ICMR and WHO HQ & SEARO, developed a randomized clinical trial protocol for assessing safety and

efficacy of the use of m102.4 monoclonal antibodies in NiV infected patients. Since NiV outbreaks involve small populations, an adaptive clinical trial protocol has been developed, which could be shifted immediately to other countries wherever the outbreak occurs. Inter-country studies would be important to meet the desired sample size for the proposed clinical trial. Before implementation of the trial, it is critical to augment the field implementation capacity as well as establish GLP compliant laboratories for conduct of Nipah clinical trial in future. Many SOPs including site selection and implementation, informed/accent process, bio-safety and containment, specimen collection-custody-transport and storage, study design for drug administration, temperature maintenance and laboratory testing protocols for NiV RCT were developed by ICMR-NIV and ICMR-NARI, Pune teams. ICMR-NIE, Chennai has developed the SOPs for data collection and data management. A regional scientific consultative workshop on NiV was hosted by the Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh from 24th to 26th September 2019 for understanding Nipah surveillance systems, clinical management, review of potential therapeutics and discussion on clinical trial with different stake holders.

6. **Crimean Congo hemorrhagic fever virus during an outbreak from Gujarat state, India during 2019 (referred sample)**

During 2019-2020, samples of suspected viral hemorrhagic fever cases with clinical manifestations *viz*., fever, rash, diarrhea, vomiting, bleeding manifestations etc., were referred from different medical colleges and hospitals of Gujarat and Rajasthan states. Thirty-four human samples from 9 districts along with livestock serum samples and tick pools from Animal Husbandry Department, Ambawadi, Ahmedabad) were tested for CCHF diagnosis. Though anti-CCHFV IgG antibody positivity was detected in a few livestock samples (22.03%), none of the tick pools tested positive for CCHFV by qRT-PCR.

Rajasthan reported six confirmed cases of CCHF during the reporting year. Clinical samples of 259 close contacts (family members/ HCWs/Paramedical staff/ ambulance drivers/BMW management workers) were tested but none tested positive for the virus. However, screening of 41 livestock samples has shown anti-CCHFV IgG antibodies by ELISA. One tick pool was also found positive for CCHFV RNA by qRT-PCR. Livestock and tick pool percent positivity from three districts of Rajasthan have shown 42.85% and 12.38% respectively.

7. **Diagnostic support for referred specimens for Kyasanur Forest disease (KFD) from Karnataka, Kerala and Maharashtra states**

During the year, 2993 clinical samples suspected of KFD were referred from Karnataka, Kerala, and Maharashtra state. Screening of the samples yielded, KFDV RNA in 91 samples while 333 showed IgM antibody positivity. Maximum positivity was observed in Maharashtra as 68 of 926 clinical samples referred from Dodamarg taluka; PHC of Sindhudurga, tested positive for viral RNA while 327 samples showed IgM antibody. Screening of 1568 tick pools and 114 monkey necropsy specimens referred from Karnataka yielded KFDV RNA positivity in 20 tick pools and three monkey specimens respectively. Clinical samples (n=4) and tick pools (n=54) referred from Waynad and Alappuzha districts of Kerala were found negative for KFD viral RNA.

HEPATITIS GROUP

Scientific staff

Dr. (Mrs.) Kavita S. Lole	Scientist F
Dr. (Mrs.) Anuradha S. Tripathy	Scientist F
Dr. Shilpa Tomar	Scientist B

Technical Staff

Mrs. Supriya L Hundekar	Sr. Technical Officer 2
Dr. Ashwini Y. Ramdasi	Sr. Technical Officer 1
Mr. Satish S. Ranawade	Sr. Technical Officer 1
Ms. Neeta C. Thorat	Sr. Technical Officer 1
Mr. Vasant Walkoli	Sr. Technician 3
Mr. Prakash B. Jawalkar	Sr. Technician 3
Mr. Shirish V. Vaidya	Sr. Technician 2
Mr. Prasad Babar	Sr. Technician 1
Mr. Pranit Ayachit	Sr. Technician 1
Mr. K. D. Ramaiah	Laboratory Assistant 1
Mr. P. D. Sarje	Laboratory Assistant 1

Ph.D. students

Ms. Diptee Trimbake	CSIR-JRF
Ms. Neha Bhise	UGC-SRF

Women Scientists Scheme

Dr. Manjita Srivastava	WOS-A

Project Staff

Dr. Namdeo Togre	RA (Till Jan 2019)
Ms. Kadambari Akolkar	SRF
Mr Siddhesh Vishwarkarma	JRF
Mr. Gajendra Shahpure	JRF
Ms. Nidhi Thakur	JRF
Ms. Varsha Mahadik	JRF
Ms. Ciyona Bastin	TA

- Computational drug repurposing studies to develop therapeutics for HEV identified 128 differentially expressed genes in HEV infection of Huh cells in-vitro in phase 1 of the study.
- Cellular autophagy was shown to play a role in virus-host interaction in HEV infection in-vitro.
- Host genetic studies identified association of NKG2A gene expression with HEV infection .
- HBeAg plays an important role in managing cellular stress during HBV infection
- Recombinant human TNF-α plays important anti-viral role against CHPV infection and could be put forward as a potential candidate for antiviral intervention against CHPV.

Hep1902: Computational drug repurposing approach for the development of therapeutics against HEV

Investigators: PI: Kavita Lole, Co-PI: Sarah Cherian, Co-Investigator: Dr. Prathama Mainkar, IICT, Hyderabad

Funding: ICMR (Extramural) **Duration**: 2019-2022

Background:

Hepatitis E virus (HEV) infection in most patients follows a self-limited course; however, occasionally it can cause acute or chronic liver failure in patients, such as those suffering from other infections/ liver injuries or organ transplant/chemotherapy recipients. Despite widespread sporadic and epidemic incidents, there is no specific treatment against HEV, justifying the need for developing a potent antiviral against it. The off-label use of ribavirin and PEG-IFN have limitations due to side effects. Drug repurposing holds the promise of rapid clinical impact at a lower cost than *de novo* drug development. The *in-silico* drug repurposing technology involves different approaches among which the systems biology approach deals with the complexity of both the pathogen and the drug host response in the form of expression patterns or molecular interaction networks whereas the structural bioinformatics approach is majorly useful for the identification of directly acting antivirals which target the viral proteins. Both computational approaches are being considered in this study for short listing potential drugs against HEV.

Objectives:

1. To generate transcriptomes data and identify signature gene profiles of genotype 1 HEV infection
2. Systems biology and structural bioinformatics approaches to shortlist drugs for repurposing
3. Evaluation of the antiviral activity of the selected drugs against HEV

Findings:

In silico studies

Systems Biology approach:

Following publically available transcriptomic datasets based on gene expression data under HEV infected conditions were considered- i) 128 differentially expressed genes (DEGs) from HEV infected Huh-7 (human hepatoma) cells, ii) 54 DEGs from infected chimpanzee liver cells, iii) 146 DEGs from the third trimester pregnant patient dataset from our lab. The connectivity scores were established for the compounds using the in-silico approach based on Connectivity Map (CMap) and drug repurposing predictions were undertaken. The FDA approved as well as investigational drugs targeting these genes/ pathways were shortlisted. These analyses identified multiple groups of drugs that included protease inhibitors, insulin receptor inhibitors, PARP inhibitors, cannabinoid receptor agonists and PPAR agonists, few compounds in the category of directly acting antivirals.

The significant host proteins differentially expressed in the proteomics and interactomics studies with HEV infection were queried using STITCH to obtain the network of interacting proteins and drugs. Seventy three drugs were found to interact with specific host proteins in the interactomics and proteomic data.

Some of the prominent proteins generated the highest number of drug hits included retinol binding proteins (RBP), eukaryotic translation factors (eEF1A1), mitogen activated protein kinases (MAPK) and Hypoxanthine guanine phosphoribosyl transferase (HPRT).

Structural Bioinformatics approach:

The docking interaction analysis of the protease inhibitor group of compounds with the available X-ray crystal structure of HEV protease revealed that, danoprevir and saquinavir have better binding affinities forming crucial residue interactions in the metal binding catalytic site (figure 1).

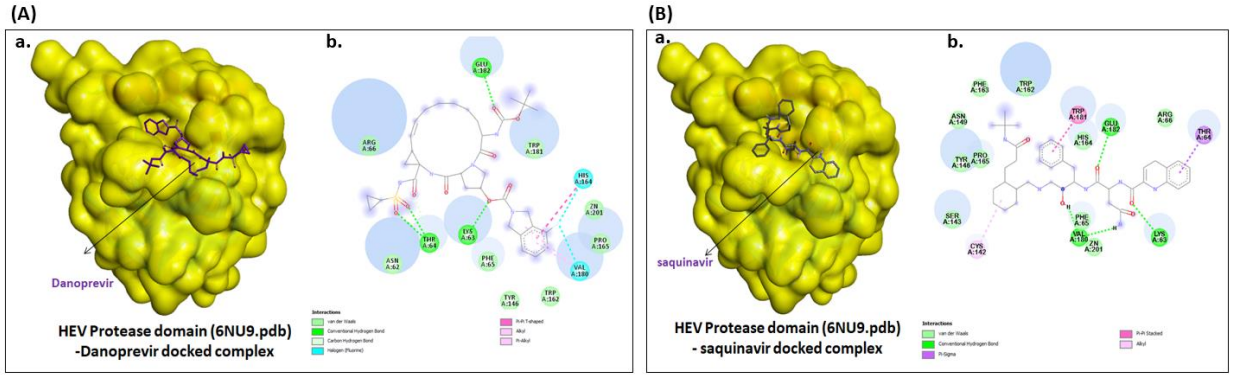


Figure 1: Docking interaction analysis with HEV protease domain, A) danoprevir (a. surface overview of docked complex, b. 2D interaction analysis; B): saquinavir (a. surface overview of docked complex, b. 2D interaction analysis

Molecular modelling of HEV RdRp (RNA dependent RNA Polymerase)

Among the several HEV target proteins, the RdRp enzyme is most crucial for the elongation and replication of viral genetic material. As the crystal structure of this protein is still not available, we modelled the HEV RdRp structure using the online structure prediction server, I-tasser. The predicted model of HEV RdRp protein will further be considered for in-silico docking and virtual screening studies.

Wet lab studies

Evaluation of the antiviral activity of the selected drugs against HEV

Analysis of cell toxicity of drugs

The shortlisted drugs were processed for determination of cytotoxic concentrations (CC_{50}) using MTT assay. For that, the Huh-7 (human hepatoma) cell line derived clonal cells, S10-3, which support HEV replication were used. We plan to use these cells for testing antiviral activity of the drugs against HEV.

For finding half maximal effective concentration (EC50), a range of drug concentrations between CC50 and conc that shows 100 % cell viability are being tested using HEV subgenomic replicon by measuring Renilla luciferase activity.

HEP1804: Hepatitis E virus replication and cellular autophagy

Investigators: Kavita Lole, Manjita Srivastava

Funding: DST WOSA-A project **Duration: 2018-2021**

Background

Autophagy plays a key role in maintaining cellular homeostasis by eliminating unwanted proteins and damaged organelles. Viruses modulate autophagy to escape the host immune response or to exploit the host's defense to their advantage. Many positive-strand RNA viruses rearrange host intracellular membrane compartments that house replication complexes and these rearrangements are prone to induce membrane stress. Autophagy plays an important role in protein homeostasis in normal liver functions. HEV is known to induce endoplasmic reticulum (ER) stress via ORF2 (capsid) protein indicating possible modulation of autophagy by the virus. ER stress, which is probably initiated as an antiviral response by the host cell has been shown to enhance HEV-1 replication. This suggests that disease conditions that induce hepatic ER stress may lead to worst outcomes of HEV-1 infection. We intent to study whether autophagy plays proviral or antiviral role during HEV infection, and if HEV modulates autophagy, then understand the mechanism.

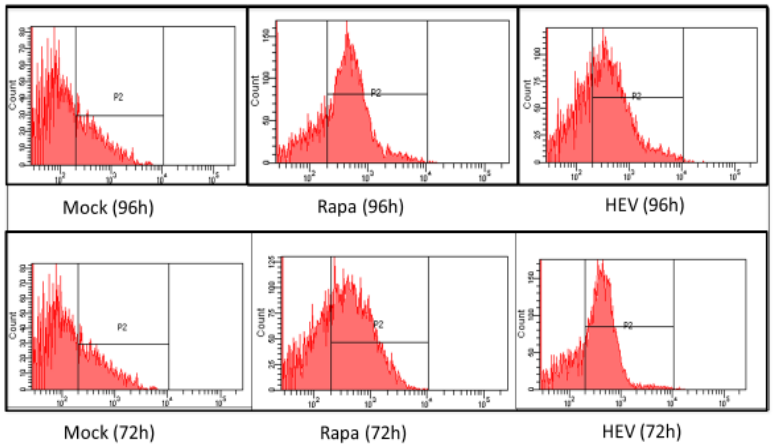
Objectives:

1) To evaluate role of autophagy in HEV replication
2) To know whether HEV replication requires autophagy machinery
3) To understand the mechanism of modulation of autophagy by HEV

Findings:

To determine whether autophagy can be induced upon HEV infection, RNA derived from subgenomic HEV-1 clone, pSK-HEV-Rluc (encoding reporter gene Renilla luciferase), was used for transfection of S10-3 hepatoma cells. Rapamycin (rapa), an autophagy inducer was used as a positive control. Cells were harvested after different time intervals and analyzed for the Rluc activity (which indirectly measured HEV RNA replication). At 96 h post tranfection, ~2-fold increased luciferase activity was seen in cells treated with rapa and transfected with HEV-Rluc RNA as compared to rapa untreated cells ($p<0.05$), indicating autophagy being favourable condition for HEV replication. To reconfirm the HEV induced autophagic flux, we used the acidotropic fluorescent drug monodansylcadaverine (MDC), a specific autophagolysosome marker. HEV infected cells showed significant increase in the formation of autophagolysosome, comparable to the cellular autophagy inducer drug, rapamycin (figure 2).

A



B

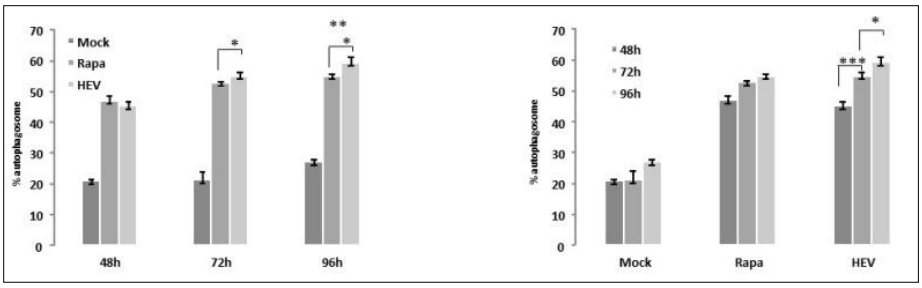


Figure 2: Flow cytometry analysis of the autophagosome formation post HEV infection, A) Flow cytometry analysis of MDC treated cells, B) Graphical representation of the data

During the initial stages of autophagy, LC3I cytosolic autophagy protein is lipidated to form LC3II, which in turn gets associated with membranes to form autophagosomes. Upon transfection of HEV infected cells with GFP tagged LC3, induction of autophagy via formation of autophagosomes and autophagolysosomes was observed. This indicated that HEV induces autophagy in infected cells and uses it favourably for replication. The siRNA-mediated knockdown of key autophagy proteins Beclin-1 and ATG-7 resulted in inhibition of autophagy as well as significant reduction in HEV replication. With these

observations it was concluded that HEV can induce autophagy in cells upon infection and is dependent on it for efficient replication.

Hep1902: Study of involvement of Host/Virus factors in Hepatitis E virus infection during pregnancy

Investigators: PI: Anuradha S Tripathy, Co PIs: Prof (Dr.) Pradip Sambarey**,** Dr. Jayanthi S. Shastri

Funding: ICMR (Extramural) **Duration**: 2019-2022

Background

More than 50% of the reported adult acute viral hepatitis cases from India are attributed to HEV. Hepatitis E is mostly a self-limiting disease, may progress to fulminant hepatic failure (FHF-E) with 20-25% mortality rate in the third trimester of pregnancy. No vaccine against Hepatitis E is available in the Indian market & ribavirin, a possible therapeutic agent for FHF-E has a long way to go. HEV pathogenesis is immune-mediated; however, has remained a puzzle in HEV infected pregnant women with differential outcomes. With this background, we plan our study to initiate in patients with self-limiting form of disease; generate data that will form basis for a further comprehensive comparison with the fulminant form of the disease to characterize both the (a) host immune response (b) infecting virus. The proposed study may generate lead towards the identification of bio markers of severity/recovery & immune targets towards treatment.

Objectives:

Primary objective: To identify the bio markers of severity and recovery in pregnant women with HEV infection. To study this, both the host immune response and the infecting virus in HEV infected pregnant women will be characterized.

Secondary objectives:

1. To evaluate phenotype and functions of NK and NKT cells.
2. To assess HLA class I & II alleles/haplotypes and polymorphisms of cytokine genes distribution
3. To assess the role of T and B regulatory cells
4. To assess the involvement of Notch signalling pathway & epigenetic role of Foxp3 locus in Hepatitis E
5. NGS based full genome sequencing & assessment of viral quasispecies among patients with differential outcomes

Findings

This study attempts to identify the process and also the key molecules regulating the immune response towards recovery in pregnant women with HEV infection. We initiated the study with non pregnant women and males with HEV infection as study subjects. A total of 211 healthy controls, 111 hepatitis E patients were genotyped for the presence of IL-1RN VNTR polymorphic alleles by PCR-RFLP method. The genotypes 1/1 and 1/2 as well as allele 1 were found to be susceptible to HEV infection while genotype 2/2 and allele 2 were identified as protective in the studied population. Similarly, genotype and allelic frequency distributions for NKG2A rs2734440 was carried out in 210 healthy controls and 105 hepatitis E patients, while NKG2D rs7980470 was carried out in 222 healthy controls and 111 hepatitis E patients. Allele A frequency in NKG2A gene was significantly higher in HEV patients compared to control group indicating its role in susceptibility towards HEV infection while frequency of allele G was found to be significantly lower in patient group suggesting a protective role. In case of NKG2D, no significant association was observed at genotype as well as allelic level.

The mechanism underlying the associations between NKG2A, IL-1RN and HEV infection is not understood. Further evaluation of the translational mechanism with reference to these polymorphisms may provide insight into their contribution to HEV pathogenesis.

HEP1602: Cellular antiviral responses against hepatitis B virus

Investigators: Kavita Lole & Bhukya Prudhvi Lal
Funding: Intramural Duration: 2016-2019

Background:

During chronic hepatitis B virus (HBV) infection, mutations in the precore (PC) or basal core promoter (BCP) region affecting HBV e antigen (HBeAg) expression are known to emerge spontaneously. The PC mutation (G1896A) creates a translational stop codon resulting in absent HBeAg expression, whereas BCP mutations (A1762T/G1764A) reduce HBeAg expression by transcriptional regulation mechanisms. These mutants represent the predominant virus species in HBeAg-negative chronic hepatitis B patients. However, primary infections with BCP and PC mutant virus are associated with severe liver injury. In the present study, we aimed to characterize host cell responses generated upon infection with wild type and BCP/PC mutant viruses isolated from a resolved case of acute hepatitis and a liver failure case respectively during an HBV outbreak that occurred in the Modasa Tehsil, Sabarkantha district of Gujarat state in India, in 2009.

Objectives:

- Analysis of cellular antiviral pathways induced in response to infection with the wild type and mutant viruses.
- To identify genomic region of the mutant virus responsible for eliciting altered antiviral response in human hepatoma cells

Findings:

We analyzed host cell responses in HepG2/C3A, hepatoma cells transfected with infectious clones developed from genotype D wild type (WT) and BCP/PC mutant (MT) viruses. Cells transfected with MT virus construct showed ~60% apoptosis and with WT construct ~30% apoptosis at 72 hr. Comparative gene expression analysis showed that the mutant virus triggers apoptosis at early phase upon infection. Both WT and MT viruses induce ER and mitochondrial stress, however, presence of HBeAg in WT virus possibly helps cells to recover by inducing metabolic and synthetic pathways.

Several studies have linked HBx with activation of apoptotic pathways in cells. Since MT clone was unable to synthesize HBe protein, we hypothesized that during HBV infection, HBx induces apotosis and anti-apoptotic activity of HBe counterbalances it. To assess this, we measured apoptosis in cells expressing HBe and HBx proteins alone and in cells co-transfected with WT+HBe/HBx and MT+HBe/HBx constructs. Cells remained healthy and no significant difference was observed in cells transfected with HBe plasmid or empty vector (pcDNA3.1). However, HBx induced apoptosis in ~25% cells (figure 3). Cells co-transfected with WT+HBx and MT+HBx showed ~17% and ~54% apoptotic cells respectively. There was a marginal improvement in cell viability with HBx co-expression (statistically insignificant). Interestingly, cells co-transfected with WT+HBe or MT+HBe showed a significant improvement in cell viability, ~7.5% and ~23% apoptosis respectively when compared to WT or MT (figure 3). Co-expression of HBe protein in cells transfected with either WT or MT rescued cells from progressing into apoptosis. HBx is known to induce apoptosis via P38MAPK and JNK pathways and treatment of cells transfected with WT and MT constructs with P38MAPK and JNK inhibitors, SB 202190 and SP600125 respectively, rescued both cells from apoptosis. These findings confirmed involvement of HBx protein in inducing apoptosis in both WT and MT virus infected cells. It also confirmed important role of HBeAg in maintaining cellular homeostasis during HBV infection.

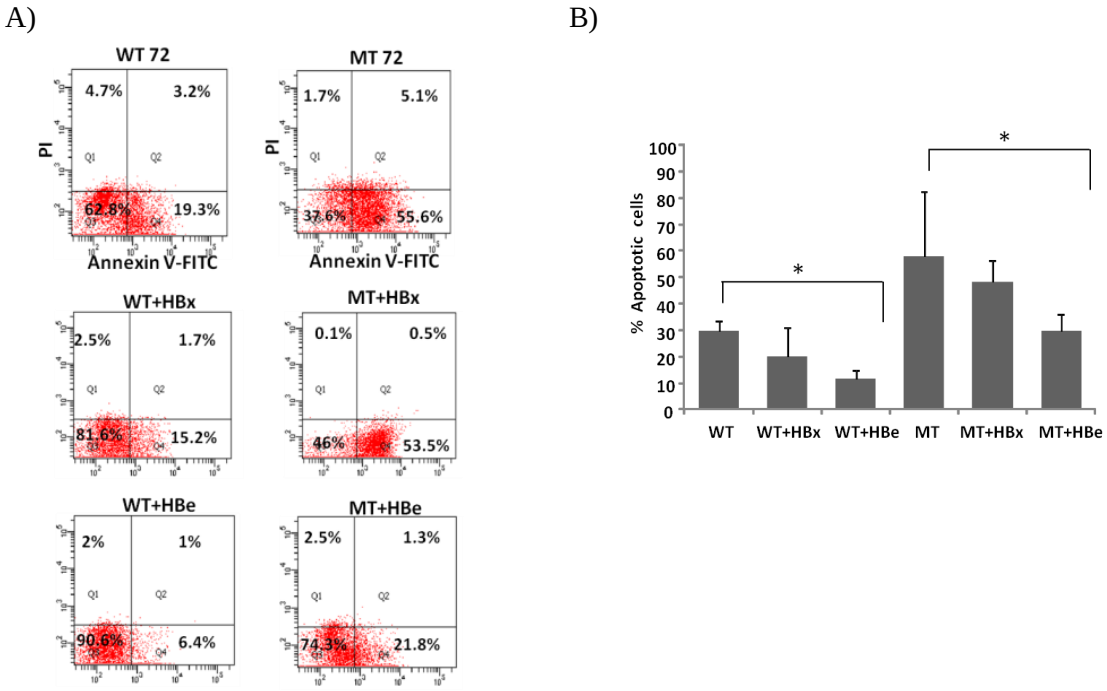


Figure 3: Apoptosis in HepG2/C3A cells co-transfected with 1.3 mer WT/ MT HBV clones and HBx/HBe clones, A) Flow cytometry analysis of transfected cells, B) Graphical representation of the data

Hep1305: Identification, characterization and validation of neutralizing monoclonal antibodies (MAbs) against Hepatitis E Virus

Investigators: Anuradha Tripathy, Namdev Togre
Funding: DST SERB (grant no: EMR/2016/004977) **Duration:** 2017-2020

Background: Till date, there is only one vaccine against HEV that is licensed in China only. An ongoing study at NIV has led to the development of a liposome encapsulated protein based HEV vaccine candidate that protected monkeys against HEV infection. However, it is at the pre clinical stage. In a nutshell, in the Indian market, no vaccine or specific therapy against Hepatitis E is available. Hence, viable approaches towards identification/development of therapeutics also need to be investigated. With this background, the current study aims at the identification, characterization of neutralizing monoclonal antibodies against HEV that may have immunotherapeutic utility for severe fulminant hepatic failure patients, pregnant women and chronic liver disease patients with HEV infection

Objectives

1. Construction of antibody phage library from the lymphocytes of HEV recovered individuals
2. Isolation of monoclonal antibodies from the cDNA library

Findings

Two HEV recovered individuals with high anti-HEV antibody titers were identified as the source for PBMCs. Taking PBMC s as the starting material, the cDNA was synthesized from mRNA isolated from the recovered individuals. The light chain and sequence for control antibody form both recovered individuals (R1 & R2) were amplified successfully by PCR usingVK3a / CK1Z and CONK / CK1a family specific primers and cDNA as a template. The heavy chain from R1 was amplified by PCR using

combination of each family specific and constant gene specific primers (VH6a/CGZ1). The purified PCR products were cloned into pCR™2.1-TOPO vector.. The cloned plasmid pCR™2.1-TOPO-*VH6a/CGZ1*vector was double digested with *Eco*RI restriction enzyme which resulted in the release of the *VH6a/CGZ1*insert (850 bp) and linearization of pCR™2.1-TOPO plasmid Pre and post-transformation sequence analysis of both R1 & R2, heavy chain genes revealed 99% sequence similarity with human heavy chain genes. In a similar manner, control antibodies from both R1 & R2 were amplified and the PCR product of the two control antibody sequences was found at750bp. The amplified PCR product was then purified and cloned into pCR™2.1-TOPO vector. The cloned plasmids (pCR™2.1-TOPO-CONGa/CGZ1-R1 and pCR™2.1-TOPO-CONGa/CGZ1-R2) for two control antibodies for both R1 & R2 were double digested with EcoRI restriction enzymes, which resulted in the release of CONGa/CGZ1 inserts(750 bp) and linearization of pCR™2.1-TOPO plasmid. The amplified gene products are now ready to be cloned into phage display vectors and further transformed to generate antibody phage library

Hep19-01: Assessment of risk factors for the development of Cardiovascular disease (CVD) in patients with active HCV infection

Investigators: Shilpa J. Tomar, Kavita S. Lole
Funding agency: ICMR (Extramural) Project duration: Three years

Background: HCV infection has been associated with increased risk of atherosclerosis, peripheral artery disease, myocardial injury, cerebro- and cardiovascular events and increased cardiovascular mortality. Till date, most studies on cardiovascular risk and HCV were conducted on patients treated with IFN based regimens, which makes it impossible to differentiate whether the effects observed, are due to virus clearance or an effect of the IFNs themselves.

Objectives

Primary:

a) Assessment of various parameters as risk factors for CVD in patients with active HCV infection
b) To assess the association of metabolic syndrome and CVD risk

Secondary:

a) Association of different HCV genotypes to CVD risk
b) Association of Hepatitis C viral load with CVD risk

Work done: The patient enrollment for the project was initiated on 1st March 2020 at NEIGRIHMS, Shillong and a total of 7 treatment-naïve patients with active-HCV infection have been enrolled in the study till date. Patient enrollment was affected heavily due to the ongoing COVID-19 pandemic as borders were sealed and travel restricted. Out of the 7 patients the HCV-RNA viral load was > 1 lac in 5 patients and <1 lac in the remaining two. The mean carotid-intima media thickness in the right artery was 0.47 mm (95% CI 0.31- 0.63) and in the left was 0.48 mm (95% CI 0.30- 0.67). The liver enzymes (AST, ALT) were both raised in 4 patients, only AST raised in 1 and normal in 2 patients. The mean AST level in the patients was 72.6 (U/L) (95% CI 35.4- 109.7) and ALT was 90.6 (95% CI 45.7- 135.4). The USG abdomen was normal in 4 patients but showed hepatosplenomegaly (fatty liver grade II) and mild diffuse fatty liver with cholelithiasis in two. The non-invasive liver indexes APRI and FIB-4 were calculated for these patients.

Findings: Out of the 7 patients, except one, the APRI index of all others reflected some degree of liver fibrosis. Five patients had significant fibrosis according to the APRI index i.e >0.5 and one had cirrhosis with APRI > 1.5. The mean APRI index was 1.09 (95% CI -0.06 - 2.26). The FIB-4 score calculated was normal in 4, showed mild fibrosis in 2 and severe in one patient. The mean FIB-4 score was 1.34 with a

range of 0.41 - 2.27 (95% CI). Additionally, 3 of the 7 patients had abdominal obesity as measured during anthropometry.

Hep1304. Studies on the roles of host factors in Chandipura virus (CHPV) infection

Investigators: Anuradha Tripathy, Ms. Pooja Gupta

Funding: Intramural **Duration**: 2016-2019

Background:

Infection with Chandipura virus (CHPV), is a public health concern in India due to its association with high mortality in children. Although it was discovered in !965 from dengue and chikungunya suspected patients, it raised to prominence when it was isolated from patients of 2003 encephalitic outbreak, affecting children below age 15 years with 55-77% fatality rate. Like other typical viral encephalitis, CHPV encephalitic cases have rapid acute onset of fever with majority of cases following fatality within 48-72 hr of appearance of symptoms. No CHPV specific treatment/vaccine is commercially available. Identifying the defense mechanisms of host leading towards recovery from Chandipura virus infection is the necessity for designing therapy/vaccine. This study had two components, (1) to examine the role of complement system in the in vitro neutralization of CHPV (2) to assess the anti CHPV action of TNF-α in the *in vitro* system.

Findings:

The present study examined the role of complement system in the in vitro neutralization of CHPV in Vero E6 cells. In vitro neutralization, real time PCR for assessing viral copies and flow cytometry based tissue culture limiting dose assay (TC-LDA) were carried out with normal human serum (NHS), heat inactivated serum (HIS), human serum deficient of complement factor, and deficient serum reconstituted with respective human complement protein to assess the effect of complement system on CHPV infectivity. Nine out of ten NHS showed complement mediated neutralization, reduced viral copies as well as reduced percentage CHPV positive cells compared to their respective HIS counterparts. Pre treatment of NHS with EGTA or EDTA specified that CHPV neutralization takes place through the alternative pathway of complement activation. There is strong reliance on C3 for CHPV in vitro neutralization. Disparity in CHPV neutralization levels between factor B deficient and reconstituted sera could be attributed to amplification loop/"tick-over" mechanism. Assays using C3, C5, C8 and C9 deficient/reconstituted human sera signified that complement dependent CHPV neutralization & repression of CHPV infectivity occur primarily through C3 & C5, and do not dependent on downstream complement factors C8 and C9. In absence of any specific anti viral treatment or vaccine against Chandipura, the current data, elucidating role of human complement system in the in vitro neutralization of CHPV, may help in designing effective therapeutics.

Towards the assessment of antiviral action of TNF α against CHPV, the current study suggested that recombinant human TNF-α inhibits CHPV titer and copy number in the CHPV infected cells by an apoptosis independent mechanism. Treatment of Rhabdosarcoma cells with 100ng/ml of recombinant human TNF-α prior to CHPV infection resulted in decreased CHPV copies and cytopathic effect. Mechanism of anti CHPV action of TNF-α was further studied by analyzing caspase 8 and 3 expressions and by apoptotic cell detection, which confirmed the antiviral activity of TNF-α and that it is through an apoptotic independent manner. This in vitro anti CHPV activity of TNF-α suggested its role in host defense against CHPV infection.

Taken together, our data suggest that recombinant human TNF-α plays important anti viral role against CHPV infection and could be put forward as a potential candidate for antiviral intervention against CHPV. Our results of in vitro complement studies indicate a protective involvement of alternative pathway of complement activation towards *in vitro* CHPV neutralization, primarily through C3 &C5.

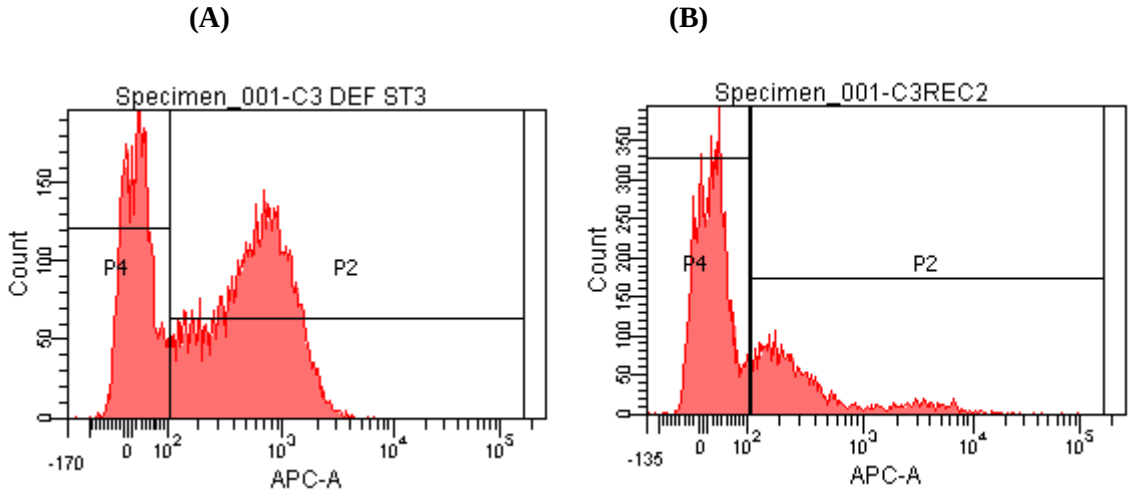


Figure 4: Vero E-6 cells were treated with (A) "C3 deficient serum + CHPV" and (B) "C3 reconstituted serum +CHPV"/ "NHS +CHPV" (100 TCID 50). Twenty four hr post infection, cells were fixed, permeabilized, and stained with mouse polyclonal purified anti CHPV antibody followed by addition of anti mouse Alexa Fluor 680. C3 reconstituted serum showed significant decrease in CHPV positive cells percentage compared to the other group. Values are depicted as mean±SD of CHPV positive cell percent of parent population. Statistical significance was determined by students's unpaired t test.

ENCEPHALITIS GROUP

Scientific staff

Dr. Vijay P. Bondre	Scientist F & Group Leader

(vpbondre@gmail.com, bondre.vp@niv.co.in)

Technical staff

Mrs. S. A. Mahamuni	Sr. Technical Officer - I
Mrs. V. Sankararaman	Sr. Technical Officer - I
Dr. Mrs. D. V. Pavitrakar	Technical Officer
Mr. V. K. Jadhav	Senior Technician - I
Mr. D. K. Butte	Senior Technician - I

Research students

Ms. D. N. Mali	Senior Research Fellow

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

Investigators: *Bondre VP, Mahamuni SA, Jadhav V, Sankararaman V, Pavitrakar D, Butte D.*
***Funding Agency*:** Intramural (Service project) Duration: Ongoing

1. Diagnosis of referred human clinical specimens:

Background:

Encephalitis group routinely provides diagnosis to referred clinical specimens collected from suspected acute encephalitis syndrome (AES) cases in addition to its primary role of investigation of encephalitis outbreaks in the country. The group also support the state health authorities in the diagnosis of other encephalitic viruses, *viz*., Chandipura, dengue, other Flaviviruses, human Herpes viruses (1, 2, 6, etc), Cytomegalovirus, Epstein Bar virus, etc., in addition to Japanese encephalitis virus (JEV). The group has also enhanced its diagnostic capacity to detect Measles, Mumps, Rubella, *Vericella Zoster*, *Orientia tsutsugamushi* and Spotted Fever Group Rickettsia by serological or molecular diagnosis assays.

***Work done and Findings*:**

During 2019-20, 735 suspected encephalitis cases were referred for investigation of JE / non-JE AES etiologies from Maharashtra, Gujarat, Andhra Pradesh, Madhya Pradesh, Tamil Nadu, Karnataka, Kerala, Rajasthan, Telangana, West Bengal, Delhi and Puducherry. All the specimens were investigated first for anti-JEV IgM and detected positivity only in 09 samples; seven received from Maharashtra and one each from Gujarat and Puducherry, predominantly pediatric cases (08/09). Samples received from pediatric age group from Chandipura endemic states (parts of Maharashtra, Gujarat and Telangana) were investigated for CHPV infection by RT-PCR and CHPV-IgM ELISA. Screening of 819 samples showed 2.19% (n=18) positivity for CHPV; 17 samples from Gujarat and one from Maharashtra. Based on the clinical symptoms 364 samples were investigated for HSV-1 and 2, VZV, EBV and CMV with specific PCR which yielded 02 pediatric cases positive for HSV-1; one positive for HSV-2 (adult); one for Vericella Zoster virus (CSF) and one for human Enterovirus (CSF). Investigations for other viruses yielded IgM positivity for dengue (14%), chikungunya (5.3%) and 11% as intermediate. Samples with intermediate results were tested for West Nile (PanBio) and other neurotropic viruses (PanBio), but all found negative. Samples were also screened for *Orentia tsutsugamushi* and Spotted Fever Group Rickettsia using specific IgM ELISA and PCR, but yielded negative results. The findings were communicated to the respective health authorities as per the ICMR guidelines.

2. Virus isolation from clinical specimens

Background:

Isolation of the etiological agent is considered as gold standard of diagnostics as isolates from different geographical areas of the country adds to the understanding on evolutionary dynamics. Specimens collected within 5 days post onset of symptoms and transported in cold chain yield best results.

Work done and findings:

Three serial blind passages of 56 CSF and sera samples from AES cases in Vero, BHK 21, MRC -5 cells and infant CD1 mice yielded 02 CHPV isolates from samples referred from Gujarat.

3. Surveillance of JEV and CHPV in endemic region:

Background:

As a part of the surveillance of CHPV and JEV, sera from close contacts of JE/CHP cases as well as pig serum and vector mosquitoes from endemic/outbreak areas referred by state health authorities were routinely tested for antibodies to different etiological agents to understand the prevalence of the viruses in nature.

