ANNUAL RESEARCH PROGRESS REPORT

ICMR-NATIONAL INSTITUTE OF VIROLOGY, PUNE

(2021-2022)

INDEX

From Director's Desk

It is indeed my pleasure and privilege to present the Annual Report of ICMR-National Institute of Virology, Pune, for the year 2021- 2022. In continuation with the pivotal COVID-19 research performed during the first year of the pandemic, ICMR-NIV remained the torchbearer of India's battle against SARS-CoV-2. The institute provided support to the nation in terms of diagnostics and training, distribution of external quality assessment panels, reagents and standard operating procedures, in addition to the evaluation of vaccine response.

During the year, nearly 20,000 SARS-CoV-2 whole genome sequences were submitted to GISAID. Mapping of the SARS-CoV-2 strain variations geographically and monitoring of the introduction of new strains into the country was an ongoing activity. The assessment of the protective efficacies of multiple vaccines against different variants of concern was performed. This along with follow up investigations in vaccinated and re-infected populations identified the need for boosting the immune response. These investigations helped the nation undertake appropriate measures to contain the pandemic. It also led to the development of upgraded technologies in diagnostics which were transferred to industry. Under the Government of India's efforts to bolster healthcare delivery through the Pradhan Mantri Ayushman Bharat Health Infrastructure Mission (PM-ABHIM), India's first Biosafety Level 3 Mobile Laboratory was dedicated to the nation by the Honorable Union Minister of State for Health and Family Welfare, Dr. Bharati Pravin Pawar, in the august presence of Prof. (Dr.) Balram Bhargava, Secretary, DHR and DG, ICMR. This mobile laboratory will aid the nation in rapid response to outbreaks of newly emerging and re-emerging viral infections with high mortality.

I am also proud of the quality of research our scientific team has carried out in various domains. Our Institute continued to provide timely diagnostic support for samples referred from different parts of the country. ICMR-NIV is always at the forefront when it comes to outbreak investigations. Over the past year, we supported the governments of several states during various outbreaks including Zika, Nipah, CCHFV and avian influenza viruses. ICMR-NIV scientists have been part of various central teams investigating outbreaks and providing active support to many states. As an apex resource centre for the Virus Research & Diagnostic Laboratories (VRDLs), the Institute has strengthened the diagnostic capacities in medical colleges across the country by conducting several trainings. The three peripheral units of ICMR-NIV in Bengaluru, Alappuzha, and Mumbai continued to provide their respective state governments with diagnostic support and trainings.

ICMR-NIV continued to provide a significant number of diagnostic kits for dengue, chikungunya and Japanese encephalitis to national programs. The National Influenza Center (NIC) continued to meet the challenges of SARS-CoV-2 testing as well as providing diagnosis for influenza and other respiratory viruses. ICMR-NIV Mumbai Unit continues to be a WHO Global Specialized Laboratory for Polio and WHO Regional Reference Laboratory for Measles Rubella (MR), while the ICMR-NIV Bengaluru unit continues activities on WHO SEARO projects for Acute Flaccid Paralysis, MR and Polio sewage surveillance.

Despite the hectic schedule, basic research continued to flourish as is evident from the high quality publications in reputed national and international journals. This year we have achieved an average impact factor of 14.3. The academic activities (M.Sc. Virology and Ph. D. courses) of ICMR-NIV have shown good progress. Additionally the syllabus of a post graduate diploma in diagnostic virology has gained approval by the Savitribai Phule Pune University that we are affiliated to.
We look forward to developing the Satellite Centre for One Health at Nagpur as well as four new Zonal ICMR-NIV Units at Bengaluru (South zone), Dibrugarh (East zone), Jabalpur (Central zone), Chandigarh (North zone), in coordination with ICMR Headquarters. I am sure that these units under the PM-ABHIM program will further augment research at ICMR-NIV.

Well-coordinated teamwork is absolutely essential for such phenomenal activities; the active support from the technical, administrative, and engineering teams was crucial in our successful pursuits. I would like to congratulate and thank each member of the ICMR-NIV family for their untiring efforts and enormous contributions. My sincere gratitude to Prof. (Dr.) Balram Bhargava, Secretary, DHR and DG ICMR, for his unstinting support. We also thank the SAC Chairman for his able and continued guidance, and team ICMR for their continuous support extended to this institute. I am confident that the institute shall meet every future challenge and live up to the expectations of the nation.

Prof. (Dr.) Priya Abraham
Director

Composition of committees

Scientific Advisory Committee

SrNo	Name	Address
1.	Lt.Gen.(Dr) Velu Nair (Retd.) MD(Med), FRCP (London) FACP (USA), FICP, FAMS, FIACM, FUICC (UK), FISHTM,	Group Head- Medical Services & Chief Consultant – Haemato-Oncology & Bone Marrow Transplant Comprehensive Blood & Cancer Center (CBCC) 1A Bhat GIDC, Gandhinagar - 382 428 Gujarat.
2.	Prof. V Ravi MBBS, MD, FAMS, FASc, FASTMH,	NODAL OFFICER FOR GENEOMIC CONFIRMATION OF SARS-CoV-2 Government of Karnataka, (Formerly Senior Professor and Head, Department of Neurovirology Registrar & Dean-Basci Sciences), NIMHANS Bangalore 560029, India
3.	Dr. Yogesh Shouche	Principal Investigator National Center for Microbial Resource National Center for Cell Science Pune University Campus Pune 411 007 INDIA
4.	Dr. M.S.Chadha	Former Senior Scientist G, ICMR- NIV, Pune
5.	Dr .T.Ramamurthy	INSA-Senior Scientist, ICMR- National Institute of Cholera and Enteric Diseases (NICED), P-33, CIT Road, Scheme XM, Beliaghata, Kolkata-700010.
6.	Dr Ashok Kumar	ADG (Animal Health) Indian Council of Agricultural Research (ICAR) 405, Krishi Bhavan, New Delhi- 110001, INDIA
7.	Prof. Amita Jain	Dr. Amita Jain, MD, PhD, FAMS, FRCPath Professor and Head, Department of Microbiology Incharge, Intermediate reference tuberculosis lab Incharge, Virus research and diagnostic lab, King George University, Shah Mina Rd, Chowk, Lucknow, UP, India 226003
8.	Prof. Dr. Mohan R. Wani MVSc (Surgery), PhD (Medicine, England) FNASc, FNA, FAMS	Tata Innovation Fellow Scientist G (Professor) National Centre for Cell Science S. P. Pune University Pune 411007, India
9.	Dr. Samiran Panda	Cheif-ECD, ICMR, New Delhi and Director, NARI, Pune
10.	Prof. Priya Abraham	Director, ICMR-NIV, Pune

Institutional Bio-Safety Committee (IBSC)

Sr No.	Name & affiliation	Role
1	Dr Priya Abraham, Director, ICMR-NIV, Pune	Chair man/Chair person
2	Dr Kavita Lole, Scientist F, ICMR-NIV, Pune	Member Secretary
3	Dr Arvind Sahu, Scientist G, National Centre for Cell Science, Pune - Maharashtra	DBT Nominee
4	Dr Rima Sahay, Scientist C, ICMR-NIV, Pune	Biosafety Officer
5	Dr Vikram Ghole, Retired Professor, SPPU (Pune University), Pune	External Expert
6	Dr Anita Shete, Scientist E, ICMR-NIV, Pune	Internal expert
7	Dr Tejaswini Deshmukh, Scientist C, ICMR-NIV, Pune	Internal expert
8	Dr Manohar Lal Choudhari, Scientist E, ICMR-NIV	Internal Expert

Institutional Human Ethics Committee

S.No	Name & Affiliation	Role
1.	Dr. Amitav Banerjee, MD Professor & Head, Community Medicine Dr D Y Patil Medical College, Pune 411 018	**Chairperson** (External)
2	Dr. Rajesh Kulkarni, MD Associate Professor (Ped) B J Medical College, Pune 411 001 B J Medical College, Pune 411001	Clinician (External)
3.	Dr Sheila Godbole, MD Scientist G ICMR-National AIDS Research Institute, Pune 411026	Clinician (External)
4.	Dr. Vikram Padbidri, MD Consultant Microbiology & Infection Control, Jehangir Hospital, Pune 411001	Basic Medical Scientist (External)
5.	Dr Abhijit Kadam Scientist C, ICMR-National AIDS Research Institute, Pune 411026	Basic Medical Scientist (External)
6.	Dr. Aarti Nagarkar, PhD Professor Interdisciplinary School of Health Sciences, Savitribai Phule Pune University,, Pune 411 007	Social Scientist (External)
7.	Dr. Jyoti S Bhakare, LLM, PhD, Dept. of Law, Savitribai Phule University, Pune 411 007	Legal Expert (External)
8.	Mr. Joseph Cherian, BA, Dip.Health, Community Medicine Dept. Dr. D.Y. Patil Medical College, Pune-411018	Person from Community (External)
9.	Dr. Anuradha Tripathy, PhD Scientist F, ICMR-National Institute of Virology,Pashan, Pune 21	Basic Medical Scientist (Internal)
10.	Dr K Alagarasu, PhD Scientist E, Dengue/ Chikungunya Group, ICMR-National Institute of Virology, Pune 411 001	Basic Medical Scientist (Internal)
11.	Mr Atul M Walimbe, M.Sc BDM Group,ICMR-National Institute of Virology, ,Pune 411 001	Statistician (Internal)
12.	Mr Santosh M Jadhav, M.Sc BDM Group, ICMR-National Institute of Virology, Pune 411 001	Statistician (Internal)
13.	Dr. Rajlakshmi Viswanathan, MD Scientist E, Bacteriology Group, ICMR-National Institute of Virology, Pashan, Pune 21	Member Secretary (Internal)

Institutional Animal Ethics Committee

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

Specimens tested during the reporting period

COVID-19 samples: Tested: 814556

Positivity: 29.46% at ICMR-NIV Kerala Unit
15.23% at NIC, ICMR-NIV Pune
8.08% at ICMR-NIV Mumbai Unit
6.5% at ICMR-NIV Bengalore Unit

Other Viral infections*

Number of samples Tested: 144463

[* Japanese encephalitis, dengue, chkungunya, hepatitis, herpes simplex- 1 & 2, chandipura, enteric viruses, influenza, respiratory syncitial virus, adenoviruses, rhinovirus, measles, rubella, zika, CCHF, KFD, etc.]

Section 1: Report on COVID 19 activities

SARS-CoV-2 Diagnostic Research

Diagnostic Services/Outbreak investigation: COVID-19 pandemic and Influenza Viruses

Investigator: Dr.VA Potdar, Dr. ML Choudhary, Dr. SD Bhardwaj, Dr. H Kaushal
Funding: Intramural Duration: 2021-22

Background: Clinical samples from patients suspected for SARS CoV-2 and influenza infection were referred for diagnosis by different clinics/hospitals from Pune.

Objective: To provide diagnosis of referred samples for SARS CoV-2 and influenza viruses.

Findings: Referred clinical samples (n=100867) were tested using combo real time RT-PCR for SARS CoV-2, Influenza A & B. A total 15368 (15.23%) samples were found positive for SARS-CoV-2. Second wave peaked in April-May 2021 and third wave started from January 2022 and subsided by the end of February 2022, with baseline SARS-CoV-2 activity observed thereafter (Fig. 1a). We received total 1175 samples for SARS CoV-2 quality control from regional VRDLs and different labs from Maharashtra and reports were uploaded on ICMR portal.

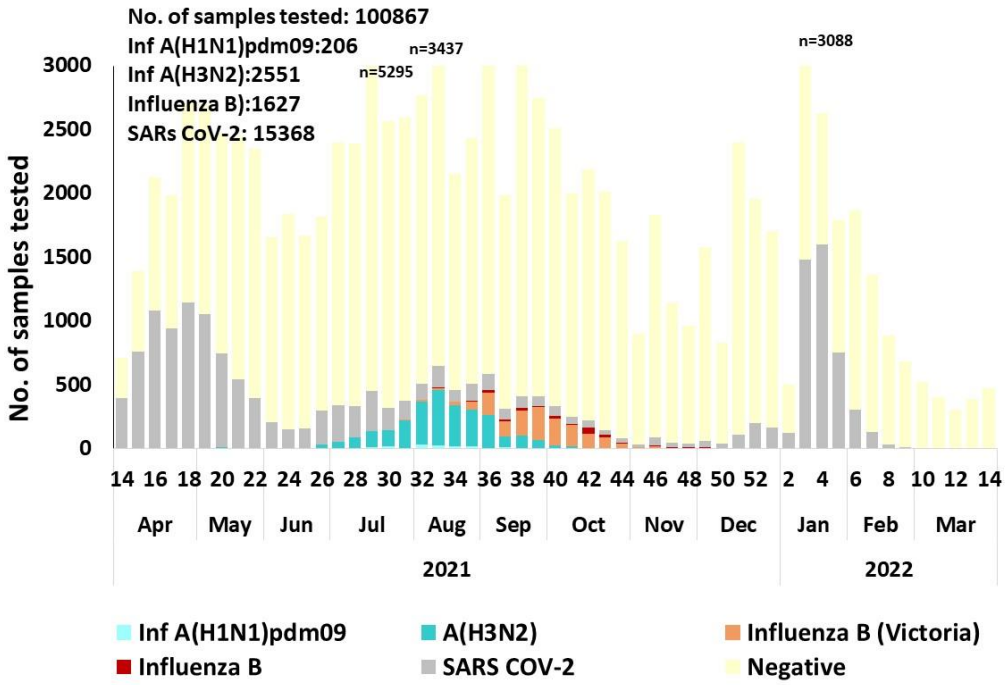


Fig. 1a: Referred samples tested for influenza viruses and SARS CoV-2

Total 100867 clinical samples were also tested for influenza viruses using real time RT-PCR and 4384 (4.34%) samples found positive for influenza viruses. Influenza A/H3N2 virus was detected in 2551 (2.52%) samples, influenza A/H1N1pdm09 virus in 206 (0.20%) samples and

influenza B in 1627 (1.6%) samples. Month wise sample tested and positives are given in fig. 1a. Influenza A/H1N1pdm09 started in circulation first followed by H3N2 and then Influenza B. A total of 206 Influenza A/H1N1pdm09 positive samples were tested for antiviral drug for H274Y mutation using allelic discrimination RT-PCR. All the samples were found sensitive to drug.

Representative influenza positive samples were inoculated in MDCK cell line (n= 500) and yielded 21 virus isolates (A/H1N1pdm09: 11; A/H3N2: 22 and Influenza B: 19). Phylogenetic analysis of HA gene of A/H1N1pdm09 viruses were antigenically similar to 2021-22 and 2022-23 vaccine component A/Victoria/2570/2019 however genetically it is forming 5A2 separate group (Fig. 1b). Phylogenetic analysis of HA gene of influenza A/H3N2 virus showed that the circulating strains grouped in Clade 3C.2a1b2a2 subclade and were antigenically similar to A/Darwin/6/2021 (H3N2) like virus which was 2022 northern hemisphere vaccine component (Fig. 1c). Phylogenetic analysis of HA gene of influenza B virus shows that B/Victoria/2/87 lineage is in circulation and antigenically similar to B/Austria/1359417/2021-like viruses (3a.2) vaccine strain (Fig. 1d).

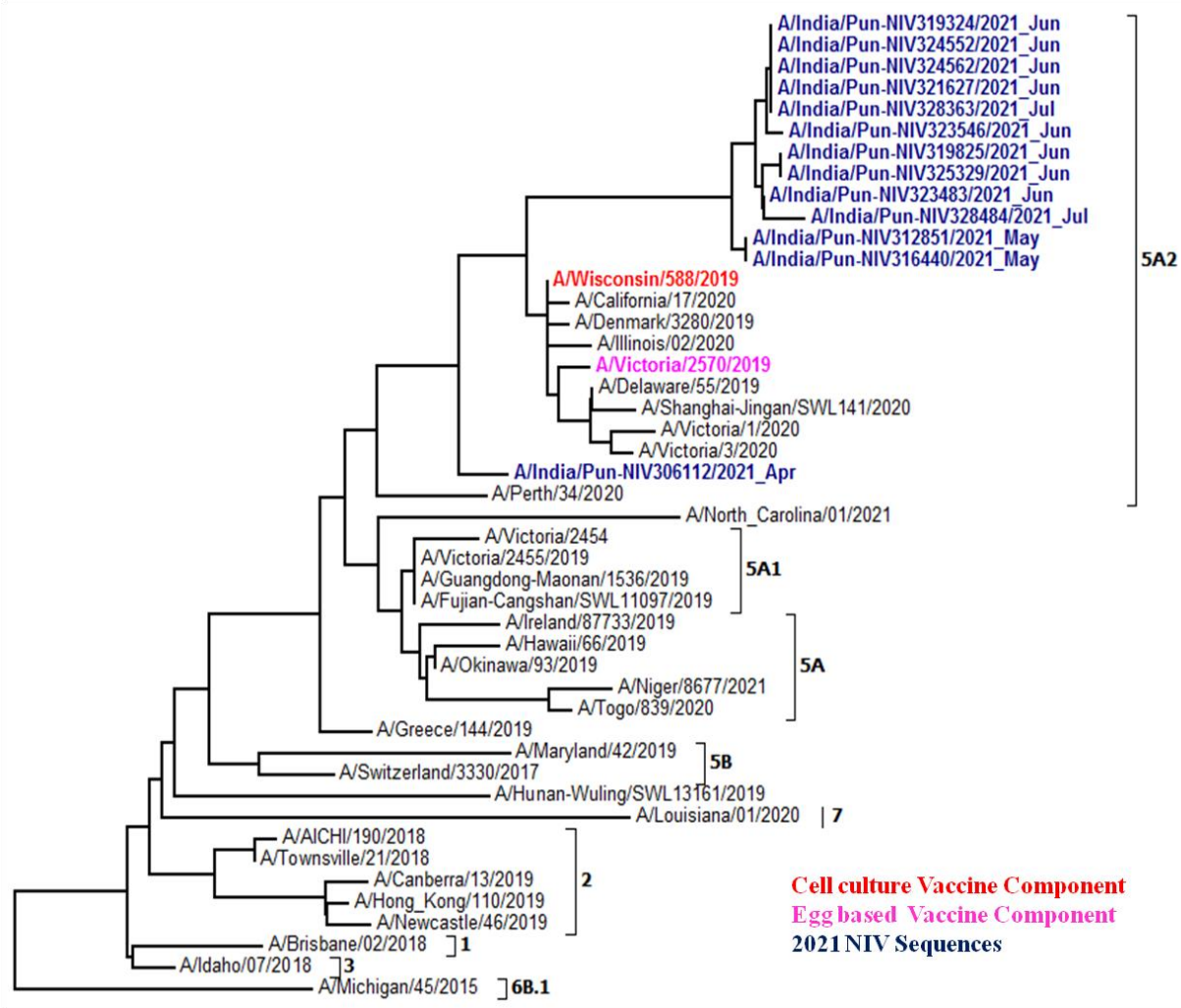


Fig. 1b: Phylogenetic analysis of Influenza A/H1N1pdm09 HA gene sequences

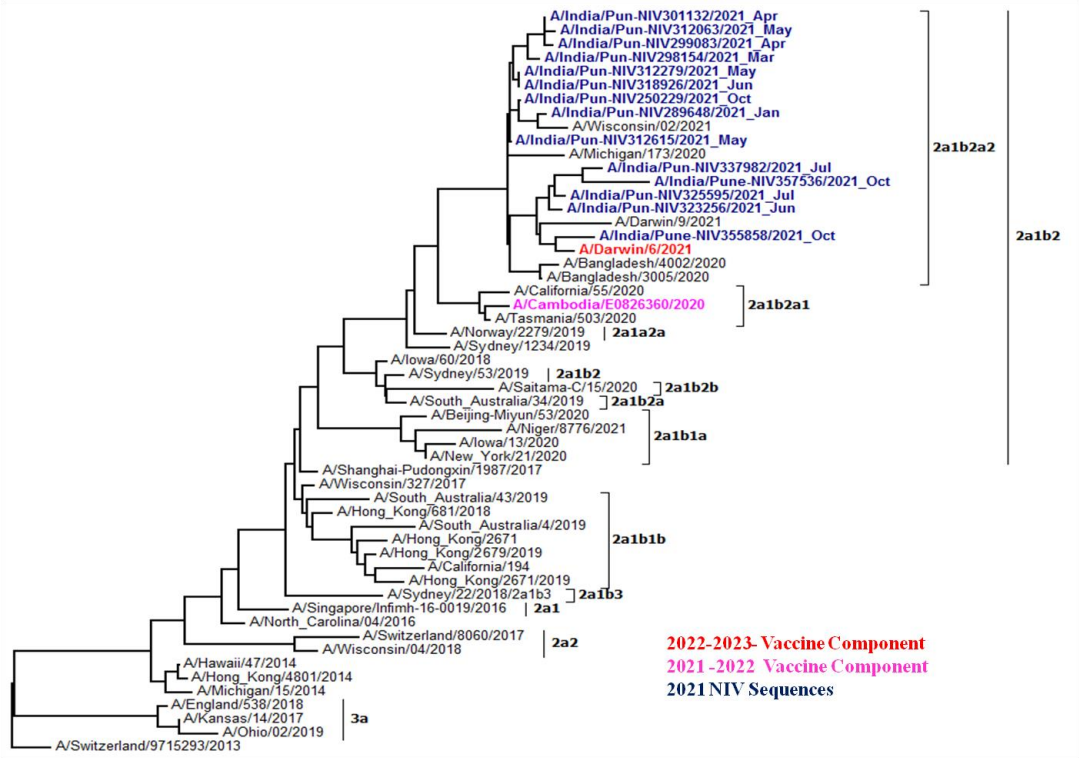


Fig. 1c: Phylogenetic analysis of Influenza A/H3N2 HA gene sequences

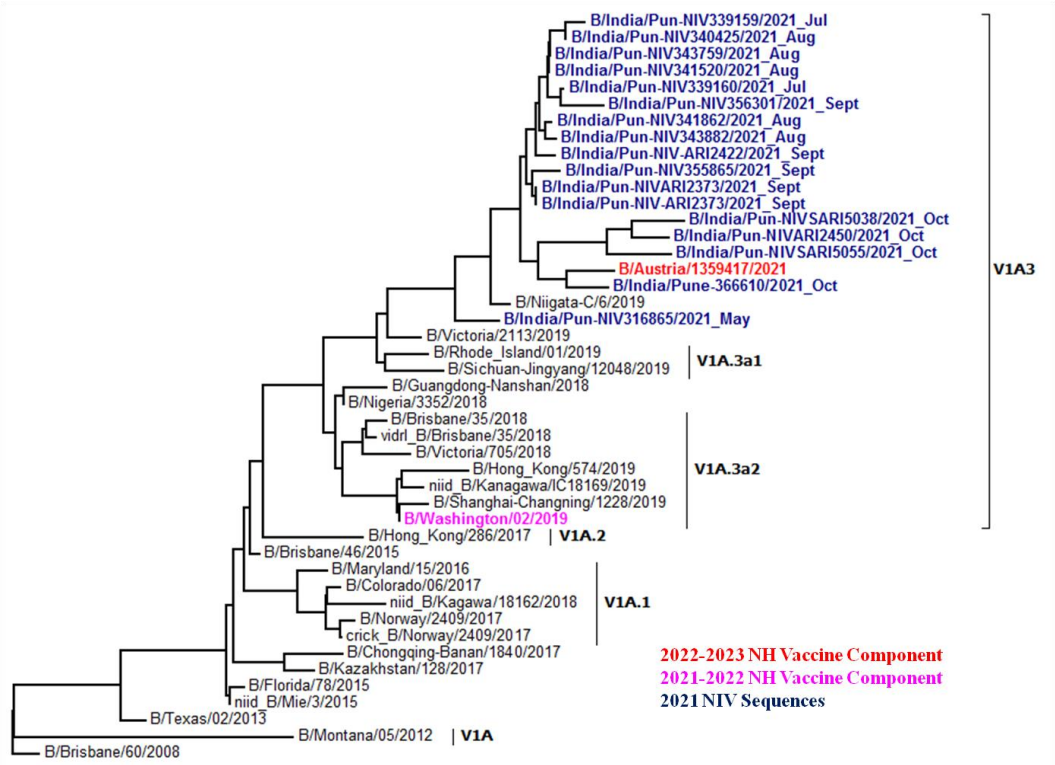


Fig. 1d: Phylogenetic analysis of Influenza B (Victoria) HA gene sequences

Development of COVID-19 laboratories for molecular diagnosis of SARS-CoV-2 virus during the pandemic period in Mumbai

Investigators: Shailesh D Pawar, Ramesh L More, Nikita Shanbhag
Funding: ICMR ***Duration*:** Ongoing
Background: In view of the pandemic caused by the SARS-CoV-2 virus, the laboratory was established to work on COVID-19.
Objective: Development of COVID-19 laboratories for molecular diagnosis of SARS-CoV-2 virus during the pandemic period in Mumbai.
Findings: During the raging COVID-19 pandemic, the ICMR-NIVMU is playing a very significant role in mitigation of the pandemic. The laboratory diagnosis to over 95,000 suspected individuals was provided. The laboratory also provided platform for various research programs. The ICMR-NIVMU lab worked as the Quality Assurance Laboratory for 40 COVID-19 labs in Mumbai and Maharashtra. The Laboratory has established environmental surveillance (ES) for SARS-CoV-2 in Mumbai and expanded ES in nine cities across the country.

SARS CoV-2 diagnosis and quality assessment of the state level laboratories in Karnataka state

Investigators: Ashok M, Manjunath MJ
Funding: NIV, Pune and Govt. of Karnataka **Duration:**Ongoing (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 deport 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. We tested 467553 suspect COVID cases and confirmed 30636 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. All received samples are tested and reported within 24 to 48 hours to the concerned hospital or district surveillance officers of the state.

Regional Depot for Karnataka state: Since April 2021 our unit was identified as regional depot by ICMR HQ. Total of 4.24 lakh PCR reaction kits, 1.27 lakh reactions of RNA extraction kit and 5.68 lakh VTMs were distributed to ICMR network laboratories in the state.

Quality assessment of SARS CoV-2 real time RT PCR for Karnataka state: Our unit is identified as state referral laboratory for Karnataka state. We do quality assessment on bianual basis for both government and private laboratories in the Karnataka state. We performed ILQC for 100 testing laboratories.

COVID-19 Sewage surveillance: We received 104 Sewage samples that was processed by phase separation method, the middle phase is taken for RNA extraction by Quiagen kit. The final extract is used for PCR testing by using NIV single plex kit. We found 37 were positive for SARS CoV-2 RNA.

SARS-CoV-2 Genetic Analysis

Distribution of whole genome sequences of SARS-CoV-2 analyzed during the period (April-2021 to March-2022) through the National Influenza Centre (NIC)

Investigators: Sarah Cherian, Varsha Potdar, Mr S.M. Jadhav, Diya Roy

Funding: Intramural **Duration:** 2021-2022

A total number of 19,953 whole genome SARS CoV-2 sequences from seven different laboratories were referred to our department. The center wise distribution of sequences is given in **Fig. 2**.

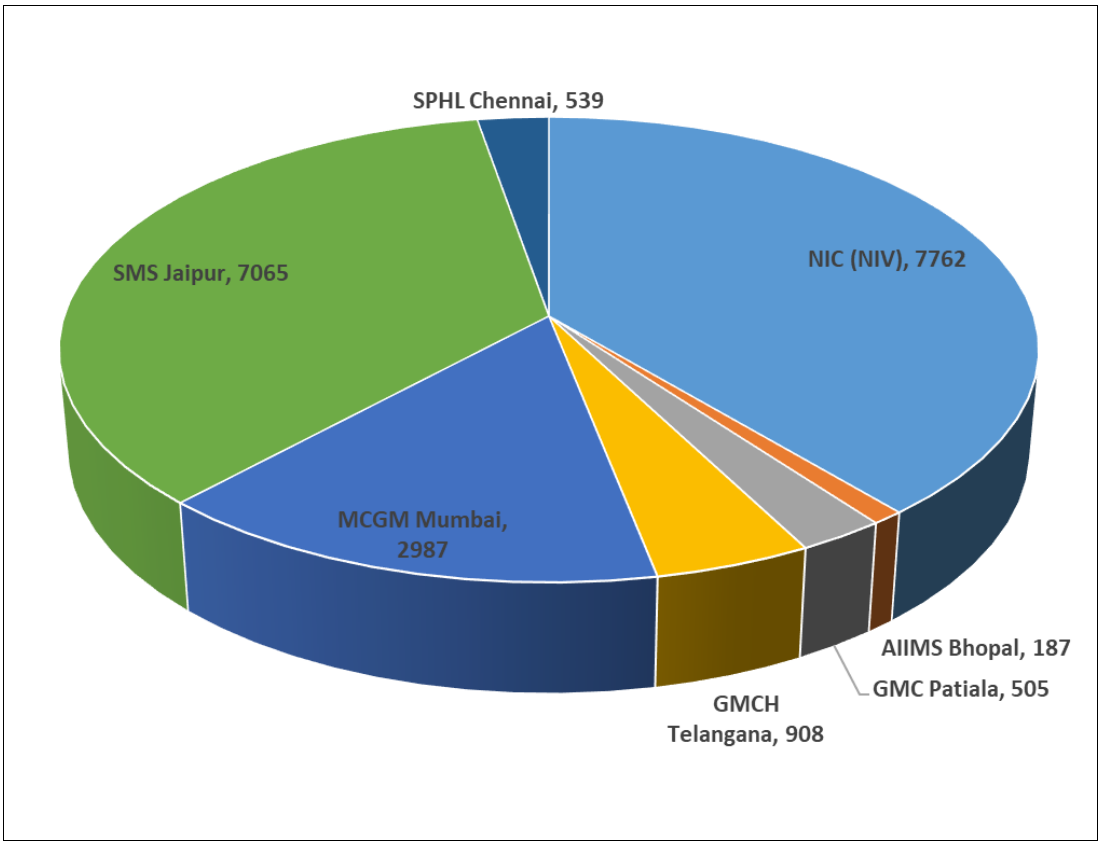


Fig. 2: Center wise distribution of SARS CoV-2 genomes sequenced during April-2021 to Mar-2022

The distribution of SARS-CoV-2 lineages and WHO variants of concern/Interest during the period between April-2021 to March-2022: The major lineages and WHO variants of SARS-CoV-2 are represented in **Fig. 3**.

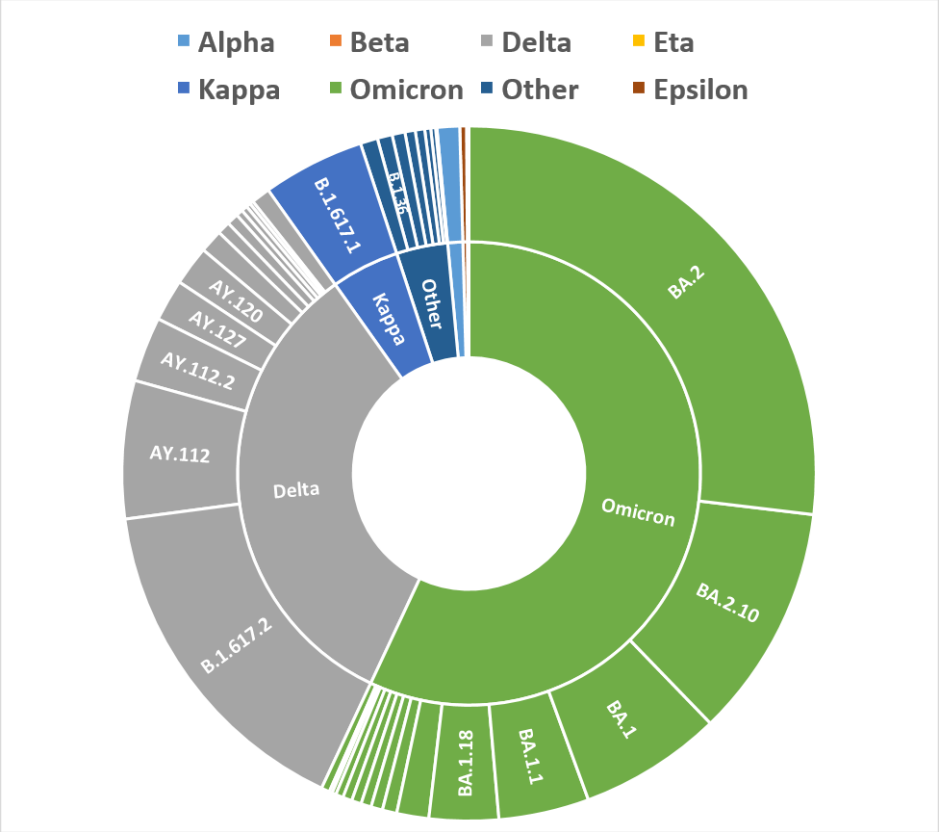


Fig. 3. Distribution of Major Lineages and WHO variants of Concern/Interest of SARS-CoV-2 during April-2021 to March-2022.

Viral Haplotype Network for the characterization of SARS-CoV-2 strains circulating in India

Investigators: Dr. Sarah Cherian, Dr. Laxmi Vandana Rongala, A.M.Walimbe

Funding: Intramural **Duration:** 2021-2022

Background: Genetic diversity analysis of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative agent of coronavirus disease 2019, is required for better understanding of the virus evolution in humans, to trace pathways of infection and develop therapeutic strategies.

Findings: Representative genome sequences of SARS-CoV-2 (n=613) were randomly selected from samples (n=1532) collected through the nation-wide VRDL labs during 2020 and from GISAID sequences of India during the period of Mar 2020 - Mar 2022, and were analyzed for genetic diversity with reference to Wuhan sequence. A population genetics based genomic surveillance tool, haplotype network, was used in comparison with the conventional phylogenetic approach for the analysis. A total number of 643 segregating sites among the

sequences revealed 420 unique and highly differentiated haplotypes on network analysis. The low variation among the viral genomes from different geographical regions found in this study was insufficient to illustrate the geographical clustering with respect to segregation. However, the variation among the viral genomes from different clades segregated the viral strains into distinct haplogroups. Nine, 45, 107, 83, 160 and 14 haplotypes, with a minimal haplotype sharing, were identified among the major haplogroups/clades: O, G, GH, GR, GK, and GRA, respectively. Strikingly, G and GR clades were found to be co-evolved and have been an epicenter for evolution of GH, GK and GRA clades (**Fig. 4**). Despite the similar topology noted in phylogeny and median-joining network, the topology of haplotype network presented more detailed illustration of genetic diversity of SARS-CoV-2 than that of the phylogenetic tree; therefore, facilitating a better visualization of the relationship among different variants and enabling efficient identification of recurrent mutations and steps in emergence of the variants.

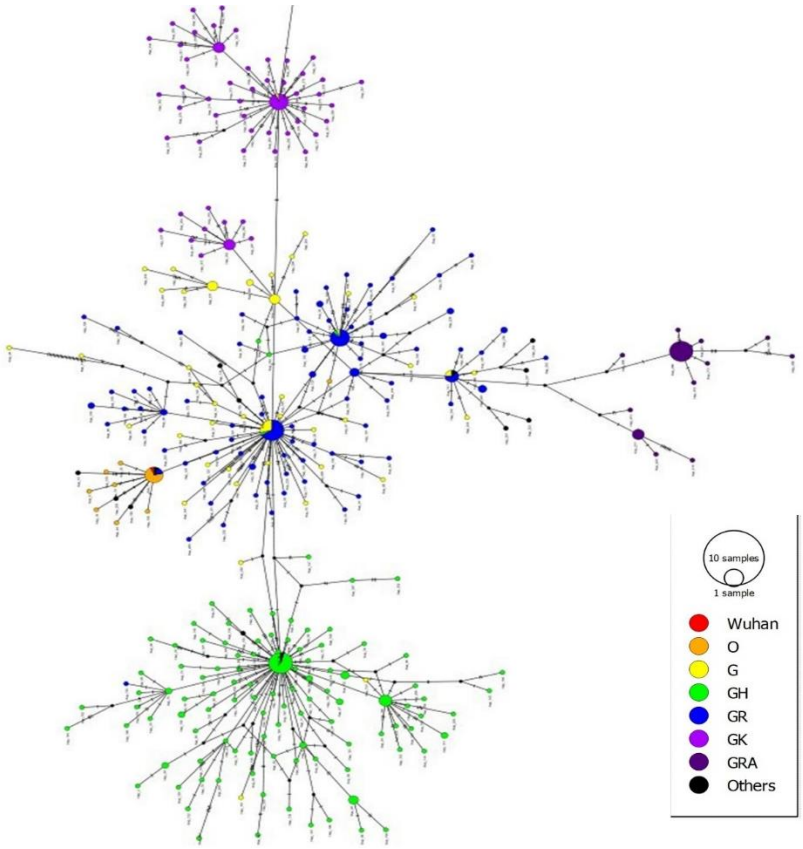


Fig. 4. Haplotype network showing details of genetic diversity of SARS-CoV-2 during the period of Mar 2020 to Mar 2022 and also highlighting the co evolution of G (yellow) and GR clades (copper blue).

Molecular epidemiological analysis of SARS-CoV-2 circulating in different regions of India

Investigators: Dr. PD Yadav, Dr. AM Shete, Dr. V Potdar, Dr. S Cherian, Dr. P. Abraham, Dr. D. Nyayanit, Dr. ML Chowdhary, Dr. RR Sahay; Contributing staff: Dr. A. Kumar, Dr. P. Pandit, Ms. M Dhudhmal, Mr. Y Joshi, Mrs. T Majumdar, Mrs. S. Patil, Dr. DY Patil, Ms. P. Gawanade, Ms. J Yemul

Funding: ICMR NIV Pune COVID-19 Fund **Duration:** 2020-2022 (ongoing)

Background: Tracking mutations in newly emerged SARS-CoV-2 variants will help in understanding viral immune defence mechanisms and learning the pathogenesis of COVID-19. This would aid in applying appropriate intervention measures.

Objectives: Whole genome sequence analysis of SARS-CoV-2 circulating in different geographical regions of India.

Findings:

a. Clinical Characterization and Genomic Analysis of Samples from COVID-19 Breakthrough Infections during the Second Wave among the Various States of India- Study on COVID-19 breakthrough infections during the second wave demonstrated the dominance of Delta variants among various states of India. Lesser hospitalization and fatality rate suggest the usefulness of vaccination.

b. Predominance of Delta variant in SARS-CoV-2 infections of pediatric cases during the second wave in India- Predominance of Delta variant in SARS-CoV-2 infections of paediatric cases during the second wave highlights the importance of genomic surveillance in children (Fig. 5).

c. Predominance of Delta variant among the COVID-19 vaccinated and unvaccinated individuals, India-The COVID-19 vaccinated and unvaccinated individuals also showed the highest rate of infection with the Delta variant during the second wave. This suggests immune escape potential of Delta; however, the vaccination reduced the severity of the disease.

d. Effectiveness of ChAdOx1 nCoV-19 Corona Virus Vaccine (COVISHIELD^{TM}) to prevent SARS-CoV2 infection, Chennai Tamil Nadu, India, 2021. Two doses ChAdOx1 vaccine regime found to be effective against the delta and delta derivatives in the community-based cohort study in Chennai during the second wave.

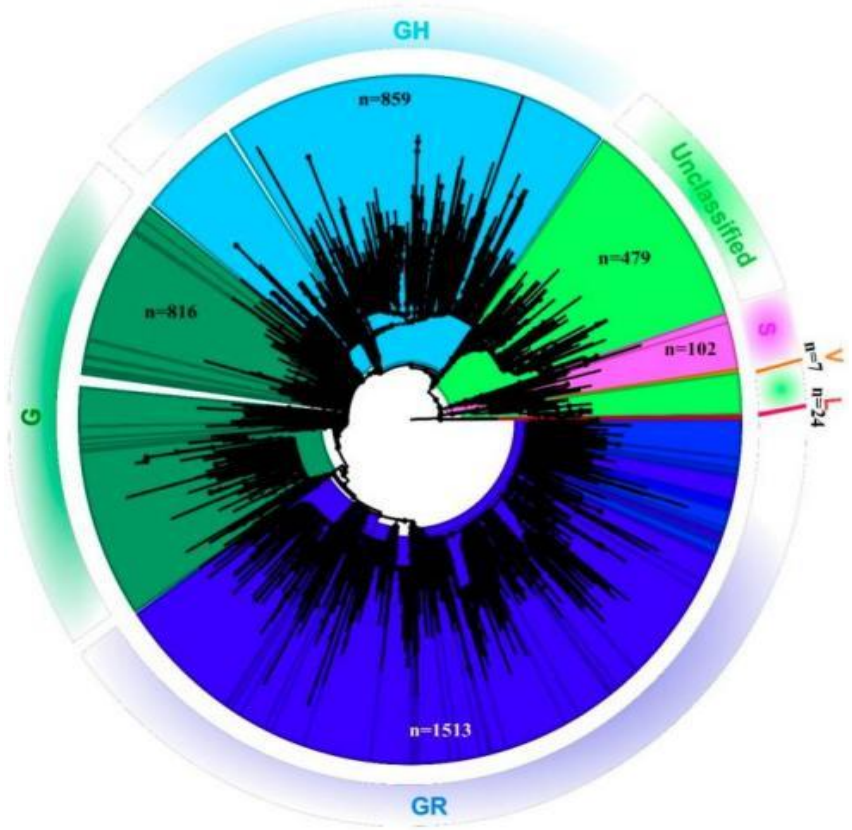


Fig. 5: Phylogenetic tree of the SARS-CoV-2 genome: Neighbor-joining tree for Indian SARS-CoV-2 sequences with a bootstrap replication of 1000 cycles. Fig. Tree v1.4.4 was used to visualize the generated tree. Various GISIAD clades of the SARS-CoV-2 are marked in different colors. The number of sequences used to generate the tree is assigned the n value.

Indian SARS-CoV-2 Genomics Consortium (INSACOG)

Investigators: Dr. VA Potdar, Dr. Sarah Cherian, Dr. Priya Abraham

Funding: Intramural Duration: 2021-22

Background: Viral genome sequencing and genetic characterization have emerged as an essential tool for the epidemiological investigation of the SARS CoV-2 virus pandemic. New SARS-CoV-2 variants have been emerging globally. The evolution of SARS CoV-2 variants

may diminish vaccine induced protective immune responses. A genome surveillance consortium was established with 10 Regional Genome Sequencing Laboratories (RGSLs) in India.

Objectives: To study SARS CoV-2 genome evolution and determine the genomic variants in unusual super-spreader events, high mortality/morbidity trend areas etc.

Findings: Under INSACOG, ICMR NIV has sequenced 6849 SARS-CoV-2 genomes from COVID-19 positive samples received from various states and UTs (Maharashtra, Gujarat, Goa, Chhattisgarh, MP, Assam, Andaman & Nicobar Islands, Lakshadweep, Dadra & Nagar Haveli). The analysis of genomic sequences of the SARS-COV-2 from Maharashtra as well as different states of India helped to detect the variants like Delta and its sub lineages, Omicron and its sub-lineages. Since April 2021, Delta variant was prominent across India. From January 2022, Omicron variant became dominant in India. Other variants like Alpha and Kappa were also circulated in Pune. Omicron variant was highly transmissible than Alpha, Delta and other sub-lineages as it was heavily mutated in receptor-binding domain (RBD) at Q493R, N501Y, S371L, S373P, S375F, Q498R, and T478K sites of ACE-2 receptor. Distribution of different GISAID clades given in Fig. 6.

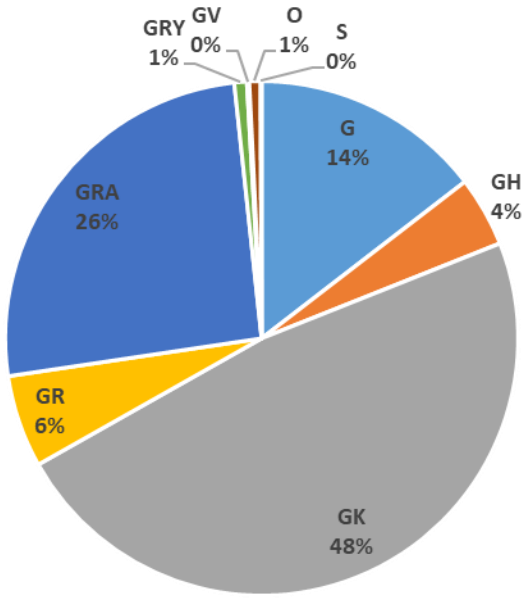


Fig. 6. Distribution of SARS CoV-2 clades circulation in Pune

SARS-CoV-2 Epidemiological Research

Pan India Epidemiological, Virological and Genomic Surveillance for Human Influenza and SARS CoV-2 through DHR-ICMR VRDL Network

Investigators: Dr.VA Potdar, Co-PI: Dr. ML Choudhary, Dr. SD Bhardwaj
Funding: DHR Duration: 2021-2022

Background: A network of 28 VRDL laboratories has been established in the country to carry out surveillance for Influenza and SARS CoV-2. ICMR-NIV Pune is an apex laboratory for the network and developed combo kit for simultaneous detection of SARS CoV-2 and influenza. Using this combo kit, the surveillance was carried out by 28 VRDL laboratories.

Objective: Detection and Genetic and antigenic characterization of Influenza viruses and Novel corona virus (SARS CoV-2) among ILI and SARI cases in different geographical regions of India through VRDL network.

Findings: Till date 18158 samples were tested for Influenza and SARS CoV 2 by 28 VRDL laboratories. The details of virus detected in ILI and SARS cases were depicted in Fig. 7a. During this period, NIC tested 1767 SARI patients' throat/nasal swabs for different respiratory viruses by duplex real time PCR. Weekly distribution of samples tested and positives were shown in Fig. 7b. RSV was predominantly detected in 21.78% samples followed by Influenza in 12.11% samples. Total 161 samples were tested from ARI cases. RSV was detected in 42.2% followed by Influenza in 34.16% samples. PIV was detected in 77 samples and adenovirus in 20 samples; rhinovirus was detected in 3 samples. Fig. 7b.

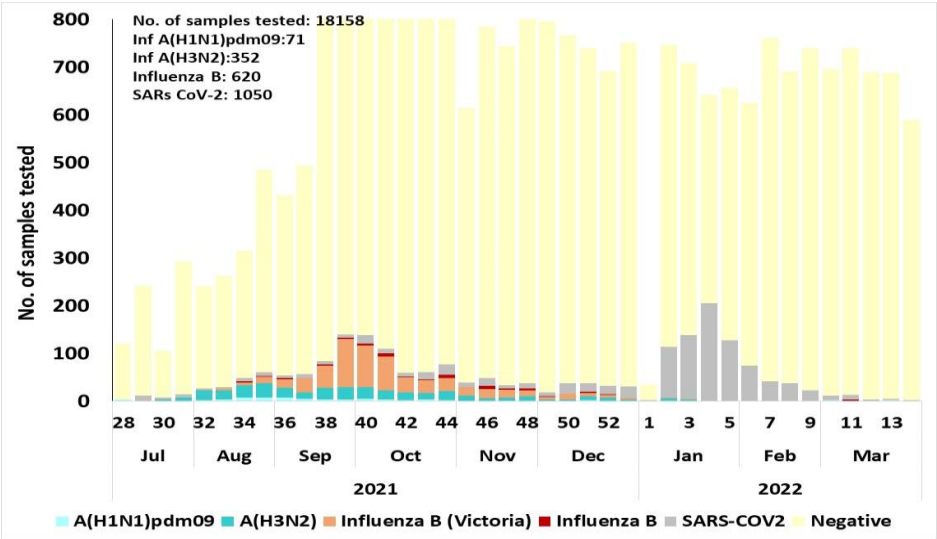


Fig. 7 a: Weekly distribution of Influenza and SARS CoV2 detected by Pan India network

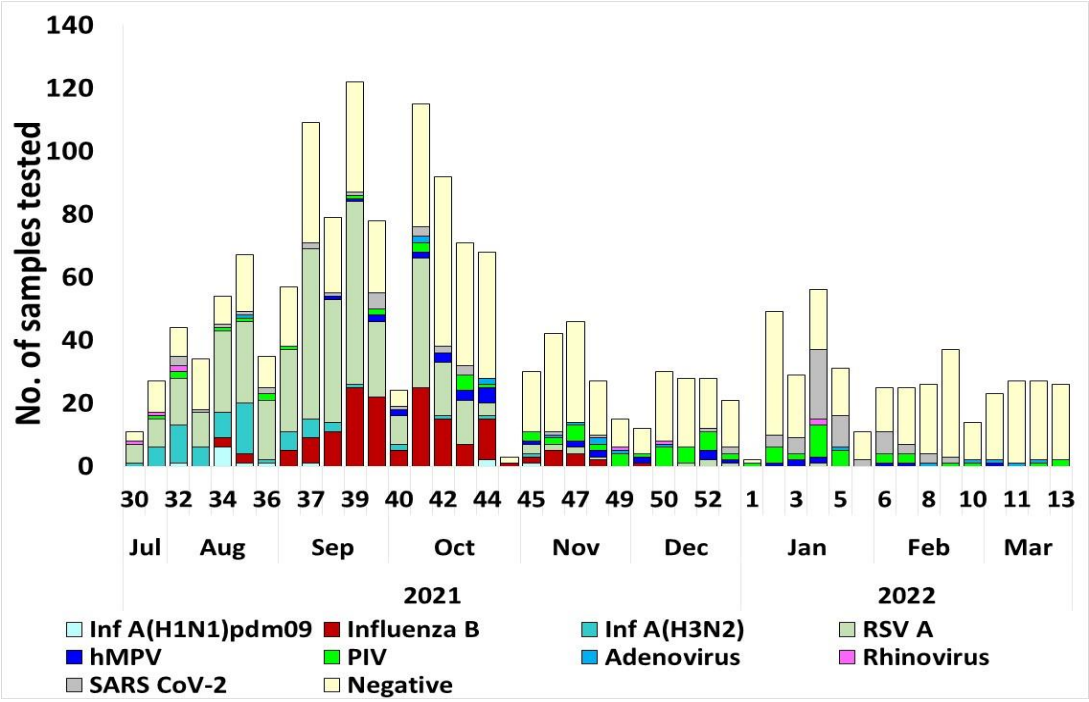


Fig. 7 b: Weekly distribution of respiratory viruses in SARI cases detected at NIC

Establishment of a network of ICMR-COVID-19 biorepositories in India.

Investigators: Dr.A.P.Sugunan & Dr.B.Anukumar

Funding DHR Duration: 2021-22 (06 months)

Background: In the backdrop of the COVID-19 pandemic, while it is of paramount importance to provide early diagnosis and treatment to all infected individuals, it is also critical to promote research and development for larger public health benefit. For development and validation of new diagnostics, therapeutics or vaccines, access to different kinds of clinical samples from infected patients is an essential requirement.

Objectives: To establish organized and dedicated bio-repositories of well characterized clinical samples of COVID-19 patients.

Findings: We collected, processed, and stored urine, serum, plasma, and throat swabs at -80 ^{o}C and -20 ^{o}C deep freezers (2-3 aliquots of each). On the initial day of the study, cross-sectional samples were taken. Following participant consent, follow-up samples were taken on days 1, 3, 7, 14, and 28 of the study. A register comprising the patient's information was maintained, and a separate CRF (case record form) was filled out for each patient. There were 636 individuals in all from whom samples were taken, 601 of which were cross-sectional samples, and 35 were follow-

up samples. 16 cross sectional samples and 7 longitudinal samples were taken from CFLTC DC Mills Kalavoor. 28 longitudinal samples and 56 cross-sectional samples were taken from the General Hospital in Alleppey and stored. From NIV, Kerala 529 cross-sectional nasal swabs are aliquoted and kept in storage.

Social Dynamics of COVID- 19 Transmission in Kerala: A retrospective Qualitative study

Investigators: Dr Retheesh Babu G, Dr.A.P.Sugunan, Rooth P John, Krishna Sarma, Haritha P and Benito Jayadas

Funding: NIV Intramural Duration: 12 Months

***Background*:** The Covid-19 outbreak has posed critical challenges for the public health and research communities. There is a need to assess the situation by conducting evidence based research for preparedness and to understand socio-epidemiological aspects along with experience of affected individual's response.

***Objectives*:** To understand the pattern of illness behaviour of Covid-19 confirmed cases with their daily living conditions. The study would also be studying about the perceived source of infection among the participants and see whether there is any linkage between the social characteristics of people and their mode of transmission. Further the study would look into the family and community response during the time of illness.

Findings: Initial levels analysis says some occupation categories like law enforcement, health department, people in local governance, people employed in un-organised sector etc. are more vulnerable. It is also seen that there is a change in the amount of discrimination based on waves at different time frame. The discrimination, fear and isolation from community was relatively high during its first phase. Apart from the discrimination, the financial difficulty that they have faced due to job loss and mandatory leave from jobs (especially with people who have been employed in unorganized sector) seems to be the major concern. The study will be completed in December 2022.

Epidemiological findings for the first and second waves of COVID-19 pandemic in Maharashtra, India

Investigators: Dr. Pratip Shil, Mr. Nitin M Atre, Dr. BV Tandale

Funding: Intramural **Duration**: 2021-2023

***Background*:** India is one of the worst affected countries during the COVID-19 pandemic. The present analyses was undertaken to estimate the impact of preventive and control measures in the epidemiology of COVID-19.

Findings: We carried out comparative analyses of the COVID-19 situation in Maharashtra for the first and second waves. Epidemiological and demographics data were obtained from open sources and from the Government of Maharashtra. Mathematical modelling and analyses were conducted to estimate the epidemiological parameters like basic reproduction number (R0) for the first wave at different times. It was observed that the districts with a higher percentage of the urban population recorded a higher attack rate during the first wave. Considering a COVID-19 seroprevalence of ~30%, we assumed that 70% of the population to be susceptible during the onset of the second wave in Maharashtra. Thus, the effective reproduction number Re was estimated for the second wave at different times based on the epidemic growth rate (**Fig. 8**). The epidemic growth rates were estimated at different times by best-fit to the exponential model. During the second wave, it was noted that the rural population was more affected across the state. The second wave affected more individuals than the first wave due to various factors such as strictness of restrictions or the lack of it and the emergence of new strains.

Such exercises, if considered during the different phases of the pandemic by public health authorities can be of use in real time to prioritize the surveillance, testing and control aspects.

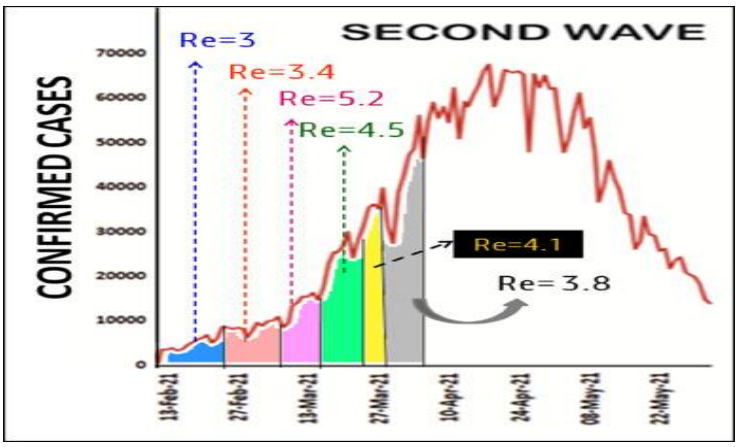


Fig. 8. Time-series of COVID-19 cases in Maharashtra state during the second wave. Effective reproduction number determined at different points in time.

SARS-CoV-2 Environmental studies

Study to assess possible route of transmission of SARS-CoV-2 through faecal material of positive patients and their potential role in persistent fecal viral shedding in the environment

Investigators: Mallika Lavania, Varsha Potdar, Pragya Yadav, Pradeep Sawant, Sreelekshmy Mohandas and Sujata Ranshing; Hospital Investigators: Vikram Padbidri (Jehangir Hospital), Patwardhan Sampada (Deenanath Mangeshkar Hospital), Sanjay Lalwani, Sujata Rege, Sonali Palkar (Bharti Hospital & Research Centre)
Staff: Madhuri Joshi, Nutan Chavan and Manohar Shinde
Funding: Intramural **Duration:** 2020-2022

Background: The primary routes of transmission of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), are through respiratory droplets and close contact of person-to-person contact. While information about other modes of transmission are relatively less, some published literature supporting the possibility of a fecal-oral mode of transmission has been accumulating. In the present pandemic of SARS-CoV-2, gastrointestinal symptoms have been reported among the patients. The detection of SARS-CoV-2 RNA in stool can remain longer after respiratory specimens became negative in patients with COVID-19. This is a hospital-based study initiated to investigate the SARS-CoV-2 shedding in excreta of COVID-19 patients during treatment and after recovery.

***Objectives*:**

To investigate the SARS-CoV-2 shedding in excreta of COVID-19 patients during infection and after recovery

To detect SARS-CoV 2 in stool samples of infected person with positive respiratory tract sample

To isolate SARS-CoV-2 virus from RT-PCR positive stool specimens

To characterize the SARS CoV-2 isolate by sequencing

Findings: Two hundred and eighty patients were recruited in the study. Fecal and Urine samples were collected from a laboratory confirmed COVID-19 patients (throat swab/nasal swab). The results showed that fecal samples of 173 of 280 patients were positive for SARS-CoV-2 nucleic acid, with a positive rate of 61.78%. Among them, the positive rate of SARS-CoV-2 nucleic acid in fecal samples of critical/severe, moderate and asymptomatic cases was 63.7% (51/80), 55.5% (60/108)and 67.39% (62/92) respectively. We obtained complete consensus SARS-CoV-2genomes from 44 of 56 samples (78.5%) processed for sequencing. NGS identified the whole genome sequence of SARS-CoV-2 from fecal specimens from COVID 19 positive patients (Fig. 9).

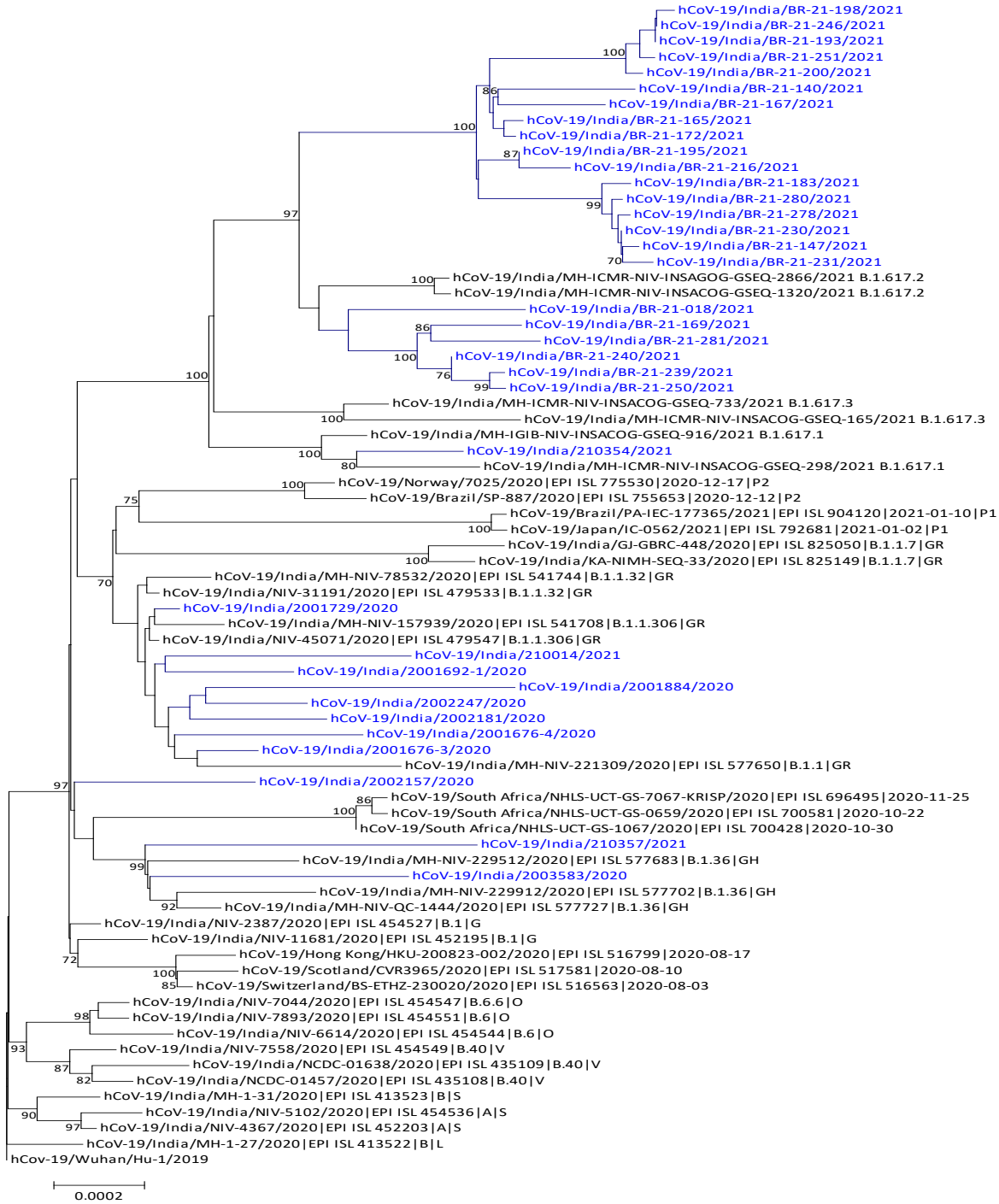


Fig. 9. Phylogeny of SARS-CoV-2 genomes from stool samples of COVID 19 positive patients from Pune, Maharashtra (In Blue text) according to lineages.

Transmission electron microscopy imaging of clarified and negatively stained feces specimens from SARS –CoV-2 infected individuals (n=10) showed the presence of distinct Coronavirus particles in two specimens (Fig. 10). The size of the virus particle was 55 nm and the spike peplomer projections were approximately 20 nm in length.

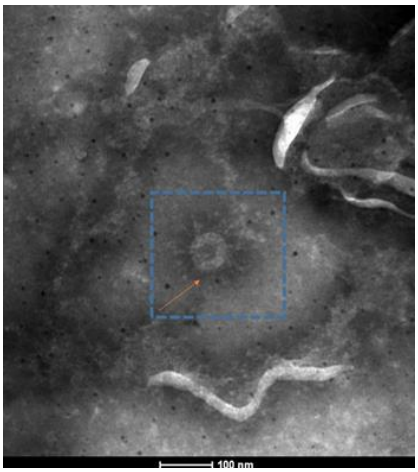


Fig. 10: Transmission electron microscopy imaging feces specimens from SARS –CoV- 2 infected individual.

Quantification of Viral load from the paired stool and urine samples of COVID-19 positive patients by using DD-PCR

Two hundred and twelve paired samples from 106 COVID-19-confirmed patients were analysed by droplet digital PCR (ddPCR) and RT-PCR based target gene (N1 and N2).

In 106 patients, all the stool samples showed 100% positive concordance by both methods, the average of 28.88 cycle threshold (Ct) of RT-PCR, was highly correlated with the average copy number of 327.10 copies/µl analyzed in ddPCR (Fig. 11). Whereas 27.3% urine samples were tested positive in ddPCR & 1.88% were positive with the average of 36.41 cycle threshold (Ct) in RT-PCR.

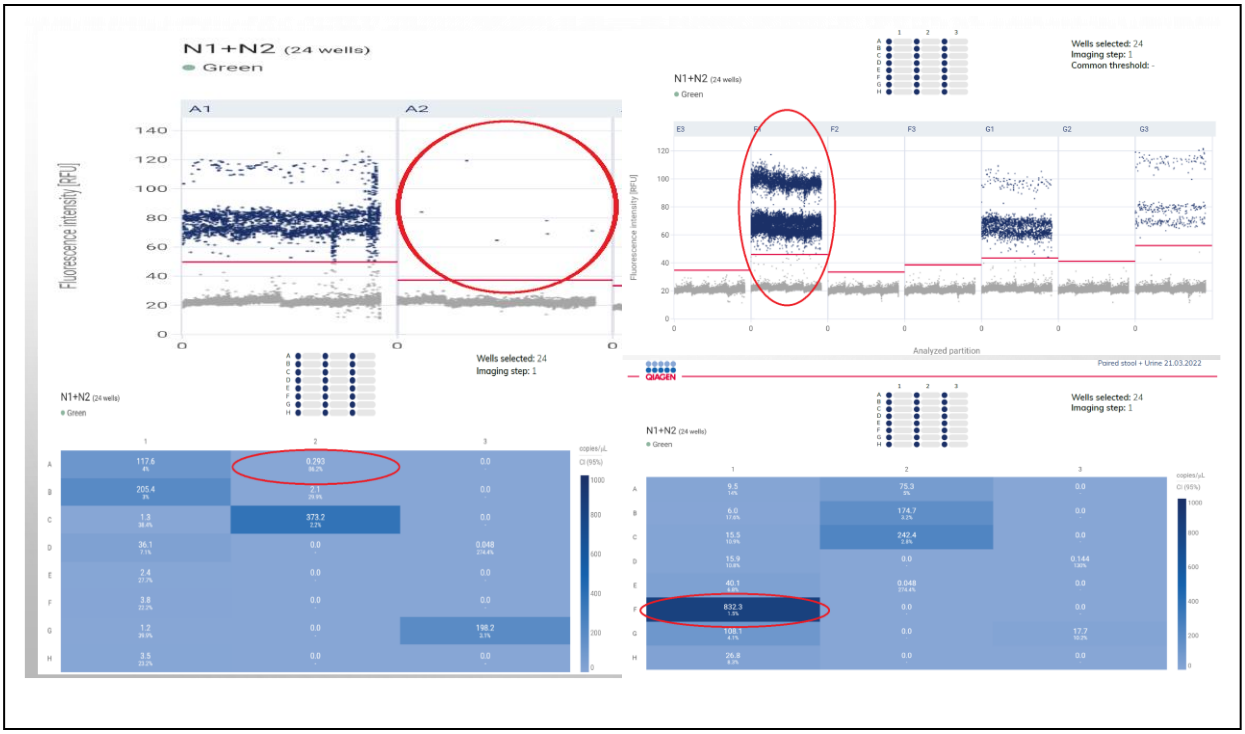


Fig. 11. Representative detection of N1 and N2 from the stool and urine samples of COVID-19 positive patients.

Environmental surveillance of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV- 2) in India

Investigators: Deepa K. Sharma, Uma P. Nalavade, Nivedita Gupta, Jagadish M. Deshpande

Funding: ICMR Duration: 2021-2022

Background: The Severe Acute Respiratory Syndrome coronavirus -2 (SARS-CoV-2) was first detected in China in December 2019 and since then has rapidly spread across the world. Though SARS-CoV-2 spreads mainly via the droplets of respiratory secretions it was also detected in stool specimens of patients indicating active infection of the gastrointestinal tract. The use of environmental surveillance for monitoring SARS-CoV-2 was explored.

Objectives: To establish supplementary surveillance for COVID-19 in various cities across India.

Findings: A total of 472 sewage samples collected from 19 wards in Mumbai were collected and processed using the two phase PEG-Dextran separation method modified by NIVMU. The middle phase was taken for RNA extraction and tested using real time PCR kit of NIV. A total of 239 samples were found to be positive for SARS-CoV-2. Apart from Mumbai, the surveillance was further expanded to 9 states across India. Seven laboratories were selected for testing sewage specimens from selected sites. Most of the sites are the one used for

environmental surveillance of Polio. The laboratories were trained virtually by NIVMU on sewage collection, processing and testing by real time PCR. The data generated from specimens collected from Mumbai as well as other states indicated good correlation between the trend in sewage specimens and that in reported COVID-19 cases at that time point. The data generated has been beneficial in monitoring prevalence of the virus in the community.

SARS-CoV-2 Clinical Research

Reinfection with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) among healthcare workers in India

Investigators: ICMR New Delhi- Dr S. Panda, Dr. A . Mukherjee, Dr. T. Anand, ICMR-NIV Pune: Dr. PD Yadav Co-PIs: ICMR New Delhi- Dr. G Kumar, Dr Madhumathi J, Dr. J Singh, Dr L Sharma, Dr. P. Sharma ICMR- NIV Pune- Dr. GN Sapkal, Dr. RR Sahay, Dr. AM Shete, Dr. Abraham.P 10 Site PIs Contributing staff: Dr. GR Deshpande, Dr. R Jain, Mrs. T. Majumdar, Mrs. S. Patil, Ms. M. Dudhmal, Mr. Y Joshi, Dr. A Kumar

Funding: DHR-ICMR **Duration**: 2021-2022 (Ongoing)

Background: Although, a history of previous vaccination has been reported to reduce severity of the disease and mortality in COVID-19, it couldn't protect the individuals from getting a reinfection. Many incidences of SARS-CoV-2 reinfection have been reported from various countries. Against this backdrop, the present research focused on building an open cohort of HCWs in selected health facilities across India to estimate the incidence of reinfections with SARS CoV-2 among healthcare workers.

Objectives:

Primary Objectives

To estimate the incidence of confirmed reinfection with SARS CoV-2 among healthcare workers (HCWs) in India

Secondary Objectives

To calculate the diagnostic accuracy of epidemiological case definition of reinfection with SARS CoV-2 developed by Indian Council of Medical Research (ICMR).

To assess socio-demographic, immunological and clinical factors associated with the confirmed cases of reinfections with SARS CoV-2.