Work done & findings:

(i) Sero - surveillance (Human):

Seventeen serum samples from contacts referred from Maharashtra (Jalna, Palghar and Solapur districts) and Gujarat (Godhara and Vadodara districts) tested negative for Anti-JEV neutralizing antibodies (PRNT). While 44/72 (60%) serum samples of contacts referred from Gujarat (Dahod, Godhara, Panchamahal and Narmada districts) were detected positive for anti-CHPV neutralizing antibodies by PRNT.

(ii) Sero - surveillance (Animal):

Pig serum samples, 6/18 from JE endemic regions of Maharashtra (Solapur & Palghar districts) and Madhya Pradesh (Sehore and Betul districts) showed the presence of anti-JEV neutralizing antibodies by PRNT.

(iii) ***Vector - surveillance (JEV / CHPV):*** Six sand fly pools (S*ergentomyia* species) referred from Gujarat (Kheda) and Maharashtra (Chandrapur dist) were screened for CHPV using CHPV specific RT-PCR. No virus could be detected. Similarly, mosquito pools referred from Kolhapur district of Maharashtra were also processed with JEV/WNV specific RT-PCR, but tested negative for both.

EPD1803: Japanese encephalitis epidemiology in Central part of India (Collaborative project)
Investigators: Tandale BV & Bondre VP
Funding: ICMR Duration: July 2018 to Jan 2021

Background:

Vaccination against JEV using SA14-14-2 has been initiated in JE endemic areas, which has shown a positive impact. A collaborative study involving hospitals located in JE / CHPV endemic areas of Maharashtra and Telangana state was undertaken to study the epidemiology of JE following vaccination.

Objectives:

(i) To estimate the incidence of AES hospitalizations and the contribution of JE and other AES etiologies in total AES cases documented

(ii) Estimation of JE vaccination coverage and its effectiveness in population.

Work done & findings:

Clinical specimens (273 sera and 117 CSF) collected from 264 hospitalized AES cases were investigated to detect IgM antibodies against JEV, CHPV and dengue viruses. Molecular diagnosis was also carried out for CHPV, JEV and HSV by virus specific RT-PCR / PCR and sequencing. Anti-JEV IgM positivity was detected in 26 (9.84%) cases, while dengue and CHPV IgM positivity was detected in 11 (4.16%) cases each. Presence of both JE and dengue antibodies were detected in 23 (8.7%) cases and the convalescent sera samples are being investigated for further confirmation. One CSF tested positive for HSV-1 by PCR.

4. Genetic characterization of CHPV isolated from human cases:

Background:

Genetic and biological characterization of infectious viruses is important to track evolutionary changes occurring with time, introduction of newer genetic and phenotypic variants and updating of existing diagnostics and vaccines. Earlier studies have yielded a Chandipura virus isolate from a fatal case, which has shown >11% genetic divergence from the prototype strain. During 2019-20, another human strain of CHPV was isolated and characterized at the genomic level and compared with existing CHPV strains reported from India and Africa.

Work done & findings:

Full genome sequence (11120 nucleotides) of CHPV isolate 196388 (Human, Gujarat, India, 2019) was obtained from both the ends using 23 pairs of overlapping primers. Comparison of the sequences of the new strain with worldwide sampled CHPV strains showed its close genetic relationship with Indian strains and grouped as Clade 1(Fig. 1). However, the strain showed 4% genetic divergence from the prototype strain grouped in Clade II (1965); 12% divergence from the only genetic variant of CHPV, *i.e.*, Gujarat human strain No. 1511584 (Clade III) and 28-29% genetic divergence from strains reported from Senegal and Nigeria (Clade IV). These findings suggest predominant circulation of Clade I strains in different parts of India.

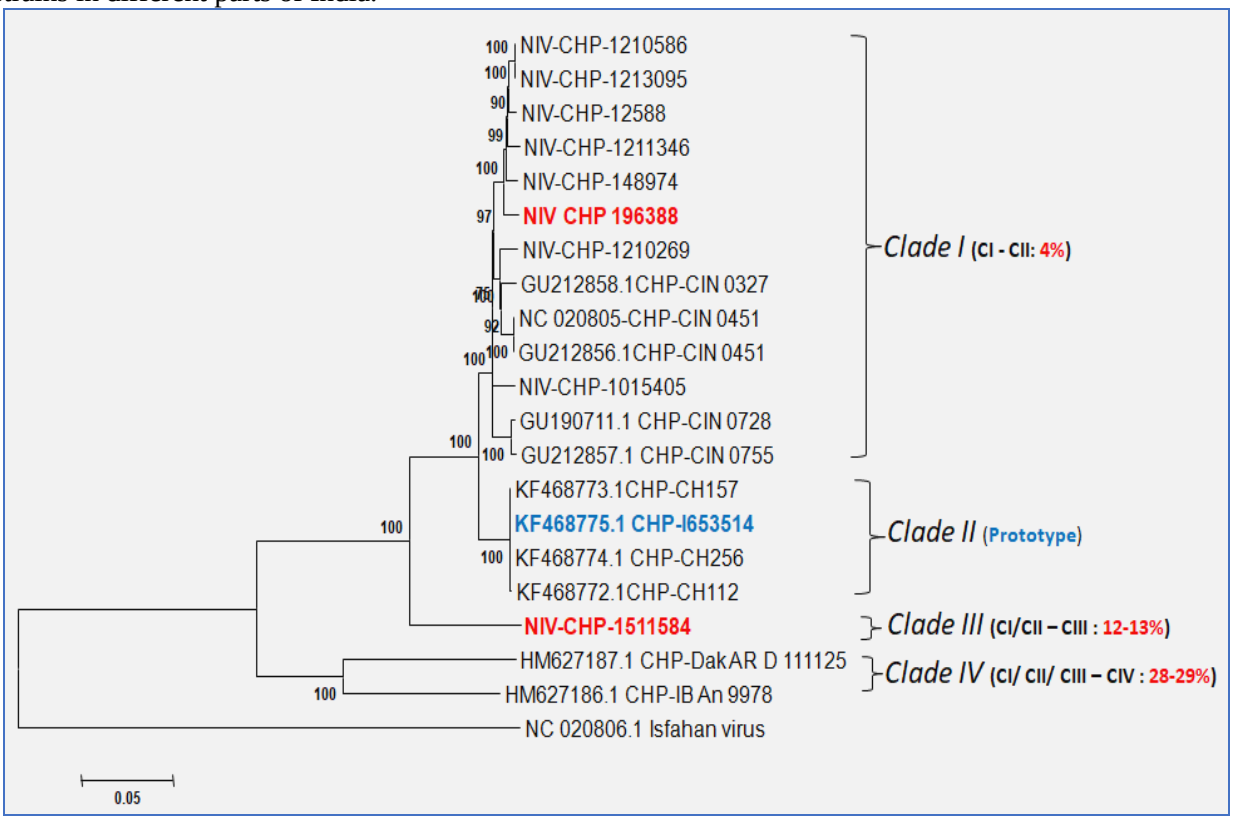


Fig. 1. Phylogenetic analysis of recently isolated CHPV strain from Gujarat.

5. Development of molecular diagnostic assays for detection of viruses associated with human central nervous system infections in India.

Background:

Availability of full genome sequences of newer strains of different encephalitic viruses prevalent in India helps to estimate the utility of existing diagnostic assays for these viruses. With the emergence of JEV genotype I, identification of a new genetic variant of CHPV and the frequent detection of HSV-2 in CNS infections, necessitated the need for new diagnostic assays. Accordingly, development of quantitative RT-PCR assays for detection of JEV (genotypes I-V), CHPV (both the prototype and newly identified strains) and PCR for simultaneous detection of HSV-1 and 2 was undertaken.

Work done & findings:

The primers and probes necessary for Taqman chemistry based amplification were designed by selecting the conserved fragments from alignment of GenBank retrieved sequences representing all genetic variants and confirmed by multiple primers / probe designing softwares. Nucleic acid extracted from respective lab grown, plaque titrated virus strains served as controls. Genomic region flanking the target sequence to be explored for Taqman assay development was amplified by RT-PCR / PCR and cloned in TOPO cloning vector. The plasmid isolated from positively transformed bacteria served as standard template for the assay.

I. Diagnostic RT-qPCR for Japanese encephalitis virus

Japanese encephalitis virus specific diagnostic RT-qPCR was designed to detect all genetic variants (genotypes) and standardized using the JEV GI isolate 1601136 (Human, 2016, UP, India) and JEV GIII 057434 (Human, 2005, UP, India) grown in BHK-21 cells. Standard RNA template for the assay was prepared from cloning of a 621bp fragment amplified from 5'UTR-C target region in TOPO-TA cloning vector. RNA transcripts generated from linear plasmid by in vitro transcription, quantified and used as quantitation standard templates. Sensitivity of the assay was determined by using viral RNA prepared from serially diluted plaque titrated JEV strains with virus titers ranging from 106 to 1 pfu / ml of dilution. The detection limit of both the strains of JEV was established to be 50 pfu / ml while the detection limit of RNA transcripts used as standard was established to be 10 copies / reaction (Fig. 2). The assay is expected to have similar detection sensitivity for the strains from GII, GIV and GV as the probe and primer sets hybridizes equally to the target sequence of all JEV strains independent of their genotypes.

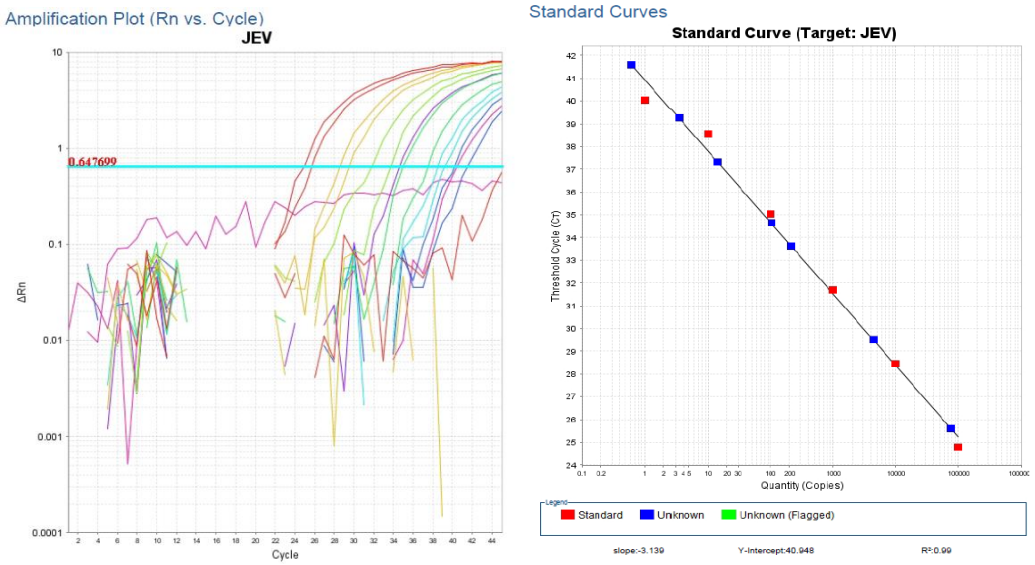


Fig. 2. Determination of sensitivity of the JEV specific RT-qPCR assay. Panel I shows the amplification graph of multiple dilutions containing JEV GI and GIII ranging from 10^6 to 1 pfu / ml while Panel II shows the standard curve of amplification.

Specificity of the newly designed JEV RT-qPCR assay was determined by using a panel of encephalitic viruses including the target JEV GI and GIII strains, West Nile virus, dengue virus, CHPV and human Herpes virus 1. The assay did not amplify the nucleic acid from any of the non-target neurotropic viruses while multiple dilutions of different JEV (GI and GIII) were specifically amplified by the assay (Fig. 3). These findings suggest that the newly designed RT-qPCR assay is JEV specific and sensitive enough to detect 10 genomic copies of JEV.

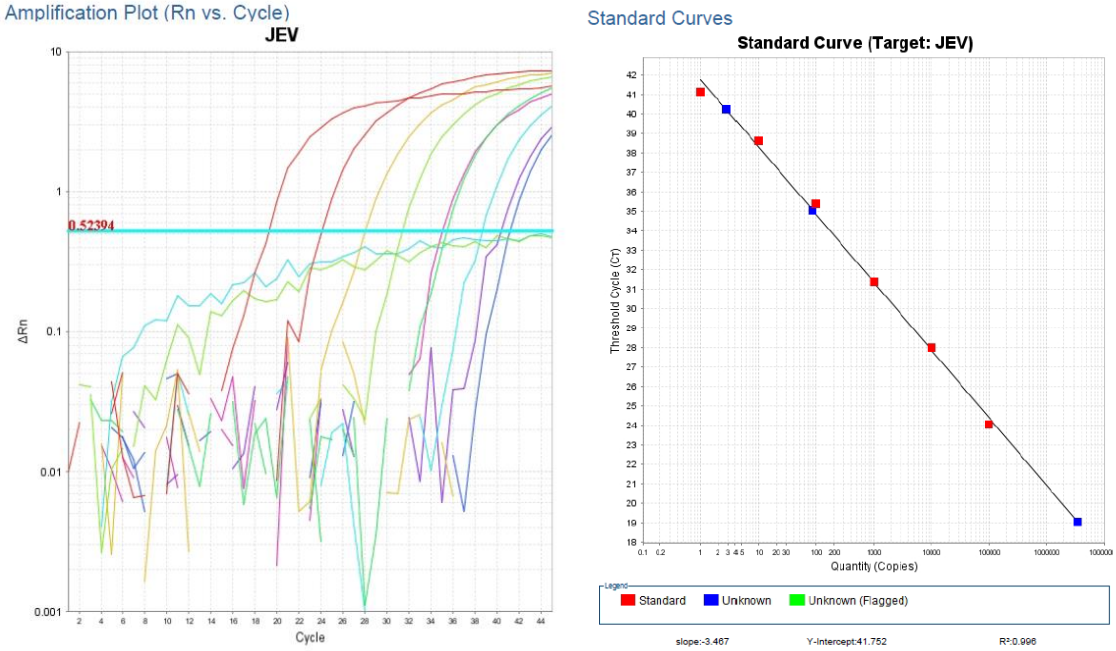


Fig. 3. Specificity of JEV specific RT-qPCR assay using human encephalitic viruses prevalent in India. The assays detected RNA from both the JEV GI and GIII strains while it did not show any amplification signal for nucleic acid isolated from other encephalitic viruses (WNV, Den, CHPV, HSV-1) used in the assay.

II. Diagnostic RT-qPCR for Chandipura virus

To support the routine diagnosis of suspected encephalitic cases and the antiviral research to evaluate effectiveness of various antivirals licensed for human use against CHPV infection, RT-qPCR assay was designed targeting the G gene. The primer / probe concentration and assay conditions were optimized to detect 10 genomic copies of CHPV /ml of the culture (Fig. 4). Sensitivity of the assay evaluated using ten-fold serial dilutions of titrated CHPV virus stock while specificity was determined by using a panel of non-related encephalitic viruses including West Nile virus, dengue virus, Japanese encephalitis virus and human Herpes virus -1. The assay specifically detected CHPV while it did not show any amplification signal from nucleic acids isolated from other viruses indicating that the Real time PCR is specific only to CHPV. On account of high sensitivity and specificity the assay can be further used for rapid detection and quantification of CHPV RNA from clinical samples.

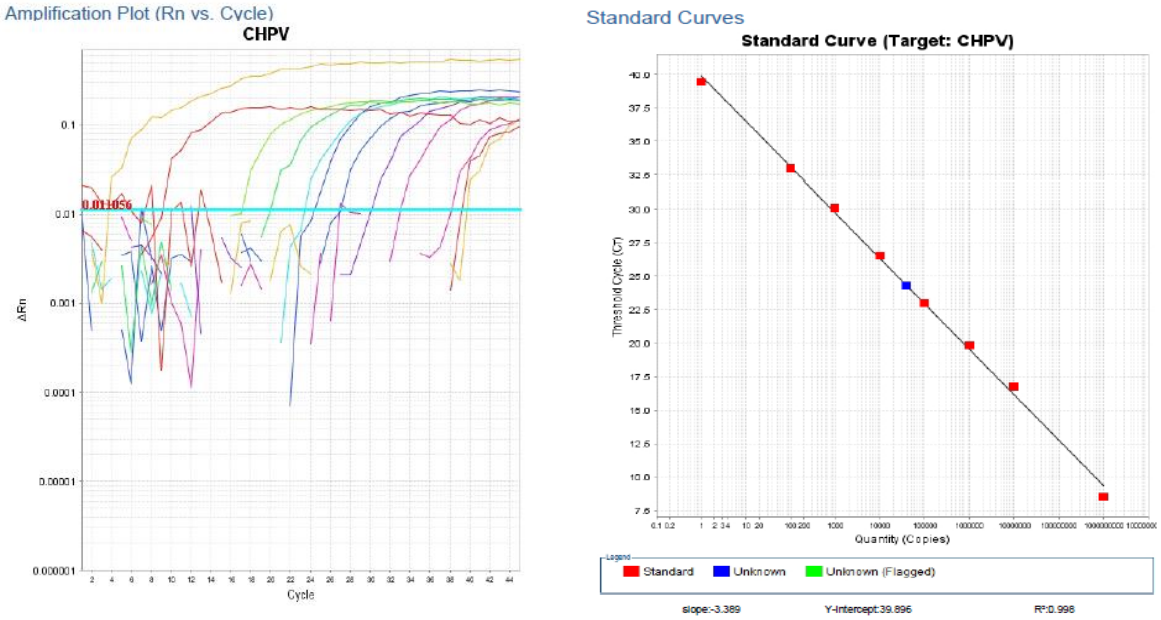


Fig. 4. Standardization of CHPV specific RT-qPCR assay

III. Diagnostic qPCR for detection of Human Herpesvirus-1 and 2

A Real time PCR assay was designed for detection / quantitation of HSV-1 and 2 using a primer set and probe from the conserved region of UL 30 (DNA polymerase) gene. DNA standards were generated by cloning in TOPO-TA vector and the assay was optimized for primer / probe concentrations and reaction parameters to detect 50 genomic copies of HSV-1 and HSV-2 DNA (Fig. 5) and human clinical specimens. Nucleic acid prepared from non-related neurotropic viruses including JEV, WNV, CHPV, DEN, etc. served as controls for determination of the assay specificity.

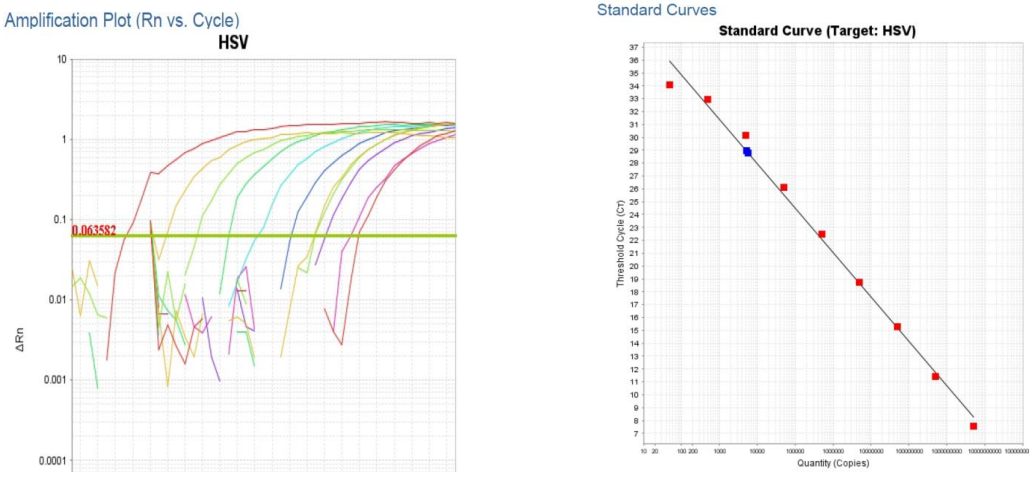


Fig. 5. Amplification plot and standard curve of qPCR using multiple dilutions of HSV-1 and HSV-2 nucleic acid.

The quantitative diagnostic assays developed for JEV, CHPV and HSV will be further validated in different laboratories before their inclusion as routine assays to be used for diagnosis of AES cases. Further work is in progress.

ENC1603: Role of Histidine residues in membrane fusion of Japanese encephalitis virus envelope protein.

Investigators: Bondre VP, Mali DN.

Funding: Institutional Funding Duration: 2016-2019

Background: Current study is proposed to unravel the role of flavivirus conserved histidine residues in E glycoprotein of JEV in membrane fusion with the host cell membrane. Time kinetics of wild type JEV tracking will serve as a standard control in the experiments that will be performed using wild type VLPs and point mutated VLPs at conserved histidine residues. Finding the exact role of conserved histidine residues in membrane fusion process during host cell infection will develop a path for new therapeutics.

Objectives:

1) Characterization of Wild type VLPs by TEM analysis.
2) Application of VLPs as an antigen in place of JE virus whole antigen to detect the IgM antibodies by JE IgM MAC ELISA kit.
3) Replacement of conserved histidine residues with alanine to generate the mutated VLPs.

Work done and finding:

As reported earlier, expression of the JEV C-prM-E construct was achieved and the clone was studied to stably express JEV VLP till 40th passage level. During analysis of all the clones for extracellular expression of E protein, clone IE_6 was found to be most efficient in secretion as established by IgM ELISA using the expressed VLP as antigen. To confirm its immunological reactivity and structural integrity, cell supernatant collected at 72 hrs was directly used as an antigen in the JE IgM ELISA kit to estimate its sensitivity and specificity. A standard panel consisting of 100 JE positive and negative samples was used in ELISA and the results were compared with the kit antigen. JEV E glycoprotein as an antigen was equally efficient in detecting the IgM antibodies in samples (Fig. 6). Further optimization is in progress to use the E glycoprotein in the JE IgM MAC ELISA kit in place of JE virus whole antigen.

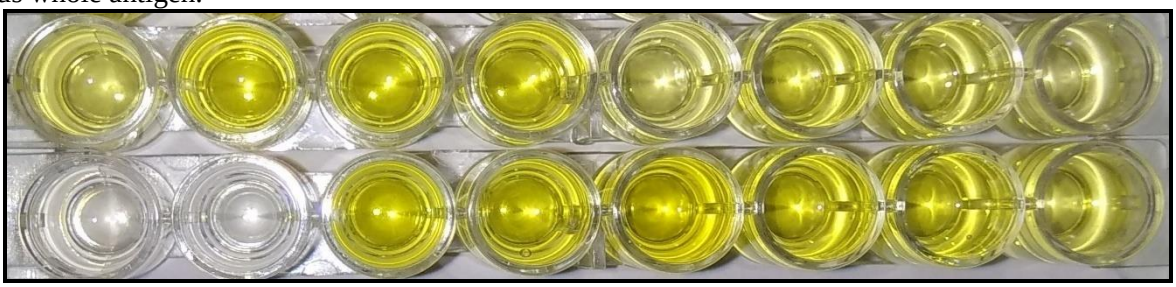


Fig. 6. Detection of anti-JEV IgM antibodies in human sera using expressed JEV E protein as antigen. Upper panel: Positive controls (well 1 and 2) and different human sera used for testing; lower panel: Negative controls (wells 1 and 2) and different human sera used for testing.

Furthermore the clone IE_6 expressing large scale VLPs in cell supernatant was studied for integrity of its confirmation by transmission electron microscopy. The TEM shows that the VLP itself is taking the morphology of virus establishing its conformational integrity and may interact with mammalian cells like the infectious virus particles (Fig. 7).

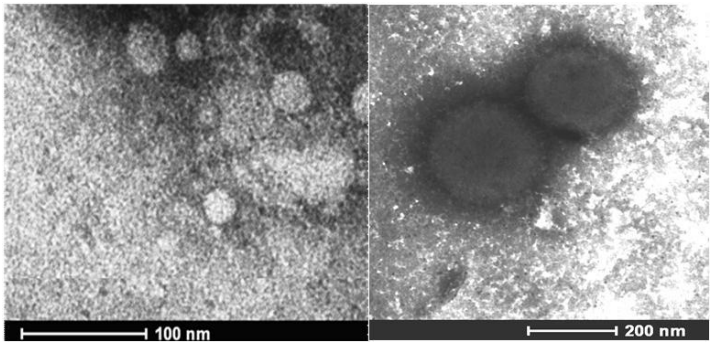


Fig.7. TEM analysis by negative staining of JEV GI VLP constitutively expressed in BHK-21 cells. The expressed E protein takes the shape of virus like particles.

Generation of VLPs with mutated His residues

Mutations in different conserved His residues (n=5) in JEV E protein was achieved in the pcDNA3.1-JEV-E construct containing partial C-prM-E by site directed mutations through introduction of Alanine in place of the His amino acid at independent positions 144, 246, 319, 395 and 397 of the E different constructs. The mutated plasmid was transformed in *E. coli* DH5α and plasmids isolated from positively selected transformants were confirmed for desired His mutations at respective sites by PCR and sequencing (Fig. 8). The sequence confirmed His mutated individual constructs were transfected BHK-21 cells and positively selected antibiotics G418. The expression of His mutated JEV E protein was confirmed by IFA using JEV specific monoclonal and polyclonal antibodies. Further, the individual His mutated VLP's will be used to study the impact of mutations in different conserved His residues in host cell virus interaction and endosomal membrane fusion in comparison to wild type virus.

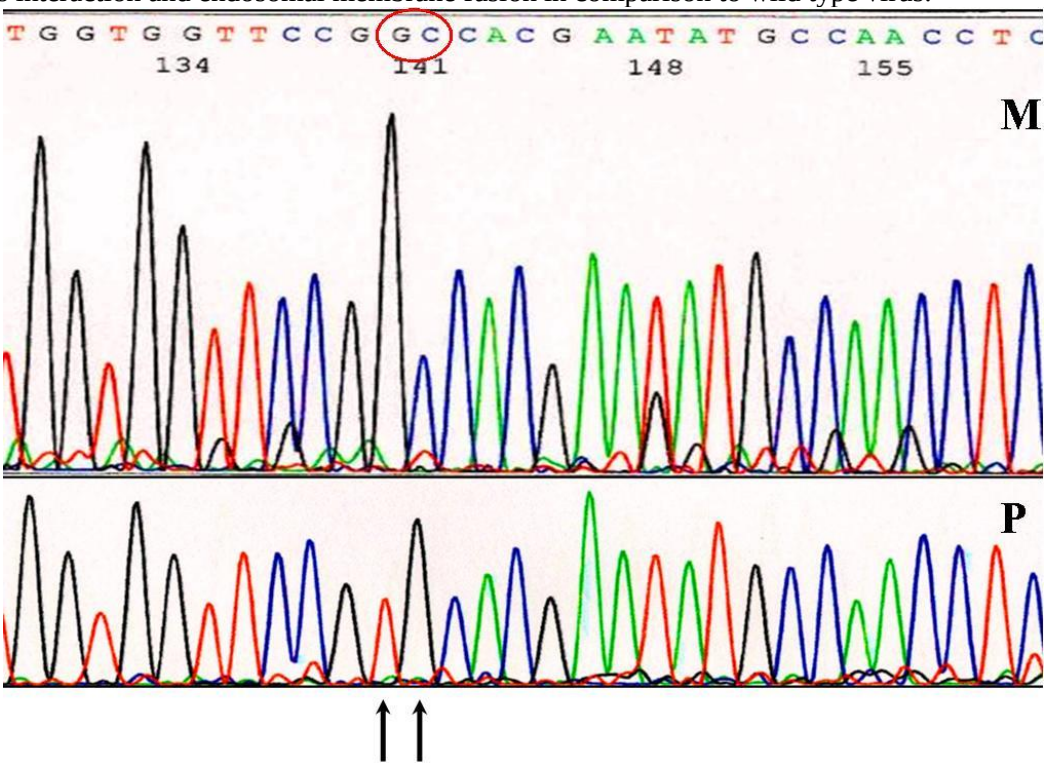


Fig. 8. Genetic alterations introduced in the JEV –E construct for mutations in His residues.

ENC1902: Expression of Japanese encephalitis virus - genotype 1 envelope and non-structural proteins to explore in early diagnosis.
Investigators: Bondre VP, Pavitrakar D, Sankararaman V, Mali DN.
Funding: Intramural Duration: 2019-21

Background:

JE infection is primarily diagnosed through detection of anti-JE IgM antibodies during the acute phase of illness as viremia is known to be transient. However, detectable titers of IgM antibodies against the virion (mainly envelope protein) are demonstrated to reach after 6-7 days of illness. In flaviviruses, nonstructural (NS) protein 1 is synthesized quite early during the infection (36-40 hrs of infection) and secreted outside the infected cell which interact and alter the membrane of non-infected cells making them more susceptible. Hence, most of the recent diagnostic assays against flaviviruses are being targeted either through demonstration of NS1 protein or antibodies against it. The existing JE MAC ELISA developed by NIV is based on cell culture derived inactivated virus as a source of antigen; this can be replaced by recombinant E protein based antigen. Replacement of the whole virus antigens with recombinant envelope (E) protein in earlier studies has eliminated the bio-safety risk but not the cross reactivity problem. Hence, it is necessary to study the cross reactive epitopes present in E protein and modify them genetically to minimize cross reactivity. Similarly, the JEV NS1 protein will also be cloned and expressed to validate its utility in early detection. Accordingly a study is planned to clone and express JEV Egp as virus like particles (VLP) and NS1 protein to explore their applications as antigens to develop NS1 and E based ELISA for early and virus specific diagnosis.

Work done and findings:

Envelope protein of JEV Genotype I strain (JEV-0945054) was cloned in pcDNA3.1 mammalian expression vector (5.4 kb) along with the viral E signal sequences and intact pre-membrane region (2 kb) to express it under the T7 promoter (Fig. 9A). Sequence analysis of the insert confirmed the directional cloning of the JEV E insert in pcDNA3.1 which was further confirmed by immunofluorescence assay using JEV specific antibodies and non-infected control cells (Fig. 9B and 9C) as well as western blotting.

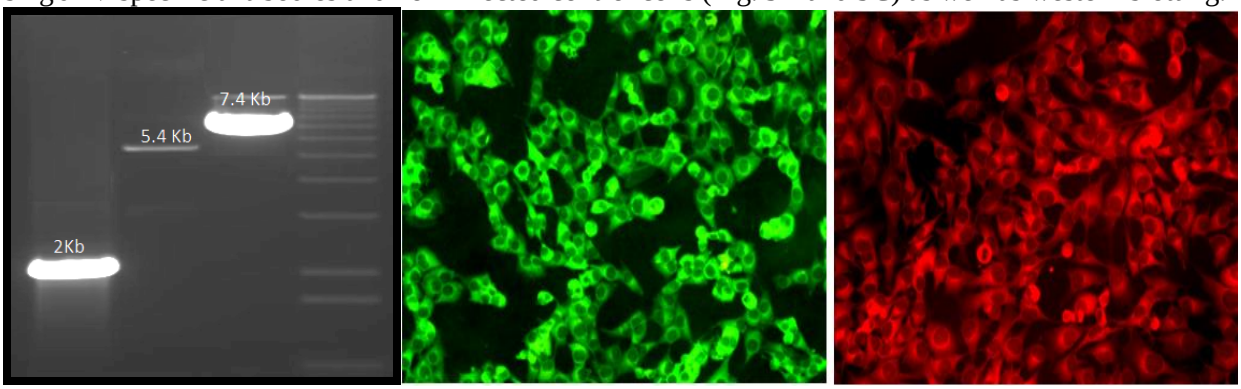


Fig. 9. (A) Cloning of JEV insert (390-2477 nt) in pcDNA 3.1+ vector under T7 promoter flanked by *Kpn*I at 5' and *EcoR*I at 3' end. (**B)** Transformed BHI-21 cells expressing JEV E protein as detected by specific antibodies and **C.** Negative cell control used in IFA.

The IgM detection ELISA performed using expressed JEV E protein as VLP antigen and the JE specific monoclonal antibodies for capture and detection yielded results compared to complete JE virion as an antigen (Fig. 10). The assays performed by capturing VLP on coated HX2 MAbs and HRP-conjugated HX2 as detector antibodies reacted with the VLP indicating it as a prominent protein that can be used as antigen to replace the complete virion which may reduce non-specific results. In addition to it, it will also minimize the risk of handling infectious / inactivated virion as antigen that is being used in current IgM ELISA kit. The JEV-VLP clone constitutively expressing E protein in BHK-21 cells is being studied for its sensitivity and specificity in JE IgM ELISA with the help of DRF Group, NIV. The

expressed VLPs will be a good source of control antigen for studying the naturally JEV infected arthropod vectors for transmission. In addition to it, a study will be planed to explore the VLP as vaccine candidate for swine use which may help to reduce JE burden in India.

Fig. 10: JE antigen capture ELISA assay performed using JEV E protein based VLP as antigen. Well 1 and 2 in upper panel represents ELISA using JEV virion as antigen and negative control.

A number of studies on flavivirus E protein cross reactive epitopes identified cross reactivity at the antigenic level due to the highly conserved amino acids in the hair pin loop of fusion peptide located domain II of E protein. Genetic modifications in the fusion peptide alter the confirmation that results in specificity to bind the antibodies. Accordingly, the mutation by site directed mutations in the flavivirus cross-reactive epitope located in fusion peptide (residues Trp101Gly, Gly104His, Gly106Lys, and Leu107Asp) were introduced and confirmed by sequencing (Fig. 11). Further expression of E protein carrying all 4 genetic mutations was confirmed by IFA using JEV specific polyclonal antibodies (Fig. 12). Studies related to establishment of stable cells expressing VLPs mutated in different fusion loop specific residues and recovery of purified VLP is in progress. The expressed VLPs will be further studied to explore in JE IgM ELISA kit to minimize antigenic cross reactivity in patients infected with other co-circulating flaviruses in different parts of India.

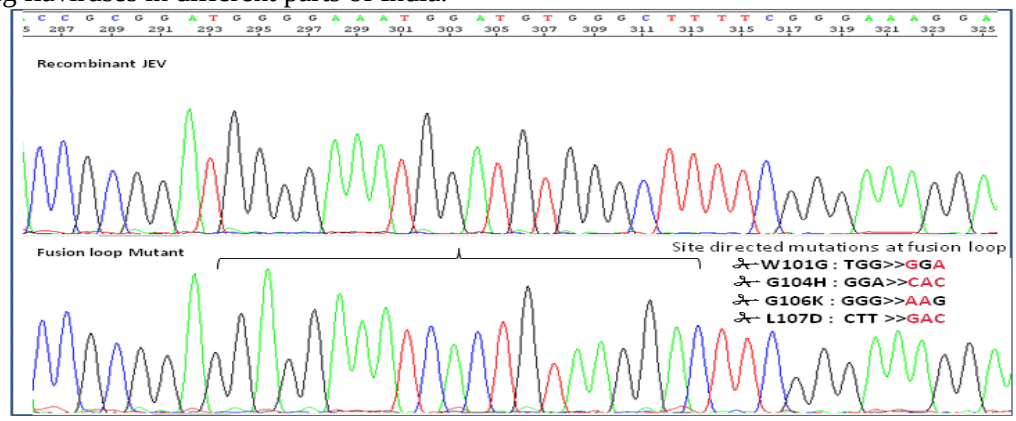


Fig. 11: Genetic modifications introduced in JEV VLP construct cloned in pcDNA3.1 as compared to the nucleotide sequence of parental JEV isolate.

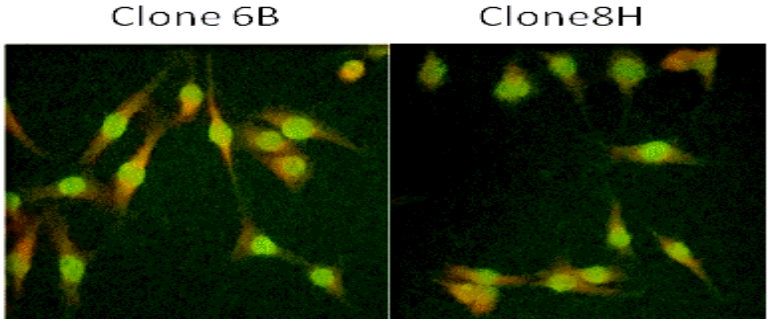


Fig. 12: Expression of JEV E protein after transfection with fusion peptide mutated JEV VLP's in BHK-21 cells.

ENC1903: To determine the antiviral activity of viral RNA dependent RNA polymerase inhibitors against Chandipura virus infection.

Investigators: Bondre VP, Pavitrakar D, Thakare Y.

Funding: Intramural Duration: 2019-21

Background: Chandipura virus (CHPV) has emerged an encephalitis causing virus with case-fatality rate >70% in children. Central India comprising Maharashtra, Gujarat, Telengana and parts of Odisha is experiencing sporadic outbreaks, but with few casualities. However, no licensed vaccines /therapeutic measures are available to-date. Negative strand RNA viruses encode an RNA dependent RNA polymerase (RdRp) enzyme required for viral genome transcription and replication. Since, this activity is restricted to virus synthesis upon infection; antiviral compounds targeting viral RdRp activity are of current research attraction as it selectively inhibits viral RdRp without affecting host cells. Accordingly, the current study is aimed to evaluate the potential of well characterized RdRp inhibitors namely, Ribavirin, Favipiravir, Remdesivir etc., against CHPV infection through *in vitro* and *in vivo* studies.

Objective: Study of antiviral activity of RdRp inhibitors: Ribavirin, Favipiravir, and Remdesivir against CHPV infection in the *in vitro* system.

Work done and findings: Stock of Chandipura virus human isolate (CHPV-1511584) was prepared in Vero cells the non-toxic dose of Ribavirin and Favipiravir in Vero and Ribavirin in HepG2 cells was estimated by MTT assay. The *in-vitro* antiviral effect of Ribavirin and Favipiravir was determined using plaque reduction assay in Vero cells and viral growth kinetics assay in HepG2 and Vero cells.

Anti-CHPV activity of Ribavirin:

The 50% cytotoxic concentration (CC_{50}) of Ribavirin in both the cells was found to be very less (> 10,000µM in Vero cells and > 8,000µM in HepG2). In Plaque reduction assay ribavirin effectively reduced the viral plaque size and caused complete inhibition of virus growth in Vero cells within non-toxic range of the drug. The gradual reduction of plaque size was observed from 500µM, the 50% inhibition in plaque number was observed at 2147µM concentration with complete inhibition of plaque development noticed at 4000µM (Fig. 13).

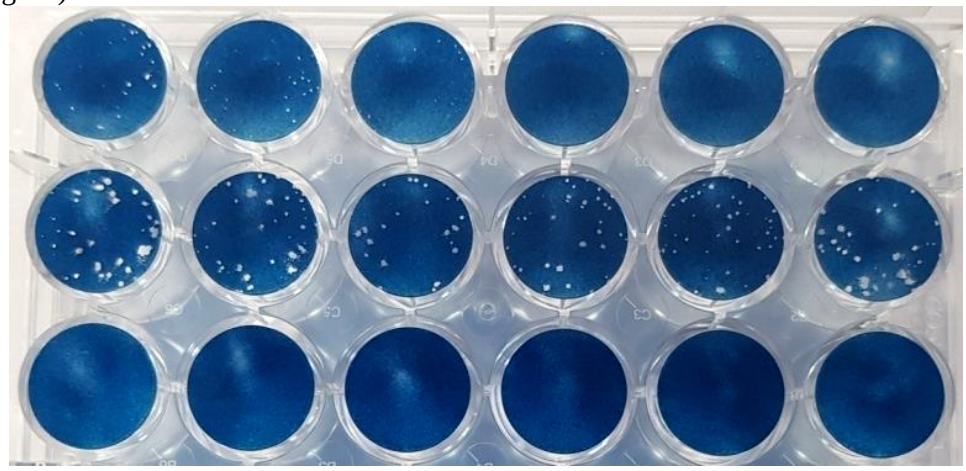


Fig. 13: Inhibitory effect of Ribavirin against CHPV infection in Vero cells: **Row 1**: Wells 1-6: Decreasing plaque number and size of CHPV against the increasing Ribavirin concentrations (100, 500, 1000, 2000, 4000, 8000 µM). **Row 2**: Wells 1-6: CHPV virus controls; **Row 3**: Drug controls of respective Ribavirin concentrations with no infection.

Growth kinetics of CHPV in the presence of ribavirin at 24hr post infection showed substantial inhibition of virus (≥4 logs) at 1000µM concentration onwards. The growth kinetics of CHPV in presence of different concentration of ribavirin using HepG2 cells (Human origin) also showed 4 log reductions at

1000µM concentration at 24hr time point. These *in vitro* studies established the antiviral activity of ribavirin against CHPV infection.

Anti-CHPV activity of Favipiravir:

The CC_{50} of Favipiravir in Vero cells was found to be 4800 µM.. The determination of EC_{50} of Favipiravir by plaque reduction assay in Vero cells showed that at 31.85 and 63.69 µM concentrations gradual reduction in the CHPV plaque size was observed although the number of plaques was same for these concentrations as that of no drug control (Virus control) as well as 6.369µM. The 127.4 µM drug concentration was found to reduce the plaque number by 50% with substantial reduction in plaque size. The concentration of 318.5 µM showed complete inhibition of CHPV plaque formation (Fig. 14) highlighting the possible antiviral effect of Favipiravir in CHPV infection.

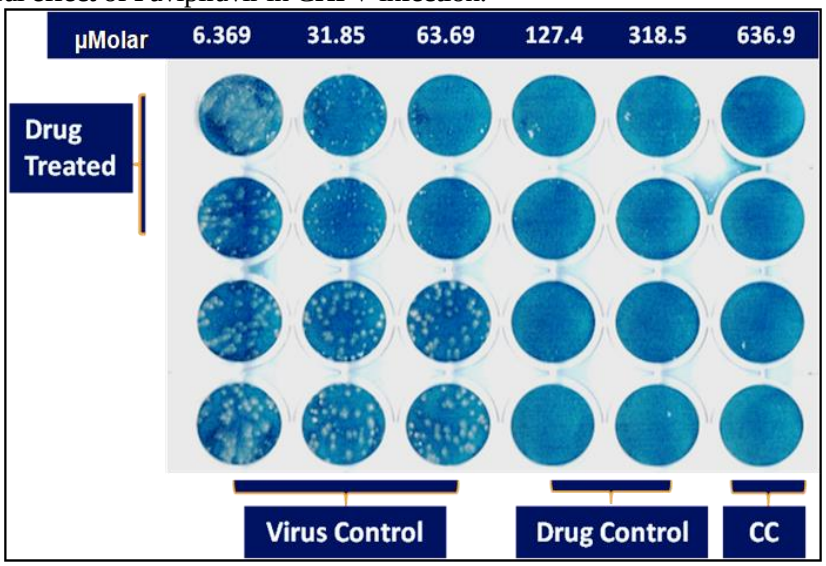


Fig. 14: Determination of 50% Effective concentration (EC_{50}) of Favipiravir in CHPV infection by plaque reduction assay.

INFLUENZA GROUP

Scientific Staff

Dr. VA Potdar	Scientist 'D' & Group Leader (potdarvarsha9@gmail.com)
Dr. ML Choudhary	Scientist 'D' (mlchoudhary@gmail.com)
Dr. SD Bhardwaj	Scientist 'B' (sumitduttbhardwaj@gmail.com)
Dr. Himanshu Kaushal	Scientist 'B' (hkarya@gmail.com)
Dr. MS Chadha	Consultant (till 30Th June 2019) (mscniv@gmail.com)

Technical Staff

Mrs. VC Vipat	Senior Technical Officer
Mrs. SY Jadhav	Technical Assistant
Mr. HK Kengale	Technician 'C'
Mr. AS Awhale	Technician 'B'
Mr. VN Autade	Technician 'B'

Project Staff

Sc. B, Medical	(One)
Sc. B, Non-Medical	(One)
Computer Programmer	(One)
Technical Assistant	(Three)
JRF	(Three)
Staff Nurse	(Seven)
Social worker	(Two)
Lab attendant	(Two)

- Providing laboratory diagnosis services for influenza and other respiratory viruses.
- A total of 2045 clinical samples were tested using real time RT-PCR and 298(14.5%) samples were found positive for influenza viruses. Of these, 155 (7.57%) were Influenza A/H1N1pdm09 virus positives, 75 (3.66%) influenza A/H3N2 virus positives and 68 (3.32%) were Influenza B virus positives. Peak influenza activity was observed during July to September and inter-seasonal peak during February-April
- The Influenza A/H1N1/pdm09 circulating in 2019-20 showed a clade 3C.2a.1 genotype.
- Served as the National Apex laboratory for SARS-CoV-2 laboratory diagnosis
- Studies on prevalence of influenza in elderly showed incidence rate in ARI was 35.97 per 1000 elderly per week. The incidence rate in ALRI was 1.38 per 1000 elderly per week. Total 468 samples were collected and influenza positivity was 4.48% and influenza-associated ARI incidence rate was 0.42 per 1000 elderly per week. The RSV positivity was 0.85%.
- Health Technology assessment studies on commercial kits for H1N1 detection was carried out.
- NIC activities on Global Influenza networking and WHO external quality assessment programs were carried out as mandated.

INF1601: Diagnostic Services/Outbreak Investigation

Investigators: VA Potdar, ML Choudhary & SD Bhardwaj
Funding Agency: Intramural Duration 2019-2020

***Background*:** Clinical samples from patients suspected for Influenza infection were referred for diagnosis of influenza and other respiratory viruses by different clinics/hospitals across Maharashtra as part of NiV's mandate.
Objective: To provide diagnosis to referred samples to Influenza and other respiratory viruses.
***Work done*:** Referred clinical samples (n=2045) were tested using real time RT-PCR and 298 (14.5%) samples were found positive for influenza viruses, of which 155 (7.57%) were positive for Influenza A/H1N1pdm09 virus, 75 (3.66%) for influenza A/H3N2 virus and 68 (3.32%) for Influenza B virus. Peak influenza activity was observed during July to September and inter-seasonal peak during February-April as given in Figure 1.

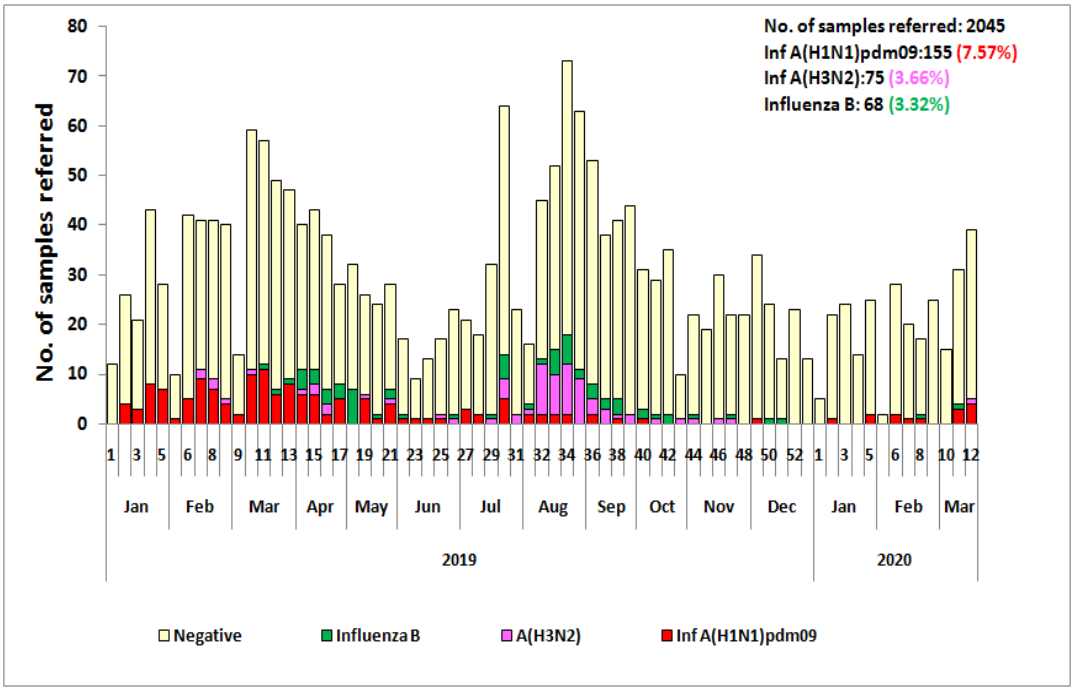
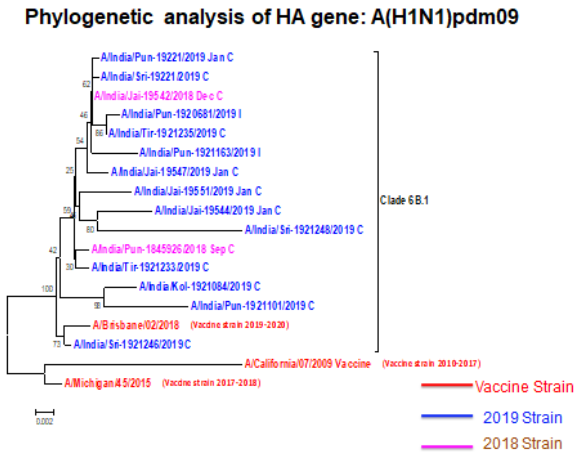
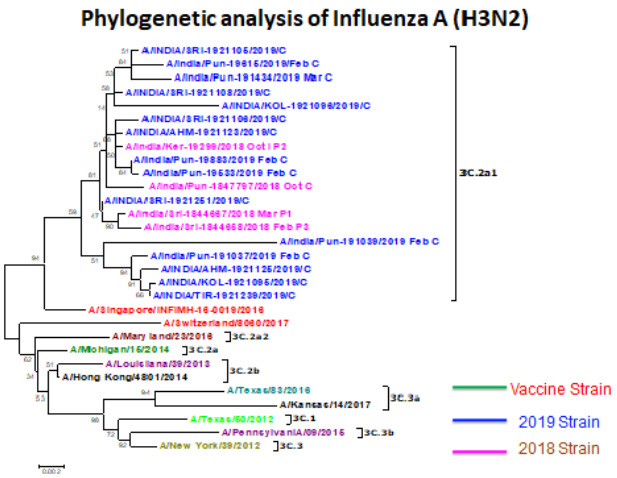


Fig 1: Influenza activity in referred clinical samples

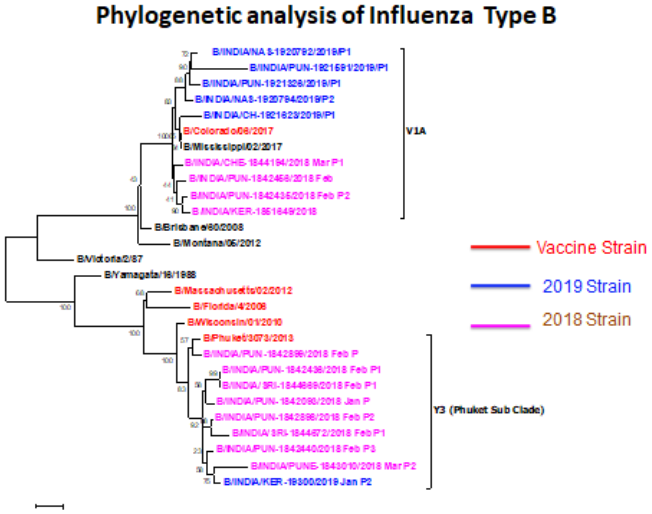
Representative influenza positive clinical samples received from Srinagar, Delhi, Jaipur, Ahmadabad and various districts of Maharashtra were inoculated in MDCK cell line and yielded virus isolates. HA gene analysis of influenza A/H1N1pdm09 virus showed that 2019-20 isolates grouped in clade 6B.1 (Fig 2a). Phylogenetic analysis of the HA gene of influenza A/H3N2 virus showed that the circulating strains grouped in Clade 3C.2a1 (Fig 2b). Phylogenetic analysis of HA gene of influenza B isolates showed that both Victoria and Yamagata lineages were in circulation and are similar to vaccine components of southern hemisphere for 2019-20 season (Fig 2c).



2a)



2b)



2c)

Fig 2: Phylogenetic analysis of the HA gene of influenza viruses: a): A/H1N1pdm09 b): A(H3N2) ; and c) Influenza B for the year 2019-20.

INF1501: Surveillance, outbreaks and epidemic investigations of high-risk group of viral pathogens causing respiratory infections

VA Potdar, ML Choudhary and SD Bhardwaj Duration 2019-2020

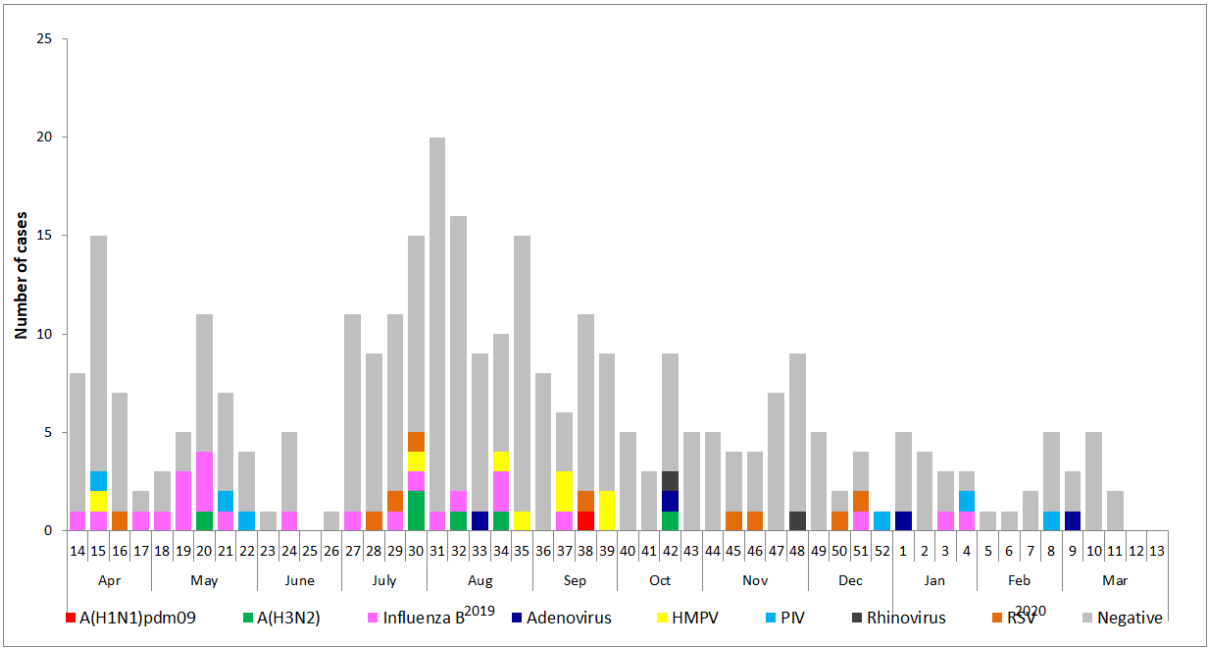
Background Respiratory viral infections are a leading cause of disease and mortality. Severity of these illnesses can vary from mild upper airway infections to severe wheezing, bronchiolitis or pneumonia.

***Objectives*:** To carry out epidemiological and virological surveillance for influenza and non-influenza respiratory viruses, *viz.*, Respiratory syncytial virus (RSV), Human metapneumovirus (hMPV), parainfluenza viruses (PIV), adenoviruses, rhinoviruses in India.

***Work done*:** During the entire period of the project, 1483 patients were enrolled and categorized; 314 throat/nasal swab samples were collected from acute respiratory infections (ARI) and 1169 from Severe Acute Respiratory Infection (SARI) patients were tested for different respiratory viruses by duplex real

time PCR. 18.1% and 37.3% positivity was detected in ARI and SARI cases, respectively. In ARI, 9.5% samples were found positive for influenza virus, hRSV (2.9%), hPIV (1.9%), adenovirus (1.3%), rhinovirus (0.6%), hMPV (2.5%). In SARI, influenza virus was detected in 9.2% samples, RSV (12.7%), Rhinovirus (2.9%), PIV (5%), hMPV (6%), Adenovirus (3.3%) and co-infection in 2.3% samples. The weekly distribution of samples tested and positives are shown in figure 4.

ARI



SARI

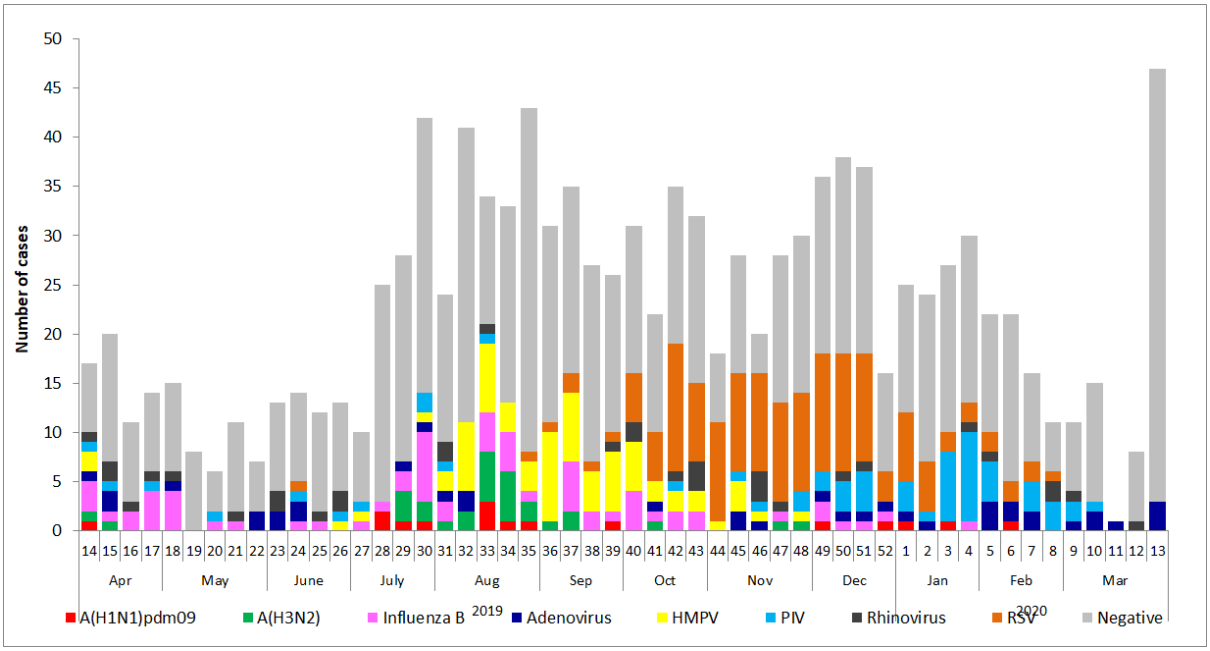


Fig 4: Weekly distribution of respiratory viruses in ARI and SARI cases

INF1602: Antiviral Susceptibility profiling of influenza virus in India
Investigators: Dr. VA Potdar, Dr. MS Chadha
Funding Agency: DHR Project Duration- 2016-20

Background: Monitoring influenza viruses for antiviral susceptibility is important for the clinical patient management as well as tracing emergence and spread of drug-resistant influenza viruses.
Objectives: To identify known and compensatory mutations in the influenza isolates, as well as to determine the sensitivity of neuraminidase inhibitor drugs.
Work done: During the reporting period, 633 A(H1N1) pdm09 positive clinical samples were assessed for H275Y mutation by allelic discrimination real-time RT-PCR. All samples were sensitive except 2 samples from Pune. Similarly, 91 Influenza A(H1N1) pdm09 isolates, 5 A(H3N2) and 37 Type B isolates were assessed for resistance to the drug Oseltamivir by Neuraminidase Inhibition phenotypic assay. Majority of A(H1N1) pdm09 and all Type B and A(H3N2) viruses remained sensitive to Oseltamivir, except two A(H1N1)pdm09 isolates which showed reduced susceptibility to the drug.

INF1604: Tracking community mortality due to respiratory syncytial virus
Dr. MS Chadha, Dr. VA Potdar.
Collaborators: Dr. Eric Simoes, University of Colorado and Dr. Ashish Satav, MAHAN Melghat
Funding Agency- Bill & Melinda Gates Foundation Duration 2016-2020

***Background*:** Respiratory synsitial virus is a major cause of morbidity and mortality among children in developing countries. There is insufficient data on RSV mortality in children below 2 years of age in India.
***Objective*:** To identify RSV associated mortality in infants/children below 2 years of age in Melghat, a tribal area in Maharashtra.
***Work done*:** A total of 1160 samples collected from children below 2 years of age from Melghat, were screened for RSV and other respiratory pathogens by real time RT-PCR. RSV was detected in 9 (0.43%) samples (Fig 4). Among other respiratory pathogens, Influenza virus was detected in 94 (8.1%) samples.

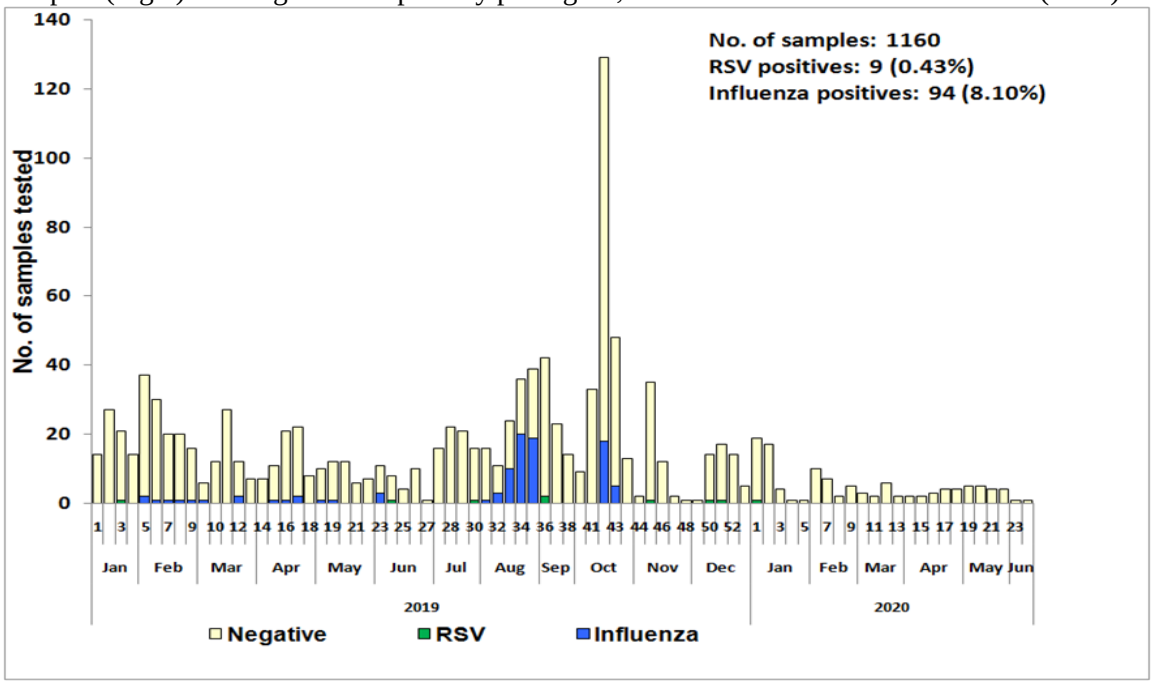


Fig 4: Month wise distribution of RSV in Melghat

Inf1701: Immunogenetics of severe pandemic H1N1pdm09 infections

Dr. ML Choudhary, Dr. K Alagarasu, Dr. MS Chadha
Funding Agency- DBT, Project Duration- 2017-2020

***Background*:** Susceptibility to severe influenza A/H1N1pdm09 virus is multi-factorial involving pathogen, host and environmental factors.
***Objectives*:** To find out 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*:** A total of 379 blood samples from influenza A/H1N1pdm09 positive patients (293 mild cases and 86 severe cases including 40 cases with fatal outcome) were collected and genotyping of single nucleotide polymorphisms (SNPs) in *TLR3* rs3775291, *FCGR2A* rs1801274, *TLR8* rs3764879, *IFIH1* rs1990760, and *CD55* rs2564978 were genotyped by real-time PCR. Genotyping of *IFITM3* (rs12252), *CD209* (rs735239 & rs2287886), 54th codon and 57th codon polymorphisms of *MBL2* gene, *TLR3* rs3775290 & *TLR3* rs5743313, *CCL2* (-2518), *DDX58* (rs10813831), *MxA* (-129 and -88) *IL10* (-592), and *IFNG* (+874) were performed by PCR-RFLP in all the 379 samples. *MBL2* (-221), *TNFA* (-308), *IL10* (- 1082), *TGFB* (25th and 10th codon) polymorphisms were genotyped by PCR–SSP based techniques.

Allele and genotype frequencies of IFITM3 (rs12252), CD209 (rs735239 & rs2287886), -221 Y/X and 57th codon polymorphisms of MBL2 gene, TLR3 (rs3775290, and rs3775291), DDX58 rs10813831, MxA (-129 and -88), CD55 rs2564978, FCGR2A rs1801274, and IFIH1 rs1990760 were not different between mild and severe influenza A H1N1pdm09 cases.
The results of the study suggest that the A allele and G/A genotype of TNFA -308 and TLR3 rs5743313 T/T genotypes were associated with susceptibility while the mutant allele (B) of 54th
codon polymorphisms of MBL2 gene was associated with protection to severe influenza A H1N1pdm09 disease. Moreover, TLR3 haplotypes were also associated with severe disease.

INF1703: Etiology of childhood pneumonia in India: ICMR Multicentre study
Dr. MS Chadha, Dr.VA Potdar
Funding Agency- ICMR Project Duration- 2017-19

Background: Considering the importance of childhood pneumonia, multisite taskforce study was initiated by the ICMR at 4 sites in India.
Objectives: To find out the etiology in childhood pneumonia.
***Work done*:** ICMR-NIV, Pune worked as reference laboratory for virology for the network and provided diagnostic support to two sites, KEM Pune and PGI, Chandigarh. A total of 371 and 198 throat/nasal swabs from KEM, Pune and PGI, Chandigarh ware shared with NIV for respiratory virus detection. Of the 569 clinical samples tested, RSV B was detected in 120 (21%) clinical samples and influenza was detected in 22 samples mainly during the rainy season. The other viruses detected were hRV (25) hPIV (16) hMPV and Adeno (11 each).

INF1801: Strengthening/promoting evidence-based advocacy for influenza prevention and control in India
Dr. SD Bhardwaj, Dr.VA Potdar
Funding Agency: CDC Project Duration- 2018-20

***Background*:** Influenza studies in India to date have largely focused on children, and those conducted among older adults are limited in sample size and geographic spread. The hospital-based studies will likely underestimate the burden of illness among older adults as they are less likely to visit the hospitals

especially in low- and middle-income countries, because of reduced mobility and other access-related issues.

Objectives: The specific objective of this component is to find the burden of illness for influenza and RSV among elderly age above 60 years in India. The community-based surveillance platform provides the necessary population denominators for this purpose.

***Work done*:** Strengthening/promoting evidence-based advocacy for influenza prevention and control in India: Community-based surveillance is being conducted among an open cohort of 1093 elderly subjects to find the burden of illness for influenza and RSV among the elderly in India. Trained project nurses conducted household surveillance five days a week to screen and enroll individuals for the presence of acute respiratory infection [ARI]. After 52 weeks of follow-up, total 47957 [94.8%] visits have been conducted. The incidence rate was 35.97 in ARI and 1.38 in ALRI per 1000 elderly per week. Total 468 samples were collected and influenza positivity was 4.48% and influenza-associated ARI incidence rate was 0.42 per 1000 elderly per week. The RSV positivity was 0.85%.

INF1802: Health Technology Assessment study on RT-PCR for H1N1 in India

Dr. ML Choudhary, Dr. Anita Aich

Funding Agency- DHR, Project Duration- 2018-19

Introduction: Limited labs are testing H1N1pdm09 virus in country and have not been able to effectively implement at large programs due to lack of adequate infrastructure, trained manpower and limited resources and high costing of kits. Sensitivity, specificity and costs vary among different tests.

Objectives: The purpose of this assessment was to appraise the current evidence for the clinical effectiveness (in terms of sensitivity and specificity) and costing of kits against CDC/WHO real time RT-PCR for diagnosis of influenza A/H1N1pdm09 in India.

Work done: Sensitivity and specificity was obtained from kits (n=4) evaluated at NIV. Summary of the data is shown in Table 1.

Table 1: Summary of the study

Parameters		Thermo Fisher Scientific Pandemic H1N1/09 Assay kit	Qiagen (artus Infl./H1 LC/RG) kit kit	3BBlackBio TruPCR kit	Cepheid Xpert® Flu kit
Cost (Rs) Per Sample		2015	1902	1660	4342
Ease of doing		Easy	Easy	Easy	Easiest
Turnaround time		4 hours	4 hours	4 hours	2 hours
Samples in one go		29 Samples	34 or 46 samples, depending upon the rotor used.	29 Samples	1 to 4 samples, depending upon the machine module.
Operational Feasibility		Open system Existing labs equipped with RT-PCR machine	Open system Can be used in existing facilities.	Open system Can be used in existing facilities.	Closed system Health system will need to buy new RT-PCR machines, if this kit is introduced.
Influenza A	Sensitivity (95%CI)	100 (91-100)	84 (76-90)	100 (95-100)	100 (88-100)
	Specificity (95%CI)	100 (96-100)	96 (88-99)	94 (87-97)	99 (95-99)

H1N1	Sensitivity (95%CI)	100 (91-100)	94 (85-98)	94 (84-98)	93 (78-93)
	Specificity (95%CI)	100 (96-100)	98 (94-99)	100 (96-100)	100 (96-100)
H3N2	Sensitivity (95%CI)	100 (85-100)			63 (for influenza A) (38-81)
	Specificity (95%CI)	100 (97-100)			100 (97-100)
Influenza B	Sensitivity (95%CI)				96 (83-99)
	Specificity (95%CI)				100 (96-100)

The testing costs of the different kits are tabulated in Table 1. In view of highest sensitivity and specificity among all the kits evaluated in this study, Invitrogen kit was found to be most suitable for diagnosis of influenza A/H1N1pdm09 virus from clinical samples.

INF1901:- Sentinel surveillance for influenza, including avian influenza, in India

Dr. VA Potdar, Dr. ML Choudhary and Dr. SD Bhardwaj

Funding Agency- WHO Duration: 2019-20

Introduction: Globally new viral respiratory tract infectious diseases with epidemic/pandemic potential that threaten global health security have emerged and had huge burden on health services. The influenza virus and the emerging novel variant like influenza A(H7N9), low pathogenic avian influenza virus (LPAIV) A(H9N2) are a continued threat to the population in the South East Asian Region.

Objectives: Monitor circulating influenza strains for novel variants with epidemic and pandemic potential and sharing them with WHO's GISRS.

Work done: A network of laboratories has been established in the country to carry out surveillance for Influenza and other viruses especially across the north-east region and migratory bird fly-ways. Of 2499 SARI patients that were enrolled, all were tested for different respiratory viruses by real-time PCR. Among the cases, influenza virus was detected in 100 (4%) of the samples, of which Influenza Apdm09 (H1N1) was 19 (0.8%), Influenza A(H3N2) 07(0.3%) and Influenza B 74(3%).

Contribution to Global Influenza Network

Virological data for 34441 clinical samples including positives 1763 influenza A(H1N1)pdm09, 1356 A(H3N2) and 1185 of Influenza B were submitted to Global Influenza Surveillance and Response System (FLUNETPLUS). Influenza isolates (n=29) (H1N1pdm09: 09, H3N2: 09, Influenza B: 15) were submitted to WHO CC, CDC, Atlanta.

WHO External Quality Assessment Programme (EQAP)

Panel number 19 (2020) for influenza A real-time PCR and Panel 1 for COVID -19 from WHO, CHP Hong Kong was received. The Influenza panel consisted of 10 coded samples of A(H3), A(H5), A(H1)pdm09, influenza B and other Influenza A. Covid panel consisted of 10 coded samples. Both the panel results were 100% concordant.

NABL Accreditation

Realtime PCR test for influenza virus diagnosis was 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). The surveillance audit and assessment of the tests conducted were done in August 27 2020 and validity of certificate was extended for two years.

DIAGNOSTIC VIROLOGY GROUP

Scientific Staff

Dr. Gajanan N Sapkal,	Scientist 'E' & Group Leader
Dr. Ullas PT	Scientist 'B'
Dr. Gururaj Rao Deshpande	Scientist 'B'

Technical Staff

Mr. Bipin N. Tilekar	(STO-B)
Mrs. Rashmi S. Gunjikar	(STO A)
Mrs. Rashi Srivastava	(TO-A)
Mrs. Asha Bhagat	(Technician B)
Mr. Shankar.M. Vidhate	(Technician B)
Mrs. Kirtee Khutwad	(Technician A)
Mr. Suresh Kamble	(MTS)

Project Staff

Dr. Suji George	Scientist C
Dr. Sharada H Sharma	Scientist B
Dr. Mandeep Kaur	Scientist 'B'
Mr. Ojas Kaduskar	Technical Assistant
Mrs.Vaishali Bhatt	Technical Assistant
Ms. Heena Shaman	Technical Assistant
Mr. Shivshankar Gaikwad	Technical Assistant
Mr. Vishal Jagtap	Technical Assistant
Ms. Ketki Deshpande	Technical Assistant
Ms. Snehal Shingade	Technical Assistant
Mr. Chetan Patil	Technician C
Ms. Kajal Jarande	Technician C
Mrs. Roshni Patil	Technician C
Ms. Sanskruti Saka	Technician C
Mr. Darpan Phagiwala	Technician C
Mr. Prasad Gomade	Data Entry Operator
Mr. Ajay Koli	Multi Tasking Staff

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

Investigators: Gajanan Sapkal, PT Ullas, G Deshpande & VA Potdar,

Support staff: Ojas Kaduskar, K Deshpande, P Gomade, D Phagiwala, R Patil, S Saka.

Funding Agency: ICMR Duration: 03 Years

Background & Objectives: RCVRDL was created in response to the recommendations of the Virology Task Force monitoring the VRDL network in India, with an objective of providing training to different categories of staff in the existing and newly establishing VRDL network, on conducting quality assurance (QA)/quality control (QC) programs as well as extending scientific and technical expertise.

Work done: During 2019-20, the RCVRDL conducted five comprehensive training programs covering the basics of bio-safety and bio-security, outbreak investigations, biomedical waste management and viral diagnosis using serological (ELISA) and molecular (conventional and real-time PCR) techniques (Table 1). The training programs were attended by 96 participants from 33 VRDLs. Results of the pre and post-tests have shown clear evidence of the effectiveness of the trainings.

Table 1: Training programs conducted during the year 2019 to 2020

Sl. No.	Topic of training	Period of training	No. of participants/ No. of VRDLs
1	Training Program for VRDLs (2019/03)	April 21-May 1, 2019	17 (05)
2	Collection of venous blood/dry blood spot samples, sample processing and transport	April 26-27, 2019	Field Staff of IMRVI Study at Dahanu, Maharashtra
3	Collection of venous blood/dry blood spot samples, sample processing and transport	May 29-39, 2019	Field staff of IMRVI Study at Agra, UP
4	Training Program for VRDLs (2019/04)	July 24-31, 2019	13 (05 VRDLs)
5	Collection of venous blood/dry blood spot samples, sample processing and transport	August 30-31, 2019	Field staff of IMRVI Study at Bhunga, Punjab
6	Collection of venous blood/dry blood spot samples, sample processing and transport	October 17-18, 2019	Field staff, IMRVI Study at Dibrugarh, Assam
7	Training Program for VRDLs (2019/05)	October 14-20, 2019	13 (04 VRDLs)
8	Capacity building in good clinical laboratory practice for laboratory site preparation for Nipah virus clinical trials	October 2019	Technical and scientific staff of ICMR-NIV, Pune
9	Fourth sub-national level training workshop on public health preparedness and response to Ebola Virus Disease	November, 2019	Rapid Response Team, Bengaluru, Karnataka
10	Collection of venous blood/ dry blood spot samples, sample processing and transport	December 2019	Bhubaneswar, Odisha
11	Training Program for VRDLs (2019/07)	Dec 11-18, 2019	19 (07 VRDLs)
12	Laboratory Training Program on Congenital Rubella Syndrome Surveillance in India	January 8-9, 2020	04 (01 VRDL)
13	Training Program for VRDLs (2020/01)	January 22-29, 2020	17 (06 VRDLs)
14	Collection of venous blood /dry blood spot samples, sample processing and transport	March 2020	Field staff of IMRVI Study at Hyderabad Chittoor (AP)

Quality Control Program for VRDLs

The RCVRDL continued to support the Quality Control Programs of VRDLs in viral diagnostics. During the period, 125 centres participated in the quarterly QA/QC program of serodiagnostic testing for 13 etiologies (Dengue, Chikungunya, Japanese encephalitis, Hepatitis A, B, C and E, Rubella, Measles, Parvo & Mumps). A total of 8211 samples were received and tested till March 2020. The average annual concordance for all the participating centres for the year was found to be 92.85% (Figure-1).