To estimate the incidence of vaccine breakthrough infections among healthcare workers

Findings: After taking IHEC and IBSC approval, the project was initiated. A total of 774 SARS-COV-2 cases are enrolled till date and enrolment is still on. This cohort would be followed to see the incidence of reinfection. Out of 774 cases samples, NGS was performed on 564 samples and full genome retrieved in 487 samples. Majority cases are infected with BA.1 and BA.2 sub lineages of Omicron.

Risk factors for Covid Associated Mucormycosis in India: a case control investigation

Investigators: ICMR New Delhi- Dr S. Panda, Dr. A . Mukherjee, Dr. T. Anand, ICMR-NIV Pune- Dr. PD Yadav, Dr. P Abraham; Contributing staff:, Dr. AM Shete, Dr. RR Sahay, Dr. R Jain, Mrs. T. Majumdar, Mrs. S. Patil

Funding: ICMR New Delhi **Duration**: 2019-2021

Background: The increased incidence of COVID-19 associated Mucormycosis (CAM) during second wave of pandemic in India led us to undertake a multi-site case control investigation examining the monthly trend of proportion of CAM cases among in-patients and to identify factors associated with CAM.

***Objective*:** A case control investigation of COVID-19 associated Mucormycosis in India

Findings: A multi-site case control investigation of COVID-19 associated Mucormycosis (CAM) conducted on 1211 patients revealed lag of a month between the peak of COVID-19 and CAM cases (Fig. 12). Risk factors independently associated with CAM were dusty working environments such as farming or gardening, longer duration of hospital stay during COVID-19 illness, newly onset diabetes mellitus, steroid usage and zinc supplementation.

The present multi-site, nationwide study clearly depicted the trend of CAM cases in India peaking during the month of May 2021. Next generation sequencing was performed on the samples from cases and controls having E gene Ct value <30; samples from 31/31 CAM cases and 53/71 controls were sequenced. Complete genome could be retrieved from 20/31 CAM cases and 49/53 controls sequenced. Next generation sequencing of the CAM and control cases revealed SARS-CoV-2 B.1.617.2 as predominant strain; various delta sub lineages were also detected in both the cases.

Genomic sequencing of cases and controls showed a comparable presence of SARS-CoV-2 variants and did not reveal any specific association of mutation in either group. Variants of concern (Alpha, Delta and Delta derivates) or Variants Under Investigation (VUI) did not show any preferential distribution among CAM cases

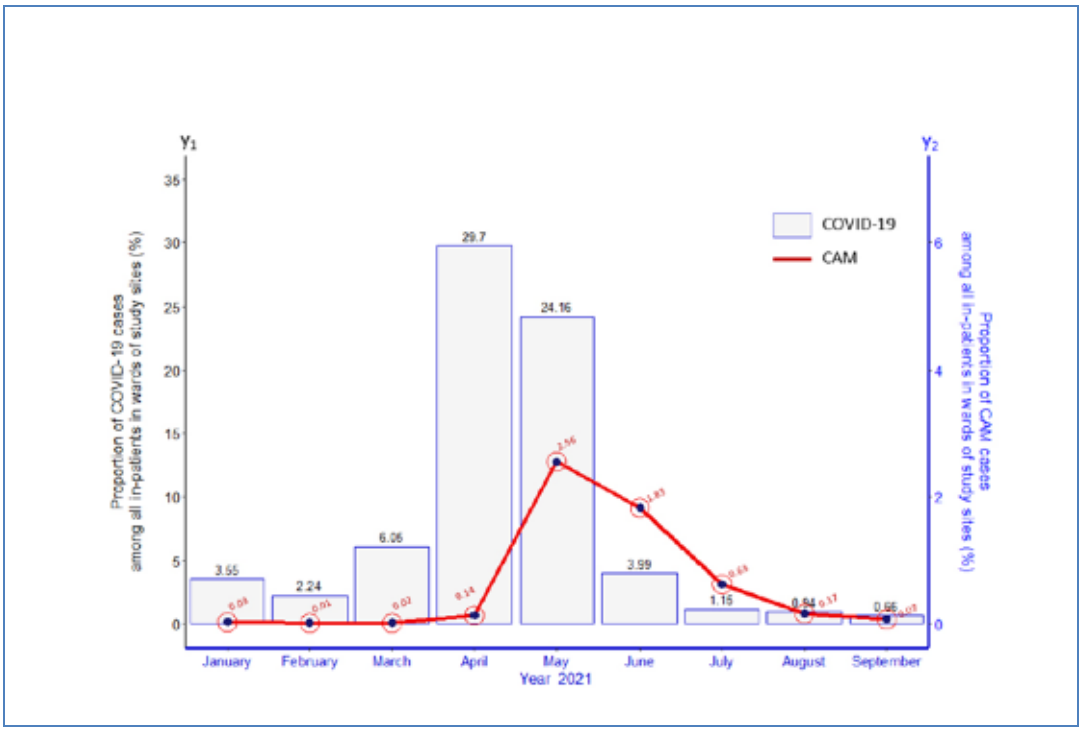


Fig.12. Monthly trends for COVID associated mucormycosis (CAM) and COVID-19 cases.

A prospective observational study to establish the duration of persistence of replication-competent SARS-CoV-2 in oncology patients with COVID-19 on chemotherapy

Investigators: Tata Memorial Hospital, Mumbai: Dr. C Dhamne, Dr. O Shetty, Dr. S. Epari, Dr. S. Biswas, Dr. G Salunke, Dr. P Ranganathan, Dr. C S Pramesh, Dr. M Sengar, Dr. G Chinnaswamy, Dr. N. Moulik, Dr. A Ramaswamy, Dr. S. Srinivas, Dr. N. Mummudi, Dr. Pr. Nayak, Dr. L. Nayak; ICMR-NIV Pune: Dr. PD Yadav, Dr. AM Shete, Dr. RR Sahay, Dr. Ullas PT, Dr. S Mohandas, Dr. DY Patil; Coordinator: Dr P. Abraham;
Contributing staff: Mrs. T Majumdar, Mrs. S. Patil, Ms. P. Gawande, Mrs. A. Waghmare, Mrs. K. Kalele, Dr. R Jain, Mrs. P. Bodke, Mrs.P. Waghmare, Mrs. S. Ray
Funding: ICMR NIV Pune COVID-19 Fund **Duration:** 2021-2022 (ongoing)
Background: The persistent delay in chemotherapy associated with COVID-19 isolation increases risk of relapse in cancer patients. Duration of isolation and chemotherapy interruption for cancer patients might have to be individualized unlike the immunocompetent individuals. Establishing biomarkers to predict resolution of COVID-19 in these patients will be immensely helpful to the oncologist to guide management of the underlying cancer in these patients.
***Objectives*:** To determine the duration of persistence of replication-competent/infectious virus and related biomarkers in oncology patients.

Findings: A total of 37 cases (age ranged from 1-76 years) were enrolled in this study and followed up to see the persistence of viremia. Cases had been diagnosed with different cancers including multiple myloma, acute myeloid leukemia, lung carcinoma, rectal carcinoma, stomach cancer, lymphoma, maxillary sinus cystic carcinoma, sarcoma, chronic lymphocytic leukemia, meduloblatoma, cancer of prostrate, tongue, pancreas, cervix, buccal mucosa, bladder and larynx. Most of the cases were infected with Delta and Delta derivatives and Kappa variant of SARS-CoV-2. The IgG response in the cases were low and majority of cases were negative at the follow up.

SARS-CoV-2 Vaccine Research and Preventive Measures

A Phase II, Open Label, Randomized Controlled Trial to Assess the Safety and Efficacy of Convalescent Plasma to Limit COVID-19 Associated Complications in Moderate Disease

Investigators: Dr. Pragya Yadav, Dr. GN Sapkal, Dr. AM Shete, Dr. G Deshpande, Dr. H Kaushal
Contributing staff: Dr. R Jain
Funding: ICMR New Delhi **Duration**: 2020-2021 (completed)
Background: Convalescent plasma supposed to have therapeutic effect in covid-19 cases. We have investigated effectiveness of convalescent plasma to treat moderate covid-19 in adults in India. A total of 464 adults (≥18 years) admitted to hospital (22 April to 14 July 2020) with confirmed moderate covid-19 having PaO2 /FiO2 ratio 200 mm Hg-300 mm Hg or a respiratory rate of more than 24/min with oxygen saturation 93% or less on room air): 235 were assigned to convalescent plasma with best standard of care (intervention arm: two doses of 200 mL) and 229 to best standard of care only (control arm).
Objective: To assess the effectiveness of convalescent plasma to treat moderate coronavirus disease 2019 (covid-19) in adults in India.
Findings: The mean age was 51±12.4 years; 76.7% were males. Admission Sequential Organ Failure Assessment score was 2.4±1.1. Non-invasive ventilation, invasive ventilation and vasopressor therapy were required in 98.9%, 8.4% and 4.0%, respectively. The 28-day mortality was 14.4%. Median time from symptom onset to hospitalization was 4 days in both survivors and non-survivors. Patients with two or more co-morbidities had 2.25 times risk of death. Interleukin-6 levels were higher in non-survivors and increased on day 3. On multivariable Fine and Grey model, severity of illness, PaO2 /FiO2 <100, neutrophil lymphocyte >10, Ddimer >1.0

mg/L, ferritin ≥500 ng/mL and lactate dehydrogenase ≥450 IU/L were significantly associated with death. Moderately and severely ill patients with COVID-19, severity of illness, underlying comorbidities and elevated levels of inflammatory markers were significantly associated with death.

Effectiveness of COVAXIN and COVISHIELD vaccines against severe COVID-19 in India, 2021: Multi-centric hospital-based case control study

Oversight: ICMR, New Delhi- Dr. B Bhargava, Dr. Panda S, Dr. Gupta N
Lead coordination: ICMR-National Institute of Epidemiology, Chennai- Dr. Murhekar M, Dr. Batnagar T, Dr. Manickam P, Dr. Jeromie, Dr. Kumar S, Dr. Chaudhari S
Lead laboratory: ICMR-NIV, Pune- Dr. Yadav PD, Dr. Sahay RR, Dr. Sapkal G, Dr. Shete AM, Dr. Abraham P

Technical support: WHO-India, New Delhi- Mohammad Ahmad
Funding: ICMR New Delhi **Duration:** May 2021-August 2021 (Completed)
Background: The real world effectiveness of COVAXIN and COVISHIELD vaccines against SARS-CoV-2 infection was not known. From a healthcare perspective it is more important to know if the vaccine is effective against severe COVID disease and eventual hospitalization.
***Objectives*:** To determine the effectiveness of vaccination with either COVAXIN or COVISHIELD vaccine against severe COVID-19 disease among individuals aged 45 years and above in India
Findings: We did a multi-centric, hospital-based, case–control study between May and July 2021. Cases were severe COVID-19 patients and controls were COVID-19 negative individuals from 11 hospitals. Vaccine effectiveness (VE) was estimated for full (2 doses ≥14days) and partial (1 dose ≥21 days) vaccination; duration between two vaccine doses and against Delta variant. We enrolled 1,143 cases and 2,541 controls. The VE of full vaccination was 80% (95% CI: 73%-86%) with AZD1222/COVISHIELD and 69% (95% CI: 54%-79%) with BBV152/COVAXIN. The VE was highest for a gap of 6-8 weeks between two doses of AZD1222/COVISHIELD (92%, 95% CI: 82%-96%) and BBV152/COVAXIN (92%, 95% CI: 26%-99%). The VE estimates were similar against the Delta strain and sub-lineages (Fig. 13). Our study concluded that the BBV152/COVAXIN and AZD1222/COVISHIELD were effective against severe COVID-19 among the Indian population during the period of dominance of highly transmissible Delta variant in second wave of pandemic. An escalation of two-dose coverage with COVID-19 vaccines is critical to control the pandemic in the country.

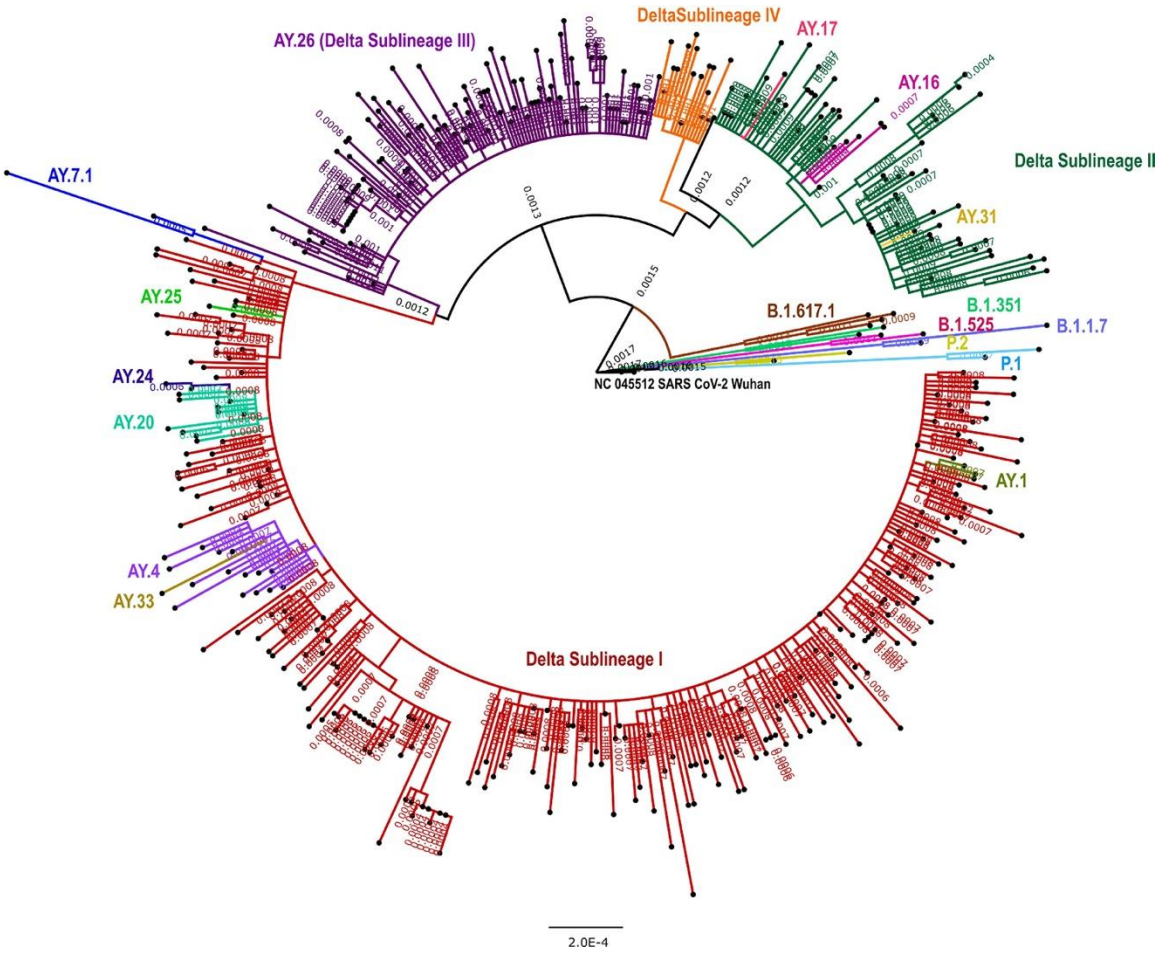


Fig. 13. A Maximum likelihood tree of the 448 SARS-CoV-2 sequences retrieved in this study, along with the representative SARS-Cov-2 sequences (n = 12) from different clades.

Assessment of prophylactic and therapeutic role of BCG vaccine against SARS-CoV-2 infection in hamster model

Investigators: Dr. M Bhaumik, [ICMR-NICED Kolkata], Dr. PD Yadav [ICMR-NIV Pune]
Co-PIs: Dr. Sreelekshmy M and Dr. M Chawla
Funding: DBT **Duration**:2021-2022 (ongoing)

Background: Although Bacillus Calmette– Guérin (BCG) does not possess any inherent antiviral activity, it engages in host immunity such that many types of viral infections are considerably reduced. Notably, countries not having universal BCG vaccine policies have higher mortality associated with COVID 19 compared to the countries having long-lasting universal

BCG vaccine policy. However, the evidence may be circumstantial and till now there is no evidence on this hypothesis.

Objective: To study the protective and therapeutic efficacy of BCG against SARS-CoV-2 infection in hamster.

Findings: Three groups (Prophylactic group, therapeutic group and virus control) of 8 hamsters each were used for the study. Four hamsters from each group were sacrificed on day 3, 7 post virus inoculation. BCG immunized hamsters showed marginal but significant decrease in viral load in throat and nasal wash on day 1 post SARS-CoV-2 infection. But no significant difference was observed in respiratory organs and nasal turbinate. There was no significant decrease in viral load with threapeutic treatment with BCG.

Antibody responses in rhesus macaques against inactivated SARS CoV-2 virus

Investigators: Dr. DR Patil, Dr. PD Yadav, Dr.S Mohandas, Dr.AM Shete, Dr. GN Sapkal, Dr. HK Kaushal; Contributing staff: Mr. M Kadam, Mr. A. Suryawnashi, Mr. A. Kumar, Dr. R Jain

Background: It is well studied known that successful vaccine doses dosage may differ in elderly as compared to infants and young children. Nonhuman primates are ideal translational models, as the immunological response in non-human primates is similar to human, so it enables to understand response of human immune system human immunogenicity of to the vaccine. Considering this, we have conducted an assessment of studied immune response in aged rhesus macaques against BBV152, an inactivated SARS CoV-2 vaccine.

Objectives: To evaluate antibody responses in Rhesus macaques using inactivated SARS-CoV-2 virus

Findings: Aged rhesus macaques were vaccinated with three dose regimen of the BBV 152 inactivated vaccine on 0, 14 and 62 days apart using three formulations BBV 152 A, 3µg+ BBV 152 B and BBV 152 C. The vaccine candidate with 6 µg with Algel-IMDG (BBV 152 CB) induced robust neutralizing antibody response in aged rhesus macaques.

Comparative assessment of BBV152 vaccine (COVAXIN^{TM}) antibody and antigen-specific responses in immunized population without past COVID-19 infection, individuals vaccinated after recovery from COVID-19 and non-vaccinated individuals with past COVID-19 infection

Investigators: Dr. RR Sahay, Dr. P.D. Yadav, Dr. AM Shete, Dr. A Tripathy, Dr. YK Gurav, Dr. H Kaushal; Coordinators: Dr. P Abraham, Dr P. Awate; Collaborative Hospital from Pune: District Aundh Hospital;
Contributing staff: Mr. A. Suryawnashi, Dr. R Jain, Dr. R. Dhawade, Mr. R Hawale)
Funding: ICMR NIV Pune COVID-19 Fund **Duration:** 2021-2022 (Ongoing)
Background: Here, we propose to assess the antibody and antigen-specific responses in population immunized with BBV152 at different time intervals and compare the antigen and antibody responses in SARS-CoV-2 infected non-vaccinated individuals as well as vaccinated individual with past COVID-19 infection.
Cohort details:

The cohort comprised of two groups viz category 1 ([ndividuals vaccinated without prior history of SARS-CoV-2 infection] and category 2 [Individuals vaccinated after recovery (one month after the last negative RTPCR test/clinical recovery) from SARS-CoV-2 infection]

Enrolment was considered at Pre-first dose (Day 0) followed by 1st follow up after 28 days of 1st dose (or on the day of 2nd dose), followed by 2nd follow after 56 days of 1st dose (or 28 days after 2nd dose), followed by 3rd follow up after 180 days of 1st dose
Objectives: To evaluate and compare specific immune responses in the individuals immunized with BBV152 vaccine without past SARS-CoV-2 infection, with SARS-CoV-2 infection, and with naturally infected non-vaccinated individuals.
Findings: In the cohort study, 246 participants were recruited with 167 males and 79 females. Out of 246, 118 adults lacked Covid history (category 1) whilst 128 reported SARS Cov 2 infection in past (category 2). Forty two percent of adults were of age 18-30. In the cohort 13% adults had co morbidities predominantly hypertension and diabetes with 10% in category 1 and 17% in category 2. During enrolment and follow ups, nasopharyngeal swab was collected to determine SARS COV 2 RTPCR positivity. Besides, levels of whole SARS Cov2 antigen Ab, S1RBD Ab and N protein AB were detected by ELISA. In 1st follow up, 218 participants turned up with 107 (49%) in category 1 group and 111(51%) in category 2 group. In 2nd follow up, 180 participants turned up with 89 (49%) in category 1 group and 91(51%) in category 2 group. In 3rd follow up, 116 participants turned up with 51 (44%) in category 1 group and 65(56%) in category 2 group. Cohort retention was found to be in 88%, 73% and 47% in 1st, 2nd and 3rd follow up respectively. No adverse reaction or event reported post vaccination throughout the

study period indicated BBV152 is a safe vaccine among adult Indian population. Vaccine effectiveness was found to be 98% as throughout the study period only 6 participants out of 246 (2%) were SARS COV 2 RT PCR. Of which 3 were breakthrough from category 1 and 3 were reinfection from category 2.

Neutralization of different SARS-CoV-2 variants with sera of COVAXIN vaccinated individuals:

- **a.** Neutralization of VUI B.1.1.28 P2 variant with sera of COVID-19 recovered cases and recipients of COVAXIN: In this study, we determined the IgG immune response and neutralizing activity of the 19 convalescent sera specimens obtained from the recovered cases of COVID-19 and confirmed for B.1.1.7 (UK) (n = 2), B.1.351 (South Africa) (n = 2), B.1.1.28.2 (n = 2), B1 lineage (n = 13) (15–113 days post positive test).Results confirm 1.92 and 1.09 fold reductions in the neutralizing titre against B.1.1.28.2 variant in comparison with prototype D614G variant with sera of COVAXIN vaccinees and natural SARS-CoV-2 infection, respectively.
- **b.** Neutralization of Variant Under Investigation B.1.617.1 With Sera of COVAXIN Vaccinees: Here, we report the immunological characteristics of a Variant under Investigation (VUI) B.1.617.1, playing a critical role in the current surge of coronavirus disease 2019 (COVID-19) in the western state of Maharashtra, India. The GMT ratio comparison of B.1.1.7 was significantly higher than the GMT for B.1.617.1. The comparison of D614G and B.1.1.7 showed equivalent responses, with a GMT ratio of 1.06 and a 95% CI of 1.02–1.10. The B.1.617.1 variant performance with vaccine sera was better than that of recovered cases. The results of B.1.1.7 variant neutralization with BBV152 vaccine sera and findings of B.1.617.1 emphasize that this vaccine is robust against emerging mutation and maintains the efficacy of the vaccine.
- **c.** Neutralization of Beta and Delta variant with sera of COVID-19 recovered cases and COVAXIN vaccinees: Here, we assessed the neutralization of sera from COVID-19 recovered cases post 5–20 weeks of infection and vaccinees 28 days after two doses of BBV152 against Beta, Delta variants and compared with prototype B.1 (D614G). The recovered cases were infected with B.1 and B.1.617.1 lineage. The GMT ratio of B.1 to Beta and Delta variants was 3.3 and 4.6. Our study demonstrated that despite a reduction in neutralization titers with BBV152 vaccinees sera against Beta and Delta variants, its neutralization potential is well established.
- **d.** Comparable neutralization of SARS-CoV-2 Delta AY. 1 and Delta with individuals sera vaccinated with COVAXIN: Here, we present the data from a cross-sectional study, where the sera of the fully vaccinated study participants with two doses of COVAXIN vaccine were evaluated for neutralizing antibodies. The participants were divided in three separate groups, namely COVID-19 naïve vaccinees (CNV), COVID-19 recovered cases (real-time RT-PCR positive) and vaccinated (CRV) and breakthrough infections post-vaccination (BTI). The present study revealed 1.5-, 3.5- and 2.8-fold reduction in NAb titer for Delta AY.1 and 1.3-, 2.5- and 1.9-fold reduction against Delta variant compared with B.1 variant in sera of CNV, CRV and BTI, respectively. The findings of the study suggest that BBV152 would still be able to protect vaccinated individuals with severe disease from Delta, Delta AY.1 and B.1.617.3 variants.

e. Elevated neutralization of Omicron with sera of COVID-19 recovered and breakthrough cases vaccinated with COVAXIN than two dose naïve vaccinees: Considering, the impact of third wave of pandemic aroused with Omicron in India, we assessed the sera of naïve, recovered and breakthrough cases vaccinated with COVAXIN for its neutralizing ability against Omicron and other SARS-CoV-2 variants. Comparative analysis of COVID naïve cases demonstrated fold-reduction of 4.9, 2.06 and 12.49 against Beta, Delta, and Omicron respectively compared to prototype strain B.1. The administration of booster or precautionary dose is of much significance as it provides better protection against COVID-19 disease.

Evaluation of immunogenicity and protective efficacy after vaccine interchangeability in a selected population of Eastern UP

Investigators: ICMR-RMRC Gorakhpur- Zaman K, Dwivedi G, Kant R, Deval H, Pandey A, Singh R; ICMR-NIV Pune- Yadav PD, Sahay RR, Shete AM, Sapkal GN, Kaushal H, Abraham P; Chief Medical Officer, Community Health Centre, Siddarthnagar, Uttar Pradesh, India- Sandeep Chaowdhary; Command Hospital (Southern Command), Armed Forces Medical College, Pune- Kumar S

Funding: ICMR NIV Pune COVID-19 Fund **Duration:** 2021-2022 (Ongoing)

Background: Recently an incidence of inadvertent vaccine interchangeability occurred in twenty individuals in Audai Kalan village under the CHC Badhni of Siddarthnagar district of eastern Uttar Pradesh, India. We studied the vaccine effectiveness, immunogenicity and reactogenicity, in this already vaccinated heterologous group (COVISHIELD followed by COVAXIN).

Objectives:

1. To evaluate the vaccine effectiveness and immunogenicity in selected group
2. To study the impact of vaccine interchangeability on immunogenicity
3. To study the short/long term immune protection due to the interchangeability.

Findings: We compared the safety and immunogenicity profile of them against that of individuals receiving either COVISHIELD or COVAXIN (n=40 in each group). Lower and similar adverse events following immunization in all three groups underlined the safety of the combination vaccine-regime. Immunogenicity profile against Alpha, Beta and Delta variants in heterologous group was superior; IgG antibody and neutralizing antibody response of the participants was also significantly higher compared to that in the homologous groups.

Immune responses following six months of administration of heterologous prime-boost COVID-19 vaccine: Of the 98 vaccine recipients, 88 individuals could be followed up at 6 months following administration of the second dose. Comparative analysis at one- and six-months post-vaccination showed modest reduction in S1-RBD IgG antibody and NAb titers

against B.1, Alpha, Beta and Delta variants in heterologous and homologous vaccine recipients groups. However, significant reduction in NAb titers against Omicron in vaccinees' sera post-six months underlines need for cautious prospective follow-up.

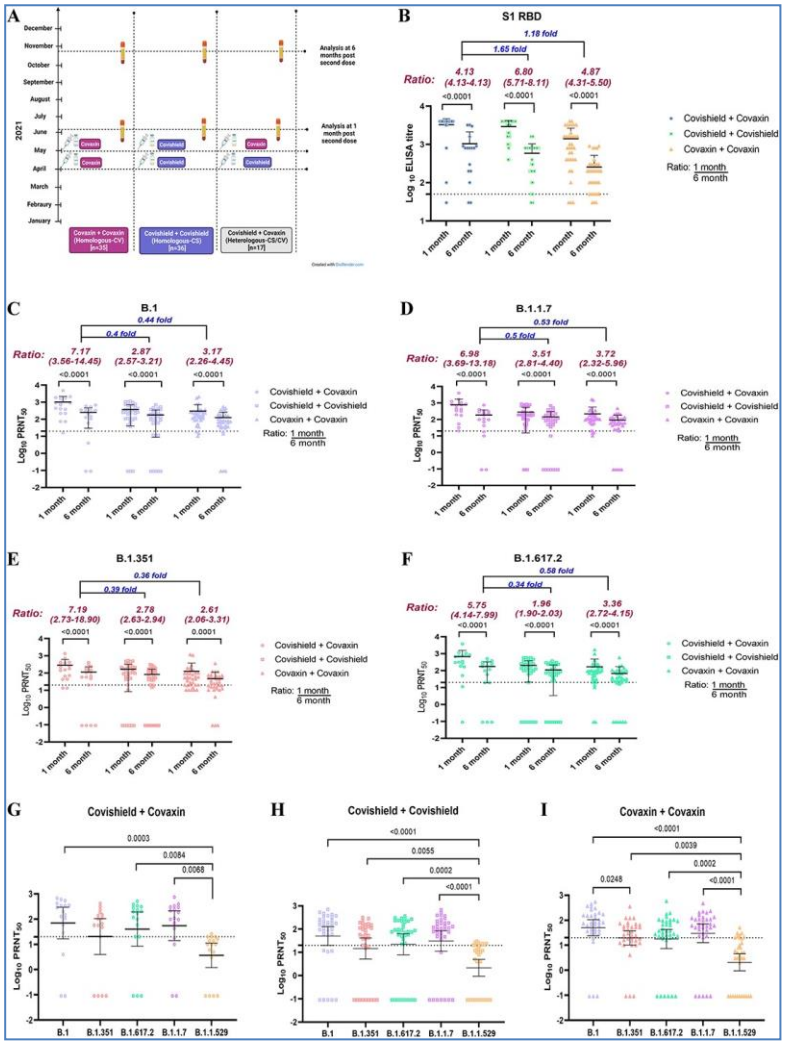


Fig. 14. The flow chart of the study design and ELISA titer of individual sera vaccinated with different vaccine regime: (A) study design of the experiment performed delineating the detailed steps of the sample collection from participants (B) anti-SARS-CoV-2 IgG antibody against S1-RBD for the different vaccine combination (CS followed by CV marked as circle; CS followed by CS as square and CV followed by CV as triangle). The fold reduction provided in magenta colour is the geometric mean titer ratio of one and 6 month. The statistical significance was assessed using a two-tailed Wilcoxon matched-pairs signed rank test; P-value < 0.05 were considered to be statistically significant. The dotted line on the Fig. s indicates the limit of detection of the assay. Data are presented as mean values ± standard deviation (SD). Comparative ratios of the neutralizing antibody geometric mean titres in CS/CV, CS/CS and

CV/CV group against B.1, Alpha, Beta and Delta at 1 and 6 months post second dose (C–F). The neutralizing antibody geometric mean titres in CS/CV, CS/CS and CV/CV group against Alpha, Beta Delta and Omicron compared with B.1 at 6 months post second dose (G–I). The fold reduction provided in magenta colour is the geometric mean titer ratio of one and 6 month. The statistical significance was assessed using a two-tailed Kruskal–Wallis with Dunn's multiple comparison test; P-value < 0.05 were considered to be statistically significant. The dotted line on the Fig. 14. indicates the limit of detection of the assay. Data are presented as mean values ± SD.

Immune response to precautionary third dose of COVISHIELD/COVAXIN among healthy adult population: an ICMR Cohort study, India

Investigators: Dr. GN Sapkal, Dr.Pradnya Shinde, Dr.Gururaj Rao Deshpande,
Funding: ICMR Duration: 2022-2024

Background*:* India's COVID-19 vaccination programme has proposed to initiate additional third dose for healthcare and frontline workers and individuals aged above 60 years with comorbidities. Limited studies from India have documented dynamics of immune response of additional third dose of COVISHIELD/COVAXIN vaccine using homologous regimen. In this context, ICMR proposes to establish a cohort of employees (Permanent and Project staff who will be available for follow up till one year) working in ICMR institutes, fully vaccinated and eligible for receiving additional homologous third dose ('precautionary dose') to characterize the humoral and cellular immune response before and after the precautionary dose vaccination.

Objectives*:* 1. Primary: Characterize SARS-CoV-2 specific humoral and cellular immune response after homologous precautionary third dose of COVISHIELD/COVAXIN vaccine at different time points. 2. Secondary: Estimate the incidence of SARS -CoV-2 symptomatic infection post third dose of COVID- 19 vaccine.

Findings*:* During the study period a total of 45 individuals were enrolled at ICMR-NIV study site. A total of 596 serum samples were received including follow up samples collected from 14 study sites and tested by Abbott Alinity anti-SARS CoV-2 IgG Quant assay for anti-SARS CoV-2 S1RBD IgG antibody measurement. Of the total 596 samples tested, 23 samples collected at baseline were negative. The IgG titers against S1-RBD proteins in booster group showed a significant increase of 2.80 fold (*P*-value: 0.0011) indicating immune responses mounted with booster dose.

SARS-CoV-2 Antiviral Research

Drug repurposing for SARS-CoV-2 using structural bioinformatics and systems biology approaches

Investigators: Dr. Sarah Cherian, Ms. Prachi Jagtap, Dr Kavita Lole
Funding: Extramural (ICMR - Sr. Res. Fellowship) **Duration**:2020-2025
***Background*:** The recent COVID-19 pandemic has become a major public health concern worldwide. We focussed on differentially expressed genes (DEGs) from the COVID-19 transcriptome based on COVID-19 samples from patients with mild, moderate, and severe disease conditions.
***Objective*:** The current study aimed to determine promising treatment options for COVID-19 through a computational drug repurposing approach.
Findings: The detected DEGs were employed in a connectivity map (CMap) analysis to identify therapeutic candidates. Results showed that drugs including prednisolone, triamcinolone, medroxyprogesterone, phorbol-12-myristate-13-acetate, amoxicillin, rimantadine, NSC-663284, depomedrol, kinetin-riboside, mepireserpate, SA-94315, prostratin, xanthohumol, sulindac, fluocinolone, naringin, vincristine, and mepyramine were found to be common for mild, moderate, and severe disease conditions. Emetine, rivaroxaban, corticosterone, vindesine were specific for mild conditions. Ivermectin, tipifarnib, methylprednisolone, fluticasone, verteporfin, terbinafine, perphenazine, sotalol, azacytidine, norgestimate were found to be specific for moderate disease, whereas nelfinavir, cycloheximide, erythromycin, exemestane, medrysone were found to be specific for severe disease. Telmisartan, an Angiotensin Receptor Blocker (ARB) and antihypertensive drug, was found to be common for mild and moderate conditions. These approved drugs, as well as many other small investigational compounds, may have the potential to be repurposed as SARS-CoV-2 therapeutic targets and need to be evaluated by *in vitro* and *in vivo* studies.

Evaluation of monoclonal antibodies as a countermeasure against SARS-CoV-2 infection in Syrian Hamster Model

Investigators: Dr. PD Yadav, Dr. S. Mohandas, Dr. AM Shete, Dr. GN Sapkal, Dr. DR Patil; Contributing staff: Mr. M. Kadam, Mr. A. Suryawanshi, Dr. R. Jain, Ms. P. Gawande, Mrs. A. Waghmare, Dr. D. Nyayanit
Funding: ICMR NIV Pune COVID-19 Fund **Duration:**2020-2021 (completed)

Background: Neutralizing antibodies are naturally produced by the body against infectious agents as a part of adaptive immune response to provide long-term immunity. Zydus has developed 1:1 cocktail of two human monoclonal antibodies (MAbs) i.e., ZRC3308-A7 and ZRC3308-B10 for the treatment of SARS-COV-2 infection. We have evaluated the ability of of these Mabs for its therapeutic as well as prophylactic role against SARS-CoV-2 in Syrian hamsters.

Objective: To evaluate therapeutic and prophylactic efficacy of ZRC3308-A7 and ZRC3308-B10 cocktail against SARS-CoV-2 infection in Syrian hamsters.

Findings: We have evaluated a monoclonal antibody (mAb) cocktail (ZRC-3308) comprising of 22 ZRC3308-A7 and ZRC3308-B10 in the ratio 1:1 for COVID-19 treatment in hamster model. The mAbs were were found neutralizing SARS-CoV-2 variants B.1, B.1.1.7, B.1.351, B.1.617.2 and 26 B.1.617.2 AY.1 in vitro. Prophylactic use of the monoclonal antibody cocktail at the dose rate of 50 mg/kg and 5 mg/kg in hamsters significantly reduced the SARS-CoV-2 load in lungs with only mild histopathological lesions comparedto placebo control. Therapeutic use of the monoclonal antibody cocktail 24 hours post infection, could not significantly reduce the viral RNA load in the lungs with a virus challenge dose of $10^{6.5}$ TCID50/ml whereas reduction could be observed with a lower challenge dose of $10^{4.5}$ TCID50/ml. Therapeutic use at 6 hours post virus infection also showed virus reduction although not statistically significant. The antibody cocktail appears to be a 28 promising candidate for the prophylactic use and for therapy in early COVID-19 cases which 29 have not progressed to severe disease.

Study on the effect of L452R mutation in the Omicron variant

Investigators: Shyam Sundar Nandi, Bishwajit Kundu, IIT-Delhi

Funding: ICMR intramural **Duration:** 1 year

Background: RBD being a major target of immune recognition for neutralizing antibodies and Omicron has accumulated 15 substitutions in the RBD region. The RBS-A, B, C, CR302 and S309 sites are multiple antigenic sites that have been categorized in RBD. The Omicron variant does not possess is the L452R. Global analysis has revealed that the L452R is nearly omnipresent in many independently emerged lineages and associated with immune escape. So in this study we have investigated the consequence of occurrence of L452R in the Omicron variant as an additional mutation.

Objective: To analyze the stability of RBDs of wild type virus and additional mutations in the Omicron variant in the RBD-ACE2 complex by using simulation modeling.

Findings: The stability of the RBD-ACE2 complex was analyzed using the RMSD profile, which indicated slight deviation in case of alpha, delta and wild type from the original structure. But in case of Omicron, the RBD structure deviates from an initial state and stabilizes with time

(Fig. 15). A similar trend was observed in the case of Rg as well as the SASA profile of Omicron as can be observed in Fig. 14 1B and 1C respectively. On the contrary, including a single mutation L452R in Omicron exhibited reversal of the RMSF profile with the rest of the variants. For a more thorough analysis, RBD-ACE2 complexes were analyzed by calculating the frequency of hydrogen bonds and points of contact. The Omicron exhibited the highest frequency of hydrogen bonds as compared to the other VOCs. Inclusion of additional mutation L452R in Omicron decreased the hydrogen bond profile and it exhibited the binding similar to the other variants. This confirmed that the L452R is a crucial mutation which can alter the properties of Omicron and bring it close to the Delta variant.

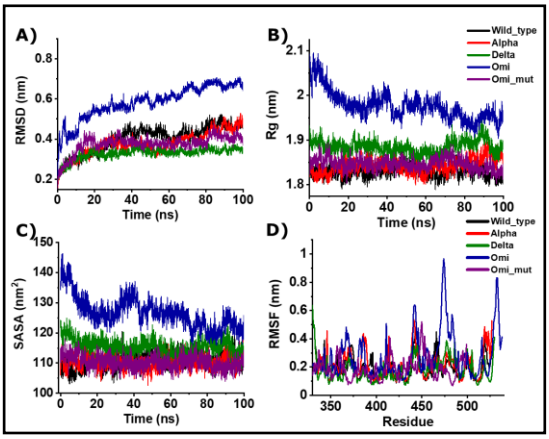


Fig. 15. The behavior of SARS CoV-2 Spike protein RBD region of different VOCs when bound to the ACE2 receptor during simulations. Black (wild type), Red (alpha), Green (Delta), Blue (Omicron), Purple (Omicron mutant L452R).

SARS-CoV-2 Immunological Research

Comparative Analysis of Host Immune Responses against Emerging SARS-CoV-2 Variants in COVID-19 Vaccinated Individuals

Investigators: Dr. H Kaushal, Dr. VA Potdar, Dr. ML Choudhary, Dr. Y Gurav
Funding: Intramural Duration: 2021-2022
Background: SARS-CoV-2 variants of concern, including delta, and omicron encompass mutations facilitating immune evasion. Neutralizing antibody recognition and function may be

variably impaired. Therefore, we investigated to understand comparative humoral responses in terms of IgG against wild/variants of SARS-CoV-2 in COVID-19 vaccinated individuals.

Objectives: To determine IgG against wild and variants of SARS-CoV-2 in COVISHIELD vaccinated individuals.

Findings: Serum samples from COVISHIELD vaccinees (n=30) collected at the pre-and post-booster dose and healthy controls, HC (n=20) groups were initially tested for IgG against B.1, delta and omicron variants of SARS-CoV-2. The results are represented as mean±SD OD_{492}. The anti-B.1 IgG level at post booster dose (0.096±0.101, $p<0.0001$) was found significantly high compared to pre-booster level (0.035±0.023). Additionally, there was a significant increase in anti-B.1 IgG at both pre-booster (0.035±0.023, $p<0.001$) and post-booster (0.096±0.101, $p<0.0001$) compared to the HC group (0.012±0.008) (Fig. 15). Similarly, anti-delta IgG level was significantly high at post-booster (0.094±0.083, $p<0.0001$) compared to pre-booster (0.029±0.018). Also, the level of anti-delta IgG at both pre-booster (0.029±0.018, $p<0.01$) and post-booster (0.094±0.083, $p<0.0001$) was significantly high compared to the HC group (0.015±0.011) (Fig. 16). Against omicron variant, the IgG level at post-booster (0.026±0.016, $p<0.0001$) was significantly high compared to pre-booster (0.014±0.010). In addition, the IgG level there was significantly raised at post-booster (0.025±0.015, $p<0.0001$) compared to the HC group (0.012±0.010). However, the anti-omicron IgG level at pre-booster (0.014±0.01) was found comparable to HC group (Fig. 15). Our study demonstrated a significant rise in the total IgG level against wild/variants of SARS-CoV-2 at post-booster dose in the vaccinees, indicating that the booster dose of COVISHIELD induced heightened humoral responses against all the investigated SARS-CoV-2 strains.

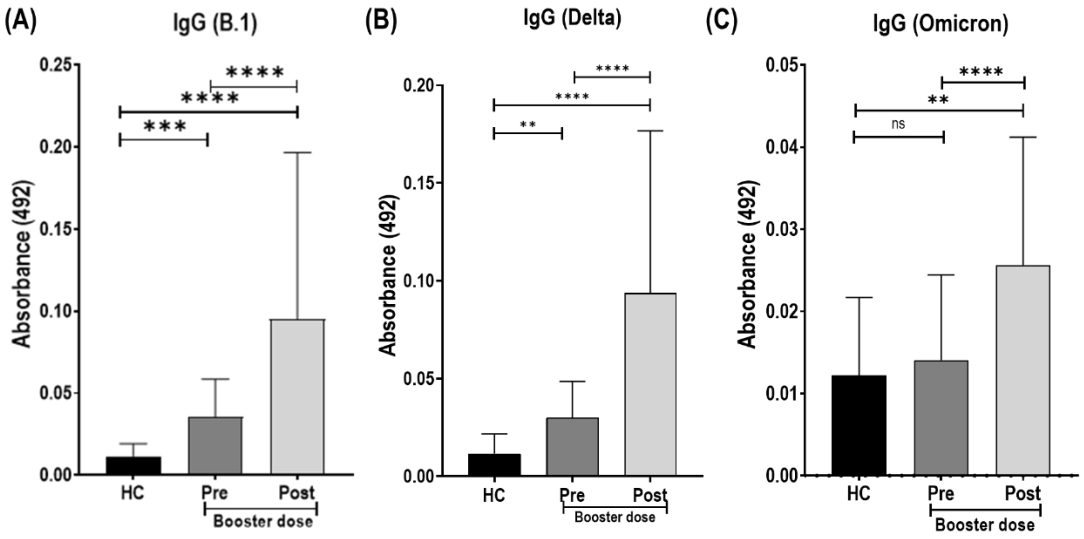


Fig. 16: Comparison of total IgG level at pre-and post-booster dose against B.1, delta and omicron variants of SARS-CoV-2 in COVISHIELD vaccinees (n=30) and healthy controls

(n=20) groups. The mean ± SD OD_{492} levels were determined by the ELISA method. The bars represent the means for the different study groups. Data were analysed by a nonparametric Wilcoxon matched-pairs signed-rank test between pre-and post-booster vaccinees groups and Kruskal–Wallis test, followed by the posthoc Dunn's multiple comparison test for more than two groups. The statistical tests were two-tailed and *p* values <0.05 were considered significant. ns, not significant, ***p*< 0.01, ****p*<0.001, **** *p*<0.0001.

In silico investigation of the T-cell based immune response against SARS-CoV-2 variant

Investigators: Dr. Sarah Cherian, Mrs. Shivangi Sharma, Ms. Rama Kulkarni, Ms. Pooja Wakchoure, Mr Amol Gayakwad)

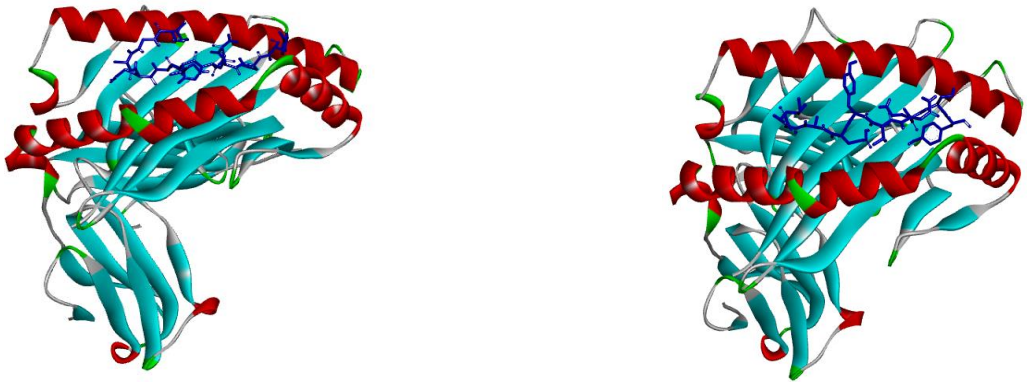
Funding: Intramural **Duration:** 2021-2022

Background: T lymphocyte responses typically regulate and frequently eliminate viral infections. As the antibody may play a potential role, it is likely to be of greater significance in the prophylaxis against repeated exposure to the same infection. Several studies have shown that viruses have developed means of evading the immune system. In November 2021, Omicron variant (BA.1) of SARS-CoV-2 was identified as a variant of concern (VOC). Omicron carries a number of changes in the spike protein not seen in earlier VOCs. According to preliminary research, Omicron significantly reduces the ability of antibodies produced by vaccination and prior infection to neutralise foreign substances. Its impact on T cell responses, however, is still unknown. In this study, we examined how the mutations in the Omicron variant sub-lineages affect experimentally reported T cell epitopes that can lead to significant changes in antigenicity and immune evasion.

Objective: (1) Prediction of T-cell specific epitopes for BA.1 and BA.2. (2) Conservancy analysis of SARS-CoV-2 epitopes in Omicron VOCs (BA.1 and BA.2). (3) Docking study of peptide-HLA complex.

Findings: Experimentally derived T cell epitopes of SARS-CoV-2) for the spike protein were extracted from the IEDB server for the analysis of Omicron-specific T cell response. Only dominant epitopes for MHC Class I that have been identified in three or more studies were taken into consideration. We found that majority of experimental CD8+ T cell epitopes of SARS-CoV-2 are unaffected and conserved in omicron variants, BA.1 and BA.2. However, 18% and 13% of CD8+ T cell epitopes comprise at least one site harbouring a mutation in BA.1 and BA.2 sequences, respectively. In order to understand the effect of these mutations on the binding affinity of these peptides to MHC Class I molecules, we undertook docking studies with respect to the wild type Wuhan strain and the BA.1 and BA.2 strains. At a global population level, HLA A*24:02, A*02:01, HLA B*35:01, and HLA A*01:01 are frequently detected and were taken

into consideration for the docking studies. Results showed that there was no significant difference in binding affinity suggesting lower possibility to T-cell escape (Fig. 17).



(A) (B)

Fig. 17. Interaction study of the dominant epitope (IGAEHVNNSY) of SARS-CoV-2 Wuhan strain (A) and BA.1 (epitope IGAEYVNNSY) (B) binding to HLA B*35:01 with affinities of -7.6 kcal/mol and -8.3 kcal/mol, respectively (PDB ID: 4LNR)

Assessment of immunological responses in breakthrough cases of SARS-CoV-2 in post COVID-19 vaccinated group

Investigators: Dr. P.D. Yadav, Dr. AM Shete, Dr. RR Sahay, Dr. A Tripathy, Dr. R Vishwanathan, Dr. H Kaushal; Collaborative Hospitals from Pune: AFMC, AICTS, Command Hospital, Star Hospital, Naidu Hospital, DY Patil, Jehangir; Coordinators: Dr. P Abraham, Dr P. Awate; Collaborative Hospitals from Mumbai: Nair Hospital, Kasturba Hospital and Seven Hills Hospital; Contributing staff: Mr. A. Suryawnashi, Dr. R Jain, Mr. V. Dhanure, Ms. P. Vedhpathak, Mrs. T Kore, Ms. P. Gawande, Mrs. K Kalele, Mrs. A Waghmare, Ms. J Yemul, Mrs. P. Bodke, Mrs. T Majumdar, Mrs. S Patil, Dr. A. Kumar

Funding: ICMR-NIV Pune COVID-19 Fund **Duration:** 2021-2022 (Ongoing)

***Background*:** Since identification of new variants of SARS-CoV-2 in India including variants of concern B.1.1.7, B.1.351 and Brazil P.1 (6,7,8), Delta, Kappa, Omicron there exist the concerns about the escape mutants that leads to moderate reduction on neutralization by convalescent and post-vaccination sera.

Objectives:

To understand the clinico-epidemiological profile of the SARS-CoV-2 infected cases post vaccination

To understand the humoral and neutralizing antibody responses to SARS-CoV-2 infection in these breakthrough cases.

To explore the cell mediated immune response to SARS-CoV-2 infection

To perform the WGS to understand the strain by which the infection had occurred.

To evaluate the effectiveness of vaccine in real world scenario outside clinical trials

Findings:

The oro/nasopharyngeal [OP/NP] samples, blood samples were collected from the COVAXIN or COVISHIELD vaccinated individuals at the time of SARS-CoV-2 infection (n=448) categorized into four groups: COVAXIN (single dose, n=9; CVSD), COVAXIN (double dose, n=40, CVDD), COVISHIELD (single dose, n=149, CSSD), and COVISHIELD (double dose, n=240, CSDD) and recovery (n=256) during April to August 2021. Complete SARS-CoV-2 genome (>98%) could be retrieved from 224 samples [CVDD (n=5), CVSD (n=22), and CSDD (n=74), CSSD (n=123)] using next generation sequencing. Majority of the cases characterized with variant Delta & Delta derivatives (AY) & all delta variants belongs to clade GK. Delta AY.50 (n=72) was the major SARS-CoV-2 lineage in infected cases followed by Delta B.1.617.2 (n=58) and other delta derivatives. Delta variant predominated in moderate and severe SARS-CoV-2 cases as compared to the mild cases which also had other variants including B.1, Kappa and Beta. In summary, we report SARS-CoV-2 breakthrough infections predominated by the Delta variant from the western part of India. Most of the cases being mild and all have recovered uneventfully with a very low fatality of 0.4%. The infections were associated with prolonged PCR positivity and low level of the IgG and neutralizing antibody titres at the time of the infection post vaccination, emphasizing the need for the booster dose.

Neutralization of different SARS-CoV-2 variants with sera of COVISHIELD vaccinated individuals**:**

Neutralization potential of COVISHIELD vaccinated individuals' sera against B. 1.617. 1:

We have evaluated neutralizing capability of the COVISHIELD vaccine recipient sera obtained 4 weeks after the second dose of COVID-19 naive subjects and COVID-19 positive recovered subjects. The results demonstrated that sera of COVID-19 positive recovered subjects who received 2 doses of COVISHIELD have higher antibody response compared to the COVID-19 naïve vaccinees with a significant difference ($P < .0001$) in NAb titer against B.1 and B.1.617.1 variants. An increase in the GMT of the sera of COVID-19 recovered cases with vaccination (29.5-fold) compared to COVID19 naive vaccinees (23.5-fold) was observed against B.1 and B.1.617.1, respectively. Although we observed a reduction in the neutralizing titer against B.1.617.1 variant, COVISHIELD vaccine-induced immunity may still limit the severity of disease and mortality in the vaccinated individuals.

Neutralization of Delta variant with sera of COVISHIELD vaccinees and COVID-19: recovered vaccinated individuals: A comparative assessment of COVISHIELD™ vaccinated individuals' (n = 116) sera in different categories were performed against prototype strain B.1 (D614G) and Delta variant. Sera under this study were grouped into five categories: (i) one dose (n = 31), (ii) two doses (n = 31), (iii) COVID-19-recovered plus one dose (n = 15), (iv) COVID-19-recovered plus two doses (n = 19) and (v) breakthrough COVID-19 cases (n = 20). NAb titers for Delta relative to B.1 were reduced in the sera of the participants belonging to Categories I (78%), II (69%), III (66%), IV (38%) and V (47%). We observed significantly lower NAb titers (3.2–4.5-fold) for the Delta relative to B.1 variant. However, NAbs in breakthrough participants and the COVID-19-recovered individuals with one or two-dose vaccination had relatively higher protection against Delta in comparison to the vaccinees with one or two-dose vaccination.

Substantial immune response in Omicron infected breakthrough and unvaccinated individuals against SARS-CoV-2 variants of concern: To evaluate the immune evasion potential of the Omicron in the individuals with natural infection and/or vaccination, we have analyzed the IgG and neutralizing antibodies (NAbs) against B.1, Alpha, Beta, Delta and Omicron variants with the sera of individuals (breakthrough infections after two dose of ChAdOx1 nCoV-19, BNT162b2 mRNA and unvaccinated individuals infected with the Omicron variant. The GMTs of neutralizing antibodies of ChAdOx1 nCoV-19 breakthrough individuals showed significant fold-reductions compared to B.1 against Alpha (3.23), Beta (2.38), Delta (3.23) and Omicron (4.31) variants respectively. Our study suggests a 3-fold reduction in the NAb titres in BNT162b2 mRNA breakthrough individuals as compared with ChAdOx1 nCoV-19. Our study demonstrated that the individuals infected with Omicron have significant immune response which could neutralize not only the Omicron but also the other VOCs including most prevalent Delta variant.

SARS-CoV-2 Delta and delta derivatives impact on neutralization of COVISHIELD recipient sera: We determined the NAb titre of sera obtained from COVID-19 naïve vaccinees (CNV) immunized with two doses of vaccine (8 weeks post vaccination), COVID- 19 recovered cases immunized with two doses of vaccine (CRV) (8 weeks post vaccination) and breakthrough infections (BTI) post immunization with two doses of vaccine against Delta, Delta AY.1 and B.1.617.3. The geometric mean titer (GMT) of NAb against B.1, Delta, Delta AY.1 and B.1.617.3 were determined with the sera of the subjects from CNV group (30.8, 1.1, 9.8, 1.6), CRV group (1248, 489.8, 403.8, 314) and BTI group (876.7, 499.8, 415.8, 235.8) respectively. This suggests the need for the booster vaccination to cope up with waning immune response among vaccinees. Delta variant has shown highest reduction of 27.3-fold in NAb titer among CNV group compared to other groups and variants. In conclusion, our findings suggest that COVISHIELD vaccine was able to neutralize Delta derivatives and prevent serious disease and fatality among breakthrough cases. A booster dose vaccination of COVID-19 naïve vaccinees would achieve protective immune response to fight against emerging SARS-CoV-2 variants.

Reduced neutralizing antibody response in naïve COVISHIELD vaccinees against Omicron emphasizes booster vaccination: Here, we report the IgG and neutralizing antibody response in individuals vaccinated with two doses of COVISHIELD vaccine against B.1, Delta, Beta and Omicron variant. Neutralization studies demonstrated reduction in the GMT titre of neutralizing antibodies (NAb) against Omicron with sera of naïve vaccinees (32.81-fold), recovered cases (28.13-fold) and breakthrough cases (46.86-fold) compared B.1 variant.

All the three groups effectively neutralized the B.1, Beta and Delta variants than Omicron. The GMT titre of NAb was lowest for Omicron with the sera of naïve vaccinees (0.11) than the recovered cases (11.28) and breakthrough cases (26.25). Even though highest fold- reduction amongst breakthrough cases was observed with Omicron variant, it also had highest NAb titre than the recovered cases and naïve vaccinees. In summary, our study demonstrated lowest IgG and NAb response in naïve vaccinees than other groups. This emphasizes the waning immune response in naïve vaccinees post second dose and warrants the administration of precautionary dose to boost the immunity.

Persistence of serum IgA response in SARS-CoV-2 naturally infected and vaccinated individuals

Investigators: Dr.GN Sapkal, Dr.Gururaj Rao Deshpande,
Contributing staff: Mrs.Rashi Srivatsav, Ms.Aparna Rakhe
Funding: Intramural **Duration**: 2021-2022

Background: The emergence of multiple variants of concerns of SARS-CoV-2 has contributed to the worldwide development of multiple waves of the formidable COVID-19 pandemic. SARS-CoV-2 evokes vigorous humoral immune responses, which includes production of virus-specific immunoglobulin mainly IgM, IgG, & IgA isotypes. IgA is the major antibody class in mucosal membranes. It's response in the early stage of the disease seems to be more pronounced than IgM. The role of IgA response in COVID-19 disease and vaccine is unclear.

Objective: 1. To detect the persistence of serum IgA antibody response against Spike Receptor Binding Domain and Nucleocapsid protein of SARS-CoV-2 in naturally infected patients. 2. To understand the antigen specific IgA antibody response in COVAXIN & COVISHIELD vaccinated individuals.

Findings: Our results suggested a linear trend in the level of IgA antibody response in natural infection. In vaccinated individuals COVAXIN groups exhibit a prominent increase in the IgA response in comparison to COVISHIELD.

Characterization and durability of COVID-19 vaccine induced antibody and cell mediated immune responses in volunteers

Investigators: Anuradha Tripathy, DS Singh, Sukeshani Salwe, Srikanth Tripathy. AL Kakrani, YK Gurav, GN Sapkal & Gururaj Despande.

Funding: Intramural Duration: 2021-2022

Background: Currently, the Indian population is being vaccinated with COVISHIELD and COVAXIN. However, the correlates of protection against COVID-19 infection, and the immunological parameters required for vaccine effectiveness are yet to be chucked out clearly. At the same time, it is not yet clear if vaccine-induced immunity B and T cell immunity will be short- or long-lived, nor how effective a vaccine will be in older people, whose immune systems often respond less. In the case of SARS-CoV-2, although the period of protective immunity is not yet clear, a recent study indicates that antibody to spike protein was relatively stable for at least 6 months, CD4+ and CD8+ T cell responses decreased with a half-life of 3–5 months. Our unpublished data in mild COVID-19 recovered patients from Pune show waning of antibodies and detectable cellular immune reactivity up to 8 months. Hence it was imperative to take a longitudinal study that follows immunity over an extended period of time, with consideration given to comorbidities that might further influence vaccine-induced immunity.

Objectives. To characterize the antibody, memory B and T cell mediated immune responses in the vaccinated (COVISHIELD/ COVAXIN vaccine) volunteers from Pune.

Findings: We have assessed the durability of antibody, SARS-CoV-2 specific immune cell frequencies, SARS-CoV-2 specific T and B and memory cell responses and their functionality in a set of 47 individuals prior to first dose of vaccination, 3 months post first dose of COVISHIELD (n=38) and at 6 months post second dose(n=31).

Assessment of the role of host immune response in Corona virus infection (COVID-19)

Investigators: Anuradha Tripathy, Priyanka Wagh, Yogesh Gurav and Varsha Potdar

Funding: Intramural Duration: 2020-2023

Background: Patients with SARS-CoV-2 infection show a complex profile with varied clinical presentations.. Based on the worldwide hospitalized patient data, approximately 80 per cent of infections were mild or asymptomatic, 15 per cent were severe and five per cent are critical infections requiring ventilation. An important public health concern is the extent to which immunity in asymptomatic patients may confer protection from re-infection and whether its breadth is lesser than/similar to that observed in symptomatic patients. In a proportion of patients, the infection results in moderate-to-severe acute respiratory distress syndrome (ARDS), requiring invasive mechanical ventilation resulting in damage of internal organs, multiple organ

failure and sometimes death. It is, therefore, important to identify the key differentiating host factors/immune molecules responsible for the differential clinical manifestation and outcome. We had studied the immune cell profiles, cytokine chemokines profiles, T cell response up to 6 month till last year in the asymptomatic, hospitalized mild symptomatic, moderate and severe cases of SARS-CoV-2 infected patients and in clinically recovered individuals. In the current year, we have followed up them up to one year. Have also assessed the frequencies of human leukocyte antigen class I and class II alleles in SARS-CoV-2 infection.

Objectives: 1**.** Assessment of durability of immune response post COVID-19 infection 2. Assessment of the frequencies of human leukocyte antigen class I and class II alleles in SARS-CoV-2 infection.

Findings: Multiple parameters of immune response in recovered individuals at different time points post recovery up to one year were analyzed. We observed that each component of SARS-CoV-2 immune response exhibited distinct kinetics. Overall compiled IgG antibody, neutralization antibody and T cell data indicated persistence of protective immune response up to 9 months post recovery from COVID-19.

HLA class I and class II genotyping was carried out in a total 216 COVID-19 patients and 228 COVID-19 negative healthy controls by PCR-SSP method. HLA class I genotyping was carried out in 101 severe, 113 mild COVID-19 patients and 225 healthy controls, whereas HLA class II, HLA-DRB1 and DQB1 were carried out in 82 severe, 134 mild COVID-19 patients and 228 and 220 healthy controls respectively. Among the MHC class I A alleles, HLA-A*01 allele was significantly high in healthy controls as compared to the total COVID-19 cases [p=0.03 with 95%CI and OR=0.59(0.4-0.88)] contrarily, HLA-A*02 was significantly high in all COVID-19 groups (total, [p=<0.001 with 95% CI and OR=2.56 (1.79-3.67); severe, p=<0.001 with 95% CI and OR=2.27 (1.47-3.50); and mild, p=<0.001 with 95% CI and OR=2.83 (1.88-4.26)]) compared to healthy controls post Bonferroni correction. Among the HLA-B, B*15 allele frequency was significantly high in control group compared to total COVID-19 patients [p=0.03 with 95% CI and OR= 0.47 (0.27-0.81)], whereas HLA-B*40 frequency was high in mild COVID-19 patients [p= 0.03 with 95% CI and OR=1.88 (1.15-3.107)] compared to healthy controls. At the Clocus, HLA-C*01 was significantly low in severe COVID-19 group compared to mild COVID-19 group [p=0.024 with 95% CI and OR=0.09(0.011-0.68)]. Among the MHC class II alleles, frequency of HLA DQB1*02 [p=0.05 with 95% CI and OR=1.57(1.06-2.29); p=0.04 with 95% CI and 1.52(1.08-2.13), respectively] and HLA DQB1*06 were significantly high in mild and total COVID-19[mild: p=0.028 with 95% CI and OR=1.51 (1.09-2.08); total: p=0.04 with 95% CI and OR=1.43(1.08-1.89)] groups compared to healthy controls, whereas HLA DQB1*03 allele frequency was significantly high in healthy controls compared to severe [p=0.02 with 95% CI and OR=0.51(0.31-0.82)], mild [p=0.002 with 95% CI and OR=0.49 (0.33-0.73)]and total [p=0.002 with 95% CI and OR=0.49 (0.35-0.70)] COVID-19 patients (Table 1).

Our finding of HLA-A*02 as a susceptible allele goes hand in hand with the global computational in-silico data. The current data suggest that HLA-A*01 and HLA-B*15 may act

as protective alleles. Further, our data of HLA-B*15 as a protective allele is in line with the global *in-silico* data.

Table 1: Association of HLA class I and II alleles in COVID-19 groups and healthy controls.

Subjects	Alleles	SARS-CoV-2 patients 2n (%)	Healthy Controls 2n (%)	*p*-value	OR (95% CI)
Total COVID-19 patients Vs Control	HLA-A*01	46 (10.75)	76 (16.9)	0.03	0.601 (0.36-0.99)
	HLA-A*02	109(25.5)	53 (11.8)	<0.001	2.56 (1.79-3.67)
	HLA-B*15	27(6.37)	29 (12.7)	0.03	0.47 0.27-0.81
	DQB1*2	99(22.92)	72(16.36)	0.04	1.52 (1.08-2.13)
	DQB1*3	62(14.35)	111(25.23)	0.002	0.49 (0.35-0.70)
	DRB1*09	1 (0)	10 (2.19)	0.038	0.10 (0.01-0.81)
	DRB1*11	20 (5)	44 (9.65)	0.012	0.46 (0.26-0.78)
Mild COVID-19 Vs Control	HLA-A*02	61(27.73)	53(11.78)	<0.001	2.83 (1.88-4.26)
	HLA-B*40	57(25.45)	35(15.35)	0.03	1.88 1.15-3.107
	DQB1*2	63(23.51)	72(16.36)	0.05	1.57 (1.06-2.29)
	DQB1*3	38(14.18)	111(25.23)	0.002	0.49 (0.33-0.73)
	DRB1*11	11 (4.1)	44 (9.65)	0.02	0.38 (0.19-0.76)
	DRB1*15	99 (36.94)	118 (25.8)	<0.01	1.67 (1.19-2.35)
Subjects	Alleles	S. COVID-19 patients 2n (%)	Mild COVID-19 2n (%)	*p*-value	OR (95% CI)
Severe COVID-19 Vs Mild COVID-19	HLA-C*01	1 (0.5)	12 (5.31)	0.024	0.09 (0.011-0.68)

Innate immune evasion by COVID-19: Evaluation of role of immune sensors in disease progression in children and adults

Investigators: Madhu Mohanty, Swapnil Varose, Unnati Sawant, Mevis Fenandes
Funding: Intramural **Duration**: 2 years (2020-2022)

Background: In our earlier study asymptomatic SARS-CoV-2 positive patients showed increased MDA5 in Nasopharyngeal cells and increased induction of type 1 IFN subsequently protecting them from further clinical severity (Mohanty et al., IJMR, 2021). In order to investigate the innate immune response in the buccal mucosa, retrospective throat swab (TS) samples of COVID-19 patients (April-September 2020) were utilized to separate throat swab cells at ICMR-NIV, Mumbai Unit and detect presence of innate immune markers.

Objectives: To delineate the role of buccal mucosal cells in innate immune responses in asymptomatic and symptomatic COVID-19 infected individuals

Findings: TLR7 plays a major role in the entry of SARS-CoV2 virus through oral cavity. MDA5 plays a role in preventing severity of the disease by triggering interferon type-I response to SARS-CoV-2 infection. Thus MDA-5 could be considered as a biomarker for prognosis of the disease at the entry points of SARS-CoV-2. The Pre-activated TLR7 in the SARS-CoV2 negative groups showed that the SARS-CoV2 virus gets detected by this PRR leading to downstream protective effects against COVID-19 via type-I/II interferon responses. Comparison of expression of MDA5 throat swab vs. nasal swab confirms that presence of MDA5 is responsible for containment of severity of the disease. The higher induction of IFNα/β in SARS-CoV2 negative as well as the SARS-CoV2 asymptomatic positive groups serves as a proof of concept confirming the roles of TLR7 as well as MDA5 seen previously (Fig. 18).

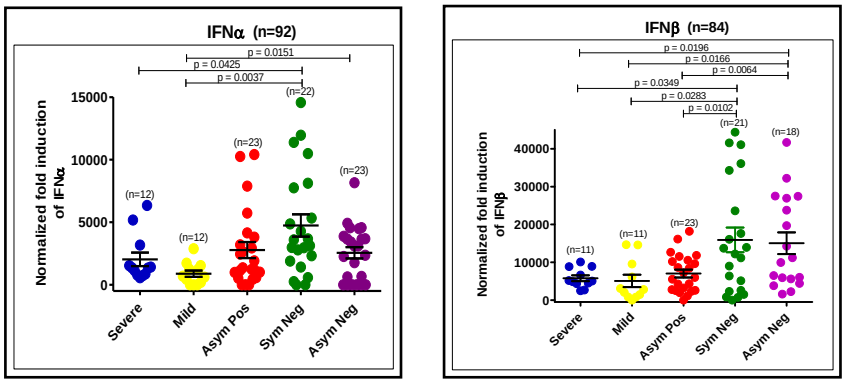


Fig. 18. Differential Expression of IFNα/β in Throat Swab cells Symptomatic and Asymptomatic SARS-CoV-2 Positive Patients.

SARS-CoV-2 Pathology Research

Propagation of new SARS CoV-2 variant isolate and characterization in cell culture and animal model

Investigators: Dr. P.D. Yadav, Dr. AM Shete, Dr. RR Sahay, Dr. GN Sapkal, Dr. S Mohandas, Dr. VA Potdar; Contributing staff: Mr. M Kadam, Mr. A. Suryawnashi, Mr. A. Kumar, Dr. R Jain

Funding: ICMR NIV Pune COVID-19 Fund **Duration:** 2021-2023 (ongoing)

Background: Considering the potential threat from emerging SARS-CoV-2 variants and the rising COVID-19 cases, SARS-CoV-2 genomic surveillance is ongoing in India. We propagated and characterized SARS-CoV-2 variants using cell culture and animal model.

Objectives: To propagate and characterize SARS-CoV-2 variants in cell culture and animal model.

Findings:

Isolation, characterization and pathogenicity of Brazil variant P2 lineage (B.1.1.28.2): The Brazil variant P2 lineage (B.1.1.28.2) was isolated from clinical specimens of travellers returned to India from the United Kingdom and Brazil using in vitro method. The pathogenesis study in hamsters demonstrated increased disease severity and neutralization reduction compared to B.1.