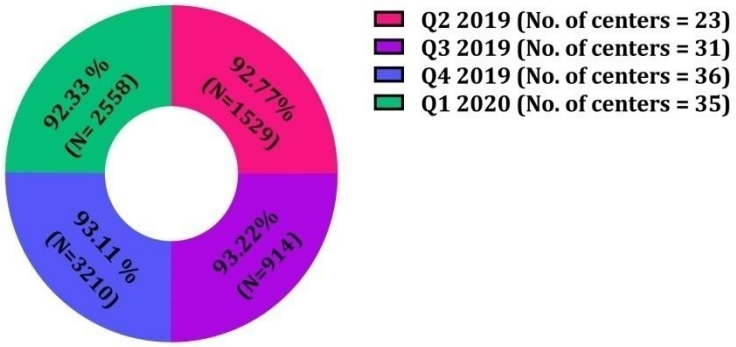


Figure-1: Data on the percentage of concordance of QA/QC programs conducted by VRDLs in comparison to ICMR-NIV data

QA/QC data for influenza by Molecular diagnostic method (Real time RT-PCR): A total of 415 samples were received from 23 VDRLs for QA/QC for influenza RT-PCR. High level of concordance was observed with ICMR-NIV study (Figure-2).

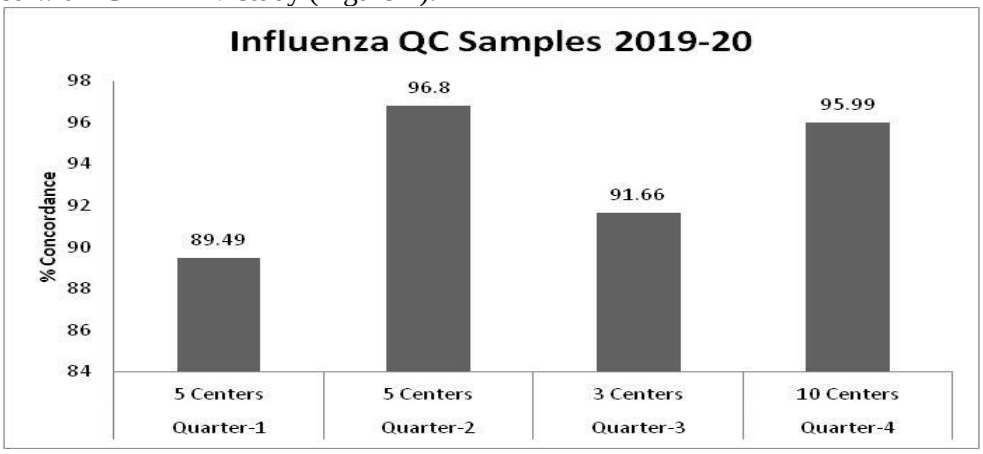


Fig 2. Quality control test results of molecular diagnosis of influenza virus by 23 VRDLs in comparison to ICMR-NIV data

External Quality Assurance (EQA) Program for VRDLs

An external quality assurance serum panel for detection of anti-Dengue, Chikungunya and Japanese encephalitis IgM by ELISA was distributed to 35 VRDLs across the country. The average concordance of 99.52 was obtained for all the three parameters.

Supply of diagnostic reagents for influenza: RCVRDL supplied reagents and test kits for influenza diagnosis to 28 VRDLs across the country, which included RT-PCR kits (23000 reactions), viral RNA extraction kits (9500 tests), viral transport medium kits (75 kits of 50 each), and primer-probe mix for 7 target genes (5750 reactions each).

i) Response to COVID-19 pandemic

a) Building laboratory capacity for COVID-19 in the country

During the early stage of the COVID-19 pandemic in India, the Group actively contributed to build National preparedness to fight COVID-19 and enhanced laboratory capacity of 10 VRDLs for COVID-19 diagnosis by shipping necessary diagnostic reagents and kits. Scientists from the Group also visited state VRDLs at Bangalore Medical College & Research Institute and Gandhi Medical College, Secunderabad and provided technical and troubleshooting assistance in COVID-19 testing, during February 2020.

b) Assistance provided for COVID-19 testing of Indian expatriates in Iran

As part of the special mission by the Govt. of India, Ministry of Health & Family Welfare and ICMR to assist testing and expatriation of the Indian nationals stranded in Iran during the COVID-19 pandemic, ICMR-NIV has deputed a team comprising six professionals. DVG group supported the move by deputing two Scientists and a Technical staff to organize collection and shipment of clinical samples from >2000 Indians citizens from various locations in Iran.

c) Development of anti-human SARS-COV-2 IgG ELISA kit

In collaboration with Maximum Containment Facility of NIV, developed anti-human IgG ELISA kit (COVID KAWACH Human IgG ELISA), and transferred the technology to Zydus Cadila, Ahmedabad and five other companies.In addition, micro-neutralization test (MNT) and plaque reduction neutralization test (PRNT) for SARS-CoV-2 were also developed subsequently. Results of both the tests were found specific and had no cross reactivity. The intra-class correlation was calculated to assess the correlation between MNT and PRNT and found to be 0.520. The PRNT assay may find application to determine the antibody titer of samples collected from NAb in recovered/vaccinated or infected COVID-19 patients while MNT may be used for large scale serosurveys.

d) Validation of serological test kits for COVID-19

During the pandemic, a pressing need was felt by ICMR to have a validation mechanism for COVID-19 test kits in the country. In view of this, the Diagnostic Virology Group developed a well-characterized sample panel for validation of the serological test kits [including Rapid Diagnostic Tests, ELISA and Chemi-luminescent Immunoassay kits for detection of IgM, IgG and total antibodies against SARS-CoV-2 from RT-PCR-confirmed cases. Till March, 2020, 07 test kits were evaluated, of which 04 Rapid Diagnostic Test kits were found satisfactory. The reports were conveyed to ICMR and the manufacturers.

DVG1606: Surveillance for Zika virus infections in India

Investigators: GN Sapkal, PD Yadav, G Deshpande, PT Ullas , RS Gunjikar, H. Shaman, C Patil.
Funding agency**:** DHR/ICMR Duration**:** 03 years

Background: to bolster the national preparedness against Zika virus (ZIKV) disease, the RCVRDL has been assigned as the apex laboratory for coordinating ZIKV surveillance activities in the country.

Objectives: Enhanced surveillance of Zika virus (ZIKV) based on:

(i) Zika virus disease symptoms and case history in retrospective and prospective samples
(ii) Congenital Zika Virus Syndrome including microcephaly in infants

Major findings: Following the September 2018 outbreak of Zika virus disease in Jaipur, active surveillance for ZIKV was undertaken in Rajasthan by SMS Medical College, Jaipur and other trained laboratories and screened 17817 serum and 1645 urine samples. A total of 832 samples were tested for anti-ZIKV IgM and IgG antibodies using commercial kits. Based on the results of IgM and IgG tests, 60

serum samples were subjected to PRNT for Zika virus of which 21were found Zika positive while 28 were flavi cross reactive and 11 were negative.

Cohorts for Zika Epidemiology in India
Investigators: GN Sapkal, T Bhatnagar B Malhotra, G Deshpande, PT Ullas,
Funding agency: ICMR **Duration:**3.5 years

***Background*:** A prospective cohort study was planned to understand the incidence and extent of fetal abnormalities as well as maternal clinical presentations among pregnant Indian women carrier of Zika virus and their uninfected counterparts. This prospective study was initiated within 6 months after the Zika outbreak in Jaipur in 2018.
Objective: To estimate the risk of adverse fetal outcome in ZIKV infected women compared to uninfected pregnant women.
Work done: Two cohorts were identified. Cohort 1 comprised of pregnant women who delivered their babies after a confirmed Zika infection during the 2018 outbreak while the second Cohort consisted of pregnant women at any stage of pregnancy regardless of trimester residing anywhere in Jaipur, irrespective of Zika infection or previous medical and obstetric history. Urine and blood samples collected from the pregnant women and newborns were tested for Zika virus by RT-PCR or anti-ZIKV IgM and IgG antibodies by ZIKV PRNT. To rule out other etiologies resulting in similar clinical presentations, sera samples were also tested for dengue, chikungunya, Japanese encephalitis, toxoplasma, Varicella Zoster virus, rubella, Herpes Simplex Virus 1 and 2, and syphilis by ELISA. Reports of live birth, still births, miscarriage and intrauterine deaths were also noted.
Findings: A total of 119 pregnant women in their first trimester with unknown Zika status were enrolled till September 2019. Follow up was conducted for 95 of the women in this cohort, of whom, 77% were in the second trimester and 18 were in third trimester. In Cohort 1 (n=95), 10 women were found to have confirmed ZIKV infection by $PRNT_{90}$, of whom 9 had typical symptoms, *viz*., nausea, muscle pain, abdominal pain headache etc. Approx. 20-25% of the cases in Cohort 1 tested positive for dengue IgM; 5-28% for JE IgM and a high positivity for Chikungunya IgM. IgM antibodies to rubella were found in approx. 10% of the cohort, while a very low rate of infection was noted for HSV1, HSV2 and CMV. None of the samples tested positive for syphilis.

In the cohort 2 (n=77), five patients tested positive for ZIKV by $PRNT_{90.}$ Of these, dual infections of Zika and dengue and Zika and chikungunya were found in 2 patients each. Seropositivity for dengue IgM antibodies was noted in 20-22% of women while >50% tested positive for chikungunya IgM. Sera of 18-22% of women tested positive in JE IgM ELISA, while 2% was positive for rubella IgM; 4, 2 and 1% showed reactivity for antibodies to CMV, HSV2 and toxoplasma respectively. None of the women tested positive for HSV-1or syphilis.

Twenty-five babies were enrolled in the newborn cohort. One baby, diagnosed with colorectal malformation died 3 days later. Urine and sera samples of all babies tested negative in Zika RT-PCR, while one sample each tested positive for anti-Zika IgM and IgG antibodies. One baby, enrolled at 3 months of age, tested positive for anti-Zika IgM and in Zika $PRNT_{90,}$ and showed thinning of corpus callosum and mild splenomegaly. Another baby delivered prematurely, tested positive in Zika $PRNT_{90}$, had imperforated anus, developed jaundice and died 2 days within birth. Two babies with microcephaly, born to mothers residing in other areas of Jaipur, were recruited into Newborn Cohort 2. The babies and their mothers tested negative for ZIKV while one baby developed jaundice and died one month later.

Laboratory investigations undertaken

Sl. No.	Test conducted	No. of samples tested
1.	CDC Trioplex Real Time PCR	706
2.	Zika IgM ELISA	706
3.	Zika IgG ELISA	706

4.	Dengue IgM Capture ELISA	414
5.	Chikungunya IgM Capture ELISA	319
6.	JE IgM Capture ELISA	237
7.	Rubella IgM ELISA	85
8.	HSV-1	5
9.	HSV-2	8
10.	CMV	22
11.	VZV	33
12.	$PRNT_{90}$ (Zika)	283
13.	$PRNT_{90}$ (Dengue)	283

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

Investigators: GN Sapkal, BV Tandale, G Deshpande & PT Ullas
Co-investigators: N Gupta, L Sangal, M Murhekar, M Ahmad, K Hayford, A Shete, W Moss, J Lessler, J Metcalf, M Ferrari.
Funding agency: ICMR (Extramural)/Johns Hopkins University **Duration: 2017-2020**

Background: A community-based, cross-sectional sero-survey was undertaken among different age groups to estimate the immune status of the population to measles and rubella viruses in districts with MRHRUs and other priority areas. Also, a facility-based sero-survey from participants in a similar population (e.g., a representative convenience or easy access sample such as children at the outpatient department) will be assessed as control population.

Objectives:

a. 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.
b. To compare the accuracy, precision and cost of estimating the age-specific measles and rubella population immunity using convenience samples from health care facilities versus community-based serosurveys.

Work done and Findings

a. Four dried blood spots (DBS) were evaluated for stability in comparison to blood collection and transportation using Hemaspot HF. Field studies conducted using DBS devices in Ghatampur (UP) and Dahanu (Maharashtra) along with paired sera had shown a strong correlation of test results for IgG antibodies against measles (R^2 =0.94) and rubella (R^2 =0.89) between the paired samples.
b. During the period, 4508 serum and 646 dried blood spot (DBS) samples were tested for IgG antibodies against measles and rubella. Screening of post Measles-Rubella vaccination samples from Dahanu (Maharashtra), Ghatampur (UP), Bhunga (Punjab) and Dibrugarh (Assam) have shown 84.77 and 80.02% IgG seropositivity rubella and measles respectively across all age groups.

DVG 1604 Congenital Rubella Syndrome (CRS) Surveillance in India
Investigators: GN Sapkal, R Viswanathan, S George, V Jagtap, O Kaduskar
Funding Agency: ICMR **Duration**: 05 years
Background: In view of the proposed introduction of rubella vaccine in the national immunization program in the country, it was proposed to establish surveillance for CRS, to provide a baseline estimate of disease burden and to help monitor the impact and progress made by rubella vaccination. Based on the guidelines outlined in the strategic plan for Measles and Rubella elimination in south East Asia Region,

six sentinel sites were established in India. Periodic sero-surveys among pregnant women attending selected antenatal clinics in areas where MMR vaccine is in use is also envisaged as part of the strategy.

Objectives:

(i) 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.

(ii) To conduct periodic serological surveys to monitor the rubella sero-surveillance among pregnant women over the time.

Findings:

Quality Control testing of 623 serum samples received from 8 sentinel sites showed 89.7 and 90.3% concordance for anti-rubella IgM and IgG antibodies respectively. Of the 550 throat swabs received from suspected rubella patients, 50 tested positive in diagnostic rubella RT-PCR. Of the 10 follow up samples all were found positive by diagnostic PCR. Annual proficiency testing panels for rubella IgM and IgG ELISA were sent to all sentinel sites while rubella PCR panels were sent to 4 sites. A score was assigned to each centre based on their performance. All except one centre scored ≥90%.

Awards/recognition received by members of the Group

Dr. Ullas PT, Scientist 'B', received a medal and certificate of appreciation from the Director General-ICMR and Secretary-DHR, for the services rendered during the 2019 Nipah outbreak in Kochi, Kerala.

NIV Field Units

BANGALORE UNIT
Annual Report - April 2019 - March 2020

Staff list

Scientific staff	
Dr. Ashok M	Scientist-B & Officer-In-Charge
Technical staff	
Mr. Hanumaiah	Principal Technical Officer
Dr. D. P. Sinha	Technical Officer-A
Mr. Jayaprakash H	Technical Officer-A
Mr. Madhava Rao	Technical Officer-A
Mrs. Asia Thounaojam	Technical Officer-A
Mr. Manjunatha MJ	Senior Technician-II
Mr. Raju M	Laboratory Assistant
Mrs. Prema BM	Senior Technician-I
Mr. Thippeswamy B	Senior Technician-I
Mr. H.M.Muninarayanappa	Laboratory Assistant
Mr. B.N. Wakde	Technician-A
Mr. Kunal Sakhare	Technician–A
Mr. Naveen G	Technician–A
Supporting Staff	
Mr. Vybhav Bichkule	MTS
Mr. Arjun Jogangari	MTS (Maintenance)
Mr. R.Basavaraju	Assistant
Kum. J.Jayajyothi	Assistant
Mr. Mahajan	Driver
Project staff	
Mr. Basavraj H. M.	Technical Assistant
Ms. Senthil kumar	DEO
Mis. Jenevi	Lab Technician-C
Mis. Mala D	Lab Technician-C
Mr. Kiran Kumar	Lab Technician-C
Mr. Madhu	Lab Technician-C
Mis. Niveditha	Lab Technician-C
Mis. Krishnaveni	Lab Technician-C
Mis. Shravya	Lab Technician-C
Mis. Nandini	Lab Technician-C
Mr. Dilip	Lab Technician-C
Mrs. Garima	Lab Technician-C
Mr. Santosh	Lab Technician-C
Mr. Geerisha	Lab Technician-C
Security – **06 and Housekeeping - 04**	

BNU9702: Surveillance of Acute Flaccid Paralysis (AFP) cases from Karnataka and Kerala states and southern parts of Bihar as a part of WHO-SEAR Polio Lab Network in the WHO's Global Eradication of Poliomyelitis Programme.
Ashok M, Hanumaiah, D P Sinha
Funding Agency – World Health Organization/GOI Duration – In service project (since 1997)

Background: Acute Flaccid Paralysis Surveillance for Global Eradication of Poliomyelitis Programme was initiated by Govt. of India in collaboration with WHO/WHO-SEARO/WHO-NPSP. As part of this program, India have one specialized and seven national laboratories and NIV-BU is one of the National Polio Laboratories (NPL). Since 1997, NIV-BU is playing an important role in polio eradication by laboratory investigation of AFP samples from Karnataka, Kerala, Uttar Pradesh and Bihar.
Objectives: To interrupt Polio virus transmission
Findings: During April-2019 to March-2020, 6386 samples were received from Bihar (n-3943, 62%), Karnataka (n-1679,26%) and Kerala (n-739,12%). Only RD cell line positives that is NPEV accounts for 13.5% (n-864). L20B cell line positive accounts for 3.2% (n-208). Of the 208 L20B positive samples, 199 samples positive for Sabin like 1 & 3, seven samples were NPEV by PCR and remaining two samples were NEV. 97.3% of samples were reported within 14 days of received date.

2. Environmental Surveillance of Polio Virus
Environmental Surveillance of sewage samples from Bangalore city as a part of WHO-SEAR Polio Lab Network in the WHO.
Investigators: Ashok M, Hanumaiah, D P Sinha, Jayaprakash
Funding Agency – World Health Organization/GOI
Project duration – Since January 2019

Background: Environmental Surveillance of Polio virus in sewage areas is a part of the Global Eradication of Poliomyelitis Programme. In Bangalore city four sewage sites have been identified for this purpose.
Objectives: To identify Polio virus in sewage plant in Bangalore city.
Findings: During April 2019 to March 2020, the unit received 105 sewage samples from four sewage zones of Bangalore city. From the samples received, 80% (n-84) were positive only in RD cell line and the remaining 20% (n-21) is L20B cell line positive. Among 21 L20B positive samples, 14 samples were found positive for Sabin like 1 & 3, while five samples were NEV positive and remaining two were NPEV positive by PCR.

BNU0603: Surveillance of Measles cases from Karnataka and Kerala State, as a part of WHO-SEAR Measles Laboratory Network in the WHO's Global Measles Elimination Programme.
Investigators – Ashok M, Hanumaiah, Manjunath MJ, D P Sinha
Funding Agency – World Health Organization/GOI Duration – In service project (since 2006)

Background: Measles & Rubella infection is a vaccine preventable disease. India is one of the country with largest number of measles & Rubella cases at the global level. In an effort to address the high disease burden caused by measles in India, WHO-SEAR Measles Laboratory Network (NML) and MeaslesNetIndia network was established and it is being expanding in phase manner. Currently there are 19 functional WHO-SEAR laboratories in India. Among them NIV, Bangalore unit is one of the national laboratory.
Objectives: To eliminate Measles & Rubella
Findings: During April-2019 to March-2020, 1200 serum samples were received from Karnataka state. Of them 156 samples were positive for Measles IgM antibodies and 50 samples were positive for Rubella IgM antibodies. 93 throat swab and 86 urine samples were received and tested for Measles and Rubella

PCR, of them 12 samples from throat swab and five samples from urine was found to be positive for Measles RNA. On sequencing D8 genotype was reported from all 17 positives samples.

BNU8801: Surveillance of dengue, chikungunya and Japanese encephalitis cases from Bangalore city, urban, rural and neighbouring areas under NVBDCP.
Investigators – Ashok M, Manjunath MJ, Hanumaiah
Funding Agency – GOI (NVBDCP) Duration – In service project (since 2010)

Background: The National Vector Borne Disease Control Programme (NVBDCP) is an umbrella programme for prevention and control of vector borne diseases. The program covers six diseases of which, Bangalore unit has been assigned the surveillance of Japanese encephalitis (JE), dengue, and Chikungunya. For dengue and chikungunya there are 31 sentinel surveillance centers in Karnataka, apart from NIV BU.
Objectives: Serological investigation of dengue and chikungunya viruses from urban and rural areas of Bangalore district.
Findings: A total of 3461 samples were screened and detected 1104 samples positive for dengue and 390 for chikungunya. Dual infection was detected in 246 samples and 12 showed anti JE IgM antibodies (Table 1). Screening of 74 JE suspected samples by real time PCR revealed two samples positive for JEV RNA.

Table1: details of samples analysed for JEV, DEN and CHIK IgM antibodies

Sl No	Virus	IgM antibodies positive
1	Dengue	1104
2	Chikungunya	390
3	Dengue & Chikungunya	246
4	JE	12

Molecular testing of Mosquito pools:

Approx. 50 *Aedes aegypti* mosquito pools received from six districts of Karnataka were analysed by NGS. A new insect specific virus, *i.e*., Phasi Charoen-Like Phasivirus (PCLV) was identified (Table 2). Twenty-six pools tested positive for PCLV. The medical importance of this virus in India is to be investigated.

.

Sl No	No. of Mosquitoes	No. of pools	Districts	Chik RT PCR positive	Den RT PCR positive	PCLV positive	PCLV+DENV +CHIKV positive
1	68	4	Bangalore Rural			1 pool	
2	113	9	Bangalore Urban	2 pool		4 pool	
3	218	15	Chikkaballapura	2 pool	4 pool	10 pools	DENV + PCLV CHIKV +PCLV
4	156	12	Kolar	1 pool	2 pool	4 pool	CHIKV +PCLV
5	81	6	Ramanagara			4 pool	
6	60	4	Tumakuru		1 pool	3 pool	

BNU1604: Congenital Rubella Syndrome (CRS) Surveillance.
Investigators – Ashok M, Hanumaiah, Manjunath MJ
Funding Agency – ICMR
Project code –
Project duration – Ongoing project (since October 2016)

***Background*:** Rubella infection is a vaccine preventable disease. In India, reliable estimates of CRS burden are not available. For country like India, 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 0-11 months of age with CRS attending health facilities and testing these infants for the rubella infection.
Objectives: To establish a facility-based surveillance for CRS in selected medical colleges/hospitals in different parts of the 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.
Findings: 72 samples fulfilling case definition for CRS were sent from Indira Gandhi Institute of Child Health hospital. Four cases were positive for IgG antibodies and seven samples were equivocal for IgM antibodies. 66 throat swabs were received for Rubella RT PCR testing, of them one sample was positive for Rubella RNA.

Laboratory investigation of Severe Acute Respiratory Infection (SARI) cases.
Investigators – Ashok M, Manjunath MJ, Hanumaiah
Funding Agency – NIV
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 [ARDS]), 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.
Objectives: To investigate SARI cases using real time RT-PCR for 13 viruses
Findings: 530 samples of SARI were received for Influenza A H1N1 PCR testing. Influenza A RNA was positive for 64 samples, of them 60 samples were H1N1 subtype and four samples were H3N2 subtype. 19 samples were Influenza B RNA positive; of them all were Victoria subtype. Two Influenza A samples was untypable for either H1N1 or H3N2 subtypes. One sample was found to have H1N1 allelic discrimination.

Human surveillance of Zika virus.
Investigators –Ashok M, Manjunath MJ, Hanumaiah
Funding Agency – ICMR
Project duration – Ongoing project (Since January 2018)

Background: VRDLN played a significant role in identification of first Zika virus report in our country from available arbo viral samples at the various laboratories. Later the health authorities wanted to explore and identify the hidden ongoing Zika virus infection in our country. So Human surveillance of Zika virus from available arbo viral samples at various VRDL was initiated.
Objectives: Laboratory screening of Zika virus from acute febrile illness samples
Findings: 638 samples were tested for Zika virus real-time RT PCR. None of the samples were positive for Zika RNA.

Laboratory diagnosis of human samples for KFD virus infection
Investigators – Ashok M, Diamond Prakash, Thippeswamy, Manjunath MJ, Hanumaiah
Funding Agency – NIV, Pune
Project duration – Ongoing project (Since December 2018)

Background: Kyasanur Forest Disease (KFD) or Monkey fever is a zoonotic disease caused by KFD Virus. This disease was first discovered in 1957 from Kyasanur forest area, Shivamogga district of Karnataka state in southern India. It is transmitted to man by bite of infected ticks. KFD infection is confirmed in laboratory by KFD IgM antibodies by ELISA and by real-time RT-PCR test, which is first line of test to confirm the infection. The human samples from various districts of Western ghats in Karnataka state was received and tested from December 2019 to March 2019.
Objectives: Laboratory diagnosis for suspect for KFD infection among human.
Findings: 351 samples were received for KFD testing from Shivamogga district. 21 samples were positive for KFD RNA and 12 samples were positive for KFD IgM antibodies. Among KFD negative samples we found that 35 samples were positive for Chikungunya IgM antibodies, 17 samples were positive for Dengue IgM antibodies and 13 samples were positive for both Dengue and Chikungunya IgM antibodies.

9. **Virus Research Diagnostic Laboratory Network projects**
Investigators – Ashok M, Diamond Prakash, Manjunath MJ, Thippeswamy
Funding Agency – DHR **Project duration –** 2019

Background: Department of Health Research (DHR) and Indian Council of Medical Research (ICMR), Government of India, have established Virus Research and Diagnostic Laboratory Network (VRDLN) to strengthen the laboratory capacity in the country for providing timely diagnosis of disease outbreaks. This network was established for enhancing India's capacity to diagnose and detect viruses of public health importance. VRDLs, which follow a uniform protocol for laboratory testing, for various viral aetiologies (hepatitis: hepatitis A, B, C and E; arboviruses: Japanese encephalitis, West Nile, dengue, chikungunya, Chandipura virus and Kyasanur Forest Disease; respiratory viruses: influenza, parainfluenza, RSV, adenovirus, rhinovirus; fever with rash: measles, rubella, varicella zoster, mumps and parvovirus B 19; herpesvirus family: EB virus, herpes simplex virus and cytomegalovirus; enteric viruses: rotavirus, enteric adenoviruses, norovirus and astrovirus).
Objective: (1) Create infrastructure for timely identification of viruses and other agents causing morbidity significant at public health level and specifically agents causing epidemics and/or potential agents. (2) Develop capacity for identification of novel and unknown viruses and other organisms, emerging and re-emerging viral strains and develop diagnostic kits.

Findings of projects that were in collaboration with VRDLN network are

Sl No	Projects	PCR test	ELISA test
1	Monitoring of dengue and chikungunya viruses circulating in India for changes in the serotypes, genotype and lineages utilizing Viral Research & Diagnostic Laboratories Network	Tested - 119 CHIKV – 16, DENV – 28 Serotype D1 – 7, D2 – 8, , D3 – 4, D4 - 1	
2	Testing for Scrub Typus for Acute Encephalitis Syndrome and Fever Rash Syndrome (FRS)		Tested – 238 IgM - 18
3	Testing for Leptospirosis for acute febrile illness		Tested – 89 IgM - 5

SARS CoV-2 diagnosis
Investigators – Ashok M, Manjunath MJ, Thippeswamy, Hanumaiah
Funding Agency – NIV & Govt of Karnataka
Project duration – Ongoing project (Since February 2018)

Background: A novel corona virus causing respiratory illness was first noted and confirmed in India on 29th February, 2020 in Kerala state and within couple of months' cases were reported all over the country. The virus is designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is transmitted through respiratory tract and causes Coronavirus Disease (COVID-19). The transmission mode and clinical presentation is similar to Influenza A virus infection, and both these viruses are prevalent all over the country. Since 3rd February 2020 we started testing for COVID-19 and providing report to the Karnataka state. From March we were identified as regional depot for the state. From April we were recognized as quality assessment laboratory for our state.
Objective: **i)** To provide timely PCR diagnosis for SARS CoV-2 suspect cases in the state. ii) To distribute PCR and RNA reagents for the state ICMR labs. iii) Quality assessment laboratory for the state level COVID-19 testing laboratories.

Findings: Diagnostic testing of SARS CoV-2 real-time RT PCR for Karnataka state
NIV BU was one of the 14th COVID-19 laboratories in our country that were initiated in February 2020. Our unit supported Karnataka state in timely providing COVID-19 real-time RT PCR diagnostic reports. Till date we tested 1195 suspect COVID cases and confirmed 33 by PCR. We mainly tested samples from Bangalore city and almost all the districts of the Karnataka state. All received samples are tested and reported within 24 to 48 hours to the concerned hospital or district surveillance officers of the state.
10.2. Regional Depot for Karnataka state
Since March 2020 our unit was identified as regional depot by ICMR HQ. Total of 2.7 lakh PCR reaction kits, 1.91 lakh reactions of RNA extraction kit and 0.5 lakh VTMs were distributed to 34 laboratories in the state.
10.3. Quality assessment of SARS CoV-2 real time RT PCR for Karnataka state.
Our unit is identified as the State Referral laboratory for Karnataka state. We perform quality assessment on quarterly basis for both government and private laboratories in the Karnataka state.

NIV KERALA UNIT

Scientific staff

Dr. A. P. Sugunan	Scientist-G and Officer in-Charge
Dr. B. Anukumar	Scientist-E
Dr. Retheesh Babu G	Scientist-D
Dr. R. Balasubramanian	Scientist-D

Technical Staff

Mr. Bestin Payyapilly	Tech. Officer-A
Mrs. Sreelekha K. P.	Senior Technician I
Ms. Kunjila Konikkara	Technician-C (TS)
Mr. Nikhil T. L.	Technician-B (TS)
Mrs. Amal Mol Peter	Technician-B (TS)
Mr. Jijo Koshy	Technician-B (TS)
Mrs. Shyma V. S.	Technician-II (TS)
Mr. Santosh Kumar Botsa	Technician-A (ES)
Mr. Uma Ganesh Pentakota	Technician-A (ES)
Mr. Vikrant Talape	MTS (TS)

Administrative staff

Mrs. Mangala Gangadharan	UDC
Mr. Prashant D. Patil	LDC
Mr. P. B. Santhoshkumar	LDC
Mr. Govindsing Patil	Staff Car Driver (OG)

Project staff

Dr. Devaki Antherjanam S.	Scientist-C (Medical)
Dr. Rajalakshmi Aiyappan	Scientist-B (Medical)
Dr. Chingtham Santhalembi	Scientist-B (Non-Medical)
Ms. Sahina S.	Women Scientist (DST Project)
Mr. Mohammed Shafi M. A.	Technical Officer-A (Social Science)
Mr. Krishna Sarma S.	Senior Investigator (Social Science)
Ms. Rooth P. John	Senior Investigator (Social Science)
Mr. Abhijith A. K.	Tech. Assistant (Social Science)
Ms. Aishwaryan Babu G.	Technical Assistant (Lab)
Mr. Basil A. Baby	Technical Assistant (Lab)
Ms. Shilpa L. S.	Technical Assistant (Lab)
Ms. Soundarya R. H.	Technical Assistant (Lab)
Ms. Rolbi Merlin	Technical Assistant (Lab)
Mr. Vivek Vijay	Technician-III
Ms. Nisari Babu	Technician-III
Ms. Feba K. Roy	Technician-III
Mr. Jithu Sreekumar	Technician-II
Ms. Moomina V. M.	Technician-II

KFU1801: Generation of an RNA vaccine candidate that protects the Chandipura virus challenge in mice.

Authors: Dr.B.Anukumar & Aishwarya Babu

Funding Agency: DHR Duration: 2019-22

***Background*:**

Chandipura virus (CHPV), a *Vesiculovirus*, belonging to family *Rhabdoviridae*, is an emerging tropical pathogen in India which inflicts high mortality in children with a case fatality rate of 55-75%. Neither specific antivirals nor vaccines are available to date against the virus. Amongst the different vaccine platforms available today, RNA vaccines have emerged popular due to their unique characteristics, *viz*., safety and immunogenicity.

***Objectives*:**

To develop glycoprotein (G) gene based RNA vaccine against Chandipura virus and to test its immunogenicity and potency in mice.

Work done and Findings:

Chandipura virus Glycoprotein (G) gene was successfully amplified using synthesised primers, cloned into pGEMT-Easy vector and subsequently to pGEM3Z(+) which has T7 promoter for *in vitro* transcription. *In vitro* transcription reaction was followed by Poly A tailing to add 150 base poly (A) to the RNA transcripts generated from the reaction. RNA transcripts were purified to remove unincorporated nucleotides and transfected into Vero cells and analysed for CHPV glycoprotein expression by western blot and immunofluorescence assay. Western blot probed with anti CHPV polyclonal antibody showed a band of expected size (~ 60 kDa). Maximum expression was observed at 48 hours in transfected cells when probed with anti CHPV polyclonal sera and FITC tagged secondary antibody (Fig 1). The synthesised mRNA will be used for vaccine study.

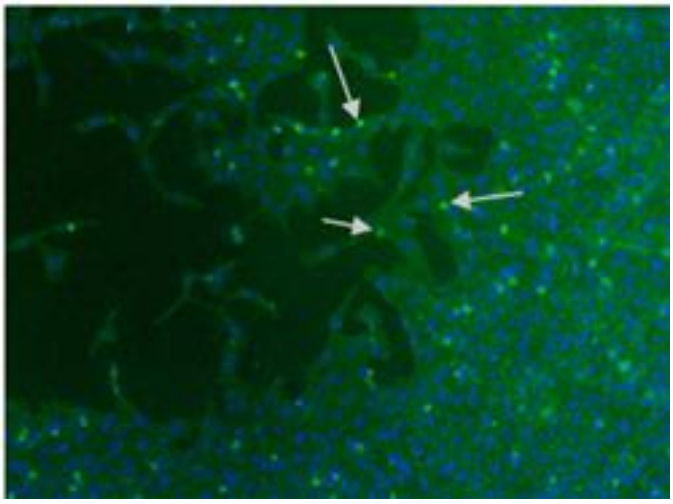


Figure 1: Vero cells transfected with CHPV G gene mRNA showing fluorescence suggestive of expression of CHPV glycoprotein

KFU 1201: Trafficking of Chandipura virus in neuronal cells

Authors: Vishal K Kavathekar & Dr. B. Anukumar

Funding Agency: NIV Duration: 2014-20

Background: Information on viral-host interactions allow the thorough characterization of viral life cycle and reveal important information that could be targeted for anti-viral drug therapy. So far, no significant effort has been made to understand CHPV interactions with neurons and its neuronal transmission.

Objectives: To profile the interaction of whole virus and individual protein components of CHPV with the neuronal proteins to deduce molecular events involved in CHPV replication.

Findings: Recombinant N-protein bound resin was used for pull-down assay to identify the cellular proteins from Neuro2a lysate interacting with CHPV-N protein. The bound proteins were separated on SDS-PAGE and analyzed by mass spectroscopy. The Q-TOF LC/MS analysis of bound proteins identified 17 proteins. Out of which ELAV like protein 2, heat shock cognate 71(HSC71), polyadenylate binding protein interacting protein 1 (PABP 1), 40S ribosomal protein SA (RPSA) and cytolasmic actin were selected for further analysis as these proteins were found involved in the life cycle of other viruses. In the CHPV infected Neuro2a cells, no significant change was observed in mRNA expression level of ELAV like 2 protein, PABP and RPSA protein up to 12 hr post infection (PI). However, expression of HSC71 and actin mRNA was found to be elevated after 10 hr and 12 hr PI respectively. Confocal microscopy confirmed the interaction of HSC71 and actin with N protein in virus infected cells (Fig 2).

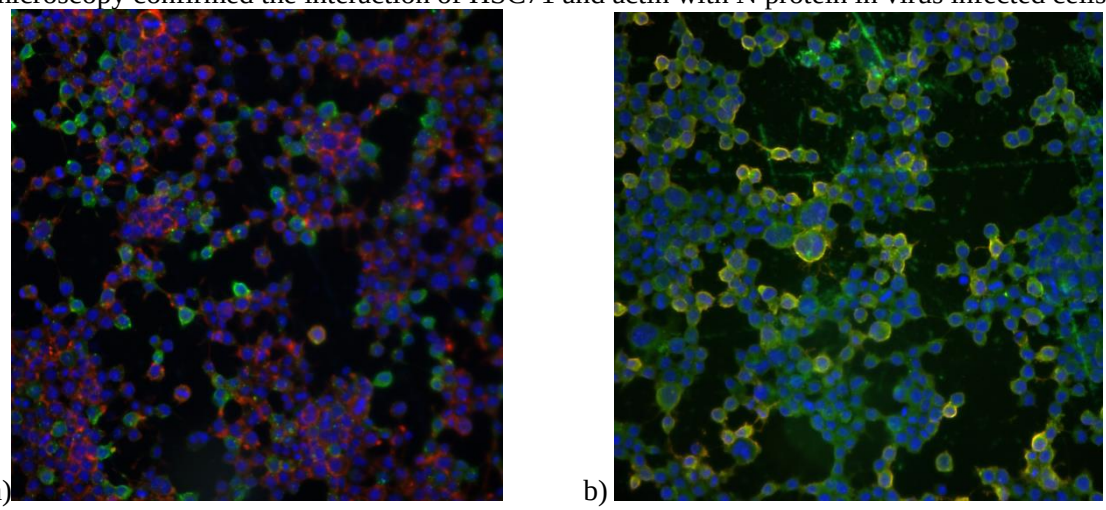


Figure 2. Confocal micrographs of Chandipura virus infected Neuro 2A cells. a) CHPV infected cells probed with anti CHPV N and anti actin antibodies. b) Infected cells probed with anti CHPV N and anti HSC71 antibodies.

KFU1401: Potential species distribution and detection of Kyasanur Forest Disease virus in tick population in forest areas of Kerala

Authors: R. Balasubramanian, Pragya Yadav, Prathius P.R

Funding Agency: DHR Duration: 2016-2019

***Background*:** Kyasanur Forest Disease (KFD) is a viral zoonotic disease which is endemic in many areas of Western Ghats of India. A new focus of virus activity was reported from tribal area of Wayanad and Malappuram districts of Kerala since 2013. Hematophagus ticks are the known vectors for the virus. A study was therefore designed to investigate the potential ticks responsible for virus transmission in the two districts.

Objectives:

(i) Surveillance of ticks to determine the distribution and relative abundance at selected forest sites in Wayanad and Malappuram districts of Kerala

(ii) To determine KFD virus presence in ticks using RT- PCR methods and virus isolation

(iii) To generate a spatial distribution map of host-seeking ticks present on vegetation.

***Findings*:** A total of 29372 ticks belonging to 11 species under five genera were collected during 148 visits to Wayanad district. Similarly, 5062 ticks belonging to 10 species in four genera were collected from Malappuram district during 50 field visits. *Hemaphysalis spinigera* and *H. turturis* were the predominant species collected and both together constituted 90% and 88% of the total collection in the two districts respectively (Table 1). Screening of 675 pools from Wayanad and 129 pools from Malappuram district yielded 20 and four pools positive for KFDV RNA respectively. Tick abundance was

found highest during December to February months. Temperature range of 28ºC to 30ºCalongwith relative humidity of 60-80% is found favorable to support questing nymph tick density.