SARS CoV-2 variant B.1.617.1 is highly pathogenic in hamsters than B.1 variant: A study on B.1.617.1 variant demonstrates higher pathogenicity in hamsters evident with reduced body weight, higher viral load in lungs and pronounced lung lesions as compared to B.1.

SARS-CoV-2 Delta Variant pathogenesis and host response in Syrian Hamsters:

SARS-CoV-2 Delta Variant pathogenesis and host response in Syrian Hamsters demonstrated high levels of SARS-CoV-2 sub genomic RNA in the respiratory tract for 14 days with lung disease of moderate severity.

Isolation and characterization of Beta and Eta variant: Isolation and characterization of Beta and Eta variant from clinical specimens of international travellers using in vitro culture.

Isolation and characterization of Kappa (B.1.617.1) variant: The pathogenicity of the Kappa variant in hamsters is evident with reduced body weight, high viral load in lungs and pronounced lung lesions.

Isolation and characterization of Omicron variant: An initial in vivo in hamsters and subsequent in vitro approach was utilized for the isolation of the virus. The pathogenesis study of the Omicron variant in hamsters demonstrated moderate to severe lung disease similar to Delta. However, the sera showed poor neutralizing ability against like Delta and earlier VOCs which suggest the possibilities of increased reinfection cases.

Study of the potential of re-infection by Delta variant in Syrian hamster model: The study on re-infection of hamsters with Delta variant shows that prior infection with B.1 could not produce sterilizing immunity but it can reduce disease severity in case of reinfection.

Characterization of the endothelial lipid metabolism and bioenergetic stress to SARS-CoV2 spike glycoprotein and virus infection in-vitro

Investigator: Atanu Basu, Co-PI: Virendra K Meena
Funding: Intramural **Duration**: 2021-2023

Background: Lipid metabolism and cellular signaling pathways are intrinsic and fundamental in regulating vascular endothelial cell function in normal and disease states. Endothelial dysnfunction has been shown to contribute in pathogenesis of infectious diseases and its role in viral diseases are rapidly emerging. In COVID, vascular endothelial dysfunction has been well documented to be a high risk factor for severe and often fatal disease outcome. The main Objective of the present study was to develop in 3D cultures, vascular endothelial cells mimicking organoid physiology and study the effect of in-vitro exposure of the full length SARS-CoV-2 spike glycoproteins on key endothelial lipid metabolic events that are important in normal/abnormal hemostatic and signaling pathways.

Objectives: In phase 1 of the study (2021-22) two Objectives were attempted (a) develop in-vitro cultures of primary vascular endothelial cells in conventional 2D and in 3D scaffold cultures(b) establish baseline data on endothelial cell lipid composition under different growth conditions using published protocols.

Findings:

***Growth of primary endothelial cells in 3D scaffolds*:** Preliminary optimization of primary endothelial cells in organoid forms within a hydrogel based scaffold matrix was successful (Fig. 19). The growth factor matrix for differentiation was worked out and current studies are ongoing towards achieving capillary differentiation.

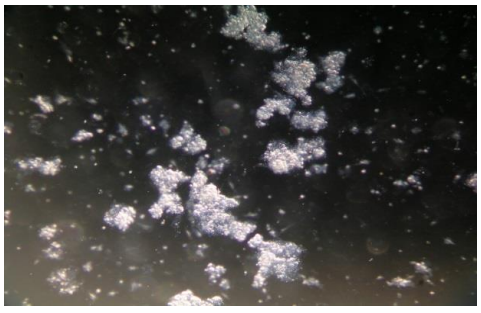


Fig. 19. Organoid differentiation morphology of endothelial cells in 3D scaffold cultures

***Lipid composition of primary endothelial cells under different growth conditions*:** Experiments have been initiated to study the whole cell lipid composition of endothelial cells under different growth conditions and correlative mitochondrial bioenergetics to generate baseline data. In the reporting year 2021-22 we have started optimization of chromatography techniques for lipid quantitation. For mitochondrial studies, a Mitosox ^{TM} based *in-vivo* staining and calculation of mitochondrial trans-membrane potential changes by JC1 dye are ongoing.

Complete transcriptome analysis of cells in throat/nasal swabs of COVID-19 patients in India

Investigators: Kavita Lole, Varsha Potdar, Yogesh Gurav, Shilpa Tomar, Sarah Cherian, Prachi Gajarmal

Funding: Intramural Duration: 2020-2022

Background: We performed whole transcriptome sequencing of human RNA obtained from nasopharyngeal swabs of patients with mild, moderate, and severe conditions with COVID-19, and identified molecular signatures associated with the disease severity. The data was generated by using the paired end approach of Illumina technique on a NOVASeq-6000.

***Objectives*: 1.** To undertake profiling of complete transcriptome of cells in oropharyngeal/ nasopharyngeal swabs of COVID-19 patients manifesting mild, moderate and severe symptoms. 2. To use the transcriptome data for computational drug repurposing.

Findings: Of the 96 processed samples 32 samples passed the QC filters. Overall, we detected 3958 upregulated and 216 downregulated genes in mild, 3884 upregulated and 189 downregulated genes in moderate, and 3875 upregulated and 318 downregulated genes in severe cases. The GO terms and Pathway enrichment analysis was performed using the iDEP tool to identify shared pathways among DEGs. We specifically identify immune-related pathways that are dysregulated, such as MAPK signalling, TNF signalling, chemokine signalling, cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor pathways that are common in mild and severe conditions. In all three conditions, pathways involved in osteoclast differentiation, neurodegeneration, and chemical carcinogenesis were dysregulated. Only patients with mild conditions had significantly altered olfactory transduction pathways. With severe conditions, NF-κB, IL-17 signalling, and rheumatoid arthritis pathways were significantly altered. The cytokines and chemokine genes, CCL2, CCL3, CCL20, CXCL1, IL1B, and CXCL12 and IL10 were significantly higher in severe cases. The findings suggest that rapid transcriptome analysis of nasopharyngeal swabs can be an effective method for quantifying host molecular response and may provide important insights into COVID-19 pathophysiology.

SARS-CoV-2 Product Development Research

Preparation, characterization and evaluation of SARS-CoV2 spike glycoprotein conjugated metal nanoparticles as SARS CoV2 mimetic

Investigators: Dr Viren Meena, **Co-PI:** Dr Atanu Basu
Funding: Intramural **Duration**: 2021-23
***Background*:** Nanoparticles are unique in the physical characteristics and have revolutionized applications in biology-specially drug delivery. In the present proposal we have projected to explore the idea of differential phase-controlled assembly of the SARS-CoV2 full length spike glycoprotein can be achieved on surface of a metal nanoparticle. As protein folding is largely driven by multiple coulombic and thermodynamic interactions between molecular side chains, an optimized situation would thus mimic a viral glycoprotein assembly on the nanoparticle surface. Such a particle would be ideal for understanding the biophysical determinants of cell surface interactions, endocytosis and further movement of the biological cargo within intracellular compartments.
***Objectives*.** Synrhesis of nanoparticles to deliver viral glycoprotein.
Findings. In the period 2021-22, we could optimize the synthesis of several nanoparticle species using gold, nickel and titanium. The particles are currently in different stages of characterization using TEM, AFM and spectral properties.

Development of recombinant adenovirus vector platform against COVID-19

Investigators: Pradeep Sawant and Mallika Lavania
Funding: Intramural **Duration:** 2020-2021
Background: The replication-defective adenovirus (AdV) vectors as a vaccine platform outperform all other approaches because of their properties to stimulate robust transgene-specific T cell and antibody responses, ability to stimulate the immune reaction, the genome can be easily modified by inserting exogenous transgenes of interest, and relatively stable viral capsid. Although many adenoviruses with very low seroprevalence: AdV2, AdV26 and AdV35 are considered promising candidates for vaccine development. However, their efficacy and immunogenic potency are lower in comparison to the most well studied and used, AdV5. The AdV carrying transgene can be rapidly developed as a vaccine candidate against the present severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic and to contain

emerging pathogens. Therefore, the present study is designed to construct a adenovirus vector containing the SARS-CoV2 gene.

Objectives: 1. To construct Ad-SARS-CoV2-S plasmid. 2.To evaluate in vitro expression of S gene encoded by recombinant Ad-SARS- CoV2-S

Findings: The 'S gene' of SARS-CoV-2 variants of concern (alpha, beta, gamma, delta, omicron) were ligated with pAdenoX-CMV vector individually. The, transformants were confirmed by colony PCR, plasmid was extracted, and sequencing was performed. After sequencing the S gene of all the variants was found in the direction of CMV promoter inside cloning site. After this the recombinant adenovirus will be produced and confirmed. Then the immunogenicity potential of virus will be evaluated in mice.

Assay for detection of epidemiologically important SARS-CoV-2 genetic variants

Investigator: Shyam Sundar Nandi

Funding: Intramural ***Duration****:* 1 year (2020)

Background: Genomic surveillance of SARS-CoV-2 variants has largely focused on mutations in the spike glycoprotein, which mediates attachment to cells and is a major target of neutralizing antibodies. There is intense interest in whether mutations in the spike glycoprotein mediate escape from host antibodies and could potentially compromise vaccine effectiveness, since spike is the major viral antigen in the current vaccines. This project demonstrates an indigenous multiplex SNP assay for the screening of epidemiologically important variants of SARS-CoV-2.

Objective: To design and develop assay(s) for screening of clinical samples for identification of epidemiologically important genetic variants of SARS-CoV-2.

Findings: For this project novel RT-PCR primers which were designed to specifically amplify the RBD of the Spike protein gene of SARS-CoV-2. The number of important mutations in the SARS-CoV-2 genome are located in this region. Along with these, the site-specific nucleotide detection primers were designed specific to several locations of mutation in the previously selected region. These primers were added with a non-specific tail of nucleotides in an ascending concerted manner. The amplified DNA was subjected to multiplex reaction to identify mutations using SNaPshot reagent in Genetic analyzer (ABI Prism 3130xl). This was followed by analysis of results in GeneMapper software (V4). The assay is modifiable and updatable in case occurrence of new variants (VOC/VOI). By using the combination of various SNP primers we designed the assay for the detection of Alpha, Delta, Delta plus and Omicron variants. A total of 50 samples representing the above-mentioned variants were tested using this assay. The assay detected all tested variants (Fig. 20). The assay can be used in the laboratories having regular capillary sequencing machine without the need of NGS, within turn-out time of four hours.

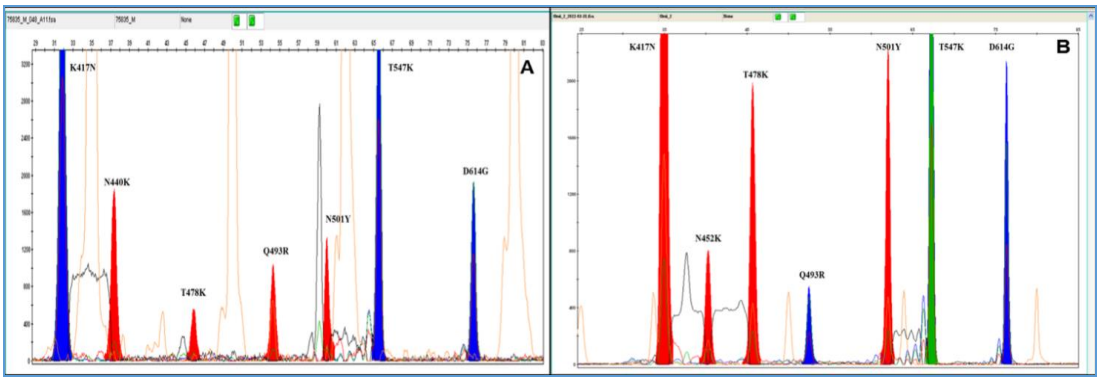


Fig. 20. : Significant sites of mutations in the Spike protein gene to identify the A: delta and B: omicron variant of SARS-CoV-2.

Section 2: Scientific work reports (NON- COVID)

ANIMAL HOUSE GROUP

Scientific Staff

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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.D. Dalvi	Lab Assistant
Mr. A. Thimanapalli	Lab Assistant
Mr. V.P. Sasane	Lab Assistant
Mr. S.B. More	Lab Assistant

Contract Staff

Mr.A.S.Shinde
Mr.M.S.Parsuram
Mr.S.Poharkar
Mr.N.M.Helonde
Mr.R.D.Tayade
Miss.Vedika G.Joshi

Breeding, Supply and Maintenance of Laboratory Animals:

Investigators: Dilip R. Patil
Contributing staff: Sidharam Fulari
Funding: Intramural **Duration:** Ongoing Institutional service project
Work done:

Animal house at the Institute comprises of small animal breeding and experimentation, primate experimentation facilities distributed over three floors. Besides that, Institute has ecofriendly monkey enclosures for rehabilitation of macaques post experimentation. The facilities and animals are maintained and taken care of by experienced and trained staff everyday including weekends and holidays. Animal house at the Institute is 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 July 2027. Ten strains of mice (Inbred: BALB/c (Jax and CRL), C_{57}BL/6, DBA/2, C3H, Outbred: CD-1, Immuno-deficient: CD-1 *nu/nu*, BALB/c *nu/nu*, RAG-1 KO and AG129) and Golden Syrian hamsters were maintained and bred. The animals were supplied under Institutional Animal Ethics Committee (IAEC) approved research projects to the scientists for in house research. Rodents for breeding as well as experimentation were housed in individually ventilated caging (IVC) system and supplied in filter top cages to prevent microbial contamination (Fig. 1). During the report period, a total of 1030 mice and Golden Syrian Hamsters were supplied to institutional scientists against 12 IAEC approved ongoing research projects. Other species of laboratory animals *viz:* guinea pig, rabbit, fowl, turkey, goose were procured from CPCSEA authorized sources as per requirement. A total of 503 ml blood from different species of laboratory animals, as diagnostic reagent in various assays was supplied to institutional scientists. Presently, 27 rhesus macaques are under post experimentation rehabilitation at the ecofriendly group housing enclosures. Health monitoring programme as per the guidelines of CPCSEA was conducted. Physical examination, tuberculin testing was done and rectal temperature, body weights were recorded. Hemogram, serum biochemistry was done through NABL accredited laboratory. Chest X ray examination was carried out onsite using portable X ray machine. Health records of the animals were maintained individually. Services of consultant veterinarian were sought for the treatment of sick animals.

Institutional animal ethics committee reviews the research protocols and also ensures compliance with the CPCSEA norms. Accordingly two meetings of IAEC were conducted (July and August 2021) for evaluation of research protocols and inspection of the animal facilities respectively.

Fig. 1: Individual Ventilated caging (IVC)

BACTERIOLOGY

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Dr Rajlakshmi Viswanathan	Scientist E (vishwanathan@gov.in)
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Dr Arun TR	Scientist B (joined 28th March 2022) (aruntr04@gmail.com)

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Mrs Savita Katendra	Senior Technician 1

Support Staff

Mr Rajendra Khedkar	Multi Tasking Staff

Project Staff

Ms Meera Prabhakar	Project Tech Support-2 (December 2021onwards)
Ms Shruti Borawake	Project Tech Support-1 (December 2021onwards
Mr Nikhil Bhongale	Project Tech Support-1 (February 2022 onwards)

Contract Staff

Mrs Shailaja Bhosale- Data Entry Operator

Bacteriological Diagnostic Services

Funding: Intramural

Duration: Ongoing (2017 till date)

Diagnostic Support for outbreak of acute watery diarrhea in village Top, Kolhapur district of Maharashtra

Investigators: Rajlakshmi Viswanathan, Mallika Lavania, Kunal Pise, Sorna Narayanan, Pradeep Sawant

Contributing staff: Savita Katendra, Prabhakar Jadhav, Manohar Shinde

***Background*:** An outbreak of acute watery diarrhea was reported in Top, a village located in Hatkanagale Taluka of Kolhapur district, Maharashtra in October 2021.

***Objective*:** To investigate the cause of acute watery diarrhea among residents of the affected village.

Findings: The index case presented with watery diarrhea, vomiting and pain abdomen to the local sub center on 25th October 2021. A total of 108 cases occurred in a population of 2299, till 1st November 2021; with a peak of 43 cases on the 28th of October (attack rate 4.69%).

An investigation was performed by ICMR-NIV, Pune with the help of the local rapid response team. The cases were clustered in an area where water was supplied directly from a public well, through pipelines. Leakages were noted in the pipelines which were in close proximity to shallow drainage areas.

Vibrio cholerae O1 Ogawa biotype El Tor was confirmed from 8 of 20 stool samples. Additionally, norovirus was detected in two and sapovirus in one case each. The patients were managed symptomatically and there was no mortality.

Field control measures included stopping the supply from the identified well and its chlorination. The damaged water supply pipelines were repaired. Alternate arrangements for drinking water were made by the Gram Panchayat. Households were provided with chlorine solution for purifying drinking water. The local Gram Panchayat Sarpanch, Gram Sevak of the village were suggested long term public health measures to prevent recurrence of such outbreaks. The outbreak was declared as over on 11th November 2021.

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

Rajlakshmi Viswanathan in collaboration with clinical partners (AIIMS Jodhpur, JIPMER, Puducherry, AIIMS, Bhubaneshwar)

Funding: The DBT Wellcome India Alliance Intermediate Career Fellowship in Clinical and Public Health

Duration: 2019-2024

***Background*:** Pertussis, is one of the most poorly controlled vaccine-preventable diseases in the world. Clinical diagnosis of pertussis in infants is challenging due to non specific presentation and absence of classical symptoms. No systematic information on pertussis is available from India.

***Objectives*:**

To generate information on pertussis in India

To estimate seroprevalence of pertussis antibodies in infant – mother and susceptibility to pertussis

To determine the role of pertussis in severe respiratory infection among young infants: strengthening laboratory capacity

Findings:

Capacity Building: Three collaborative sites in the study - AIIMS, Jodhpur, AIIMS, Bhubaneshwar and JIPMER, Puducherry are functionalized. Quality Control Program has been successfully initiated for serology with good concordance (98%)

Laboratory assays: Very few laboratories in India possess the capability to isolate these organisms Culture methods for isolation of *Bordetella pertussis, B.parapertussis* and *B.holmesii* were established using simplified techniques, standardized using ATCC strains of the three organisms and validated using patient samples. *B. pertussis* was successfully isolated in a case of neonatal pertussis. *B. parapertussis* was also successfully isolated from a four month old child.

Seroprevalence of pertussis antibodies in infant – mother dyads The second Objective was completed and reported last year from Pune site. Across the other three study sites a total of 393 participants were recruited. Of the 340 samples tested by ELISA for anti-pertussis toxin IgG, seropositivity was 7.05% (95% CL 4.79 -10.29). The women who participated in the study did not report any pertussis immunization after childhood vaccination.

Table1: Maternal seropositivity for pertussis in study sites across India.

Study Site	**Participants Recruited(n)**	**Maternal Serum Samples Tested(n)**	**Positive (n)**
AIIMS Jodhpur	220	211	20
AIIMS Bhubaneshwar	117	90	3
JIPMER, Puducherry	56	39	1

Understanding the role of pertussis in severe respiratory infection among infants

A total of 45 participants were recruited across three sites (Pune, Jodhpur and Puducherry) during the reporting period. *B. pertussis, B holmesii* and *B. parapertussis* were confirmed in one case each.

Table 2: *Bordetella* species in respiratory infection among young infants (2021-22).

Study Site	Participants Recruited (n)	Positive N (%)
ICMR-NIV, Pune AIIMS Jodhpur	10 34	3* 0 (0)
AIIMS Bhubaneshwar	Not initiated	-
JIPMER, Puducherry	1	0 (0)

***** One each for *B.pertussis*, *B. parapertussis* and *B. holmesii*

Number of samples tested during the year

Type of specimen	Number	Tested for
Stool	91	Diarrheagenic *E.coli*, *Salmonella* spp, *Shigella* spp and *Vibrio cholerae* by culture and PCR
	30	*Campylobacter jejuni*
Serum (mother/infant)	68	*Bordetella pertussis* IgG Quality Control
Nasopharyngeal swab	10	*Bordetella pertussis, B parapertussis,B.holmesii*
Water (from outbreak affected area)	4	Microbial contamination
Bacterial isolates	16	For identification

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Core facility services

Investigators: Sarah Cherian, Pratip Shil, Diya Roy, 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 modelling etc. are provided. Management and maintenance of computers, servers, laptops, printers, computer peripherals, and network and internet services are 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. A new NIV website has been designed and launched (https://niv.icmr.org.in) and is being maintained on a regular basis. Audio and video conferencing units are maintained on a regular basis. Technical support has been provided for conferences and workshops held by NIV.

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

Investigators: Dr. Sarah Cherian, Dr. Deepti Parashar, Dr. K. Alagarasu, Ms. Bhagyashri Kasabe, Ms. Diya Roy

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

Background**:** 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, multi-target 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 protein 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.

Findings:

The transcriptomic analysis of chikungunya virus using GSE49985 microarray GEO dataset showed 379 differentially expressed genes (DEGs). Further, the online database, Connectivity Map (CMap) was accessed that determined 75 drugs candidates for the DEGs. Several studies related to proteomic analyses of cells affected by CHIKV revealed 233 proteins showing >1.5-

fold change in up/down regulation expression in CHIKV infection. Additionally, literature review has shown the experimental evidence of the interactions (PPIs) present between human and CHIKV. In this study, the interactomic reports highlighted four proteins (nsP2, nsP3, nsP4 and E1) that interacts with around 233 different human proteins. Further, STITCH was used as a target-based method to identify drugs, which can interact with the signature viral proteins /human proteins identified. A representative interaction network of the protein ANXA5 that shows interaction with several compounds including Cisplatin and its derivatives containing tilorone and a tilorone derivative, which are reported to be effective in inhibiting DNA and RNA viruses (**Fig. 1a**). The top CHIKV proteins involved in the interactions with human proteins were nsP2, nsP3, nsP4, E1, E2, E3 and capsid that displayed 64, 56, 37, 13, 16, 6 and 4 interactions, respectively, as shown in **Fig. 1b**. The comparison with the PPIs in DENV is in progress.

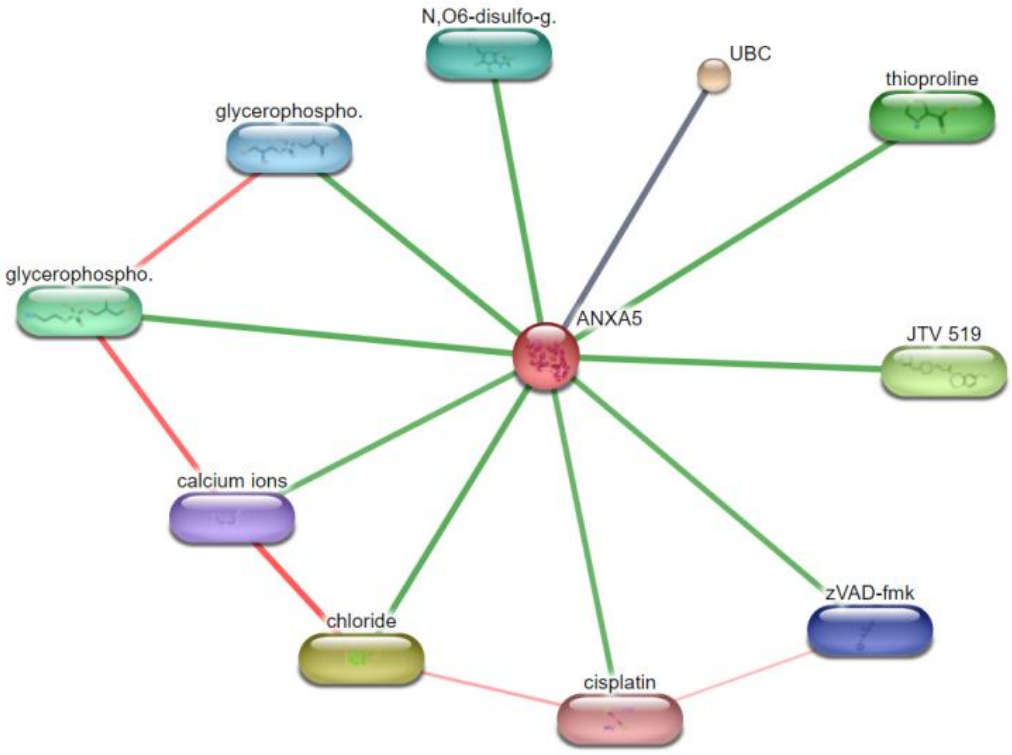

Fig. 1a: STITCH network generated for ANXA5, which shows interactions with several compounds including Cisplatin, its derivatives containing tilorone and a tilorone derivative.

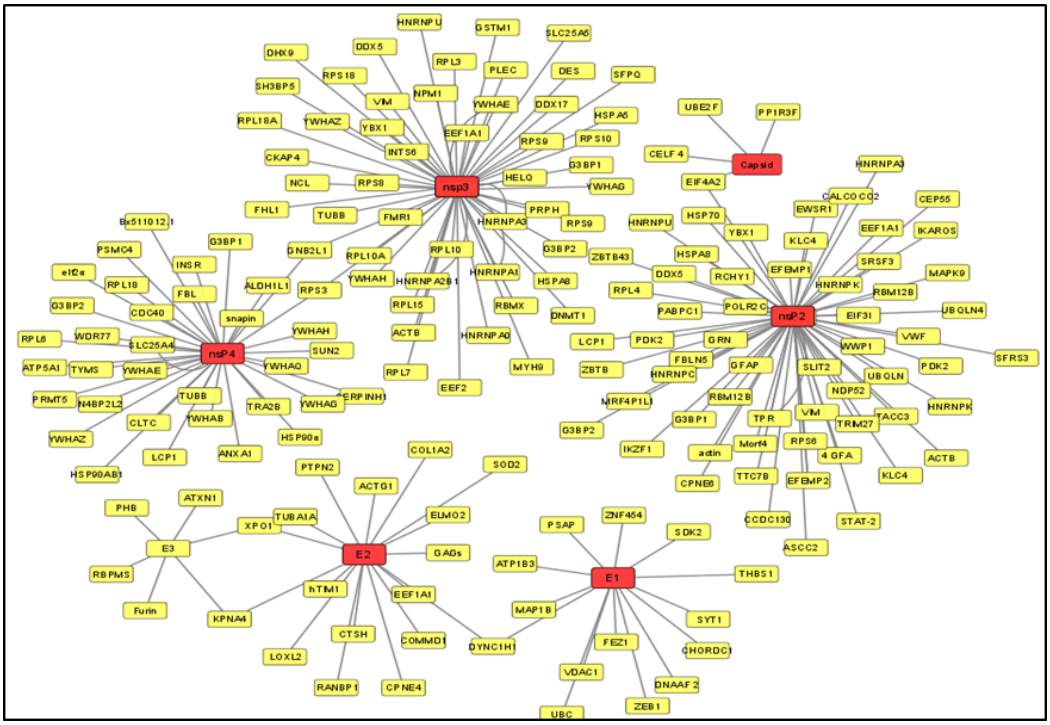

Fig. 1b: Yellow nodes represent the human proteins and red nodes represent Chikungunya viral proteins. Human proteins are labelled with the corresponding UniProt ID. The black edges show the interactions between human proteins and CHIKV proteins as determined by our interactomics analysis.

ArVirInd: A database of Arboviral proteins from the Indian subcontinent

Investigators: Dr. Pratip Shil, Dr. K Alagarasu

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

***Background*:** The recent increase in the burden of vector-borne diseases in India has brought about challenges including diagnostics, mitigation and management of outbreaks. The immunoinformatics studies on these viruses possess limitations towards development of diagnostic tools, vaccines and antivirals as searching for location specific amino acid sequences in the databases is cumbersome due to the non-existence of country wise enlisting of viral proteins. Thus, it has become necessary to have a single platform consisting of the information of arboviral proteins particularly of India and bordering countries.

***Objectives*:** To develop a database of antigenic proteins from arboviral strains isolated from India and neighbouring countries.

Findings: We have compiled a knowledge-based database called ArVirInd - of antigenic arboviral proteins from the Indian subcontinent. The database has curated amino acid sequences covering Dengue (all serotypes), Japanese Encephalitis, West Nile, Chandipura and

Chikungunya viruses. The database has been launched in 2020 and is available online at http://arvirind.co.in/. The countries included in the database are India, Bangladesh, Bhutan Maldives, Nepal, Pakistan and Sri Lanka. The ArVirInd homepage enables the user to search by "Virus Name", "Country of Origin", "Year of Origin" or a combination of these options. This search option is facilitated with AJAX based auto-search and auto-suggest query for user. The ArVirInd database has an epitope-mapping tool called "EpiMapAr", which helps to display the predicted and known B-cell epitopes on the database sequences. As an output, it presents all the available sequences in the database for the predicted B-cell epitope (Kolaskar method), Confirmed Epitope (Experimental), and Variability (Sequence with Mutation/s). The ArVirInd database provides an opportunity for the user to submit sequences of viral strains isolated from emerging outbreaks of Dengue, Chikungunya, West Nile, Japanese encephalitis, and Chandipura using the sequence submission tool called, ***SeqKosh***. The ArVirInd database also provides an interactive dashboard available at http://arvirind.co.in/dashboard/ (**Fig. 2**). The database will be updated and expanded periodically.

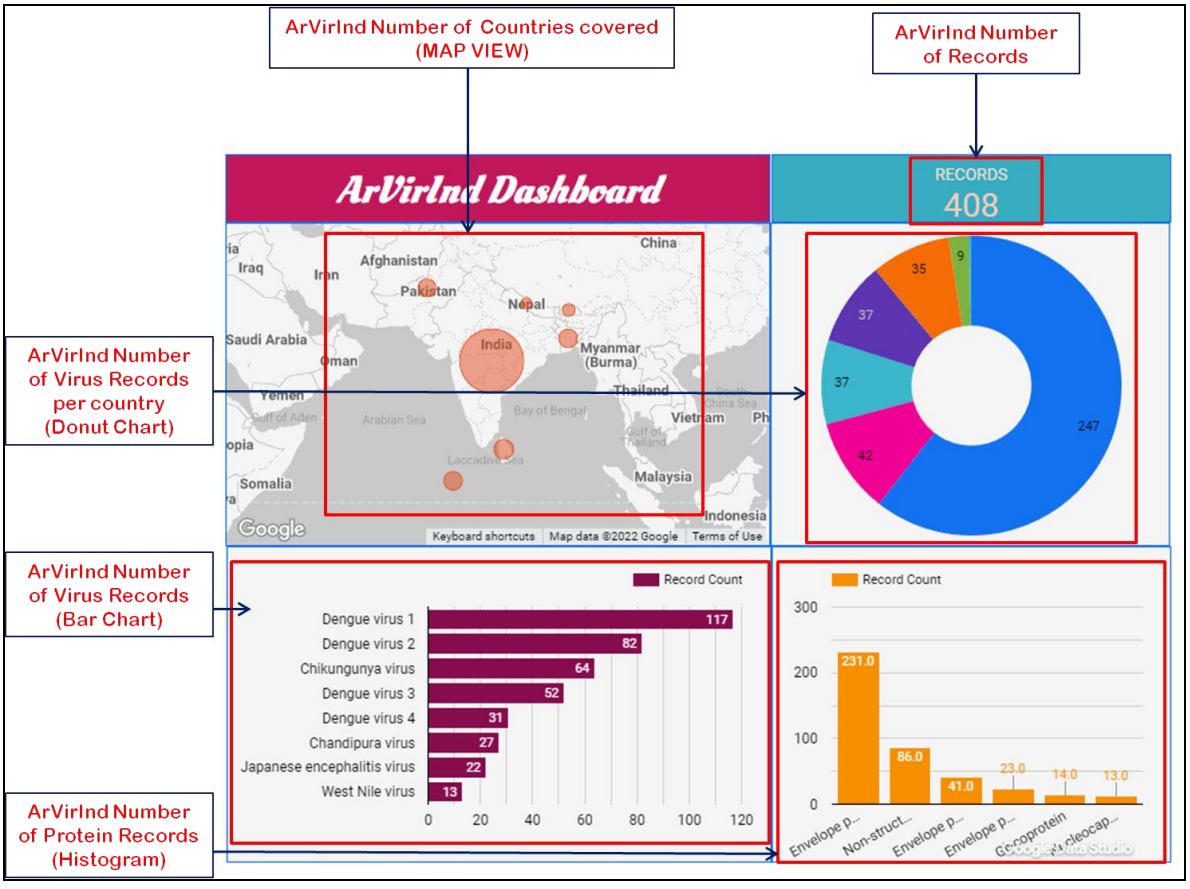

Fig. 2: The ArVirInd dashboard

DENGUE - CHIKUNGUNYA GROUP

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Apex referral laboratory activity for National Vector Borne Disease Control Programme

Investigators: K. Alagarasu, D. Parashar, J.A. Patil, M.B. Kakade, M. Bote, Y.K. Gurav
Funding: NVBDCP/Intramural D**uration:** 2021-2022

A. 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 2021. ICMR-NIV is an apex referral laboratory which provides information on the cirucalting serotypes and genotypes of dengue virus (DENV).
***Objective*:** To find out the distribution of serotypes of DENV and genotypes within serotypes in Maharashtra and other states during the 2021 dengue season.
Findings: During the year 2021, 2352 samples were tested by real-time RT-PCR from Maharashtra among which 468 were positive for DENV. Among the positive samples, DENV-1 was observed in 145, DENV-2 in 233, DENV-3 in 25 and DENV-4 in 18 samples. The samples tested from other states and the number of samples positive for each serotype is provided in table 1.

Table 1: Prevalence of DENV serotypes in different states.

State	Number tested	Samples positive for DENV	DENV-1	DENV-2	DENV-3	DENV-4	Mixed serotype infections
Andhra Pradesh	201	58	2	39	13	-	2
Bihar	34	19	18	1	-	-	-
Goa	207	140	23	101	14	1	1
Gujarat	17	2	1	1	-	-	-
Sikkim	90	83	-	83	-	-	-
Telangana	91	49	1	29	13	3	3

The results revelaed that DENV-2 is the dominant serotype all states from where samples were tested except Bihar, wherein dominance of DENV-1 was observed.Envelope gene sequencing of dengue viruses in samples was performed. Results revealed circulation of Asian and American African genotypes of DENV-1, cosmopolitan genotype (GV) of DENV-2 and DENV-3 genotype III (Fig. 1).

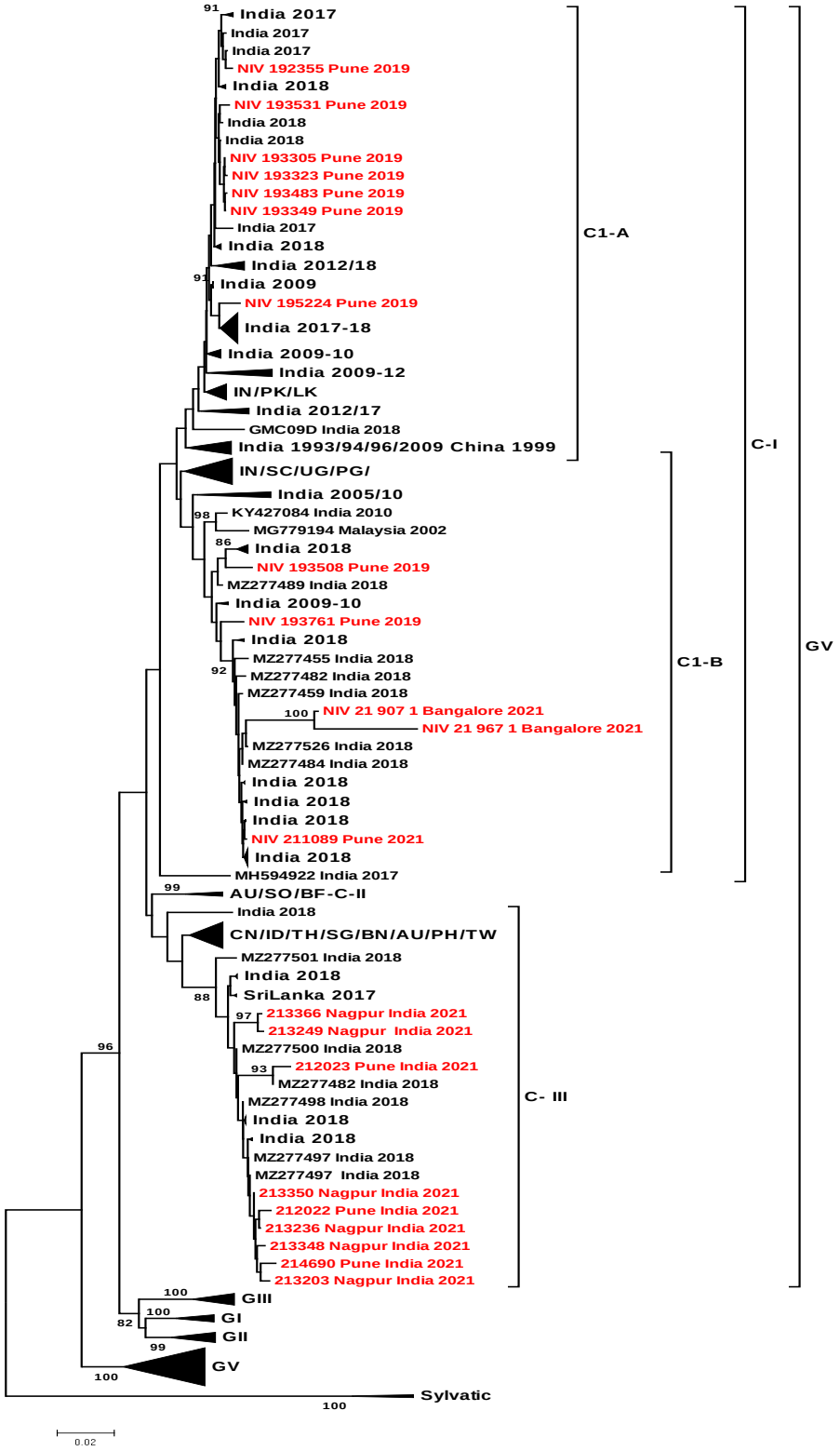

Fig. 1: Phylogenetic tree of DENV-2 based on envelope gene sequences. 2021 sequences are highlighted in Red colour.

B. Molecular characterization of chikungunya viruses

***Background*:** A large number of chikungunya cases were reported from Pune district during 2021-2022

***Objective*:** To perform molecular characterization of chikungunya virus (CHIKV) cuirculating in Pune district during 2021

Findings: A total number of 1766 samples were tested for CHIKV by real-time RT-PCR among which 273 were found to be positive for CHIKV. Eleven samples were processed for whole genome sequencing. Phylogenetic analysis revealed that the all the isolates belonged to the Indian ocean lineage of East Central South African genotype. Sequences belonging to 2020 and 2021 samples clustered separately (Fig. 2).

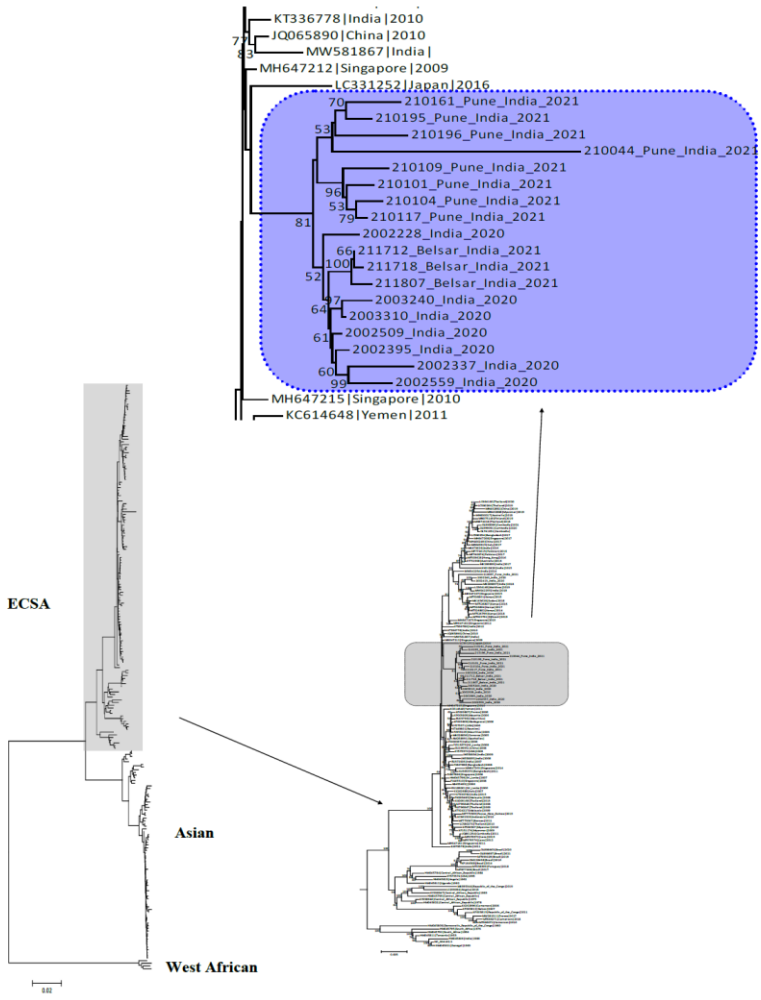

Fig. 2: Phylogenetic tree based on the whole genome of CHIKV. Sequences from 2021 are highlighted in Violet color.

Development and evaluation of an in-house developed multiplex real time RT-PCR assay for simultaneous detection of dengue, chikungunya and zika viruses in clinical samples

Investigators: K. Alagarasu, D. Parashar, G. Sapkal, P. Yadav, Y.K. Gurav.
Contributors: M.B. Kakade, M. Bote,
Funding: Intramural **Duration:** 2020 - 2023

Background: Early diagnosis of dengue, chikungunya and Zika viruses is essential for controlling virus outbreaks. Multiplex assays offer the advantage of testing for more than one virus in a single test.

Obejctive: To develop and evaluate a multiplex real time RT-PCR assay for simultaneous detection of dengue, chikungunya and zika viruses in clinical samples.

Findings: New Primers and probes for Zika viruses were designed. A fourplex assay involving detection of dengue, chikungunya, and zika viruses and internal control (B-actin) was standardized. The assay was tested in 103 dengue real-time PCR positive samples and 34 chikungunya PCR positive samples. The assay had a sensitivity of 96% (90.3 to 98.9) for detection of dengue virus and 100% (89.2-100) sensitivity for detection of chikungunya virus. The assay was test for cross reactivity against Japanese encephalitis virus, West Nile virus, Kyasanur Forest Disease virus, Influenza A and B viruses, PIV and respiratory syncytial virus. The assay did not not show any cross reactivity with the tested viruses. Moreover, there was also no cross reactivity between dengue, chikungunya and zika viruses. Plan for testing more number of dengue, chikungunya and zika samples are in progress.

Influence of vitamin D and vitamin D receptor gene polymorphisms on antibody dependent enhancement mediated dengue virus infection, replication and cytokine response in monocytic cells

Investigators: K. Alagarasu, D. Parashar.
Contributors: S. Dubey, A. Telmore, J.A. Patil
Funding: Intramural **Duration:** 2022 – 2025

***Background*:** Vitamin D is an immunomodulatory hormone. Studies have shown that vitamin D is know restrict the growth of dengue virus in monocytes, macrophages and dendritic cells. The effect of the active form of vitamin D, 1, 25 dihydroxy vitamin D_3 (1,25 $(OH)_2$ vit D_3) on antibody dependent enhancement of DENV in monocytes/macrophages is not studied.

***Objective*:** To study the effect of 1,25 $(OH)_2$ vit D_3 on the ADE of dengue virus infection in K562 cell line

Findings: Preinfection treatment and post in fection treatment of K562 cells with 1,25 $(OH)_2$ vit D_3 reduced dengue virus RNA copy number in K562 cells infected with dengue virus through ADE mode (Fig.). The reduction was significant when the cells were treated with 1000 nM 1,25 $(OH)_2$ vit D_3 in case post infection treatment while the decrease in viral load was significant in cells pretreated with 1000 nM 1,25 $(OH)_2$vit D_3 (Fig. 3).

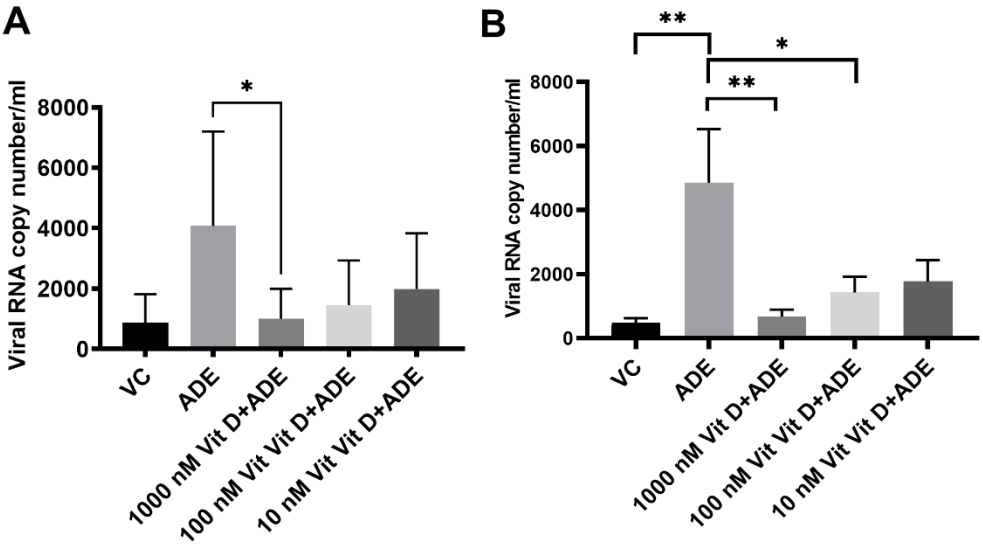

Fig. 3: Effect of preinfection treatment (A) and post infection treatment (B) of vitamin D on ADE of dengue virus infection in K562 cells.

Antiviral activity of approved drugs and natural peptides against dengue and chikungunya

Investigators: K. Alagarasu, D. Parashar, S. Cherian

Contributors: P. Patil, M.B. Kakade, D. Chowdury

Funding: Intramural **Duration:** 2020 – 2023

Studies on the antiviral activity of chebulinic acid against dengue and chikungunya viruses and *in silico* investigation of its mechanism of inhibition

***Background*:** Chebulinic acid (CA), originally isolated from the fower extract of the plant Terminalia chebula, has been shown to inhibit infection of herpes simplex virus-2 (HSV-2), suggestively by inhibiting the host entry step of viral infection. Like HSV-2, the dengue virus (DENV) and chikungunya virus (CHIKV) also use receptor glycosaminoglycans (GAG) to gain host entry.

***Objectives*:** To explore the activity of CA against these viruses.

Findings:, Co-treatment of 8 µM CA with DENV-2 caused 2 log decrease in the virus titer (4.0 log10FFU/mL) at 120 h post infection, compared to virus control (5.95 log10FFU/mL). In contrast, no inhibitory efect of CA was observed against CHIKV infection under any condition. The mechanism of action of CA was investigated *in silico* by employing DENV-2 and CHIKV envelope glycoproteins. During docking, CA demonstrated equivalent binding at multiple sites on DENV-2 envelope protein, including GAG binding site, which have previously been reported to play a crucial role in host attachment and fusion, indicating blocking of these sites. However, CA did not show binding to the GAG binding site on envelope protein-2 of CHIKV. The in vitro and *in silico* fndings suggest that CA possesses the ability to inhibit DENV-2 infection at the entry stage of its infection cycle and may be developed as a potential therapeutic agent against it (Fig. 4).

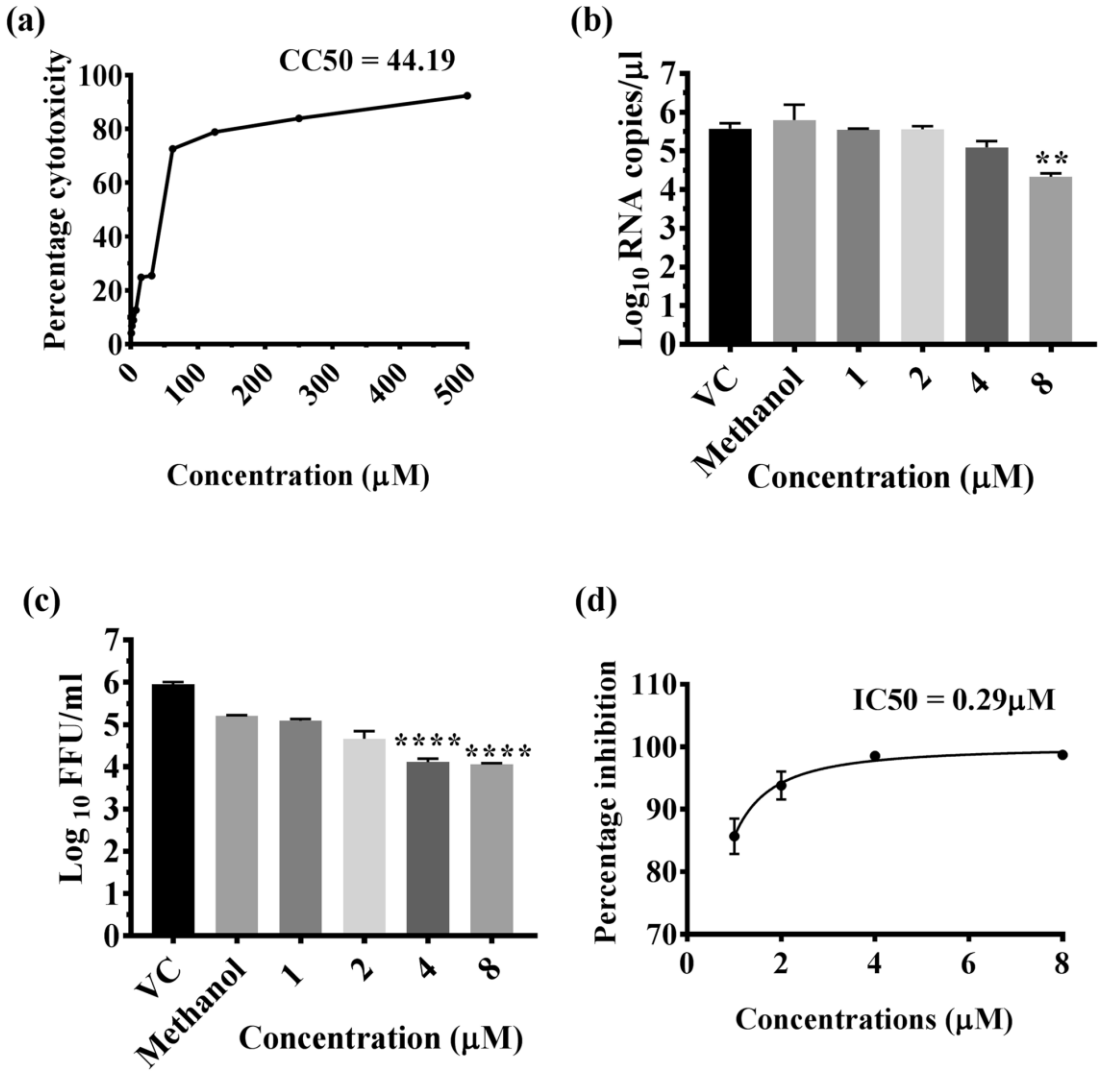

Fig 4. Effect of Chebulinic acid (CA) on Vero E6 cells and dengue virus. (a) Cytotoxic effect of CA at various concentrations on Vero E6 cells. (b, d) Direct virucidal effect of CA at various concentrations on dengue virus infection at MOI = 0.1 in Vero E6 cells. Virus titer was assessed at 120 h post infection and expressed in terms of (b) Log10 viral RNA copies/μL and (c) Log_{10} focus forming units/ml, ***P value < 0.0001, *P < 0.05. (d) Percentage inhibition of DENV focus forming units/mL by CA compared to virus control (VC). All the experiments were performed in triplicates, and the data values are expressed as mean ± SEM.

A. Antiviral activities of Sauropus androgynus L. Merr. leaf extract against dengue and chikungunya

***Background*:** *Sauropus androgynus* L. Merr, a perennial shrub belonging to the family Euphorbiaceae. Different parts of this plant have been known to cure several medical conditions like cholestasis, cough, ophthalmia, soreness, coryza, and erythrina. Above all, the content of different vitamins in *S. androgynus* was higher than in other vegetables.

***Objective*:** To evaluate the antiviral activities of extract of the plant *Sauropus androgynous*.

Findings: DENV infection reduction was observed under pre and post-infection treatment conditions at a concentration of 31.25 μg/ml (Fig. 5). Anti-CHIKV activity was not observed. The chemical composition of the extract is provided in Table 2. The autodock-vina-based *in-silico* docking study revealed that a compound named β-Sitosterol could form a strong interaction with both the DENV E glycoprotein and NS2B-NS3 protease domain domain which might explain the observations of *in vitro* studies. Our study findings suggest that *Sauropus androgynus* L. Merr can inhibit DENV infection and might act as a potent prophylactic/therapeutic agent against DENV-2.

Table 2. Chemical composition of *S. androgynus* leaves hexane extract

Compound	RI	%	Identification
β-Caryophyllene	1421	0.1	RI, MS, CI
E-β-Ionone	1488	t	RI, MS
Methyl hexadecanoate	1927	0.5	RI, MS
Hexadecanoic acid	1976	5.6	RI, MS
Linolenic acid, ethyl ester	2158	10.2	RI, MS
Squalene	2831	36.9	RI, MS
δ-Tocopherol	2943	8.9	RI, MS
β-Tocopherol	3033	2.2	RI, MS
γ-Tocopherol	3049	7.6	RI, MS
Vitamin E	-	12.5	MS, CI
β-Sitosterol	-	5.9	MS, CI
Phytonadione	-	0.7	MS
Sesquiterpene hydrocarbon		0.1	

Phenyl derivative	t
Long chain oxygenated hydrocarbons	16.3
Triterpenoids	42.8
Chromane terpenoids	31.2
Quinine terpenoid	0.7
Total	91.1

RI = retention index relative to C_8-C_{30}*n*-alkanes on ZB-5 column, MS = NIST and Wiley library and the literature, CI=Co-injection of commercial samples, t = trace (<0.1%).

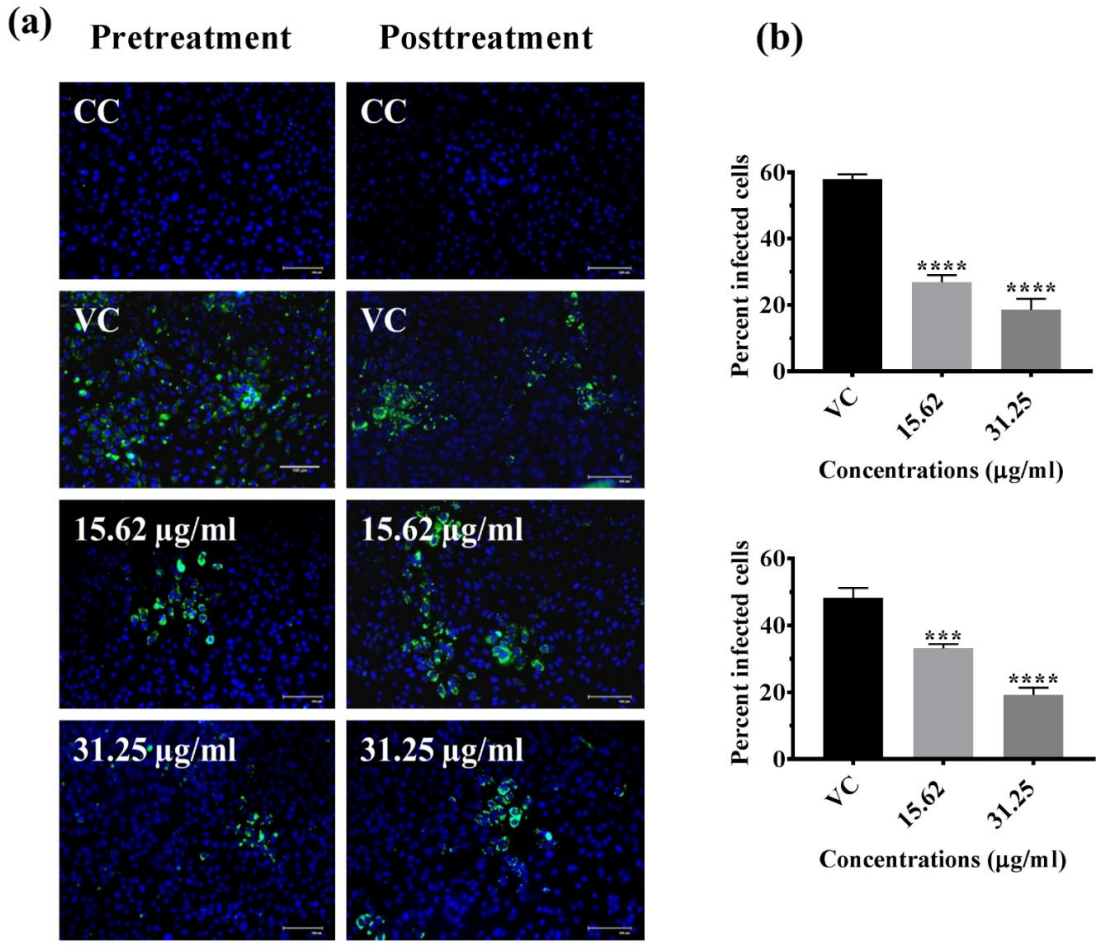

Fig 5. Effect of *S. androgynous* extract on the percent of infected cells under pretreatment and post treatment conditions. Microscopic images of Vero CCL-81 cells infected with DENV under pre and post infection treatment conditions respectively (a and c). Green color is exhibited by virus-infected cells; Percent of infected cells in cultures infected with DENV and treated with different concentrations of extract under pre and post infection treatment conditions respectively

(b and d). All the values are expressed as mean±SD. The experiments were performed in triplicates in three independent trials. ****, $p < 0.0001$ and ***, $p = 0.0002$ vs. control.

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

Investigators: S. Cherian, K. Alagarasu, D. Parashar

Contributors: B. Kasabe, M. Punekar, P. Patil, M. Kakade

Funding: Extramural **Duration:** 2020 - 2022

***Background*:** Rising incidence of dengue virus (DENV) infections in the tropical and sub-tropical regions of the world emphasize the need to identify effective therapeutic drugs against the disease. Repurposing of drugs has emerged as a novel concept to combat pathogens. In this study, a transcriptomics-based bioinformatics approach was employed for drug identification. Gene expression omnibus datasets from patients with different grades of dengue disease severity and healthy controls were used to identify differentially expressed genes in dengue infected subjects which were then applied to the query tool of Connectivity Map, which is a library containing over 1.5M gene expression profiles from ~5,000 small-molecule compounds, to identify inverse drug-disease relationship. Identified drugs were further screened for *in vitro* antiviral activity against DENV-2 and in silico binding to DENV-2 proteins

***Objectives*:** Evaluation of *in-vitro* screening of FDA-approved drugs for repurposing against dengue virus-2

Findings: Results revealed that five compounds viz. resveratrol, doxorubicin, lomibuvir, elvitegravir and enalaprilat have significant anti-DENV activity. Doxorubicin, lomibuvir, and elvitegravir showed anti DENV activity under pretreatment condition. Under cotreatment condition, only doxorubicin showed anti DENV activity. Resveratrol, doxorubicin and enalaprilat showed anti DENV activity under post treatment conditions (Fig. 6).

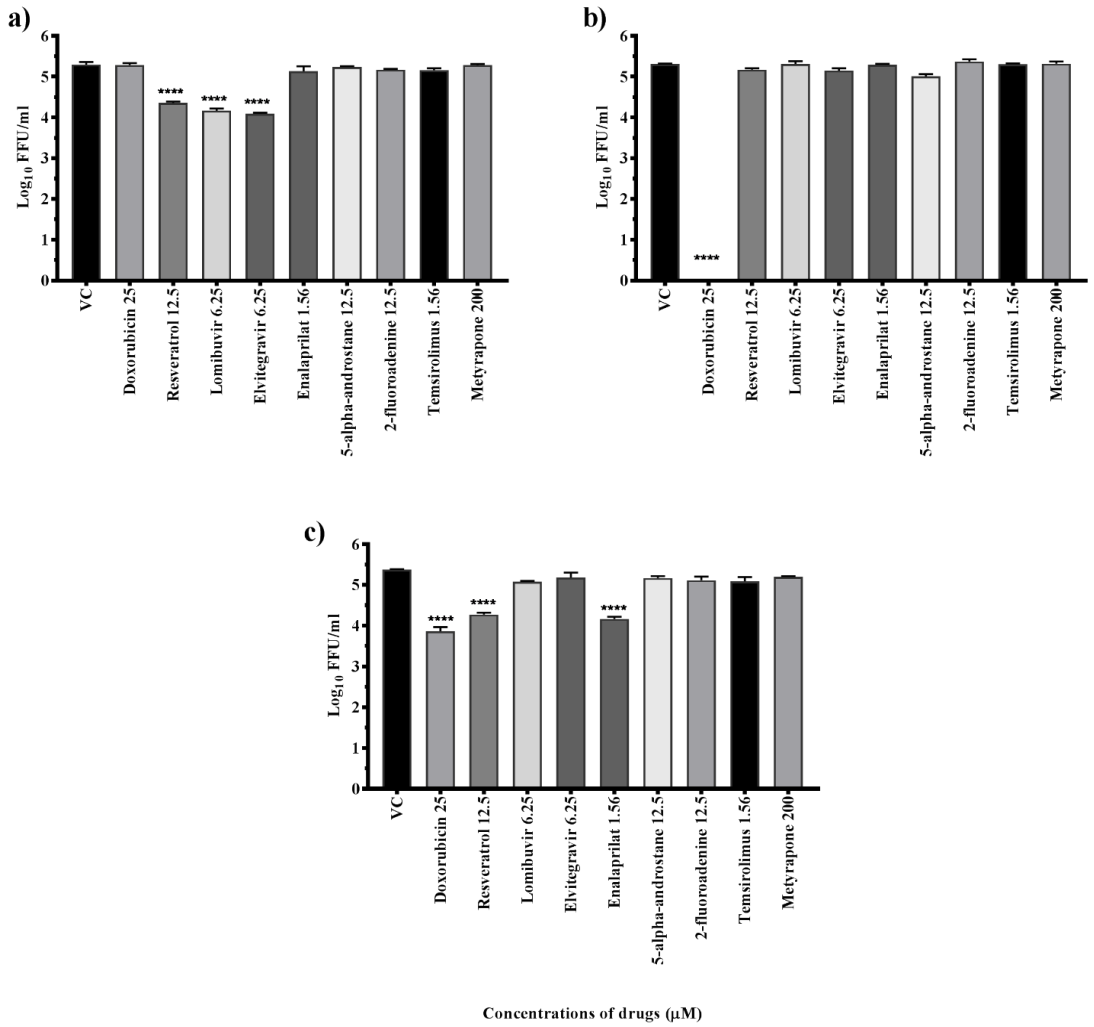

Fig 6. Antiviral screening of drugs for repurposing at maximal nontoxic concentration against DENV under pre (a), co (b) and post (c) treatment conditions. Vero CCL-81 cells were treated with a maximum non-toxic dose of drugs for 24 hours in a pre, co and post-infection manner and incubated for 120 hours with DENV. Post incubation, plates were frozen and culture filtrates were used for different assays. Experiments were performed in triplicates in two independent trials. Results were plotted based on mean $\log_{10}$ of focus-forming unit/ml ± standard error. All the treatment groups were compared with a control group (VC) (which did not receive drugs). ****$p < 0.0001$.

Targeted *in vitro* gene silencing of E2 and nsP1 genes of chikungunya virus by biocompatible zeolitic imidazolate framework

Investigators: K. Alagarasu, D. Parashar

Contributors: R. Tagore, M. Kakade, P. Patil

Funding: Intramural **Duration:** 2021- 2022

***Background*:** The lack of any licensed vaccine or antiviral agents against CHIKV warrants development of effective antiviral therapies. Earlier, we have reported that siRNAs targeted against nsP1 and E2 genes lead to inhibition of CHIKV replication in CHIKV infected cells and mice models. The therapeutic efficiency of siRNA can be improved by using an efficient delivery system. Recent studies have indicated that metal organic framework (MOF) biocomposites can protect and deliver nucleic acids.

***Objectives*:** To explore ZIF-C (a polymorph of zeolitic imidazolate framework- 8, ZIF-8) biocomposites to deliver siRNAs targeted against E2 and nsP1 genes of CHIKV to achieve a reduction in viral replication and infectivity.

Findings: Cellular transfection studies of E2 and nsP1 genes targeting free siRNAs and ZIF-C encapsulated siRNAs in CHIKV infected Vero CCL-81 cells were performed. Our results reveal a significant reduction of infectious virus titre, viral RNA levels and percent of infected cells in cultures transfected with ZIF-C encapsulated siRNA compared to cells transfected with free siRNA. The results suggest that delivery of siRNA through ZIF-C enhances the antiviral activity of CHIKV E2 and nsP1 genes directed siRNAs (Fig. 6).

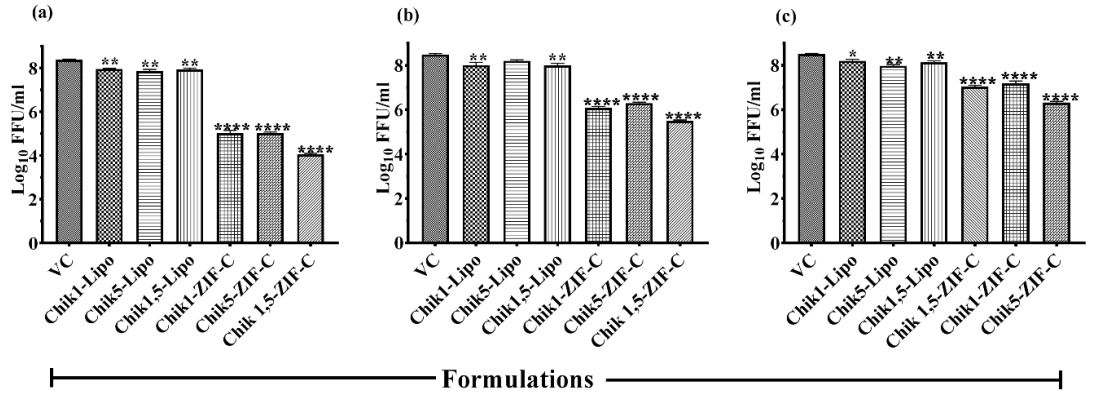

Fig. 7. Inhibitory effects of siRNA-ZIF-C and Lipofectamine mediated delivery of siRNA at treatment after 0h.p.i; (a) 3h.p.i and (b) 6h.p.i (c) on production of virus particles as assessed by FFU assay. All the values are expressed as mean±SEM of three experiments. The FFU titres were compared between VC and different siRNA formulations using one-way ANOVA with multiple corrections (****p<0.0001,***p<0.001,**p<0.01, *p <0.05).

No. of samples tested:

Dengue IgM: 1899 tested, 602 (31.7%) positive

Chikungunya IgM 1967 tested, 719 (36.5%) positive

DEN IgM Kit validation: 56 batches

CHIK IgM Kit validation: 13 batches

DIAGNOSTIC REAGENT FACILITY

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Scaling up facilities for production of Diagnostic kits/ Reagents for detection of JE, DEN & CHIK viruses

Investigators: Director, ICMR-NIV, Pune

Funding: NCVBDCP ***Duration*****:** Ongoing

OBJECTIVE**:**

The National Vector Borne Disease Control Center (Formerly National Vector Borne Disease Control program) has identified around 700 sentinel surveillance hospitals/ centers (SSHs) and 17 Apex laboratories (ARLs) throughout the country for diagnosis of JE, Dengue and Chikungunya. NCVBDC has given the responsibility of kits production and supply to ICMR-NIV so that the uniform data can be generated on the Vector Borne Viral Diseases throughout the country. Also, these kits are being supplied to VRDLs throughout the country for outbreak investigations and surveillance purpose. The kits are being supplied as per the need of the Sentinel centers/ VRDLs, outbreak of these diseases in the vicinity/ community.

WORK DONE:

Production & supply of MAC ELISA diagnostic kits

During the year 2021-2022, the MAC ELISA kits were produced and supplied to SSH and Apex labs under the national program (Table 1).

Table 1: Supply of MAC ELISA kits in 2021-2022

	JE	DEN	CHIK	TOTAL
National Program	508	8913	2763	12184
VRDL (DHR)	170	360	201	731
Others*	9	12	10	31
Total	687	9285	2974	12946

* NIMHANS-Bangalore, AIIMS-Bibinagar, ICMR-RMRC Bhubaneshwar

The kits were provided to the Centers based on their requirements and additional kits were supplied in case of outbreak in the neighboring area and therefore the kits are to be provided throughout the year (Fig. 1).