Table.1. Tick species collected from Wayanad and Malappuram district during the study period from December 2016 to 2019

Sl. no	Tick species	Wayanad	Malappuram
1	*Amblyomma integrum*	806(2.74)	255 (5.03)
2	*Dermacentor auratus*	1(0.003)	0
3	*Haemaphysalis bispinosa*	1671(5.68)	276 (5.45)
4	*H. cuspidata*	8(0.02)	6 (0.11)
5	*H. kyasanurensis*	62(0.21)	10 (0.19)
6	*H. spinigera*	16319(55.55)	2400 (47.41)
7	*H. turturis*	10423(35.48)	2097 (41.42)
8	*H. wellingtoni*	18 (0.06)	0
9	*Hyalomma anatolicum*	32(0.10)	2 (0.03)
10	*Rhipicephalus microplus*	10(0.03)	2 (0.03)
11	*R. sanguineus*	22(0.07)	13 (0.25)
Total		**29372**	**5062**

KFU1602: Impact of climate change on mosquito abundance in coastal brackish water and etlands of Alappuzha district Kerala

Authors: Dr. R. Balasubramanian

Funding: DST-SERB, New Delhi Duration: 2017-2020

***Background*:** Kerala had experienced massive outbreaks due to Japanese encephalitis and West Nile viruses during the last decade. Mosquito vectors of both the viruses are highly prevalent in the district. A study was therefore proposed to determine the temporal distribution, spatial pattern and abundance of vector mosquitoes in the coastal areas of the district with special emphasis on virus detection/isolation.

Objectives

(i) To determine the temporal distribution, spatial pattern, salinity tolerance of JE and WN virus vectors in relation to environmental factors

(ii) To screen the mosquitoes for JE and WN virus as well as to study the biology of immature mosquitoes under different salinity parameters

Findings:

A total of 81696 mosquito larvae belonging to five genera were collected, *viz*., *Anopheles* (2.32%), *Culex* (93.8%), *Aedes* (1.23%), *Armigeres* (0.58%) and *Lutzia* (0.33%) (Table-2). In the coastal areas *Cx. sitiens* (69.48%) was the predominant species encountered and they survived at an average pH of 6.4 with 25 PPM of salinity. However, other important vector mosquitoes, *viz.*, *Cx tritaeniorhynchus* and *Cx gelidus* survived only at an average pH of 7.6 to 8.3 and a salinity range from 0 to 7 ppt. During the year, 60, 963 female mosquitoes were collected and identified. Culex was the predominant genus (82.07%) collected during the study followed by *Mansonia* 10.44%, *Armigeres* 3.96%, *Anopheles* 3.4% and *Aedes* .08%. Among the genus Culex, *Cx. tritaeniorhynchus* was the predominant species collected with 65.8%.

Table-2. Species composition and abundance of mosquitoes of Alappuzha district from March-2017 to December-2019

l. No	Mosquito species	Immature		Adult	
		Coastal	Rice field	Coastal	Rice field
1	*Aedes albopictus*	452 (0.78)	0	8(0.04)	0
2	*Ae.vexans*	0	0	29(0.14)	14(0.034)
3	*Ae.vittatus*	560 (0.97)	0	2 (0.01)	0
4	*An. barbirostris*	47 (0.08)	1	11(0.05)	8(0.02)
5	*An.peditaniatus*	62 (0.10)	104(0.43)	126(0.63)	1828(4.47)
6	*An. philipiniensis*	0	0	5(0.02)	6(0.01)
7	*An. subpictus*	1658 (2.87)	27 (0.11)	0	89 (0.22)
8	*An. vagus*	1	0	1 (0.02)	0
9	*Ar. subalbatus*	16	460 (1.91)	1603 (8.00)	812(1.98)
10	*Cx.bitaeniorhynchus*	2	70 (0.29)	0	15(0.04)
11	*Cx. crossipes*	0	0	42 (0.21)	7(0.02)
12	*Cx. gelidus*	3642 (6.32)	1245 (5.17)	1885 (9.41)	3357(8.20)
13	*Cx. infula*	0	0	16 (0.08)	36(0.09)
14	*Cx. quinquefasciatus*	1431 (2.48)	0	3166 (15.81)	854(2.09)
15	*Cx. pseudovishnui*	31 (0.05)	0	32 (0.16)	0
16	*Cx. sinensis*	0	0	53 (0.26)	0
17	*Cx. sitiens*	40035 (69.48)	0	376 (1.88)	22(0.05)
18	*Cx.tritaeniorhynchus*	8258 (18.10)	21933 (91.08)	10109 (50.48)	30010(73.31)
19	*Cx. vishnui*	1391 (2.41)	0	43 (0.21)	14(0.03)
20	*Mansonia annulifera*	0	0	1008 (5.03)	896(2.19)
21	*Ma. indiana*	0	0	637 (3.18)	1312(3.20)
22	*Ma. uniformis*	0	0	862 (4.30)	1652(4.04)
23	*Lutzia fuscanus*	29 (0.05)	241 (1.0)	0	1(<0.1)
	Total	**57615**	**24081**	**20026**	**40937**

KFU 1701: Public trust in vaccine: A qualitative study on the determinants of acceptance and hesitancy towards JE vaccines in various Blocks in Alappuzha District

Authors: Dr Retheesh Babu G, Rooth P John & Krishna Sarma

Funding Agency: ICMR Duration: 2020 to 2022

Background:

Vaccinations have a significant role in the history of public health. Though Universal Immunization Program in was launched in 1985, full immunization coverage has been a mirage and the current coverage is estimated to be around 65 per cent. Japanese encephalitis virus (JEV) is the most common agent causing epidemic encephalitis in India. Alappuzha district despite being endemic for JEV, has >300 unimmunized children below the age of five unimmunized against JEV and other viruses.

Objectives:

(i) To analyse the underlying determinants of JE and other vaccine hesitancy among parents and to understand the decision pathways, explore peer-to-peer communication influence in vaccine hesitancy. **(ii) T**o understand the perspective of stakeholders towards vaccination hesitancy as a public health challenge.

Findings:

The study is exploratory by nature. Data was collected from households who are not keen to vaccinate their children with the help of Block Panchayats in Alappuzha district using Key Informant Interview, In-depth Interview and focus group discussion.

The project team created a database of over 400 articles that included previous studies, journal articles, reports, research papers and systematic reviews. After review, the database was classified into different groups considering the methodology, geographical context and theme discussed in the studies. Based on the literature review, data collection tool was prepared. The recent data of non-immunized children were collected from DMO, Alappuzha. The initial work for primary data collection is being done based on the information collected from the grass root level ASHA workers.

KFU 1802: Response of urban health service systems to road traffic injuries

Authors: Dr. Retheesh Babu G, Mohammed Shafi M.A & Abhijith A K

Funding Agency: ICMR Duration: 2019-2021

Background:

The study was designed to examine to what extent the road traffic injuries are rendered in primary, secondary and tertiary care by the urban health care system. Mapping of existing mechanisms and gaps in services within the health system will be carried out. Thus the broad methodology adopted is a case study method. Although the study is conducted in two different settings, Mumbai (undertaken by TISS Mumbai) and Alappuzha, the design is descriptive and not comparative. For convenience of the study, it was divided into three phases, *i.e*., pre-ambulance phase, ambulance phase and post ambulance phase.

Objectives:

The study aims to map the range of urban health care systems and their capacities within the government sector for road traffic injuries and to interpret the challenges faced by persons with RTIs while accessing post- crash emergency care within the golden hour. Thus suggest ways to improve the responsiveness of the existing health care facilities to cater to more cases of RTIs with requisite quality of care.

Findings:

The research team completed the collection of initial two phases, *i.e*., pre- ambulance and ambulance phase. The third phase, post-ambulance, is withheld due to COVID-19 pandemic; as the data collection process in this phase primarily involved with different hospital settings and health care workers. So the research team has been working with the literature of the study area and initiated data entry and analysis of data collected for the pre ambulance and ambulance phase. Interviews conducted have been transcribed and translated.

KFU 2001: Establishment of a network of laboratories for managing epidemics and natural calamities.

A.P.Sugunan, Devaki Antherjanam & Rajalakshmi Aiyappan (organization)

Funding Agency: DHR Duration: 60 Months (2019-24)

Background: The 2018 and 2019 Nipah virus outbreaks and the consequent flood situation in Kerala have warranted the need of the health care system for preparedness to meet the eventualities. The emergence of novel corona virus and its subsequent spread in large scale is also another example for which the state needs to be prepared. ICMR-NIV field unit at Alappuzha, needs to be prepared for these emergencies and the state is looking at NIV unit for their needs.

Work done: The project has been designed and discussions are in progress with state health departments to develop and enhance preparedness to meet the challenges of viral outbreaks. The unit has already developed diagnostic reagents and tests for at least 20 viruses of local importance. Further optimization and standardization are in progress.

NIV MUMBAI UNIT

Scientific staff

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

Technical staff

Mr. R L More	Sr. Technical Officer (2)
Dr. (Mrs.) D K Sharma	Sr. Technical Officer (1)
Mrs. S V Rane	Sr. Technical Officer (1)
Mrs. R H Bhavasar	Sr. Technical Officer (1)
Mrs. N R Shanbhag	Sr. Technical Officer (1)
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 (2)
Mr. C B Sawant	Laboratory Assistant
Mr. G J Bane	Laboratory Assistant
Mr. P P Gurav	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

Dr. U Lambe	Research Associate (EV71 Receptor Project)
Mr. A P Salunke	Technical Officer (NPSP Project)
Mrs. S S Sarjine	Res. Asst. (NPSP Project)
Ms. N A Karkare	Sr. Lab. Tech (NPSP Project)
Ms. S S Kawale	Sr. Lab. Tech (NPSP Project)
Ms. D Bhoye	Technical Assistant (NPSP Project)
Mr. M G Narkar	Lab. Tech (NPSP Project)
Ms. D N Jatekar	Lab. Tech (NPSP Project)
Mr. K Kalgutkar	Lab. Tech (NPSP Project)
Ms. S Meshram	Lab. Tech (NPSP Project)
Ms. S Mulye	Lab. Tech (NPSP Project)

Ms. S T Padelkar	Asst. Data Manager (NPSP Project)
Ms. T S Gohil	Junior Research Fellow (DHR)
Ms. M M Fernandes	Technical Officer (WHO-PID)
Ms. U P Sawant	Technical Assistant (WHO-PID)
Mr. V S Dhanawade	Lab. Asst. (NPSP Project)
Mr. R G Bane	MTS (NPSP Project)

Administration (Permanent)

Mrs. A B Palkar	Section Officer
Mr. A R Magade	Assistant
Mr. D V Muneshwar	Assistant
Ms. Y C Bhandare	Lower Division Clerk

Administration (Project)

Ms. D Rane	Clerical Asst (NPSP Project)
Mrs. R Sheshware	DEO (NTF Project)

NIV MU 1997-01: National Polio Surveillance Project (NPSP), India
Shailesh Pawar, Uma Nalavade, Deepa Sharma, Vinay Saxena
Funding agency: Extramural (WHO, India) **Duration:** Ongoing (since 1997)

Background:
ICMR-National Institute of Virology, Mumbai Unit (NIVMU) is one of the WHO global specialized laboratories (GSL) for polio surveillance since 2000. India has been certified free of wild poliovirus by WHO as the last case of wild poliovirus was detected in India in January 2011. This has necessitated stringent surveillance as there is always a potential risk of importation of wild poliovirus from polio-endemic countries *viz.*, Pakistan and Afghanistan.
Objectives: to carry out rigorous surveillance with continuous monitoring of polioviruses until global eradication of poliovirus is achieved.
Findings: During April 2019 to March 2020, 9028 stool specimens from AFP cases received from Maharashtra, Madhya Pradesh, Goa, Bihar, Gujarat, Dadra and Nagar Haveli, Rajasthan, Karnataka and Telangana were screened and isolated 202 Sabin-like (SL) polioviruses (P1SL=58; P3SL= 75; P1+P3SL= 69 and NPEV= 24). Under the environmental surveillance, 365 sewage specimens/concentrates were tested at NIVMU as per WHO protocol for Mumbai, Hyderabad and Patna and isolated 245 polioviruses. All the isolates were found to be Sabin-like poliovirus type 1 and type 3 and non-polio enteroviruses (P1SL-46, P3SL-100, P1SL+P3SL-99 and NPEV-119). Wild or circulating vaccine derived poliovirus was not detected during this period. Being a GSL for polio, NIVMU also received 23 VDPV1 isolates from Myanmar for sequencing which were timely reported. PV2 which is under containment was not detected in India during the mentioned period.

Dynamics of enteroviruses in sewage waters before and after oral polio vaccination (OPV) drives in Mumbai, India, 2018, was studied. There was a significant rise in poliovirus isolation rate as compared to NPEVs after OPV drives. The findings of the present study could help studying effectiveness of OPV drives.

A novel method for detection of polio and enteroviruses from sewage samples using poly-electrolytes was developed, which has been accepted by ICMR HQ as a novel innovation. This has potential for environmental surveillance of polio and other water borne viruses.

NIV MU 2016-01: Measles and Rubella Surveillance
Shailesh Pawar, Deepa Sharma, Uma Nalavade
Funding agency: Extramural (funded by National Polio Surveillance Project, WHO, India)
Duration: Ongoing (since 2016)

Background: Nineteen measles and rubella WHO network laboratories exist in India, of which NIVMU is the WHO reference laboratory for sequencing. The measles and rubella surveillance programme is of utmost priority to achieve the goal of elimination of these viruses by 2024. NIVMU receives serum specimens from suspected cases of measles and rubella reported in various districts of Maharashtra, Madhya Pradesh, Chhattisgarh and Goa.
***Objectives*:** The objective of the surveillance programme is the elimination of measles and rubella.
Findings: A total of 2228 serum samples were tested using Measles and Rubella IgM ELISA and found 212 (9.51%) and 149 (6.69%) samples positive for measles and rubella virus antibodies respectively. The remaining specimens were either found equivocal for measles (1.8%) and rubella (2.15%) or negative for both (80.3%). Screening of 1581 urine/throat swabs from Maharashtra, Madhya Pradesh, Goa, Chhattisgarh, Gujarat, and Daman and Diu using RT-PCR have yielded 161 (D8 and D4 genotype) samples positive for measles and 69 for rubella (2B genotype). Being a reference laboratory for sequencing, NIVMU received 744 PCR products from WHO MR network labs, of which 620 measles PCR positive products were genotyped successfully (D8:446; D4:18 and B3:2). Of the 07 rubella positive PCR products, 11 were identified as 2B.

NIV MU 2018-04: Strengthening laboratory quality management systems including EQA of DHR/ICMR VRDL laboratories for confirmation of outbreaks including measles and rubella.
Shailesh Pawar, Deepa Sharma, Uma Nalavade
Funding agency: Extramural (DHR) **Duration:** 2018-2021

***Background*:** NIVMU, being a WHO reference laboratory for measles and rubella sequencing, was identified as a nodal laboratory for capacity building of VRDLs by providing training and technical support to make them proficient in testing Measles-Rubella surveillance specimens.
Objectives: 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.
***Findings*:** To initiate the project, a workshop on "Strengthening of Measles and Rubella case confirmation by serology IgM detection and conventional RT-PCR" was conducted at NIVMU in February 2018 for the first six VRDLs selected by ICMR/DHR. In 2018, these laboratories successfully completed the first three phases of the study. The on-site review of these six VRDLs was conducted by WHO and these were successfully integrated as WHO MR network laboratory. In continuation with the expansion process, another nine VRDLs were trained in a workshop on "Serological and Molecular diagnosis of Measles and Rubella" conducted at NIVMU from 5– 9 August 2019. Post-training, these laboratories were provided with molecular and serology panels and the first phase was successfully completed by all the nine labs. As part of the phase II, these laboratories will be provided with molecular panels by NIVMU and serology panels by KIPM, Chennai (WHO reference laboratory for serology).

NIV MU 2019-01: Cross-sectional serologic assessment of immunity to polioviruses post tOPV-bOPV switch period' (India Polio Seroprevalence Study- 2019).
Shailesh Pawar, Uma Nalavade, Deepa Sharma

Funding agency: World Health Organization (WHO) Duration: 2019-20
***Background*:** As per the Polio Eradication and Endgame Strategic Plan 2013–2018, a global switch from trivalent OPV (tOPV) to bivalent OPV (bOPV containing types 1 & 3) and introduction of inactivated poliovirus vaccine (IPV) into routine immunization schedules was initiated. The tOPV-bOPV switch was carried out in India in April 2016. In the cohort born post-switch, type-2 immunity will come only from the IPV taken in routine schedule, as bOPV provides immunity only against types 1 & 3. The 27th Meeting of the India Expert Advisory Group on polio eradication had recommended polio seroprevalence study be conducted in India in 2019. Keeping in view of this, WHO India in collaboration conducted a polio seroprevalence study among infant population in selected high risk states.
Objectives:

- To assess the seroprevalence against all three serotypes of poliovirus among infants (6-11 months of age) from selected high risk states in India.
- To compare seroprevalence between different study states.
- To analyze vaccine (bOPV/IPV) dose/response relationship to polio seroprevalence.

***Findings*:** Serum samples (n=1440) received for testing under this study were tested against poliovirus type 1 and 3 and the results were submitted to NPSP, WHO for further analysis.

NIVMU 19-02: *In-vivo* potency evaluation for the hexavalent, a combination vaccine containing diphtheria, tetanus, pertussis, hepatitis B, Hib PRP-TT and IPV (Type 1, 2 & 3 antigens) and IPV trivalent vaccine formulation containing IPV (Type 1, 2 & 3)
Shailesh Pawar, Uma Nalavade, Deepa Sharma
Funding agency: Indian Immunologicals Ltd **(**IIL), Hyderabad **Duration:** 2019-20

Background: Indian Immunologicals Ltd is in the process of developing a standalone trivalent inactivated polio vaccine and a hexavalent vaccine containing DPT + Hep B + Hib +IPV. For both the vaccines the polio component is based on Sabin strains. IIL would like to collaborate with NIVMU for performing serum neutralization assays for *in-vivo* potency for the IPV components in the two vaccines being developed by them.
Objectives: to test serum specimens using micro-neutralization assay against Sabin-like Polio viruses types 1, 2 & 3.
***Findings*:** During the year, 1600 sera were received for testing of which, 1140 sera were tested against poliovirus type 1 and type 3 using microneutralization assay as per the WHO guidelines and the results were submitted to IIL. The remaining 460 sera are being tested against poliovirus type 1and type 3 serotypes.

Development of the Polio Essential Facility in line with Global Action Plan III at ICMR-NIV, Pune to support work on Polio covered at one place
SD Pawar, J Mullick, B Mathapati, SS Kode, SS Keng
Funding agency**:** Intramural. Duration: 2019-2021

Background: The competent authority has decided to develop the Poliovirus-Essential Facility (PEF) at the ICMR-NIV, Pune.
Objectives: To establish poliovirus essential facility at ICMR-NIV, Pashan.
Findings: Additional charge was given to Dr. Shailesh D Pawar as the 'Nodal Officer' by the Director, ICMR-NIV and ICMR HQ, for development of PEF. Certificate of participation was forwarded to the National Authority for Containment (NAC) for its onward submission to the WHO. NAC expert visits were arranged, PEF layout and perimeter were finalized. List of facility and documentation work was made. Various PEF committees were formed. Several meetings with the Top Management were conducted. Laboratory and facility documents, which included standard operating procedures, risk assessments, policies, plans and executive procedures were made as per the GAP III guidelines. However, due to COVID-19 pandemic situation and lockdown, facility work and training could not progress as per the timelines.

Studies on poliovirus infections in children with immune-deficiency.
Madhu Mohanty, Manisha Madkaikar, Mukesh Desai, Swapnil Varose, Prasad Taur
Funding Agency: Intramural **Duration**: 2015-2019

Background: As the potential reservoir for neurovirulent VDPV (Vaccine Derived Poliovirus) strains, children with Primary Immunodeficiency (PIDs) represent a global risk to unimmunized contacts and to the Global Polio Eradication Initiative.
Objective: To screen excreted enteroviruses from PID children of India
In order to address the above issue we had initiated a screening facility & further studies on the in collaboration with from the year 2015 to 2019.
Findings: The study was undertaken in collaboration with NIIH, Mumbai and Wadia Children's Hospital, Mumbai and screened 282 stool samples of 98 PID patients, which included 12 SCIDs (Severe Combined Immunodeficiency), 9 CVIDs (Common Variable Immunodeficiency), 13 XLAs (X linked Agammaglobulinemia) and 64 other PID patients. Seventeen patients (17.34%) tested positive for enteroviruses of which 12 (12.24%) tested positive for NPEVs and 5 (5.10%) for polioviruses (Table 1).

Table 1: PID patients detected positive for polio and non-polio enteroviruses from Bai Jerbai Wadia Hospital, Mumbai (2015-2019).

Poliovirus						
Sr. No.	ID Type	Months from Last OPV	IVIG Therapy	Result	Period of Poliovirus excretion (days)	Final Status
1	SCID	37	Yes	P3VDPV	774	Died
2	FLH	NA	No	P1SL	241	Died
3	SCID	4	Yes	P1SL	29	Died
4	SCID	6	Yes	P1SL	-	Died
5	SCID	NA	Yes	P3SL	50	Died

Non- polio enteroviruses (NPEV)					
Sr. No.	ID Type	IVIG Therapy	Result	Period of NPEV excretion (days)	Final Status
1	IgG Sub class Def.	Yes	EV75	62	Negative
2	Hyper IgE	No	E13	-	Loss to follow-up
3	ALPS	No	E5	-	Negative
4	CGD	No	E14	-	Died
5	XLA	Yes	EV76, E11	337	E11
6	CVID	Yes	E14	710	E14
7	CGD	No	E21	-	E21
8	CVID	Yes	E21	350	E21
9	CVID	Yes	E25	-	E25
10	Hyper IgM	Yes	EV80	-	EV80
11	CGD	No	E33	107	Negative
12	CGD	No	E15	-	E15

Note: SCID: severe combined immunodeficiency, P3VDPV: Type 3 vaccine derived polioviruses, P1SL: Type 1 Sabin like polioviruses, FLH: familial lymphohistiocytosis, XLA: X-linked agammaglobulinemia, CGD: chronic granulomatous disease, ALPS: autoimmune lymphoproliferative syndrome, CVID: common variable immuno-deficiency, E13: Echovirus 13.

One SCID child identified as a prolong excreter of P3VDPV who abruptly stopped excretion after 2 years. Occurrence of an episode of Norovirus diarrhoea associated with increased activated oligoclonal cytotoxic T cells significantly elevated pro-inflammatory cytokines and subsequent clearance of the poliovirus suggests a possible link between inflammatory diarrheal illness and clearance of iVDPV (Figure-1).

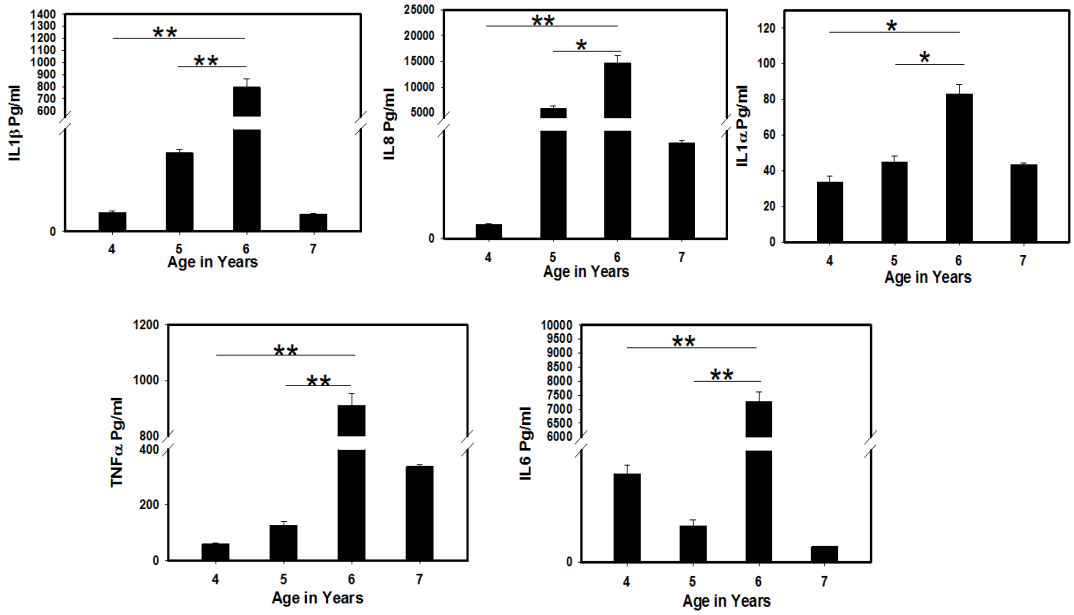


Figure 1: Cytokine/chemokine secretion in SCID patient identified as prolonged VDPV3 excreter. Significantly high release of pro-inflammatory cytokines such as IL-6, IL-8, IL-1b, IL-1 and TNF- indicating role of pro-inflammatory cytokines in poliovirus clearance.

Study on polio and non-polio enterovirus infections in children with primary immunodeficiency at multiple medical institutes in India.

Madhu Mohanty, Manisha Madkaikar, Ahmad Mohammad, Swapnil Varose, Mevis Fenandes, Unnati sawant, Shailesh Pawar and the study site investigators
Funding Agency: Extramural (WHO) Duration: 2 years (2019-2021)

Background: Based on the significant information obtained from the single center study on polioviruses in children with immunodeficiency, a research proposal was submitted to WHO to expand the scope and intensity of iVDPV (immunodeficiency associated Vaccine Derived Poliovirus) study with six additional selected medical institutes in different states of the country. The proposal was approved by WHO-Polio Research Committee and the study was initiated from December 2019.
Objective: (i) to address the unknown number of PID patients who excrete poliovirus but have not been captured by the AFP surveillance system (of non-AFP cases) (ii) to correlate the immunological parameters with virus excretion (iii) to align the study to the global guidelines of PID surveillance and to learn lessons for its implementation in the national program.
Findings:A total number of 56 stool samples from 36 PID patients with 15 different Primary Immunodeficiency types were received from six collaborating hospitals during Dec 2019 to March 2020. Five samples tested positive for NPEV (from the sites:Hyderabad, Lucknow and Mangalore) and none for polioviruses. The study will estimate the proportion of poliovirus infection in patients with immunodeficiency, investigate Immunological parameters responsible for enterovirus replication/clearance and contribute to global surveillance database on iVDPV and long-term excretors of poliovirus.

Table 2: Number of cases diagnosed with PID and tested for enterovirus excretion from six study sites across India (Dec 2019 –March 2020).

Study Site	Total no of PID cases	NPEV positive	NPEV types
Bai Jerbai Wadia Hospital for Children, Mumbai	11	0	-
Sanjay Gandhi PGIMS, Lucknow	7	3	E16, CVA2, EVB75
Nizam's Institute of Medical Sciences, Hyderabad	5	1	CVA8
Kasturba Medical College Hospital Mangalore	4	1	CVA2
Aster CMI, Bangalore Bangalore	4	-	-
Government Medical College, Calicut	4	-	-

Note: No poliovirus has been detected from the samples. E16 - Echovirus16, CVA2- Coxsackievirus A2, EVB75- Enterovirus B75, CVA8- Coxsackievirus A8

Seroprevalence of enterovirus 71 antibody among Indian children

Madhu Mohanty, Swapnil Varose, Sneha Rane, Babasaheb Tandale, Shailesh Pawar

Funding agency: Intramural **Duration**: 2 years (2019- 2021)

Background:

Though circulation of multiple genotypes of EV-71 causing HFMD has been reported in India, no data is available on the seroprevalence of EV-71 antibodies in Indian children. Keeping in mind the severity of the outbreaks caused by EV-71 across the world, a study was initiated to investigate the seroprevalence of EV71 antibodies in Indian children.

Objective: to conduct a sero-surveillance study to determine the exposure of Indian children to EV-71.

***Findings*:** Screening of 500 samples of children of different age groups of Pune collected in 2019 (NIV repository) for EV71 antibodies revealed that 37.93% of children in the age group of 2-5 yr; 64.90% of 6 -10 yr age group and 76% in the age group of 11-15 yr were positive for EV-71 genotype D, which is the major circulating genotype in India. The gradual increase in sero-positivity with age indicated the presence of circulating EV-71. However, antibody to EV-71 could not be detected in children up to the age of 2 years when 113 serum samples of children aged 1- 5 years were tested (Figure-2). Since infection with EV-71 occurs mostly in children from 2 to 4 years the data needs to be confirmed by increasing the sample size of this age group. Further studies are being carried out with other EV71 genotypes circulating in India.

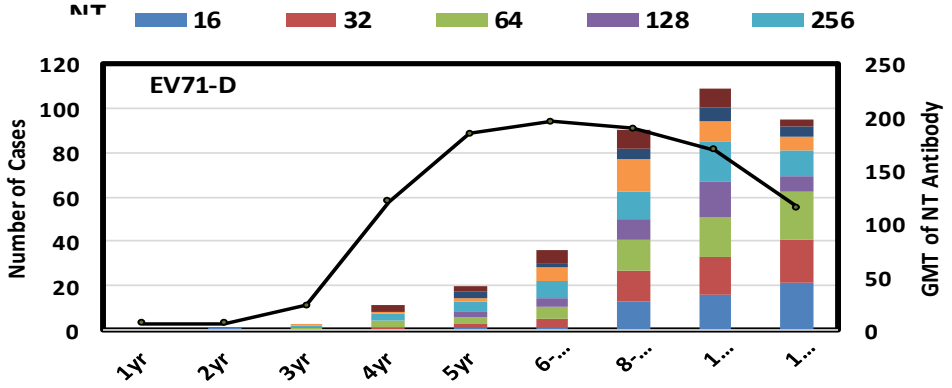


Fig 2: Distribution of neutralizing antibody and geometric mean titers against EV-71-D by age group.

Cytokine/ chemokine responses to EV-71 in human cultured cells

Madhu Mohanty, Swapnil Varose, Vinay Saxena

Funding agency: Intramural Duration: 3 years (2017- 20)

Background: Enterovirus-A (EV-A71) associated hand-foot-mouth disease (HFMD) is a major threat to public health in the Asia-Pacific region. In India, EV-A71 has been isolated from stool samples of acute flaccid paralysis cases as well as asymptomatic children, but so far no recognizable outbreaks of EV-A71 caused HFMD, AFP or encephalitis have been reported. EV-A71 isolates have been reported at NIVMU from cases of AFP, HFMD and encephalitis in Mumbai, which has been classified as genotype D, genotype C and a new genotype G.

Objective: to study cellular immune responses to different genotypes of EV-71 isolated in India.

Findings: THP-1 cells were further differentiated into macrophages by culturing with PMA (phorbol 12-myristate 13-acetate) and infected with C1, C2, G and D genotypes of EV-71 isolates. Infection of human macrophages with indigenous EV-71 D genotype and the pathogenic C genotype (HFMD) produced significantly higher IL-6 and TNF- than C (Enc) and G genotypes. Significant amount of IP-10 could be released by all the four isolates. The EV-71 virus isolated from CSF sample of an encephalitis patient did not induce substantial amount of IL-6 or TNF- or IP-10 or IL-1 compapring the other three isolates. IL-1 and IL-6 were comparatively higher in the viruses isolated from AFP cases as compared to others. The data was further confirmed with cytokine gene expression assay (Fig 3).

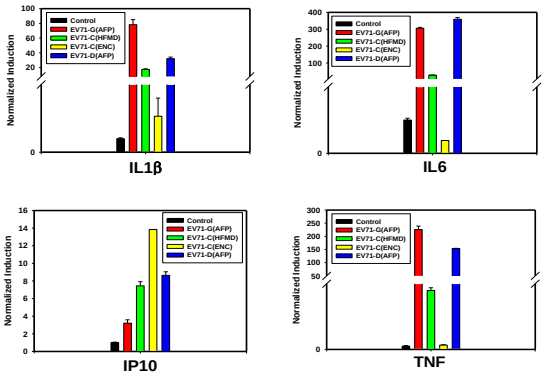


Figure 1: Cytokine gene expression in human monocyte derived macrophages (THP-1) infected with different genotypes of EV71.

CD155/PVR knockout cell strains from human rhabdomyosarcoma cell line (RD) for use in polio and non-poliovirus diagnostics and research.

Shyam Nandi, Sonali Sawant, Trupti Gohil, Jagadish Deshpande

Funding agency**:** Extramural (DHR) Duration: 3 years **(**2018-21)

Background: Achieving poliovirus containment concomitant with certification of global polio eradication is the highest priority of the Global Polio Eradication Initiative. WHO GAP III recommends laboratories wanted to culture viruses from suspected poliovirus infectious materials in poliovirus permissive cell lines will have to establish bio-safety and bio-risk management systems and obtain certification by National Containment Authority.

Objectives**:** to develop a human RD cell line with CD155-PVR knockout or deletion and to check the growth of polio and non-polio enteroviruses in the newly generated RD cell strains.

Findings**:** The CD155/PVR knockout RD cell line developed using CRISPR/Cas9 technology renders resistance to poliovirus growth while susceptible to non-polio enteroviruses. Poliovirus non-permissive RD cells did not express CD155-specific surface immunofluorescence. A selected clone (RD-SJ40) was field tested against 626 stool samples of AFP cases by three National Polio Laboratories, *viz*., BJMC, Ahmedabad; SGPGI, Lucknow; KIPM, Chennai. The RD-SJ40 cells did not support the growth of poliovirus from positive stool samples while all the NPEV types, isolated in parental RD cells were also isolated in RD-SJ40. Thus, RD-SJ40 cells are safe for NPEV isolation from poliovirus PIM without derogating GAPIII containment requirements, suggestive of applications in laboratories worldwide. It is envisaged that WHO Global Polio Laboratory Network will also support the use of CD155 knockout RD cells for enterovirus/enteric virus work in all the network laboratories across the World. RD-SJ40 will be deposited to ATCC, USA soon.

Investigation of host genetic susceptibility markers to Enterovirus A-71 infection in Indian population.

Shyam Nandi, Sonali Sawant, Trupti Gohil, Jagadish Deshpande

Funding agency: ICMR Intramural Duration: 3 Years (2018-2021)

Background: EV-71 is endemic in India. However, outbreaks of EV71 caused HFMD or aseptic meningitis and encephalitis or AFP have not been reported in India. There may be two hypothesis: a) the indigenous EV71 genotypes are naturally less virulent and b) the Indian population is genetically less susceptible to EV71 caused diseases. We plan to use the prior knowledge of genetic susceptibility to EV71 to develop a multiplex SNP assay to explore mutations in several genes. It would be interesting to understand genetic contribution to susceptibility to EV71 infection in Indian population.

Objectives**: (i)** to develop a multiplex single nucleotide polymorphism assay for investigation of host genetic risk factors in different genes by Enterovirus 71 infection.

(ii) to carry out population based surveys of SNPs in genes contributing to EV71 susceptibility.

Findings**:** A single nucleotide polymorphism (SNP) multiplexed assay was developed targeting 15 genetic markers from 12 genes against EVA-71 infection and validated with 100 blood samples collected from healthy adult individuals, in collaboration with Central Railway Hospital, Kalyan. The polymorphisms observed for each of the 12 selected genes were analyzed and the frequency of the individual alleles was calculated. Bio statistical interpretation is to be performed to gain insights on the susceptibility of Indian population to EVA-71 infection

Studies on the role of different cell surface receptors of Enterovirus A71

Shyam Nandi, Upendra Lambe, Sonali Sawant, Jagadish Deshpande

Funding agency: Extramural (ICMR) Duration: 2019-22

***Background*:** EV-A71 utilizes several cell surface receptors, of which Scavenger Receptor B2 (SCARB2), P-selectin Glycoprotein Ligand-1 (PSGL-1) and Fibronectin (FN) are used for virus attachment and internalization. Identification and over expression of the most preferred receptor by Indian strains will be attempted during the study. This will lead to high titer virus production which can be utilized for purposes like antigen production, vaccine development etc.

***Objectives*: (i)** to dysfunction of EV-A71 receptors sequentially and in combinations to identify most preferred receptors by different genotypes of EV-A71.

(ii) to study the effect of over expression of the preferred cell surface receptor on per cell yield of EV-A71.

Findings: Sequence analysis of all the three receptors through literature search has been performed. PAM (protospacer adjacent motifs) sites were identified from the structurally important regions of the receptors. The PAM site for Cas9 was identified by 5' NGG 3'. This was followed by designing of sgRNAs by addition of four nucleotides, CACC before the 20-mer guide sequence and AAAC before the guide's reverse complement at 5'-end to create Bbs1 cloning site. Vector pX330 has been selected for the cloning of the sgRNA. The clones of SCARB2 and PSGL-1 have been prepared. The other cloning experiments are in progress.

POLIO VIROLOGY GROUP

(Former Avian Influenza Group)

Scientific Staff

Dr. Jayati Mullick	Scientist-F & Group Leader (mullick.j@niv.co.in; jayati_mullick@hotmail.com)
Dr. Basavaraj S. Mathapati	Scientist-C (basavaraj.mathapati@gmail.com)
Dr. Shailesh D. Pawar	Scientist E (Nodal Officer for Polio Essential facility-PEF)

Technical Staff

Mrs. Sadhana S. Kode	Senior Technical Officer-3
Dr. Vaishali S Tatte	Senior Technical Officer-1 (December 2019 onwards)
Mr. Sachin S. Keng	Technical Officer-A
Mr. Dinesh K. Singh	Senior Technician-1
Mr. Rameshwar P Khedekar	Technician-3 (December 2019 onwards)
Mr. Javvaji P. N. Babu	Technician-2
Mr. Ratnadeep More	Technician-1 (December 2019 onwards)
Ms. Vaishnavee Bagde	Technician-1 (December 2019 onwards)

Research Scholar

Ms. Rewati Kasbe	Ph.D. Fellow (UGC)
Ms. Deeksha S. Tare	SRF (ICMR)

AVI1001: Avian Influenza outbreak investigations and diagnostic services (SERVICE PROJECT)
Jayati Mullick (PI)

Sadhana S Kode, Sachin S. Keng, Dinesh K Singh, JPN Babu (Contributors).
Funding Agency**:** Intramural Duration: Ongoing

Avian Influenza animal-human interface studies*:*
Diagnosis of avian influenza (AI) for referred human samples: Nasopharyngeal samples from suspected people (n=14) during a confirmed AI H5N1 poultry outbreak in a poultry farm in Ambikapur, Chattisgarh were referred to ICMR-NIV from the Government Medical College, Ambikapur, Chattisgarh for diagnosis of AI H5N1 on 30th December 2019. Real-time RT-PCR tests were performed and all the samples were found negative for AI H5N1 virus. Report of the diagnosis was sent to GMC, Ambikapur & Department of Animal Husbandry, Dairy & Fisheries, Krishi Bhavan, New Delhi with a copy to ICMR on 31st December, 2019.