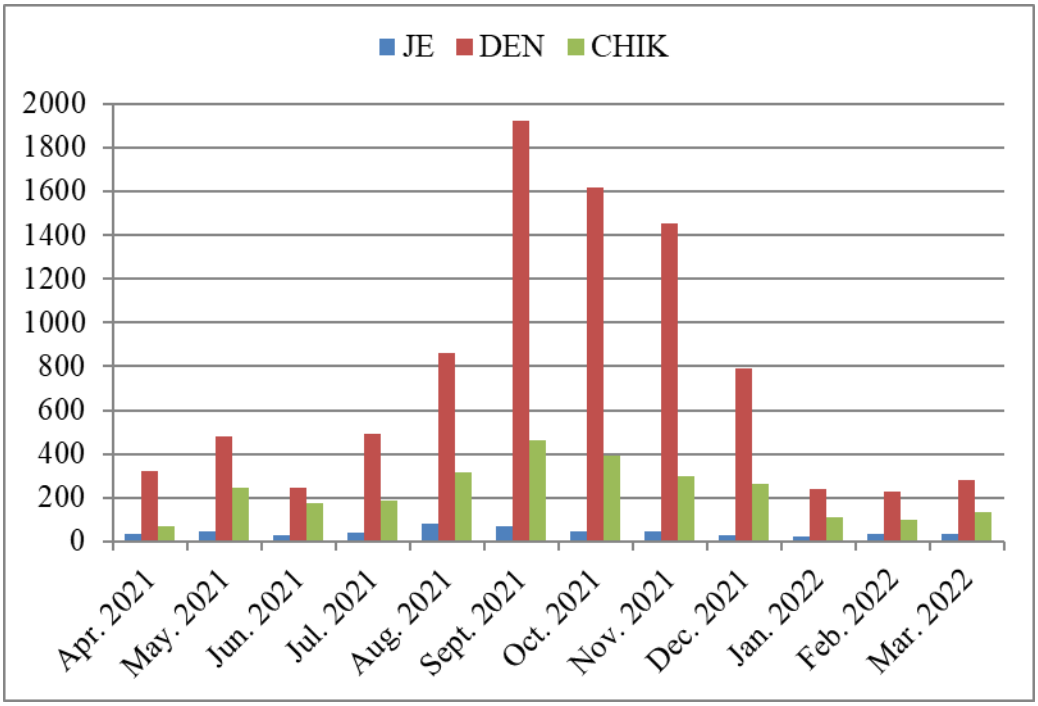

Fig. 1: Month-wise supply of MAC ELISA kits under National Program

DIAGNOSTIC VIROLOGY GROUP

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Dr.Pradnya Shinde	Scientist 'B'

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Mrs. Rashmi S. Gunjikar	(STO A)
Mrs. Rashi Srivastava	(TO-A)
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Ph.D Students

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Mr. Jose Antony Jenish R	Ph.D Student

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

Investigators: G Sapkal, G Deshpande, P. Shinde

Support staff: N Tadkalkar, R Patil, S Saka, P Gomade, Keshaw Sharma, Manjusha Gopale, Ms. Pooja Pawara

Funding: DHR/ICMR Duration: 2022 -2023

Background & Objectives:

Resource Centre for Virus Diagnostic Research Laboratories (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.

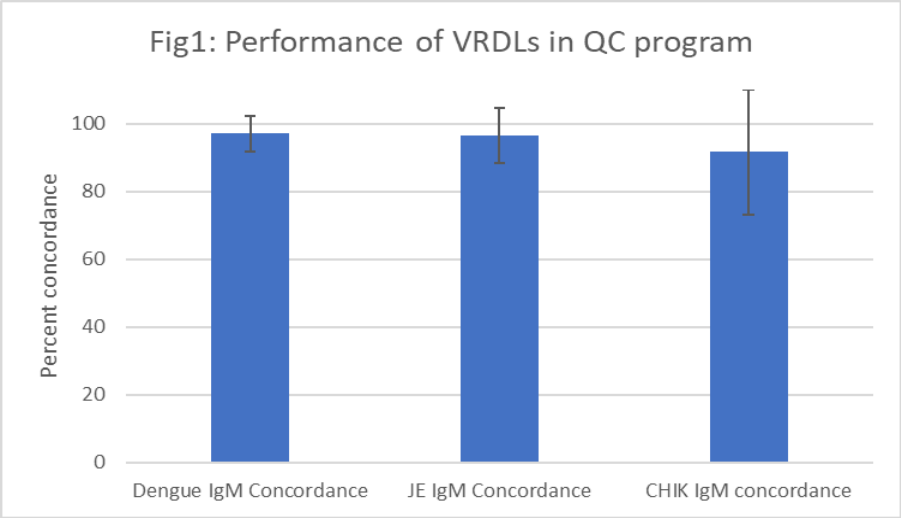
Work done:

Training programmes:

A total of six training programs were conducted for Viral Research and Diagnostic Laboratory (VRDL) network in the country (n=42). Among them 04 trainings were 07 days' consolidated hand's on training on virus diagnosis by serodiagnostics, molecular biology tools, Biosafety accepts & outbreak investigation procedures. A total of 111 staff members were trained. Details of the trainings are as below. Other two trainings were "Hands-on training session on whole-genome sequencing of SARS-CoV-2 and the use of bioinformatics tools in data analysis".

Quality Control Program for VRDLs

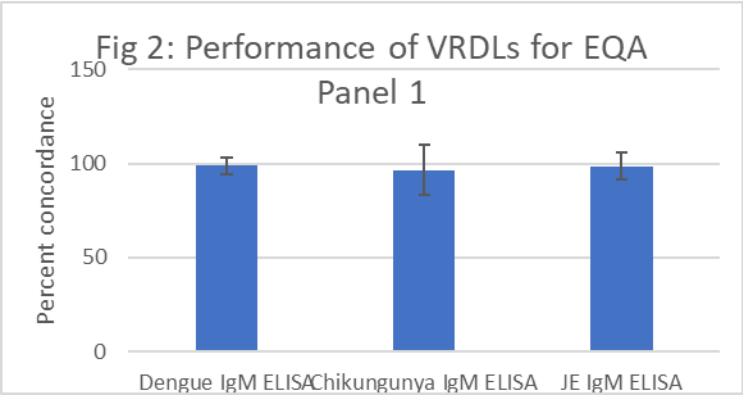
The RCVRDL continued to support the Quality Control Programs of VRDLs in viral diagnostics. During the period, 18 centres participated in the quarterly QA/QC program of serodiagnostic testing for 10 etiologies (Dengue, Chikungunya, Japanese encephalitis, Hepatitis A, B, C and E, CMV, Measles and Mumps). A total of 18 VRDLs participated in quality control program in the year 2021 to 2022. 993 samples were received from the participating VRDLs. RCVRDL tested 942 samples and reported the results to respective VRDLs. The summary of results of quality control testing is indicated in Fig. -1.



The overall results of QC program ascertained that most VRDLs were maintaining high quality of testing as demonstrated by the percent concordance scores. VRDLs with lower scores were provided with information for trouble-shooting and root-cause analysis.

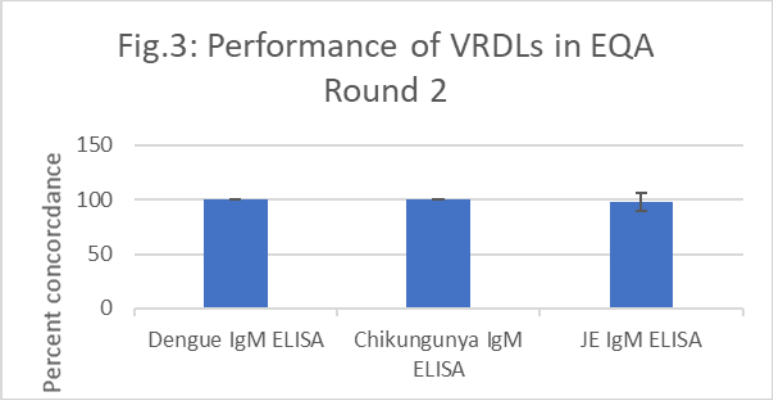
External Quality Assurance (EQA) Program for VRDLs

As a part of continuous improvement program, prepared a plan, designed and performed experiments for executing the External Quality Assurance panel (EQA). Serum panel for detection of anti-Dengue, Chikungunya and Japanese encephalitis IgM by ELISA was distributed to 64 VRDLs across the country. The average concordance of test results was 97.92%. This reassured the quality of testing in pandemic situation. Reports of the program have been submitted to the ICMR and individual VRDLs (Fig. -2).



Similarly, in EQA Round-2 (October 2021): External Quality assurance panel was sent to 40 VRDLs for detection of anti-Dengue, Chikungunya and Japanese encephalitis IgM by ELISA. Of them 27 VRDLs participated in the program. The average concordance of test results was

99.37%. This reassured the quality of testing in pandemic situation. Reports of the program have been submitted to individual VRDLs (Fig. -3).



In conclusion, 55, 53 and 42 VRDLs were showing 100% concordance for Dengue, Chikungunya and Japanese encephalitis IgM capture ELISA respectively. Further 02 and 02 VRDLs were showing 83.33% of concordance for Dengue and Chikungunya IgM ELISA respectively. Although all VRDLs were primarily contributing to COVID-19 diagnostics during the duration of the report, the high levels of concordance indicate continued quality of serological testing.

Surveillance for Zika virus infections in India

Investigators: GN Sapkal, PD Yadav, G Deshpande

Contributors: RS Gunjikar, CA Patil.

Funding: DHR/ICMR Duration: 2016-2022

***Background*:** As a part of 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:

Zika virus disease symptoms and case history in retrospective and prospective samples

Congenital Zika Virus Syndrome including microcephaly in infants

Major *Findings:* To accomplish continuous monitoring of Zika virus infection in humans in the country. With a view to enhance the laboratory capacity for Zika surveillance in India, on-site hands on training programmes were conducted. Additionally in the middle of pandemic during 2021-2022, online training followed by diagnostic reagent supply and verification of validity

test run by respective VRDLs on real-time RT-PCR diagnosis of Zika infection enhanced the Zika surveillance network to a total of 132 VRDLs.

Coordinated timely shipment of Zika virus diagnostic reagents/Kits to 140 network laboratories and shipped 35000 reactions. This ensured in screening continous screening of samples from all over country by the network laboratories. During the COVID-19 pandemic,virus disease (ZVD) outbreaks were reported in Kerala (May–July, 2021), Maharashtra (July, 2021), and Uttar Pradesh (October, 2021). Considering these outbreaks a retrospective study was undertaken (May – October 2021) by VRDLs to screen dengue and chikungunya negative samples for ZVD. This revealed the circulation of ZIKV in additional states of the country.

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

Investigators: GN Sapkal, G Deshpande, BV Tandale

Co-investigators: N Gupta, L Sangal, M Murhekar, M Ahmad, K Hayford, A Shete, W Moss, J Lessler, J Metcalf, M Ferrari.

Funding: ICMR (Extramural)/Johns Hopkins University **Duration: 2017-2022**

***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*:**

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.

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

During this period, a total of 5132 samples from pre and post campaign pediatric sites were tested for measles IgG antibody. A community-based cross-sectional serosurveys in four selected districts of India before and after implementation of measles-rubella containing vaccine serosurveys: Hoshiarpur (Punjab), Palghar (Maharashtra), Kanpur Nagar (Uttar Pradesh), and Dibrugarh (Assam). The surveys were conducted between two age groups: 9 months to <5 years and 5 to <15 years and collected sera were tested for IgG antibodies against measles and rubella viruses using Euroimmun quantitative IgG ELISA.

The overall measles and rubella seroprevalence for MH and UP facility pediatric group was also obtained to compare with respective community seroprevalence observed during serosurveys. The overall measles seroprevalence observed 86.3% and 74.7% for MH and UP facility respectively. The overall rubella seroprevalence observed was 70.8% and 65.8% for MH and UP facility respectively.

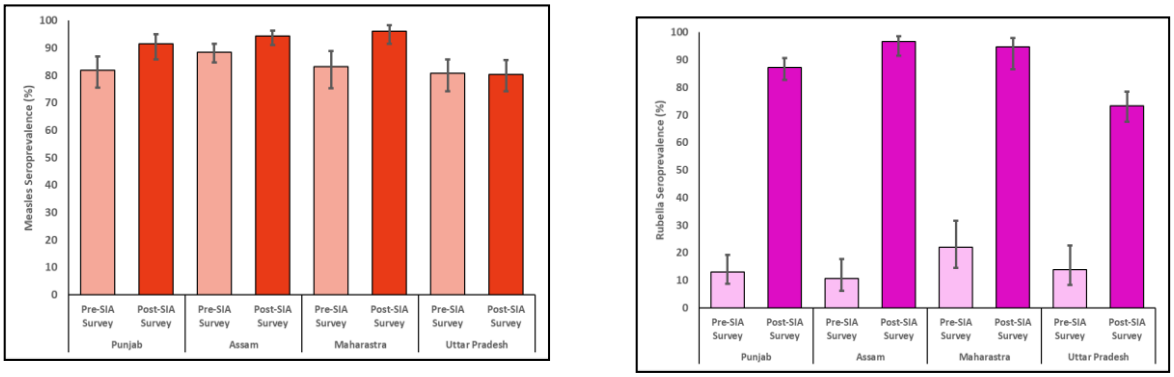


Fig. : Representative seroprevalence of IgG antibodies against measles and Rubella among children aged 9 months - <5 years and 5 - <15 years.

Congenital Rubella Syndrome (CRS) Surveillance in India

Investigators: G Sapkal, R Viswanathan, **ICMR-NIE Chennai:** M Murhekar

Contributors: R Patil

Funding: ICMR Duration: 2016-2022

***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:

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.

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

Findings:

A total of testing of 185 serum samples received from 9 sentinel sites showed and concordance 87.33% with anti-rubella IgM ELISA results and 100% concordance for anti-rubella IgG antibodies respectively. Of the 383 throat swabs received from suspected rubella patients, 169

tested negative and 1sample was positive in diagnostic rubella RT-PCR and further confirmed as genotype 2B.

Development of serodiagnostic assays for Nipah Virus

Investigators: Dr. GN Sapkal, Dr.Pragya Yadav, Dr.Gururaj Rao Deshpande

Contributors: Mrs.Rashi Srivatsav, Mr. Shankar Vidhate, Mrs. Kirti Khutwad

Funding: DHR **Duration: 2019-2022**

***Background*:**

Nipah virus (NiV) is an emerging paramyxovirus capable of causing lethal infections in a number of mammalian species including humans. NiV is classified as a Biosafety level 4 pathogen due to the high morbidity and mortality associated with the virus and lack of an approved vaccine or treatment. NiV has also been established as the cause of fatal human encephalitis in Bangladesh and India during different outbreaks from 2001 to 2018. An indication of involvement of *Pteropus* bats as a reservoir for NiV in India was observed during 2010. Followed by the same a multi-site virological survey of NiV carried out by ICMR-NIV, Pune confirmed the presence of NiV among *Pteropus giganteus* bat from Cooch Bihar district, West Bengal and Dhubri district, Assam in 2015. In the year 2018, a dreadful outbreak of NiV was observed in Kozhikode, Kerala State that claimed the lives of 17 individuals. The unprecedented emergence of NiV in the Kerala State created waves of threat and panic throughout the country and even across the world. Accurate diagnosis is critical for providing appropriate care in infectious diseases and cutting down the chain of transmission and saving human lives. The detailed outbreak investigation confirmed the role of the *Pteropus* bats in the transmission of NiV by ICMR-National Institute of Virology (NIV), Pune.

Objectives:

Development of anti-Nipah Human IgM antibody detection ELISA for screening human samples.

Development of anti-Nipah IgG antibody detection ELISA for screening human, bat, and Swine serum samples using characterized monoclonal and polyclonal antibody.

Findings:

During the reporting period, among a total of 43 hybrids generated 8 were found to be strong positive (9C9 was found to be IgG1 and 05 MAbs (9H5, 7G1, 610, E10, 3F2 & 7F9) were found to be IgG3 and 02 Mabs were mix of IgG1 & IgG3 isotypes (7D5 & 7F9) by ELISA, further characterization with isotyping and Western blotting indicated the potential applications of these MAbs for diagnostic utility for increasing the sensitivity and specificity of the Nipah ELISA.

ELECTRON MICROSCOPY & HISTOPATHOLOGY GROUP

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Mr Ulhas Gadekar	Multitasking staff (pathology)

Core facility activities

As a part of institutional core facility high resolution electron microscopy imaging and analysis services were provided to in-house scientific and also non-institutional researchers from other national laboratories/industries on request. The application areas included negative staining, virus morphodiagnosis, resin embedding, ultramicrotomy and Tokyasu imaging and image analysis services. As a part of routine quality control, the 23rd Virus EQA was carried out with Robert Koch Institute, Germany.

Characterization of vascular endothelial stress response induced by Japanese encephalitis virus (JEV) in an in-vitro model

Investigators: Dr Preksha Jain WOS-A fellow Co-PI: Dr Atanu Basu

Funding: Extramural DST, completed 2021

Summary

Japanese encephalitis virus (JEV) is pathogenic flavivirus with significant public health importance. The study examined the role of JEV infection cultured endothelial cells with respect to virus susceptibility, vascular integrity and stress response profiling of the cells after JEV infection with respect to vasoactive molecules. Cultured HUVEC cells were susceptible to JEV (Nakayama strain) without prominent cytopathic effect. JEV antigen expression was detectible by using immunofluorescence microscopy showing 30-40 % infectivity and immunoblots using polyclonal rabbit sera against JEV. When compared with BHK21 and PS cell lines, JEV replication as detected by real time based polymerase chain reaction using a TaqMan probe showed early rise in virus RNA (2 fold high than BHK21 and PS) and a rapid drop after 36 hours post infection. The important morphological change was observed as reorganization of the actin distribution in the infected cells that resembled stress fiber assemblies. Such changes were not observed in control HUVEC, BHK21 and PS infected cells. Importantly, the SK Hep1 cell, a cell line of endothelial origin was also susceptible to JEV and showed similar changes.

Transmission electron microscopy imaging of resin embedded thin sections of the infected cells were consistent with these observations and replicating virus particles could be see in well defined cytoplasmic multivesicular compartments.

Profiling vasoactive molecules after JEV infection showed significant changes in vWF, IL6, IL8 and angiopoietin levels. A rather pertinent observation that needs further studies was the early rise and subsequent fall in VEGF levels after JEV infection that is a novel finding and possibly implicates an abnormal angiogenic signaling in the pathophysiologic events associated with JEV disease biology.

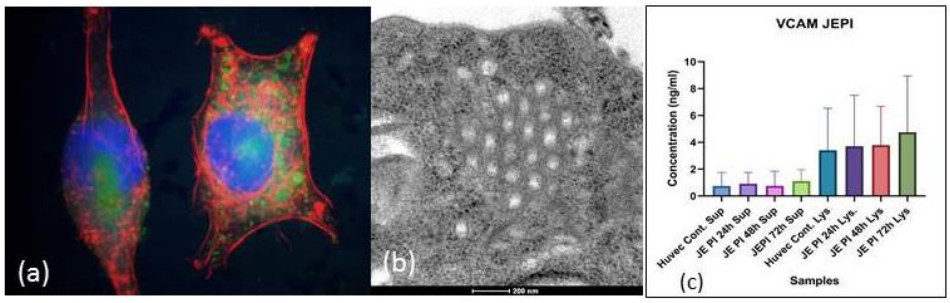

Fig. 1: Infection of endothelial cells by JEV (a) a representative fluorescence micrograph (dual color) showing changes in actin framework (red) and perinuclear JEV antigen localization in infected cells (b) a transmission electron micrograph showing formation of sub-membrane multivesiculate structures (c) significant changes in VCCAM expression after infection.

Other work

(i) ***Raman spectroscopy of dengue virus NS1 proteins from different serotypes*:**

Vibrational spectroscopy technique has emerged as an important tool for non-invasive analysis of spectrum of materials. Raman Spectroscopic methods are being used for plethora of application in life science research. These methods are now used for identification and screening of various proteins in their natural environment.

In the current work, we have attempted to identify and map Raman spectral signatures of DENV non-structural protein from four different virus serotypes. In first set of experiment, the SERS fingerprints of purified DENV NS1 proteins on an Aluminium foil substratum were generated independently, which shows different signature spectrum peaks for the NS1 proteins from each serotype. Results also indicate that the NS1 protein from the different serotypes could be identified in a mixture of serum proteins. The signature peaks of NS1 represent unique peaks that stands out in a cocktail of serum protein even at high concentration of serum proteins. Two-fold serial dilution gives the highest resolution spectrum with characteristic peaks corresponding to NS1 protein.

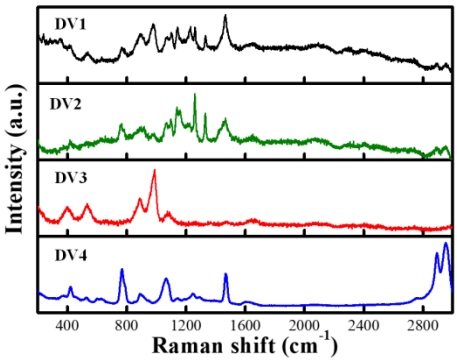

Fig. 2: SERS spectrum of purified DENV NS1 from different serotypes (5µg protein adsorbed on Al Substrate for each spectra).

Gene expression profiling of vascular endothelial cells infected with DENV 2 virus and viral NS1 protein

***Electron tomography imaging of blood platelets*:** 3D electron tomography imaging of whole mount blood platelets can provide significant clues towards understanding activation physiology. Earlier studies from our laboratory had shown that purified DENV NS1 protein can activate platelets *in-vitro* and to understand these findings at an ultrastructural level 3D electron tomography experiments were carried out on normal platelets. Whole mount platelets were negative stained and imaged using low dose electron beam and data collected on an automated tomography platform through ± 55^{o} tilt range. Rebin factor corrected software codes were developed using Inspect 3D software (FEI, Netherlands) and 3D volume reconstructions done on an offline AMIRA workstation. Preliminary results could achieve resolution of alpha and deep granules (Fig. 3) and further studies are ongoing.

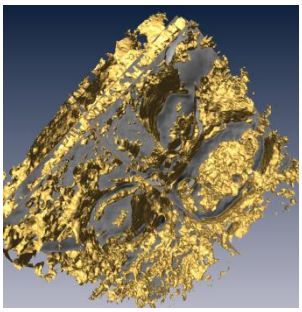

Fig. 3: A representative 3D volume reconstruction of human blood platelet using electron tomography.

Human resource developments

Ms Nitali Tadkalkar, senior research fellow (CSIR) received her doctorate degree PhD in Biotechnology from the Savitribai Phule University, Pune. Mr Girish and Ms Vaishali completed their MSc dissertation projects. Online workshops were carried out on electron microscopy principles and applications training for ICMR EM lab personnel.

Other activities

ICMR Electron Microscopy Consortium (IEMC)

The IEMC was created with an objective to bring all EM laboratories of ICMR institutions on in one platform, pool resources, develop manpower, standardize operational protocols, troubleshoot technical and instrumentation issues and develop into zonal resources for ICMR for electron microscopy application in biomedical research. These activities have been initiated under ICMR initiative on infrastructure development. In the period 2021-22 the following activities were carried out.

Online meetings to identify issues, define areas of application.

Basic understanding of the principles and operations of EM

Protocol and SOP developments

Site visits

ENCEPHALITIS GROUP

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Ph.D. Students

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Mrs. Sonal Sujit Bhansali

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

Investigators: Bondre VP

Contributors: Mahamuni SA, Sakhare KS, Sankararaman V, Butte DK

Funding: Intramural **Duration:** Ongoing Institutional Service Project

***Background*:** Acute encephalitis syndrome (AES) is an important cause of morbidity, mortality and permanent neurologic sequelae in affected population globally. Etiological identification of the cause helps in appropriate timely treatment and performing prevention and control as well as surveillance activities. In India, neurotropic arboviruses, herpesviruses, and members of Rhabdoviridae family are known to be primary cause of AES with occasional association of other viruses including the entero, orthomyxo, paramyxo, and adenoviruses. Since CNS is largely affected during AES, independent of the cause the clinical symptoms are largely similar making its clinical diagnosis difficult. Japanese encephalitis (JE) is established to be a major cause of AES in India along with non-JE causes. To support the public health systems AES outbreak investigations became one of the major responsibilities of the Group. In addition, the Group also support the entomologic investigations in keeping track of various arthropod borne viruses capable of causing neurological manifestations. All referred / outbreak cases are investigated for JEV infection and the non-JE cases are investigated for cause (Chandipura virus, other neurotropic members of *Flaviviridae* and *Herpesviridae* families, measles, mumps, rubella and other bacterial pathogens including rickettsiae) based on the demographic and clinical details received, using appropriate serological and molecular methods. A subset of these samples also processed for virus isolation and genomic characterisation to study their molecular evolution.

***Objectives*:** Investigation of AES cases and disease surveillance.

Findings:

a) **Investigations and surveillance on AES**

During 2021-2022, 877 (CSF=452; sera=425) clinical specimens from 574 suspected AES cases referred from different parts of the country were investigated for common AES associated viral etiologic agents. JE IgM antibodies were detected in 08 cases (1.4%) while specimens from 109 cases showed indeterminate results in JEV IgM MAC ELISA. Investigation of these samples at the Dengue & Chikungunya Group identified 26 (23.85%) cases to be positive for dengue IgM antibodies. These findings clearly indicate an increasing trend of neurological complications in dengue infection and necessity to improve upon the specificity of JE IgM Kit. AES cases referred from the Chandipura endemic areas were investigated by Chandipura virus (CHPV) specific RT-PCR and IgM ELISA. CHPV RNA was detected in sera of 09 (4.89%) cases while anti-CHPV IgM antibodies were detected by ELISA in an additional 03 cases.

Depending on the clinical recommendations, seasonality, age group affected and geographic region of specimen source, CSF available from undiagnosed cases (n=361) were investigated by molecular diagnostic assays for non-JE, non-CHPV and non-dengue AES associated etiologies. These investigations identified HSV-1 infection in 05 cases and human enterovirus infection in 02 cases as confirmed by sequencing. Investigations performed for diagnosis of West Nile and other flaviviruses, Human herpesviruses 2 & 6, Cytomegalovirus (CMV), Epstein Barr virus (EBV), Measles, Mumps, Rubella, Varicella Zoster virus (VZV), *Orientia tsutsugamushi* (OT) and Spotted Fever Group (SFG) *Rickettsia* yielded negative results.

Surveillance for JEV and CHPV transmission in endemic areas:

Plaque Reduction Neutralization Tests (PRNT) were performed on 92 animal samples referred from Chandrapur and Aurangabad regions of Maharashtra to detect anti-JEV and anti-CHPV neutralizing antibodies. Thirty out of the 72 samples (41.6%) processed for JEV PRNT demonstrated positive results. Anti-JEV neutralizing antibodies were detected in blood of buffalos (10/16), bulls (1/1), cows (14/18), dogs (2/14), donkey (1/1), goats (1/1), horse (1/1), and pigs (2/20). Anti-CHPV neutralizing antibodies were not detected in any of the animal blood samples. Horse tissue samples referred from Gujarat state tested negative for JEV and other flaviviruses by PCR. As part of routine surveillance activity, sand fly pools (S*ergentomyia* species) were referred from Kheda district of Gujarat for investigation of CHPV infection by RT-PCR. In addition, mosquito pools were also received from Maharashtra and Madhya Pradesh and tested negative for JEV, other flaviviruses, Bunyavirus, Nairovirus, and Alphavirus in RTPCR assays.

Virus isolation attempts from human samples

Virus isolation is often considered to be the gold standard in viral diagnostics. Human clinical samples which test positive in PCR assays are processed for virus isolation, along with a subset of PCR negative samples. During this period, all 16 PCR positive samples were processed for isolation. Two isolates of CHPV were obtained (one each in Vero and BHK-21 cell lines); both samples were from Gujarat. Genomic characterization on these isolates is in progress.

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

Different members of *Flaviviridae* family like JEV, WNV, Dengue, Kyasanur forest disease virus, etc. are predominantly circulating and are known to be associated with AES in different parts of the country. To support the molecular diagnosis of neuroviral infections, a new set of primers from the conserved NS5 region of flaviviruses designed and analyzed *in silico* to detect AES associated flaviviruses. Conventional nested RT-PCR was developed and standardized to detect different genetic variants of flavivirus strains available in the laboratory (Fig. 1). RT-PCR specifically detected different genetic variants of JEV including the genotype III strain 733913

(Human, WB, 1973), 057434 (Human, UP, 2005), genotype I strain 0945054 (Human, UP, 2009); West Nile virus lineage 1 strain 68856 (bat, India, 1968), lineage 5 strain 804994 (human, Karnataka, 1980), the lineage 2 Wrangler strain UGA (human, Uganda) and the Bagaza virus strain. Nested PCR further amplified these strains specifically. The assay will be used in routine diagnosis after further validation.

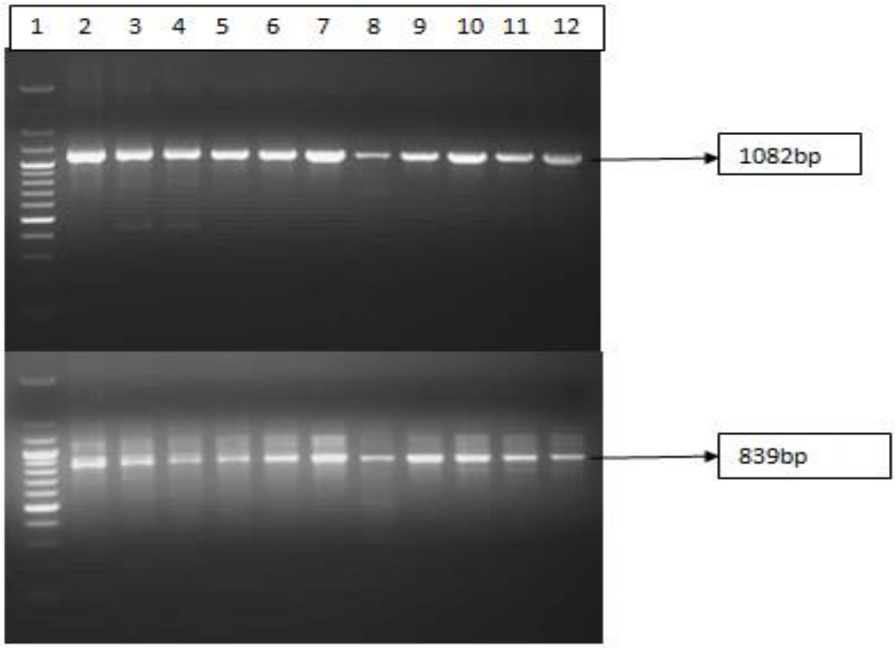

Fig. 1. Universal RT-PCR (upper panel) and nested PCR developed to detect multiple flaviviruses from clinical specimens. Specific amplification of JEV – 733913 (lane 2), 057434 (lane 3), 0945054 (lane 4); West Nile virus – 68856 (lane 5), 804994 (lane 6), Wrangler strain UGA (lane 7); Dengue virus – serotype 1 (lane 8), serotype 2 (lane 9), serotype 3 (lane 10), serotype 4 (lane 11); and Bagaza virus (lane 11).

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

PI: Bondre VP and Mali DN. Duration: 2016-2021

Project Completion Report

Current thrust area in JE research focuses on the host-virus interaction at the molecular level to unravel the infection regulating pathways, which can be further explored to devise therapeutic strategies. JEV infection begins with the specific receptor mediated entry which is a multifaceted process governed by conformational changes in the viral and host cell membrane that result in successful delivery of virus nucleocapsid for further synthesis. The fusion process triggered by change in pH is a necessary step for productive infection. The membrane fusion is

initiated with the sensing of a low pH environment by viral membrane, which further dictates the process of viral membrane conformational changes and fusion with endosomal membrane. In enveloped viruses, Histidine (His, pKa ~6.5) residues plays key role in fusion as its side chains titrate with a pKa in the ~ 5-7 range which can respond to the alterations in pH conditions. JEV GI E protein contains 13 His residues distributed throughout the sequence; of which 4 His residues are conserved among all the members of *Flaviviridae* family while 5 are conserved among the members of JEV-serocomplex, suggesting some of these must undergo protonation against altered pH triggering the membrane fusion process. Identification of the viral factors governing host-virus interaction during virus entry will allow development of therapeutic agents targeting virus entry pathway. Accordingly, this study was carried out to identify role of conserved His residues in host cell – viral membrane fusion that governs the entry process.

This study performed to identify the specific His residue on JEV E protein instrumental in triggering the membrane fusion process during virus entry. Selection of the five most interactive His residues (Flavivirus conserved 3 His and JEV sero-complex conserved 2 His residues) was performed through assessment of hydrogen bonding pattern with neighboring residue allowing its protonation in low pH environment. Cloning prM-E coding cassette along with nucleocapsid C-terminal signal sequence and its stable expression in mammalian cells resulted in extracellular expression of JEV E protein in the form of virus like particles (VLPs) biologically mimicking the parental virus as established through IFA, WB, antigen capture ELISA and TEM analysis. Molecular interaction between reporter labelled (DID, a lipophilic fluorescent dye) parental JEV and VLP with mammalian cells using cellular organelle specific labelled antibodies established that JEV GI enters the mouse neuroblastoma cells (Neuro 2A cells mimicking CNS) via caveolin-mediated endocytosis accomplishing the membrane fusion process within 30 min of exposure. Furthermore, site-directed mutagenesis of the individual His residues in VLP construct resulted in generation of VLPs genetically altered for different His residues. Comparative studies on membrane fusion process of parental JEV, native VLP and five different VLPs individually mutated for different His residues in Neuro2A cells identified important role of E His 319 in membrane fusion. The process was completed within 30 min of infection with parental JEV and exposure with native as well as 4 VLPs mutated for H144A, H246A, H395A and H397A resulted in fusion. However, membrane fusion does not occur in case of VLP mutated with H319A even after 90 min of exposure indicating its role in triggering the process upon protonation in low pH environment. The study suggests that JEV E His 319 might be instrumental in sensing low pH in endosome where it undergoes protonation that triggers the conformational changes in JEV E protein and host membrane to accomplish the process of membrane fusion and delivery of nucleic acid for viral synthesis. The knowledge generated in this study can be applied to other JEV strains and related flaviviruses and targeted to plan research on developing antivirals hampering/abolishing the virus infection and further pathological outcomes in JE cases.

To determine the antiviral activity of viral RNA-dependent RNA polymerase inhibitors against Chandipura virus infection

Investigators: Bondre VP,
Contributors: Pavitrakar DV, Deshmukh GD and Sakhare KS
Funding: Intramural **Duration**: 2020-2022

***Background*:** Chandipura virus **(**CHPV) is a well-known cause of an acute neurological illness with high case-fatality rate (CFR ~75%) in pediatric population. Upon re-emergence of CHPV with epidemic potential in 2003, it has been associated with several outbreaks and annual sporadic cases in central India. In the absence of a protective vaccine and effective therapeutic measures, it causes high morbidity and mortality in the endemic region. Currently, development of broad-spectrum antiviral therapies is gaining considerable research attention as it provides protection against a range of viruses while mitigating risks of resistance and reducing costs associated with drug development. RNA-dependent RNA polymerases (RdRps) from different families of viruses are highly conserved in their structural and functional features. Nucleoside analog polymerase inhibitors are the most common antivirals among the licensed antivirals and those, which are undergoing clinical trials against different viral diseases. Due to their broad spectrum of activity, the RdRp inhibitors have been most successfully repurposed directly acting antivirals. This study carried out to explore the potential of approved RdRp inhibitors for treatment against CHPV infection. Our earlier studies established the limited inhibitory potential of Ribavirin (EC50 = 2147µM against CC50 = 10,000µM) and Galidesivir (EC50 = 300 µM against CC50 = 600 µM) CHPV infection at higher concentrations but below the cytotoxic concentrations in Vero cells. On contrary, Remdesivir did not exert any inhibitory effect on CHPV infection in Vero cells as the inhibitory potential exceeds the cytotoxic concentration. Favipiravir estimated to be a promising drug against CHPV infection in Vero cells as it inhibited virus multiplication to 50% (EC50) at 20 µg as compared to the 50% cytotoxic concentration (CC50) of 750µg. In vitro studies on molnupiravir (RdRp inhibitor) and in vivo studies on favipiravir performed to estimate protective efficacy against CHPV infection.

***Objective*:** To study the antiviral potential of Favipiravir and Molnupiravir RdRp inhibitors against CHPV infection using *in vivo* and *in vitro* systems.

Findings:

Effect of time of initiation of Favipiravir in CHPV infection:

The *in vitro* growth kinetics of CHPV studied in Vero cells (infected with 0.01 MOI) after addition of Favipiravir at 0, 4 and 8 hours post-infection (PI). Favipiravir treatment of cells simultaneously with CHPV infection showed complete inhibition of viral infectivity at of 320µM and higher concentrations. Significant reductions in virus titer (3 and 5 log reduction) documented in CHPV infected cells treated with 80 and 160 µM Favipiravir. Treatment with 160

μM and higher concentrations after 4 hrs of infection showed 2-2.5 log reduction in virus titer at 18 hrs PI (Fig. 2). Addition of drug at 4 and 8 HPI did not exert complete inhibitory effect on CHPV replication independent of the drug concentration added.

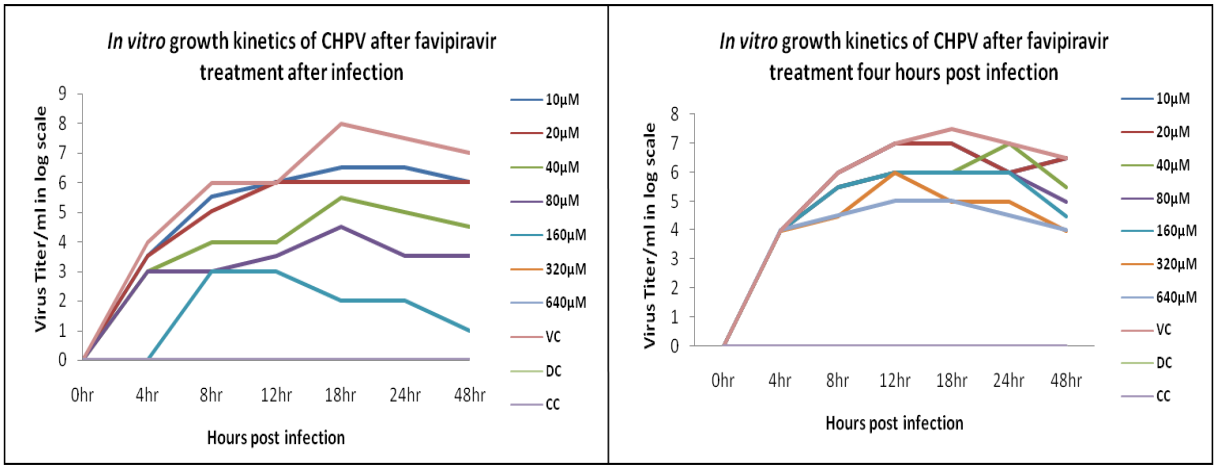

Fig. 2: Effect of time of drug treatment on growth kinetics of CHPV.

In vivo antiviral activity of Favipiravir:

Ten-day-old CD1 mice inoculated with 10000 PFU of CHPV by intraperitoneal route were treated with low (100mg/kg/day), medium (300mg/kg/day), and high (600mg/kg/day) oral doses of Favipiravir for seven days. The 100mg/kg/day dose showed 60% protection (6/10 mice survived), while 300mg and 600mg doses showed 100% protection till 21st day post infection (PI). The untreated group succumbed to infection by 6th day post-infection. Blood from survived animals showed presence of anti-CHPV neutralizing antibodies with ND_{50} titer of 8807, 9936 and 90 in animal groups receiving 100mg, 300mg and 600mg doses, respectively. CHPV was un-detectable in brains harvested from survived mice in 300mg/kg/day dose group, while a CHPV titer of $10^{7.3}$ PFU/ml was detected in brains of untreated mice harvested on day 4 PI. This study emphasizes the potential utility of Favipiravir in the treatment of CHPV infection.

Anti-CHPV activity of molnupiravir in Vero cells:

The antiviral activity of molnupiravir against CHPV infection was studied in Vero cells. The 50% cytotoxic (CC_{50}) and 50% effective (EC_{50}) concentrations were determined to be 5000μM and 3000μM, respectively. However, a gradual reduction in CHPV plaque size detected at 1000μM and higher concentrations (Fig. 3). Growth kinetics of CHPV in presence of molnupiravir (EC_{50} concentration) added simultaneously with infection and four hours post - infection showed 3.5 and 1 log reduction in virus titer at 18hrs of incubation, respectively (Fig. 4).

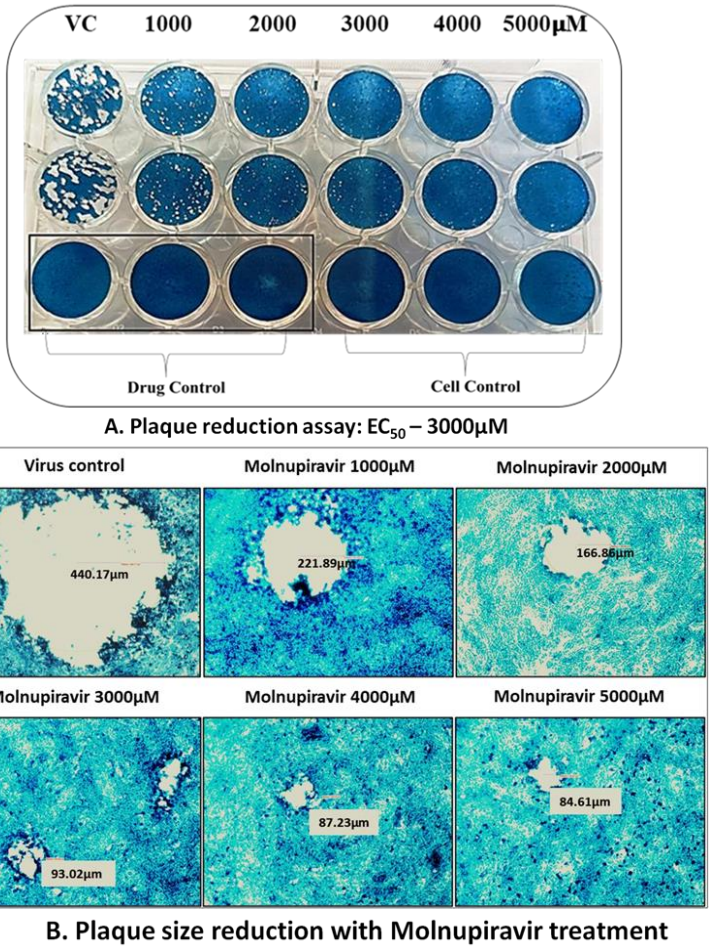

Fig 3:.Panel 1) Plaque reduction assay to determine the $EC_{50.}$ (Panel 2) Effect of Molnupiravir on CHPV plaque size in Vero cells.

The RdRp inhibitors interfere in viral RNA synthesis which is further translated to viral encoded proteins. Inhibitory effect of molnupiravir on CHPV was further established by evaluating the viral protein expression in infected cells treated with drug by IFA using CHPV monoclonal antibodies. Reduction in the viral protein expression was evident with the increasing concentrations of molnupiravir in Vero cells (Fig. 4). These findings clearly suggest interference in viral synthesis by molnupiravir but complete inhibitory effect was not exerted below the cytotoxic concentration in Vero cells.

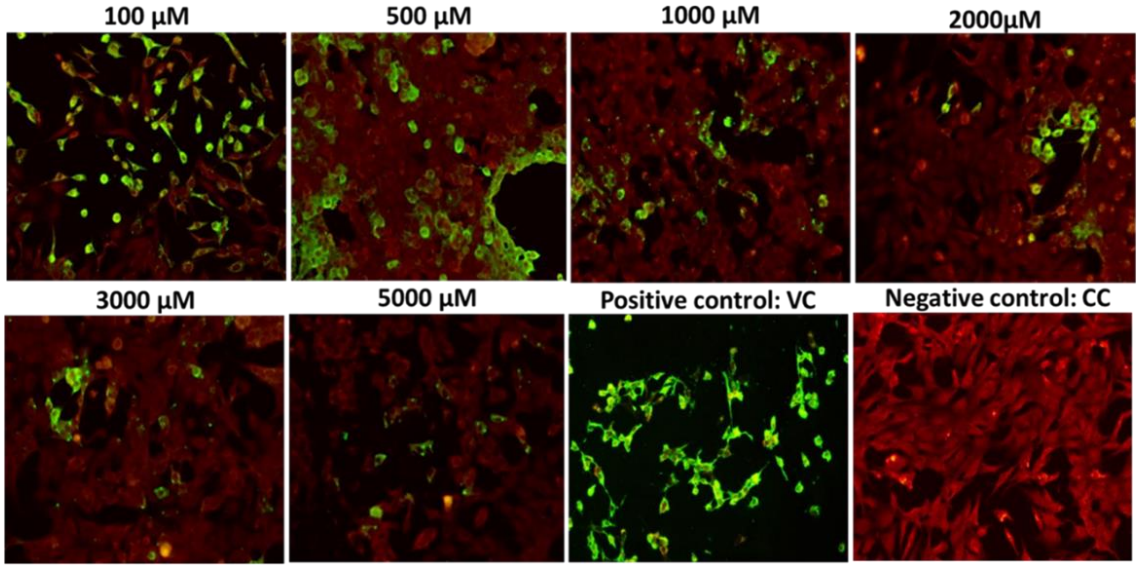

Fig. 4: Effect of molnupiravir treatment on expression of CHPV proteins in infected Vero cells.

Expression of Japanese encephalitis virus-genotype 1 envelope and non-structural proteins to explore in early diagnosis

Investigators: Bondre VP

Contributors: Pavitrakar D, Sankararaman V, Mali DN

Funding: Intramural **Duration**: 2020-22

***Background*:** WHO recommends JEV IgM ELISA as front line diagnostic assay, which mainly rely on antibodies generated against envelope protein. However, co-circulation of flaviruses sharing antigenic cross reactivity poses a major difficulty in JE diagnosis based on detection of IgM antibodies. The existing JE MAC-ELISA makes use of inactivated cell culture grown virus as antigen, which may exhibit low specificity resulting in false positive results in cases reported from areas where the population is frequently exposed to multiple flaviviruses. Since, the flavivirus cross reactive antibody response is mainly directed against the conserved epitopes located at fusion loop of E protein, replacement of the whole virus antigen by purified E glycoprotein mutated for the flavivirus cross reactive epitope thus not only eliminates the associated bio-safety risk but also minimizes the assay cross reactivity. Earlier data generated using virus-like particles (VLPs) generated from JEV genotype I strain as an antigen showed to increase specificity of the than the existing whole virus antigen used in JE MAC ELISA Kit.

***Objective*:** Development of flavivirus conserved cross-reactive epitope (fusion loop) mutation in JE-VLP to explore its utility as specific antigen in MAC-ELISA.

Findings:

a) Recombinant JEV E protein with mutations in fusion loop as an antigen:

The JEV PrM-E protein coding gene cassette genetically altered in the conserved flavivirus cross-reactive epitope residues located in the hairpin loop of E fusion peptide. Using site directed mutagenesis; mutations in E protein residues 106 G-K (GGG-AAG) and AA107 L-D (CTT-GAT) located on the fusion loop generated in the VLP construct. Culture supernatant from single cell clones expressing modified E protein tested in JEV MAC ELISA using JEV specific detector monoclonal antibody (HS-3). Flavivirus cross-reactive MAb (HX-2) failed to detect the JEV fusion loop mutated E protein by IgM ELISA and IFA (Fig. 5). Even though the VLPs mutated for 106 G-K and 107 L-D residues did not react with flavivirus cross-reactive antibodies, JEV specific MAb (HS-3) specifically detected the extracellularly expressed mutated VLP by ELISA and IFA using HS-3 (Fig. 5).

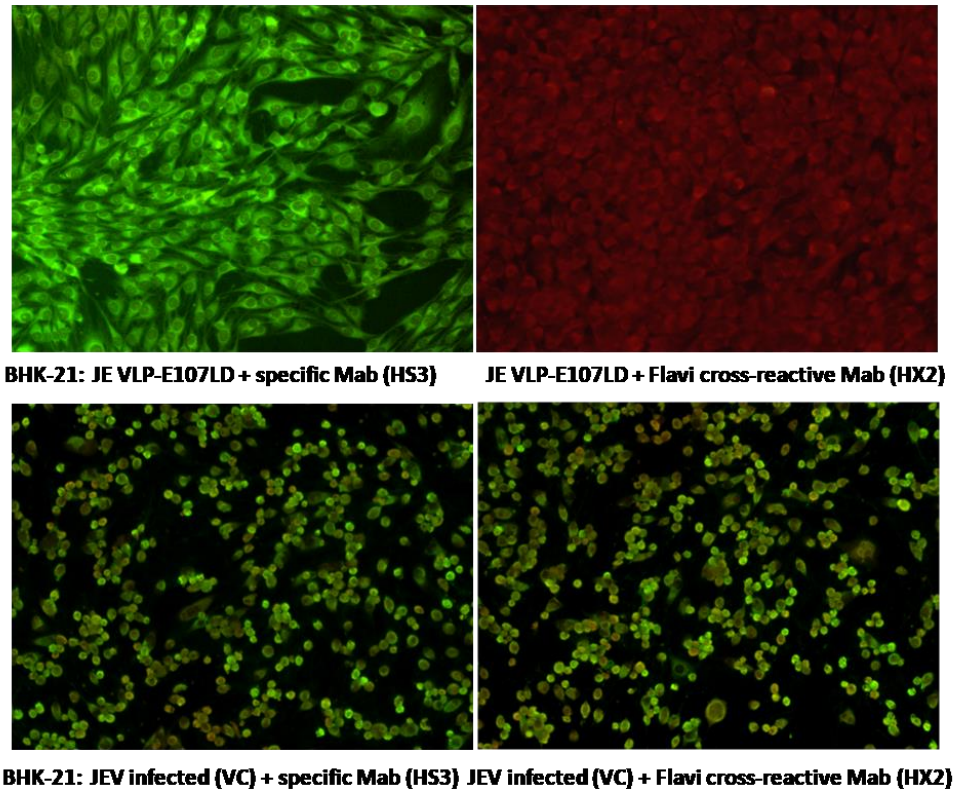

Fig. 5: Inhibitory effect on JEV VLP mutated for 107L-D residue on binding with Flavivirus-cross-reactive Mab (HX-2) as against JEV specific Mab (HS-3).

Specificity and sensitivity of the newly developed JE VLP-IgM ELISA employing VLP as an antigen and JE mE-IgM ELISA employing L107D residue mutated VLP as antigen along with JEV specific HS-3 MAb as detector antibody were evaluated using a panel of JE IgM positive

(n=37), equivocal (n=15) and negative (n=39) specimens tested by conventional JE IgM ELISA. The OD values obtained for the standard kit controls in the assays were comparable. Among the 37 JE IgM positive samples, both the newly developed assays yielded positive results only for 26 samples. Among the 37 JE positive samples, JE VLP-IgM ELISA yielded negative results for 7 while JE mE-IgM ELISA yielded negative results for 6 samples. The negative results obtained for 6 samples by JE mE-IgM ELISA were further confirmed by the commercially available InBios JE MAC-ELISA kit. In both the assays 4/37 and 5/37 samples, respectively, turned to be equivocal (Table 1). Among the 15 JE IgM equivocal samples identified by conventional JE IgM ELISA, 2 tested as positive in both (JE VLP-IgM ELISA and JE mE-IgM ELISA, while 6 and 7, respectively, tested negative, and 6 and 7, respectively, remained as equivocal.) Similarly, among the 39 negative samples detected by conventional JE IgM ELISA, 2 and 1 samples, respectively, tested equivocal due to slighter change in OD values. The overall results indicated a slight enhancement of assay specificity with the use of recombinant antigen (as against the whole virus) and the JEV specific Mab HS-3 as the detector antibody.

Table 1: Comparative analysis of suitability of different antigens in JE MAC ELISA.

Conventional JE IgM ELISA kit	Standard panel	JE VLP-IgM ELISA			JE mE-IgM ELISA (L107D mutation)		
		Positive	Negative	Equivocal	Positive	Negative	Equivocal
Positive	37	26	07	04	26	06**	05
Equivocal	15	02	06	07	02	07	06
Negatives	39	00	37	02	00	38	01

** - Confirmed to be negative by commercially available InBios JE MAC-ELISA kit.

Future Directions:

Mutation of the E-107LD did not participate in reducing antigenic cross reactivity as compared to the parental native VLP. Efforts will be made to introduce mutations in E protein residues E101, E106 and E107. The mutation established to reduce cross reactivity in MAC-ELISA will be introduced in JEV infectious cDNA clone to further explore it as a specific antigen. VLP based ELISA established in this study will be further evaluated using clinical specimens from lab-confirmed JE cases in the Gorakhpur (UP) through a collaborative effort.

Development of an indirect ELISA assay for surveillance of Japanese encephalitis

Investigators: Bondre VP
Contributors: Shubhangi Mahamuni, Datta Butte, Mali DN
Funding: Intramural Duration: 2021-23

***Background*:** JE is currently endemic in 24 countries, which are implementing JE immunization program either nationwide or targeting the risk group population in endemic areas. In India, JEV vaccination program covers only high risk and sentinel areas. To assess the immunity levels post infection or sero-conversion post vaccination, several serological methods are used to study antibody responses to JE. However, antibody measured by HI, ELISA, and IFA do not correlate with protection while the neutralizing antibodies (NAbs) correlate with protection offered by vaccination or immunity acquired through natural infection. Although the neutralization test (NT) is the most specific measure of antibody, it essentially involves the risk of handling infectious virus, as it has to be grown on a cell monolayer. Final readout of NT is the number of plaques reduced upon mixing of the titrated virus with serum containing NAbs, and this reduction in plaques measures the antibody titre. The assay needs lab infrastructure and expertise to handle and grow the virus making it cumbersome, time consuming and limited to apex labs. Detection of IgG antibodies is measure of pre-exposure of population either through vaccination or through natural infection. IgG ELISA assay enables the detection of JE specific IgG antibodies, which helps in determining their prevalence in human populations. The assay can be performed even in primary health settings, which will helpful for JE surveillance activity in endemic regions. It helps in assessment vaccination impact in population, efficacy of vaccination and prevention measures implemented.

***Objective*:** Development IgG ELISA to detect exposure to JEV in population.

Findings: The JEV GI 1601136 (Human, India, 2016) isolate specific antigen was prepared to develop an indirect ELISA to detect anti-JEV IgG antibodies in convalescent sera tested to be positive by plaque reduction neutralization test (PRNT). The OD values of negative controls lies <0.13 while in IgG positive cases the OD exceeded 0.35 and above. The newly developed JE IgG ELISA was evaluated using 62 archived convalescent sera collected from JE IgM positive hospitalized cases from the endemic region. Among them 36 (58%) sera tested positive for IgG ELISA; 45 (72.6%) tested positive by PRNT (titre >20) with 30 sera positive in both tests while 11 (18%) sera tested negative by both the assays. Furthermore, investigation of 21 convalescent sera referred from suspected AES cases from non-endemic areas yielded IgM positive results in 5 (24%); PRNT positive results in 6 (28.6%) with 4 positive common in both assays. In areas where multiple flaviviruses co-circulates, antigenic cross-reactivity among them poses difficulty in diagnosis often leading to false positive findings. Hence, the newly developed JE IgG ELISA was evaluated for specificity using a panel of 25 dengue PRNT positive sera (kindly shared by DRF Group). However, almost 88% dengue PRNT positive sera yielded positive results in JE IgG ELISA (Table 2). The comparative results obtained by JE IgG ELISA and PRNT clearly indicates the limitations of the newly developed JE IgG assay for detection of exposure to JE in endemic areas. Furthermore, false positive results obtained with sera collected from dengue-exposed population suggest that the JE whole virus based antigen suffers antigenic cross reactivity.

Table 2. Comparative analysis of the newly developed JE IgG LISA using sera from JE and Dengue exposed population.

Comparison of JE IgG ELISA versus PRNT									
Samples	Sera tested	JE IgG ELISA		PRNT		Common + ve by both	IgG +ve PRNT negative	PRNT +ve IgG negative	Negative by both
Archived sera (endemic region)	62	36 Positive	26 Negative	45 Positive	17 Negative	30	06	15	11
Convalescent serum samples from suspected cases	17	4 Positive	13 Negative	5 Positive	12 Negative	04	00	01	12
Dengue PRNT positive Sera	25	22	03	25	00	22	00	03	00

We recently generated JE virus like particles and genetically altered them to minimize antigenic cross-reactivity strongly elicited by the flavivirus conserved epitope located at the fusion loop of E protein. Applications of the recombinant VLP with mutations introduced in immune-dominant flavivirus cross-reactive epitope will be evaluated using the standard panel of sera from JE and Dengue cases. Furthermore, these findings will be compared with global standard panel of sera obtained from NIBSC.

Establishment of a Rabies Diagnostic and Research Laboratory in ICMR-NIV.

Investigators: Ullas PT, Bondre VP, Chandhu Balachandran

Funding: Intramural **Duration:** Ongoing Institutional Service Project

***Background*:** Molecular and immunofluorescence assays for rabies diagnosis were previously established and validated in the laboratory, and steps were initiated to establish a rabies virus neutralization assay.

***Objectives*:** Establishment of Rabies diagnosis and research center at ICMR-NIV.

Findings: During the period, Rapid Fluorescent Focus Inhibition Test (RFFIT) for detection of rabies virus neutralizing antibodies (RVNA) successfully optimized. Cell culture adapted standard rabies virus reference strain (PV3462) obtained from Pasteur Institute of India,

Coonoor, used to prepare virus stocks in BHK-21 cell line. RFFIT assay standardized using BHK-21 cells, using pre- and post-vaccination serum samples from staff volunteers and a reference anti-rabies serum calibrated against the WHO International Serum Standard. In addition, the full range of molecular and serological assays for laboratory diagnosis of rabies successfully established. Subsequently, an Inter-Laboratory Comparison of rabies diagnostic assays undertaken with the WHO Collaborating Centre for Reference and Research on Rabies at National Institute of Mental Health & Neurosciences, Bengaluru. Coded sets of samples received from them tested and reported 100% concordance in test results for DFAT, RTPCR and RFFIT assays. Subsequently, the fully established Rabies Laboratory was commissioned at the Encephalitis Group of ICMR-NIV, by Prof. Dr. Balram Bhargava, Hon. Secretary, Department of Health Research & Director General, ICMR, on 28th September, 2021 (the 15th World Rabies Day).

Upon its establishment, Rabies Laboratory began to support the public health system for investigations of clinical cases. Rabies suspected 21 human cases referred from Maharashtra, Madhya Pradesh, Kerala and Jammu & Kashmir were investigated. Fifteen (71.43%) and 04 (19.05%) among these patients had Category III and II bite exposures, respectively, from stray dogs, while the nature of the exposure could not be ascertained in two (9.52%) patients. Five (23.81%) patients had bite exposures on the head/neck. Ten (47.62%) patients had received at least a few doses of rabies vaccine, while 07(33.33%) did not receive any rabies vaccine, and vaccination status was unknown in 04(19.05%) patients. Only 05(33.33%) out of the 15 patients with Category III exposure had received rabies immunoglobulin.

A total of 66 samples (including 16 saliva, 17 CSF, 07 neck skin biopsy, 19 sera, 01 plasma, 05 urine and 01 corneal scraping) were received for testing. Rabies encephalitis could be identified by laboratory testing in 09(42.86%) out of the 21 suspected cases: by positivity for rabies virus RNA alone in 04(19.05%) cases; RVNA alone in 04(19.05%) and for both in 01(4.76%). The overall positivity for rabies virus RNA (by real-time RTPCR and conventional semi-nested RTPCR) was as follows: saliva: 04(25%); neck skin biopsy: 02(28.57%), and CSF: 01(5.88%). A total of 07(41.17%) CSF samples (including 3 sequential samples from one patient) tested positive for RVNA. One serum sample was also received for evaluation of sero-protection following pre-exposure rabies vaccination, and showed protective levels (>0.5IU/mL) of RVNA.

In addition, we also identified a probable case of survival from clinical rabies in a 4-year old male child from Aurangabad, Maharashtra. The child had suffered a Category III bite exposure from a stray dog, and received four doses of rabies vaccine and equine rabies immunoglobulin. He subsequently developed meningoencephalitis, and RVNA detected in his CSF sample (collected on day 3 of illness) in our laboratory. The RVNA titres in CSF subsequently increased 8-fold by day 34, and declined by day 86, which, in correlation with the clinical illness, was highly suggestive of rabies encephalitis. Serial saliva, CSF and neck skin biopsy samples, however, were negative for rabies virus RNA. The child received only intensive and supportive care, and demonstrated substantial clinical improvement during the course of hospitalization. He

was finally discharged from the hospital, albeit with neurological sequelae, and is being monitored at present. A manuscript detailing this case is currently in preparation/review.

Generation of murine monoclonal antibodies to rabies nucleoprotein and exploring their utility in laboratory diagnosis of rabies

Investigators: Ullas PT, Bondre VP
Contributors: Pavitakar DV, Butte DK
Funding: Intramural **Duration:** 2021-2023

***Background*:** Detection of rabies virus nucleoprotein antigen in infected nervous tissue by Fluorescent Antibody Test (FAT) remains the gold standard for laboratory confirmation of rabies. The test requires the use of an anti-rabies nucleoprotein-fluorochrome antibody conjugate, which is currently available from only a few international distributors, and faces a global supply shortage.

***Objectives*:**

1. Development of murine monoclonal antibodies against rabies virus nucleoprotein.
2. Exploring utility of anti-RV N – protein MAbs to develop immunofluorescence- and/or immunohistochemistry-based assay to detect RV antigen in brain tissue from cases.

Findings: BHK-21 cells infected with a standard laboratory 'fixed' rabies virus strain (PV3462) and viral nucleoprotein purified from cell lysate by cesium chloride density gradient ultracentrifugation. Purity and quantity of the protein was assessed by SDS-PAGE. Screening ELISA for detection of IgG antibodies against RV nucleoprotein was developed using the purified protein and validated using mice sera available in the laboratory. Subsequently, four weanling BALB/c mice immunized subcutaneously with 100µg of the purified protein on days 0 (adjuvanted), 14 and 28 (non-adjuvanted). Blood samples collected from the immunized mice by retro orbital puncture on days 14 and 35, and the sera tested for IgG antibodies against RV nucleoprotein using the screening ELISA developed earlier. Low levels of IgG antibodies against RV nucleoprotein detected in sera of 3 out of 4 immunized mice, on days 14 and 35. In order to improve the antibody titer, an additional dose of the purified immunogen was administered to all mice on day 42, and the sera will be tested after 1 more week to identify the best responder to be selected for harvesting of spleen and fusion experiment.

Details of sample testing done for referred human samples

Assays performed for diagnosis of referred AES cases.

S. N.	Tests Performed	Positive / total CSF investigated	Positive / Total Sera investigated
1	JE IgM ELISA	02/452	06/425
2	CHPV IgM ELISA	00/305	03/286
3	West Nile virus IgM ELISA	Not done	00/27
4	Chandipura RT-PCR	00/140	09/184
5	Flavivirus generic RT-PCR	00/155	Not done
6	Herpes simplex virus 1 & 2 PCR	05/361	Not done
7	Enterovirus PCR	02/162	Not done
8	Varicella Zoster virus PCR	00/21	Not done
9	Cytomegalovirus PCR	00/55	Not done
10	Epstein – Barr virus PCR	00/55	Not done
11	*Orientia tsutsugamushi* PCR	00/55	Not done
12	Dengue IgM ELISA (DEN & CHIVK Group)	*JE IgM ELISA Indeterminate sera	26/109*

No. of samples tested for rabies

Tests performed	No. of samples tested (No. of positives)					
	Saliva	CSF	Neck skin biopsy	Urine	Corneal impression	Serum/ plasma
Real-time RTPCR	16 (04)	17 (01)	07 (02)	05 (0)	Not suitable	Not suitable
Rapid Fluorescent Focus Inhibition Test	Not suitable	17 (07)	Not suitable	Not suitable	Not suitable	20 (8)
Fluorescent Antibody Test	Not suitable	Not suitable	Not suitable	Not suitable	01 (0)	Not suitable

Validation of JE IgM MAC ELISA Kits

Validation of 07 batches of IgM Capture ELISA kits for JEV were completed using a standard panel of samples, and the results communicated to Diagnostic Reagent Facility.

JE IgM Kit Validated since April 2019 to August 2020		
Sr. No.	Kit No.	Date of validation
1	21-016	27.04.21
2	21-019	14.06.21
3	21-027	18.08.21
4	21-034	03.09.21
5	21-044	24.09.21
6	21-081	17.12.21
7	22-002	20.01.22

RT- PCR Testing for COVID-19

In addition to the routine activities, Encephalitis Group was entrusted with the responsibility of RTPCR testing for COVID-19 for the staff and students of Pashan Campus, reporting with clinical symptoms/re-joining work after outstation travel. Following an initial training in the National Influenza Centre, the Group started processing nasopharyngeal/oropharyngeal swabs of symptomatic individuals/travel returnees from January 14th, 2022. Until August 10, 2022, the Group processed 632 samples, out of which 114 (18.03%) tested positive for SARS-CoV-2. 62 samples among these were handed over to NIC for genome sequencing and further studies.

ENTERIC VIRUS GROUP

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Hospital based surveillance of enteric viruses and strains in children with acute gastroenteritis (AGE) at ICMR-NIV

Investigators: Mallika Lavania, Pradeep Sawant
Staff: Madhuri Joshi, Manohar Shinde and Nutan Chavan
Funding: Intramural **Duration:** 2020- 2023

Background

Acute gastrointestinal (AGE) infections are a major source of morbidity and mortality in preschool children and lead to approx. 446,000 deaths every year, mainly in low and middle-income countries. The enteric viruses associated with acute gastroenteritis include rotaviruses (RVA, RVB, RVC), Caliciviruses (Norovirus (NoV genogroup I & II) and Sapovirus), enteric adenovirus (AdV), human astroviruses (AstV), aichiviruses, toroviruses, coronaviruses, picobirnaviruses, enteroviruses (EV), and Sali/Klassi viruses.RVA infections are the major cause of severe dehydrating diarrhea in children <5 years old despite the availability of two oral vaccines. In India, RotaVac and ROTASIIL vaccines have been implemented in phased manner in the National Immunization program since 2016. Epidemiological and molecular studies of RVA and non-RVA viral agents would be helpful to define the effect of rotavirus vaccines in diarrhea control.

Objective

- To identify cases of rotavirus among hospitalized AGE patients and determine the circulating genotypes using sentinel hospital-based active surveillance.
- To characterize all enteric viruses through molecular methods to understand their diversity.
- To identify risk factors among AGE cases compared to controls and to evaluate the association of enteric pathogens with AGE

Findings

Investigation of fecal specimens from 89 children hospitalized with AGE at Pune city (Maharashtra) showed viral etiology in 53% (Fig. 1). Predominance of RVA(35%; n=34) with total of nine different G-P types of RVA in circulation was identified. The dominance of the G3P[8] genotype of RVA was observed in 59% specimens. The samples tested for NoV-genogroup I and II by RT-PCR. Nucleotide sequencing and phylogenetic analysis using partial ORF1 and ORF2 regions, confirmed presence of GII.P16-GII-4 genotype of Genogroup II in five [GII P16.GII4 (n=2); GIIP31.GII4 (n=2), GIIP7.GII9 (n=1) and GI (GIP4.GI4) in one specimen. Using hexon gene based PCR five specimens were positive for AdV and their typing using penton region showed presence of Ad 40 (n=2), Ad 41 (n=2) and Ad C (n=1). AstV was detected in two specimens and nucleotide sequencing analysis using partial ORF1 and ORF2 regions confirmed presence of HAstV-1 genotype. Dual infection was observed in two specimens with

RVA and AdV and one with RVA, NoV and AstV. Overall, the study highlights dominance of RVA followed by NoV, AdV and AstV. Rotavirus vaccination status was available for 40% patients and among them 44% were vaccinated. Comparison of RVA detection rate in the vaccinated (37%) showed a decline as compared with unvaccinated (55%) group of individuals.

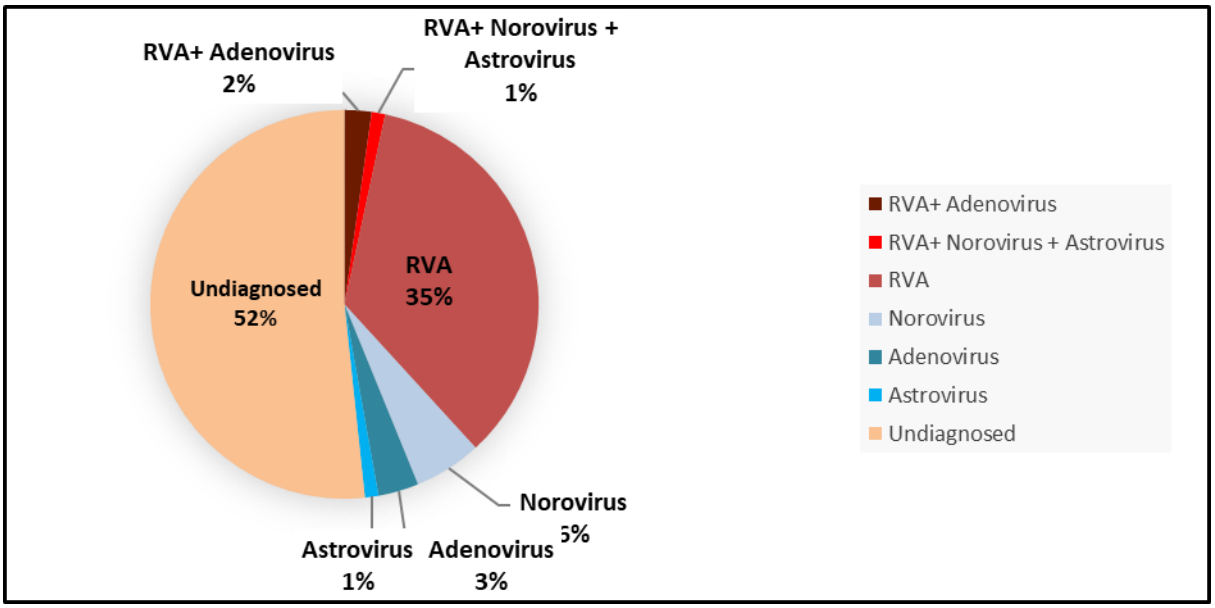

Fig 1: Rotavirus A, Norovirus, Adenovirus & Astrovirus Percent Positivity during April 2021 to March 2022.

Referred samples tested for Enteric Viruses.

Investigators: Mallika Lavania

Staff: Madhuri Joshi, Manohar Shinde and Nutan Chavan

Funding: Intramural **Duration:** Ongoing

Sr. No.	Provisional diagnosis	Type (number) of clinical specimens	Viruses Tested / Result
1	Acute gastroenteritis (AGE) (n=1)	Faecal specimen (n=1)	RVA, Norovirus GI, GII, Adenovirus, Astrovirus, Enterovirus : All Negative
2	Acute diarrhoeal disease (n=2)	Faecal specimen (n=2)	RVA : Positive (n=1) Norovirus GI, GII, Adenovirus, Astrovirus, Enterovirus : All Negative (n=1)
3	Viral encephalitis (n=1)	CSF (n=1)	Enterovirus RT-PCR: Negative

4	Conjunctivitis (n=1)	Eye Swab (n=1)	Enterovirus RT-PCR: Negative
5	Viral Myositis (n=4)	Blood/Serum (n=2) Nasal Swab (n=1) Faecal specimen (n=1)	Enterovirus RT-PCR: Negative
6	Myocarditis (n=3)	Nasal Swab (n=1) Blood (n=1) Endomyocadial biopsy (n=1)	Enterovirus RT-PCR: Negative
7	Acute Febrile illness (n=2)	Nasal Swab (n=1) Serum (n=1)	Enterovirus RT-PCR: Negative
8	Guillain-Barre syndrome (n=28)	CSF (n=10) Serum (n=9) Throat Swab(n=8) Faecal specimen (n=1)	Enterovirus RT-PCR: Negative
9	Viral Panel Study (n=7)	CSF(n=2) Serum (n=1) Throat Swab(n=3) Faecal specimen (n=1)	RVA, Adenovirus, Astrovirus, Enterovirus: All Negative

Evaluation of antiviral agents against Coxsackie virus A-16 (CV-A16) causing hand, foot and mouth disease (HFMD)

Investigators: Mallika Lavania and Sanjay Tikute

Funding: Intramural **Duration:** 2021-2023

Background

In India, earlier HFMD cases were diagnosed based on clinical features and symptomatic conditions. Several cases were reported from Kerala, Maharashtra, Assam, West Bengal, Orrisa and Tamil Nadu. The investigations carried out previously in Enteric Viruses Group revealed that CV-A16 is the major strain circulating in India. Therefore, it is the necessity for medical intervention to treat HFMD disease caused by CV-A16 strains to avoid further severity and any complications. At present there are some antiviral drugs are in trial phase against EV-A71 and CV-A16 strains. In this study attempt will be done by selecting suitable herbal product / extract which will be easily available for the treatment without any side effects.

***Objective*:**

Evaluation of herbal product / extract as antiviral candidate against HFMD caused by CV-A16 infection.

Findings:

This study was initiated by selecting latest (collected in 2018) HFMD positive clinical specimens of CV-A16, confirmed by molecular typing for isolation. Total 30 vesicular swabs were selected for isolation in RD cell line. The isolation attempt was continued till passage number 6. One of the isolates was successfully done from vesicular swab. The isolate was propagated on large scale and $TCID_{50}$ was estimated in RD cell line using 96 well plate. Isolates of CV-A16 from 2013 and 2018 were further confirmed by NGS. Phylogenetic analysis of VP1 gene (891bp) of CV-A16 isolates from 2013 and 2018 were analysed. Both the isolates were clustered and were identified to B1c sub genotypes. Further work for antiviral will be done by using both the fully characterized isolates.

Study on the prevalence of Human Papillomavirus infection and distribution of genotypes in cervical cancer patients from NEIGRIHMS, Shillong from North East India

Co-ordinator: Prof. Priya Abraham
Principal Investigator: Dr. Mallika Lavania
Site PI: Dr. Manika Agarwal (NEIGRIHMS, Shillong)
Co-PI (if any): Dr. Pradeep Sawant (NIV, Pune), Dr. Evarisalin Marbaniang (NEIGRIHMS, Shillong)
Staff: Manohar Shinde and Nutan Chavan
Funding: Intramural **Duration:** 2021-2024

Background

In contrast to developed countries, cervical cancer continues to be an important cause of death in India (Cancer Registry 2017). Most cervical cancers result from human papillomavirus (HPV) infection and therefore are preventable through screening and vaccination. The main viral etiological agent identified in the development of cervical cancer is considered to be the infection with high risk types of HPVs. About 96,922 new cervical cancer cases are diagnosed annually in India and the prevalence of human papillomaviruse (HPV) infection in women with cervical cancer is 83.2% (Human Papillomavirus and related Disease report 2018). More than 140 types of HPV have been identified among these only 40 types are sexually transmitted. Of these, two high risk HPV types 16 and HPV 18 are responsible for more than 80% of cervical cancers in India. In spite of having high burden of cervical cancer, there is no organized cervical cancer screening programme especially in North Eastern states in India. The concept of routine screening of asymptomatic women is almost nonexistent. Pap smear cytology facilities for early cancer detection are available at only selected laboratories in urban areas, although their quality varies widely.

Aim

To investigate the prevalence of high risk and low risk types of HPV infection and their association with cervical cancer and analysis of clinico-pathological characteristics with early cancerous lesions prone to develop cervical cancer from North East India

Findings

Cervical Pap smears were obtained by colposcopy in 25 subjects recruited from Jan-March 2022. Cytological Bethesda classification was coupled with HPV typing. HPV subtypes were assayed by Xpert® HPV system alongside pathological cytology analysis of cervical tissue samples. Eighteen women showed negative for intraepithelial lesion or malignancy (NILM) with inflammation, while six showed atypical squamous cells with negative results of HPV (Table 1). One woman showed positivity for HPV 16 and another participant showed HPV DNA positivity with NILM. Focused rescreening of such cases who are NILM and high-risk HPV DNA positive will be done at the interval of every three months.

Table 1: Cervical cytological grades in HPV infected women.

Cytological findings	Frequency of types (n=25)		
	HPV-16	HR [3]	HR [4]
NORMAL	1	1	1
ASCUS	0	0	0
HSIL	1	0	0
Total	2	1	1

Number of samples tested

Sr. No.	Viruses Tested	No. of Samples Tested	Total Positive
1	COVID-19	492	308
2	HPV	25	4
3	Rotavirus A (RVA)	120	36
4	Norovirus Genogroup GI	124	2
5	Norovirus Genogroup GII	124	6
6	Astrovirus	125	2
7	Adenovirus	125	5
8	Enterovirus	83	-

List of Outbreaks investigated

Sr. No.	Outbreak Area	Type (Number) of specimens	Viruses Tested / Result
1	Tirupati AP, Nov 2021	Faecal specimen (n=4)	RVA, Norovirus GI, GII, Adenovirus, Astrovirus, Enterovirus : All Negative
2	Bengaluru, Dec 2021	Faecal specimen (n=8)	RVA, RVB, RVC, Norovirus GI, GII, Adenovirus, Astrovirus, Enterovirus : All Negative with single exception of one specimen positive for RVA

Details of investigations on outbreak in Tirupati & Bengaluru

Investigators: Mallika Lavania and P. N. Sorna
Staff: Madhuri Joshi, Manohar Shinde and Nutan Chavan
Funding: Intramural **Duration:** 2021-2022 (Ongoing)

Background

Outbreak samples were sent from Tirupati, Andhra Pradesh & Bengaluru Karnataka to test for Enteric viruses in November & December 2021 respectively. Cases presented with symptoms of diarrhoea, vomiting, abdominal pain and decreased Urine output.

Objective

To investigate the viral etiology.

Findings

A total of four (Male n=3, Female n=1) faecal specimens were received from Tirupati & eight (Male n=4 , Female n=4) from Bengaluru. None of the samples from Tirupati tested positive for any of the enteric viruses.

ENTOMOLOGY GROUP

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Metagenomics analysis of viromes of *Aedes*mosquitoes in India (In continuation to last year's report)

Investigators: Dr. AB Sudeep, Dr. Sarah Cherian & Dr. Kavita Lole

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

***Background**:* Mosquitoes harbor a large number of insect specific viruses (ISV) that play important roles in altering the susceptibility of the mosquitoes to pathogenic viruses either by enhancing or inhibiting virus replication. Also, there is a potential threat of these ISVs evolving into human pathogens by genome alterations. Recent studies have shown abundance and diversity of arthropod-associated RNA viruses, demonstrating the role of arthropods in viral evolution, potentially serving as hotspots where ISVs have evolved into dual-host viruses.

Objectives: To study mosquito viromes of *Aedes* collected 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: The study revealed predominance of Phasi Charoen-like virus (PCLV, Family: *Phenuviridae*) contributing to >60% of the total reads in *Aedes aegypti* mosquitoes collected from Pune district of Maharashtra using next generation sequencing based metagenomic analysis of viromes. Similar results were also obtained with mosquitoes from Assam, Tamil Nadu and Karnataka. Sequence comparison of Pune mosquito sequences with PCLV Rio (Brazil) isolate showed 98.90%, 99.027% and 98.88% homologies in the S, M and L segments respectively indicating less genetic heterogeneity of PCLV (Fig 1). The study also demonstrated occurrence of transovarial transmission of PCLV in *Aedes aegypti* mosquitoes as PCLV viromes were detected in F_1 generation eggs, larvae, pupae and male mosquitoes. *Ae. aegypti* mosquitoes collected from Pune also showed a large number of reads for viruses belonging to *Baculoviridae, Rhabdoviridae, Genomoviridae* and *Bunyaviridae* families. The role of PCLV in the replication of dengue and chikungunya virus in *Ae. aegypti* is yet not clear and needs further studies.

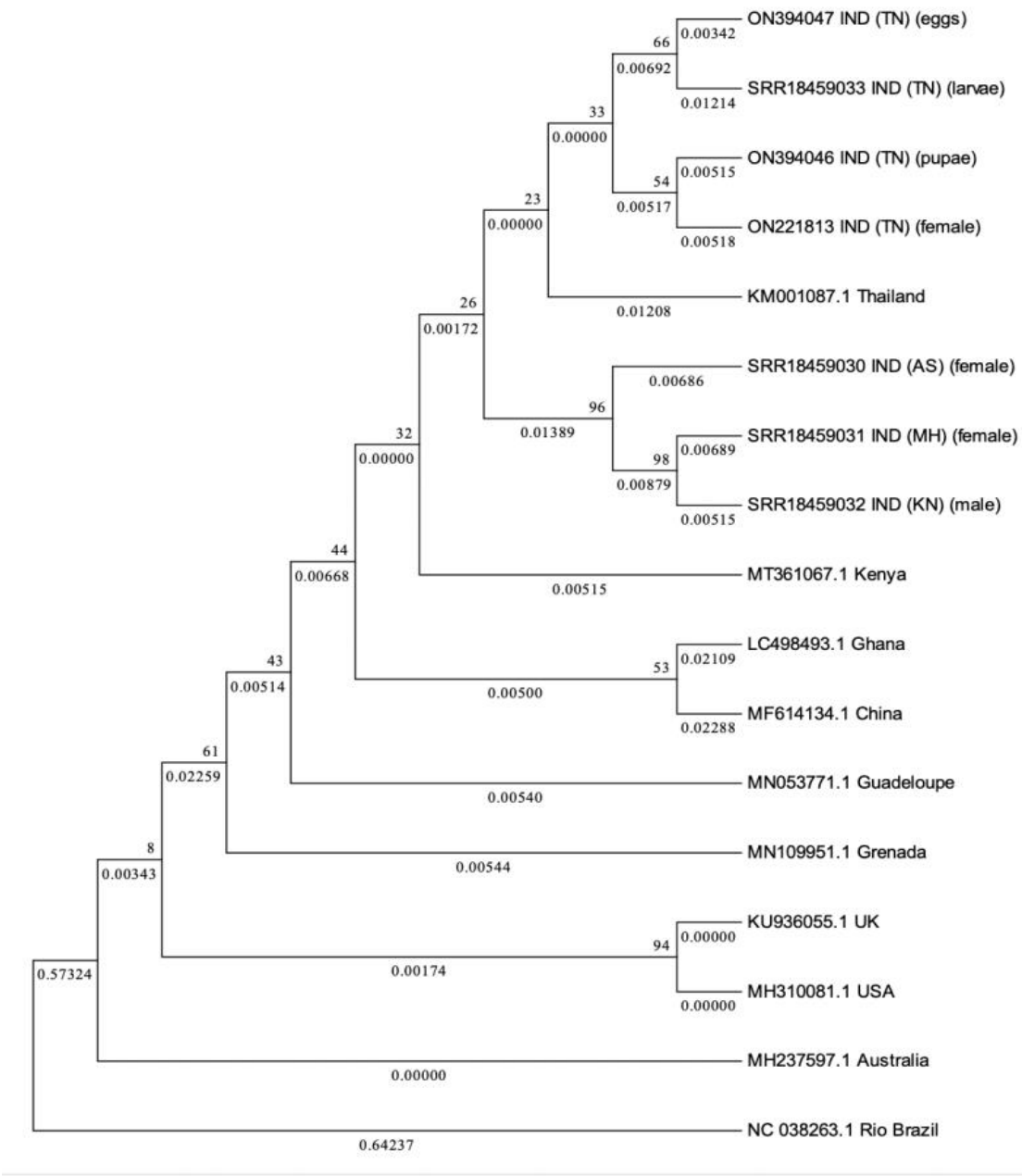

Fig. 1: Phylogenetic tree for PCLV S segment sequences obtained from *Aedes aegypti* mosquitoes from different states of India (MH: Maharashtra, TN: Tamil Nadu, KN: Karnataka, AS: Assam), from different stages of development, along with reference sequences from across the world.

Dual infection studies of dengue and chikungunya viruses in *Aedes aegypti* mosquitoes using molecular approach (Work related to metagenomics analysis of viromes of *Aedes* mosquitoes in India).

Investigators: Dr. AB Sudeep, Dr. Kavita Lole, Dr. Amol Nath

Funding: Intramural (Ph. D. project) **Duration:** 2020-2023

Background: Dengue and chikungunya viruses are important human pathogens transmitted by *Aedes aegypti* mosquitoes. Dual infection of dengue and chikungunyain humans are known to cause high morbidity and mortality. A study was initiated to understand the growth kinetics of the viruses in *Aedes aegypti* mosquitoes using molecular approach to reveal the replication kinetics.

Objectives:To understand themechanism of replication of dengue and chikungunya viruses in *Aedes aegypti* mosquitoes.

Work done &Findings: To understand the mechanism of action, five different sets of experiments were carried out. *Aedes aegypti* mosquitoes were infected intrathoracially with one virus followed by oral infection with other virus after an incubation period of five days.The experiment was replicated by reversing the sequence of the two viruses. In another set, mosquitoes were infected with both viruses (with equal titers) together. Harvesting of samples was done on 0, 5, 10 and 15 days post infection (PI) and the titers of both the viruses were determined. The study has shown rapid replication of CHIKV in the mosquitoes irrespective of the mode of infection. When dengue-2 (DEN-2) infected mosquitoes were super infected (on the 5^{th} day PI) with CHIKV, a surge in CHIKV replication was seen on 5^{th} day PI followed by a sharp decline in subsequent days PI. There was no increase in DEN-2titres in comparison, except for maintaining the original titer.Interestingly, superinfection of CHIKV infected mosquitoes with DEN-2 showed increase in titers of both the viruses on subsequent days PI, though the former showed predominance. More studies are needed to understand the exact mechanism of modulation of virus replication.

Aedes aegypti survey in Pune city and suburbs in relation to dengue virus detection

Investigators: Dr. AB Sudeep, Dr. Kavita Lole, Dr. Amol Nath

Funding: Intramural (Ph. D. project) **Duration:** 2020-2023

Background: Dengue virus has become endemic to Pune and a progressive increase in dengue cases with high morbidity and mortalitywas observed.No recent update on either the mosquito fauna or their breeding habitats in Pune is available despite the increase in mosquito borne viruses.

Objectives: (i) To determine the distribution, abundance and seasonal prevalence of *Aedes aegypti* mosquitoes in different parts of Pune city.

(ii) Characterization of dengue virus isolated from field collected mosquitoes to study the strains circulating in the city.

Work Done: A year-long mosquito survey was conducted in 15 different localities of Pune city The *Aede*s mosquito survey was carried out at various places in Pune (Table 1). Initially different locations were screened and those showing high indices for larvae were selected for the follow up visits. While some places were visited after reporting of dengue cases from that area via print media, hospital-based reporting etc. Of these, four places viz. Dehu Road, Patil Estate, Kala Khadak and Kondhawe Dhawade areas were visited every month from Jun 21 to Mar 22. After analysis of preliminary data, it was found that *Aedes* mosquito larval density as depicted by entomological indices (viz. House, Container and Breteau indices) are closely linked to Pune's seasonal patterns. Larval density spiked during monsoon from Jul to Sep, followed by Jun and Oct. There was a moderate increase in larval density during the start of summer (March), most probably due to scarcity of water supply, resulting in an increased tendency of water storage in different containers. This is an ongoing study and a comparative analysis of *Aedes* mosquito larval density will be carried out with the occurrence of Dengue/Chikungunya cases in these areas. None of the collected larvae/ adults tested positive for DENV or CHIKV.

Table 1: Details of Aedes larval collection and entomological indices in different areas of Pune.

Sr. No.	Name of area	Date of survey	No. of houses searched	No. of houses +ve	No. of containers searched	No. of containers found +ve	House index (%)	Container index (%)	Breteau index (%)
1	Dehu Road Market Area	03 Jun 21	24	6	34	7	25	20.59	29.17
2	Dehu Road Parsi Colony/Chawl	17 Jun 21	24	9	53	16	37.5	30.19	66.67
3	Sanjay Nagar, Aundh (Survey No. 12)	13 Jul 21	28	4	44	4	14.19	9.09	14.19
4	Kala Khadak area (Near Dange Chowk)	20 Jul 21	28	5	47	6	17.86	12.77	21.42
5	Matoba Nagar, Wakad	27 Jul 21	19	3	37	3	15.79	8.1	15.79
6	Laxminagar & Harikanagar, (BhavaniPeth)	03 Aug 21	21	5	40	7	23.8	17.5	33.3
7	Phulenagar, Yerwada	17 Aug 21	26	3	48	3	11.54	6.25	11.54
8	Gunjalnagar, Warje	08 Sep 21	25	3	49	3	12	6.12	6.12
9	Landewadi, Bhosari	22 Sep 21	23	6	29	6	26.07	20.68	20.68
10	MajiSainik Nagar, Vishrantwadi	05 Oct 21	21	1	32	2	4.76	6.25	9.52
11	Darji Galli, Khadaki	18 Oct 21	20	3	49	3	15	6.12	15

12	Mahatma Gandhi slum, Patil Estate area	03 Nov 21	25	13	30	13	52	43.33	52
13	Kondhawe Dhawade , Uttam Nagar	27 Nov 21	23	3	34	4	13.04	11.76	17.39
14	Sidhharthanagar, Tadiwala Road	14 Dec 21	26	2	37	2	7.69	5.4	7.69
15	Juna Bazar area, Mangalwar Peth	10 Jan 22	25	3	47	5	12	10.63	20

Title: Vector competence of *Aedes aegypti, Culex quinquefasciatus and Culex tritaeniorhynchus* mosquitoes to West Nile virus: a comparative study.

Investigators: Dr. A.B. Sudeep, Dr. M.D. Gokhale

Funding: Intramural **Duration:** 2021-2022

Completion summary: This study was undertaken to understand the vector competence of *Aedes aegypti*, *Culex quinquefasciatus* and *Cx. tritaeniorhynchus* mosquitoes to West Nile Virus(WNV). The mosquitoes were infected with WNV (E-101) and the growth kinetics and virus dissemination was studied on different post infection days. Growth kinetic study revealed similar levels of replication of WNV in the three mosquitoes. *Cx. tritaeniorhynchus* and *Aedes aegypti* showed titer of 10^7TCID$_{50}$/ml while *Cx. quinquefasciatus* showed$10^{6.5}$TCID$_{50}$/ml on the 6th day PI. Virus remained detectable in all mosquitoes till 12th day PI (last day of experiment) with titer of ~10^3 TCID$_{50}$/ml. Vector competence was confirmed by detection of WNV in saliva on different days PI using TCID$_{50}$ assay. WNV was detected in the saliva of both the *Culex* mosquitoes from 6th day PI onwards while *Ae aegyti* was found to be non-competent to transmit the virus. The study revealed that in India, both *Cx. quinquefasciatus*and *Cx. tritaeniorhynchus* mosquitoes are potential vectors for WNV.

Field studies:

***Aedes aegypti* collection from different parts of the country for metagenomic analysis of viromes (Part of Metagenomics project).**

As part of the metagenomics analysis of *Aedes aegypti* mosquitoes from different parts of the country, field trips were carried out to Assam, West Bengal, Punjab and Rajasthan during March and April 2022for mosquito collection. Adult mosquitoes collected from the above states were brought live to the laboratory and laboratory colonieswere established at ICMR-NIV. Both the wild caught adults and F_1 generation adults of the strains were used for metagenomics analysis of mosquito viromes.

EPIDEMIOLOGY GROUP

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Mobile Application for Immunization Data in India

Investigators: Tandale BV (Site PI), Gupta N (PI) ICMR Delhi and Others
Funding: Extramural – ICMR (Sponsor - BIRAC-DBT through the BMGF under Grand Challenges Initiative) **Duration:** 2020 – 2021.
***Background*:** The project was initiated earlier in four subcentres of Khadakwasala Primary Health Centre for development, improvements, field testing and piloting of mobile application.
***Objective*:** We studied operational feasibility of mobile application implementation for immunization.
Methods: The ASHAs and ANMs were invited for participation by seeking consents for assessing usage and feasibility assessment. Infants were registered on the app at immunization session and at door step visit by ASHAs. The vaccination dates were entered on app for the timeliness of vaccination.
Results: We identified challenges like lack of records, deficits in enumeration of beneficiary infants and delay in vaccination as per schedules. The application provided digital records, household enumeration and tracking of children including timely report generation. Application also helped healthcare workers in monitoring vaccination uptake along with timeliness as per the schedules. On-time vaccination of infants during study period indicated decline in timeliness - 124 (63.3%) of 196 infants were vaccinated timely at 6 weeks, 109 (43.6%) of 250 at 10 weeks, and only 86 (31.9%) of 269 at 14 weeks. However, 270 (90%) of 300 infants were vaccinated on time at 9 to 12 months.
Conclusion: Application improvements were considered based on the feedback of the users that may be helpful for wider dissemination. Delayed vaccination needs to be addressed by sensitizing beneficiary parents and healthcare workers along with assurance on infection control practices. The mobile application may be helpful for healthcare workers re-orientation, awareness of parents and performance monitoring and related improvements by the health system managers.

Hepatitis A outbreak investigation at Hingnole, Satara, Maharashtra, August 2021

Investigators: Dr AR Deoshatwar, Dr KS Lole
Funding: Intramural **Duration:** August 2021 (Outbreak)
***Background*:** On 13/08/2021 six blood samples were received by NIV from Indoli primary health center [PHC], Satara. All six samples tested positive for anti-HAV IgM and negative for anti-HEV IgM.
***Objectives*:** 1) To investigate the source of infection and suggest control / prevention measures, 2) To characterize the outbreak on epidemiological and environmental parameters.

Methods: Data were collected by the NIV team in assistance with the PHC team from the villagers on online platform [Google form]. Blood samples were drawn from 174 participants who consented [from all age groups]. Samples were also taken from all the ante-natal cases in the village. Stool samples were collected from five individuals who had experienced symptoms during previous 4-5 days. The line list of the symptomatic individuals [19] was obtained from the medical officer. The list of ante-natal cases was also obtained. Water samples were taken from 10 different sources for analysis. Soil samples were taken from the area close to main well.
Results: Out of total 174 samples collected, 25 sera were positive for IgM against HAV. None of the samples were positive for HEV IgM. Stool samples were tested for presence of HAV RNA, four out of six samples were found positive. Of the 10 water samples, only water from the main well was found HAV positive. All soil samples tested negative for presence of HAV.
Conclusion: IgG testing of the samples showed that the decline in seroprevalence in this population probably started 15-20 years ago.
Table-I: Serological analysis of samples collected during HAV outbreak in Hingnole, Satara, Western Maharashtra, August 2021

Age group	Age-group specific population of the village	Number of participants	IgM Positive (% of participants)	IgG Positive AND IgM Negative (% of participants)	IgM & IgG Negative (n)
0 to 9 yrs	130	6	3 (50%)	1 (16.7%)	2
10 to 15 yrs	85	52	13 (25%)	21 (40.4%)	18
16 to 25 yrs	186	34	9 (26.5%)	20 (58.8%)	5
25+ yrs	790	80	0 (-)	80 (100%)	0
Total	1191[#]	172	25 (14.5%)	0.9%)	25

[[#] Ages of 10 individuals not known, total population 1201].

Development of automated chlorination monitoring system

Investigators: ICMR-NIV Pune: - Dr AR Deoshatwar, Dr. KS Lole, Dr Jayati Mullick, Dr Rajlakshmi Viswanathan, Dr Mallika Lavaniya.
COEP: - Dr Vishal Bhalla, Dr Rashmika Patole
IDSP – Maharashtra: Dr Pradeep Awate, State surveillance officer.
Funding: Intramural **Duration:** 2021-2022

***Background*:** Ensuring that the water reaching the people is chlorinated may avert many waterborne outbreaks. There is a need to develop a system that will monitor the chlorination of drinking water supply and inform about it to all concerned.

***Objectives*:**

Primary ***Objectives***:

Design an automated chlorination monitoring device that can be easily integrated into existing water supply systems.

Informing the authorities, stake holders and end-users about the chlorination status in real time via wireless messaging methods [e.g. SMS].

Methods: Design and assembly of the experimental prototype:

Fig. 1: Experimental Prototype of the test section

Work done:

Control over flow rate of the water

As the detection of the chlorine is to be done time to time (at a particular time), a solenoid valve-based flow controlling valve has been used in the system where at time of the day the water is collected in the chlorine sensing unit and the level of the chlorine is detected.

Generation and delivery of the data

For the generation and delivery of chlorine level in the water to the authorities a system has been prepared which has three main components, the sensing mechanism, on-site controller & the cloud channel.

The sensing mechanism is the sensor which is converting the Analog signal to the digital signal. For the controlling part Raspberry Pi controller has been purchased and the programming of the controller is under process.

Uploading data to the cloud: To demonstrate sending data to the cloud over a wireless network, CPU temperature as input has been considered. It involves uploading data to a ThingSpeak channel using a unique API key. **Analysing data on the cloud to send email alerts:-** At present, temperature data is being uploaded and relayed to stake holders using cloud.

Conclusions: The experimental prototype has been successfully assembled and data is being successfully uploaded and relayed. The system development is under further processing.

MAXIMUM CONTAINMENT FACILITY GROUP

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Surveillance for Zika virus infection in humans, India

Investigators: Dr. G Sapkal, Dr. PD Yadav, Dr. Viswanathan

Contributing staff: Dr. AM Shete, Dr. RR Sahay, Mrs. T Majumdar, Mrs. S. Patil, Ms. P Gawande. Coordinators: Dr. N Gupta and Dr. H Kaur and VRDLs

Funding: DHR-ICMR **Duration**: 2017-2022 (ongoing)

***Background*:** India initiated sentinel surveillance of ZVD in March 2016 through its network of virus research and diagnostic laboratories (VRDLs) by ICMR across the country. The sentinel ZIKV surveillance was initiated with 10 laboratories in 2016, the VRDLs were up-scaled to 56 in 2018 and 132 by 2021.

***Objectives*:** Enhanced surveillance of Zika virus (ZIKV) based on symptoms and case history in retrospective and prospective Samples, and Congenital Zika virus Syndrome including microcephaly in infants.

Findings: In 2021, ZIKV outbreaks were reported from Kerala [May-July], Maharashtra [July] and Uttar Pradesh [October] states of India. Since these outbreaks were reported from distant locations and over a period of six months, we conducted a retrospective screening of dengue and chikungunya negative clinical samples [stored with VRDLs], from May to October 2021, to understand the extent of spread of ZIKV in India. The clinical samples of 1475 patients, across 13 states and a union territory of India were screened and re-tested for Dengue, Chikungunya and Zika by CDC Trioplex Real time RT-PCR. The positivity was observed for Zika (67), Dengue (121) and Chikungunya (10) amongst screened cases. The co-infections of Dengue/Chikungunya, Dengue/Zika and Dengue/Chikungunya/Zika were also observed. All Zika cases were symptomatic with primary symptoms of fever (84%) and rash (78%). The Asian Lineage of Zika was found in circulation.

Our study indicates the spread of Zika virus to several new states of India (Delhi, Jharkhand, Rajasthan, Punjab, Telangana) and an urgent need to strengthen its surveillance (Fig. -1).

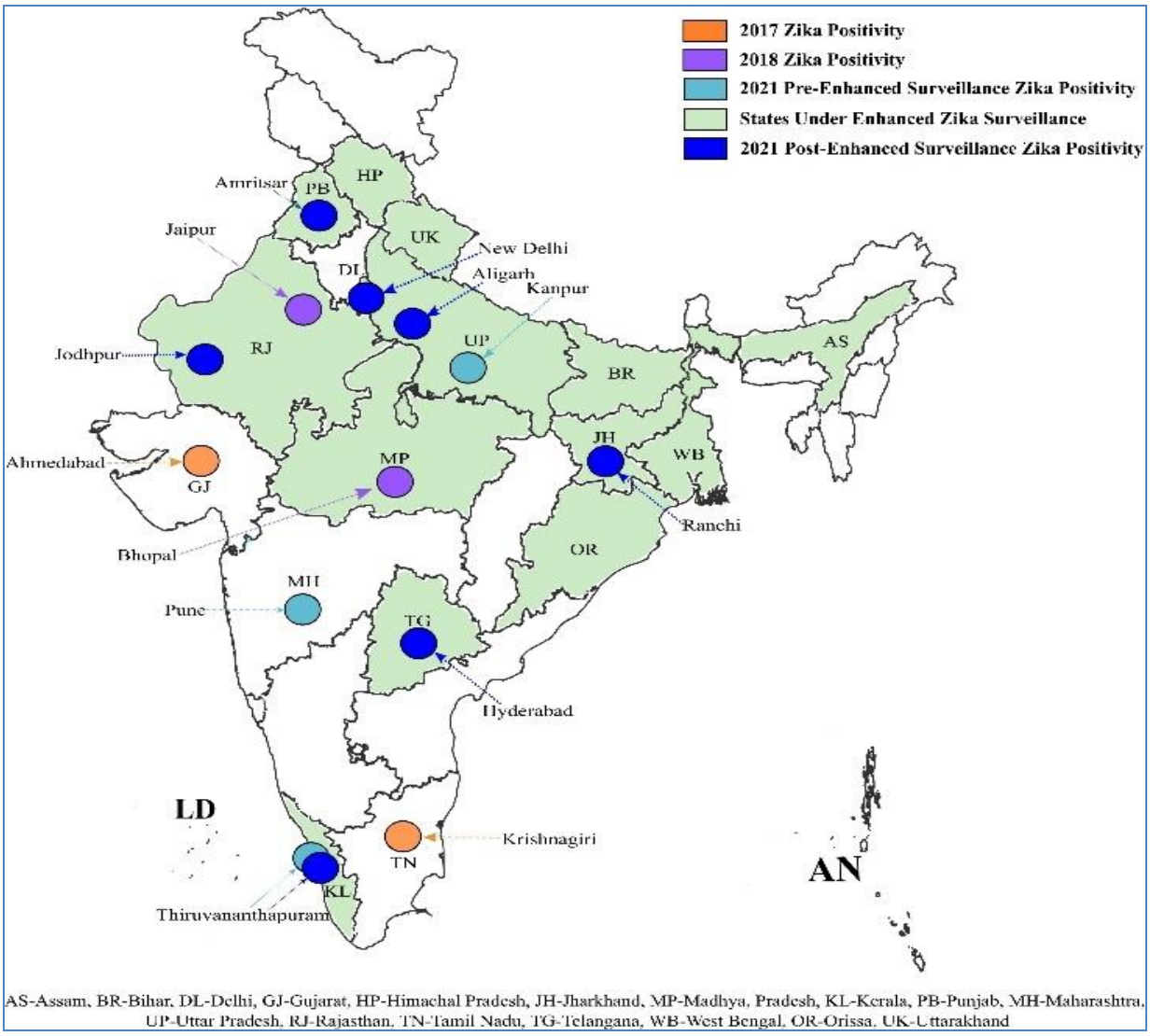

Fig. 1: *Geographical distribution of Zika positive cases in India*

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

Investigators: Dr. PD Yadav, Dr. GN Sapkal, Dr. AB Sudeep

Contributing staff: Dr. R. Jain, Dr. RR Sahay, Dr. S Mohandas, Mr. P. Sarkale, Mr. H. Dighe, Mr. S. Baradkar

Funding: DHR-ICMR **Duration:** 2017-2022 [ongoing]

Background: Positive controls are essential to molecular diagnostics, and it can fall short or become unavailable during public health emergency. Viruses propagated and inactivated during the year 2020 were supplied as positive controls for different viruses along with reagents, to

national network laboratories/other institutes/companies during outbreaks/emergency preparedness for various viral threats. ICMR-NIV, Pune has capacities for rapid manufacturing and distribution of these positive controls for viruses

***Objective*:** Preparation of positive and negative standard controls for molecular diagnosis of different viruses of public health importance.

Findings: Reagents for Real time RT-PCR as well as ELISA reagents along with positive controls for KFDV were provided to 11 VRDLs. Reagents for SARS-CoV-2 IgG ELISA was supplied to multiple commercial companies. Zika real time reagents were supplied to GMC, Thissue and Thiruanthapuram. Details of supply of reagents are listed in Table-1.

Table-1: Details of reagents supplied to the Bio-medical loabrtaories in India.

Sr No	Antigen/other details	Total Volume of virus stock supplied & purpose	VRDL centers/field units of NIV Pune/companies/ other institutes
1	SARS CoV-2	SARS CoV-2 Inactivated antigen for 2800 kits	J Mitra & Co Pvt Ltd, New Delhi
2	SARS CoV-2	SARS CoV-2 Inactivated antigen 8000ml	VINS Products Limited, Hyderabad
3	Zika	Zika Real Time PCR reagents 300 rxn	GMC, Kozhikode
4	Zika	Zika Real Time PCR reagents 300 rxn	ICMR-NIV Kerala
5	Zika	Zika Real Time PCR reagents 300 rxn	GMC Thrissur
6	Zika	Zika Real Time PCR reagents 500 rxn	GMC, Thiruvanthapuram
7	SARS CoV-2	SARS CoV-2 Inactivated antigen for 2000 kits	Trivitron, Chennai
8	SARS CoV-2	SARS CoV-2 Covid Kavach ELISA -1 kit	ICMR-NIV Mumbai
9	SARS CoV-2	SARS CoV-2 300ml Inactivated antigen	Central Research Institute, Kasauli
10	Zika	Zika Real Time PCR reagents 1000 rxn	GMC, Thiruvanthapuram
11	SARS CoV-2	SARS CoV-2 Real Time PCR reagents 400 rxn	GMC, Kozhikode
12	Nipah	Nipah Human IgM ELISA -5 kits	GMC, Kozhikode
13		Laboratory Consumables (PPE, Gloves)	GMC, Kozhikode
14	Nipah	Nipah Real Time PCR reagents 500 rxn	ICMR-NIV Kerala
15	Nipah	Nipah Real Time PCR reagents 1000 rxn	GMC, Kozhikode
16	KFDV	KFD Real Time PCR reagents 500 rxn	Virology Lab, Wayanad
17	KFDV	KFD Human IgG ELISA 100 kits reagents	Zydus Cadilla, Ahmedabad
18	Zika	Zika Real Time PCR reagents 1000 rxn	KGMU, Lucknow

19	SARS CoV-2	SARS CoV-2 2ml Inactivated antigen	NICED Kolkatta
20	SARS CoV-2	SARS CoV-2, 10ml	NIHSAD, Bhopal
21	KFDV	KFD Real Time PCR reagents- 500 rxn; KFD Human IgM ELISA -11 kits	ICMR-NIV Bangalore
22	KFDV	KFD Real Time PCR reagents- 500 rxn; KFD Human IgM ELISA -11 kits	ICMR-NIV Kerala
23	KFDV	KFD Real Time PCR reagents- 100 rxn; KFD Human IgM ELISA -02 kits	Kasturba Hospital, Mumbai
24	KFDV	KFD Real Time PCR reagents- 100 rxn; KFD Human IgM ELISA -02 kits	KIPMR, Chennai
25	KFDV	KFD Real Time PCR reagents- 100 rxn; KFD Human IgM ELISA -02 kits	GMC, Kozhikode
26	KFDV	KFD Real Time PCR reagents- 100 rxn; KFD Human IgM ELISA -02 kits	GMC, Bambolim, Goa
27	KFDV	KFD Real Time PCR reagents- 100 rxn; KFD Human IgM ELISA -02 kits	GMC, Miraj
28	KFDV	KFD Real Time PCR reagents- 100 rxn; KFD Human IgM ELISA -02 kits	GMC, Nagpur
29	KFDV	KFD Real Time PCR reagents- 100 rxn; KFD Human IgM ELISA -02 kits	District Hospital, Mapsua, Goa
30	KFDV	KFD Real Time PCR reagents- 100 rxn; KFD Human IgM ELISA -02 kits	District Hospital, Margao, Goa
31	KFDV	KFD Real Time PCR reagents- 100 rxn; KFD Human IgM ELISA -02 kits	VDL, Shimoga

Development of Serodiagnostic Assays For Nipah Diagnosis & Surveillance

Investigators: Dr. P.D.Yadav, Dr. GN Sapkal, Dr. AM Shete, Dr.G Deshpande, Dr. S Mohandas, Dr. R Jain. **Contributing staff:** Dr. RR Sahay, Mr. P Sarkale

Funding: ICMR New Delhi **Duration:** 2019-2022 [Ongoing]

***Background*:** Nipah is one of the priority diseases that need urgent action. During last two decades (2001 – 21), India has reported five Nipah outbreaks among human population in West Bengal and Kerala. Low cost, easy-to-use and sensitive diagnostic assays would be useful to curb the Nipah infection and would help possible interventions.

***Objective*:**

Development of anti-Nipah Human IgM antibody detection ELISA for screening human samples.

Development of anti-Nipah IgG antibody detection ELISA for screening human, bat, and swine serum samples using characterized monoclonal and polyclonal antibody.

Findings: Nipah virus was propagated in BSL-4 laboratory and used for development of serodiagnostic assay for Nipah. The developed Nipah Human IgM assay indicated sensitivity and specificity of 100 % and 99.64 %. For Nipah IgG ELISA, the sensitivity and specificity was 100 % and 99.28 % respectively. External validation of the assay was performed in IEDCR, Dhaka, Bangladesh. Anti Nipah Bat IgG ELISA was also optimized which showed a sensitivity and specificity of 73 % and 87.6% respectively. Similarly anti NiV swine IgG ELISA was optimized using the NiV antigen for coating and anti Nipah swine IgG conjugate as detection antibody. More than 100 swine serum samples were screened collected from different parts of country. This assay showed a sensitivity and specificity of 100.0 % compared to ELISA using CDC reference test.

These in house developed and validated assays were used successfully during 2019 and 2021 Nipah outbreak in Kerala and samples of humans and bats were screened using the same.

Prospective investigation of transmission of Crimean Congo Haemorrhagic Fever (CCHF) amongst close contacts of confirmed CCHF cases

Investigators: Dr. RR Sahay, Dr. PD Yadav, Dr. AM Shete, Dr. K Upadhyay

Contributing staff: Dr. R Jain, Mrs. T. Majumdar, Mrs. S. Patil

Funding: ICMR New Delhi **Duration**: 2019-2021 (Completed)

***Background*:** Several outbreaks of CCHF were reported from Gujarat state from 2011 to 2020 and from Rajasthan state in 2014, 2015 and 2019. Cosidering the paucityof data on viral kinetics and subclinical infection, this study was performed.

***Objectives*:**

To study the clinico-epidemiological profile of symptomatic/asymptomatic close contacts of CCHF positive cases

To study the possible (likelihood) of transmission of CCHF amongst symptomatic/asymptomatic close contacts of positive CCHF cases

Findings: The persistence CCHF viral RNA was observed till 76th POD in one of the survivors. We also observed that the anti-CCHFV IgM detection in the serum samples starts as soon as 2nd POD but anti-CCHFV IgG antibody could be detected in the majority of the cases only after the 20th POD (Fig. -2). The elevated IL-6 level, IL-10 level and IFN-γ were observed during the acute phase of the CCHFV infection. Interestingly, serum IL-10 level subsided to normal, upon recovery while TNF-α cytokine was found to be increased. This study also identifies the circulation of the reassortment of Asian-African genotype and Asian genotypes from human

cases for the first time from India. Livestock and tick pool percent positivity recorded from Gujarat and Rajasthan states in these two years were 31.9 % and 10.03 % respectively.

A total of 705 close contacts were followed and only one asymptomatic close contact showed anti-CCHF IgM and anti-CCHF IgG antibodies positivity after 14 days, suggestive of very low subclinical infections (1/705 [0.14%]).

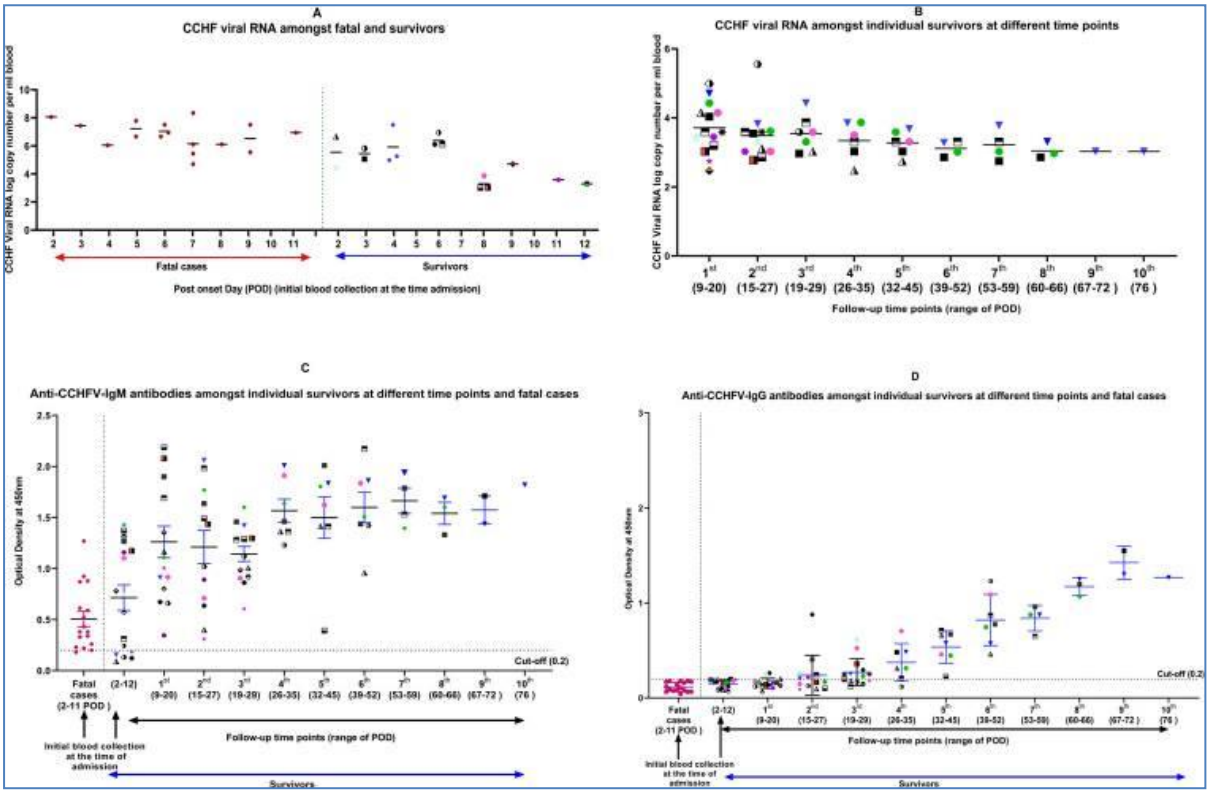

Fig. 2: *CCHF viral RNA, anti-CCHFV IgM and anti-CCHFV IgG kinetics of CCHFV cases*

Sustainable Laboratory Network for Monitoring of Hemorrhagic Fever viruses and mitigation for high risk pathogen

Investigators: Dr. P.D. Yadav, Dr. GN Sapkal, Dr. AM Shete, Dr. RR Sahay, Dr. S Mohandas VRDLs PI:17 VRDLs

Coordinators: Dr. P Abraham, Dr. N Gupta, Dr. N Aggarwal, Dr. H Kaur

Contributing staff: Dr. R. Dhawade, Dr. A Kumar, Dr. R Jain, Mrs. T. Majumdar, Mrs. S. Patil

Funding: ICMR New Delhi **Duration**: 2021-2024 (ongoing)

***Background*:** India, being a country of extreme geo-climatic diversity, faces a constant threat of emerging and re-emerging viral infections of public health importance. There is a need for strengthening disease surveillance in the country focusing on the epidemiology and disease burden. Highly infectious viruses like Crimean Congo hemorrhagic fever [CCHF], Kyasanur forest disease [KFD] have reportedly caused catastrophic outbreaks and sporadic cases in different parts of India. Apart from CCHF and KFDV, other viral hemorrhagic fever (VHF) causing pathogens like Ebola, Marburg, Lassa, Rift Valley Fever, Yellow fever has shown several outbreaks in the African continent.

***Objective*:**

Conducting intensive bio-risk mitigation training health care workers and laboratory staff in the VRDL network to enable public health emergency preparedness for known and unknown pathogens.

Strengthening laboratory capacity of VRDLs for detecting high-risk pathogens already known in the country viz., CCHF and KFDV and also pathogens which pose a significant risk to India: Ebola, Marburg, Lassa fever, Rift Valley, Yellow Fever.

Identification of all unknown agents which may have an epidemic potential using in-vivo and in-vitro approach and characterization using Next Generation Sequencing (NGS) platform

Validating indigenous developed Point of care for KFDV and CCHFV diagnosis at selected centres and implementation in assigned centres

Findings: In order to strengthen the capacity for the diagnosis for emerging and re-emerging viruses a network of 17 laboratories were created. A virtual training on bio safety, molecular and serological methods for KFD and CCHF were conducted for the 11 KFDV and 7 CCHF laboratories. Post training a quality assurance and quality control (QAQC) check for KFDV RTPCR and ELISA testing was conducted with previously trained eleven laboratories.Through extensive training, QAQC activities along with supply of reagents and PPE kits, the laboratories trained for KFDV developed confidence to perform the diagnosis.

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

Investigators: Yadav PD, Shete AM, Sahay RR, Mohandas S, Nyayanit D

Contributing staff: Mrs. T. Majumdar, Mrs. S. Patil, Ms. M. Dudhmal, Dr. A. Kumar, Mr. Y Joshi, Dr. P. Pandit

Funding: ICMR-NIV, Pune **Duration:** 2020-2022 [ongoing]

***Background*:** Next generation sequencing (NGS) is presently the most advanced approach for the identification of unknown/untyped viruses independent of prior sequence information.

***Objective*:** Identification of unknown etiological agent from the specimens of a Tiger.

Findings:

Investigation of samples of Orangutan and Gorilla received from Sri Chamarajendra, Zoological Gardens, Mysuru, Karnataka

Next-generation sequencing did not show any reads of Simian AIDS, Polio and Rabies virus genomes.

Investigation of the Horse samples received from Bangalore, Karnataka

Next-generation sequencing (NGS) revealed high relevant reads for Clostridium botulinum from 3 horses. Besides this, partial Equine Herpesvirus 2, Equine Herpesvirus 5 virus genome and complete 16rRNA gene of Bordetella species was retrived from one horse.

Sequencing of 3 isolates of Virbrio Cholarae received from Bacteriology department, ICMR-NIV, Pune

Next generation sequencing (NGS) retrieved 93% genome in chromosome 1 and 83% in chromosome 2 of vibrio cholarae of one isolate.

Sequencing of 18 Avian Influenza isolates were received from Polio Group department, ICMR-NIV, Pune.

Whole genome sequence from all samples were mapped with reference genome.

Whole genome sequencing of 5 Stool samples RNA received from Enteric virus department of ICMR NIV Pune.

Coxsackievirus A16 whole genome sequence was retrieved from all samples.

Investigation of 5 tick pools received AFMC.

No relevant viral, bacterial or fungal reads could be obtained.

Investigation of a suspected H5N1 Human Specimen received From Influenzae Department, ICMR-NIV, Pune and Isolates:

The complete genome of H5N1 was retrieved from clinical sample and isolates.

A Study On pathogenicity Of Nipah Virus (Kerala Isolate) In Syrian Hamster Model

Investigators: Dr. S Mohandas, Dr. PD Yadav, Dr. A Shete, Dr. RR Sahay, Dr. HK Kaushal

Contributing staff: Mr. M Kadam, Mr. A. Suryawnashi, Mr. A. Kumar, Dr. R Jain

Funding: ICMR NIV Pune COVID-19 Fund **Duration:** 2019-2022 (ongoing)

***Background*:** Nipah virus (NiV) is a highly fatal disease in humans, and the various outbreaks have been reported from India during 2001 to 2021. The first virus NiV isolate from India was obtained from a NiV patient during a recent outbreak in Kerala state, which shows close homology to the Bangladesh strain. The Syrian hamster is a rodent model that closely mimics multiple aspects of human NiV disease.

***Objectives*:**

To determine susceptibility, tissue tropism and viral kinetics of Nipah virus isolate from Kerala in the Syrian hamster model.

To study the host immune response to NiV by intra-nasal and intra-peritoneal route of infection in hamster model

Findings: The isolate induced respiratory and nervous signs in hamsters by intraperitoneal infection characterized by prominent vascular lesions in lungs, brain, kidney and extravascular lesions in brain and lungs. Congestion, haemorrhages, inflammatory cell infiltration, thrombosis and rarely endothelial syncitial cell formation were seen in the blood vessels. Intranasal infection resulted in respiratory tract infection characterised by pneumonia probably due to the lower virus dose used for infection and the faster clearance from the respiratory tract. The model showed disease characteristics resembling the human Nipah Virus infection except that of myocarditis. Cytokine mRNAs were measured by real time RT-PCR in brain, lungs and spleen. The cytokine levels in brain samples were found slightly upregulated compared to the lungs and spleen samples at various time points. The upregulation was observed in case of IL-4, IL-6, IL-12 and IFN-Gamma.

NABL accreditation ISO/IEC 17025:2017

Investigators: Dr. PD Yadav, Dr. AM Shete, Dr. RR Sahay

Contributing staff:, Dr. R Jain, Mrs. Patil S, Mrs. Majumdar T, Mr. Mohite M, Ms. Gawande P and Mr. Gaikwad V

Funding: ICMR-NIV Pune **Duration:** 2016-Till date

***Background*:** Maximum containment facility has diagnostic assays for KFD, CCHF under the scope of NABL. Recently the NABL has updated the standards from ISO/IEC 17025:2005 to ISO/IEC17025:2017. For compliance to the standards audits were conducted by NABL.

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

Findings: Internal audit for continuation for the accreditation system was conducted on 23rd August 2021. NABL renewal audit was conducted on 18-19th September 2021. Non Conformities raised during the same were closed by making the corrective actions. Laboratory received the continuation of accreditation till 29th September 2023.

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

Investigators: Dr. PD Yadav, Dr. AM Shete, Dr. RR Sahay, Dr. S. Mohandas

Contributing staff: Mr. M. Mohite, Mr. V. Gaikwad, Mr. N Sharma

Funding: ICMR-NIV, Pune **Duration:** Service Project [ongoing]

Findings:

Annual Inspection of IBR Boiler from "Directorate of Steam Boiler Department" visited the site in the month of October 2021. The latest certificate is valid until October 2022.

Installation of new equipments in the facility.

Routine and annual maintenance contract visits including the servicing of the major utility equipment (Diesel Generator, Boiler, UPS, HT & LT panels) completed as per the schedule.

Annual Shutdown for re-validation was carried out for performing the maintenance work. All the supply and exhaust filters were tested for any leakage / damage. The Bio safety doors, autoclaves, Biological Effluent Decontamination (BLED) tanks and tissue digester were also validated during the period. All the pressure / temperature sensors, transducers were calibrated. Servicing of 14 no's. AHU's were performed.

All the major maintenance activities including thermal insulation and cladding of IBR boiler and steam line, laying of new fiber optics cable for Ethernet, UPS installation for major machines, repair and replacement works were performed.

Outbreaks investigations and containment responses:

Nipah outbreak containment response, Kozhikode Kerala August-September 2021:

- **Confirmation of the index case of Nipah virus infection by ICMR –NIV, Pune**

  12 year boy, from Chathamangalam gram panchayat, Kozhikode had fever followed by encephalitis like symptoms was confirmed as Nipah virus case and outbreak was declared on September 5, 2021.

- **Establishment of onsite Nipah diagnostic facilities at Government Medical College, Kozhikode, Kerala State**
  - As per the directives from ICMR-HQ, a team of 5 scientists and four technical staff was deputed immediately on September 6, 2021 to GMC Kozhikode to set up a field laboratory with the facility of testing of Nipah virus by Point of Care assay (PoC), Real time RT-PCR and ELISA
  - A total of 125 suspect/contact high risk cases were tested for Nipah virus infection at the on-site laboratory at (GMC), Kozhikode
- **Bat survey to track the source of infection for Nipah virus infection**
  - The trained field team from ICMR-NIV, Pune and Kerala unit including two scientific staff and 6 technical staff were deputed to capture the bats using specialized nets and equipment.
  - With the permission from the Principal Conservator of Forest and Principal Secretary Health, Kerala state and coordination with the local health officials.

- The bat capturing activities were undertaken to find the source of infection to the index case. A total of 102 bats were captured by the ICMR-NIV team.

- **Capacity building**
  - The capacity building of the existing lab was enhanced up to the mark and all diagnostics SOPs, Biosafety concerns/ issues were taken up including the proper donning and doffing protocols.
  - Intense training on Biorisk mitigation, donning and doffing of Personal Protective Equipment was imparted to the VRDL team of GMC Kozhikode (n=10) and the field team members of the National Health Mission (n=6).

- **Risk Categorization and Contact Tracing**

A total of 240 contacts were listed and among them 64 close contacts [33 women/31 men] were identified and grouped into primary high-risk (n = 50) and low-risk (n = 9) contacts; secondary high-risk (n = 3) and low-risk (n = 2) contacts. All the contacts were screened and found negative for Nipah virus **[Fig. -3]**

Considering the ongoing COVID-19 pandemic, all the close contacts were also screened for SARS-CoV-2. The throat/nasal swab of the 12 close contacts (symptomatic-8 and asymptomatic-4) was found positive for SARS-CoV-2 by qRT-PCR. On sequencing of SARS-CoV-2 positive samples (n = 12), Delta variant (B.1.167.2) and its derivatives (AY.26) were detected in 10 and two cases, respectively.

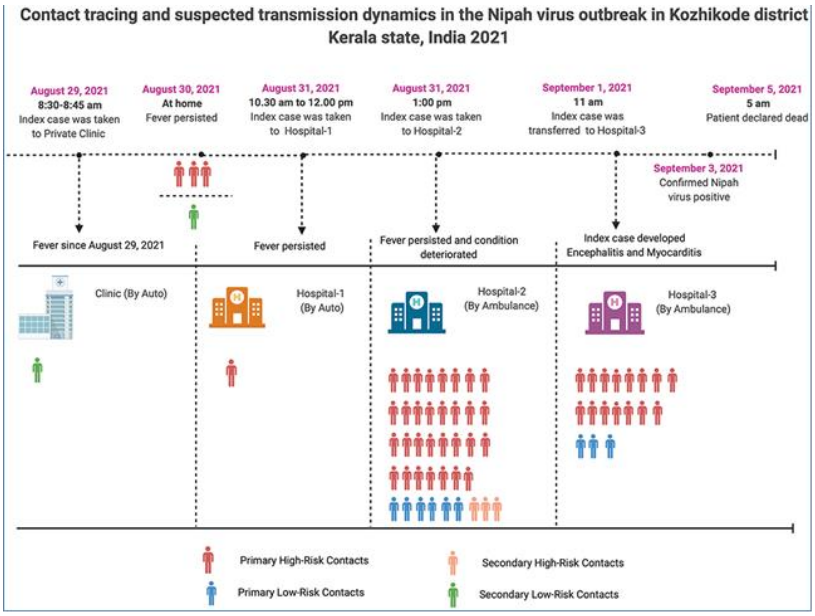

Fig. -3: Contact tracing and probable transmission dynamics in the Nipah virus outbreak in Kozhikode district, Kerala state, India, 2021.

Outcome:

Rapid establishment of an onsite NiV diagnostic facility and contact tracing helped in quick containment of the outbreak.

NiV sequences retrieved from the clinical specimen of the index case formed a sub-cluster with the earlier reported Nipah I genotype sequences from India with more than 95% similarity.

Anti-NiV IgG positivity could be detected in 21% of Pteropus medius (P. medius) and 37.73% of Rousettus leschenaultia (R. leschenaultia). Neutralizing antibodies against NiV could be detected in P. medius.

Stringent surveillance and awareness campaigns need to be implemented in the area to reduce human-bat interactions and minimize spillover events, which can lead to sporadic outbreaks of NiV.

Zika virus detection for the first time in Thiruvananthapuram Kerala (July 2021) and Kanpur Uttar Pradesh (October 2021):

Detection of Zika cases from Thiruvananthapuram district of Kerala state, India (July 2021)

In the midst of the second wave of the COVID-19 pandemic, an increase in the number of undiagnosed exanthematous fever were observed from Thiruvananthapuram during the month of May 2021. The cases presented with maculopapular morbilliform rash involving the face, trunk and upper limb (94.74%), mild fever (68.42%), myalgia (47.37%), arthralgia (26.32%), conjunctival congestion (26.32%), sore throat (31.58%), headache (15.79%), rhinitis (5.26%), and posterior cervical lymphadenopathy (5.26%).

Out of 19 cases, 13 cases were tested positive for Zika viral RNA (C t ranged: 26.75–37.47) and 10 cases were positive for anti-Zika IgM antibodies. The complete genomes (>97%) could be retrieved from two Zika positive samples using next-generation sequencing with 99.33% and 99.4% nucleotide similarities with Zika strain from Rajasthan, India respectively.

Detection of Zika cases from Kanpur, Uttar Pradesh (October 2021)

A 56 years old male with high grade fever, with worsening respiratory distress and acute tubular necrosis and thrombocytopenia was confirmed to be Zika case with positivity in serum, blood and TS/NS specimens. The serum was also positive for anti-Zika IgM antibodies. The partial sequence retrieved from the clinical sample of the ZIKV case matched with sequences of Rajasthan outbreak 2018. With the detection of this case, the surveillance was enhanced and till date 126 cases of ZIKV are reported from Kanpur district UP.

Considering these outbreaks, Zika virus diagnosis were strengthened in the VRDLs and diagnostic reagents were supplied.

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

Investigators: Dr. PD Yadav, Dr. AM Shete, Dr. RR Sahay, Dr. S. Mohandas
Contributing Staff: Dr. R. Jain, Mrs. T Majumdar, Mrs. S. Patil, Mr. P Sarkale, Mr. H. Dighe, Mr. S. Baradkar, Mr. R. Lakra, Ms. P. Gawande, Mr. A. Kumar
Funding: ICMR-NIV, Pune **Duration:** Service Project (ongoing)
***Background*:** The mandate of BSL-4 facility is preparedness and diagnosis of emerging and reemerging viral infections in the country. It is the only facility in India and Apex center for diagnosis of VHF causing high risk viral pathogens like KFD, CCHF, Ebola, Marburg, Rift valley fever, Nipah, etc.
***Objectives*:** Provide diagnostic support to the referred samples and outbreak investigations (Fig. -4 and Fig. -5)

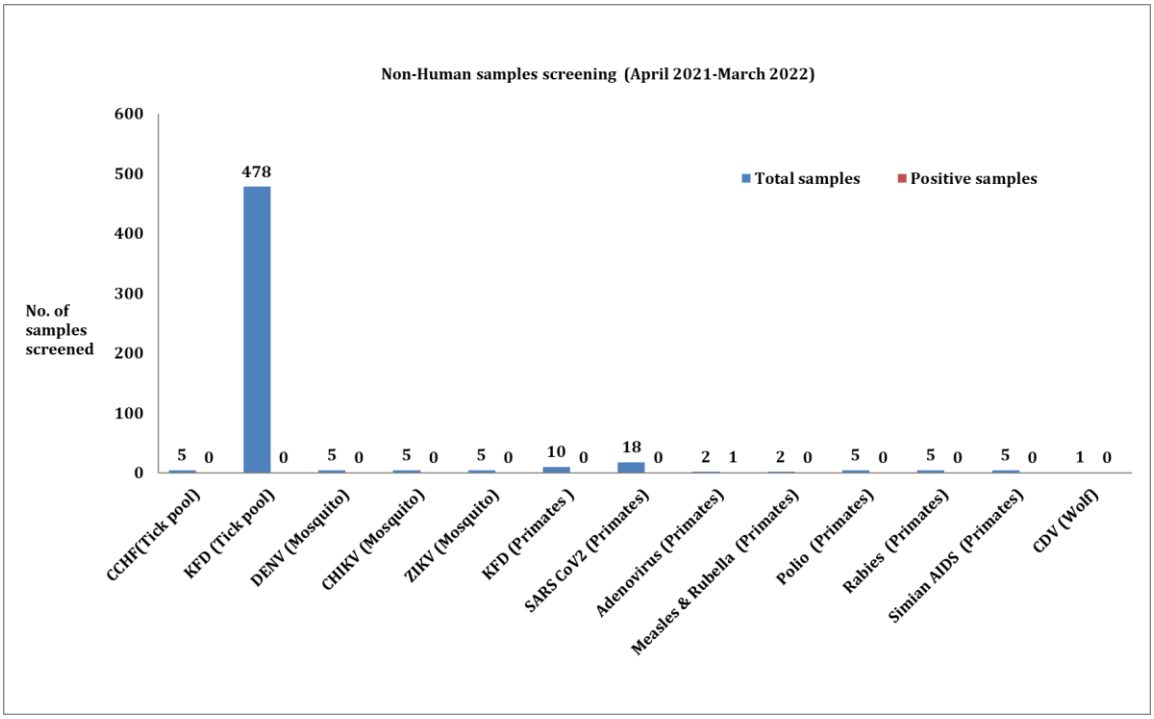

Fig. -4: Details of nonhuman samples screened from April 2021 to March 2022.

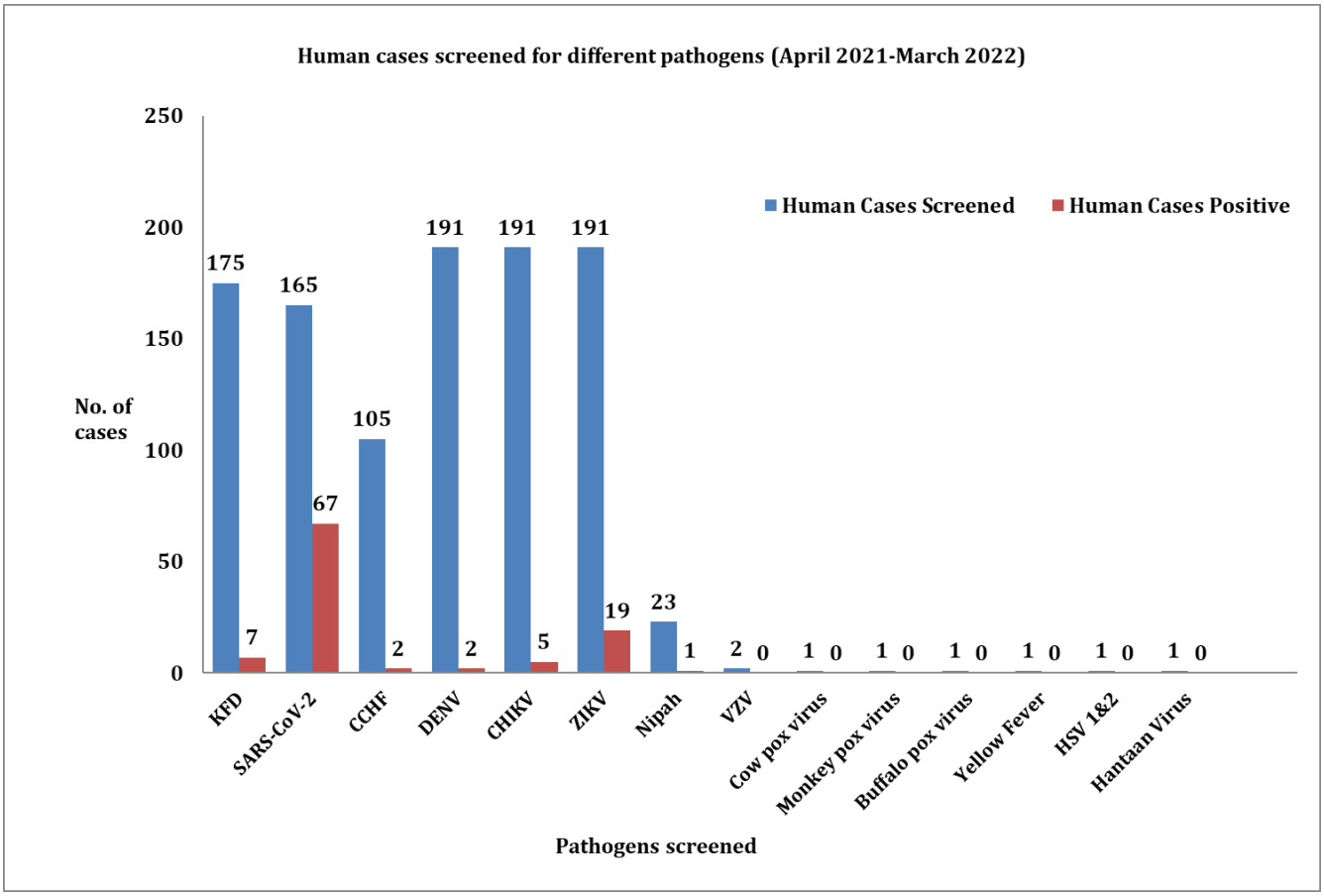

Fig. -5: Details of human cases screened for different viral pathogens from April 2021 to March 2022

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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 mostly follows a self-limited course; however in certain conditions antiviral therapy is required. Despite several in vitro studies, to date there has not been a systematic effort to identify drugs for HEV. In recent years, high-throughput technologies such as microarray and RNA-sequencing have produced gene expression profiles of drugs available in public domains. These profiles can be used to construct a detailed map of connections between diseases and drug actions and identify drugs that could affect the metabolic pathways crucial for the pathogen replication and pathogenesis. In the current study we are using two methods to identify candidate drugs against HEV. First to analyse transcriptome, proteome and interactome data and identify pathways as the drug targets and second, to target virus protein functions by using structure based virtual drug screening approach.

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: Transcriptome analysis of HEV infected cells was done to identify significant pathways or processes in HEV infection. The FDA approved as well as investigational drugs targeting these genes and corresponding pathways were shortlisted using Connectivity Map (CMap). A total of 40 drugs were selected based on transcriptome data generated in the current study as well as from the datasets available in the public domain. Drugs (n=20) targeting viral proteins such as polymerase, helicase and protease were selected from drugs in use for other viruses. After testing against HEV following drugs were found to be effective, acarbose, miglitol, voglibose, metformin, rosiglitazone (anti-diabetic), artesunate (antimalarial), fenofibrate (used along with cholesterol reducing diet) and zinc acetate (Fig. 1A). It was seen that for drug testing, HEV-1 full genome (FG) replicon based hepatoma cell culture model was superior as compared to subgenomic replicon. Screening of direct acting antivirals was done by using enzymatic assays of HEV helicase and polymerase proteins in presence of drugs. Following drugs showed significant inhibition of HEV polymerase activity, Ribavirin (20%), Sofosbuvir (SOF), elvitegravir and acarbose (ACA) (>50%), daclatasvir (DAC), miglitol (MIG), metformin (MET) and voglibose (VOG) (>30%), Zinc acetate (ZnA) and artesunate (~25%) (Fig. 1B). All drugs showing inhibitory effect on polymerase also showed effective inhibition of HEV helicase. Direct-acting antivirals showing significant inhibition of HEV-1 replication were sofosbuvir

(HCV NS5B polymerase inhibitor), daclatasvir (HCV NS5A inhibitor) and elvitegravir (HIV-1 integrase inhibitor).