AVI1302: Operation and maintenance of High Containment Laboratory. (SERVICE PROJECT)
Jayati Mullick (PI), Basavaraj S Mathapati, Ajay B. Khare

Dinesh Singh, Sachin Keng, JPN Babu (Contributors).
Funding Agency: Intramural **Duration:** Ongoing

Background:
The High Containment laboratory (BSL-3) is a specialized laboratory designed to work on high risk pathogens, providing protection to workers and the community. Regular maintenance is an integral part for smooth functioning of the facility. During the reporting period, the facility has undergone major maintenance work and was non functional for most of the time. PU painting and epoxy painting has been completed and calibration of equipments and other relevant engineering systems are in progress. The laboratory side of the BSL-3 has been proposed as a designated Polio Essential Facility (PEF), and is undergoing modification as per Global action plan III (GAPIII).

Poliovirus Essential Facility (PEF)
Jayati Mullick, Basavaraj Mathapati, Shailesh D Pawar (Nodal Officer)

Avian Influenza group has been renamed as the Polio Virology Group in January 2019 as per ICMR directive and the mandate of the group has been revised. Since renaming, in addition to the research activities of avian influenza, the group has been actively involved in the activities of PVG for the preparatory audit of PEF as per Global action plan III (GAP III). The whole group has been involved in preparing the outlines/framework for work flow for modification of the Bio-safety Level 3 (BSL-3) laboratory settings and Standard Operating Procedures required for audit of PEF as per GAP III in the near future on the basis of assessment reports of the mock audit. Due to the proposed designated PEF in the laboratory side of the BSL-3 facility, the animal side of the facility will be reoriented and modified for the laboratory work.

AVI1101: **Studies on host-virus interactions of low-pathogenic and high-pathogenic avian influenza viruses.**
Jayati Mullick (PI), Shailesh D. Pawar (Co-PI), Tripti Sanjeevi (Contributor)
Funding Agency: ICMR Intramural **Duration:** April 2012- March 2018 (extended)

Background: The non-structural protein 1 (NS1) of avian influenza (AI) viruses are critical IFN antagonist. One of them manifested the difference in type I IFN response inhibition in highly–pathogenic AI (HPAI) and low-pathogenic AI (LPAI) viruses, and HPAI NS1 is known to inhibit IFN response to a greater extent, even when the levels of NS1 were low (recent studies from the lab). This hints a fact that

there are other viral proteins (PB2, PA, PB1/PB1-F2) assisting NS1 protein in antagonizing the type I IFN response. Few studies have demonstrated the role of PA-X protein in inhibiting IFN response. On the basis of these studies, it has been hypothesized that influenza virus PA-X protein may function in the inhibition of type I IFN response in synergy with NS1 protein. Using two representatives of each HPAI viruses (H5N1 [2.3.2.1 and 2.2 clades)] and LPAI virus [H9N2 (Pune) and H11N1] strains, to determine whether PA-X proteins from the respective viruses possess any synergistic effect with NS1 in attenuation of the type 1 IFN response, molecular cloning of the respective PA-X gene in mammalian expression system using pcDNA 3.1/His A have been initiated. These will be used for functional assays such as transfection of PA-X along with the respective NS1 from the HPAI and LPAI viruses in A549 cells. The antagonistic effects of PA-X and NS1 proteins will help in better understanding of characteristics of avian influenza infection and host-pathogen interaction.

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

Shailesh D. Pawar (PI), Jayati Mullick (Co-PI)
Sadhana S Kode, Deeksha Tare, Sachin S Keng (Contributors)

Funding Agency: ICMR Intramural **Duration:** April 2016- March 2019

Abstract: **Amantadine resistance markers among low pathogenic avian influenza H9N2 viruses isolated from poultry in India, during 2009–2017**

Avian influenza (AI) H9N2 viruses are the most prevalent and economically important viruses in poultry in the world. Antiviral susceptibility screening of AI H9N2 viruses is crucial considering their potential to cause human infections. Resistance to amantadine, an M2 inhibitor drug has been widely reported. To understand the current situation in India, Matrix genes of 48 H9N2 viruses isolated from India during 2009–2017 were sequenced and M2 trans-membrane region sequences were screened for mutations which are known to confer resistance to amantadine, *viz*., L26F, V27A, A30T/V, S31N and G34E. Twenty-nine out of the 48 H9N2 virus M2 sequences showed presence of amantadine resistance markers. Among the 29 viruses, S31N mutation was observed in 24 isolates, V27A + S31N dual mutations in 4 isolates and V27A mutation in one isolate were seen. According to a previous report, all influenza H9 variants with adamantane resistance were distributed only in Asia. Comparison of the M2 sequences from other Asian countries showed different patterns of amantadine resistance wherein phylogenetic analysis of the M genes of the strains from Pakistan formed a separate cluster (**Figure 2**). In conclusion, prevalence and gradual increase of amantadine resistance among AI H9N2 viruses in India, emphasizes the importance of the antiviral surveillance.

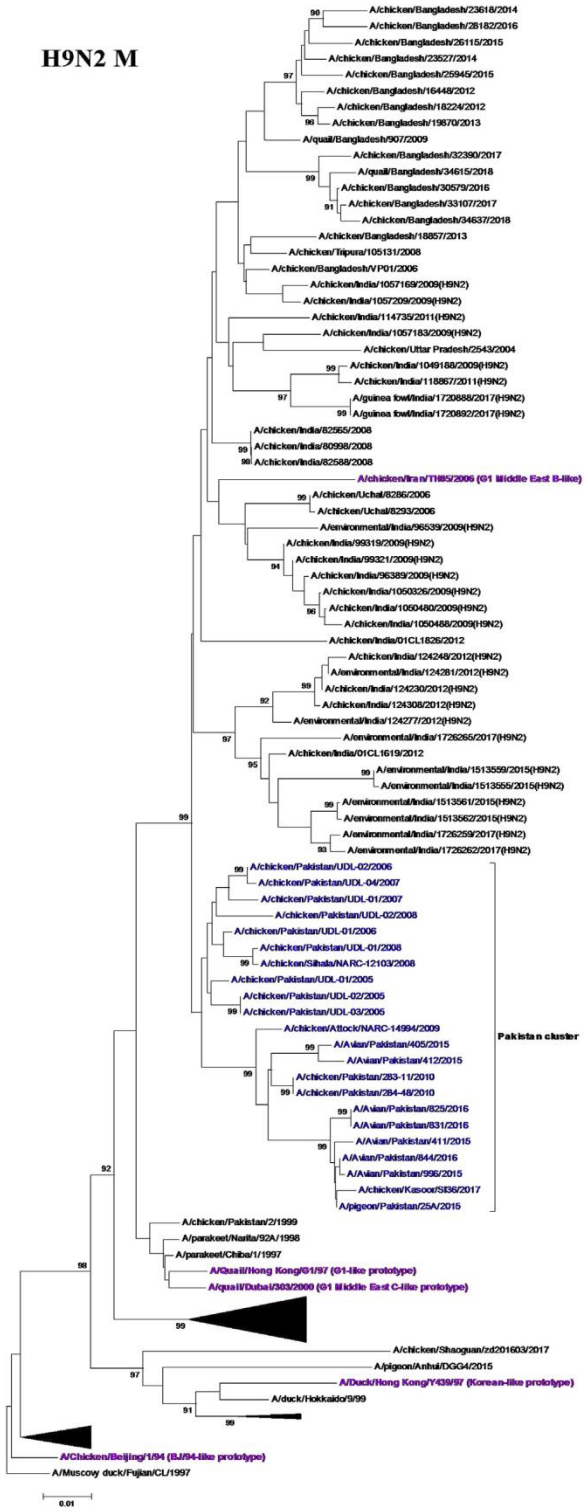


Figure 2. H9N2 M gene phylogenetic tree

The phylogenetic tree for M gene was constructed using Neighbor-Joining method with 1000 bootstraps using MEGA v5.05. Bootstrap values > 90% are shown next to the branches. The scale bar indicates the number of base substitutions per site. The tree was rooted to the sequence of A/Muscovy duck/Fujian/CL/1997. Strains from Pakistan are shown in blue to highlight their separate cluster.

Prototype strains of each HA lineage of H9N2 viruses are highlighted in pink. The collapsed strains in the tree are from China and Japan (1997–2018).

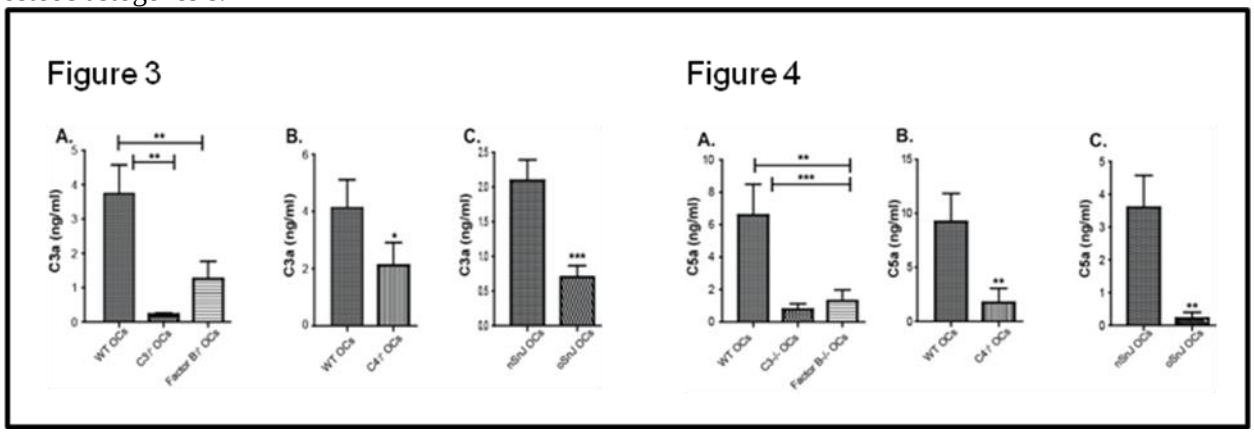
AVI1008: **Studies on the inflammatory mediators in osteoclast differentiation.**

Jayati Mullick (PI), Rewati Kasbe (Ph.D. Scholar), MR Wani (Co-PI; NCCS), AS Tripathy & A B Sudeep

Funding Agency**:** ICMR Intramural Duration: April 2012- March 2019 (extended)

Abstract

The distinct role of complement system in primary defense has been well established in the haematopoiesis, skeletal, vascular development etc., in the last decade. Studies from our lab have shown that complement protein and complement pathways play a crucial role during osteoclastogenesis from a lymphocyte and stromal free milieu though the underlying mechanism by which these complement proteins mediate the osteoclastogenesis is still under mask. Using different complement knock-out mice ($C3^{-/-}$, $FB^{-/-}$, $C4^{-/-}$, and $C5^{-/-}$ (oSn/J) mice) and Wild type (C57BL/6, C5 sufficient (nSn/J) mice we have substantiated the involvement of the classical and alternative complement pathways in osteoclast (OC) differentiation in a stromal free environment. These data have been substantiated by TRAP staining of the osteoclasts and studying the gene expression by real-time PCR. Our preliminary replenishment experiments by exogenously adding human C3a and/or C5a to the $C3^{-/-}$ osteoclast cultures, intriguingly demonstrated that both C3a as well as C5a alone were able to drive the M-CSF and RANKL-induced osteoclast differentiation in a dose-dependent manner. These results along with the previous reports have corroborated that OC differentiation and maturation are modulated in the presence of C3a and C5a. Therefore, we assessed the levels of complement activation products C3a and C5a by ELISA in the OC precursors (OCPs) and the differentiated OC from different complement KO mice. In brief, the culture supernatants from OCs of $C3^{-/-}$, factor $B^{-/-}$, $C4^{-/-}$, and oSn/J KOs and their respective WTs were collected and subjected to ELISA analysis. The results in **Figure 3** show that compared to the respective WT OCs the C3a levels were significantly diminished in the $C3^{-/-}$ ($p<0.01$) factor $B^{-/-}$ ($p<0.01$) and oSn/J ($p<0.001$) mice, while in the $C4^{-/-}$ ($p<0.05$) mice the C3a levels were marginally reduced. The ELISA data in **Figure 4** show that similar to C3a, the C5a levels were diminished. As expected the level of C5a was significantly reduced in the oSn/J ($p<0.01$) and $C3^{-/-}$ ($p<0.001$) mice, followed by factor $B^{-/-}$ ($p<0.01$) and $C4^{-/-}$ ($p<0.01$). Complement anaphylatoxins C3a and C5a generated locally play a key role in osteoclastogenesis.



Figures 3 & 4: Evaluation of complement activation product C3a and C5a in the culture supernatant of differentiated osteoclasts by ELISA: culture supernatants from WT and respective KO groups were concentrated and subjected to ELISA. The analyzed data were plotted as bar diagrams. A. Comparative analysis between WT (C57BL/6) versus $C3^{-/-}$ and factor $B^{-/-}$ KO, B. Comparative analysis between WT (C57BL/6) versus $C4^{-/-}$ KO and C. Comparative analysis between WT (nSn/J) versus oSn/J. The t-test and Annova were applied for statistical analysis. Asterisks indicate the statistical significance of C3a and C5a expressions among the WT and KO groups (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$).

SRF Project: Gene pool analysis of highly pathogenic H5N1 and low pathogenic H9N2 avian influenza viruses isolated from India

Shailesh D. Pawar, Deeksha S. Tare (SRF)

Funding: Indian Council of Medical Research, Senior Research Fellowship grant to Deeksha Tare (VIR/Fellowship/1/2008-ECD-I) starting 17th August 2018

Project duration: August 2018 to August 2021

Background: High pathogenic avian influenza (HPAI) H5N1 and low pathogenic avian influenza (LPAI) H9N2 viruses are known to cause human infections and have pandemic potential. LPAI-H9N2 viruses are prevalent in poultry in India and the first human case in India was reported in 2019. There is no data on the genome analysis, evolution and characterization of LPAI-H9N2 viruses from India.

Objectives:

(i) Gene pool analysis of avian influenza viruses isolated from India

(ii) Full genome sequencing of the pool of LPAI and HPAI viruses isolated by ICMR-NIV

(iii) Characterization of the AI viruses by antigenic analysis, receptor binding specificity, and antiviral susceptibility.

Findings: Phylogenetic analysis revealed that the LPAI-H9N2 viruses isolated from India in 2015 and 2017 formed a separate cluster for all genes along with the LPAI-H9N2 human virus from India and were found related to viruses from Bangladesh, Pakistan and Iran. The internal genes bore resemblance to HPAI-H7N3 and H5N1 viruses. Substitutions associated with increased virulence, mammalian adaptation and high polymerase activity were observed. These findings suggest that there are independent introductions and continuous evolution of LPAI-H9N2 viruses towards mammalian adaptation. This highlights the potential risk posed by AI viruses, underscoring the need of rigorous surveillance.

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* ***Group Leader****: Dr. Jayati Mullick*

The HA & HI tests from the Avian Influenza group along with other tests from the Maximum Containment Laboratory and Human influenza groups of the ICMR-NIV have been accreditated by NABL since 2016. During this period, NABL conducted desktop surveillance audit. Internal audits of Avian Influenza, Human influenza, MCL and Engineering Support groups as per NABL requirement were conducted with Management Review meetings as per the criteria of NABL by the Quality Cell. In addition, the Scope extension assessment of ICMR-NIV as per ISO/IEC 17025:2005 was conducted and NABL has granted accreditation of ICMR-NIV in the discipline of Mechanical testing as per ISO/IEC 17025:2005. One-day training on "Transition program from ISO/IEC 17025:2005 to ISO/IEC 17025:2017 of the Laboratory Quality Management" at ICMR-NIV, Pashan and NABL awareness meeting was conducted along with Dr. S. D Pawar for Dr. Gururaj Deshpande and Ms. Rashi Srivastav of DVG group for the extension of scope under NABL accreditation. The members of the Quality cell participated in NABL training program on "General Requirements for Proficiency Testing as per ISO/ IEC 17043" from 21 to 24 January 2020 at Kolkata.

Quality Cell Team

Dr. Shailesh D. Pawar	: Quality manager	Mrs. Sadhana S. Kode	: Dy. Quality Manager
Mr. Sachin S. Keng	: Technical Manager	Mr. Dinesh K. Singh	: Dy. Technical Manager

POLIO ESSENTIAL FACILITY

Development of Polio Essential Facility in line with the Global Action Plan III at ICMR-NIV, Pune to support work on Polio

Shailesh D Pawar, (Nodal Officer), Jayati Mullick (Group Leader), Basavaraj Mathapati, A B Khare, Sadhana S Kode, Vaishali S Tatte, Sachin S Keng, Dinesh K Singh, Rameshwar P Khedekar , JPN Babu, Ratnadeep More, Vaishnavee Bagde

Funding agency: Intramural. Proposed for funding to ICMR **Duration:** 2019-2021

***Background*:** The competent authority has decided to develop the Poliovirus-Essential Facility (PEF) at the ICMR-NIV, Pune.

***Objectives*:** To establish poliovirus essential facility at ICMR-NIV, Pashan.

***Findings*:** Additional charge was given to Dr. Shailesh D Pawar as the 'Nodal Officer' by the Director, ICMR-NIV and ICMR HQ, for development of PEF. Certificate of participation was forwarded to the National Authority for Containment (NAC) for its onward submission to the WHO. NAC expert visits were arranged, PEF layout and perimeter was finalized. List of facility and documentation work was made. Various PEF committees were formed. Several meetings with the Top Management were conducted. Laboratory and facility documents, which includes standard operating procedures, risk assessments, Policies, Plans and Executive Procedures were made as per the Global Action Plan III guidelines. However, due to COVID-19 pandemic situation and lockdown, facility work and training could not progress as per the timeline.

- A mock audit of PEF at ICMR-NIV was done by a team of auditors from the WHO during 1st to 5th April 2019. Documents such as SOPs of the work and equipment, Training plan and training records of staff, competency records, validation documents of the facility, equipment list, records of incidence, records related to security were arranged for the audit.
- WHO auditors visited BMS of BSL-3 on 3rd April 2019. Auditors reviewed documents, records and witnessed some tests related with engineering elements.
- WHO auditors visited BSL-3 laboratory on 4th April 2019. Auditors reviewed documents, records related with biosafety and biosecurity practices. They visited laboratory area under the scope of PEF and raised issues related to biosafety and biosecurity elements. Satisfactory answers and documentary evidences were given to the auditors by the Group Leader and team. Some suggestions were given by WHO auditors for improvement as per PEF.
- A review meeting after mock audit was held on 8th April 2019. Dr. D.T. Mourya briefed about the mock audit findings. He instructed to take corrective actions on non-conformities and to prepare the necessary documents as early as possible.
- A meeting of PVG group along with Dr. Pragya Yadav, Dr. Anita Shete and Mr. A. B. Khare was held on 11th April 2019 to discuss the further progress on documentation required for PEF. A roadmap was prepared for work flow for modification of laboratory settings, and SOPs for audit of PEF as per GAP III in near future on the basis of assessment reports of the mock audit.
- PEF documentation

Various SOPs of the work carried out inside the PEF such as entry-exit protocol, handling of spillage, handling of needle stick injury, emergency evacuation, sample receipt etc., were prepared and revised.

- Project Proposal:Project proposal formulated for functional PEF as per GAP III at NIV-Pashan, Pune (*Development of the Polio Essential Facility in line with the Global Action Plan III at ICMR-NIV, Pune to support work on Polio*).
  - NAC visit: The National Authority for Containment (NAC) members visited and inspected the designated Poliovirus Essential Facility (PEF) in November 2019 (**Figure** 1).

List of facility and documentation work was made. Various PEF committees were formed. Several meetings with the Top Management were conducted. Laboratory and facility documents, which includes standard operating procedures, risk assessments, Policies, Plans and Executive Procedures were made as per the Global Action Plan III guidelines. However, due to COVID-19 pandemic situation and lockdown, facility work and training could not progress as per the timeline.

VIRUS REGISTRY AND VIRUS REPOSITORY

Scientific staff

Dr. Sunil R. Vaidya,	Scientist-E & Group Leader (sr.vaidya@icmr.gov.in)

Technical Staff

Mrs. Neelakshi S. Kumbhar	Sr Technician-3
Mr. Madhukar B. Kamble	Sr Technician-1
Mrs. Divya R.Bhattad	Technician-C
Mr. Sarang S. Kamble	Lab Assistant-1
Mr. Roben P. George	Lab Assistant-1
Mr. Raju M. Rahurkar	Lab Assistant-1
Mrs. Surekha S. Mohol	Lab Assistant-1

MMR1201: Outbreak Investigations/ Diagnostic services provided to Local/State health authorities
Investigator: Vaidya SR
Funding agency: ICMR-NIV **Duration: 2012-2022**

1. Laboratory support for Mumps Diagnosis: During the reporting period, 29 cases of fever with parotid swelling were referred for laboratory diagnosis at NIV, Pune. Altogether, 16 cases were referred from Dadra and Nagar Haveli, 2 from Mumbai based hospital, 9 from Akola district and 2 from Pune based hospitals. 26 of 29 cases were laboratory confirmed for mumps by either IgM EIA or RT-PCR. Also during March-2020, 31 serum samples, six throat swabs and six urine specimens were referred from Primary Health Centre, Wadhona Bazar (Yavatmal District) for mumps laboratory diagnosis. Majority of the cases were under 15 years except one, who is 32 years old. Also, six serum samples were received from five contacts aged between 28-42 years. Overall, 29 cases and two contacts were confirmed by mumps IgM EIA.

Altogether, 14-suspected mumps cases (including sporadic) were subjected to mumps virus RNA detection and genotyping. Six of 14 cases were positive for mumps virus. SH gene RT-PCR and sequencing revealed circulation of mumps genotype C strains. Attempts are being made to sequence the full genomes directly from the cerebrospinal fluids, throat swabs and urine specimens.

2. ***Laboratory support for chickenpox diagnosis:*** During the 1stquarter of 2019, 64 suspected cases were referred for chickenpox diagnosis from Dadra and Nagar Haveli (n=58), Maharashtra (n=5) and Gujarat (n=1). Altogether, 31 suspected cases were laboratory confirmed for chickenpox (VZV IgM EIA). Seven chickenpox isolates were successfully propagated in Vero cells without showing evident cytopathic effect, but VZV DNA PCR confirmation up to passage-3. Five TCFs were subjected to full genome sequencing by NGS platform but unable to obtain genome sequences.

3***. Chickenpox outbreak in a tribal population***: Between 44th week of 2018 and 16th week of 2019, 247-fever with skin rash cases were observed in a tribal dwelling in 14 villages of Silvassa, Dadra and Nagar Haveli, India. Detailed epidemiological and laboratory investigations were performed in the affected 14 villages to understand the characteristics and determinants of the outbreak. For the laboratory investigations, 33 serum samples and three blister swabs were collected from 36-suspected Chickenpox cases (Table 1). Two suspected cases had both serum and blister swabs. Overall, the age of the suspected cases ranged from 5 months to 48 years. Altogether, 5-skin rash cases were confirmed by VZV (ORF-28 gene) DNA PCR and remaining 28 by VZV IgM EIA. Both anti-VZV IgM and IgG antibodies were detected in 23-skin rash cases. Five skin rash cases showed absence of IgM and IgG antibodies to VZV but two cases showed presence of VZV DNA, whereas, three cases showed anti-VZV IgM antibodies but not anti-VZV IgG antibodies. Overall, 33 of 36-suspected cases (inclusive of equivocal case) were laboratory confirmed. The partial sequencing of VZV ORF-28 gene revealed circulation of clade-1 viruses in Silvassa. Study emphasizes more investigations on skin rash cases to detect the viral etiology (if any) in the tribal population.

Table 1: Village-wise details of Chickenpox cases from the 8 of 14 villages of Silvassa block.

Case(s)	Village	Age (years)	Sex	Fever onset (days)	Vesicular skin rash	IgM EIA/ RT-PCR Result
1	Karachgam	16	F	12	Yes	Positive
2	Karachgam	1.5	M	11	Yes	Positive
3	Karachgam	10	F	12	Yes	Positive
4	Karachgam	24	F	10	Yes	Positive
5	Karachgam	6	F	8	Yes	Positive
6	Karachgam	12	M	6	Yes	Positive
7	Karachgam	6	M	5	Yes	Positive

8	Karchond	9	M	25	Yes	Positive
9	Karchond	10	M	10	Yes	Positive
10	Karchond	6	M	8	Yes	Positive
11	Karchond	5	M	8	Yes	Positive
12	Karchond	7	M	3	Yes	Positive
13	Nana Randha	9	F	27	Yes	Positive
14	Nana Randha	9	M	28	Yes	Positive
15	Nana Randha	5.9	F	15	Yes	Positive
16	Nana Randha	6.9	M	12	Yes	Positive
17	Mota Randha	7.5	M	12	Yes	Positive
18	Silli	9	M	6	Yes	Positive
19	Kilvani	9	F	9	Yes	Positive
20	Kilvani	9	M	10	Yes	Positive
21	Kilvani	9	M	10	Yes	Positive
22	Kilvani	9	F	9	Yes	Positive
23	Kilvani	7	F	8	Yes	Positive
24	Kilvani	9	M	6	Yes	Equivocal
25	Surangi	10	M	4	Yes	Positive*
26	Surangi	6	M	11	Yes	Positive
27	Velugam	1	F	7	Yes	Negative
28	Velugam	6	M	2	Yes	Negative
29	Velugam	7	M	2	Yes	Negative
30	Velugam	6	M	2	Yes	Positive*
31	Velugam	7	M	2	Yes	Positive*
32	Velugam	10	M	1	Yes	Positive*
33	Velugam	6	M	1	Yes	Positive*
34	Veugam	7	M	3	Yes	Positive
35	Velugam	6	M	13	Yes	Positive
36	Velugam	6	M	12	Yes	Positive

***VZV RNAPCR Positive case(s).**

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

Investigator: Vaidya SR

Funding agency: ICMR-NIV Duration: 2019-2022

(i) Genome and antigenic characterization of Indian rubella viruses

The goal of 'measles elimination and control of rubella/ congenital rubella syndrome (CRS) by 2020' has been revised to 'measles and rubella elimination by 2023'. Hence, for the confirmation of suspected rubella cases and genotyping of circulating viruses, a role of laboratory is crucial. The complete genome data of rubella virus (RuV) isolates was not available from India. Hence, nine RuV isolates obtained during the years 1992, 2007, 2008, 2009, 2015, 2016 and 2017 from the States of Karnataka, Kerala, Maharashtra and Odisha were completely sequenced and analyzed. The Indian RuV genomes

were compared with the reference genotypes and vaccine strains. Additionally, antigenic characterization of RuV wild type and vaccine viruses was performed using a rapid and reliable neutralization test. Genome analysis of Indian RuVs revealed three clusters (Figure1) i.e. Pune-1999 (n=1), Kannur-2009 (n=5) and Chitradurg-2007 (n=3), interestingly no major variations evident in crucial epitopes present on E1, E2 and capsid proteins. Results showed significantly higher neutralizing antibody titers to RuV vaccine strain (RA 27/3) compared to the wild type (Kannur-2009). The antigenic studies by neutralization tests showed 91.9% agreement between two FRNTs (RuV genotypes 2B and 1a) when 99 serum samples were challenged. The FRNT titers to RuV vaccine strain was significantly higher than the RuV wild type strain (1.6171 vs 1.0117, P<0.001, Paired t-test). Overall, the mean FRNT titers to RuV wild type and vaccine strains were 1.0117 (SE Mean: 0.058) and 1.6171 (SE Mean: 0.088) respectively (highly significant for vaccine strain, p<0.001, Paired t-test). However, comparison of rubella IgG EIA with FRNTs revealed slightly higher agreement when challenged with RuV 2B (86.8%) than RA 27/3 vaccine strain (82.8%). The quantitative titers between vaccine and wild type virus showed good correlation (R^2 = 0.909), indicating antigenic similarity amongst RuVs. This is first study from India that describes complete genomes of wild type RuVs (genotype 2B) and its comparative neutralization activity to RuV vaccine (genotype 1a) strain.

(ii) Cross-neutralization between measles wild types and vaccine strains

This study aims to determine likely reasons for clinical measles in the immunized individuals. Generally, neutralization tests are applied to measure the neutralizing antibody response (or protective immunity levels) to live virus. A study has been performed using two different serum panels. 1st panel consisted of 57 serum samples obtained from 'suspected measles cases' with history of measles immunization at childhood. These sera were challenged in FRNTs using measles virus (MeV) D8-Jamnagar & MeV Edmonston Zagreb (EZ) vaccine strain. The 2nd panel consisted of 50 serum samples obtained from 'suspected measles cases' without history of measles immunization. These sera were challenged in FRNTs using genetically divergent MeV D8-New Delhi & D8-Jamnagar strains. Both these serum panels were tested for measles specific IgM and IgG antibodies using commercial EIAs. Amongst the 1st panel, 52 sera showed slightly higher titer to wild type (MeV D8 i.e. mean titer 2.38) compared to vaccine (MeV EZ *i.e.*, mean titer 2.19) whereas, 5 sera showed higher titer to vaccine compared to wild type strain. The quantitative titers to MeV EZ and MeV D8 virus showed good correlation (R^2 = 0.99), indicating *in vitro* antigenic similarity. In the 2nd panel, both challenge viruses (D8) showed relatively similar neutralizing antibody titers (2.30 & 2.28) with R^2 value of 0.97. This is the first study from India that describes neutralization activity of wild type MeVs and vaccine strain. Overall, no major antigenic differences were noted in vaccine strain and circulating wild types from India.

Figure 1: Whole genome phylogenetic tree of global and Indian RuV wild type isolates generated using maximum-likelihood method. Indian isolates sequenced in this study are shown in bold. Black circles indicate branches >70% bootstrap support.

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

Vaidya SR

Funding agency: ICMR-NIV Duration: 2019-2022

It is well known that commercial EIAs may provide false positive or negative results and sometimes difficult to interpret equivocal results in the clinically suspected cases. And for the confirmatory tests, collection of convalescent serum sample is not easy. Hence, a study was designed to understand the qualitative or quantitative correlation of IgM, IgG and neutralizing antibody in the suspected measles or rubella cases (fever with rash). Altogether, 300 serum samples (age ranged between 1 to 15 years) referred for measles and rubella diagnosis were included in the study. Preliminary results showed 16.89% (50/296), 59.47% (160/269), 69.45% (141/203) positivity for rubella IgM, rubella IgG and RuV FRNT, respectively. Whereas, 41.66% (125/300), 82.57% (218/264) and 97.06% (265/273) samples were positive for measles IgM, measles IgG and measles FRNT, respectively.

During this period, 188-serum samples were tested in IgM EIAs, IgG EIAs and FRNTs for both measles and rubella. The presence of measles and rubella specific IgM, IgG and neutralizing antibodies is depicted in Figure 2. All three measles and rubella specific antibodies were detected in 23.93% and 15.42% cases, respectively. For measles, 29.78%, 85.63% and 96.80% samples were positive in IgM, IgG and FRNT respectively. For rubella, 17.02%, 54.78% and 67.55% samples were positive in IgM, IgG and FRNT respectively. Overall, significantly higher positivity noted by FRNTs for both measles (96.80% 182/188) and rubella (68.08%128/188) compared to IgM and IgG EIAs, indicating usefulness of neutralization tests in diagnosis of suspected measles/ rubella cases. The presence of neutralizing antibodies was higher, followed by IgG antibodies and IgM antibodies amongst suspected cases. The quantitative correlation of different antibodies is in progress.

REG1401 & REP1401: Virus registry and Virus Repository activity

The responsibility of virus registry has been taken on 9th September 2019. During this reporting period, Virus Registry received 7153 clinical samples for diagnosis of dengue (n=2212), chikungunya (n=567), influenza-COVID-19 (n=2371), Kyasanur Forest disease- Crimean Congo haemorrhagic fever (n=861), Japanese encephalitis-Chandipura-cytomegalovirus (n=462), hepatitis (n=345), measles-mumps (n=31) and bacterial markers (304). As per ICMR headquarters initiative involved in the project title “Establishment of a network of ICMR-COVID-19 biorepositories in India”.

Also, request for procurement of prototype viruses from various institutes/ organization was received and processed as per the standard protocol of NIV, Pune.

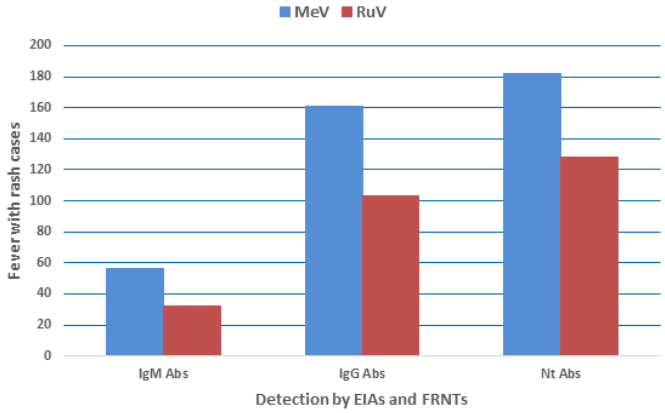


Figure 2: Detection of measles-rubella virus specific IgM, IgG and neutralizing (Nt) antibodies in 188-fever with skin rash cases.