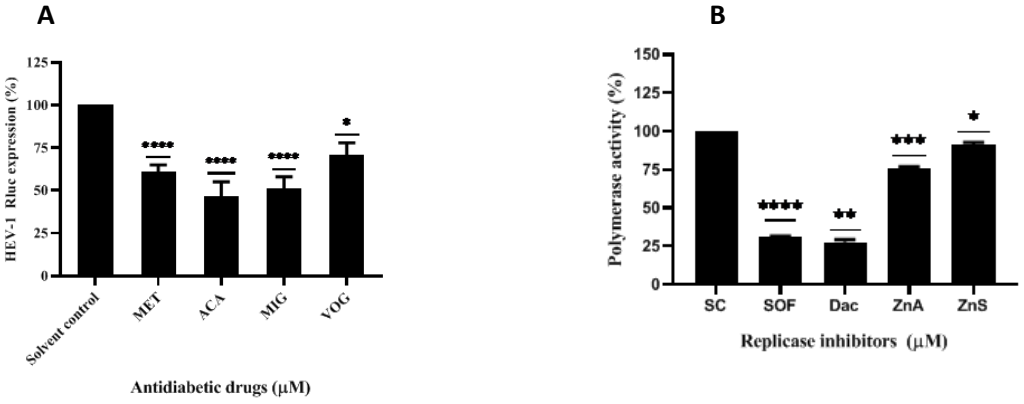

Fig. 1: A) Testing of drugs using subgenomic replicon, B) HEV polymerase inhibition

Hepatitis E virus replication and cellular autophagy

Investigators: Kavita Lole, Manjita Srivastava

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

***Background*:** Several aspects of HEV pathogenesis remain unexplored due to lack of efficient replication models. Viral infections are known to activate autophagy in host cells, either by virus-encoded proteins or due to cellular stress. Hepatitis E virus (HEV) egresses out from infected hepatocytes as quasienveloped particle containing open reading frame 3 (ORF3) protein. HEV ORF3 (small phosphoprotein) that interacts with host proteins to establish favorable environment for virus replication. It is a functional viroporin that plays important role during virus release.

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: HEV ORF3 pull-down assay revealed that it directly interacts with host proteins involved in modulation of autophagy such as mTOR, Beclin1, DAPK1, ATG2B, ATG16L2 and HDAC2. Canonical autophagy activation is initiated by the formation of Beclin1-PI3K complex. Western blot analysis of HEV infected cells as well as ORF3 expressing cells showed higher level expression of DAPK1 and Beclin1 phosphorylation, indicating activation of Beclin 1 by

DAPK1 (Fig. 2A & B). Further analyses showed that ORF3 utilized non-canonical NF-κB2 pathway, sequestered p52NF-κB and HDAC2 to up-regulate DAPK1 followed by Beclin1 phosphorylation and induction of autophagy. HEV requires exosomes derived from multivesicular bodies for virus release. It appeared that ORF3 induces autophagosome formation that helps in virion egress.

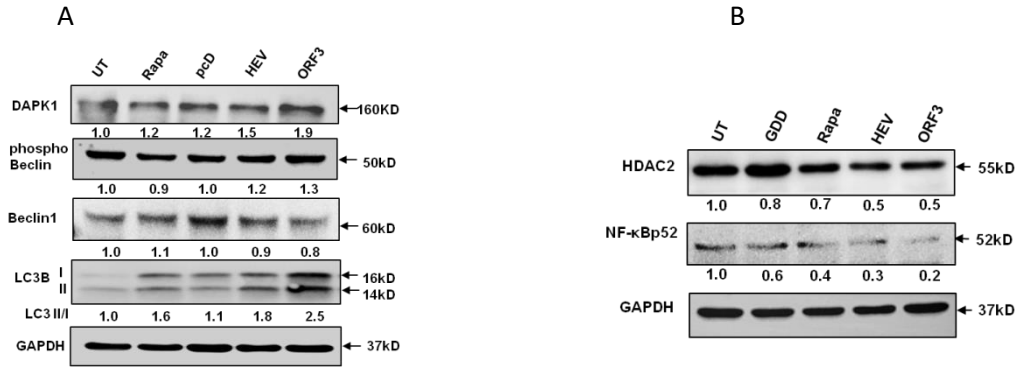

Fig. 2: A) HEV and ORF3 up regulate DAPK1 expression and Beclin1 phosphorylation, B) Western blot analysis of total cell lysates from cells infected with HEV or transfected with ORF3 plasmid.

Study of Anti-Chikungunya Virus-Specific Antibody-Dependent Cellular Cytotoxicity (ADCC) Immune Response

Investigators: Anuradha Tripathy, Mohini Ganu and Diptee Trimbake

Funding: Intramural Duration: 2019-2023

***Background*:** NK cells lyse target cells and secrete interferon gamma to recruit adaptive immune cells and are defined for their ability to kill virally infected and malignant cells without priming. Human NK cells can express both FcγRIIIA/CD16a and/or FcγRIIC/CD32c which can bind to the Fc portion of immunoglobulins, transmitting activating signals within NK cells. The binding of FcγR to the Fc domain induces the release of both granzyme and perforin from effector cells, leading to target cell lysis. This mechanism of lysis of target cells coated with antibodies by effector cells with cytotoxic activity and specific Fc immunoglobulin receptors is describe as "Antibody dependent cell mediated cytotoxicity (ADCC)". ADCC has been reported as an important mechanism for resistance and clearance of various viral infections. Our current study of chikungunya pathogenesis has established robust involvement of NK cells in chikungunya virus (CHIKV) infection .We have elucidated the potential role of activation receptors on cytotoxic NK cells in influencing the pathogenesis of early CHIKV infection. Since, NK cells play a key role in chikungunya pathogenesis, hence, it is important to find whether ADCC also plays a role in CHIKV pathogenesis and if yes, to what extent. A typical ADCC response is NK cell mediated. This is a collaborative project with Sanjeevan hospital, Latur, Maharashtra.

***Objective*:** The current study aims to assess NK cell-mediated ADCC response in chikungunya infection.

Findings and conclusions:. A total of 80 chikungunya patients' blood samples were processed to assess NK cell-mediated ADCC response in chikungunya infection. For anti-CHIKV specific heterologous NK cells mediated ADCC assay, the study population comprised of 30 acute chikungunya patients[7 males,23 females],24 prolonged chikungunya patients [4 males ,20 females] and 26 post chikungunya chronic arthritis (PCCA) [7 males,19 females] patients and 30 chikungunya negative individuals[14 males,16 females] as healthy controls. Besides clinical diagnosis, the confirmed diagnosis of chikungunya was based on the presence of IgM antibodies against the virus (anti-CHIKV IgM) as determined by ELISA and/or nested PCR using CHIKV RNA. The characteristics of the study groups are depicted in Table I.

Table 1: Characteristics of study population**.**

Parameters	Study population			
	Acute	Prolonged	PCCA	Healthy Controls
Study population	n=30	n=24	n=26	n =30
Sex ratio (Male: Female)	0.12	0.2	0.31	0.87
Age (years): Median (range)	50(23-68)	44(18-73)	42(19-68)	36(19-55)
Post Onset Days of illness: median (range)	6(1-14)	39(16-90)	3 months (2 months-15 years)	NA
Anti-CHIKV IgM	Positive, n= 24 Negative, ,n=3 Indeterminate, n=3	Positive, n= 22 Negative, n=2	NA	All negative.
CHIKV Nested PCR	Positive 6	NA	NA	NA
Anti-CHIKV IgG	Positive ,n=19 Negative, n= 11	Positive, n= 23 Negative, n=1	Positive, n = 25 Negative, n=0	Negative

PCCA: Post Chikungunya Chronic Arthritis, NA: not applicable

Magnitude of the anti-CHIKV specific NK-mediated heterologous ADCC response was measured among patients' categories. Fifteen of 30 acute chikungunya patients (50%), 2 of 24 prolonged chikungunya patients (8.33%) &1 of 26(3%) PCCA patients showed antibody-dependent NK cell activation compared to 30 CHIKV-negative healthy controls clearly

indicating higher magnitude of heterologous NK cells-mediated ADCC response in acute chikungunya patients. We then characterized ADCC responses in the plasma from 30 acute chikungunya patients, 24 prolonged chikungunya patients, 26 post chikungunya chronic arthritis patients (PCCA) & 30 healthy controls using a flow cytometry based antibody-dependent NK cells activation assay with CD107a & IFN-γ as surrogate ADCC markers. Acute chikungunya patients displayed significantly higher heterologous NK cells-mediated ADCC responses compared to healthy controls (*p*-value=0.032). It is important to note that acute chikungunya patients shown significantly higher percentages of antibody-dependent activation of NK cells with ADCC surrogate markers CD107a & IFN-γ compared to prolonged chikungunya patients (*p*-value < 0.000 in each).. PCCA patients showed significantly lower NK cells-mediated ADCC response compared to acute & prolonged chikungunya patients (*p*-value< 0.000 in each) [Fig. . 3]. Overall, our data elucidate potential role of NK cells-mediated ADCC response for protective immunity in acute chikungunya patients. Our study also suggests the activation of NK cells through an FcγR-mediated ADCC process with effector functions. This may have a role towards future therapeutics & vaccine development.

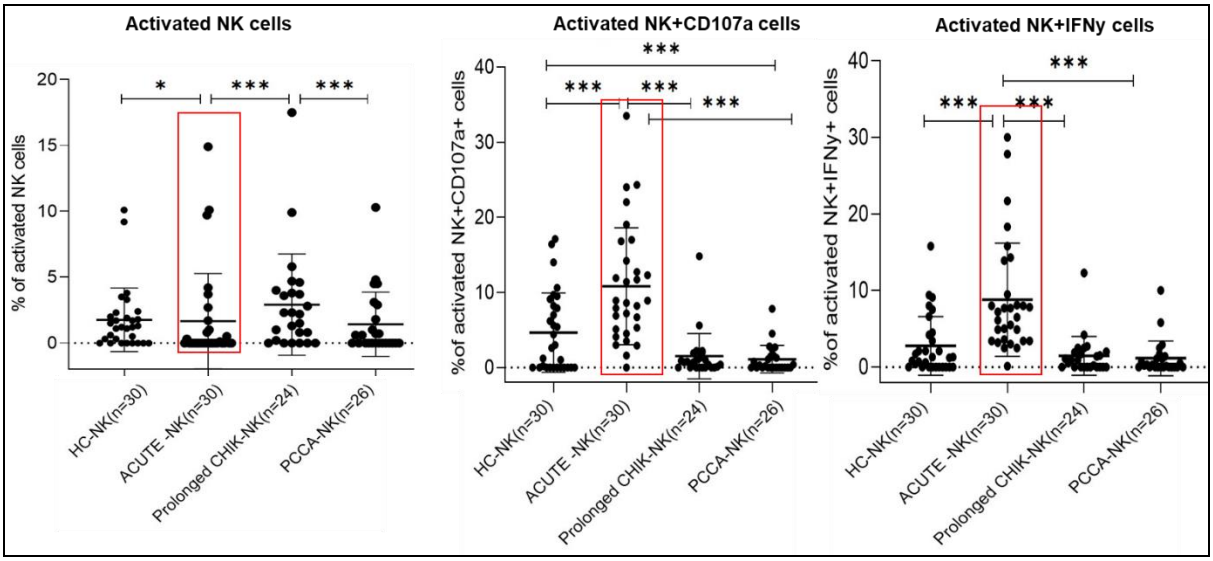

Fig. 3. Anti-chikungunya specific NK cell mediated heterologous ADCC response**.** ICS-based anti-CHIKV specific heterologous NK mediated ADCC response in chikungunya patients and healthy controls were studied. Percentages of an activated NK cells expressing CD107a & IFN-γ are represented as mean (range)+2SD. Vertical scatter plots denote the comparisons of percentages of an activated NK cells and their ADCC surrogate markers among chkungunya patients and healthy controls. The dots represent individual values and bars represent median values. (**p*-value <0.05, ***p*-value <0.005, ****p*-value <0.0001)

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: ICMR (Extramural) Duration: 2019 – 2022

***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.

Aim: To assess risk factors for the development of Cardiovascular disease (CVD) in patients with active HCV infection

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: Till date, a total of 78 HCV infected patients and 70 healthy controls have been enrolled in the study.

Findings: Analysis of clinical data of active HCV patients and healthy participants revealed that there was no statistically significant difference of carotid-intima media thickness(CIMT) in HCV infected patients and healthy controls in both right (p-value=0.2347) and left carotid (p-value=0.0752) artery. In active HCV patients, the mean AST value was 85.3 U/L (95% CI 64.47 – 106.12) and in healthy individuals it was 39.7 U/L (95% CI 26.31- 53.10) *(p value=0.0004)*, mean ALT enzyme levels 83.2 U/L (95% CI 63.68 – 102.68) and 29.3 U/L (95% CI 23.30 – 35.27), *(p value <0.0001)*, mean γGT levels 76.0 U/L (95% CI 54.05– 98.03) and 37.6 U/L (95% CI 26.59– 48.70),(p value=0.0017) respectively. In the active HCV patients, the mean APRI score was 1.53 (95% CI 0.91 – 2.15) and in healthy individuals it was 0.47 (95% CI 0.32 - 0.63) and this difference was statistically significant *(p-value= 0.0015)*. This difference in the FIB-4 index was found also statistically significant *(p-value= 0.0101)*, thus HCV patients were significantly more prone to develop liver fibrosis including cirrhosis, in contrast to healthy controls.

Active HCV patients had significant hypocholesterolemia *(p-value 0.0013)* at baseline as compared to the healthy individuals and the mean LDL levels were significantly much lower. IL-10 and IL-1β levels were significantly raised in HCV infected patients as compared to healthy individuals (Fig. 4).

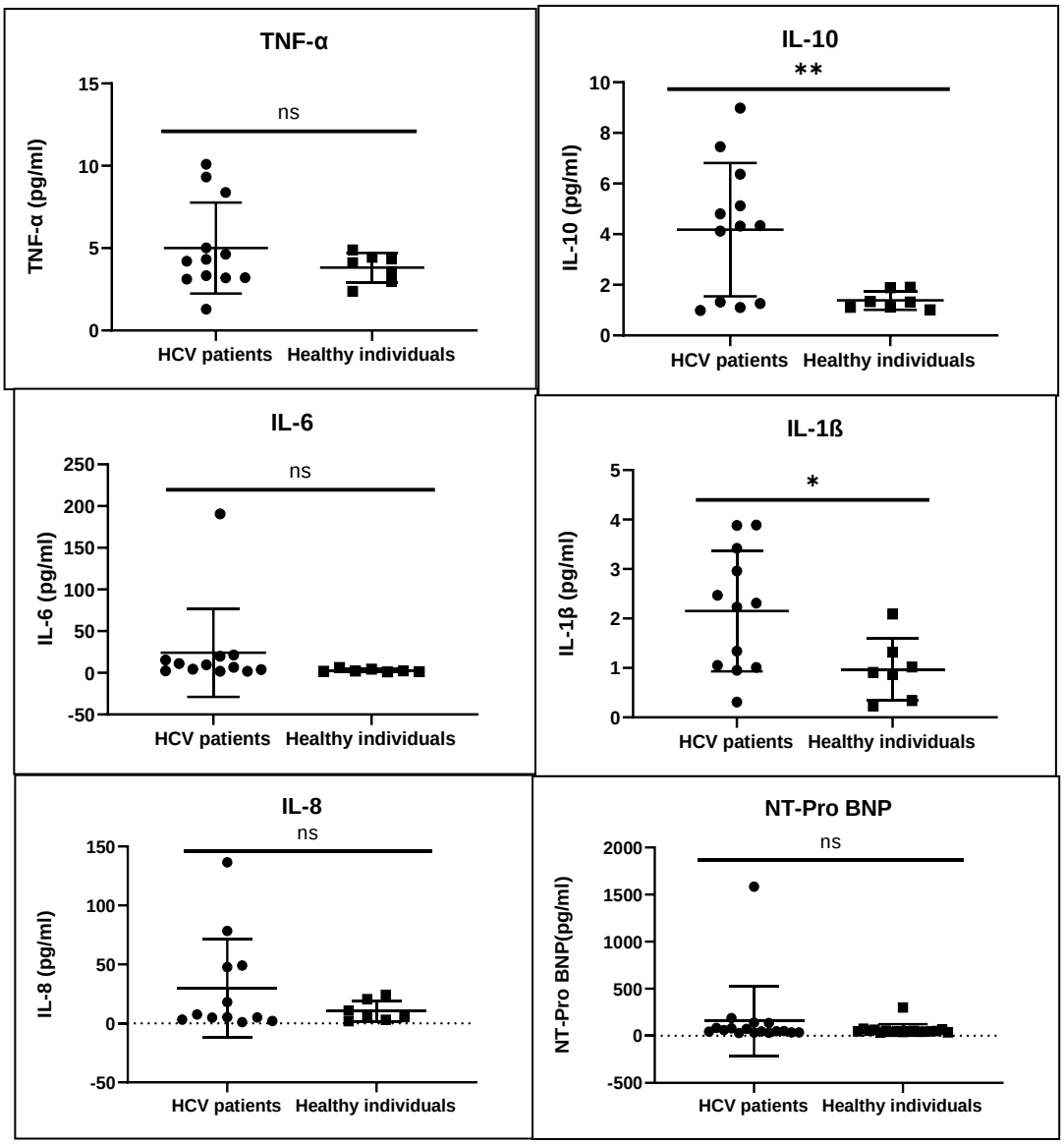

Fig. 4. Serum biomarkers in HCV patient's vs healthy individuals.

In the HCV infected patients, 11.9% were identified to be suffering from Metabolic syndrome, whereas in the healthy individuals 3.8%, but this difference (8.10%) was not found to be statistically significant (p-value=0.1204). Mean value of HOMA-IR in active HCV patients was 3.03 (95% CI 1.05 – 5.02), and in healthy individuals it was 1.46 (95% CI 1.31 – 1.61), thus HCV infected patients were significantly more prone to develop Insulin resistance, whereas healthy controls had insignificant insulin resistance.

Number of samples tested: April 2021 to March 2022.

Test	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	TOTAL
HAV IgM	3	2	1	3	5	2	1	1	4	1	4	4	31
HEV IgM	8	6	1	3	3	2	1	1	4	1	3	3	36
HBsAg						1			9	60	5		75
HAV RNA			1										1
HEV RNA			1			2	2						5
HCV RNA							20	1	1			19	41
HCV GENOTYPING							20					19	39
HAV IgG										60			60
HEV IgG		4											4
ANTI-HBS				22					9				31
DCI SAMPLES	55	48	39	34	41	43	35	37	49	30	31	41	483
HBV DNA QUANT	1		7	5	9	3	3	2	7	3	10	2	52
WATER SAMPLES	13	4	1	4	6	2	2	6	5		2	5	60
COVID19 SAMPLES, NAVALE HOSPITAL	6	4											10
HEPATITIS A OUTBREAK, INDOLI, SATARA					182								182
STOOL SAMPLES, INDOLI, SATARA					11								11
DR GANU LATUR CHIKV PROJECT						8	5		1				14
TOTAL	86	68	51	71	257	63	49	48	89	155	55	55	1125

INFLUENZA GROUP

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Technical Officer (Two)
Technical Assistant (Twelve)
Technician (Four)
JRF (Two)
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Social worker (Five)
DEO (Five)
Lab attendant (Six)

Strengthening/promoting evidence-based advocacy for influenza prevention and control in India (INSPIRE-II):

PI: Dr Sumit Dutt Bhardwaj, Co-PI: Dr Varsha A Potdar

Funding: CDC, USA Duration- 2018-2022

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*:** To find the burden of illness for influenza and RSV among elderly age above 60 years in India.

Work done: Community-based surveillance is being conducted among an open cohort of 1000 elderly at an urban slum named Janata Vasahat, Parvati, Pune to find the burden of illness for influenza and RSV. Trained project nurses conducted household surveillance five days a week to screen and enroll individuals by filling the forms on ODK platform based on handheld devices for the presence of acute respiratory infection [ARI]. After 52 weeks of follow-up, total 45138 [97.4%] visits have been conducted. The incidence rate in ARI was 18.3 per 1000 elderly per week. The incidence rate in ALRI was 0.47 per 1000 elderly per week. Total 329 samples were collected of which 7 were positive for influenza and no RSV positivity was observed. Also during this period 270 samples of COVID-19 like illness [CLI] has been collected of which 26 [9.6%] were positive for SARS CoV-2. Frailty follow-up round-6 has been completed by trained project medical social worker from 01/10/2021 to 31/12/2021 with 907 [97.3%] follow up visits done.

Tracking Community Mortality Due to Respiratory Syncytial Virus In Collaboration with University of Colorado and MAHAN Melghat

PI: Dr. VA Potdar

Funding: Bill & Melinda Gates Foundation Duration: 2016-2021

Background: RSV 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.

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

Work done: During this period, 520 samples were tested for respiratory viruses. RSV A was predominantly detected in 226 samples (43.46%), followed by Influenza B in 29 (5.57%) samples, influenza A in 24 (4.61%) samples, out of which 20 were positive for influenza

A(H3N2), 4 for Influenza A(H1N1)pdm09 and SARS CoV-2 detected in 19 (3.65%) samples. (Fig. 1 RSV A G gene Phylogeny)

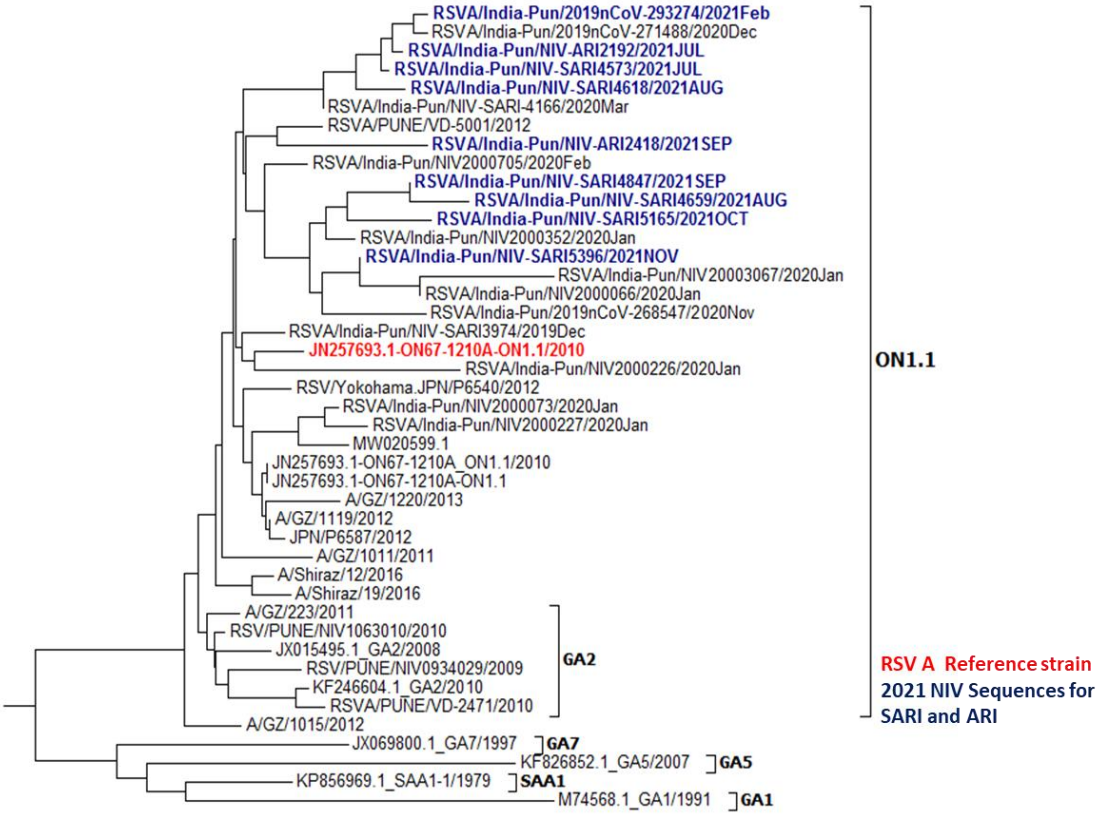

Fig. 2: Phylogenetic analysis of RSV-A 'G' gene sequences.

Establishment of serology platform for Respiratory Syncytial Virus

PI: Dr. ML Choudhary, Co-PI: Dr.VA Potdar, Dr. SD Bhardwaj
Funding: ICMR Duration: 2021-2023

Background: Respiratory Syncytial Virus (RSV) is the most common cause of bronchiolitis in infants and young children, it may lead to pneumonia and fatal outcomes. Respiratory syncytial virus-specific neutralizing antibodies (RSV NAbs) are an important marker of protection against RSV.

Objectives: Standardization of PRNT and FRNT based neutralization assays to detect anti RSV antibodies and comparison of different neutralization assays.

Work done: Blood from RSV positive patients have been (n=13) collected and more patients recruitment is ongoing. RSV A isolate has been grown in Hep2 cell line. Virus titration and PRNT standardization work is under progress.

Burden of respiratory viral infection in persons with human immunodeficiency

PI: Varsha A Potdar, Scientist E

Co-PI: Dr Manohar Lal Choudhary, Dr. Sumit Dutt Bhardwaj

Clinical collaborator: Dr. Amit Dravid, Consultant Nobel Hospital, Pune

Funding: ICMR, New Delhi Duration: 2021 - 2023

Background: Pulmonary complications, including respiratory tract infections, are a major cause of morbidity and mortality among people living with HIV (PLH). While bacterial and fungal pathogens have been well -described as important etiologies of opportunistic lung disease, less is known about respiratory viruses in PLH. Studies from antiretroviral therapy (ART)-limited regions revealed a 20-fold higher risk of respiratory viral infections among paediatric and adult PLH compared with HIV-uninfected subjects. In these settings, HIV infection was also a risk factor for higher rates of hospitalization, pneumococcal coinfection, and the need for supplemental oxygen in lower respiratory tract infection. The aim of our study is to determine the prevalence of respiratory viruses in a cohort of PLH hospitalized with a respiratory complaint.

***Objectives*:** To estimate the burden of respiratory viral pathogens among an existing cohort of PLH and to evaluate the outcome among PLH infected with respiratory viruses

Work done: In total 153 samples were received from the HIV positive patients having respiratory infection. Respiratory viruses were tested by using Real-Time RT-PCR. Influenza was predominantly detected in 34 samples, out of which 13 (8.49%) were Influenza A and 24 (15.68) Influenza B. 10 samples (6.53%) were detected positive for influenza A/H3N2 virus and 3 (0.20%) influenza A/H1N1pdm09 virus. SARS CoV-2 and RSV positivity was found in 17 (11.11%) and 19 (12.41%) samples respectively, while the other respiratory viruses including hMPV, PIV-1, PIV-2, PIV-3, PIV-4 and adenovirus was detected in 1 sample each.

Effectiveness of the inactivated influenza vaccine among pregnant women: A Cohort Study

PI: Dr. Sumit Dutt Bhardwaj, Co-PI: Dr. Varsha A Potdar

Funding: ICMR, New Delhi Duration: 2021 - 2023

Background: National technical advisory group on immunization sub-committee, GOI has recommended Department of Health Research- Indian Council of Medical Research (ICMR), to generate supporting data on estimating influenza vaccine effectiveness in high-risk groups. Pregnant women constitute one of the risk groups. The risk of hospitalization in pregnant women has been observed to be 18-fold higher compared to non-pregnant women. Pregnant women are designated as a priority group for seasonal influenza vaccination by the World Health Organization (WHO).

***Objectives*:** Measure the effectiveness of in-activated influenza vaccine among pregnant women.

Work done: The study received ethical clearance in October 2021. A MoU was signed with a private maternity facility to recruit pregnant women and got administrative approval from the municipal corporation of Pune. Study project staff were recruited and trained in data collection for weekly surveillance among pregnant women, sample collection, and sample transport. A digitalized data collection tool and a data management platform were established. Participant enrollment started in March 2022. At enrollment, project nurses provided participants with information sheets and took written informed consent from the participants. At enrollment, demographic, household, and baseline individual characteristics of participants' past medical history, gynecological and obstetrical history, and influenza vaccine status were collected electronically through a hand-held tablet. A total of 93 in the vaccinated arm and 9 in the non-vaccinated arm were recruited between March 11 and March 31, 2022. After recruitment, trained nurses performed a weekly telephone/physical surveillance to determine the incidence of acute respiratory illness among the cohort. Till March 31, 2022, two-weeks of surveillance were performed with a 204-person-week follow-up. 100% of weekly surveillance was conducted among the cohort. During this period, no cases of ARI or influenza were identified. The surveillance found no instances of acute respiratory disease or lab-confirmed influenza. There were no fatalities, abortions, stillbirths, or deliveries in the cohort.

Comparative Analysis of Host Immune Responses in Symptomatic and Recovered cases of Kyasanur Forest Disease

Investigators: Dr. H Kaushal, Dr. R Kartaskar, Dr. T Chiplunkar, Dr. VA Potdar, Dr. P Awate

Funding: ICMR Duration: 2020-2023

***Background*:** Kyasanur Forest disease virus (KFDV) is a tick-borne flavivirus that causes life-threatening hemorrhagic fever in humans with case fatality rates of 3–5%. Relatively little is known about the mechanism of its pathogenesis or host immune responses against KFDV. Here, we investigated KFDV-specific host immune responses in the recovered KFD cases.

***Objectives*:** To determine cellular and humoral immune responses in the recovered KFD cases.

Findings: Cellular immune responses were analysed in recovered KFD cases, KFD-R (n=13) and healthy control, HC (n=9) groups in terms of lymphoproliferative index (PI) to γ-inactivated KFDV antigen *in vitro*. The data are presented as mean±SD. Group PI mean of KFD-R (1.766±0.827, $p<0.05$) was found significantly high compared to the HC group (1.082±0.403). Furthermore, the intracellular cytokine analysis revealed percentage IFN-γ^+ $CD4^+$ T cells in KFD-R was significantly high (0.274±0.117, $p<0.01$) compared to the control unstimulated cells (0.167±0.089). However, the percentage of IFN-γ producing $CD8^+$ T cells in the KFD-R (0.914± 0.51) was comparable to the control unstimulated cells (0.575±0.371). No significant increase was found in the percentage of IFN-γ producing $CD4^+$ or $CD8^+$ T cells in the HC group.

The anti-KFDV IgG level was significantly elevated in KFD-R (0.058±0.021, $p<0.01$) compared to HC (0.028±0.011). The levels of anti-KFDV IgG1 were significantly high in KFD-R (0.091±0.042, $p<0.05$) compared to HC (0.046±0.026). Similarly, both IgG2 and IgG3 were found significantly raised in KFD-R (IgG2, 0.183±0.098, $p<0.001$; IgG3, 0.056±0.03, $p<0.001$) compared to respective values in the HC (IgG2, 0.034±0.059; IgG3, 0.010±0.012). In contrast, IgG4 level was found comparable between KFD-R (0.051±0.035) and HC (0.058±0.137). Overall, the study demonstrated the generation of strong protective host immune responses in the recovered cases of KFD.

Contribution to Global Influenza Network

Virological data for 115646 clinical samples including positives 236 influenza A(H1N1)pdm09, 2735 A(H3N2), 1767 Influenza B, detected at NIC were submitted to Global Influenza Surveillance and Response System (FLUNETPLUS). In addition Pan India data was also submitted to WHO. Influenza isolates (n=77) (H1N1pdm09: 14, H3N2: 32, Influenza B: 31) were submitted to WHO CCs, CDC, Atlanta and Melbourne Australia.

WHO External Quality Assessment Programme (EQAP)

Panel number 20 (2021) for influenza A real time PCR from WHO, CHP Hong Kong was received; this contained 10 samples of A(H3), A(H5), A(H1)pdm09, influenza B and other Influenza A and the results were 100% concordant. We also participated in WHO external quality assessment system (EQAS) panel for detection of SARS-CoV-2 and got 100% concordance.

Under Pan India VRDL project NIC performed Quality control of VRDLs. All the positives for Influenza and SARS CoV 2 were submitted by the labs. Over all the performance of the VRDLs were satisfactory. NIC also dispatched WHO SARS CoV-2 EQAS Panel to 649 different labs all over India

NABL Accreditation

Real Time PCR test for influenza virus diagnosis has been assessed and accredited in accordance with the standard ISO/IEC17025:2017 in the discipline of biological testing by National Accreditation Board for Testing and Calibration Laboratories (NABL). Reassessment of the test has been conducted in 18-19 Sep 2021.

Procurement, inventory, packaging and distribution of reagents and kits validation

NIV, Pune functions as ICMR's central depot as well as a regional depot for supply of kits to government laboratories in Maharashtra. Also validations were conducted for the batch testing of reagents purchased by ICMR and Maharashtra government.

Kit Validation: NIV Pune received various kits as mentioned below for validation and reports were uploaded on ICMR portal.

Items	Total kit received	Satisfactory	Not Satisfactory
Real Time RT-PCR Kit	80	45	35
RNA Extraction kits	25	24	1
VTM and Swabs	15	15	0
Total	120	84	36

Samples tested for different viruses and their positivity

Virus	No. of samples tested	No. of samples positive	% Positivity
SARS CoV-2	103154	15468	15.00%
Influenza A	103154	2853	2.7%
Influenza A/H1N1pdm09	2853	221	7.7%
Influenza A/H3N2	2853	2632	92%
Influenza B	103154	1798	1.74%
RSV	2287	611	26.72%
Rhinovirus	1967	5	0.25%
Adenovirus	1967	21	1.07%
Parainfluenza	1967	85	4.32%
HMPV	1967	35	1.78%

POLIO VIRUS GROUP

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

Investigators: Jayati Mullick (PI), Basavaraj S Mathapati, Ajay B. Khare
Dinesh Singh, Sachin Keng, JPN Babu (Contributors).
Funding: Intramural (Institutional Service project) **Duration:** 2013 onwards.

Abstract: The High Containment laboratory (BSL-3) is a specialized laboratory designed to work on high-risk pathogens, providing protection to workers and the community. During the report 3 staff, one from maintenance and two from our groups were trained for working in BSL-3. This were done at the animal side of the BSL-3 laboratory as the laboratory side of the BSL-3 has been proposed as a designated Polio Essential Facility (PEF) and is under consideration for modification as per Global action plan III (GAPIII). Further one day biosafety training was also provided to Ph. D students from both campuses (Fig. -1). The facilities, newly modified BSL-3 and PEF are shut down for modification and repair work. Installation of new digital cameras in the PEF perimeter, new LED light fixtures and water proofing of the Biological Liquid Effluent Decontamination (BLED) tank for PEF and installation of new pass box and dunk tank in the BSL-3 area is in progress.

Fig. 1: BSL-3 training and General biosafety trainings were conducted for the staff and the newly joined Ph.D. students

Development of Polio Essential Facility in line with the Global Action Plan III at ICMR-NIV, Pune to support work on Polio

Investigators: Shailesh D Pawar, (Nodal Officer), Jayati Mullick (Group Leader), Basavaraj Mathapati, A B Khare
Contributors: Sadhana S Kode, Vaishali S Tatte, Sachin S Keng, Dinesh K Singh, Rameshwar P Khedekar , JPN Babu, Ratnadeep More, Vaishnavee Bagde
Funding: Intramural (Proposed for ICMR funding) **Duration:** 2019-2023

***Background*:** The competent authority has decided to develop the PoliovirusEssential Facility (PEF) at the ICMR-NIV, Pune. The Polio Virus Group has been functioning since January 2019 as per ICMR directive and the mandate of the group has been revised. The group has been actively involved in the establishment of the PEF as per Global Action Plan III (GAP III), in addition to the research activities of avian influenza.

***Objectives*:** To establish poliovirus essential facility at ICMR-NIV, Pashan.

Findings: Laboratory and facility documents which include standard operating procedures, risk assessments were made as per the Global Action Plan III guidelines. The development work of the facility is in progress.

The physical verification of the inventory of biological materials in the designated PEF Lab was performed.

Installation of Deep Freezer (-40°C), water baths and Data Logger System for PEF was completed.

Adult Basic Life Support training was conducted by the Department of Anaesthesiology, MIMER Medical College & Dr. BSTR Hospital, Talegaon (D) on 4th December 2021 at ICMR-NIV, Pune.

List of facility and documentation work was prepared. Various committees for the functioning and coordination of the PEF establishment were formed. Several meetings with the Senior 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. Physical changes in the facility have commenced.

Screening of non-polio enteroviruses in respiratory samples: A Pilot study.

Investigators: Dr. Jayati Mullick

Co- Investigators: Dr. Varsha Potdar and Dr. Mallika Lavania

Contributor: Dr. Vaishali Tatte

Funding: ICMR-NIV Duration: Jan - March 2022

***Background*:** Enteroviruses (EVs), beyond poliovirus, are important pathogens. The detection of a large number of non-polio enteroviruses (NPEVs) in patients with respiratory illnesses has also confirmed their role as respiratory pathogens. Hence, the present study was undertaken for the surveillance of NPEVs in respiratory cases during the COVID-19 period, to gain a better insight on the prevalence and genetic diversity of these viruses. Limited data is available on diversity of these viruses from India.

***Objectives*:** Detection and molecular characterization of Non-Polio Enteroviruses (NPEVs) from respiratory samples during COVID-19 pandemic period 2021-2022 as an exploratory study.

Findings: COVID-19 negative ARI (n=105) and SARI (n=148) samples from cases during the period 2021-22 were screened for the presence of NPEVs by RT-PCR targeting the 5'UTR region (Fig. 2). Total positivity of NPEVs was noted in 35.23% and 31.08% of the ARI and SARI cases, respectively. Comparison in the two groups studied, showed significant difference in the age-wise distribution for cases >18 years of age, along with differences in gender and seasonality.

Sequencing of representative samples from both the groups showed prevalence of *Rhinovirus A-C (RVA-RVC)* and Echovirus in ARI cases. Predominance of *Rhinovirus C* followed by *Rhinovirus A* was noted in the SARI cases. High diversity was noted in RVC strains studied. Circulation of a rare *Echovirus-29* strain was noted in the ARI cases (Fig. 3).

The study highlights significant divergence in the Rhinovirus strains studied. It warrants the need for surveillance of NPEVs, whole genome sequencing of the circulating strains for better understanding of biodiversity among the NPEVs and the potential health burden.

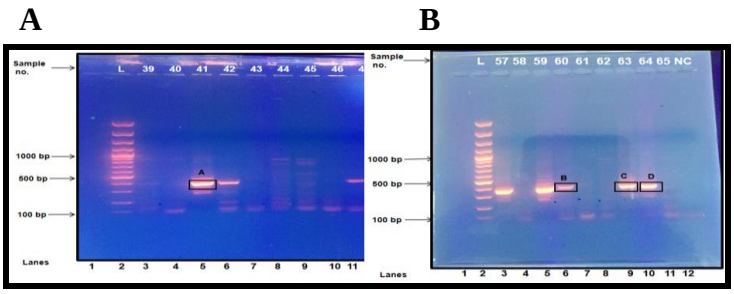


Fig. 2: Representative Agarose Gel Electrophoresis of clinical samples. Lane 2 has 100 bp Ladder and lanes 3-12 are PCR products of clinical samples and NC. The boxed products A, B, C and D were sequenced.

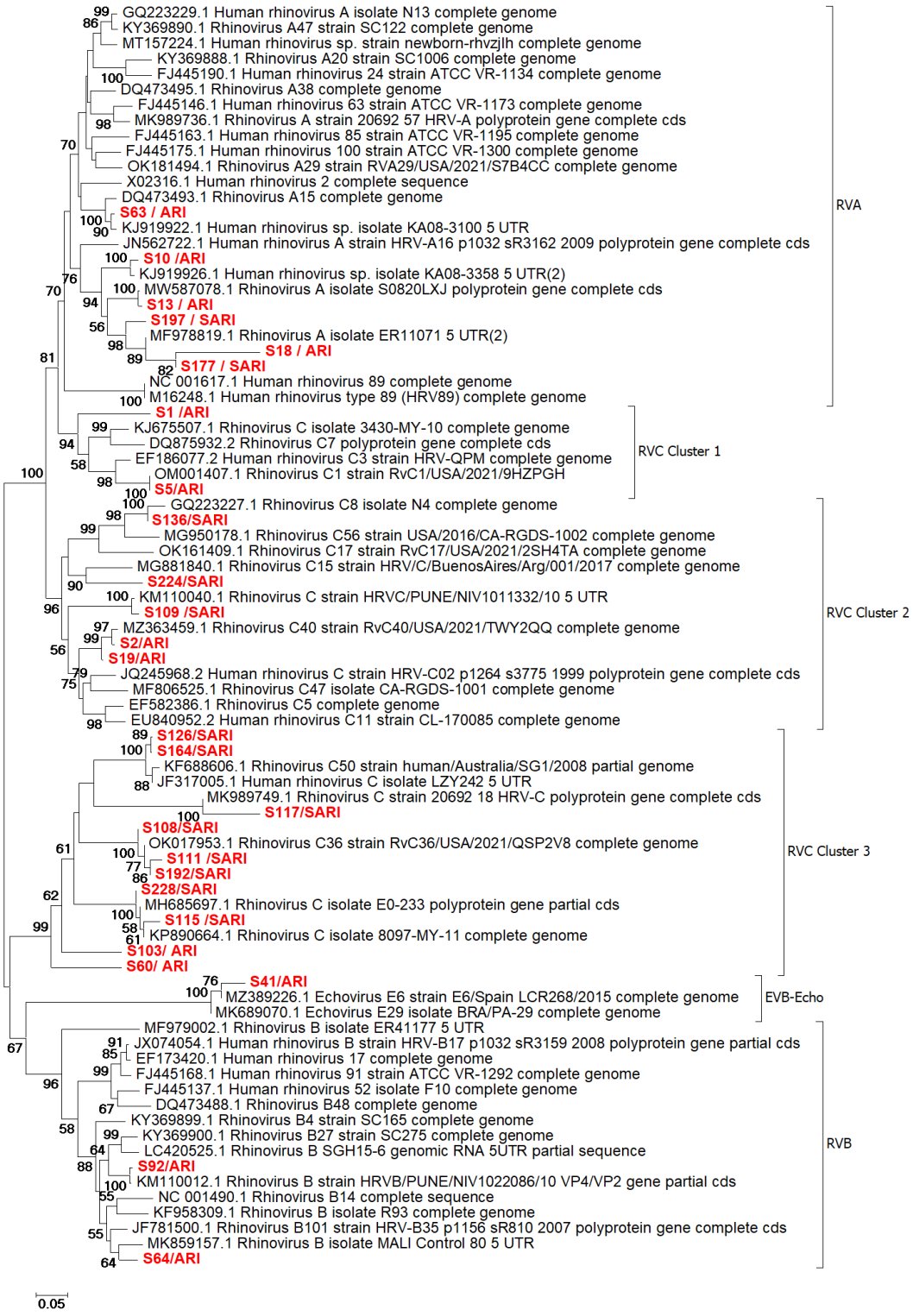


Fig. 3: The phylogenetic tree was generated by neighbor-joining method based on a final dataset of 300 bp nucleotide sequence of 5' UTR region of NPEVs. The study strains are highlighted in red. The evolutionary distances were computed using the Kimura 2-parameter method.

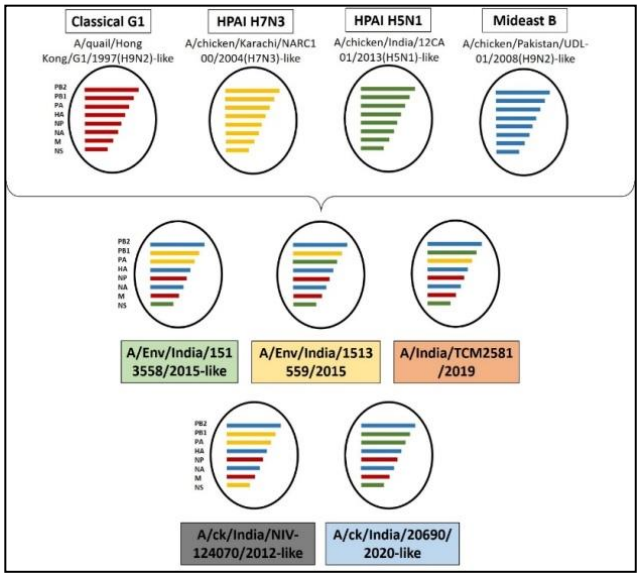
NABL (National Accreditation Board for Testing and Calibration Laboratories) Accreditation of human Influenza, Avian Influenza, Maximum Containment Laboratories and Engineering Support Group as per ISO/IEC 17025:2017.

Investigators: Shailesh D Pawar, Jayati Mullick
Contributors: Sachin S Keng, Sadhana S Kode, Dinesh K Singh,
Funding: Intramural **Duration**: Ongoing
Background: The ICMR-NIV is maintaining the Quality Management System since 2016 as per the ISO/IEC 17025:2017 guidelines.
Objectives: To continue the Quality Management System at ICMR-NIV.
Findings:
Preparation of AI laboratory and documentation in compliance with ISO/IEC 17025:2017.
Preparation for internal audit of AI Group, August 2021.
Submission of documents for renewal application of accreditation.
Attended NABL reassessment audit of ICMR-NIV as per ISO/IEC 17025:2017, through video conferencing, 18^{th} – 19^{th} September 2021.
Conclusion: The NABL granted continuation of accreditation to ICMR-NIV as per ISO/IEC 17025:2017.

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

Investigators: Shailesh D. Pawar, Deeksha S. Tare
Contributors: Sadhana S. Kode, Sachin S. Keng, Atul M. Walimbe, Vinayak V. Limaye
Funding: ICMR, SRF grant to Deeksha Tare (VIR/Fellowship/1/2018-ECD-I)
Duration: 2018-2022
Background: Low pathogenic avian influenza (LPAI) H9N2 viruses are known to cause human infections and have pandemic potential. These viruses contribute genes to highly pathogenic avian influenza viruses. H9N2 has been prevalent in India for more than two decades and recently the first human case was reported. There is no data on the gene pool analysis and genetic characterization of H9N2 viruses from India.
Objectives: Gene pool analysis of avian influenza viruses isolated from India and Full genome sequencing and characterization of the pool of H9N2 viruses isolated at ICMR-NIV
Findings: Full genome sequences were analyzed (viruses reported from 2003-2020) and several amino acid markers for increased pathogenicity and/or mammalian phenotype were observed. We found five novel reassortant genotypes of H9N2 viruses in India. The HA, NA and PB2

genes of all viruses belonged to the Middle Eastern B sublineage; NP and M to the classical G1 lineage; whereas PB1, PA and NS bore resemblance to either HPAI H7N3 or H5N1 viruses (**Fig. 6**). The viruses showed dual receptor specificity and high growth potential. Molecular clock and phylogeography revealed that the introduction of all the genes to India took place around the years 2000 to 2001 indicating complex transmission patterns. The present study is the first detailed account of the phylogeography, evolution and virological characterization of the H9N2 viruses from India. The viruses have undergone inter- and intra-subtype reassortments and show a gradual shift towards mammalian adaptation. Thus, highlighting the urgent need to carry out virological and molecular surveillance.



(Env - environment, ck - chicken)

Fig. 6. Genetic composition and circulating genotypes of H9N2 viruses from India

Correlation of structural and enzymatic characteristics of avian influenza H9N2 virus neuraminidase showing unique properties of elution, substrate binding and enzyme kinetics

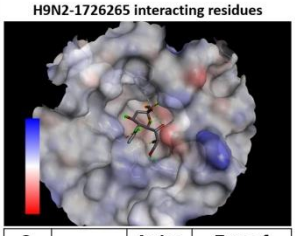
Investigators: Shailesh D. Pawar, Pratip Shil

Contributors: Deeksha S. Tare, Nitin Atre

Duration: September 2021 to February 2022

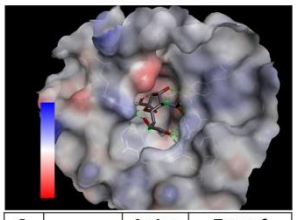
***Background*:** Avian influenza H9N2 viruses are of zoonotic importance and have been undergoing evolution in nature, leading to mammalian adaptation and increased pathogenicity. The virus neuraminidase (NA) protein plays a key role in the viral replication and is responsible for enzymatically cleaving the sialic acid bonded with the viral hemagglutinin. In the present study we describe the properties of a unique H9N2 virus with novel substitutions in the NA.
***Objective*:** To elucidate the structural and enzymatic properties of a unique H9N2 virus isolated in 2017.
Findings: The virus isolate namely A/Environmental/India/1726265/2017 (H9N2-1726265) bore a unique two amino acid substitution (position 76-77) in the NA stalk region, and was characterized further. A previously isolated and characterized virus, A/chicken/India/99321/2009 (H9N2-99321) was used for comparison. H9N2-1726265 possessed several amino acid substitutions. Homology modelling revealed several differences in surface contour and hydrophobicity. Molecular docking revealed more binding interactions of H9N2-1726265 to sialic acid as compared to H9N2-99321 (**Fig. 7**). The virus also showed an enhanced capability to elute Turkey red blood cells. NA enzyme kinetics revealed higher MUNANA substrate binding affinity of H9N2-1726265. Thus, it was found that the concerted effect of two amino acid deletion and several substitutions conferred better elution capability and enhanced substrate binding properties to the H9N2 virus.

H9N2-1726265 interacting residues



S. No.	Position	Amino acid	Type of interaction
1	152	Arg	Attractive H bonds
2	224	Arg	C-H bond
3	292	Arg	H bonds
4	294	Asn	H bond
5	396	Tyr	H bonds

H9N2-99321 interacting residues



S. No.	Position	Amino acid	Type of interaction
1	118	Arg	H bond
2	224	Arg	C-H bond
3	407	Tyr	H bond C-H bond

Fig. 7. Differences in the binding of sialic acid substrate with neuraminidase protein.

Use of one and half-year-old glutaraldehyde-fixed turkey red blood cells for hemagglutination and hemagglutination inhibition assays of influenza viruses

Investigators: Shailesh D. Pawar

Contributors: Sadhana S. Kode, Deeksha S. Tare

Duration: January 2022 to April 2022

***Background*:** Hemagglutination (HA) and hemagglutination inhibition (HAI) assays are the most widely used tools for the detection and serology of influenza viruses. Freshly prepared red blood cells (RBCs) suspension, with a shelf-life of about a week, is essential in these assays, for the detection of the hemagglutinating viruses. We developed a novel methodology to preserve turkey RBCs (tRBCs) and to enable their use in HA and HAI assays, even after 1.5 years.

***Objective*:** To study the utility of frozen glutaraldehyde-fixed turkey RBCs stored for a period of 1.5 years in HA and HAI assays.

Findings: Previously stored glutaraldehyde-fixed tRBCs were used for the detection of influenza viruses of various subtypes in HA assays; and for the detection of homologous immune serum specimens of representative viruses in HAI assay. 0.5% freshly prepared tRBCs were used parallelly for comparison. Influenza viruses of various subtypes were used in HA and HAI assays. It was found that there was no significant difference in the virus or antibody titers of any of the viruses using either of the two RBC preparations, indicating the utility of the fixed RBCs. Fig. 8 shows a representative HA assay of four H9N2 virus specimens using fresh and stored tRBCs. Thus glutaraldehyde-fixed tRBCs can be a useful tool in influenza virus detection in resource-poor settings and also for laboratories which do not have access to animal facilities.

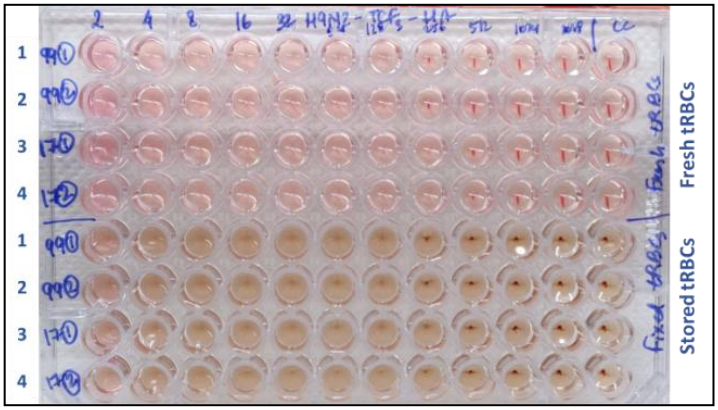


Fig. 8. Comparison of fresh and stored tRBCs in HA assay.

The seasonality of highly pathogenic avian influenza H5N1 and H5N8 outbreaks in India, 2006-2021

Investigators: Shailesh D. Pawar

Contributors: Sadhana S. Kode, Deeksha S. Tare

***Background*:** The highly pathogenic avian influenza (HPAI) outbreaks cause severe economic losses posing pandemic threat. We report the spatio-temporal distribution and seasonality of HPAI outbreaks in India from 2006 to 2021.

***Objective*:** To study the seasonal patterns and the distribution of HPAI H5N1 and H5N8 viruses in India.

Findings: A total of 284 H5N1 and 57 H5N8 virus outbreaks were reported in poultry; wild resident and migratory birds from India from 2006 to 2021. Maximum outbreaks occurred between December to March, with a peak in January. Understanding the seasonality of HPAI outbreaks would help in their prevention and control. In view of recent human infections of H5N1 in India, the present report highlights the need for monitoring avian influenza viruses in poultry, wild and migratory birds.

VIRUS REGISTRY AND VIRUS REPOSITORY

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Ms. Vaikhari N. Bagul,	MSc Virology student

Genetic and antigenic characterization of measles, mumps and rubella virus isolates.

Investigators: Vaidya SR
Contributors: Ms. Vaikhari N. Bagul, Ms. Payal Kelkar
Funding: ICMR-NIV Duration: 2019 to 2023

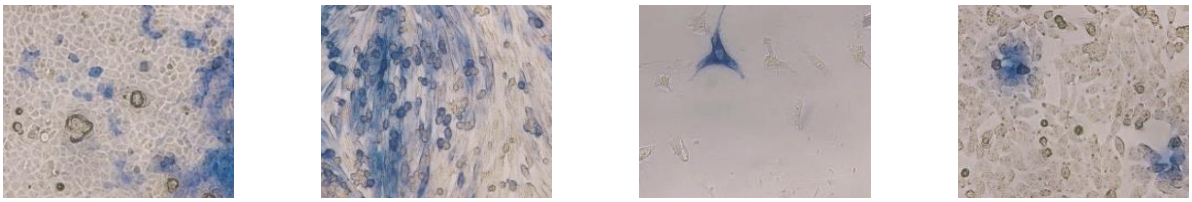
1.1. *Wild type rubella virus studies*

Background: Previously four rubella strains (three wild type and one vaccine strain) were grown on Vero, Vero/hSLAM and RK cells and a comparative growth pattern was studied. This study showed detectable cytopathic effect (CPE) in 24 hrs old Vero/hSLAM and RK cells and detection of virus was more evident by ICA and IFA compared to routine PA. Recently full genome sequencing of rubella viruses revealed three lineages of RuVs (2B genotypes) in India. In addition, a few changes noted in the capsid, E1, E2 proteins of RuV Pune, Kannur and Kolar isolates.

Objectives: Therefore, a study was designed to understand the growth pattern of these genetically diverse viruses (isolated from Pune, Kannur and Kolar) in different cell substrates i.e. epithelial (Vero hSLAM), endothelial (SK HEP-1), fibroblast (MRC-5) and neuroblastoma (Neuro 2a).

Work done and *Findings:* Our preliminary study showed varied cytopathic effects in these cells up to passage-3. Cell rounding and aggregates in Vero hSLAM, changed cell morphology noted in MRC-5, shrunken and floating cells noted in SK HEP-1, however convincing CPE was not evident in Neuro 2a cells (Fig. 1 and 2).

Conclusion: RuV presence was confirmed by RT-PCR and ICA in tissue culture fluids of epithelial and fibroblast cells but limited growth in endothelial and neuropblastoma cells.



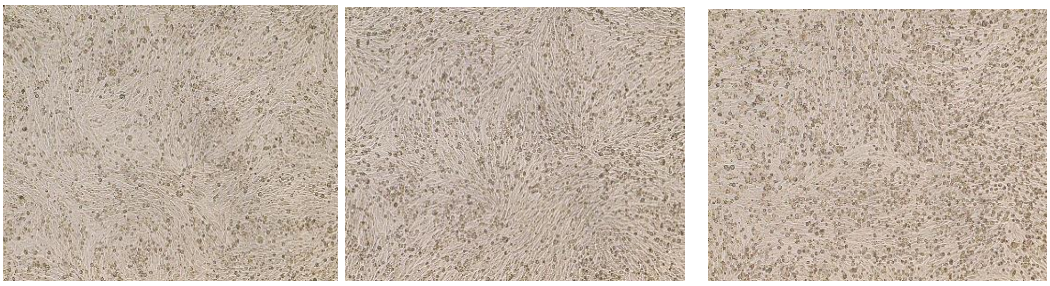
Vero/hSLAM MRC-5 SK-HEP-1 Neuro-2a

Fig. 1: RuV detection by ICA on different cell substrates.

A] Vero hSLAM Cells (day-6 post infection)

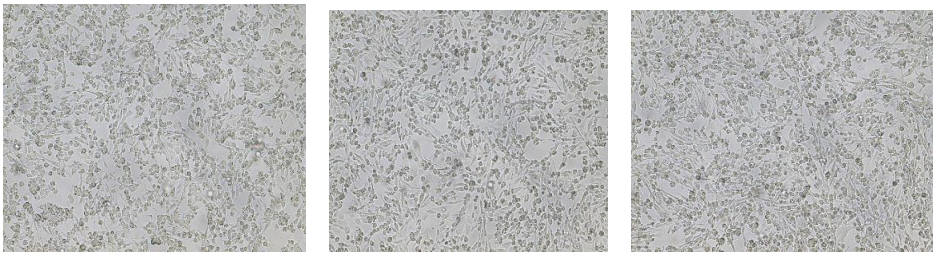
Kannur-2009 Kolar-2007 Pune-1992

B] MRC-5 Cells (day-6 post infection)



Kannur-2009 Kolar-2007 Pune-1992

C] SK-5 HEP 1 Cells (day-3 post infection)



Kannur-2009 Kolar-2007 Pune-1992

D] NEURO-2a Cells (day-3 post infection)

Kannur-2009 Kolar-2007 Pune-1992

Fig. 2: Cell cytopathic effect on different cell substrates (A, B, C and D) by three RuVs.

Wild type mumps virus studies:

Background: As part of genetic characterization of wild type mumps viruses, 11 suspected mumps cases referred during 2019-20 were subjected to MuV RT-PCR and subsequently positive specimens (i.e. cerebrospinal fluid (CSF), throat swabs (TS) and urine samples) were directly utilized for genome sequencing.

Objectives: To sequence mumps complete genomes from the clinical specimens that does not yield virus isolation.

Work done and Findings: Total of three each throat swabs, CSFs and urine specimens were taken for gene specific RT-PCRs and of which seven specimens were further processed for the genome sequencing (Table 1). The complete genomes could not obtain from the clinical specimens due to major gaps in. V/P, M, F, HN and L genes. However, partial sequences of various genes were obtained using Sanger's dye-deoxy sequencing method.

Conclusion: The attempt to retrieve MuV complete genome sequence from clinical specimens was not successful, hence NGS platform may be explored in future studies.

Table 1: Suspected mumps cases and various MuV genes sequenced by the Sanger's method.

Case details			Mumps virus genes sequenced by Sanger's method						
Age*/ Sex	Specimen	Place	N	V/P	M	F	SH	HN	L
9/ M	Throat swab	Pune	Y	Y	Y	P	MZ359747	P	P
12/ M	CSF	Mumbai	Y	P	P	P	MT395309	P	P
10/ M	CSF	Mumbai	Y	Y	P	P	MT395310	P	P
12/ M	Urine	Akola	Y	P	P	P	MT395311	P	P
9/ F	Urine	Akola	N	N	N	N	MT395312	N	N
8/ M	Throat swab	Pune	Y	P	P	P	MT395313	P	P
7.4/ M	CSF	Mumbai	Y	P	P	P	MZ359746	P	P

* Age in years, Y; yes, P; partial, NA; Not available.

1.3. Measles and mumps genome Sequencing by Sanger's and Next Generation Platform

Background: Recently, the field of genome sequencing has been completely evolved due to availability of various sequencing approaches and its rapid output time. In addition, bioinformatics and immunoinformatics branches of science has been grasping attention due to different applications and advanced developments in the computing. Project aimed for comparative sequencing of MeV isolates using 'gold standard Sanger's method' and next generation sequencing (NGS) platform, and its comparison at the nucleotide or amino acid levels.

Objectives: To study genomes of wild type measles and mumps viruses by Sanger's and NGS method.

Work Done and Findings: Altogether, 14 each measles and mumps viruses were subjected to Sanger's and next generation sequencing (Illumina platform, BSL-4 group) and output sequences were compared. Four each isolates were unable to sequence due to low virus titer in NGS but Sanger's method provided quality genome sequences.

Conclusion: The NGS method found to be rapid, sensitive and cheaper compared to Sanger's however, nucleotide read and length of genome was comparatively better in later.

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

Investigators: Vaidya SR

Funding: ICMR-NIV Duration: 2019 to 2023

Background: The appearance of different measles/ rubella virus specific antibodies amongst suspected cases essential to strengthen laboratory diagnosis for case confirmation.
Objectives: Two hundred and eighty-five serum samples with a history of fever with skin rashes were subjected to measles/rubella virus specific IgM, IgG and neutralizing antibody detection and their results were compared with commercial IgG antibody-avidity tests.
Work done and *Findings:* Of this serum panel, 126 (44.2%), 235 (82.4%) and 278 (97.5%) samples were positive for measles IgM, IgG and neutralizing antibodies, respectively. Altogether, 6.3%, 57.2% and 36.5% serum samples showed equivocal (18), high (163) and low (104) IgG antibody avidity respectively. As per kit cut offs, low avidity (>45%) indicates a recent infection (primary) acquired within the past 2 months, high avidity (>55%) indicates either a past infection or reinfection whereas it is difficult to interpret equivocal (45-55%) and recommend to screen a second sample after 2 weeks. Interestingly, 97.5% samples showed presence of measles neutralizing antibodies except seven samples (three equivocal avidity, two low avidity and two high avidity). Similarly, this serum panel was subjected to rubella IgG avidity. Of this serum panel, 17.2%, 59.6% and 69.1% were positive for IgM, IgG and neutralizing antibodies, respectively. Amongst these 1.7%, 24.2% and 74% serum samples showed equivocal (5), low (69) and high (211) IgG antibody avidity respectively. Interestingly, 69.1% samples showed presence of rubella neutralizing antibodies except 88 serum samples (one equivocal avidity, 20 low avidity and 67 high avidity). Overall, 69% (87/126) and 81.6% (40/49) fever with skin rash cases showed recent measles and rubella infection, respectively and results corroborate with measles/ rubella IgM antibody detection. Interestingly, 27% (34/126) and 14.2% (7/49) cases suggests a past infection with persisting IgM or reactivation of measles and rubella, respectively. Conclusion: IgG avidity provides information about the recent or past infection in the clinically suspected cases and may be a useful tool for MR elimination goal-2023.

Virus Registry Routine Service Activities

Investigators: Vaidya SR

Funding: ICMR-NIV **Duration:** 2014 onwards

***Background* and *Objectives*:** Majority of clinical specimens received at Virus registry for laboratory diagnosis of various viral etiologies. Initially, case details and referring doctors letter has been checked and after registration of request, a unique sample number (NIV ID) has been provided and samples were handed over to the main laboratory with utmost precautions. Sometimes, selected specimens were referred to the concerned/ specialized laboratories, hence not registered.

Work done: During this reporting period, various clinical specimens referred to ICMR-NIV Pune for the laboratory diagnosis through Virus Registry. Altogether, 5276 clinical samples were

referred for the diagnosis of Dengue (n=3262), Chikungunya (n=331), Kyasanur Forest Disease (n=162), Zika (n=4), Haemorrhagic Fever (n=4), Japanese Encephalitis-Chandipura-Cytomegalovirus-Rabies (n=987), Hepatitis (n=601, HAV-55; HEV-63; HBSAg-420; HBV/HCV PCR-63), Enteric (n=247) and Measles-Varicella (n=9) viruses. All the laboratory reports were handed over to the concerned via email or in person. A collection of Rs. 216850/- was done against various paid tests and cash deposited in the account section.

Virus Repository Audit and Supply of Prototype viruses

Investigators: Vaidya SR

Funding: ICMR-NIV Duration: 2014 onwards

***Background*:** ICMR-NIV is holding large number of archived serum samples, stock viruses and immune sera since its beginning and it requires intermittent audit to cross check all the available records. Also, records of various prototype viruses provided to different government and non-government organizations needs to be regularly updated. During this period, requests received from various institutes/ organizations were processed as per the standard protocol.

***Objectives*:** To understand real stock of viruses (stock bank) and stored serum samples, internal audit was undertaken. A team of four technical staff physically verified freezer rooms where Virus Registry and Virus Repository (VRVR) racks were kept.

Work done and Findings: All the available records and specimens details were physically checked and re-confirmed. Both freezer rooms were kept in a proper order and cleaned. All the unwanted materials/ belongings, broken vials were discarded as per the biosafety rules by keeping up to date record. Some group/ departments specific archived specimens/ reagents were handed over to the respective groups. The details of summarized activity provided herewith.

Stock viruses: Of 64 viruses available in the Virus repository, 12307 lyophilized ampoules are placed in 27-racks and 131-drawers were physically verified. Overall, 206-archived positions were found vacant. Altogether, 2103 lyophilized ampoules (of 166 likely virus isolates) were unidentified as per the records. Of which, 25% from human, 14% derived from bat-bird-monkey-horse-other animals, 35.6% from tick-mosquito-sand fly and remaining 25.2% from unknown sources. Request sent to NIV group leaders for the identification of same (previously identified but record not available).

Immune sera: Total 15304 immune sera from human or animal origin were physically verified. Of which, 2583-vials were found at proper location whereas 81-vials were wrongly labelled and positioned, subsequently properly recorded in the database. Altogether, 486 archived positions were found vacant. It has been noted that 12154-vials were not entered in the available old records, so verified and re-entered in the database.

Epidemiological sera: Altogether, 229804-vials containing archived sera found at their respective locations as per the old records. Of which, 226063 vials were physically located

whereas; 1671-vial positions were vacant. Ten broken vials were discarded as per the biosafety guidelines. Altogether, 910-vials were mismatched with old records and subsequently details of the vials and its archived positions were properly recorded in the database. It has been noted that the records of 1150-vials were not available in the old records but physically verified and freshly entered in the database. Altogether, 11 leather bound registers of archived serum samples were prepared for future use (Fig. 3).

Fig. 3: Leather bound registers of archived serum samples with available details.

Altogether, eight requests received for Dengue, Japanese encephalitis virus, Rotavirus, Respiratory syncytial virus and Influenza viruses. Of which, one request has been processed and other requests were kept on hold due to policy of ICMR HQ (since 22nd November 2021). Also, other requests (n=4) for procurement of prototype viruses [Dengue- (serotypes1, 2, 3 & 4), Chikungunya, Encephalomyocarditis virus and Vesicular stomatitis virus] from various ICMR institute (VCRC Puducherry), government organizations (NCDC Delhi, KSTM Kolkata) and private organization (Meril Endosurgery Pvt Ltd Vapi) were received and documents processed. Prototype Dengue serotype-1 (Hawai), serotype-2 (TR-1751), serotype-3 (NIV strain: 633798) and serotype-4 (NIV strain: 611319) strains were supplied to Jamia Millia Islamia University, New Delhi.

Genetic characterization of clinically suspected Measles, Mumps, Rubella and Chickenpox cases reported to the local hospitals.

Investigators: Vaidya SR

Funding: ICMR-NIV **Duration:** 2022 to 2025

***Background* & *Objectives*:** As a part of laboratory based outbreak investigation or diagnostic support during Feb 2022, chickenpox cases were reported from Surangi village (PHC Amboli) situated in Dadra & Nagar Haveli (UT). For the case confirmation, seven male cases aged between 18-28 years were referred for laboratory diagnosis.

Work done and *Findings:* All cases showed presence of VZV specific IgM antibodies. Reports were sent to concerned health officials. In addition, the protocol of VZV DNA real time PCR (CDC USA) was successfully standardized (procured primers-probes) and compared with available DNA PCR (ORF-28). For this purpose, cell culture passaged wild type VZV and 5

blister swabs collected from the suspected chickenpox cases (Dadra & Nagar Haveli) were utilized. In future, clinical specimens referred from suspected cases will be tested and sensitivity and specificity of VZV DNA real time PCR will be studied.

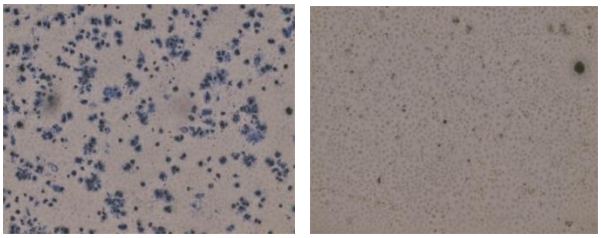
Genetic and antigenic characterization of unidentified, culturable and non-culturable viruses available at ICMR-NIV Virus repository

Investigators: Vaidya SR, Bondre VP & Potdar VA

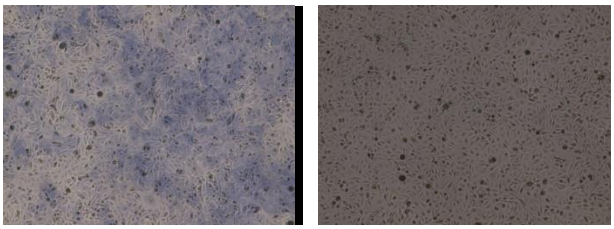
Funding: ICMR-NIV Duration: 2022 to 2025

***Background* & *Objectives*:** In connection with this project, preliminary standardization of Cell culture-based Immunocolorimetric Assays for rotavirus and Japanese encephalitis virus isolates were carried out.

Work done and *Findings:* Rotavirus (Group A, SA-II deposited by Enterovirus Group) was grown on MA104 cells and infected cells detected using immunocolorimetric assay (ICA) after 1-3 days of post-infection. Similarly, Vero adapted Japanese virus strains (JEV 733913, JEV 0945054 & JEV 057434) were procured from Encephalitis Group and ICA was standardized. Assay standardization/ optimization is in progress and subsequently, ICA based neutralization test will be developed that will be useful to understand vaccine-induced immune response in the vaccinated individuals. In addition, attempts to grow/ revive prototype strains of dengue serotypes (all four) is in progress.



Rota-ICA on MA 104 cells b) MA 104 cells



JE-ICA on Vero E6 cells d) Vero E6 cells

Fig. 4: Rota virus-ICA on MA 104 cells (a & b) and JE virus-ICA on Vero E6 cells (c & d) on 2-days post-infection.

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AFP Surveillance

Title – (i) 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. **(ii)** Intratypic differentiation of poliovirus isolates from AFP cases received from National Polio Laboratory as a part of WHO-SEAR Polio Lab Network in the WHO's Global Eradication of Poliomyelitis Programme.

Investigators – Ashok M, Sakib Akther

Funding – WHO ***Duration –*** In service project (since 1997)

***Background*:** Acute Flaccid Paralysis Surveillance for Global Eradication of Poliomyelitis Programme was initiated by the Govt. of India in collaboration with the WHO/WHO-SEARO/WHO-NPSP. As a part of this program India have one specialized and seven national laboratories. NIV-BU is one of the National Polio Laboratory (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: Total of 4874 samples were received from Bihar (n-2721,56%), Karnataka (n-982,20%) and Kerala (n-509,10%). In virus isolation RD cell line positives were 11.3% (n-554) that is NPEV. L20B cell line positives were 1.5% (n-73), among them 49 L20B positive isolates were positive for Sabin like 1 & 3. Remaining 24 samples were NPEV by PCR. 99.67% of samples were reported within 14 days of received date. For more information refer Table 1.

Table 1. Summary of AFP samples investigated by NIV BU during April 2021 to March 2022.

Region	Total No. of cases	Total No. of Samples	L20B	NPEV	ITD				VDPV		
					S1	S3	S1 + S3	NPEV PCR	S1	S3	S1 + S3
Karnataka	491	982	13	46	0	6	0	7	0	6	0
Kerala	255	509	7	5	0	2	2	3	0	2	2
Bihar	1366	2721	50	439	9	12	14	14	9	12	14
Others	349	662	3	64	1	3	0	0	1	3	0
Total	2461	4874	73	554	10	23	16	24	10	23	16

NPEV-Non Polio Entero Viruses; S1-Sabin like 1; S3-Sabin like 3; VDPV – Vaccine Derived 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, Sakib Akther

Funding – WHO Duration – Since January 2019

Background: Environmental Surveillance of Polio virus in sewage is a part of Global Eradication of Poliomyelitis Programme. In Bangalore city four sewage sites have been identified for this purpose.

Objectives: To identify Polio virus circulation in sewage in Bangalore city.

Findings: We received 102 sewage samples from four sewage zones of Bangalore city. 66.6% (n-68) of samples were positive in RD cell line and remaining 33.3% (n-34) is L20B cell line positives. All 34 L20B positive samples were positive for Sabin like 1 & 3. For more details refer Table 2.

Table 2. Summary of EV samples investigated by NIV BU

Sites name	No. Sample	L20B	NPEV	ITD				VDPV		
				S1	S3	S1 + S3	NPEV PCR	S1	S3	S1 + S3
V Valley	26	9	17	6	9	6	0	6	9	6
HBL	26	10	16	8	7	6	0	8	7	6
KCV	25	4	21	3	3	1	0	3	3	0
RCL	25	11	14	6	9	2	0	6	6	0
Total	102	34	68	23	28	15	0	23	28	15

NPEV-Non Polio Entero Viruses; S1-Sabin like 1; S3-Sabin like 3; VDPV – Vaccine Derived Polio Virus

Measles Rubella Surveillance

Title – (i) 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, Sakib Akther

Funding – WHO ***Duration –*** In service project (since 2006)

***Background*:** Measles & Rubella infection is a vaccine preventable disease. India is one of the countries with largest number of measles & Rubella cases. To address the high disease burden caused by measles in India, WHO-SEAR Measles Laboratory Network (NML) and Measles NetIndia network was established and it is being expanded in phased manner. At present there

are 19 functional WHO-SEAR laboratories in India. Among them NIV, Bangalore unit is one of the national laboratories.

***Objectives*:** To Identify population at risk and guiding vaccination strategies. To determine genotype of virus circulating in the country. Detecting cases in timely manner in order to carry out control and preventive measures.

Findings: We received 1321 serum samples from Karnataka state. Of them 24 samples were positive for Measles IgM antibodies and 84 samples were positive for Rubella IgM antibodies. Also we received 507 Throat swab samples that was tested for Measles and Rubella RNA, of them none were positives.

Arboviral testing for Human samples under National Vector Borne Disease Control Program (NVBDCP)

Title – Surveillance of Dengue, Chikungunya and Japanese Encephalitis cases from Bangalore city, urban, rural and neighbouring areas under NVBDCP.

Investigators – Ashok M, Sakib Akther

Funding – 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 transmission of vector borne diseases depends on prevalence of infective vectors and human vector contact, which is further influenced by various factors such as climate, sleeping habits of humans, density and biting of vectors etc. The program covers six diseases, of them, we perform serology testing for Japanese Encephalitis (JE), Dengue, & Chikungunya.

Objectives: Serological investigation of Dengue, Chikungunya and JE suspected cases from urban and rural areas of Bangalore district.

Findings: We received 766 samples from Bangalore district and its neighbouring districts. Among received samples 110 samples were positive for Dengue IgM antibodies, 142 samples were positive for Chikungunya IgM antibodies, 18 samples were positive for both Dengue and Chikungunya IgM antibodies.

Arboviral testing for Mosquito pools under National Vector Borne Disease Control Program (NVBDCP)

Title – Surveillance of Dengue, Chikungunya and Japanese Encephalitis virus in Mosquito samples from Bangalore city, urban, rural and neighbouring areas under NVBDCP.

Investigators – Ashok M, Sakib Akther

Funding – NVBDCP **Duration –** In service project (since 2019)

***Background*:** The transmission of vector borne diseases depends on prevalence of infective vectors and human vector contact, which is further influenced by various factors such as climate, sleeping habits of human, density and biting of vectors etc. Bangalore unit is identified as state molecular laboratory for testing mosquito samples for Dengue, Chikungunya and JE virus.
***Objectives*:** Conventional PCR investigation of Dengue, Chikungunya and JE viruses in Mosquito samples collected from various districts of the state.
Findings: About 277 Mosquito pools were received from 24 districts of Karnataka state. Each pool constituted of 10 to 20 mosquito, males and females were separated. Of total Aedes aegypti accounted for 266 mosquito pools. Eleven pools were Aedes Albopictus. All pools were tested by conventional PCR for Dengue and Chikungunya RNA and found that 15 pools were positive for Dengue virus.

Congenital Rubella Syndrome (CRS) Surveillance.

Investigators – Ashok M
Funding – ICMR **Project code –** BNU1604
***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 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: We received 130 samples fulfilling case definition for CRS from IGICH hospital. Six samples were IgM positive and 10 samples were IgG antibodies for Rubella virus. Eighteen Throat swabs were tested for Rubella RNA by conventional PCR and found no positives.

Laboratory investigation of Severe Acute Respiratory Infection (SARI) cases

Investigators – Ashok M
Funding – Intramural ***Duration* –** Ongoing (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 multiplex real time RT-PCR
Findings: Total of 1010 SARI samples were received for Influenza molecular testing. Influenza A H1N1 RNA was positive for 20 samples from nine districts, Influenza A H3N2 was positive among 28 samples from ten districts and Influenza B was positive among 75 samples from 13 districts. RSV A was positive among 281 samples from 21 districts of the state.

Virus Research Diagnostic Laboratory Network projects

Investigators – Ashok M
Funding – DHR **Duration –** From 2020
***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
7.1. Monitoring of dengue virus and its serotype circulating in India for changes in the serotypes, genotype and lineages utilizing Viral Research & Diagnostic Laboratories Network.

For Dengue serotyping by real time PCR 398 samples were received from 22 districts of the state. All samples were NS1 positive by ELISA. Dengue serotype 2 was positive among 174 samples, Dengue 3 was positive among 46 samples, 44 samples were positive for Dengue serotype 2 and one case was positive for Dengue 4 serotype.

7.2. Testing for Scrub Typus for Acute Encephalitis Syndrome (AES) and Fever Rash Syndrome (FRS)

We received 20 samples fulfilling criteria for Scrub Typus and tested for Scrub Typus IgM antibodies and found that 02 were positive.

7.3. Testing for Leptospirosis for AFI

We received 20 samples fulfilling criteria for Leptospiross and tested for Leptospirosis IgM antibodies and found no positive.

KFD serology and Molecular testing

Investigators – Ashok M

Funding – Intramural (NIV Pune) **Duration –**Since 2019

***Background*:** KFD is endemic in five districts of Karnataka. Every year suspect samples from these five districts are reported regularly during November to May months. The human suspect samples are handled in BSL-2 level whereas tick and monkey autopsy samples need to be handled in BSL-2 level. In Karnataka state VDL at Shivamogga district test Human samples suspected for KFD our unit is reserve and cross checking laboratory in the state.

***Objectives*:** 1. To perform Serology and molecular diagnostics on suspect KFD human samples

Findings: We received 58 samples and tested for KFD virus. Of 22 samples tested for KFD IgM antibodies eight were positive and 36 samples were tested for KFD RNA and found six were positive.

Lyme Disease Human surveillance

Investigators – Ashok M

Funding – ICMR D**uration –** 2020

***Background*:** Data on seropreavalence, complications of Lyme disease among population and etiological agent causing LD is unknown in our geographical area. Clinical case studies on LD currently available are not sufficient for a definite proof on the existence of LD in India. The reported numbers may be an underestimate due to lack of community-based data and non-availability of laboratory tests for proper diagnosis. The disease is most common in resource-limited settings such as forest and rural areas.

***Objective*:** Screening of patients with clinical evidence of Lyme disease using recombinant ELISA followed by confirmatory recombinant immunoblot.

Findings: Five suspect samples were received and tested for Lyme Disease serology. We found one case was positive for Borrelia IgG antibodies.

Number of samples tested (virus-wise details)

Sl No	Viruses	Total samples	Sample Type	Virus Isolation	Serology Positive	Molecular Positives
1	SARS CoV-2	467553	Throat/Nasal swab	-	-	30636
2	SARS CoV-2 Sewage surveillance	104	Sewage samples	-		37
3	Polio AFP surveillance	4874	Stool	627	-	49
4	Polio Sewage Surveillance	102	Sewage	102	-	34
5	Measles/Rubella	1312	Serum	-	108	-
6	Measles/Rubella	507	Throat swab	-	-	-
7	Dengue/Chikungunya	2766	Serum	-	270	-
8	Rubella	130	Serum	-	16	-
9	Influenza A & B	1010	Throat swab	-	-	403
10	Dengue serotyping	398	Serum	-	-	265
11	Scrub Typhus	20	Serum	-	02	-
12	Leptospirosis	20	Serum	-	-	-
13	KFD	58	Serum	-	08	06
14	Lyme Disease	5	Serum	-	0	-
15	Zika Virus	72	Serum	-	-	-
16	Mosquito surveillance	277	Mosquito samples	--	--	15

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Establishment of a network of laboratories for managing epidemics and natural calamities

Investigators: Dr. AP Sugunan & Dr.B.Anukumar

Funding: DHR **Duration:** 2019-24

***Background*:** The project is in response to DHR's timely initiative on setting up of State Level Virus Research and Diagnostic Laboratory (VRDL) at ICMR-National institute of virology in Kerala state to supplement the existing national facilities in surveillance, outbreak investigations and research in medically important viruses.

***Objectives*:** To establish a state level diagnostic virology laboratory that includes sequencing facility to investigate the viral diseases of regional and national importance.

Findings: The attention was given to diagnoses of COVID -19 because of the third wave of the SARS-CoV-2 pandemic. For COVID-19, almost 145000 samples were tested. The facility is an ICMR-recognized laboratory for the antigen, RT-PCR, RNA, and VTM kit validation. One RNA kit, one LAMP PCR kit, seven RT-PCR kits, and three antigen detection kits have all been validated. We have samples for the diagnosis of additional viral diseases in addition to COVID-19. A total of 3434 samples from 1057 individuals were received for viral detection throughout the reporting period (Table 1). Investigative work was done on an acute gastroenteritis outbreak. In cases of acute gastroenteritis, norovirus GII was confirmed. The norovirus was first discovered in Kerala. The virus was sequenced, a phylogenetic tree was built, and it was similar to the virus from Japan from 2015. We have been conducting case-based surveillance for measles, rubella, and influenza A and B virus infections as a part of this project. We got 112 samples for MR surveillance, processed them for IgM and PCR tests, and received a perfect score on the global proficiency test.

Table 1: The number of samples screened for different viruses using various diagnostics (Total number/total positives).

Sl. No	Virus/Bacteria	Conventional PCR	Real time- PCR	IgM ELISA
		Tests/ Positive	Tests/ Positive	Tests/ Positive
1	Japanese encephalitis		19/0	122/2
2	West Nile virus		11/0	116/1
3	Enterovirus		205/3	0/0
4	Dengue virus		358/8	10/3
5	Chikungunya virus		234/2	1/0
6	Leptospira		522/106	14/2
7	KFD		11/0	2/0
8	Scrub typhus		63/0	0/0

9	Herpes simplex virus (HSV)		256/1	8/0
10	Rotavirus	23/0	0/0	0/0
11	Hepatitis A (HAV)	0/0	0/0	8/0
12	Hepatitis E virus (HEV)	0/0	0/0	8/0
13	Hepatitis C (HCV)	4/0	0/0	0/0
14	Hepatitis B (HBV)	5/1	0/0	0/0
15	Cytomegalovirus CMV		79/15	1/0
16	Varicella-Zoster (VZV)	5/2	1/0	0/0
17	Respiratory Syncytial Virus (RSV)	94/52	0/0	0/0
18	Epstein-Barr virus (EBV)		32/4	2/0
19	Adenovirus		26/0	0/0
20	Influenza A (p H1N1)		910/1	0/0
21	Influenza A (H3N2)		910/104	0/0
22	Influenza B		910/33	0/0
23	Parvovirus		4/0	0/0
24	NIPAH		46/01	0/0
25	ZIKA VIRUS		271/9	0/0
26	FTD RESP		8/2	0/0
27	FTD NEURO		17/2	0/0
28	NOROVIRUS		40/7	0/0
29	MEASLES	41/0	0/0	109/3
30	RUBELLA	41/0	0/0	106/3
31	CHANDIPURA VIRUS	0/0	0/0	0
32	MUMPS	6/0	0/0	10/5
31	**SARS-CoV2**		**146097/44667**	
TOTAL		**219/55**	**150224/44967**	**517/19**

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

Investigator: Dr. B. Anukumar.

Funding: DHR **Duration:** 2019-2022

***Background*:** Chandipura virus (CHPV) belongs to genus *Vesiculovirus*, family *Rhabdoviridae* is an emerging tropical pathogen in India with a case fatality rate of 55-75%. Neither specific

antiviral nor vaccines are available to date against CHPV infection. RNA vaccines are popular nowadays, several vaccines, including ZIKA, influenza, HIV virus are already in clinical trials. RNA based vaccines are safer than a DNA vaccine because no issue of integration of DNA material into the human genome.

***Objectives*:** The aim of this project is to develop glycoprotein (G) gene based RNA vaccine against Chandipura virus and test its immunogenicity and potency in mice.

Findings: In the first year, the CHPV glycoprotein (G) gene mRNA was synthesised and its expression in Vero cells was confirmed. This year the liposome based delivery system was developed and characterized. Two types of cationic liposomes were prepared; cationic lipid liposomes and cationic adjuvant formulation (CAF01). Both the liposome formulations were prepared by thin film hydration method. The encapsulation efficiency and transfection efficiency was studied by using the green fluorescent plasmid pEGFP C3 for cationic liposome and plasmid pDsRed-Express C1 for CAF01 in Vero cells. The results confirmed that the cationic liposome has 70-80% encapsulation efficiency and green fluorescent positive cells were noticed in transfected cells (Fig,1A). Similarly the CAF01transfected Vero cells emitted red fluorescence (Fig.1B) under fluorescence microscope. The study concludes that both the liposomes can be used for delivery of CHPV mRNA. The liposome with CHPV mRNA will be prepared and will be tested its immunogenicity, and potency in mice.

(B)

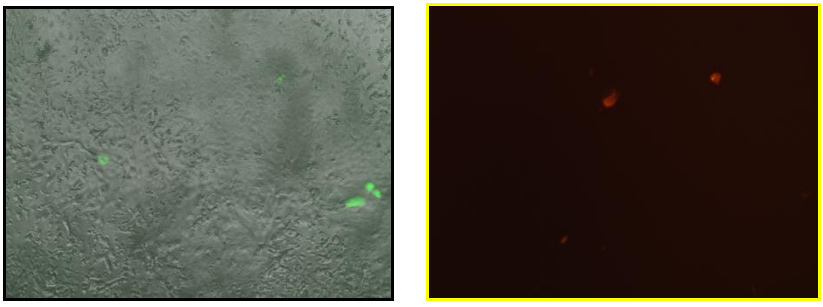

Fig. 1 Vero cells transfected with different types of liposomes. (A)Vero cells transfected with cationic liposomes encapsulated with10µg of pEGFP C3. (B) Vero cells transfected with 50µg of CAF01 complexed with 4µg of pDsRed-Express C1.

Development and Efficiency evaluation of tick repellent from plant extract: A scientific and social intervention on tribal health against ticks and tick-borne diseases in Kyasanur forest disease endemic area of Kerala.

Investigators: Dr. R. Balasubramanian & Mrs. Sahina S

Funding: DST (Women Scientist Project), New Delhi. Duration: 2019-2022

Background: Ixodidae ticks are considered as the most economically important tick species parasitizing livestock, wild animals and humans in India. Ticks are causing many diseases.
Objectives: (i) To develop and standardize plant based repellent cream against tick bite and (ii) To assess the Knowledge, Aptitude and Practice (KAP) among tribal peoples towards tick borne diseases and their traditional practice against tick bite.
Findings: A total of 16 different prototypes of 39 cream were prepared and evaluated against ticks bite. An optimized tick repellent cream was formulated with 2% *T.erecta* flower extract and validated against benzyl benzoate and lemon grass oil under laboratory condition. The extract-based formulation exhibited maximum relative repellency in both larva and adult (97.5 & 87.5%) at 1-3 hours after application. Repellency of the reference product was lower after 1 hour (Benzyl benzoate 71.1 & 26% and lemon grass oil 45.1 & 6.4%). The formulated cream is under standardization for physical appearance. A total of 499 tribal and 2 non tribes (KFD infected), individuals were surveyed in four panchayat. Most of the respondents can recognize tick (89.7%), however only 55.5% respondents were aware about the consequences of tick bite. Among the population, 41.7% individuals are taking precautions to avoid tick bites and about 29.9% populations were used to synthetic repellents, hence 11.8 % of populations were acquainted with natural plants for preventing tick bites.

Title: Clinical and epidemiological study of Lyme disease: A Multi-Centric taskforce study in India.

Investigators: Dr. AP Sugunan & Dr. R. Balasubramanian

Funding: ICMR **Duration:** 2020-2323.

Background: Prevention and management of tick borne Lyme disease used to obtain marginal attention until it become a major public health challenge. The reported numbers may be an underestimate due to lack of community-based data.

Objectives is to (i) To study the abundance and distribution of *Ixodes* tick population in selected study areas toidentify hotspots for Lyme disease (ii) To find out the clinical case of Lyme disease by screening patients using recombinant ELISA followed by confirmatory recombinant immunoblot (iii) Feasibility of application of Real time PCR for the direct detection of *Borrelia* from clinicalspecimens.

Findings: Field collection was carried out in Wayanad district from August 2021 to March 2022. A total of 764 ticks were collected by dragging method which contain 8 species from 4 genera; Haemaphysalis, Rhipicephalus, Hyalomma, and Amblyomma. The abundant species were *Haemaphysalis bispinosa* (71.46%), *H. turturis* (2.10%) and *H. spinigera* (25.40%), and the remaining species were collected below 1% (Table 2). In addition to dragging, 789 ticks were collected from various domestic animals in which 79.81% were positive with tick infestation. The most abundant species were *H. bispinosa* (56.27%), *H. turturis* (33.59%) and *R. microplus* (2.91%), and the remaining species were collected below 1%. A total of 27 pools were prepared.

Five *H. bispinosa* positive for *Anaplasma phagocytophilum*, *H. bispinosa* and *R. microplus* each positive for *Borrelia* spp. and *Anaplasma bovis* respectively. From the 35 rodent traps placed, 5 rodents were captured only in wonder trap and were examined for ectoparasites. From which tick, lice and fleas were collected.

Table 2. Species distribution and pathogen detection from field collected ticks.

Sl. no	**Tick species**	**Dragging (%)**	**Animal collection (%)**	**Pathogen detected in pools**		
				***Borrelia* spp.**	***Anaplasma* spp.**	***Ehrlichia* spp.**
1	*Haemaphysalis spinigera*	194(25.40)	20 (2.53)	0/5	0/5	0/5
2	*H. bispinosa*	546 (21.46)	444 (56.27)	**1/8**	**3 /8**	*0/8*
3	*H.turturis*	16 (2.10)	265 (33.59)	0	0	0
4	*H. shimoga*	1(0.13)	-	0	0	0
5	*H. acculeata*	1(0.13)	-	0	0	0
6	*H.intermedia*	-	14 (1.77)	0	0	0
7	*Rhipicephalus microplus*	1(0.13)	23 (2.91)	**0/10**	**1/10**	*0/10*
8	*R.sanguineus*	-	13 (1.68)	0/1	0/1	0/1
9	*R.decolaratus*	-	7 (0.89)	0/5	0/5	0/5
10	*Amblyomma intergrum*	1(0.13)	3 (0.38)	0/3	0/3	0/3
11	*Hyalomma spp.*	3(0.39)	-	0	0	0
12	*Hy. anatolicum*	1(0.13)	-	0	0	0
	Total	**764**	**789**	1/32	4/32	0/32

Title of the project: Response of urban health service systems to road traffic injuries

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

Funding: ICMR, New Delhi **Duration:** 2019-2021

Background: Road traffic injuries in India contribute to more than 400 deaths daily and like other LMICs, the failure of health services to adequately respond to RTI is identified as the major reason for high mortality. It is in this context, the response of urban health services to RTIs were studied in two different locations- (Mumbai and Alappuzha - first representing a metro city and second in urban nature).

***Objectives*:** The Objective of the study was to map the existing health services in urban areas, their infrastructure and services offered with a view to understand the challenges faced by RTI victims during an event of accident. A case study method was adopted to accomplish the study Objective using observation method of multiple sites of road traffic accidents, This was supplemented by in-depth interviews among RTI victims, relatives, police personnel and among health care professionals.

Findings: One of the major findings is the insignificant contribution of EMS (108 ambulance) in providing medical care to RTI victims. Their proportionate contribution towards transportation of RTI victims were 2% and 7% in metro city and in Municipalities. On the availability of health services within the public sector, there is complete absence of primary level care and only partial availability of secondary level care (during day time) as they offer only primary level care to RTI patients followed by referral to higher levels. This has led to overburdening of tertiary level facility that is already mired with shortage of infrastructure, specialist and other staff.

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

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

Funding: ICMR **Duration**: 2020 -2021

***Background*:** Though vaccinations contribute, there is skepticism and fear, and doubts are being raised about the legitimacy of these many immunizations and their public health importance. WHO (World Health Organization) has identified vaccine hesitancy as one of the top ten global health threats of 2019.

***Objectives*:** To analyze and understand the underlying determinants and decision pathways of vaccine hesitancy. And also explore how the peer-to-peer communication influence vaccine hesitancy and perspective of stakeholders towards vaccination hesitancy as a public health challenge or problem. The study is exploratory by nature. The qualitative methodology has been used and a total of 130 interviews were done.

Findings: The data is thematically analyzed in six phases and descriptive analysis (narrative analysis) regarding acceptance, hesitancy, and trust about vaccines was considered. Faith in doctors, the health system, and interpersonal relationships are intertwined. The alternative therapies adopted by research participants feel that CAM (complementary and alternative medicine) procedures and treatments are safe and free of side effects and they lack trust in modem medicine. There is a lack of understanding among the parents regarding JE and the immunization against it. Few parents didn't aware that vaccination is available against JE and none of the parents could talk about the usual signs and symptoms of the illness. Healthcare organizations and the government must take aggressive measures to soothe public fears about

AEFI because it has been a source of worry among vaccination users. Along with this, stringent action should be taken against false propaganda through social media.

Nipah outbreak in Kerala: An exploratory study on experiences of survivors and responses of the community.

Investigators: Dr Retheesh Babu G, Benito Jayadas & Haritha P

Funding: ICMR **Duration:** 18 months

***Background*:** WHO has identified the Nipah virus as a priority disease for Research and Development blueprint. The first Niv outbreak was reported in Malaysia and then collapsed the pig industry with significant impact on the social and economic aspects of human life. Nipah was first reported in Kerala on 2018 May and it resurfaced in 2019 and 2021. This study focuses on the community response towards Nipah infection in details along with the experiences of the survivors and affected family members which was never done before anywhere in the world.

Objectives: To explore survivor's and their families' experiences (fear, insecurity and mistrust) they had during NiV outbreak and the way people at Nipah affected area perceive the outbreak in three different time frames (2018,2019 & 2021) . To investigate the responses of the community groups towards outbreak issues and to understand the ripple effect in the community. The study is exploratory and qualitative methodology has been used.

Findings: As of now total of 104 (One Survivor/11 Affected Family Members/15 Primary Contacts/28 Community Members/27 Health Professional/22 LSGIs members) in-depth interviews and four FGDs were done. Lack of community preparedness epidemiological placism, fear, mistrust and isolation are being experienced by the survivors, affected family members and people who were actively involved in containing activities in the ground level. The data collection has been completed from Kozhikode districts (first and third outbreak--11 Panchayats and 1 Corporation). Data needs to be collected from Malappuram and Idukki district in the next phase.

List of outbreaks Investigated: Two

1. An acute gastroenteritis outbreak in Alappuzha district, Kerala with Norovirus GII etiology
2. The third Nipah virus outbreak in Kerala

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Dr. Shyam Sundar Nandi	Scientist 'E'
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Mrs. Rupa H Bhavasar	Technical Officer - C
Mrs. Nikita R Shanbhag	Technical Officer - C
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Ms. Ketki S. Jawade	Junior Research Fellow
Mr. Sagar Tivrekar	IT Engineer

National Polio Surveillance Project (NPSP), India

Investigators: Deepa Sharma, Uma Nalavade, Shailesh D Pawar
Funding: Extramural (National Polio Surveillance Project, WHO, India)
Duration: Ongoing (since 1997)
***Background*:** ICMR-National Institute of Virology, Mumbai Unit (NIVMU) has achieved the status of being one of the seven WHO global specialized laboratory for polio since 2000. In India, the last case of wild poliovirus was detected in January 2011. Following this in 2014, the South East Asia Region (SEAR) was certified wild poliovirus free. The surveillance system has therefore become more stringent as there is always a risk of importation of wild poliovirus from neighboring polio-endemic countries, Pakistan and Afghanistan.
***Objectives*:** To carry out rigorous surveillance with continuous monitoring of polioviruses until global eradication of poliovirus is achieved.
Findings: During April 2021 to March 2022, 2406 stool specimens received from AFP cases reported in Maharashtra, Madhya Pradesh and Goa from which 86 Sabin-like (SL) polioviruses (P1SL=38; P3SL=35; P1+P3SL=13 and NPEV=6) were isolated. Under environmental surveillance, 174 sewage specimens/concentrates were tested as at NIVMU as per the WHO protocol for Mumbai, Hyderabad and Patna, from which69 polioviruses were isolated. All the isolates were found to be Sabin-like poliovirus type 1 and type 3 and non-polio enteroviruses. Wild or vaccine derived poliovirus were not detected during this period in India. Also PV2, currently under containment was not detected in India from AFP cases or sewage during the mentioned period. Being a reference laboratory, NIVMU also receives AFP stool specimens and sewage concentrates from National Polio Laboratory, Nepal. A total of 461 stool specimens and 120 sewage concentrates were received during this period from which 49 polioviruses were isolated. All the isolates were found to be Sabin-like poliovirus type 1 and type 3 and non-polio enteroviruses.

Measles and Rubella Surveillance

Investigators: Deepa Sharma, Uma Nalavade, Shailesh D Pawar
Funding: Extramural (National Polio Surveillance Project, WHO, India)
Duration: Ongoing (since 2016)
***Background*:** The WHO Measles and Rubella (MR) network in India comprises of 27 laboratories and NIVMU is the WHO reference laboratory for sequencing. The MR surveillance programme has the goal of elimination of MR by 2024. Under this surveillance activity, NIVMU receives serum specimens, urine and throat swabs from suspected cases of measles and rubella

reported in various districts of Maharashtra, Madhya Pradesh and Goa. The serum specimens are tested using the IgM ELISA whereas the urine and throat swabs are tested using conventional RT-PCR.

***Objectives*:** Elimination of measles and rubella.

Findings: A total of 3254 serum samples were tested for presence of antibodies against measles and rubella viruses. Out of these, 62 (1.90%) were positive for measles and 113 (3.47%) were positive for Rubella. The remaining specimens were equivocal for measles (0.76%) or rubella (1.29%) or negative for both (92.74%). A total of 2715 Urine/throat swabs from Maharashtra, Madhya Pradesh and Goa were tested using RT-PCR. Out of these, 5 (D8 genotype) were found to be positive for measles whereas rubella genotype could not be detected. Being a reference laboratory for sequencing NIVMU received 451 PCR products from WHO MR network labs. A total of 106 measles PCR positive products could be genotyped as D8 (91 no.), D4 (12 no.) and B3 (03 no.) whereas one rubella positive PCR products belonged to genotype 2B.

Development of Tools to prevent Outbreak or Epidemics

Investigators: Deepa Sharma, Uma Nalavade

Funding: Department of Health Research **Duration:** 2021-2022

***Background*:** To increase the sensitivity of MR surveillance in India, a phase-wise switch from outbreak to case-based surveillance was initiated in 2017. This switch resulted in the significant increase in the serology workload. In June 2021, fever and rash surveillance was initiated countrywide leading to further increase in workload. Thus, a need to expand the WHO MR laboratory network in India (MRLN) was identified. To expand the existing laboratory network, 15 VRDL laboratories were selected, trained for MR diagnosis and integrated into the MRLN upon evaluating their proficiency as per WHO standards.

***Objectives*:** To maintain the WHO proficiency of the 15 VRDLs by providing support in terms of reagents/consumables, assuring quality and troubleshooting any issues related to assays/diagnosis.

Findings: The NIVMU procured reagents required for testing of samples by serology and molecular diagnosis. These were supplied to these laboratories by NIVMU as per the request. Before receiving the WHO serology EQAS panel, a virtual orientation was provided to the 9 VRDLs in collaboration with ICMR and WHO. The 15 laboratories tested the WHO EQAS panel for serology and scored 100% for both measles and rubella. Also the initially selected 6 VRDLs also tested WHO molecular EQAS panel and achieved 100% scores. The PCR products of positive measles samples were also provided to NIVMU for genotype characterization. These were sequenced, analyzed and results were provided to the respective laboratories. In addition, support was also provided if any issues related to MR diagnosis were faced by the laboratories.

In summary, efforts were made to monitor the performance of these laboratories and maintain their proficiency.

Study on Polio and Non-Polio enterovirus infections in children with Primary Immunodeficiency at multiple medical institutes across India-Phase-I

Investigators: Madhu Mohanty, Manisha Madkaikar, Ahmad Mohammad, Swapnil Varose, Mevis Fenandes, Unnati Sawant, Shailesh Pawar and the study site investigators

Funding: Extramural (WHO) ***Duration***: 2 years (2019-2021) **Completed**

***Background*:** iVDPV surveillance system initiated by this study to supplement the current AFP and environmental surveillance systems, will help identifying all poliovirus excretors and thus achieve and maintain eradication of all polioviruses.

***Objective*:** To screen patients with primary immunodeficiency across the selected medical institutes in India for poliovirus and non-polio enterovirus excretion and identify any long term excreters among them. Also to characterize the virus isolates and to correlate the virus excretion with host immunological parameters.

Findings: The study assessed 535 stool Samples, 154 patients (65 PADs, 49 CIDs, and 40 others). The samples included 14 SCIDs, 20 CVIDs, 16 XLAs and 104 other PIDs. From 154 cases investigated, 33 patients (21.42%) tested positive for enteroviruses. Out of 33 patients tested positive for enteroviruses, 4 patients (2.59%) tested positive for polioviruses (PVs) and 29 patients (18.83%) were positive for Non-Polio Enteroviruses (NPEVs) (Table 1). A 3 years 6 month old male child, a case of Hyper IgM & Hypo gamma was detected positive for type1 VDPV (iVDPV1) with 16 (1.6%) nucleotide divergence from parent Sabin strain. NPSP involved for sample collection due to challenges faced by sites and steps taken for integration. Immunological experimentations reveal very high IL-8 during the time of clearance of poliovirus. SCID showed highest appearance (31%) among all the Combined Immunodeficiency (CID) categories with higher number of enterovirus infections as compared to others. Poliovirus infections observed in CID categories viz. SCID, WAS and Hyper IgM syndrome. Therefore, PID patients with CID deficiencies could be at higher risk of contracting poliovirus infections.

Table 1: Detection of Polio and Non-Polio Enteroviruses from study sites (Dec 2019-Dec 2021).

Study sites	Total cases enrolled	Total samples received	Total cases deceased	Enteroviruses detected			
				PV positive	Types of PV	NPEV positive	Types of NPEV
B.J.W.H.C, Mumbai	71	224	13	03	iVDPV1 P3SL, P1SL	14	E30, E5, E14, E11, E20, EVB97, E3, E12, EVB75, E13, E3, E32, E7, E29

ICMR- NIIH, Mumbai	21	60	00	00	-	05	E18, E13 (2), CVA2, E21
SGPGIMS, Lucknow	14	63	00	00	-	04	E16, CVA2, EVB75, E21
GMC, Calicut	14	48	00	00	-	-	-
ASTER CMI, Bangalore	13	33	02	00	-	01	CVA2
KMC, Mangalore	12	34	00	01	P3SL	01	CVA2
NIMS, Hyderabad	09	28	01	00	-	02	CVA8, E15
Total	154	484	16	04 (2.59%)	-	27 (17.53 %)	-

Study on Polio and Non-Polio enterovirus infections in children with Primary Immunodeficiency at multiple medical institutes across India-Phase-II

Investigators: Madhu Mohanty, Manisha Madkaikar, Ahmad Mohammad, Swapnil Varose, Unnati Sawant, Manogat Tatkare, Shailesh Pawar and the study site investigators

Funding: Extramural (WHO) *Duration*: 2 years (2022-2023)

Background: Based on the results of phase I study it was recommended by the Polio Research Committee, WHO HQ, Geneva to expand the study to Phase II to include Pan India PID diagnostic hospitals/facilities.

Objectives: To expand iVDPV surveillance across India, to align the study to the global guidelines of surveillance, learn lessons for its implementation in the national program and to explore potential merging of future iVDPV surveillance into the ongoing AFP surveillance.

Findings: The phase II study included a total number of 21 study sites across India (Fig. 1). Meeting of Experts and investigators, site visits for preparedness and training at study sites, ethical clearance and initiation of study sites were conducted and testing of samples started from Jan 2022 onwards. More than 150 Clinicians/ immunologists at 10 major hospitals/ institutes were trained and the site preparedness was assessed during the period. From Jan 2022 to March-2022, 49 stool Samples were tested from 35 patients. From 35 cases investigated during the period, 5 patients (14.28%) tested positive for enteroviruses and out of 5 patients tested positive for enteroviruses, 3 patients (8.57%) tested positive for polioviruses (PVs) and 2 patients (5.7%) positive for Non-Polio Enteroviruses (NPEVs). The three patients found positive for Sabin polioviruses inadvertently vaccinated with bivalent OPV during National pulse polio Immunization day on 27th of Feb, 2022. These children are being followed up for prolonged

excretion. Treating clinicians have been informed to counsel PID patients/parents to not to vaccinate these children with live viruses, and the consequence of the same.

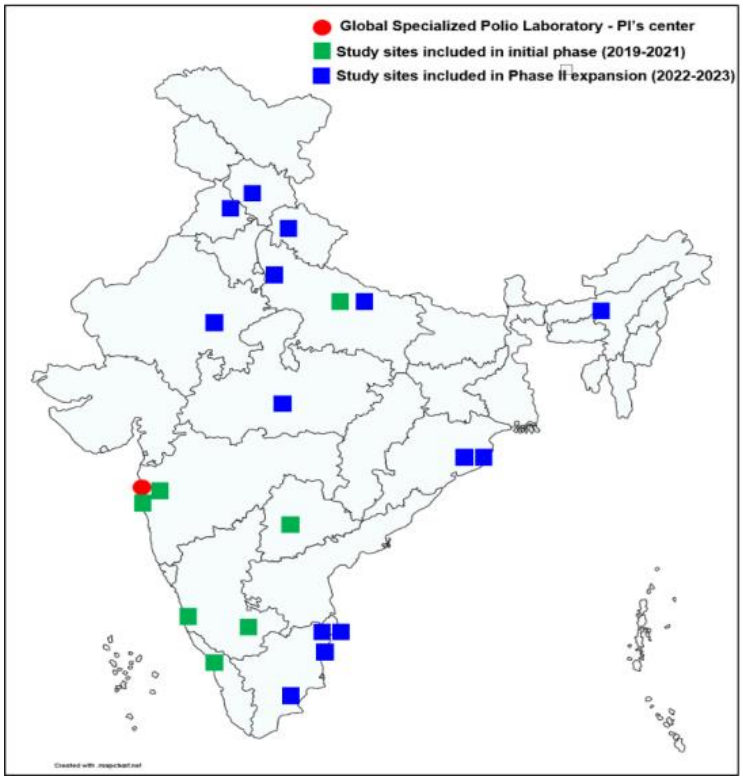


Fig. 1: Map of India depicting the study sites of Phase-I, Phase-II and the PI's Institute.

Seroprevalence of Enterovirus 71 antibody among Indian children

Investigators: Madhu Mohanty, Swapnil Varose, Sneha Rane, BV Tandale, Shailesh Pawar
Funding: Intramural ***Duration***: 2 years (2019- 2020) Extended to 2021.
Background: Circulation of multiple genotypes of EV71 (D, G, C) has been reported in India but there is no reported information available on sero-prevalence of EV71 antibodies in Indian children. In the absence of readily available vaccine and effective therapy, EV71 could replace poliomyelitis as a cause of acute paralytic disease and death in children.
Objectives: To conduct serum antibody survey to determine the exposure of Indian children to EV71, to estimate EV71 antibody sero-prevalence to all four genotypes including the three indigenous genotype (C, D, G) and one prototype genotype (A) in Indian children.

Findings: Comparison of sero-positivity against EV71 prototype A, D, G and C genotype revealed that sero-positivity against all three circulating genotypes significantly increases with increase in age. There is no significant difference between genotypes when compared gender wise. All three circulating genotypes showed similar sero-positivity with Genotype D with significantly higher overall GMT as compared to other genotypes (Fig. 2). Sero-positivity is lowest in the age group of 1 to 4 years which may be a matter of concern. The data confirms the circulation of EV71 in the community.

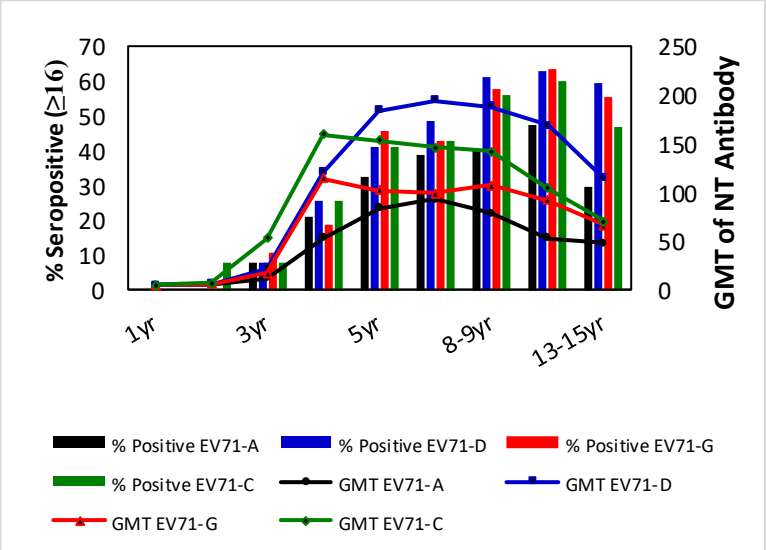


Fig. 2. Comparison of % seropositivity and GMT to four different genotypes of EV71 in children up to 15 years

Understanding the spectrum of viral infections associated with specific humoral or cell-mediated immunodeficiency and its correlation with clinical implications

Investigators: Madhu Mohanty, Mukesh Desai, Manisha Madkaikar, Prasad Taur, Swapnil Varose, Pooja Vengurlekar, Trupti Gothankar

Funding: ICMR-Extramural ***Duration***: 2021-2024 (**Initiated May-2021)**

***Background*:** Recurrent or persistent infection is the major manifestation of Primary Immunodeficiency Disorders (PIDs). Chronic viral shedding makes these patients with PID an important reservoir for infection and can cause spread and persistence of infection in the community.

***Objectives*:** To detect the viruses involved in respiratory, gastrointestinal and CNS infections in children with PIDs in order to identify the spectrum of viral pathogens in children with PID in India and to analyse viral infections associated with specific immune deficiency

Findings: There was a total of 10 different viral infections observed during the one year study period and the infections were predominated by Adenovirus (24.13%), Noroviruses (13.79%) and SARS-CoV-2 (16.12%) in the PID patients. In the respiratory panel, viruses detected were hAdV (10.34%), RSV (6.89%), InfA (3.44%). In the neuro panel hCMV (10.34%), hAdV (3.44%) and B19V (3.44%) were observed. Most viruses were detected in Gastroenteritis panel wherein Adenovirus (13.79%), Norovirus (13.34%) and Astrovirus (10.34%) were predominant. Patients with Combined B cell and T cell Immunodeficiencies were detected with different types of viral infections as compared to primary antibody deficiency or innate immune deficiency. HAdV infection was most predominantly detected in all three patients with Hyper IgE syndrome, suggesting a correlation between high IgE and adenovirus infection. A patient with congenital defects of Phagocyte or function was detected with Parvo B19V infection.

Development of a RT-LAMP assay for detection of Nipah virus

Investigators: Shyam Sundar Nandi, Pragya Yadav, Anita Shete-Aich, Upendra Lambe, Sonali Sawant, Jagadish Deshpande

Funding: Intramural. **Duration:** 1 year (2022-2023)

***Background*:** Nipah virus (NiV) is a new paramyxovirus that can cause acute central nervous system disease in humans and animals. The Nipah virus infection is a Zoonotic disease transmitted to humans via Bats. The virus can also be transmitted through contaminated food or direct contact with infected individuals. There is no vaccine or other medical antiviral treatment available and the mortality rate is around 60 to 90%. Therefore, the Nipah virus is classified under Biosafety Level 4 (BSL4) virus. The development of a rapid molecular is of prime importance here because there are no effective field tests available currently for the detection of the Nipah virus. This study involves RT-LAMP for the detection of the Nipah virus. The present invention can be applied to the suspected clinical samples that can be used in the field as a rapid detection assay. This assay is easy to perform, rapid and the results can be interpreted visually and do not require any instruments.

***Objectives*:** To develop an RT-LAMP assay for the detection of Nipah virus.

Findings: In this project, a novel colorimetric LAMP assay has been developed for the detection of the Nipah virus. This project involves designing six novel sets of LAMP primers, especially specific to the nucleocapsid (N) and matrix (M) genes of the Nipah virus. The conserved genomic segments amongst the isolates of Nipah virus from India and abroad by performing

multiple sequence alignment. The viral RNA was extracted from the known positive samples and cell culture propagated Nipah virus isolated. The RT-LAMP reaction was set up using the extracted RNA, the specific primer mix and the commercially available colorimetric RT-LAMP master mix. The reaction was incubated at 62^{o}C for 40 minutes and the results were interpreted with the naked eye (**Fig. 3).**

Sr. No.	Sample ID	M Gene N Gene B Gene	Sr. No.	Sample ID	M Gene N Gene B Gene
1	NTC		2	PTC	
3	Sample 1 Confirm negative sample		4	Sample 2 Confirm positive sample	

Fig. 1: Interpretation of results for detection of Nipah virus based on M and N gene specific primers with Beta actin as an internal control. NTC: showing all the reactions negative; PTC: showing all the reactions positive; Sample 1: showing negative result for Nipah virus; Sample 2: showing positive result for Nipah virus RNA.

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

Investigators: Shyam Sundar Nandi, Upendra Lambe, Sonali A. Sawant
Funding: ICMR Extramural Project. Duration: 3 years (2019-2022)

Background: EV-A71 utilizes several cell surface receptors, out these Scavenger Receptor B2 (SCARB2), P-selectin Glycoprotein Ligand-1 (PSGL-1) and Fibronectin (FN) are used for virus attachment and internalization. EV-A71 genotypes D and G appear to be confined to India only. There are no reports of outbreaks of HFMD, encephalitis/ meningitis or acute flaccid paralysis of EV-A71 etiology. This may be interpreted as low virulence (naturally attenuated) of the Indian genotypes. Identification and over expression of the most preferred receptor by Indian strains will be attempted. This will lead to high titer virus production which can be utilized for purposes like antigen production, vaccine development.

Objectives: Project was developed with following *Objectives* - (1) To dysfunction of EV-A71 receptors sequentially and in combinations to identify most preferred receptors by the different

genotypes of EV-A71 (2) To study the effect of over expression of the preferred cell surface receptor on per cell yield of EV-A71.

Findings: EV-A71 utilizes several cell surface receptors, out of which scavenger receptor B2 (SCARB2), P-selectin Glycoprotein Ligand-1 (PSGL-1) and Fibronectin (FN) are used for virus attachment and internalization. The sequence analysis of all the three receptors (SCARB2, PSGL-1 and Fibronectin) through literature search has been performed. This was followed by designing of sgRNAs and checking the off targets. The sgRNAs designed were successfully cloned in the pX330 vector, these were further used for transfection of HEK293T cells. Successful knock-out of SCARB2, PSGL-1 as well as FN was obtained in transfected cells. The confirmation of knock-out was done by sequencing and confocal microscopy (Fig. 5). Furthermore, over expression of the target receptors was also carried out in HEK293T cells using Lentiviral packaging system and transcriptional activation. Over expression was confirmed using infection studies with EV71. Transduction and over expression was also carried out in the knock-out cell lines obtained in the study earlier. The confirmation of these was also done by infection studies. Titrations are being performed to evaluate the increase in the titer of EV-71.

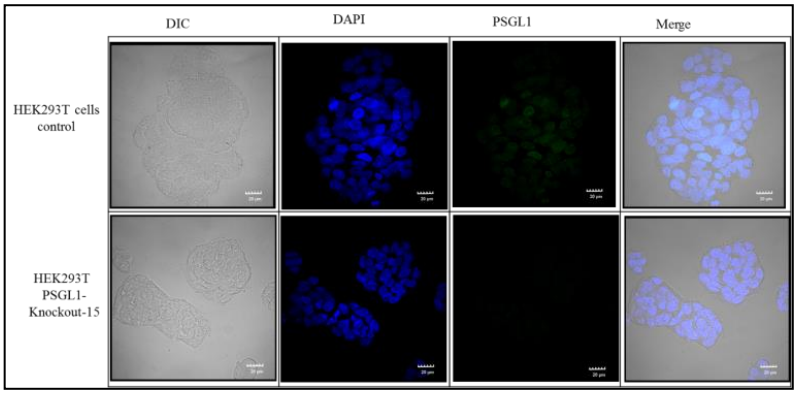


Fig. 5: Illustrates immunofluorescence staining of PSGL1 receptor on A) HEK293T cells and B) HEK293T PSGL1-Knockout cells. Anti-PSGL1 polyclonal antibody and Alexa488 labeled anti-rabbit antibodies were used for visualization of PSGL1 (green) on the cell surface by confocal microscopy (Zeiss). Cell nuclei (blue) were stained with DAPI. Each panel shows DIC, DAPI stain, PSGL1 and composite of PSGL1 immunofluorescence and DAPI staining.

Development of poliovirus non-permissive cell line by CRISPR/Cas9 technology for enteric virus diagnostics and research as per WHO Global Action Plan III for Polio Containment

Investigator: Shyam Sundar Nandi

Funding: ICMR Extramural Project. **Duration:** 2 years (2021-2022)

***Background*:** WHO GAP III that laboratories wanting to culture viruses from poliovirus potentially infectious materials (PIM) in poliovirus permissive cell lines will have to establish biosafety and bio-risk management systems and obtain verification/ certification by NAC for polio. This may prove expensive for the laboratories. A poliovirus non-permissive cell line supporting growth of a variety of different viruses (Non polio enteroviruses /enteric/ respiratory viruses) would be a boon to these researchers. Thus, poliovirus non-permissive cell line will be beneficial to the Researcher who are working on Non polio enteroviruses, enteric viruses such as rotavirus, norovirus, hepatitis viruses, adenoviruses etc.

***Objectives*:** To develop poliovirus non-permissive cell line by CRISPR Cas9 technology for Enteric virus diagnostics and research as per Global Action Plan III for Polio Containment

Findings: The sequence analysis of CD155 receptors through literature search has been performed. The virus binding domains along with secondary structural elements such as di-sulfide bridges, signal peptides, folds and turns and cross-links have been identified using various bioinformatics tools such as NCBI, Uniprot etc. The PAM (protospacer adjacent motifs) sites were identified from the structurally important regions of the receptors. This was followed by designing of sgRNAs. The sgRNAs were designed and off targets were checked. The designed sgRNAs were cloned in the pX330 vector. This was further used for transfection of the HEK293T cell line. Successful knock-out of the gene for CD155 receptor was obtained and confirmed by sequencing.

Development of genetically tailored organotypic model of human intestine for enterovirus study

Investigators: Shyam Sundar Nandi, Alpana Moghe, Sakshi Phalke

Funding: ICMR Extramural Project. **Duration:** 2 years (2021-2022)

***Background*:** Human enteroviruses are emerging viruses causing a spectrum of diseases, including meningitis, encephalitis, paralysis, and skin rash. As humans are the only host for these viruses, availability of appropriate experimental systems to mimic *in vivo* scenarios is a barrier to current understanding of pathogenesis and host interactions. There are no preventive vaccines for nonpolio-enterovirus (NPEV) infections. They therefore continue to pose a threat to human health. We propose a three dimensional organotypic culture model of human tissue grown in

NASA RWV bioreactor that mimics the differentiated structure and function of parent tissue which are often not exhibited by two – dimensional monolayers.

Objectives*:** The ***Objectives are: 1.To genetically modify HCT-8 intestinal epithelial cell line using lentivirus packaging and CRISPR/Cas 9 technique for expression of enterovirus specific receptors, 2. Use genetically modified HCT-8 cell line with human colonic fibroblast, endothelial cell lines and lymphocyte/monocytes cells to prepare three dimensional intestinal organotypic culture using a rotating wall vessel (RWV) bioreactor, 3. Application of 3 D intestinal organotypic model for enterovirus infection study and its comparison with 2 D cell line.

Findings: Application of 3-Dimensional culture model of intestinal mucosa is expected to serve as a promising tool for more predictive in vitro investigations of enterovirus pathogenesis. HCT-8 cell line was successfully modified using Lentiviral packaging and transcriptional activation. Overexpression of PSGL-1 and SCARB-2 receptors, in HCT-8 cell line was confirmed using confocal microscopy as well as infection studies with EV71 virus. Additionally, the three dimensional organotypic culture was successfully designed using human colonic fibroblast, endothelial and HCT-8 cell lines, to form tissue constructs. These constructs were analyzed by biopsy, sectioning and staining of tissues.

Number of samples tested at NIVMU

Virus	Samples	Number of cases	Number of specimens
Poliovirus	Stool	1223	2406
	Stool specimens received from Nepal	233	461
	Isolates received from other labs	08	10
	Isolates (for Banking)	409	409
	Sewage	174	174
	Sewage received from Nepal	120	120
	Sewage Isolates received from other labs	27	29
PID	Stool	187	290
Humoral, cellular immunodeficiency	Stool, serum, respiratory specimens	30	39
Measles/ Rubella	Serum	3254	3254
	Urine/ Throat	2660	2716
	PCR product received from other labs	451	451
COVID-19	Respiratory specimens	49338	49338
Total		58114	59697

OTHER ACTIVITIES

ACADEMIC CELL

List of Staff

Dr. Kavita S. Lole, Scientist F and Group Leader Hepatitis/Academic Cell In-charge
lolekavita37@yahoo.com, lole.ks@gov.in
Dr. Tejaswini M. Deshmukh, Scientist C
deshtejas1972@gmail.com,

Coordinator
Prof. MV Kulkarni, Prof and Head (Retd.), Chemistry Dept., SPPU. (Joined Academic Cell in December 2021)

Technical Staff
Mrs. Swati Bohodkar (Sr. Tech. 3)
Mr. Hemant R. Band (Sr. Tech. 1)
Mrs. Neethi Jayaram (Consultant from 06-07-2020)
Mr Vaibhav Shelke (Project Technical support II since February 2022)

M. Sc. Virology Program:

M. Sc. Virology post-graduate program was initiated by the ICMR-National Institute of Virology in June 2005 and is affiliated to the Savitribai Phule Pune University (SPPU, formerly Pune University, Pune) through the Institute of Bioinformatics & Biotechnology (IBB, SPPU, Pune). Till date, fifteen batches of students have completed the course successfully.

Academic year 2021-2022

Twenty of 22 enrolled students from 2019-21 batch (15th batch) passed the of M.Sc. Virology program.

Grade	Number of students
'O' - Outstanding	6
'A+' - Excellent	4
'A' - Very Good	7
'B+' - Good	3

Toppers of the 2019-21batch -

Rank	Name of the student
1	Mr. Abhranil Gangopadhayya (Gold medalist)
2	Ms. Unnati Bhalerao

MoU between SPPU and ICMR-NIV for continuation of M.Sc. Virology program has been renewed for a period of five years (15-06-2021 to 15-06-2026). University has granted permission to conduct admission process at all India level as per the Central Government reservation norms.

Twenty one students were enrolled in December 2021 (2021-23, 17th batch) for M.Sc. Virology. The admission for this batch was conducted at a National level.

Thirty students from 2020-22 batch and 2 (backlog) students from 2019-21 batch have been enrolled in the Second year of M.Sc. Virology.

All lectures were conducted online via Zoom till March 2022. All theory and practical examinations were conducted online via SPPU Moodle software.

Classroom teaching resumed from March 2022 as per the directives received from SPPU.

Syllabus for Post Graduate Diploma in Diagnostic Virology was approved by SPPU on 27-11-2021. The MoU draft for starting the new course PG Diploma in Diagnostic Virology was submitted to SPPU and is under processing.

Students' Achievements-

Ms. Anjali Shrivastava won the award for Best thesis for her M.Sc. Virology dissertation titled 'To determine the role of innate immune modulators on the efficacy of HDAC inhibitor to

reactivate HIV expression in the latently HIV infected cell line model". The work was done under the guidance of Dr Vanadana Saxena, Scientist D at ICMR-NARI.

Students participated in various online webinars, seminars and quizzes during the reporting period.

Award presented to First Rank Student and Best thesis for, 2019-2021 batch

M.Sc. Virology, Batch 2019-21 student, Mr. Abhranil Gangopadhayya stood first among the outgoing batch of 2021. He was presented with certificate and Gold Medal by the Director, NIV in a virtual ceremony held on 08-10-2021.

Ms. Anjali Shrivastava won the award for Best thesis for her M.Sc. Virology dissertation. She was presented with a certificate and Cash prize of Rs 5000/- on behalf of NIV Research Foundation.

Details of the program are available on-

1. https://icmr.nic.in/institutes
2. www.niv.co.in

Ph. D. Program:

NIV has 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- 20

Number of Ph.D. Degrees awarded during the last year- 4

Mr. Vishal Kavathekar (Degree awarded on 12.05.2021 in Biotechnology under the guidance of Dr. B. Anukumar, Scientist E).

Mrs. Nitali Tadkalkar (Degree awarded on 11.06.2021 in Biotechnology under the guidance of Dr. A. Basu, Scientist G).

Mr. Prudhvi Lal Bhukya (Degree awarded on 31.01.2022 in Biotechnology under the guidance of Dr. Kavita S. Lole, Scientist F)

Ms. Daya Pavitrakar (Degree awarded on 09.03.2022 in Basic Medical Science under the guidance of Dr. Pratip Shil, Scientist E, Co-Guide-Dr. Anuradha Tripathy, Scientist F)

Enrollment to Ph.D. programme:

ICMR-NIV conducted online interviews for admission into Ph. D. program 2021-22 through independent advertisement, from 4th to 6th August 2021. Out of 146 shortlisted candidates, 86 appeared for the interview.

Number of students enrolled for Ph.D. program:- 07

Name of student	Subject	Guide
Ms. Shivangi Sharma	Basic Medical Science	Dr. Sarah Cherian-Scientist G
Mr. Samarpan Bhattacharjee	Biotechnology	Dr. Jayati Mullick-Scientist F
Mr. Rohan Roy	Biotechnology	Dr. Gajanan Sapkal- Scientist F
Mr. Jose Antony Jenish R	Microbiology	Dr. Gajanan Sapkal- Scientist F
Ms. Ketki Jawade	Microbiology	Dr. Madhu Mohanty- Scientist F
Ms. Tanvi Shinde	Microbiology	Dr. Sunil Vaidya- Scientist F
Ms. Aishwarya Telmore	Microbiology	Dr. Alagarasu Kalichamy- Scientist E

Research:

Patent application titled, “A METHOD FOR DEVELOPING A RAPID IMMUNOCHROMATOGRAPHIC ASSAY FOR IDENTIFYING HEPATITIS E INFECTION” filed on 17-08-2020 (No. 202011035352) (Inventors: Dr. Tejaswini M. Deshmukh, Ms. Manisha T. Dudhmal, Dr. Kavita S. Lole; Applicant: ICMR) was evaluated in a meeting (PCT) conducted at ICMR Head Quarters. Presented the application in PCT meeting (VC), submitted patent checklist and report on queries raised by PCT committee. Application was published on 18-02-2022; Status: Application awaiting examination.

LIBRARY & INFORMATION SERVICES

Scientific Staff

Dr. M.D. Gokhale	Scientist "D" & Library in Charge (Upto February 2022)

Technical Staff

Mrs. Vandana Chandere	Senior Technical Officer (2)
Mr. V. R. Mali	Senior Technical Officer (1)
Mrs. Ekta Jain	Technical Officer 'A'

ICMR-NIV Library continues to provide pinpointed information and services to its Users viz. Scientists, Technical staff, Project staff, M.Sc. Virology and Ph.D. students of ICMR-NIV and its three Field units Bangalore, Kerala and Mumbai 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 Books, Bound Volume of Journals, Annual Reports, Thesis, Dissertations, etc in the current financial year.

ICMR-NIV Library is well equipped with modern facilities. The management of the Library is fully computerized by using LIBSYS 4.0 version software. Check-out and Check-in of books are carried through Barcode Scanner with automatic email system for transaction of books, renewals, overdue of books to the users.

The Library renders services such as Newspaper clippings on daily basis, Citation analysis of Publications, Reference service, Literature search, Document delivery service, Current awareness Service (CAS), Selective Dissemination of Information (SDI), Reprography, Binding and Lamination. It also provides Anti-plagiarism services through Turnitin iThenticate software (an anti-plagiarism web tool) for the Ph.D. thesis, M.Sc. dissertations and Manuscripts of scientists. The Library provides Inter Library Loan Service for resource sharing with National Centre for Cell Science, Pune. Library imparts orientation and information literacy programs to M.Sc. virology students, and newly joined scientists and staffs. The Library also conducts trainings, seminars, conferences on various topics for its users on time to time.

We have access of online 239+ e-journals under ERMED Consortium through National Medical Library, on IP basis subscribed from five publishers i.e., BMJ Publishing Group, Cambridge University Press, Lippincott Williams & Wilkins, Oxford University Press, Wiley Blackwell in NIV, MCC Campus and three field units Bangalore Kerala and Mumbai. Library has subscribed 22 Print Journals in virology and other allied subjects.

Library has its own Web OPAC to retrieve online Library collection effectively.

We have provided following services related to the Covid-19 Pandemic:

- Provided list of Scientific Literatures published on Covid-19 (Weekly).
- Online News published on Coronavirus in various English, Hindi and Marathi newspapers via Newspaper Clipping Service (Daily).
- Provided List of Papers Published by ICMR-NIV Scientists on Covid-19 for uploading on NIV website, SAC Meeting and other purposes.
- Selected News on NIV provided for Twitter/Facebook and other social media.
- Provided NIV News for the Quarterly NIV Newsletter.

During the year, the following activities were Conducted:

- Subscription of Journals and renewal of Magazines, Print and Online Newspapers.
- Citation Analysis of publications for Scientist's Assessment, Promotion and Award purpose.
- Reference Service, Document Delivery Service, Inter Library Loan, Literature Search, Anti-plagiarism, Xeroxing, Lamination and Binding service to users.
- Procurement of Turnitin iThenticate Software for the plagiarism detection of Ph.D. thesis, M.Sc. dissertations and Manuscripts of NIV scientists.
- Online news on NIV provided for twitter/facebook for Azadi Ka Amrit Mahotsav
- Newspaper Clipping Service on daily basis from English, Hindi and Marathi Newspapers for ready reference for NIV Scientists through email and displayed on Notice board of Library for students and others.
- Scanning of in-house publications of ICMR-NIV for preservation and future use.
- Updated and maintained in-house NIV Scientific Publications from 1953-2022.

Updated list of NIV holdings (Books and Bound Volume of Journals from 1953-2022) for ready reference.

<u>Information and Library Services Continued, Added/Started:</u>

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

Description		Quantity
Books	Purchased/Gifts/Gratis	76
	Bound Volumes	49

	Annual Reports Received	5
Journals	Print (Subscribed)	21
	Print (Gratis)	20
	ERMED Consortium	239+
	Loose Issues	257
Ph.D. Theses		3
M.Sc. Dissertations		-
Others; CDs, Microfilms, Floppies		9
Papers Sent for Publication		28
Papers Published by NIV Scientists		121
Reprints		119

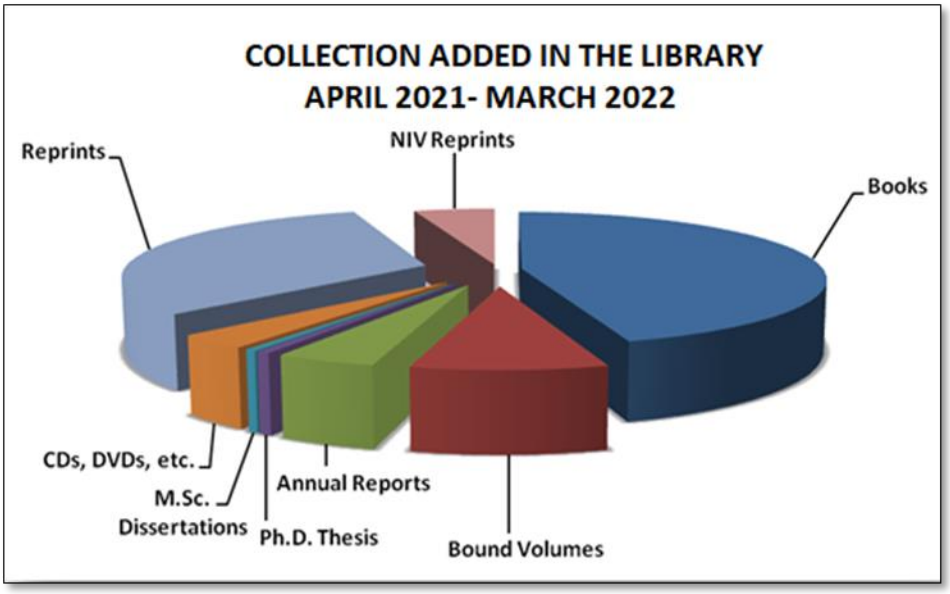


Fig. 1: Collection Added During the Period in the ICMR-NIV Library

Selective Dissemination of Service/Current Awareness Service, Reference Services, Inter Library Loan (ILL) and Document Delivery Services (DDS) Services Provided:

Table 2: Service Provided- Circulation, Reference, Inter Library Loan, Document Delivery Service, etc. by the Library.

	Book & Bound Volumes Issued	Staff: 93 Students:59
	Book & Bound Volumes Returned	Staff: 71 Students: 127
	Newspaper Clippings	5769
	Inter Library Loan Received and Sent	3

	Photocopy Service	3433
	Binding (Thermal & Spiral)	1
	Lamination	1
	Reference Service	2713
	NIV Annual Reports, Compendiums, Handbooks, Manuals Distributed	5
	Citation Analysis	9
	Anti-Plagiarism Service	57

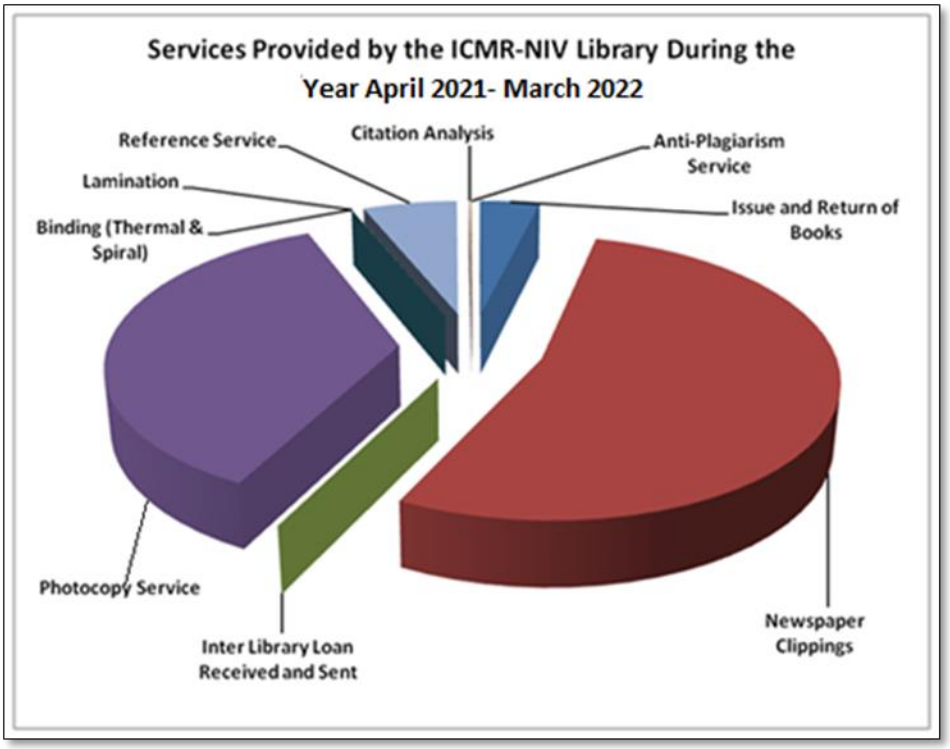


Fig. 2: Services Provided by ICMR-NIV Library

ENGINEERING SUPPORT GROUP

Staff at NIV, Pune

Mr. A.B.Khare	HOD, Sr. Tech. Officer-3 (ES)
Mr. D.R. Kumbhar	Sr. Tech. Officer-1 (ES)
Mr. A.K.Kasar	Tech. Officer- B, (ES)
Mr. V.J. Bhosale	Sr. Technician-2 (ES)
Mr. A.J. Suresh	TA (ES)
Mr. V.D. Jagtap	Sr.Technician-1 (ES)
Mr. Y.M. Taru	Sr.Technician-1 (ES)
Mr. B.S. Shelar	Laboratory Asst.- (ES)
Mr. A.B. Kelkar	Sr.Technician-1 (ES)
Mr. S.S. Utale	Laboratory Asst.- (ES)
Mr. S.S. Holkar	Sr. Technician-1 (ES)
Mr. D. V. Sutar	Technician-B
Mr. D.K. Jagtap	Laboratory Asst. (ES)
Mr. M.V. Gadhave	Technician-A (ES)
Mr. A.M. Pawar	Technician-3 (ES)
Mr. A.N.Kale	Technician-A (ES)
Mr. S.R.Jagtap	Technician-A (ES)
Mr. Md Nazim Akhtar	Technician-1 (ES)
Ms. Komal Jangid	Technician-1 (ES)

Apprentice staff NIV, Pune

Miss. Akshata Bade	Electrician
Mr. Onkar Dhale	Electrician
Mr. Satish Wagh	Electrician
Mr. Kokare Shrihari	Electrician
Mr. Kartik Oraon	Electrician
Mr. Darshan Dhanke	Ref & ACM
Mr. Promod Jadhav	Ref & ACM
Mr. Sandesh Waghmare	Plumber

Staff at Pashan unit

Mr. M. S. Mohite	Technical Assistant (ES)
Mrs.P.C. Lokhande	Technical Assistant (ES)
Mr. S.D.Pote	Sr.Technician (1)(ES)
Mr. S. N. Surbhaiya	Sr.Technician (1)(ES)
Mr. S.D.Bathe	Sr.Technician (1)(ES)
Mr. G.R.Ghogare	Technician-B (ES)
Mr. V.T.Ishte	Technician (2)(ES)
Mr. I.R.Dedunda	Technician-A (ES)
Mr. Mritunjay Singh	Technician-1 (ES)
Mr. Nand Kumar	Technician-1 (ES)
Mr. Niteesh Kumar Yadav	Technician-1 (ES)
Mr. Vishal Ashok Gaikwad	Technician-1 (ES)
Mr. Govind Sharan Meena	Technician-1 (ES)

Staff at Kerala unit

Mr. Uma Ganesh Pentakota	Technician-1 (ES)
Mr. Santosh Kumar Bosta	Technician-1 (ES)

Staff at Bangalore unit

Mr. Akash M. Jagtap	Technical Assistant (ES)
Mr. Arjun Jogangiri	MTS

Staff at Mumbai unit

Mr. Kamlesh Pawar	Technician-1 (ES)

The Engineering Support is from various trades like Electrical, Refrigeration & Air Conditioning, Carpentry, Civil, Plumbing works together to achieve the ***Objectives*** of Engineering Support department.