Section 4:
Other Activities

ICMR-NIV ADMINISTRATION, PUNE

STAFF LIST

Senior Administrative Officer Mr. Anil S. Gaikwad

Administrative Officer

Mrs. Shibi Jacob Administrative Officer	Mrs. S. H. Khamkar Administrative Officer(Stores)	Mrs. A. S. Palshikar Administrative Officer

Accounts Officer

Mrs. V. V. Shendye Accounts Officer	Mr. C. Gopalkrishnan Account Officer

Section Officer

Mr. H. S. Pasalkar (Admin Pashan)	Mrs. A. S. Bakare (Admin)
Mrs. A. B. Palkar (NIV Mumbai)	Mrs. A. A. Bapat (Bills)
Mrs. S. S. Pathak (Purchase)	Mrs. S. P. Mulay (Stock Room)
Mrs. A. R. Nair (Establishment)	Mrs. R. S. Moghe (Pension & Project)
Mr. D. V. Muneshwar (NIV Mumbai)	Mrs. R. K. Amale (PS) (Accounts)

Assistant

Mrs. P. S. Joshi	Mrs. D. D. Marathe	Ms. Jayajyothi J.
Mr. R. Basavaraju	Mr. J. R. Kumbhare (PA)	Mrs. S. M. Bhave (PA)
Mr. S. R. Vasam	Mr. K. S. Galange	Ms. P. B. Aher
Ms. Shakila Choudhari (PA)		

Upper division clerk

Ms. MJA Shaikh	Mr. Y. C. Pote	Mrs. S. B. Chakole
Mrs. T. T. Yadav	Mr. P. N. Chabukswar	Mr. A. E. Matkar
Mrs. D. N. Gujar	Mrs. Mangala Gangadharan	Mr. M. S. Malvankar
Mrs. M. L. Rupnar	Mr. H. D. Raut	Mrs. M. R. Kannalu
Mr. Prashant D. Patil	Mr. Prem P. Khandagale	Ms. Madhuri S. Tandan
Mr. Amol S. Lohbande	Ms. Prajakta A. Bapat	Mrs. Sadhana Veer
Mr. R. R. Jaiswal	Mr. P. B. Santhoshkumar	Mrs. Roshan B. Patel
Mr. Ajay S. Wable		

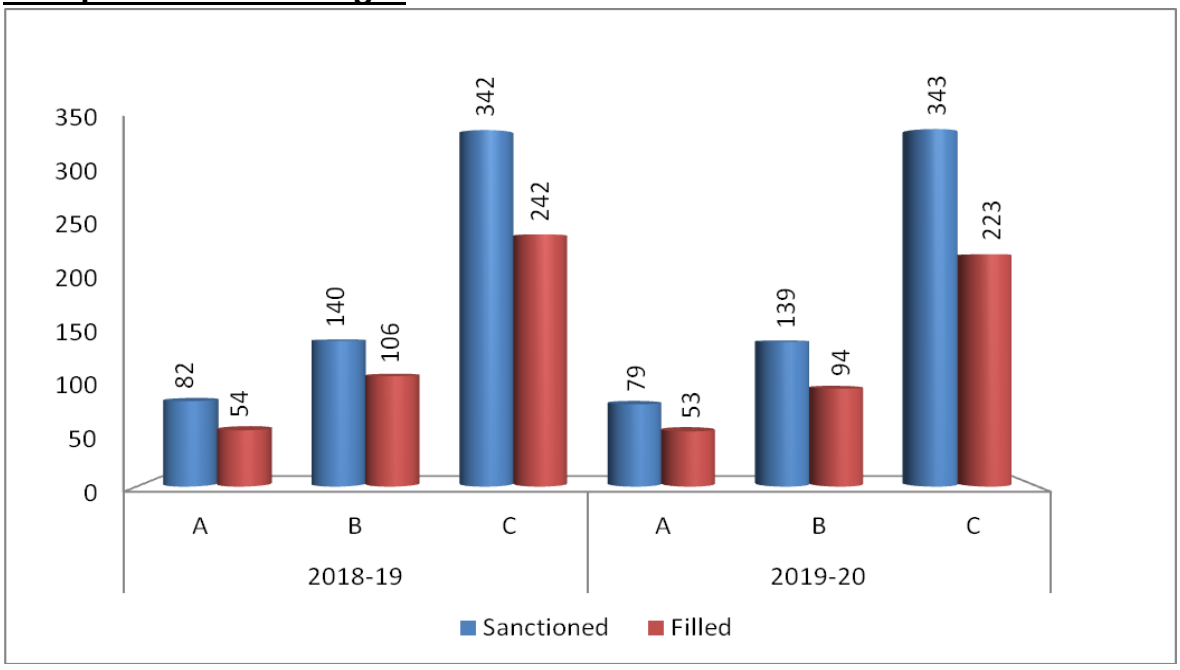
Lower division clerk

Mrs. Ashwini Dudhane Ms. Y. C. Bhandare

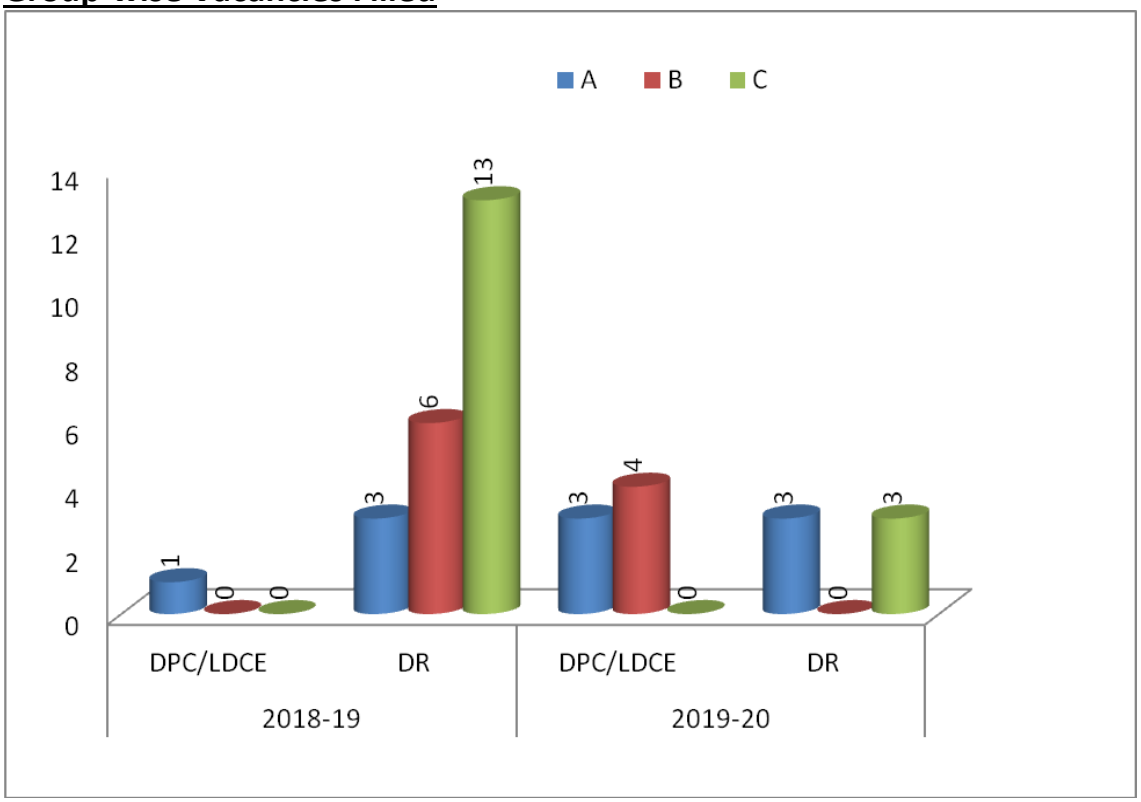
Director's office

Mrs. A. V. Shendrikar (Retd STO) Mrs. Deepika Chaudhari (TO-B)

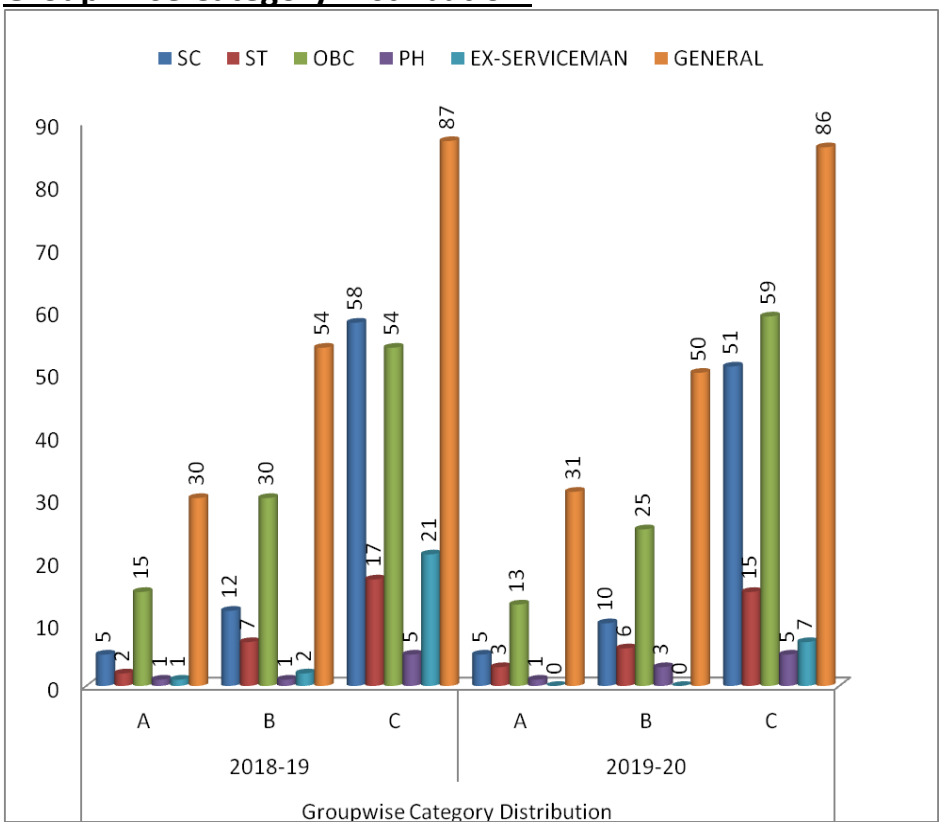
Group-wise Staff Strength



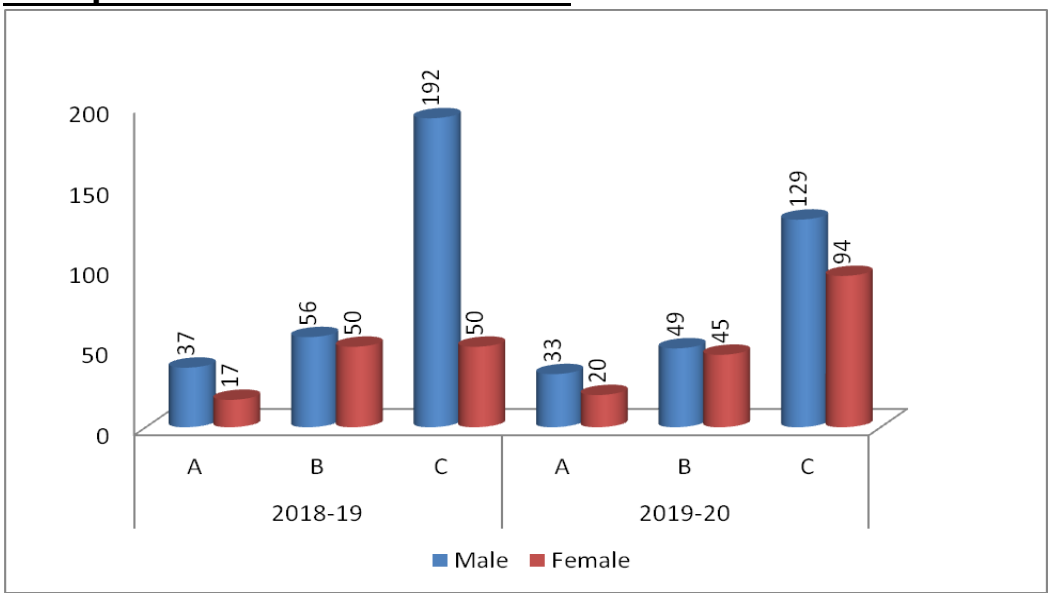
Group-wise Vacancies Filled



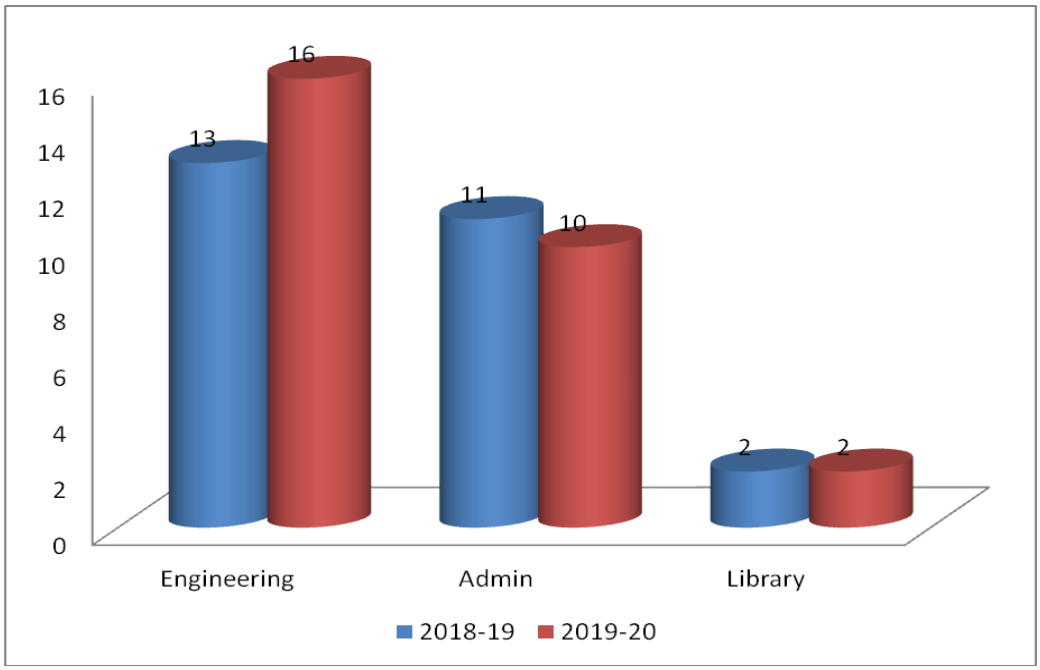
Group-wise Category Distribution



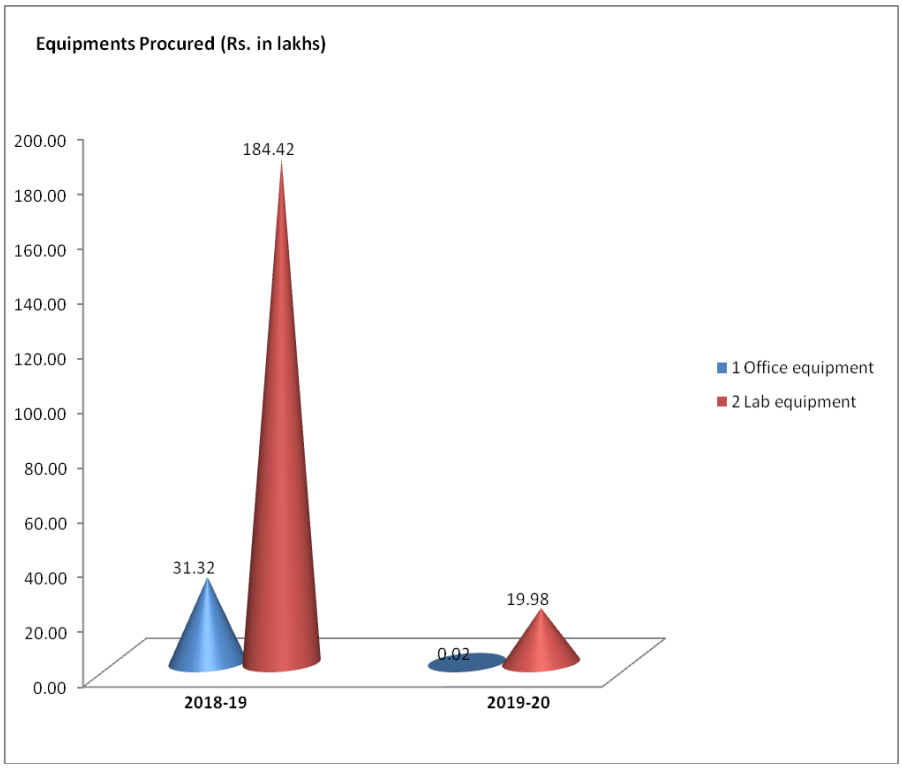
Group-wise Gender Distribution



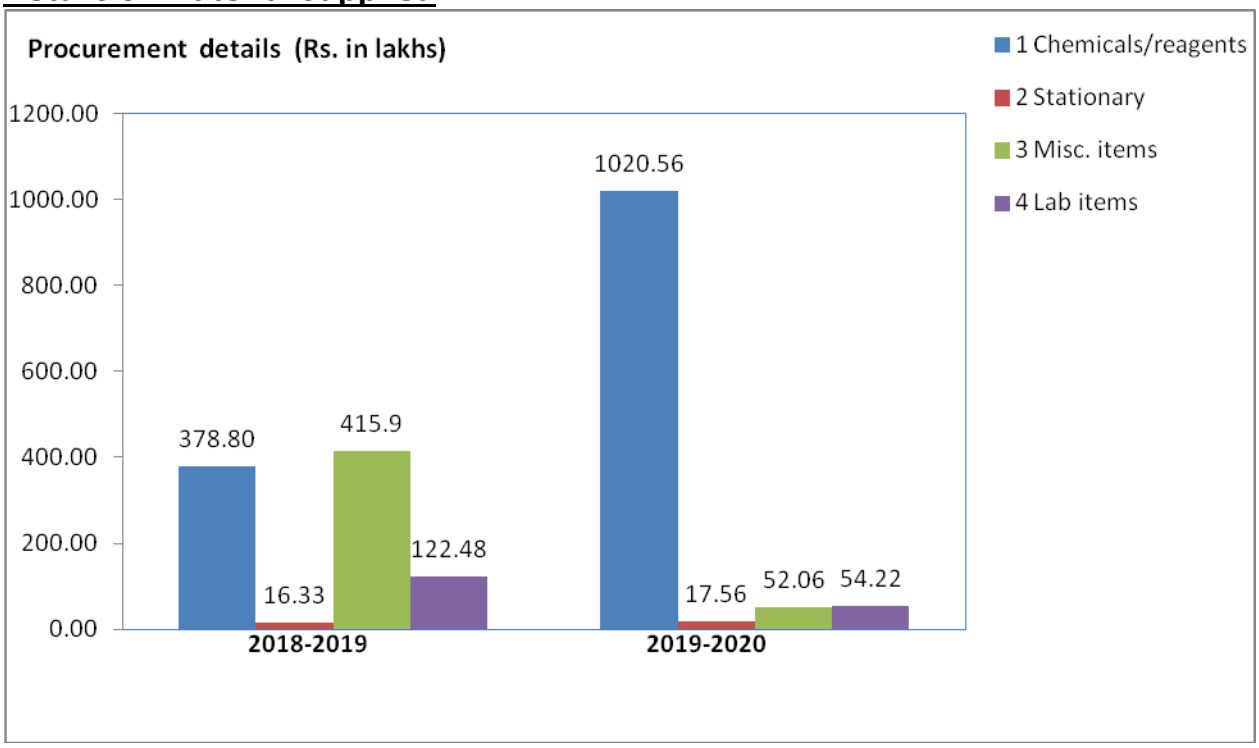
Apprentices Engaged



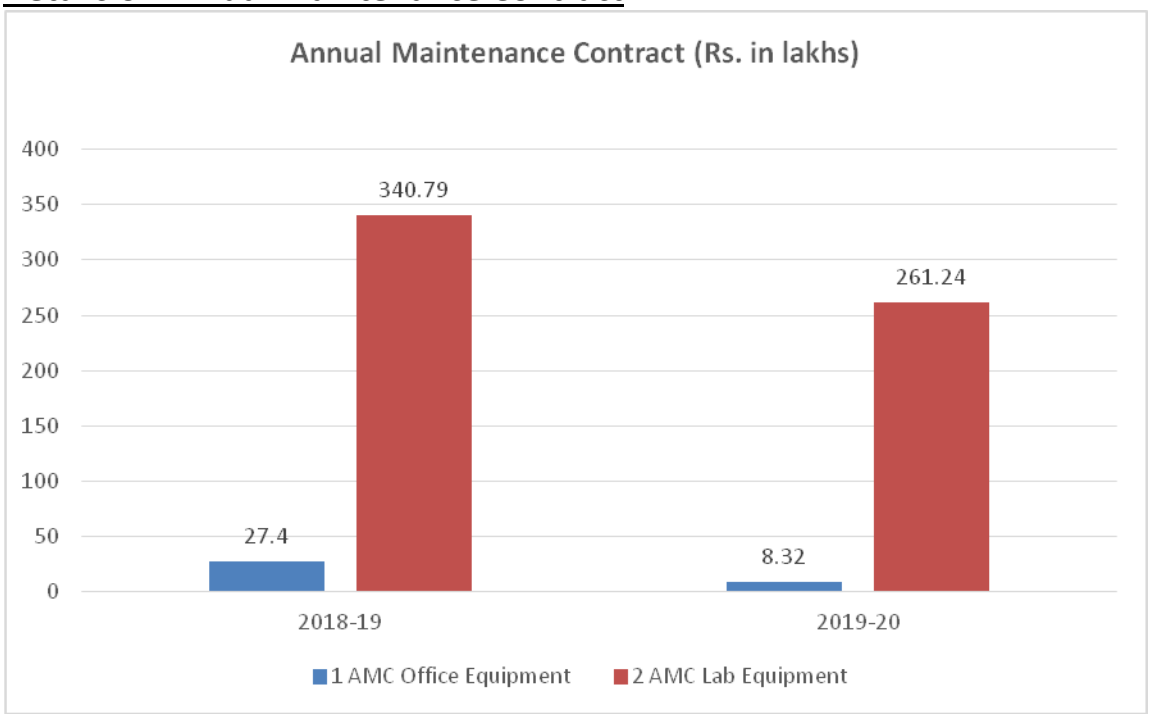
Details of Equipment supplied



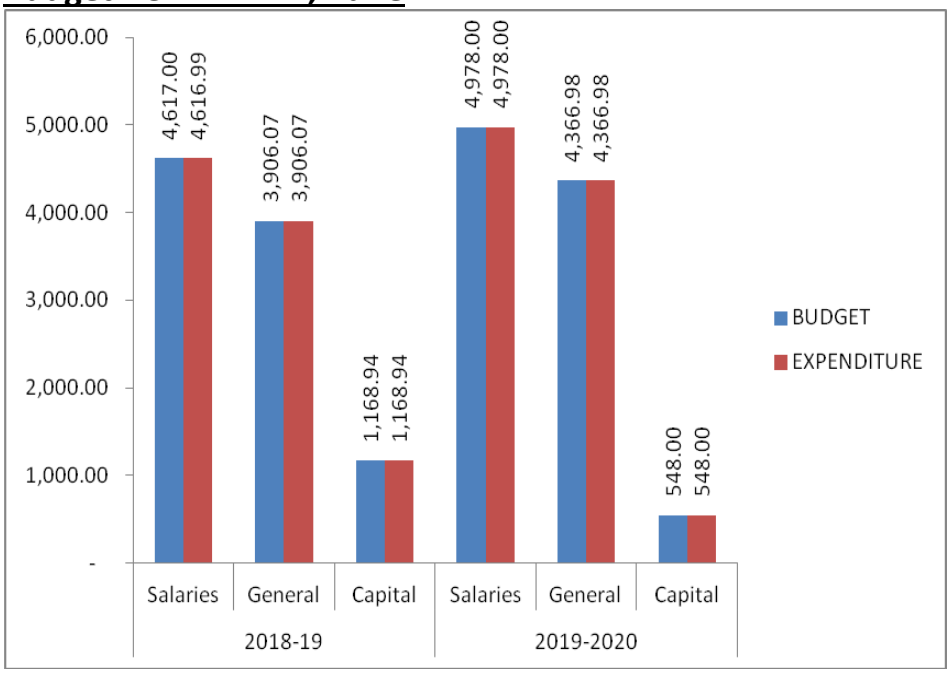
Details of Material Supplied



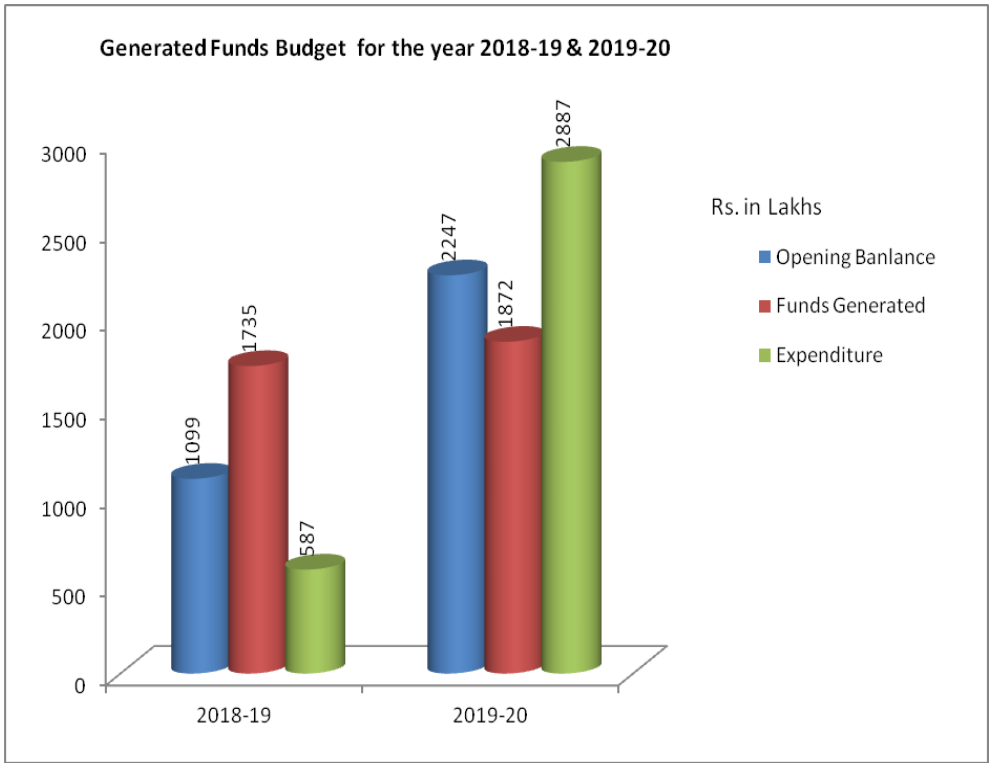
Details of Annual Maintenance Contract



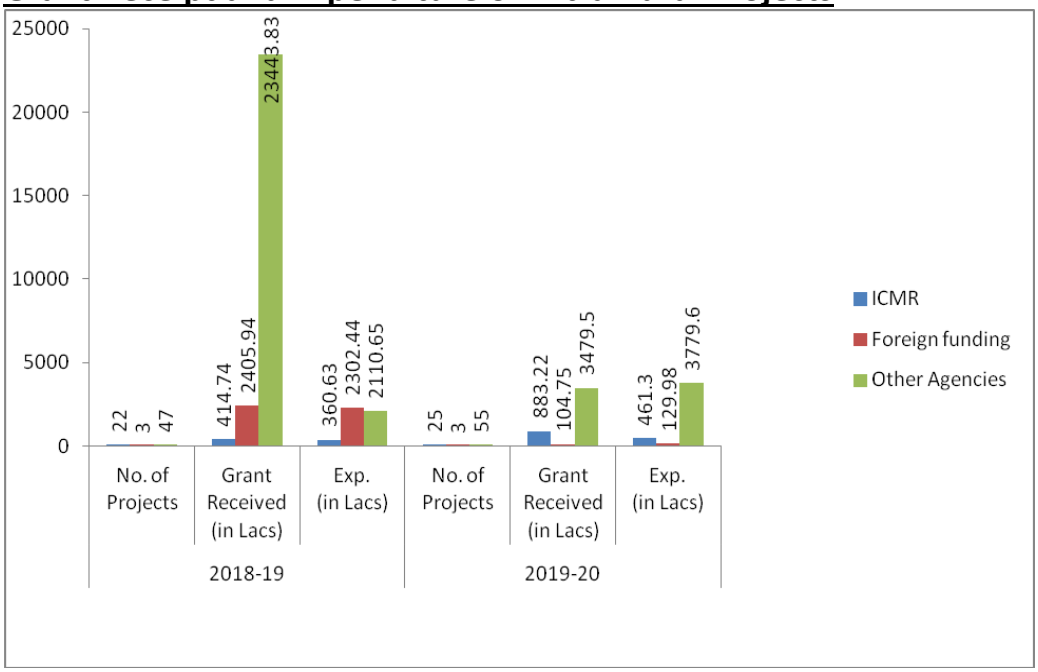
Budget: ICMR – NIV, Pune



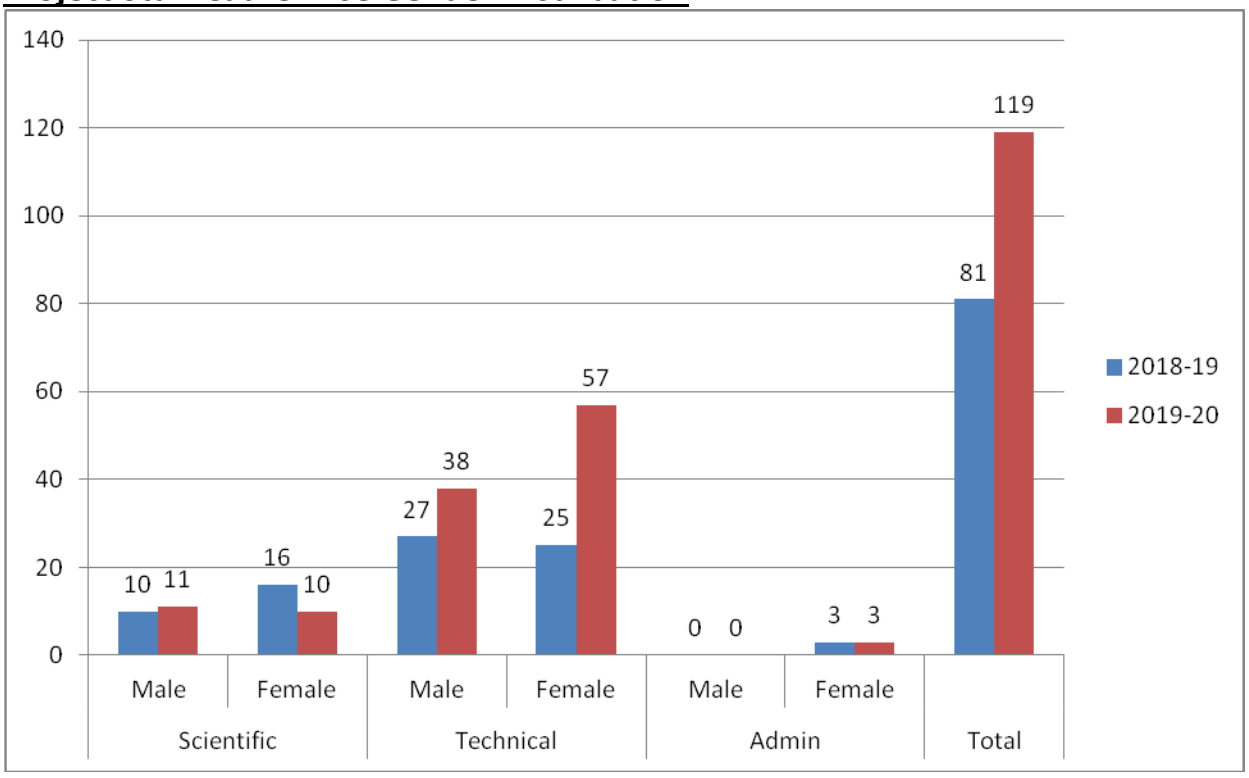
Budget: Generated Funds



Grant Receipt and Expenditure of Extramural Projects



Project Staff Cadre wise Gender Distribution



ENGINEERING SUPPORT GROUP

List of staff members

Sr. No.	Name of Staff	Designation
Staff at NIV, Pune		
1.	Mr. A.B.Khare	HOD, Sr. Tech. Officer-3 (ES)
2.	Mr.A.R. Sable	Sr. Tech. Officer-2 (ES)
3.	Mr. D.R. Kumbhar	Sr. Tech. Officer-1 (ES)
4.	Mr. A.K.Kasar	Tech. Officer- A (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. V. Sutar	Technician-B
17.	Mr. D.K. Jagtap	Laboratory Asst. (ES)
18.	Mr. M.V. Gadhave	Technician-A (ES)
19.	Mr. A.M. Pawar	Technician-3 (ES)
20.	Mr. A.N.Kale	Technician-A (ES)
21.	Mr. S.R.Jagtap	Technician-A (ES)
22.	Mr. Md Nazim Akhtar	Technician-1 (ES)
23.	Ms. Komal Jangid	Technician-1 (ES)
Apprentice staff NIV, Pune		
24.	Miss. Pratiksha Shete	Electrician
25.	Mr. Alwyn Kamble	Electrician
26.	Mr. Tushar Surve	Electrician
27.	Mr. Parag Nandanvar	Electrician
28.	Mr. Rushikesh Devkar	Electrician
29.	Mr. Santosh Jagtap	Electrician
30.	Mr. Sachin Chorge	Electrician
31.	Mr. Pavan Chaudhari	Electrician
32.	Mr.Aditya Deshmukh	Ref & ACM
33.	Mr.Amit Shaikh	Ref & ACM
34.	Mr.Kersing Changal	Carpenter
35.	Mr. Aniket Pachngane	Plumber
Staff at Pashan unit		
36.	Mr. M. S. Mohite	Technical Assistant (ES)

37.	Mrs.P.C. Lokhande	Technical Assistant (ES)
38.	Mr. S.D.Pote	Technician-C (ES)
39.	Mr. S. N. Surbhaiya	Technician-C (ES)
40.	Mr. S.D.Bathe	Sr.Technician(1)(ES)
41.	Mr. G.R.Ghogare	Technician-B (ES)
42.	Mr. V.T.Ishte	Technician (2)(ES)
43.	Mr. I.R.Dedunda	Technician-A (ES)
44.	Mr. Mritunjay Singh	Technician-1 (ES)
45.	Mr. Nand Kumar	Technician-1 (ES)
46.	Mr. Niteesh Kumar Yadav	Technician-1 (ES)
47.	Mr. Vishal Ashok Gaikwad	Technician-1 (ES)
48.	Mr. Govind Sharan Meena	Technician-1 (ES)
Project Staff Pashan Unit		
49.	Mr. Y. Gondane	Technical Officer A
Apprentice staff Pashan Unit		
50.	Mr. Amol Surwade	Carpenter
51.	Mr. Gaurav Jadhav	Plumber
Staff at Kerala unit		
52.	Mr. Uma Ganesh Pentakota	Technician-1 (ES)
53.	Mr. Santosh Kumar Bosta	Technician-1 (ES)
Staff at Bangalore unit		
54.	Mr. Akash M. Jagtap	Technical Assistant (ES)
55.	Mr. Arjun Jogangiri	MTS
Staff at Mumbai unit		
56.	Mr. Kamlesh Pawar	Technician-1 (ES)

The maintenance department works 24x7 for operation and maintenance of various electrical, HVAC, mechanical installations. Carrying out routine / preventive / breakdown maintenance works. Regular servicing, overhauling of machines and equipments were under taken to extend their performance and life. The maintenance staff is from various trades like Electrical, Refrigeration & Air Conditioning, Carpentry, Civil, Plumbing works together to achieve the objectives of maintenance department. The maintenance department of NIV renders the services to various field units such as Gorakhpur, Bangalore, Kerala and Mumbai.

Works carried out by Engineering Support

- **Achievement**
  - **Received NABL accreditation as per ISO/IEC-17025 for certification of Biosafety cabinet**

Major works undertaken during the year

Pashan Campus

Carry out Internal audits for NABL accreditation for testing of biosafety cabinet.
Repairing of refrigerated centrifuge of Hepatitis section.
Installation of Standby Circuit breaker (2000 Amp) of LT panel
Repairing of freezer room of ENC group (replacement of Compressor)
Replacement of 240 sqmm faulty cable of MDP-2 at main building ground floor
Joining of underground electrical cable of store admin building
Preparation of pictorial SOP for Fire alarm system and 600 KV DG set of BSL3.
Provision of alarm under voltage alarm for all UPS at BSl3
Oil filtration and testing of earth pits at substations
Replacement of speed control unit of 600 KVA DG set
De-scaling of condensers of central AC plant and BSL3 AC plant
Replacement of damaged door of autoclave at ENC
Repairing of damaged street light pole in front of staff quarter B.
Simulation of Cook tank for leakage of water

Pune Campus

Annual inspection of lifts
Modification of lights in passenger lift
Replaced control panel of fire alarm system at Influenza division, labeling to all zones.
Servicing of 1250 amp ACB of LT panel
Repairing of – 80 freezer of Measals, Dengue and Chikunguniya lab.
Structural audit of terrace of admin building for installation of new chillers carried out by Prof. Birajdar, HOD of Engineering Div.COEP.
Termination of 95 sqmm cable to APFC panel

Capital works:

Renovation of Electrical wiring with replacement of main panel of Admin building
Replacement of ACB at central AC plant
Installation of new In line water pumps in place of 30 years old condenser water pumps.
Provision of stand by ACB for AMF panel of 600 KVA DG set of BSL3
Laying of standby 240 Sqmm underground cable for admin building.
Repairing of parapet wall of admin building at Pashan
Renovation of Dengue lab
Plinth protection of STP plant
Fixing of interlocking blocks near exit door of main building

Sealing of cracks at duct floor of BSL3 laboratory
PU coating of BSL3 laboratory
Installation of pre-coated roofing with MS structure at terrace of main building
Renovation of Type-A and Type V staff quarter at Pashan.
Demarcation of Land allotted for SCOH, Nagpur, through private agency.
Quarterly process report of capital work sent to ICMR for June, Sept, Dec and March
Requirement of Capital funds for 2020-21 sent to ICMR.
Details of land and buildings sent to ICMR.
Details of amount capitalized sent to ICMR
Reply to Audit Para: Sr. No. 8 (09-10): Delay in completion of BSL4 and Sr. No. 12: Para no. 1(16-17): Slow progress of Capital works for which funds of Rs. 1.51 cores re appropriated from the multipurpose hall

- **Contribution in "Swachh Bharat Companion":**
  - Participated in "Swachh Bharat Companion" on 4th OCT 19
- **Emergency Attended:**
  (i) On 18th July 2019 at around 10:30 p.m. underground cable developed sparks. Engineering staff immediately put off all the electrical supply and isolated the faulty cable from LT panel and resumed supply around 11:15 p.m.
  (ii) Breakdown of freezer room at ENC failed on 28.05.19. Replaced faulty compressor and restarted the freezer room without shifting of material.
- **Other works :**
  - Electrical meter readings of Staff quarter in every month.
  - Preparation of BSNL electrical charges bill in every month
  - Distribution of Treated water (KL) of STP prided for garden (3630 KL)
  - Preparation for SAC meeting
  - Preparation for Foundation day program
  - Arrangement of MIC system for various programs/lectures at conference hall.
  - Submission of annual report of Bio Medical waste to MPCB

ACADEMIC CELL

List of Staff

Dr. Kavita S. Lole, Scientist F and Group Leader Hepatitis/Academic Cell In-charge
Dr. V S. Ghole, Co-ordinator Academic Cell (Retd Professor, Savitribai Phule Pune University)
Dr. Tejaswini M. Deshmukh, Scientist C

Technical Staff

Mrs. Swati Bohodkar (Sr. Tech. 3)
Mr. Hemant R. Band (Sr. Tech. 1)
Ms. Anuradha Vaidyanathan (Technician C)
Mrs. Neethi Jayaram (Project TO)
Ms. Manisha T. Dudhmal (DHR Project TA)

M.Sc. Virology Programme:

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

Since its launch in 2005, the M.Sc. Virology course has been progressing very well. Till date, thirteen batches of students have completed the course successfully.

20 students of M.Sc. Virology batch 2017-19 passed out in July 2019.

Grade	Number of students
'O' - Outstanding	6
'A+' - Excellent	8
'A' - Very Good	2
'B+' - Good	4

Toppers of 2017-19 batch:

Rank	Name of the student
1	Ms. Manasi N Nadkarni (Gold medalist)
2	Mr. Aniket C Gondhalekar

22 students were admitted to M.Sc. Virology 2019-21 batch in August 2019.

Student Achievements:

1. After completion of the course in 2019, five students were placed on various projects in ICMR-NIV, three were placed on projects in ICMR-NARI and four students at IRSHA, BVDU. Some of the students are working in industries and other institutes in India.
2. Ms. Manasi Nadkarni secured admission to Masters of Public Health at University College Cork, Ireland.
3. Avirup Sanyal (2018-20 batch) was selected under **Khorana Program for Scholars 2019** (awarded by DBT, Govt. of India, Indo-U.S. Science and Technology Forum (IUSSTF) and WINStep Forward) and worked at the Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, (December 20, 2019 to June 20, 2020) under Prof. James Chodosh

Scientific data generated during their dissertation work has been published in reputed national and international journals along with faculty members

Details of the program are available on

1. https://icmr.nic.in/institutes
2. www.niv.co.in

Ph.D. Programme:

Recognition for M. Phil and Ph.D. programs with SPPU for the following subjects:
1. M.Phil Biotechnology
2. M.Phil Basic Medical Science
3. Ph.D. Biotechnology
4. Ph.D. Basic Medical Science
5. Ph.D. Microbiology
6. Ph.D. Zoology
7. Ph.D. Biochemistry

Number of M. Phil/Ph.D. SPPU Recognized guides: **16**

Number of students enrolled for Ph.D.: **1**- (Ms. Prachi Jagtap in Basic Medical Science)

Number of Ph.D. Degrees awarded: **2**
1) Mr. Abhisek Behera (Basic Medical Science)
2) Ms. Asha Mathew (Biotechnology)

Research Work:

Project Title: Development of a rapid immunochromatographic assay for the diagnosis of hepatitis E
Investigators: Tejaswini Deshmukh, Manisha Dudhmal, Kavita Lole
Funding:DHR (~Rs. 25 lacs for 2 years sanctioned vide DHR letter No. R.11013/4/2017-HR dated 22-06-2017**)**
Project Duration: 15-07-2017 to 14-07-2019, No Cost Extension 15-07-2019 to 14-12-2019
Status: Completed

Background:

Hepatitis E (HE) continues to be a significant economic and health burden endemic to India. Hepatitis E virus (HEV), cause of HE is mainly responsible for water-borne epidemics of varying magnitudes occurring in different parts of India. More than 50% of reported sporadic acute viral hepatitis cases are due to HEV. HEV causes significant mortality (10-30%) in pregnant women. A three year (2011-13) surveillance report from India indicated HEV as a major cause of acute viral hepatitis in laboratory confirmed cases and outbreaks amongst the other known viral causes [hepatitis A (HAV), B (HBV) and C (HCV) viruses]. The report highlighted importance of viral hepatitis surveillance with strengthening of laboratory component. Effective management of acute viral hepatitis is a function of its virus specific mode of transmission (enteric vs. parenteral) making rapid diagnosis mandatory. A simple rapid HE diagnostic tool is much needed in resource limited settings.

HEV genome (~7.2kb) consists of three overlapping open reading frames (ORF1-3). ORF2 codes for capsid protein (ORF2p 660 a.a.); the major diagnostic/vaccine development target. A region within ORF2p (458-607 a.a., 150 a.a.) of mammalian genotype 1 HEV (HEV1) was reported to contain putative neutralizing epitope/s (T1NEp).

Objective:

- To develop simple rapid immunochromatographic assay (RICA)/strip test for hepatitis E diagnosis using IgM capture format

Findings:

1) A simple rapid immunochromatographic lateral flow based strip test/point-of-care (POC) has been developed in IgM capture format for diagnosis of HE, **ICMR-NIV HEV IgM Rapid Test**.
2) Performance of strip test was evaluated using a total of 731 human serum samples in comparison with *in house* ORF2 and T1NE based indirect ELISAs; Wantai (China) and MP Diagnostics (Singapore) ELISAs; CTK (USA) and ASSURE MP Diagnostics (Singapore) rapids tests.
3) ICMR-NIV HEV IgM Rapid Test was 99.6%, 100% and 99.6% sensitive and 100% specific as compared to ORF2, T1NE and Wantai ELISAs, respectively.
4) It showed 99.8%, 100% and 99.8% (κ=1.0, P=0.00 for all three comparisons) concordances with ORF2, T1NE and Wantai ELISAs, respectively.
5) The test did not detect anti-HAV IgM antibodies, anti-HCV antibodies, anti-DENV IgM antibodies and RA factor.
6) The test showed reproducible results for randomly coded known anti-HEV IgM antibody positive and negative serum samples in 3 internal labs of ICMR-NIV, representative shown in Figure 1.
7) Stability performance of strip test was carried out using two different types of NCMs, NCM8 and NCM2 for 12 weeks and 14 weeks, respectively. NCM8 showed better stability at all the 3 temperatures as compared to NCM2.
8) An application for filing of process patent has been forwarded to ICMR through ICMR-NIV with title: "Process for development of a rapid immunochromatographic assay for hepatitis E diagnosis" (Inventors: Dr. T. M. Deshmukh, Ms. M. T. Dudhmal, Dr. K. S. Lole).

Fig. ICMR-NIV HEV IgM Rapid Test showing results for known anti-HEV IgM antibody positive and negative serum samples used as controls (PC and NC, respectively) (Serum sample required: 10µl, Turnaround time: 10 minutes) (strips were printed and cut manually)

LIBRARY & INFORMATION SERVICES

Scientific Staff

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

Technical Staff

Mrs. Vandana Chandere	Senior Technical Officer (2)
Mr. Vishal R. Mali	Senior Technical Officer (1)
Mrs. Ekta Jain	Library & Information Assistant
Mrs. Reshma A. Rade	Library & Information Assistant

Library Apprentices

Ms. Akshada Bhalerao	(Till 31/10/2019)
Mrs. Madhusmita Dash	(Till 31/10/2019)
Mrs. Prabin Das	(From 01/11/2019 to 31/12/2019)
Mrs. Pallavi A. Parshuramkar	(From 01/11/2019 to 31/03/2020)

ICMR-NIV Library major aim is to provide pinpointed information and services to its users *viz*. Scientists, M.Sc. and Ph.D. students of ICMR-NIV as well as scientists and students of other research institutions of ICMR, Universities, Medical Colleges, and Private Colleges etc. During the year NIV Library has been able to add a collection of approx **60** books, bound volume of journals, annual reports, thesis, dissertations, etc in the current financial year.

Library services are automated using the Integrated Library Management Software and are leveraged through extensive ICT based applications to support biomedical users in education and research. The barcode technology has been implemented for all books and bound volume of journals for smooth functioning of circulation section and stock verification purpose. Library activities take place in a fully automated environment using LIBSYS Library Management Software. The library is scanned by CCTV Cameras for security and monitoring purposes.

The Library is providing very important service of Newspaper Clippings service on daily basis to the scientists of NIV and its Field Unites about outbreaks, diseases like COVID-19, dengue, hepatitis, chikungunya, swine flu, influenza, virus like Zika, Ebola, Nipah, Canine Distemper virus, etc via email and the hard copy of the news is displayed on the Notice Board of Library for the students and other staff of NIV.

Library renders others services like citation analysis of publications of scientists, reference service, literature search, document delivery service, CAS, SDI on demand, reprography, binding and lamination. We also provide anti-plagiarism services through Urkund Software (an anti-plagiarism web tool) to the Ph.D. students for thesis, M.Sc. students for dissertations and scientists for manuscripts. Library imparts orientation and information literacy programs to M.Sc. virology students, research scholars and newly joined staffs regularly. The Library also conducts trainings, seminars, conferences on various topics for its users on time to time.

Online e-journals can be accessed through IP in ICMR-NIV, MCC Campus and three field units Bangalore, Kerala and Mumbai Field Unit. Library has its own Web OPAC to retrieve Library resources effectively.

Library Collection

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

Description		**Quantity**
Books	Purchased/Gifts/Gratis	19
	Bound Volumes	-
	Annual Reports Received	13
Journals	Print (Subscribed)	14
	Print (Gratis)	53
	ICMR Consortium	4
	J Gate	4394
	ERMED Consortium	239+
	Loose issues	425
Ph.D. Theses		3
M.Sc. Dissertations		21
Others; CDs, Microfilms, Floppies		3
Papers sent for Publication		15
Papers Published by NIV Scientists		81
Reprints		NIL

Library Services Provided:

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

1.	Book & Bound Volumes Issued	Staff: 211 Students: 895
	Book & Bound Volumes Returned	Staff: 203 Students: 887
2.	Newspaper Clippings	5476
3.	Inter Library Loan Received and Sent	70
4.	Photocopy Service	5314
5.	Binding (Thermal & Spiral)	6
6.	Lamination	21
7.	Reference Service	290
8.	NIV Annual Reports, Compendiums, Handbooks, Manuals Distributed	5
9.	Citation Analysis	9
10.	Anti-Plagiarism Service	22

HINDI REPORT 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 द्विभाषी - हिंदी और अंग्रेजी भाषा में अग्रेषण किया जाता हैं ।

राजभाषा नियम 1976 के नियम 10(4) के अनुसार संस्थान के कर्मचारीयोंको हिंदी का कार्यसाधक ज्ञान प्राप्त करने हेतु हिंदी शिक्षण योजना तथा अनुवाद प्रशिक्षण केंद्र(मुंबई) द्वारा आयोजित प्रशिक्षण वर्गों में नामित किया जा रहा है । इस साल हिंदी शिक्षण योजना तथा अनुवाद प्रशिक्षण केंद्र(मुंबई) द्वारा आयोजित परीक्षाओं में उत्तीर्ण कर्मचारिओं की संख्य निम्नवत है :

हिंदी टंकण / हिंदी शब्द संसाधन – 2 और अनुवाद प्रशिक्षण केंद्र, मुंबई द्वारा आयोजित प्रारंभिक अनुवाद प्रमाणपत्र प्रशिक्षण - 2

नगर राजभाषा कार्यान्वयन समिति द्वारा आयोजित की जा रही तिमाही बैठकों में संस्थान के अधिकारी उपस्थित रहते हैं ।

परिषद् द्वारा प्राप्त निर्देश तथा संस्थान की राजभाषा कार्यान्वयन समिति द्वारा लिए गए निर्णयानुसार संस्थान में हिंदी सप्ताह दिनांक 14 से 20 सितंबर 2019 को मनाया गया । सप्ताह के अंतर्गत दिनांक 19 और 20 सितंबर 2019 को "जल संकट और संरक्षण" इस विषय पर दो दिवसीय कार्यशाला संपन्न हुई । कार्यशाला में कुल 43 प्रतिभागियों ने सहभाग लिया । केन्द्रीय भूमि जल बोर्ड, पुणे में जल-भूविज्ञानी पद पर कार्यरत श्री उपेंद्र धोंडेजी को बतौर प्रमुख अतिथि आमंत्रित किया गया था । कार्यशाला में प्रमुख अतिथि श्री धोंडेजी तथा उनके सहयोगियों द्वारा प्रस्तुतीकरण तथा संवादात्मक सत्र के द्वारा कार्यशाला के प्रतिभागियों को संबोधित किया गया । अतिथि व्याख्याता पर्जन्य रेनवॉटर हार्वेस्टिंग कंसल्टंसी के प्रमुख निवृत्त कर्नल शशिकांत दलवीजी ने अपने प्रस्तुतीकरण द्वारा प्रतिभागियों को महत्वपूर्ण जानकारी दी ।

हिंदी सप्ताह के दौरान "जल संकट और संरक्षण" इस विषय पर पोस्टर प्रस्तुतीकरण और प्रश्नोत्तरी प्रतियोगिता का भी आयोजन किया गया था । पोस्टर प्रस्तुतीकरण प्रतियोगिता में 7 प्रतिभागीयों ने पोस्टर प्रस्तुतीकरण में सहभाग लिया तथा पोस्टर के बारे में जानकारी प्रस्तुत की । प्रश्नोत्तरी प्रतियोगिता हेतु कार्यशाला में उपस्थित प्रतिभागियों को समूह ए, बी, सी और डी में विभाजित किया गया था । कार्यशाला के विषय से संबंधित प्रश्नों पर आधारित प्रश्नमंजूषा रोचक ढंग से संपन्न हुई । कार्यशाला के समापन समारोह में संस्थान के प्रभारी निदेशक डा. आर आर गंगाखेडकरजी द्वारा उत्कृष्ट तीन पोस्टर प्रस्तुतियोंको नकद पुरस्कार से सम्मानित किया गया ।

संस्थान में आतंकवाद विरोध दिन, सतर्कता जागरूकता सप्ताह, कौमी एकता सप्ताह, स्वच्छ भारत अभियान सप्ताह के उपलक्ष्य में हिंदी भाषा में शपथ ग्रहण संपन्न करवाते हुए व्याख्यान, संगोष्ठी तथा विभिन्न प्रतियोगिताओं का आयोजन कर मनाया गया ।

हिंदी कार्यशाला – 2019 के उपलक्ष्य में "जल संकट और संरक्षण" विषय पर व्याख्यान, पोस्टर प्रस्तुतीकरण प्रतियोगिता तथा प्रश्नोत्तरी प्रतियोगिता का आयोजन किया गया ।

List of papers published by ICMR-NIV Scientists
1st April 2019 to 31st March 2020

- **No. of Total Publication: Articles = 80** **Book Chapter = 2**
- **No. of Publication with Impact Factor: 73** **Average Impact Factor: 3.021**

Sr. No.	Article Details	Impact Factor
1.	Agarwal A, Nagi N, Chatterjee P, Sarkar S, Mourya D, Sahay RR, Bhatia R. Guidance for building a dedicated health facility to contain the spread of the 2019 novel coronavirus outbreak. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 177-83.	**1.503**
2.	Alagarasu K, Patil JA, Kakade, More AM, Bote M, Chowdhury D, Seervi M, Rajesh NT, Ashok M, Anukumar B, Abraham AM, Parashar D, Shah PS. Spatio-temporal distribution analysis of circulating genotypes of dengue virus type 1 in western and southern states of India by a one-step real-time RT-PCR assay. ***Infect Genet Evol***. 2019 Nov; 75: 103989.	**2.773**
3.	Alam A, Taye N, Patel S, Thube M, Mullick J, Shah VK, Pant R, Roychowdhury T, Banerjee N, Chatterjee S, Bhattacharya R, Roy R, Mukhopadhyay A, Mogare D, Chattopadhyay S. SMAR1 favors immunosurveillance of cancer cells by modulating calnexin and MHC I expression. ***Neoplasia.*** 2019 Aug; 21(10) : 945-62.	**5.696**
4.	Arunkumar G, Chandni R, Mourya DT, Singh SK, Sadanandan R, Sudan P, Bhargava B; Nipah Investigators People and Health Study Group. Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. ***J Infect Dis.*** 2019 24;219(12):1867-78.	**5.022**
5.	Bagepally BS, Gurav YK, Anothaisintawee T, Youngkong S, Chaikledkaew U, Thakkinstian A. Cost Utility of Sodium-Glucose Cotransporter 2 Inhibitors in the Treatment of Metformin Monotherapy Failed Type 2 Diabetes Patients: A Systematic Review and Meta-Analysis. ***Value Health.*** 2019 Dec;22(12):1458-69.	**4.748**
6.	Balasubramanian R, Yadav PD, Sahina S, Nada VA. Distribution and prevalence of ticks on livestock population in endemic area of Kyasanur forest disease in Western Ghats of Kerala, South India. ***J Parasit Dis*** 2019 Jun; 43(2): 256-62.	**NA**
7.	Balasubramanian, R, Sahina, S, Nadh, V Arathy, Sreelekha, K P, Nikhil, TL. Effects of different salinity levels on larval growth and development of disease vectors of Culex species. ***J Environ Biol*** 2019 Sep; 40(5) : 1115-22.	**0.781**
8.	Bhardwaj SD, Potdar VA, Yadav PD, Chaudhary ML, Chadha MS, Mourya D. A case report of the enterovirus-D68 associated severe acute respiratory illness in a pediatric case from India. ***J Infect Public Health.*** 2019 Nov-Dec; 12(6): 900-3.	**2.447**
9.	Bhatia R, Abraham P. Time to revisit national response to pandemics. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 111-3.	**1.503**
10	Cherian SS, Agrawal M, Basu A, Abraham P, Gangakhedkar RR, Bhargava B. Perspectives for repurposing drugs for the coronavirus disease 2019. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 160-71.	**1.503**
11	Choudhary ML, Vipat V, Jadhav S, Basu A, Cherian S, Abraham P, Potdar VA. Development of in vitro transcribed RNA as positive control for laboratory diagnosis of SARS-CoV-2 in India. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 251-4.	**1.503**
12	Deval H, Alagarasu K, Mittal M, Srivastava N, Bachal R, Gondhalekar A, Chaudhary U, Chowdhary D, Bondre VP. Association of single nucleotide polymorphisms in TNFA and CCR5 genes with Japanese Encephalitis: A study from an endemic region of North India. ***J Neuroimmunol.*** 2019 Nov; 336: 577043.	**3.125**

13	Dharmapalan D, Saxena VK, Pawar SD, Qureshi THH, Surve P. Clinical and Molecular Investigations of Hand, Foot and Mouth Disease Outbreak in Navi Mumbai, India. ***Indian Pediatr.*** 2019 Dec 15;56(12):1052-4.	**1.186**
14	George S, Viswanathan R, Sapkal GN. Molecular aspects of the teratogenesis of rubella virus. ***Biol Res.*** 2019 Aug; 52(1) : 47.	**3.092**
15	Gopalkrishna V, Joshi M, Viswanathan R, Malu G, Ganorkar N, Chavan N, Shinde M, Kumar A, Dhurandhare S. Cholera outbreak in Aurangabad, Maharashtra, western India. ***Indian J Med Res.*** 2019 Dec;150(6):640-643.	**1.503**
16	Gupta N, Potdar V, Praharaj I, Giri S, Sapkal G, Yadav P, Choudhary ML, Dar L, Sugunan AP, Kaur H, Munivenkatappa A, Shastri J, Kaveri K, Dutta S, Malhotra B, Jain A, Nagamani K, Shantala GB, Raut S, Vegad MM, Sharma A, Choudhary A, Brijwal M, Balakrishnan A, Manjunatha J, Pathak M, Srinivasan S, Banu H, Sharma H, Jain P, Sunita P, Ambica R, Fageria B, Patel D, Rajbongshi G, Vijay N, Narayan J, Aggarwal N, Nagar A, Gangakhedkar RR, Abraham P. Laboratory preparedness for SARS-CoV-2 testing in India: Harnessing a network of Virus Research & Diagnostic Laboratories. ***Indian J Med Res.*** 2020 151(2-3): 216-25.	**1.503**
17	Gurav YK, Retheesh Babu G, Vinu KP, Lole KS. Suspected spread of hepatitis A virus from a restaurant among adults in rural area of the Kerala state, India. ***Epidemiol Infect.*** 2019 29;147:e210.	**2.152**
18	Jadhav NJ, Patil PS, Alagarasu K. Effect of full-length truncated variants of LL-37 on dengue virus infection and immunomodulatory effects of LL-37 in dengue virus infected U937-DC-SIGN cells. ***Int J Pept Res Ther.*** 2020 Mar;26(1): 547-55.	**1.500**
19	Joshi MS, Deore SG, Walimbe AM, Ranshing SS, Chitambar SD. Evaluation of different genomic regions of Rotavirus A for development of real time PCR. ***J Virol Methods.*** 2019; 266:65-71.	**1.786**
20	Joshi MS, Lole KS, Barve US, Salve DS, Ganorkar NN, Chavan NA, Shinde MS, Gopalkrishna V. Investigation of a large waterborne acute gastroenteritis outbreak caused by group B rotavirus in Maharashtra state, India. ***J Med Virol.*** 2019 Oct;91(10):1877-81.	**2.021**
21	Joshi MS, Walimbe AM, Dilpak SP, Cherian SS, Gopalkrishna V. Whole-genome-based characterization of three human Rotavirus C strains isolated from gastroenteritis outbreaks in Western India and a provisional intra-genotypic lineage classification system. ***J Gen Virol.*** 2019 Jul;100(7) :1055-72.	**3.376**
22	Joshi P, Yadav P, Mourya D, Sahare L, Ukey M, Khedekar R, Patil D, Barde PV. Laboratory surveillance of chikungunya in Madhya Pradesh, India (2016-2017). ***Indian J Med Res.*** 2020 Jan;151(1):87-92.	**1.503**
23	Khare AB, Shejwalkar R, Mourya DT. Fast Communication Design of indigenous bat trapping device. ***Int J Innov Eng Tech.*** 2020 Jan; 15(2): 5-7.	**NA**
24	Kode SS, Pawar SD, Cherian SS, Tare DS, Bhoye D, Keng SS, Mullick J. Selection of avian influenza A (H9N2) virus with reduced susceptibility to neuraminidase inhibitors oseltamivir and zanamivir. ***Virus Res.*** 2019 May; 265 :122-6.	**2.934**
25	Kode SS, Pawar SD, Tare DS, Keng SS, Hurt AC, Mullick J. A novel I117T substitution in neuraminidase of highly pathogenic avian influenza H5N1 virus conferring reduced susceptibility to oseltamivir and zanamivir. ***Vet Microbiol.*** 2019 Aug; 235 : 21-4.	**3.030**
26	Kode SS, Pawar SD, Tare DS, Keng SS, Mullick J. Amantadine resistance markers among low pathogenic avian influenza H9N2 viruses isolated from poultry in India, during 2009-2017. ***Microb Pathog.*** 2019;137:103779.	**2.914**

27	Kumar CPG, Sugunan AP, Yadav P, Kurup KK, Aarathee R, Manickam P, Bhatnagar T, Radhakrishnan C, Thomas B, Kumar A 5th, Jayasree J, Philomina B, Kumar KGS, Thulaseedharan NK, Gupta N 5th, Rajendran R, Saritha RL, Mourya DT, Gangakhedkar RR, Murhekar MV. Infections among Contacts of Patients with Nipah Virus, India. ***Emerg Infect Dis.*** 2019 May;25(5):1007-10.	**6.259**
28	Kurup KK, Manickam P, Gurav YK. Infected food handlers led to an outbreak of hepatitis A in Ernakulam district, Kerala, Southern India, 2016. ***Clin Epidemiol Glob Health.*** 2020 Mar;8(1):308-12.	**NA**
29	Kushwaha AS, Kotwal A, Biradar CI, Ajoy Mahen, Kumar M, Pawar SD, Chadha M, Patrikar S. Seroprevalence of influenza A H1N1 (swine) infection in the human population in a cantonment. ***Med J Armed Forces India.*** 2019 Jul;75(3):312-7.	**NA**
30	Lasure N, Gopalkrishna V. Changing pattern of genotypic circulation of Human Bocaviruses (HBoV) variants associated with acute gastroenteritis in Pune, Western India: A five years retrospective study. ***J Med Virol.*** 2019 Oct; 91(10) :1887-90.	**2.021**
31	Mohanty MC, Madkaikar MR, Desai M, Aluri J, Varose SY, Taur P, Sharma DK, Nalavade UP, Rane SV, Gupta M, Shabarish S, Dalvi A, Deshpande JM. Natural Clearance of Prolonged VDPV Infection in a Child With Primary Immunodeficiency Disorder. ***Front Immunol.*** 2019 Jul; 10: 1567.	**4.716**
32	Mourya D, Yadav P, Rout M, Pattnaik B, Shete A, Patil D. Absence of Nipah virus antibodies in pigs in Mizoram State, North East India. ***Indian J Med Res.*** 2019 May;149(5):677-9.	**1.503**
33	Mourya DT, Sapkal G, Yadav PD, Belani SKM, Shete A, Gupta N. Biorisk assessment for infrastructure & biosafety requirements for the laboratories providing coronavirus SARS-CoV-2/(COVID-19) diagnosis. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 172-6.	**1.503**
34	Mourya DT, Yadav P, Sudeep AB, Gokhale MD, Gupta N, Gangakhedkar RR, Bhargava B. Spatial Association Between a Nipah Virus Outbreak in India and Nipah Virus Infection in Pteropus Bats. ***Clin Infect Dis.*** 2019 Jul; 69(2):378-9.	**9.055**
35	Mourya DT, Yadav PD, Mohandas S, Kadiwar RF, Vala MK, Saxena AK, Shete-Aich A, Gupta N, Purushothama P, Sahay RR, Gangakhedkar RR, Mishra SCK, Bhargava B. Canine Distemper Virus in Asiatic Lions of Gujarat State, India. ***Emerg Infect Dis.*** 2019 Nov; 25(11): 2128-30.	**6.259**
36	Mourya DT, Yadav PD, Ullas PT, Bhardwaj SD, Sahay RR, Chadha MS, Shete AM, Jadhav S, Gupta N, Gangakhedkar RR, Khasnobis P, Singh SK. Emerging/re-emerging viral diseases & new viruses on the Indian horizon. ***Indian J Med Res***. 2019 Apr; 149(4):447-67.	**1.503**
37	Murhekar M, Verma S, Singh K, Bavdekar A, Benakappa N, Santhanam S, Sapkal G, Viswanathan R, Singh MP, Nag VL, Naik S, Munivenkatappa A, Abraham AM, Shanmugasundaram D, Sabarinathan R, Verghese VP, George S, Sachdeva RK, Kolekar J, Manasa S, Ram J, Gupta M, Rohit MK, Kumar P, Gupta PC, Ratho RK, Munjal SK, Nehra U, Khera D, Gupta N, Kaushal N, Singh P, Gadepalli R Vaid N, Kadam S, Shah S, Mahantesh S, Gowda VK, Haldar, P, Aggarwal MK, Gupta N. Epidemiology of Congenital Rubella Syndrome (CRS) in India, 2016-18, based on data from sentinel surveillance. ***PLoS Negl Trop Dis.*** 2020 Feb; 14(2): 1-11.	**3.885**

38	Murhekar MV, Kamaraj P, Kumar MS, Khan SA, Allam RR, Barde P, Dwibedi B, Kanungo S, Mohan U, Mohanty SS, Roy S, Sagar V, Savargaonkar D, Tandale BV, Topno RK, Sapkal G, Kumar CPG, Sabarinathan R, Kumar VS, Bitragunta S, Grover GS, Lakshmi PVM, Mishra CM, Sadhukhan P, Sahoo PK, Singh SK, Yadav CP, Bhagat A, Srivastava R, Dinesh ER, Karunakaran T, Govindhasamy C, Rajasekar TD, Jeyakumar A, Suresh A, Augustine D, Kumar PA, Kumar R, Dutta S, Toteja GS, Gupta N, Mehendale SM. Burden of dengue infection in India, 2017: a cross-sectional population based serosurvey. ***Lancet Glob Health***. 2019 Aug; 7(8): e1065-73.	**21.597**
39	Nyayanit DA, Sahay RR, Sakpal GN, Shete AM, Chaubal GC, Sarkale P, Srivastava R, Mohandas S, Yadav PD. Identification and phylogenetic analysis of herpes simplex virus-1 from clinical isolates in India. ***Access Microbiol.*** 2019 Aug ;1(6):1-11.	**NA**
40	Parashar D, Rajendran V, Shukla R, Sistla R. Lipid-based nanocarriers for delivery of small interfering RNA for therapeutic use. ***Eur J Pharm Sci.*** 2020 Jan; 142:105159.	**3.616**
41	Pawar SD, Keng SS, Tare DS, Thormothe AL, Sapkal GN, Anukumar B, Lole KS, Mullick J, Mourya DT. A virus precipitation method for concentration & detection of avian influenza viruses from environmental water resources & its possible application in outbreak investigations. ***Indian J Med Res.*** 2019 Dec; 150(6):612-9.	**1.503**
42	Potdar V, Cherian SS, Deshpande GR, Ullas PT, Yadav PD, Choudhary ML, Gughe R, Vipat V, Jadhav S, Patil S, Nyayanit D, Majumdar T, Walimbe A, Gaikwad S, Dighe H, Shete-Aich A, Mohandas S, Chowdhury D, Sapkal G, Basu A, Gupta N, Gangakhedkar RR, Giri S, Dar L, Jain A, Malhotra B, Abraham P, NIC Team. Genomic analysis of SARS-CoV-2 strains among Indians returning from Italy, Iran & China, & Italian tourists in India. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 255-60.	**1.503**
43	Potdar V, Hinge D, Satav A, Simões EAF, Yadav PD, Chadha MS. Laboratory-Confirmed Avian Influenza A(H9N2) Virus Infection, India, 2019. ***Emerg Infect Dis.*** 2019 Dec; 25(12):2328-30.	**6.259**
44	Potdar V, Vijay N, Gupta N, Arunkumar G, Borkakoty B, Malhotra B, Rabha D, Hinge D, Kaur H, Chadha M; for VRDL Team. Molecular characterization of influenza A(H1N1)pdm09 viruses circulating at various geographical locations in India, 2017. ***Indian J Med Res.*** 2019 Jun; 149(6):783-9.	**1.503**
45	Prasad S, Potdar V, Cherian S, Abraham P, Basu A. Transmission electron microscopy imaging of SARS-CoV-2. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 241-3.	**1.503**
46	Ramdasi AY, Arankalle VA. The expression patterns of immune response genes in the Peripheral Blood Mononuclear cells of pregnant women presenting with subclinical or clinical HEV infection are different and trimester-dependent: A whole transcriptome analysis. ***PLoS ONE*** 2020 Feb; 15(2):1-32.	**2.740**
47	Ranshing SS, Bavdekar AR, Vaidya UV, Behera MK, Walimbe AM, Gopalkrishna V. Clinical and genetic characteristics of unusual G12P[11] rotavirus strains recovered from neonates: A study from Pune, Western India. ***Infect Genet Evol.*** 2019; 70:45-52.	**2.773**
48	Ranshing SS, Cherian SS, Agarwal MS, Jagtap AS, Patil CM, Gopalkrishna V. Full genome based sequence and structural characterization of an unusual group A rotavirus G12P[11] isolated from neonates in Pune, western India. ***Vaccine.*** 2020 Feb; 38(10): 2275-91.	**4.086**
49	Sajjajadi S, Shirode A, Vaidya SR, Cherian SS. Molecular mechanism by which residues at position 481 and 546 of measles virus hemagglutinin protein define CD46 receptor binding using a molecular docking approach. ***Comput Biol Chem.*** 2019 May; 80: 384-9.	**1.850**

50	Sarkale P, Patil S, Yadav PD, Nyayanit DA, Sapkal G, Baradkar S, Lakra R, Shete-Aich A, Prasad S, Basu A, Dar L, Vipat V, Giri S, Potdar V, Choudhary ML, Praharaj I, Jain A, Malhotra B, Gawande P, Kalele K, Gupta N, Cherian SS, Abraham P. First isolation of SARS-CoV-2 from clinical samples in India. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 244-50.	**1.503**
51	Sarkale P, Shrivastava A, Mohandas S, Patil SL, Kore P, Soman V, Yadav PD. Growth kinetics of Kyasanur Forest Disease virus in mammalian cell lines and development of plaque reduction neutralization test. ***Vector Borne Zoonotic Dis***. 2019;19(8):630-6.	**2.041**
52	Saxena VK, Pawar SD, Qureshi THIH, Surve P, Yadav P, Nabi F, Mendadkar R. Isolation and molecular characterization of coxsackievirus A6 and coxsackievirus A16 from a case of recurrent Hand, Foot and Mouth Disease in Mumbai, Maharashtra, India, 2018. *VirusDis.* 2020 Mar; 31(1): 56-60.	**NA**
53	Shah PS, Alagarasu K, Karad S, Deoshatwar A, Jadhav SM, Raut T, Singh A, Dayaraj C, Padbidri VS. Seroprevalence and incidence of primary dengue infections among children in a rural region of Maharashtra, Western India. ***BMC Infect Dis.*** 2019 Apr 2; 19(1): 296.	**2.688**
54	Singh R, Gupta V, Malhotra B, Singh S, Ravindran P, Meena D, Gupta J, Mathur VK, Mathur RP, Singh S, Sharma P, Sharma H, Bhandari S, Gupta N, Sapkal G, Mourya DT, Speer MD. Cluster containment strategy: addressing Zika virus outbreak in Rajasthan, India. ***BMJ Glob Health.*** 2019 Sep; 4(5):e001383.	**4.280**
55	Singh RK, Dhama K, Chakraborty S, Tiwari R, Natesan S, Khandia R, Munjal A, Vora KS, Latheef SK, Karthik K, Singh Malik Y, Singh R, Chaicumpa W, Mourya DT. Nipah virus: epidemiology, pathology, immunobiology and advances in diagnosis, vaccine designing and control strategies - A comprehensive review. ***Vet Q.*** 2019 Dec; 39(1) : 26-55.	**2.341**
56	Sudeep AB, Shil P, Charmode MM, Mohandas S, Bansod S, Gokhale MD, Jagtap M & Shah PS. Involvement of dual serotypes during a severe dengue outbreak in Wadi area, Nagpur district, Maharashtra 2017. *J Vector Borne Dis* 2019; 56: 48-58	**1.76**
57	Sudeep AB, Vyas PB, Parashar D, Shil P. Differential susceptibility & replication potential of Vero E6, BHK-21, RD, A-549, C6/36 cells & Aedes aegypti mosquitoes to three strains of chikungunya virus. ***Indian J Med Res.*** 2019 Jun;149(6): 771-7.	**1.503**
58	Tadkalkar N, Prasad S, Gangodkar S, Ghosh K, Basu A. Dengue virus NS1 Exposure affects von willebrand factor profile and platelet adhesion properties of cultured vascular endothelial cells. ***Indian J Hematol Blood Trasfus.*** 2019 Jul; 35(3): 502-6.	**1.615**
59	Tare DS, Kode SS, Hurt AC, Pawar SD. Assessing the susceptibility of highly pathogenic avian influenza H5N1 viruses to oseltamivir using embryonated chicken eggs. ***Indian J Med Res.*** 2019 Nov; 150(5): 486-91.	**1.503**
60	Tatte VS, Maran D, Walimbe AM, Gopalkrishna V. Rotavirus G9P[4], G9P[6] and G1P[6] strains isolated from children with acute gastroenteritis in Pune, western India, 2013-2015: evidence for recombination in genes encoding VP3, VP4 and NSP1. ***J Gen Virol.*** 2019 Dec; 100(12): 1605-30.	**3.376**
61	Tikute SS, Wangikar PB, Varanasi G. Pathological and molecular studies on Coxsackie virus A-16 (CVA-16) isolated from Hand, Foot and Mouth Disease (HFMD) cases in India: Approach using Neonatal mouse model. ***J Med Virol***. 2019; 91(10) : 1765-75.	**2.021**
62	Tripathy AS, Ganu MA, Sonam Lata, Thanapati S. Alagarasu K, Puranik S. Association of IL-1RN VNTR Polymorphism with Chikungunya infection: A study from Western India. ***J Med Virol.*** 2019 Nov; 91(11) : 1901-8.	**2.021**

63	Tripathy AS, Puranik S, Sharma M, Chakraborty S, Devakate UR. Hepatitis E virus seroprevalence among blood donors in Pune, India. ***J Med Virol.*** 2019 May; 91(5) : 813-9.	**2.021**
64	Tripathy AS, Sharma M, Deoshatwar AR, Babar P, Bharadwaj R, Bharti OK. Study of a hepatitis E virus outbreak involving drinking water and sewage contamination in Shimla, India, 2015-2016. ***Trans R Soc Trop Med Hyg.*** 2019 Dec 1;113(12):789-96.	**1.868**
65	Vaidya SR, Kamble MB, Kumbhar NS. Laboratory-based investigation of fever with rash cases in the Maharashtra State - India, 2014 to 2017. ***J Med Virol.*** 2019; 91(6): 972-8.	**2.021**
66	Vaidya SR, Kasibhatla SM, Bhattad DR, Ramtirthkar MR, Kale MM, Raut CG, Kulkarni-Kale U. Characterization of diversity of measles viruses in India: Genomic sequencing and comparative genomics studies. ***J Infect.*** 2020 Mar; 80(3):301-9.	**4.842**
67	Vaidya SR, Kulkarni AS, Bhattad DR, Raut CG. Revealing True Diversity of Measles viruses Circulating in India, 2012-17. ***J Infect.*** 2019 Sep; 79(3) : 283-85.	**4.842**
68	Viswanathan R, Bafna S, Mergu R, Deshpande G, Gunjikar R, Gaikwad S, Mullick J. Direct Saliva Real-time Polymerase Chain Reaction Assay Shows Low Birth Prevalence of Congenital Cytomegalovirus Infection in Urban Western India. ***Pediatr Infect Dis J.*** 2019; 38(4): e65-8.	**2.126**
69	Viswanathan R, George S, Murhekar MV, Abraham AM, Singh MP, Jadhav SM, Nag V, Naik S, Raut C, Munivenkatappa A, Gupta M, Jagtap V, Kaduskar O, Gupta N, Sapkal GN. Comparison of two commercial ELISA kits for detection of rubella specific IgM in suspected congenital rubella syndrome cases and rubella IgG antibodies in serosurvey of pregnant women. ***Diagn Microbiol Infect Dis.*** 2019 Jul; 94(3): 243-7.	**2.499**
70	Viswanathan R, Kumar A. Frontier Warriors-Combating cholera in Rural India. ***Am J Trop Med Hyg.*** 2019 May; 10(5): 1071-2.	**2.126**
71	Yadav P, Chaubal G, Jena S, Shil P, Mourya D. Assessment of NS1 protein as an early diagnostic marker for Kyasanur forest disease virus. ***J Vector Borne Dis.*** 2019 Jul-Sep;56(3):212-220	**1.127**
72	Yadav PD, Gurav YK, Shete AM, Jain R, Nyayanit DA, Pardeshi PG, Viswanathan R, Chiplunkar TR, Awate P, Majumdar TP, Sahay RR, Mourya DT. Kinetics of viral RNA, immunoglobulin-M & G antibodies in Kyasanur forest disease. ***Indian J Med Res.*** 2019 Aug;150 (2):186-93.	**1.503**
73	Yadav PD, Malhotra B, Sapkal G, Nyayanit DA, Deshpande G, Gupta N, Padinjaremattathil UT, Sharma H, Sahay RR, Sharma P, Mourya DT. Zika virus outbreak in Rajasthan, India in 2018 was caused by a virus endemic to Asia. ***Infect Genet Evol.*** 2019 Apr; 69:199-202.	**2.773**
74	Yadav PD, Mauldin MR, Nyayanit DA, Albariño CG, Sarkale P, Shete A, Guerrero LW, Nakazawa Y, Nichol ST, Mourya DT. Isolation and phylogenomic analysis of Buffalopox virus from Human and Buffaloes in India. ***Virus Res.*** 2020 Feb; 277:197836.	**2.934**
75	Yadav PD, Pardeshi PG, Patil DY, Shete AM, Mourya DT. Persistence of IgG antibodies in survivors of Crimean Congo hemorrhagic fever virus infection, India. ***J Infect Public Health.*** 2019 Jul-Aug; 12(4): 598-9.	**2.447**
76	Yadav PD, Patil S, Jadhav SM, Nyayanit DA, Kumar V, Jain S, Sampath J, Mourya DT, Cherian SS. Phylogeography of Kyasanur Forest Disease virus in India (1957-2017) reveals evolution and spread in the Western Ghats region. ***Sci Rep.*** 2020 Feb;10(1):1966.	**3.998**

77	Yadav PD, Potdar VA, Choudhary ML, Nyayanit1 DA, Agrawal M, Jadhav SM, Majumdar TD, Shete-Aich A, Basu A, Abraham P, Cherian SS. Full-genome sequences of the first two SARS-CoV-2 viruses from India. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 200-9.	**1.503**
78	Yadav PD, Shete AM, Kumar GA, Sarkale P, Sahay RR, Radhakrishnan C, Lakra R, Pardeshi P, Gupta N, Gangakhedkar RR, Rajendran VR, Sadanandan R, Mourya DT. Nipah Virus Sequences from Humans and Bats during Nipah Outbreak, Kerala, India, 2018. ***Emerg Infect Dis.*** 2019 May;25(5):1003-6.	**6.259**
79	Yadav PD, Shete-Aich A, Nyayanit DA, Pardeshi P, Majumdar T, Balasubramanian R, Ullas PT, Mohandas S, Dighe H, Sawant P, Patil S, Patil D, Gokhale MD, Mathapati B, Sudeep AB, Baradkar S, Kumar A, Kharde R, Salve M, Joshi Y, Gupta N, Mourya DT. Detection of coronaviruses in Pteropus & Rousettus species of bats from different States of India. ***Indian J Med Res.*** 2020 Feb-Mar; 151(2-3): 226-35.	**1.503**
80	Yadav PD, Whitmer SLM, Sarkale P, Ng TFF, Goldsmith CS, Nyayanit DA, Esona MD, Shrivastava-Ranjan P, Lakra R, Pardeshi P, Majumdar TD, Francis A, Klena JD, Nichol ST, Ströher U, Mourya D. Characterization of Novel Reoviruses [Wad Medani virus (Orbivirus) and Kundal (Coltivirus)] collected from Hyalomma antolicum ticks in India during CCHF surveillance. ***J Virol.*** 2019 Jun; 93(13): e00106-19.	**4.501**

	Book Chapter
1.	Tandale BV, Chowdhary PK, Rajderkar SS, Shrivastava N. Epidemiology of Dengue. ***In:*** Dengue Monograph. Jaypee Brothers; 2019 May: p.5-19.
2.	Chowdhary PK, Tomar S, Mandal T. Changing Epidemiology of Dengue in Southeast Asia. ***In***: Dengue Monograph. Jaypee Brothers; 2019 May: p.20-6.