The Engineering Support department works 24*7 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 Engineering Support department of NIV renders the services to various field units such as Bangalore, Kerala and Mumbai.

Works carried out by Engineering Support during 2021-2022

Routine works:

Operation of BSL-3 with maintaining Log book.
Operation of AC plant with maintaining Log book.
Operation of DG sets with maintaining Log book.
Operation of Water pumps with maintaining Log book.
Operation of Cold room plant.
Operation of Freezer room plant.
Checking round (Lab equipment).
Operation of Incinerator with maintaining Log book.
Preparation of Monthly summary of log books of all installations.
Preparation of quarterly progress report of capital works.
Operation of various valves to distribute water to all Campus.
Shift duty and Holiday duty (16.00 to 00.30) and (00.30 to 9.00) / 8.00 to 16.00.
HT metering Tri vector meter readings in the logbook.
Influenza Laboratory HVAC readings recorded in the log sheet.
Electrical meter readings of Staff quarter in every month.
Preparation of BSNL electrical charges bill in every month.

Major works :

Pune Unit:-

Telephone MDF box renovation work has been completed at NIV, Telephone system.
Projector cable laying work has been carried out in house at Director's meeting hall.
Replacement of Basement AC plant with new chiller units works has been completed.
Prepare the Note for Engineering staff Role and Responsibility and handing over the responsibility to existing staff who transferred to the other division and Institutes, Note approved by Director.
Old Basement AC plant units with its sub equipments dismantling work is in progress that was considered on buyback offer by CPWD estimates in the replacement of chiller units work.

1600 Amp. Air Circuit Breaker of LT panel repairing and servicing work has been done in-house at 11 KV HT Substation, NIV, Pune.

First floor Cold room control wires has shorted internally in the old metal electrical conduit pipes, hence, replaced the all wires with new multistrand copper wires through PVC conduit from Machine room to First floor cold room.

Standard quality Four blade ceiling Fan with LED Light and adjustable Light intensity arrangement through remote control operation ceiling fan arrangement has been done in the Director's cabin in place of Lighting decorative Zumber.

Provision of 3 phase power supply to the newly installed AHU unit for Influenza division on the loft of First floor bathroom.

Prepare the reply to the MPCB show cause notice and Note with Final Letter Put up to the Director through Dr. Patil HOD, Lab Animal.

Old Basement AC plant units cooling tower and worn-out piping of condenser water line dismantling work is in progress by CPWD contractor M/s Siddhivinayak Enterprises.

Air handling Unit and ducting work has been carried out by CPWD of chikungunya division.

The AHU unit replacement work has been done of Influenza division on the loft of First floor bathroom.

Provision of new Acrylic shutter for laminar hood at EVG division.

LED Lighting panel's installation work has been completed by CPWD contractor in the chikungunya lab.

Dr. Cherian Madam Cabin renovation work has been completed and internal electrification, AC Dismantling and re-installation as well as LAN and Telephone wiring completed.

Replacement of faulty cable of Condenser water circulation pump of Old building chiller Plant.

Reconditioning/repairing of faulty water pump and re-installed the standby pump in place of faulty.

Arrangement of PA System and Air conditioning operation as well as Lighting for Pre-SAC/SAC – 2021 which held on 26.11.2021, 27.11.2021.

Prepared the List of Unwanted / Unserviceable items and kept it at Basement plant room and List of Items submitted to Stock room for further process.

Transformer Oil filtration and Earth testing work has been carried out by Trafo Filter Services.

New Advanced Research Facility the equipment Room Industrial Power Points work has been carried out in house.

Provision of Building Illumination on occasion of Republic Day celebration at both the campuses in Tri color as requested by Director Office and care taker Job card.

Installation of New freezer room work has been carried out in-house for storage of Ice packets of DRF division.

Renovation of Chikuenguniya lab at Pune completed.

External painting work of new building carried out.

Renovation of Dr. Sarah Cherians Cabin completed.

Repair of rain water drain pipe carried out in campus
Conference hall sliding windows repair works carried out.
Platform repair and Painting work of animal house completed.
Repair work of underground water supply line leakage carried out without disturbing regular water supply and which finally resulted in 50 % water bill saving.
Repair work of compound wall barbed wire fencing carried out.
Glass filming and PVC paneling work done in reception area.
New vertical blinds fixed for Conference hall Partitions.
Mosquito net fixed for canteen windows.
Renovation of Dr. Vaidya's cabin completed.
PCC Platform work done for walk in freezer Room.
Shifting of Drain line below new generator carried out.

MCC Campus, Pashan Unit:

Renewal of MPCB Authorization for "Generation and handling of Biomedical waste" up to 13.02.2025.

Replacement of all old street lights with new LED Street light: Reduced around 40% electric consumption.
Repairing work of 10" water line and main valve replacement work of OHT: To stop the wastage of water.
Follow up with PMC and transfer 6" main water line connection to new water line of PMC to resolve the problem of water qty.: Increase the water qty and resolve the water crisis.
Repairing work of PUF panels of freezer room of ENC.: Resolve the water leakage issue.
Inspection of substations in Pashan and Pune campus through Central Electric Authority (CEA): As per IE rule it is mandatory to inspect the electrical installations through CEA.
Testing of incinerator Stack, DG set stack and water of STP: As per the MPCB norms it is mandatory to carry out these tests.
Testing of earth resistance of all earth pits: To confirm the resistance of earthings for safety purpose
Transformer oil filtration work: To remove the contamination, dirt and moisture to avoid the failure of transformer.
Prepared the budgetary estimate for Dibrugarh & Jabalpur.
Prepared revised drawings of BSL-3 & BSL-4 for Dibrugarh & Jabalpur.
Prepared equipment electrical load list for Dibrugarh.
Cleaning of water tanks in campus :To provide clean water
Preparation for inauguration ceremony of mobile lab at Nasik from 14th to 19th Feb 2022.
Inspection of Mobile lab with Dr. Shailesh Pawar & Mullick madam on 24.02.22.
Installation of data logger at BSL3.
Review of documents received from Dr. Mourya regarding Dibrugarh.

Review of proposal received from Cherian madam for Virus repository.

Capital works:

Renovation of Type-A, Type- B and Type V staff quarter at Pashan: These quarters are around 30 years old and their condition is very bad. Hence renovation was proposed and completed in time. All staff living in the quarters is now satisfied.

Development of the area near boys and girls hostel, OHT, staff quarter, AC plant and substation no-2: In this area the grass growing due to which cleaning was not possible and also snakes observed frequently. Maintenance staff has to operate the valves of water lines and also children of the staff members playing in this area. To avoid any incidence of snake bite, it was proposed to develop this area. Now the area has been developed and staff utilizing the facilities provided like as open gym equipment, playground etc.

Construction of concrete roads in Pashan Campus: The roads in the Pashan campus were damaged hence proposed to repairs. But ICMR suggested to go for concrete road instead of repairing. The construction of concrete road has been completed. While executing the work, cement pipes laid across the road at every 15 mtr. Distance to avoid the breakage of road while laying the utilities i.e. electric & network cable, water lines etc. in future expansion.

Construction of Canteen shed: Existing dining hall is not sufficient for the staff. Hence proposed to construct new shed for 200 staff. The work will be completed shortly.

External painting of all buildings in Pashan Campus: All the buildings in Pashan campus were externally looking shabby due to painting.

Renovation of Chickunguniya lab: The internal electrification work and AHU replacement with ducting work has been completed

Replacement of Chiller Plant at Pune: The chiller plant in Pune campus was very old and frequently goes under breakdown and proper repairing work not possible due to availability of spares. Due to this we have not able to maintain the temp. The electric consumption of this plant is also heavy. To come out all these issues, New Air-cooled chillers has been installed on civil structure.

Replacement of old 400 KVA DG set: This DG Set was very old hence proposed to Install new 750 KVA DG set with AMF panel in Pune Campus. The Installation and testing work has been completed awaiting handover procedure with documentation.

Construction of compound wall at SCOH, Nagpur.

Progress report of capital work: As per instructions of ICMR, prepared quarterly progress report of capital works with the help Er. Kasar, Er. Kumbhar and account section and sent to ICMR.

Follow up with PWD for settlement of accounts of completed works: Account settlement is at final stage and will be completed within June 22.

Approval awarded:

Establishment of ICMR-NIV unit at RMRC, Dibrugarh Assam.

Construction of Satellite center for One Health at Nagpur.
Establishment of ICMR-NIV, Bangalore unit.
Construction of Kitchen building and development work.
Upgradation of BSL-3 lab into PEF: Pass box & dunk tank, Water proofing of cook tank room , Repairs of Autoclave, repairs of Biosafety door, replacement of emergency door, replacement of aluminum window, replacement of PVC flooring, dismantling of isolators etc.
Appointment of consultant for HVAC work of PEF.
Upgradation of BSL-3 lab into PEF: a) Electrical, HVAC, BMS and b) Providing CCTV.

Funds Received:

Establishment of ICMR-NIV, Bangalore unit: 15.00 Cr.
Establishment of ICMR-NIV unit at RMRC, Dibrugarh Assam: 27.00 Cr.
Construction of Satellite center for One Health at Nagpur: 50.00Cr.
Establishment of ICMR-NIV, Bangalore unit: 17.00 Cr.
Construction of Kitchen building and development work: 33.00 Lakh.
Upgradation of BSL-3 lab into PEF: Pass box & dunk tank, Water proofing of cook tank room, Repairs of Autoclave, repairs of Biosafety door, replacement of emergency door, replacement of aluminum window, replacement of PVC flooring, dismantling of isolators, Structural audit etc.: 80.84 lakh.
Appointment of consultant for HVAC work of PEF: 1.15 Lakh.
Upgradation of BSL-3 lab into PEF: a) Electrical, HVAC, BMS: 63.00 lakh.
b) Providing CCTV: 9.00 lakh.

Emergency Attended:

Failure of Freezer room in DVG & ENC due to mains cable got faulty. Temporary supply provision was done with the help of engineering staff.

Statement of Job Cards:

Location	**JC Received**	**JC pending for material**	**JC pending towards Maint**	**JC Completed**
PASHAN	2527	214	123	2190
PUNE	1449	12	55	1382

ADMINISTRATION

SENIOR ADMINISTRATIVE OFFICER

Mrs. Shibi Jacob

ADMINISTRATIVE OFFICER

Mrs. Amruta S Bakare Mrs. A. B. Palkar

Administrative Officer (Stores) Administrative Officer (DDO)

ACCOUNTS OFFICER

Mrs. V. V. Shendye

SENIOR PRIVATE SECRETARY

Mrs. R K Amale

SECTION OFFICER

Mrs. A. A. Bapat (Bills)	Mr. S R Vasam (Accounts)
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. P B Aher (Accounts)
Mr. P N Jadhav (Pashan-Admin)	Mrs. Jayajyothi (Bangalore)

Mrs. D.D. Marathe (Stock Room)

PRIVATE SECRETARY

Mr. J R Kumbhare

ACCOUNTS OFFICER (Jr.Gr.)

Mrs. P S Joshi

ASSISTANT

Mrs. S. M. Bhave (PA) Ms. ShakilaChoudhari (PA)

Ms. MJA Shaikh Mr. Y C Pote

UPPER DIVISION CLERK

Mrs. T. T. Yadav Mr.	P. N. Chabukswar	Mr. A. E. Matkar
Mrs. D. N. Gujar	Mrs. MangalaGangadharan	Mr. M. S. Malvankar
Mrs. M. L. Rupnar	Mrs. M. R. Kannalu	Mrs. S. B. Chakole
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	Mr. Imran Jagirdar	Mrs. AshwiniDudhane

Ms. Y. C. Bhandare

DIRECTOR'S OFFICE

Mrs. A. V. Shendrikar (Former STO) Mrs. R V Bachal, STO-2

Mr. RohitPawar (MTS)

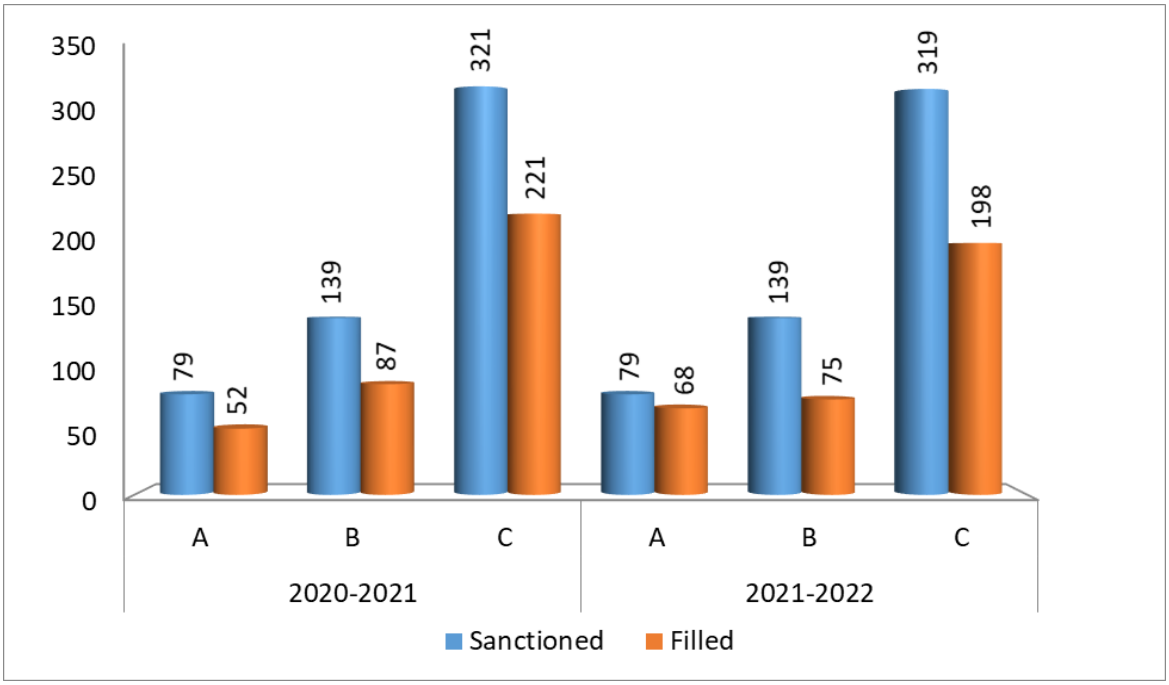
MULTI TASKING STAFF

Mr. Vikrant D. Talpe Mr. Shivam A. Jadhav

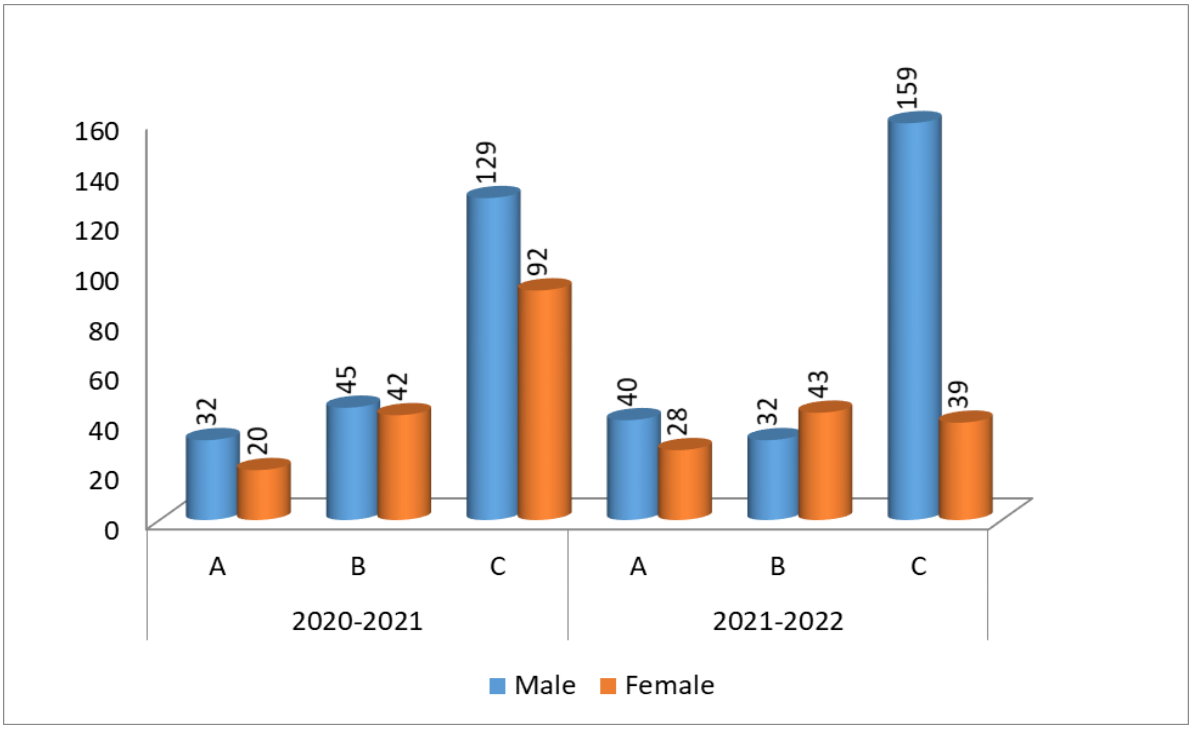
PEON

Mr. Sanjay R. Adagale

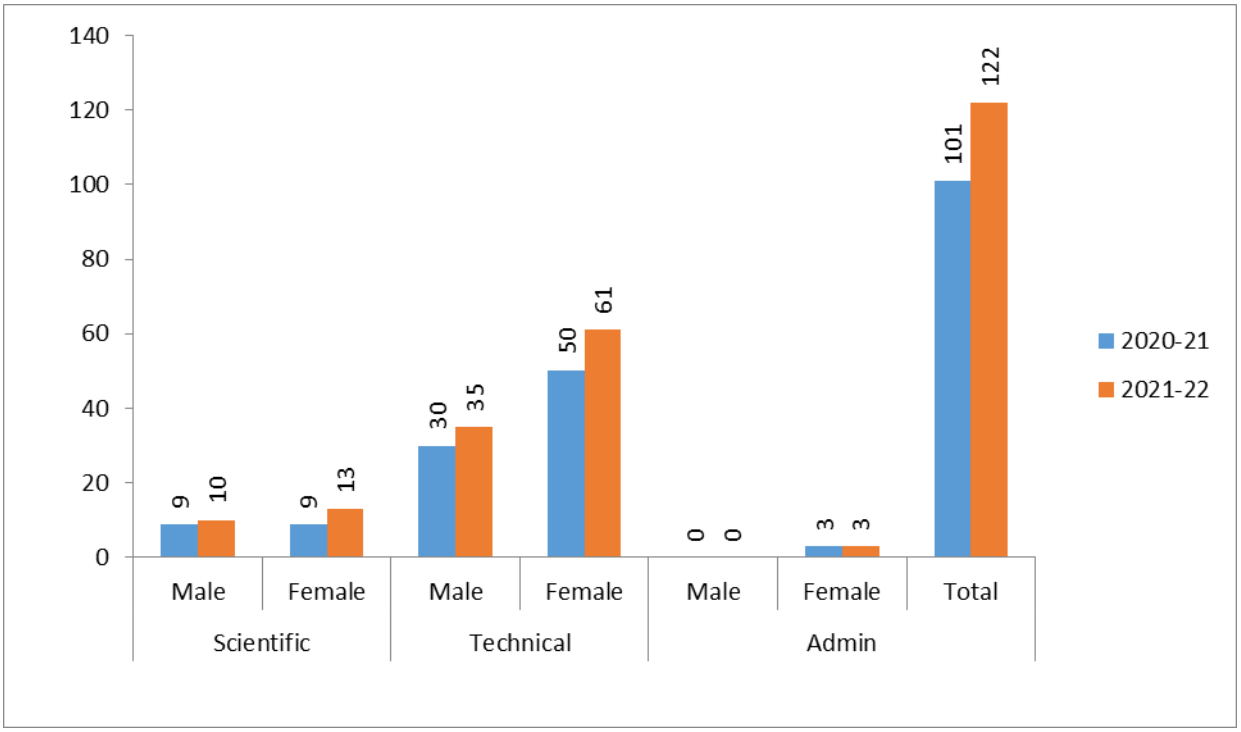
Group wise NIV staff strength



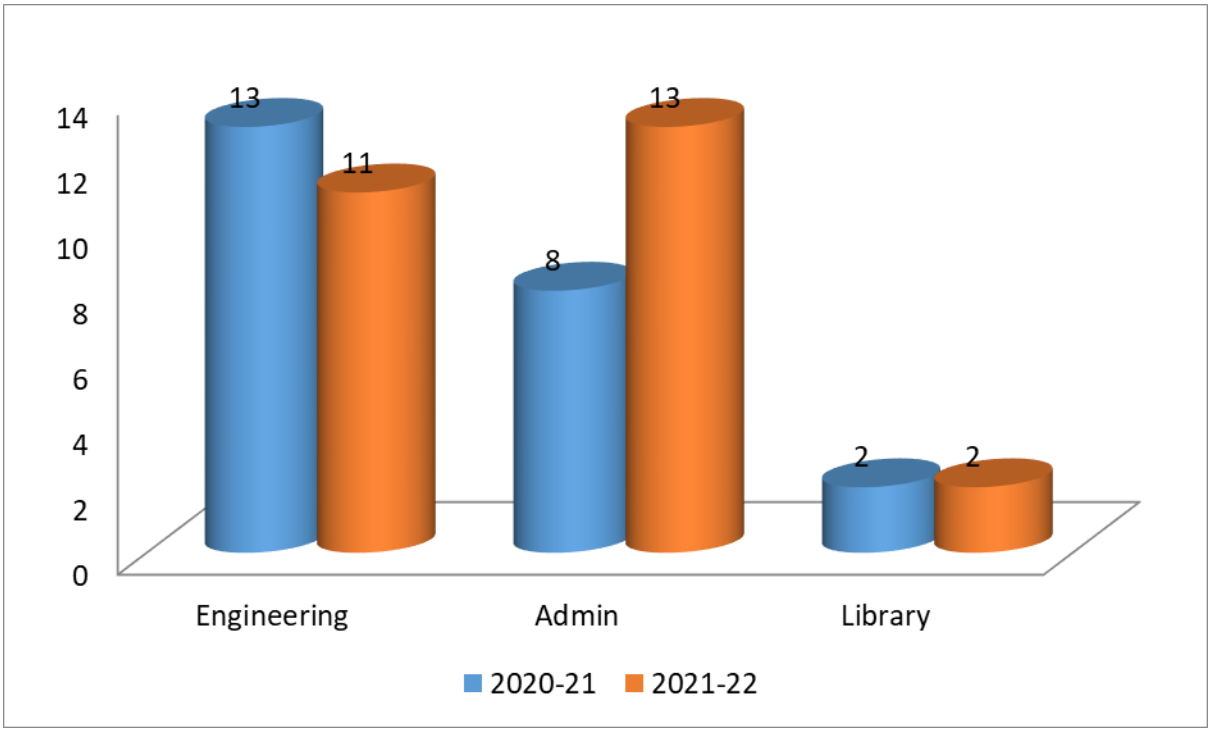
Group wise gender distribution



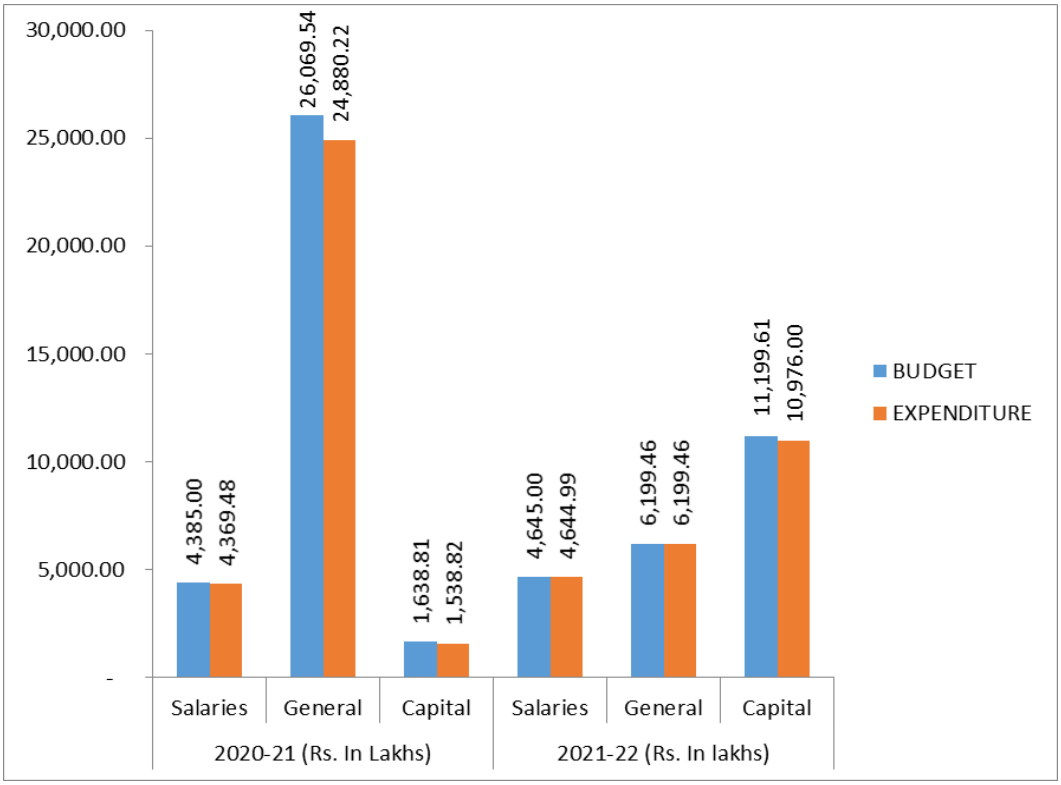
Project Staff Cadre wise Gender Distribution



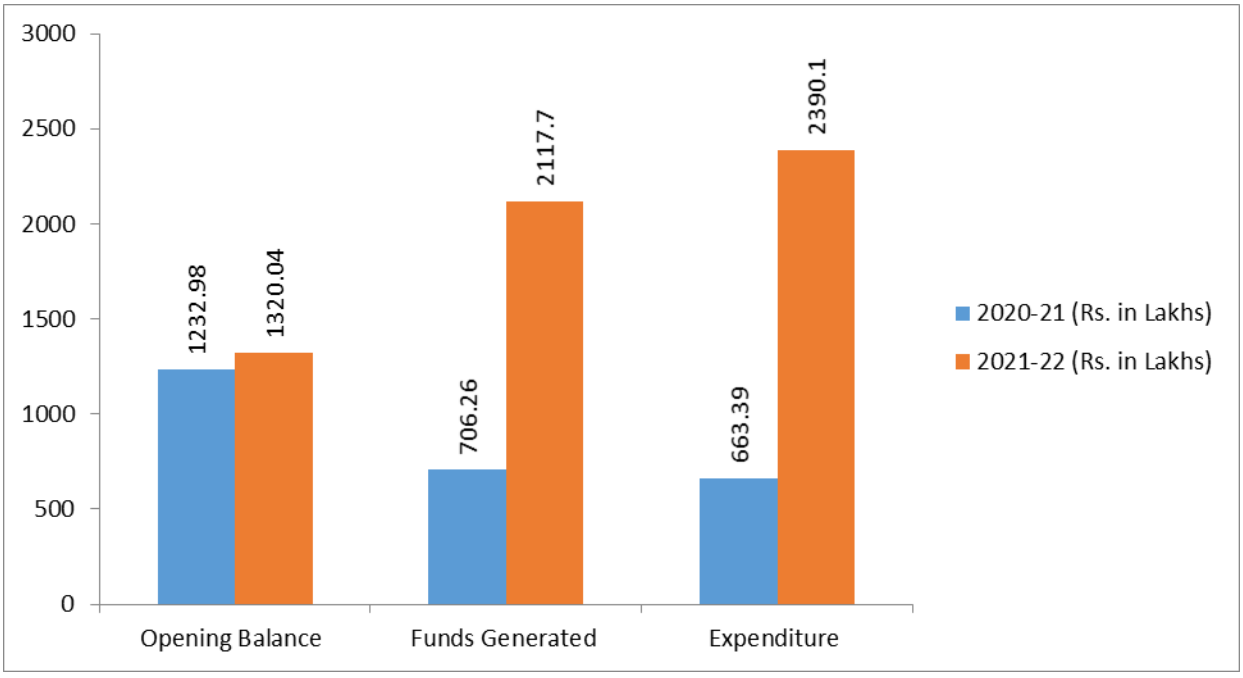
Apprentice Engaged



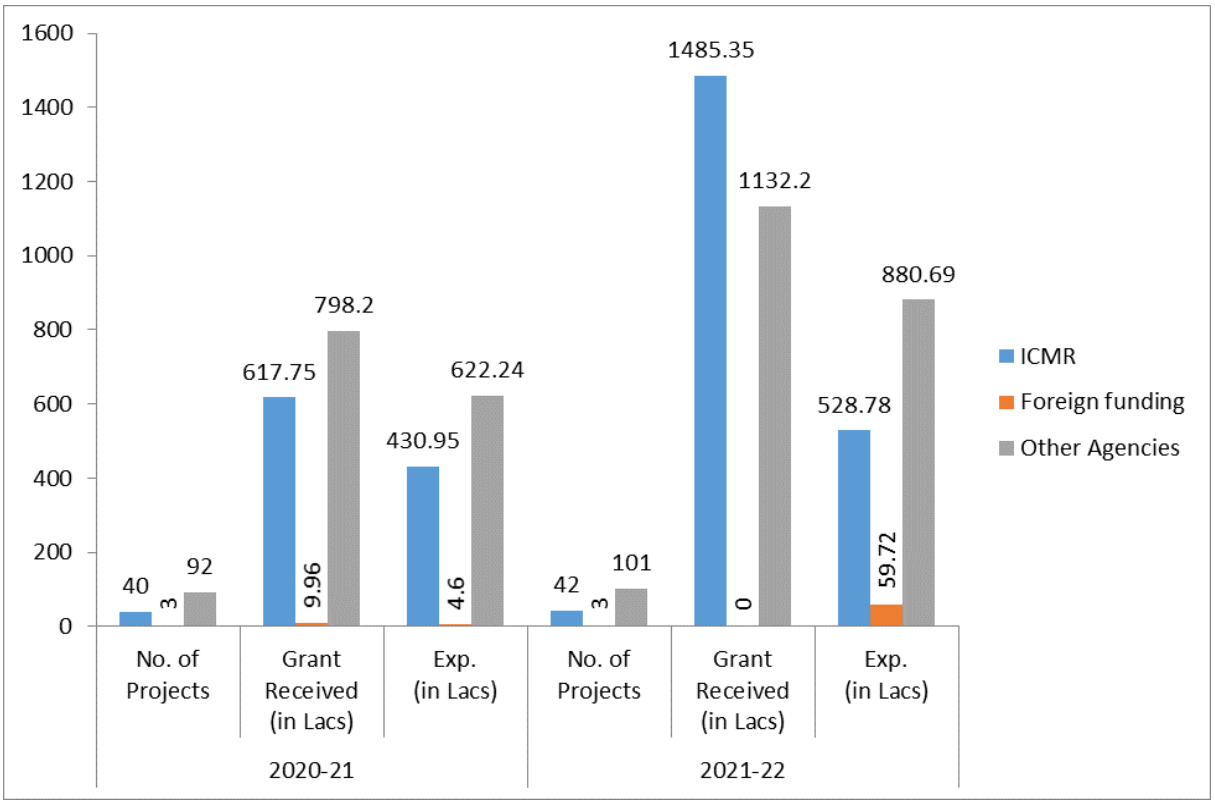
Budget ICMR-NIV, Pune (GRANTS-IN-AIDS)



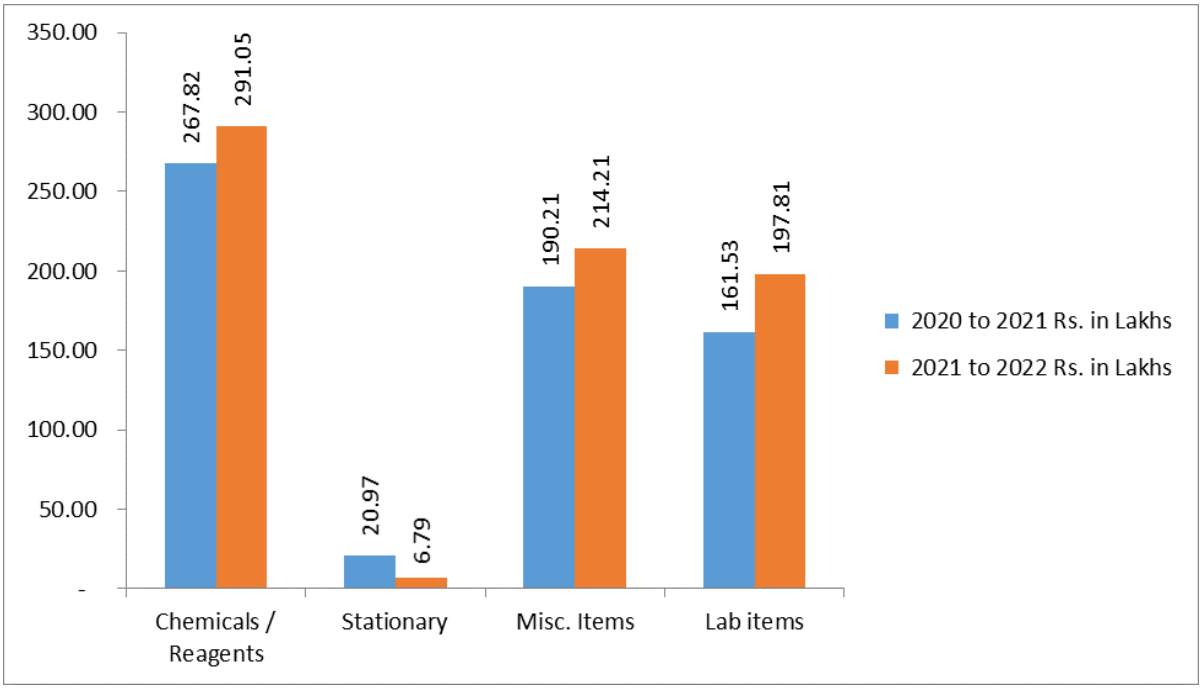
ICMR - NIV GENERATED FUNDS



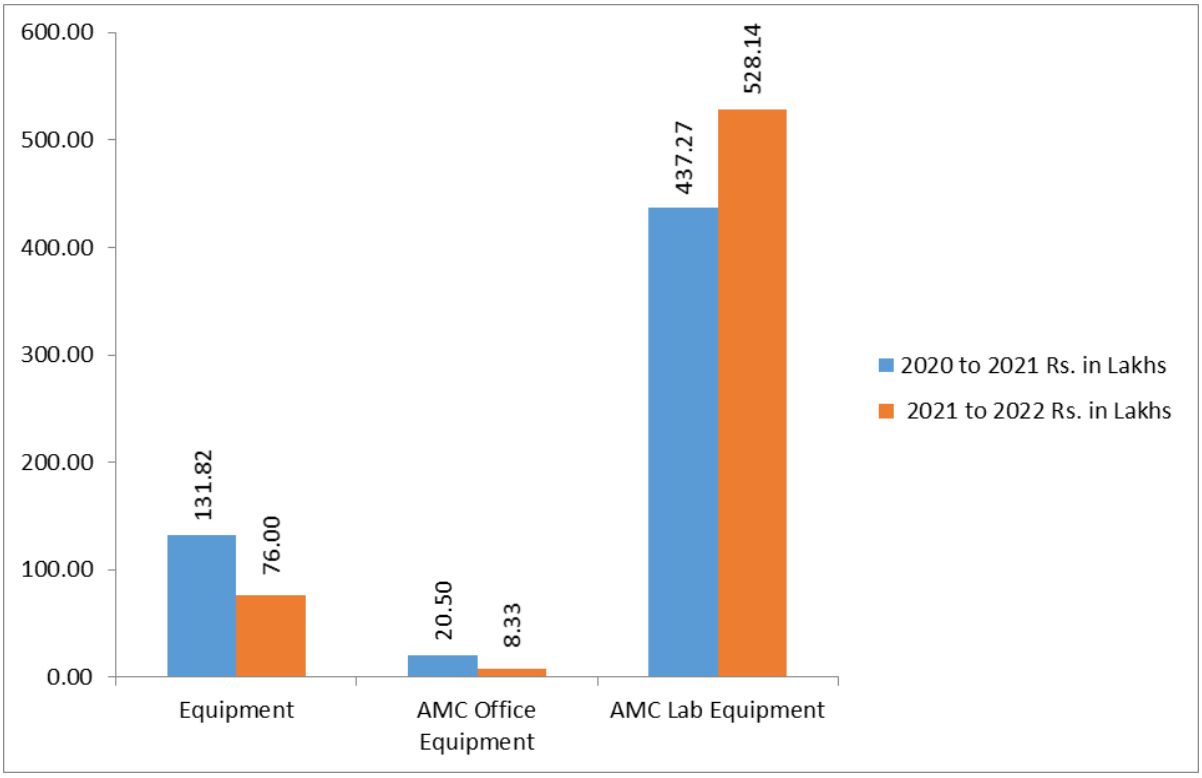
ICMR - NIV PROJECT GRANTS



Procurement details (Other Supplies)



Procurement Equipment and AMC



Training courses attended by Administrative Staff at **Regional Training Centre, Mumbai (2021-22)**				
Sr.No	**Name & Designation of the Official**	**Course Tittle**	**From**	**TO**
1	Mrs. Archana .B.Palkar. A.O	Procurement of Goods and Services through GeM	22.10.2021	
2	Mrs. Swati Pathak, S.O			
3	Mr.D.V.Muneshvar, S.O			
4	Mr. P.N Jadhav, S.O			
5	Mr. Mangesh Malvankar, UDC			
6	Ms. Madhhuri Tondan, UDC			
7	Mrs. Ajay.S.Wable, UDC			
8	Ms.Yogita Bhandare, UDC			
9	Mr. Imran Jagirdar, UDC			
10	Mr. Prashant Chabukswar, UDC			
11	Mr. Amol Lobhande , UDC			
12	Mr. Shivam Jadhav, MTS			
13	Mrs. Savita Chakole, UDC	Income Tax on Salaries	25.10.2021	26.10.2021

14	Mr. Vikrant Talapade, MTS	and Tax Savings		
15	Mrs. Madhuri R Kannalu, UDC	Income Tax on Salaries and Tax Savings	23.12.2021	24.12.2021
16	Ms.Prajakta Bapat, UDC			
17	Mrs. Suhasini M Bhave, PA	Pay Fixation and MACP	11.11.2021	12.11.2021
18	Mrs. Dhanashree N Gujar, ,UDC			
19	Mrs. Sadhana A Veer, UDC			
20	Mr. Ajay .S. Wable, UDC			
21	Mr. A.E. Matkar, UDC	Stress Management at Work	03.12.2021	
22	Mrs. Manisha Rupnar, UDC			
23	Mr. A.E.Matkar, UDC	TA / LTC	09.12.2021	10.12.2021
24	Mr. Yogesh Pote, Assistant			
25	Mr. Prem P Khandagale, UDC			
25	Mr. Prashant Patil, UDC	Seniority Promotion & DPC	27.02.2022	28.02.2022
26	Mrs. Sadhana. A. Veer	New Pension Scheme	18.02.2022	
27	Mr. Rambachan Jaiswal, UDC	Right to Information Act	21.02.2022	
28	Mr. Prashant Patil , UDC	APAR & FR (56 I)	11.03.2021	

Training courses attended by Administrative Staff at
Institute of Secretariat Training & Management (2021-22)

Sr .No	**Name & Designation of the Official**	**Course Tittle**	**From**	**TO**
1	Mr. H.S.Pasalkar, SO	1. Workshop on Citizen Centric & Services Delivery Approach. 2. Handling Parliament Matters	01. Nov 2021 17th Feb 2021	03.Nov 2021 18th Feb 2021
		3. Orientation Training Programme on CPPP & GeM for dealing hands.	15th Nov 2021	17th Nov 2021
2	Mr. K.S. Galange, Assistant	1. Budget – Formation & Implementation	15. Nov 2021	
		2. Workshop on Noting & Drafting	11th Oct 2021	12th Oct 2021
		3. Workshop on e-Procurement	14th March 2021	15th March 2021
3	Mrs. A.A. Dudhane , UDC	1. Training on e-Tendering Procedure	24th Dec 2021	
		2. Workshop on Pay Fixation	01.Sept 2021	03 Sept 2021
		3. Workshop on Public Procurement Under GRF-2017	02th Feb 2021	04.Feb 2021

RAJ BHASHA REPORT

राजभाषा रिपोर्ट 2021-2022

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 हिंदी और अंग्रेजी दोनों मे तैयार किए जा रहे हैं ।

राजभाषा नियम 1976 के नियम 10(4) के अनुसार संस्थान के कर्मचारी हिंदी का कार्यसाधक ज्ञान प्राप्त करने हेतु हिंदी शिक्षण योजना द्वारा आयोजित प्रशिक्षण वर्गों में नामित किया जा रहा है ।

नगर राजभाषा कार्यान्वयन समिति द्वारा आयोजित की जा रही बैठकों में संस्थान के अधिकारी उपस्थित रहते हैं ।

भारतीय आयुर्विज्ञान अनुसंधान परिषद नई दिल्ली से प्राप्त निर्देशों तथा दिनांक 12.08.2021 को आयोजित संस्थान की राजभाषा कार्यान्वयन समिति की बैठक में लिए निर्णयानुसार, दिनांक 14 सितंबर 2021 को मनाए गए हिंदी दिवस के अवसर पर संस्थान तथा सभी इकाइयों में दिनांक 14.09.2021 से 21.09.2021 को हिंदी सप्ताह का आयोजन किया गया था। संस्थान की राजभाषा कार्यान्वयन समिति की बैठक में लिए निर्णय के अनुसार इस वर्ष हिंदी दिवस के अवसर पर हिंदी निबंध प्रतियोगिता तथा हिंदी पोस्टर प्रस्तुतीकरण प्रतियोगिता का आयोजन किया गया था। इस संदर्भ में समिति के सदस्य के सुझाव के अनुसार निम्नलिखित विषय पर हिंदी निबंध तथा हिंदी पोस्टर प्रस्तुतीकरण प्रतियोगिता के आयोजन किया गया था।

निबंध प्रतियोगिता (किसी एक विषय पर निबंध)

1. स्वतंत्रता के 75 वर्ष, सार्वजनिक स्वास्थ्य और अनुसंधान की दृष्टि से।
2. ओलंपिक में भारत - कल आज और कल ।
3. आगामी महामारियों की संभावना और राहत के उपाय ।

पोस्टर प्रस्तुतीकरण प्रतियोगिता (किसी एक विषय पर पोस्टर प्रस्तुतीकरण करें)

1. आओ सब मिलकर नोवल कोरोनावायरस (COVID-19) को हराए ।
2. कोरोना मुक्त विश्व एक चुनौती ।

निबंध प्रतियोगिता में कुल 26 प्रतिभागियों ने निबंध प्रस्तुत किया था। प्रतिभागियों मे से तीन विजेताओं को नकद पुरस्कार दे कर सम्मानित किया गया । साथ ही हिंदी पोस्टर प्रस्तुतीकरण प्रतियोगिता में कुल 11 प्रतिभागियों ने प्रस्तुतीकरण प्रस्तुत किया था। प्रतिभागियों से तीन विजेताओं को नकद पुरस्कार दे कर सम्मानित किया गया।

अत: संस्थान में आतंकवाद विरोध दिन, सद्भावना दिन, सतर्कता जागरूकता सप्ताह, कौमी एकता सप्ताह के उपलक्ष्य में हिंदी में शपथ ले कर व्याख्यान, संगोष्ठी, विभिन्न प्रतियोगिता का आयोजन करके मनाया गया ।

<u>हिंदी निबंध प्रतियोगिता के उपलक्ष्य में प्रतिभागियों ने सहभाग होते हुए चित्र।</u>

Trainings/ Workshops conducted by various departments

BACTERIOLOGY GROUP

1. Congenital Rubella Syndrome Refresher Lab training for 7 sentinel sites of the project during October 27 to October 28, 2021. Participants = 14.
2. Onsite training on pertussis diagnostics and Epi Info at AIIMS, Jodhpur during December 20 to December 24, 2021. Participants = 05.

BIOINFORMATICS AND DATA MANAGEMENT GROUP

1. High Performance Computing (HPC) technology – Using an HPC cluster, including schedulers such as pbs and slurm, Execution of Bioinformatics codes such as gromacs, etc, July 11-12, 2022 . Number of participants- 10.
2. Online HPC training & demo for Anvaya tool, June 23, 2022 & July 1, 2022. Number of participants – 10; C-DAC Pune.

DENGUE - CHIKUNGUNYA GROUP

1. A Ph.D student from AIIMS, New Delhi was trained in Tissue Culture Techniques for 15 days (8th November 2021 to 18th November 2021).
2. Two DM (Virology) students from Christian Medical College, Vellore were trained in "In-vitro testing of antiviral" on January 14 to 15, 2022.

ENCEPHALITIS GROUP

1. A webinar on rabies conducted on 28th September 2021, in connection with the inauguration of the Rabies Laboratory in ICMR-NIV, and the 15th World Rabies Day. Prof. Dr. Balram Bhargava, Hon. Secretary, DHR and DG-ICMR, delivered the inaugural address in the event. Several eminent speakers from ICMR, NIMHANS, and IDSP delivered talks on the epidemiology, laboratory diagnosis, disease biology, clinical management and prevention of rabies, during the program. The event was attended by 400 clinicians, researchers and students from across the country.

MAXIMUM CONTAINMENT FACILITY GROUP

1. Online training EXPERT'S CONCLAVE: DECODING THE SECOND organized by The Pathologists Association and delivered a session on topic "ABC of SARS-CoV-2, mutations, genomic sequencing and RTPCR on May 16, 2021. Participants = 100.
2. Online training on Training on sample collection, biosafety, samples storage and shipment to the entire site PIs and Coordinators of the study Effectiveness of Covaxin and Covishield vaccines against severe COVID-19 in India, 2021: Multi-centric hospital-based case control study on May 17, 2021. Participants = 40.
3. Online webinar Subsequent to the Zika virus outbreak in Kerala, an online training of the 4 VRDLs (ICMR-NIV Kerala unit, GMC Thrissur, GMC Thiruvanathapuram and GMC Kozhikode) conducted for the detection of Zika virus by real-time RT-PCR on July 9, 2021. Participants = 20.
4. Online webinar Training including the cases enrollment, study protocols, sample and data collection, biosafety, samples storage and shipment was provided under the study

'Reinfection with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) among healthcare workers in India on May 16, 2021. Participants = 100.

5. Online webinar Training including the cases enrollment, study protocols, sample and data collection, biosafety, samples storage and shipment was provided under the study 'Reinfection with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) among healthcare workers in India on July 12, 2021. Participants = 48
6. Intense training on Biorisk mitigation, donning and doffing of Personal Protective Equipments was imparted to the VRDL team of GMC Kozhikode (n=10) and the field team members of the National Health Misison (n=6) during Nipah outbreak 2021 at GMC Kozhikode during September 7-8, 2021. Participants = 16.
7. Online training The two days "virtual training on Biosafety and KFDV molecular and serological diagnosis" for the participants from three laboratories in Goa on September, 30 and October 1, 2021. Participants = 17.
8. The six days "Orientation and Hands-on Training on Illumina NGS platform" from BMCRI at ICMR-NIV, Pune during October, 4-9, 2021articipants = 02.
9. Online training for three days "Second virtual training on Biosafety and KFDV molecular and serological diagnosis for participants (including microbiologist, consultant, technical team members) from eight laboratories from Kerala, Karnataka, Tamil Nadu and Maharashtra had participated in this virtual training. The laboratories included were Kerala (GMC Kozhikode; ICMR-NIV Kerala unit); Karnataka (VDL Shivamogga; ICMR-NIV Bangalore unit); Tamil Nadu (KIPMR Chennai) and Maharashtra (Kasturba infectious diseases hospital Mumbai, GMC Nagpur and GMC Miraj) during October 27-29, 2021. Participants = 41.
10. A half day 'Virtual Training on Zika virus detection by real time RT-PCR assay' was conducted for 26 VRDLs all over the country during December 1, 2021. Participants = 350.
11. The half day Adult Basic Life Support workshop was conducted by Department of Anaesthesiology MIMER Medical College and Dr. BSTR Hospital Talegoan at ICMR-NIV Pune Ambedkar Road campus and was attended by participants/staff from different departments of ICMR-NIV Pune on December 4, 2021. Participants = 22.
12. Online training Under the ICMR Pandemic Preparedness Program, ICMR-NIV Pune being one of the training site for the preparedness; training session on the "Implementation of Research during Health Emergencies" for participants from 6 institutes on February 24-25, 2022. Participants = 28.

INFLUENZA GROUP

1. Training: Isolation of Human Influenza viruses and antigenic characterization-HA & HAI on 29-11-2021 at ICMR-NIV. 06 Participants from KGMU Lucknow, SMS Jaipur, KIPM Chennai, NICED Kolkata, RMRC Dibrugarh and AIIMS New Delhi.
2. Human Influenza HA Gene Sequencing by Sanger Method during 13-12-2021 to 20-12-2021 at ICMR-NIV. 06-Participants from KGMU Lucknow, SMS Jaipur, KIPM Chennai, NICED Kolkata, RMRC Dibrugarh and AIIMS New Delhi.
3. Hands on training on whole genome sequencing of SARS CoV-2 using ION Torrent platform and use of bioinformatics tool in data analysis during 21-02-2022 to 26-02-2022 at ICMR-NIV, Pune.
4. Trainings of Microbiologist for Whole Genome Sequencing and Cell Culture Techniques during 07-03-2022 to 13-03-2022 at ICMR-NIV, Pune. 4- Participants from SMS Jaipur.

DIAGNOSTIC VIROLOGY GROUP

1. Training program for VRDLs 2022/01 during 05-01-2022 to 12-01-2022 for 15 participants from 05 VRDLs.
2. Training program for VRDLs 2022/02 during 03-03-2022 to 10-03-2022 for 15 participants from 04 VRDLs.
3. Training program for VRDLs 2022/03 during 23-03-2022 To 30-03-2022 for 12 participants from 04 VRDLs.
4. Online training for COVID-19 diagnostics to VRDLs during April 2021 for 390 participants.
5. Whole-genome sequencing on NGS platforms for detection of SARS CoV-2 variants during 21-02-2022 to 26-02-2022 for 24 participants.
6. Hands-on training for COVID-19 diagnostics to medical scientists from CMC Vellore on 17-01-2022 for 02 participants.

POLIO VIRUS GROUP

1. Five days training for working in High Containment Laboratory/PEF from 12th to 16th July, 2021 (3 trainees- 2 from PVG & 1 from Engineering) for Organizer and faculty
2. Online Joint Services Disaster Management Course on 'Emerging viral Diseases and management" as part of the Disaster Management Course for the Tri-services (Army, Navy & Air-Force). Guest lecture (Online) conducted for the officers from all over India at CME, Dapodi, Pune -411031 on 31-07-2021 (20 Trainee Officers) for faculty.
3. Training on 'Biosafety issues while handling high risk pathogens' to CMC Vellore DM (Clinical Virology) students (2) on 20th January 2022 during onsite visit.
4. Biosafety training for the team of Microbiologists from SMS Group of Medical College, Jaipur, Rajasthan on 7th March 2022 during their Physical visit and training program.

BENGALURU UNIT

1. Training on Serology testing for Arboviruses for Bangalore Urban from 8th March 2022 to 10th March 2022 for the Helath Dept Bangalore rural staff.
2. Training on Serology testing for Arboviruses for Belgavi during 15th March 2022 to 17th March 2022 for Health dept Belgavi staff.
3. Training on SARS CoV-2 molecular testing during 5th Jan 2022 to 7th Jan 2022 at Indira Gandhi Institute of Child Health.
4. Virus Isolation, Cell culture, Serology and Molecular testing during 26th April 2021 to 11th June 2021 at Jaya Deva Cardiology Institute

KERALA UNIT

1. Training on tick collection and identification was conducted in DVC Unit, Wayanad 22 health workers participated. Date of training – 17-18 th January – 2022.

MUMBAI UNIT

2. Virtual training on NABL Awareness as per ISO/IEC 17025:2017 guidelines for Dengue & Chikungunya Group, ICMR NIV, 5th May 2021.
3. Virtual training on Environmental surveillance of SARS-CoV-2 for Ministry of Housing & Urban Affairs, GOI at ICMR Hq, ICMR-NIVMU, 7th May 2021.
4. Virtual training on environmental surveillance of SARS-CoV-2 at ICMR Hq, WHO, ICMR-NIVMU, 19th April 2021.
5. Environmental surveillance of SARS-CoV-2 in a virtual training programme for Central Pollution Control Board, GOI at ICMR Hq, ICMR-NIVMU, 18th May 2021.
6. Training on sewage sample collection for detection of poliovirus for sites in Rajasthan state, WHO and NIVMU, 2nd September 2021.
7. Virtual training on "sewage water testing for SARS-CoV2" for Surat city in coordination with Urban affairs ministry/ICMR/WHO, ICMR Hq, WHO, ICMR-NIVMU, 24th November 2021.
8. Virtual ILQC training/meeting with 38 COVID-19 diagnostic laboratories, ICMR-NIVMU, 25th November 2021.
9. Organized virtual Experts and Investigators Meeting on Long-term Poliovirus Excretors in Patients with Primary Immunodeficiency Disorders (PIDs) on 24th September 2021 at ICMR-National Institute of Virology, Mumbai Unit.
10. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at 21 study sites across India and assessment of site preparedness, initiation of study sites in ICMR-WHO-iVDPV study King George Medical University, Lucknow, UP, 22nd October 2021.
11. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at Super Speciality Hospital, Noida, UP, 26th October 2021.
12. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at PGIMER, Chandigarh, 27th October 2021.
13. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at Dr. Rajendra Prasad Government Medical College, Kangra, HP, 28th October 2021.
14. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at AIIMS, Rishikesh, Uttarakhand, 14th December 2021.
15. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at Christian Medical College, Vellore, TN, 22nd December 2021.
16. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at Sawai Man Singh Medical College, Jaipur, Rajasthan, 29th December 2021.
17. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at AIIMS, Rishikesh, Uttarakhand, 10th February 2022.
18. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at AIIMS, Bhubaneswar, Odisha, 16th February 2022.
19. Conducted training of paediatricians, clinical immunologists, clinicians of Institutes / hospitals at Kalinga Institute of Medical Sciences, Odisha, 17th February 2022.
20. Training at AcronnoLife Genomics, Chennai, India for company personnel regarding production and manufacture of “RT-LAMP kit for detection of SARS-CoV-2”, 28th June 2021-1st July 2021.

21. Training at Rapid Diagnostic Group of Companies, Delhi, India for company personnel regarding production and manufacture of "RT-LAMP kit for detection of SARS-CoV-2", 2nd July 2021-9th July 2021.
22. Hands on training on performance of RT-LAMP assay for detection of SARS-CoV-2 at Chhatrapati Shivaji Maharaj International Airport, Mumbai on 11th January 2022.
23. Hands on training on performance of RT-LAMP assay for detection of SARS-CoV-2 at Kasturba Health Society Medical Research Centre, Mumbai on 12th January 2022.
24. Hands on training on performance of RT-LAMP assay for detection of SARS-CoV-2 at Municipal Corporation of Greater Mumbai, F-South Ward on 1st February 2022.
25. Training at AXIVA Sichem Biotech, Delhi for company personnel regarding production and manufacture of lateral flow assay kit for semi-quantitative detection of CC16 for Silicosis or Silico-Tuberculosis, 18th October 2021-21st October 2021.
26. Detection of SARS-CoV-2 RNA from sewage to ICMR- NIV, Bangalore and SGPGIMS, Lucknow on 19th April 2021.
27. "Molecular diagnosis of measles and rubella" to National Polio Laboratory, Nepal from 26th April 2021 to 28th April 2021.
28. "Sewage water collection and testing for SARS-CoV-2" to PGIMER, Chandigarh on 27th September 2021.
29. "Sewage water testing for SARS-CoV-2" organized by Urban Affairs Ministry on 7th and 11th October 2021.
30. "Sewage water testing for SARS-CoV2 detection" organized for Surat city on 24th November 2021.
31. "Sewage water processing for poliovirus isolation and reporting through PLIFA" for CRI, Kasauli and PGIMER, Chandigarh on 9th Dec 2021.
32. Environmental Surveillance for SARS-CoV-2 and poliovirus organized for AIIMS, Patna on16th February 2022.

ENGINEERING SUPPORT GROUP

1. Training on Efficient Maintenance & operation in switch Gear on 18/06/2021 for 6 participants.
2. Training on Energy efficient Steam System on 10/06/2021 to technical staff.
3. Training on Awareness on energy conservation on 04/06/2021 to Engineering staff.
4. Training on Bio - STP and Modern Solid Waste Management Technologies on 16 July 2021 to Engineering staff.

Awards received

1. Dr. Varsha Potdar received award "Rising Star Award" by Rotary Club of Pune for the outstanding contribution during COVID Pandemic.
2. Dr. Varsha Potdar received Sharda Shakti Vishesh Sanman Puraskar 2021 from Shakti, 23 November 2021.
3. Dr. Pragya Yadav received the Dr. K. M. Bhansali Oration award in 69th Annual Indian Association of Occupational Health, Mumbai Branch on 12 July 2021.
4. Dr. Pragya Yadav honored with the prestigious National Academy of Medical Sciences Dr. Vinod Kumar Bhargava Award for her work on "Immunogenicity and protective efficacy of inactivated SARS-CoV-2 vaccine candidate, BBV152 in rhesus macaques "presented in the 61st Annual Conference of National Academy of Medical Sciences at the Banaras Hindu University on 28 November 2021.
5. Dr. Pragya Yadav received the National Academy of Medical Sciences Amritsar Award for the best orator during the 61st Annual Conference of National Academy of Medical Sciences at the Banaras Hindu University on 28 November 2021 for her work on "Immunogenicity and protective efficacy of inactivated SARS-CoV-2 vaccine candidate, BBV152 in rhesus macaques".
6. Dr Pragya Yadav received the Sharda Shakthi Visesh Samman Purskar for being the Women Warrior in the COVID-19 pandemic from the Sharda Shakti (A National Movement of Women), Maharashtra Unit on 4 December 2021.
7. Dr Pragya Yadav was conferred with the 'Award of Honor to The COVD-19 Frontline Warrior' for her outstanding work and scientific contributions towards prevention and control of COVID-19 pandemic by the National Academy of Sciences, India (NASI) during the 91st Annual Session and Symposium on Interface between Biological and Physical sciences towards Atmanirbhar Bharat on 4 December, 2021.
8. Dr. Pragya Yadav received the National Academy of Agricultural Sciences fellowship award in January 2022.
9. Dr. Pragya Yadav was awarded with Rising Star award from Rotary Club Pune on March 2, 2022
10. Dr. Pragya Yadav, Dr. Anita Shete, Dr. Rima Sahay and Dr. Sreelekshmy Mohandas and 31 technical staff and 3 Project scientists of BSL-4 facility felicitated and awarded with appreciation certificate for the exemplary work during COVID-19 pandemic by the Rotary club Pune on March 5, 2022.
11. The WEEK appreciated the "Sheroes" on International Women's Day 2022 by giving Momento of Honor to Dr. Pragya Yadav on March 8, 2022.

12. Dr. Pragya Yadav awarded with Bharat Bhagya Vidhata Samman 2022 by Shri J.P. Nadda, National President, Bharatiya Janta Party on March 30, 2022 at Bharat Bhagya Vidhata Red Fort Festival New Delhi. The award is in recognition of her valuable contribution in isolation of SARS-CoV-2 virus and conducting animal studies for development of indigenous vaccine (Covaxin and ZyDCoV).
13. Dr. Gajanan N Sapkal received "RISING STAR AWARD" from Rotary Club of Pune Kothrud for the year 2021-22 for the outstanding contribution made during COVID pandemic and making a difference in the lives of the community.
14. Dr. Gururaj Rao Deshpande felicitated from Rotary Club of Pune Kothrud for the year 2021-22 for the outstanding contribution made during COVID pandemic and making a difference in the lives of the community.
15. Dr. Gururaj Rao Deshpande received First E-poster prize (Under-Medical Virology) for poster entitled "Development of anti-SARS-CoV-2 mouse monoclonal antibodies of diagnostic and therapeutic potential" Virocon-2021 in "National Conference of Virology (Virtual Mode) " Emerging and Remerging viral diseases-Climate change Impact and Mitigation" conducted by AIIMS, Bibinagar, Hyderabad, India held between March 26th to 28th, 2022.
16. Dr. Ullas PT received a Certificate of Appreciation from the Rotary Club of Pune, Kothrud, for his contributions during the COVID-19 pandemic.
17. Dr. Mallika Lavania received ICMR-JALMA Trust Fund Oration Award from ICMR for the work carried out the field of Leprosy and other mycobacterial diseases.
18. Mrs. Veena Vipat participated and secured 2nd place in the "E –Poster Competition for Regular Staff of Technical Cadre" in "COVID-19 Category", organized by ICMR National Institute of Virology, Pune as part of National Science Day 2022 celebrations.
19. Mrs. Vandana Chandere, Awarded 3rd Prize in Hindi Poster presentation on subject "आओ सब मिलकर (COVID-19) को हराये" during हिन्दी सप्ताह from 14/09/2021 to 20/09/2021.
20. Mr. Vishal Kavathekar awarded Ph. D. degree on 12.05.2021 in Biotechnology (Guide: Dr. B. Anukumar, Scientist E).
21. Mrs. Nitali Tadkalkar awarded Ph. D. degree on 11.06.2021 in Biotechnology (Guide: Dr. A. Basu, Scientist G).
22. Mr. Prudhvi Lal Bhukya awarded Ph. D. degree on 31.01.2022 in Biotechnology (Guide: Dr. Kavita S. Lole, Scientist F)
23. Ms. Daya Pavitrakar awarded Ph. D. degree on 09.03.2022 in Basic Medical Science (Guide: Dr. Pratip Shil, Scientist E).

24. Dr. B. Anukumar received Dr H.G. Sharma & Mrs. Kanti Devi award on Medical Entomology-Laudable Arboviral Research and Service to both Science & Society during the 14th International Conference of Medical Arthropodology on "Moving toward the elimination of malaria from India: challengrs and opportunities" on November 27, 2021 (via Webinar) by Society of Medical Arthropodology.
25. Dr. Shailesh D Pawar received First Prize for Poster Presentation: on "Corona Mukt Vishwa: Ek Chunowti", Corona-free World: A Challenge" in Hindi Workshop, held at the ICMR-NIV, Pune, 14-20 Sept 2021.
26. Dr. Shailesh D Pawar received Certificate of Appreciation for the significant contribution for establishing the Lab Quality Management System for NABL accreditation as per ISO/IEC 17025:2005 requirements, as the Quality Manager, May 2021.
27. Dr. Shailesh D Pawar became Member of the Technical Expert, National Accreditation Board for Testing and Calibration Laboratories, Quality Council of India, Government of India.
28. Dr. Shyam Sundar Nandi selected for "Young Achiever in Science Award 2020-21" by Bencos Research Solutions.
29. Ms. Sonali Sawant received Best poster presentation award on Science day celebration at ICMR-NIV 28th February 2022 at ICMR-NIV Pune.
30. Mr. Prasad Sarkale, Techncial Officer, Maximum Containment Facility had received performer of the year Late SN Ghosh Memorial Award under the technical cadre category- best performer for year 2020 during Foundation Day Celebration on Feb 4, 2022.
31. Mrs. Triparna Majumdar, Technical Officer from BSL-4 facility was awarded with cash prize and certificate for bagging the first prize for the best poster presentation on 'Development and Validation of Nipah PoC" during celebration of National Science Day at ICMR NIV Pune on February 28, 2022
32. Mrs Poonam Patil: Received 2nd prize for poster presentation '*In-vitro* antiviral activity of *Carica papaya* formulations against dengue and chikungunya viruses' during celebration of National Science Day at ICMR NIV Pune on February 28, 2022
33. Dr. J. Patil: Received 3rd prize for poster presentation 'An unusual outbreak of chikungunya along with dengue and zika virus infection in a rural region of Pune, Maharashtra' during celebration of National Science Day at ICMR NIV Pune on February 28, 2022.

List of publication: From 1st April 2021 to 31st March 2022

	NIV List of Publications From 1st April 2021 to 31st March 2022 (n=123)	
	Average Impact Factor	14.314
	No. of Book Chapters	6
1	Aggarwal N, Potdar V, Vijay N, Mukhopadhyay L, Borkakoty B, Manjusree S, Choudhary ML, Chowdhury D, Verma R, Bhardwaj SD, Sarmah N, H SK, Kumar P, Gupta N. SARS-CoV-2 and Influenza Virus Co-Infection Cases Identified through ILI/SARI Sentinel Surveillance: A Pan-India Report. Viruses. 2022 Mar 17;14(3):627. doi: 10.3390/v14030627. PMID: 35337033	5.818
2	Ahmad M, Verma H, Deshpande J, Kunwar A, Bavdekar A, Mahantashetti NS, Krishnamurthy B, Jain M, Mathew MA, Pawar SD, Sharma DK, Sethi R, Visalakshi J, Mohanty L, Bahl S, Haldar P, Sutte RW. Immunogenicity of Fractional Dose Inactivated Poliovirus Vaccine in India. J Pediatric Infect Dis Soc. 2022 Feb 23;11(2):60-68. doi: 10.1093/jpids/piab091.	5.235
3	Alagarasu K, Kaushal H, Shinde P, Kakade M, Chaudhary U, Padbidri V, Sangle SA, Salvi S, Bavdekar AR, D'costa P, Choudhary ML. TNFA and IL10 Polymorphisms and IL-6 and IL-10 Levels Influence Disease Severity in Influenza A(H1N1)pdm09 Virus Infected Patients. Genes (Basel). 2021 Nov 28;12 (12):1914. doi: 10.3390/genes12121914.	4.141
4	Alagarasu K, Patil JA, Kakade MB, More AM, Yogesh B, Newase P, Jadhav SM, Parashar D, Harmanmeet K, Nivedita G, Neetu V, Jitendra N, Shah PS (for VRDL team). Serotype and genotype diversity of dengue viruses circulating in India: A multicentre retrospective study involving virus research diagnostic laboratory network during 2018. Int J Infect Dis. 2021 Oct;111:242-52. doi.org/10.1016/j.ijid.2021.08.045	12.074
5	Alagarasu K. Immunomodulatory effect of vitamin D on immune response to dengue virus infection. Vitam Horm. 2021 Jul;117:239-252. doi: 10.1016/bs.vh.2021.06.001.	2.247
6	Anukumar B, Asia Devi T, Koshy J, Nikhil NT, Sugunan AP. Molecular characterization of chikungunya virus isolates from two localized outbreaks during 2014-2019 in Kerala, India. Arch Virol. 2021 Oct;166(10):2895-9. doi:10.1007/s00705-021-05186-9.	2.685
7	Ashok M, Sahay RR, Deshpande GR, Patil DY, Shete AM, Sapkal GN, Kumar R, Narayana M, Yadav PD, Shettar V. A case with SARS-CoV-2 reinfection from India. Indian J Med Microbiol. 2022 Jan-Mar; 40(1):166-8. doi.org/10.1016/j.ijmmb.2021.09.010.	1.347

8	Babu GR, Sundaresan R, Athreya S, Akhtar J, Pandey PK, Maroor PS, Padma MR, Lalitha R, Krishnappa L, Shariff M, Manjunath C, Sudarshan MK, Gururaj G, Ranganath TS, Vasanth KD, Banandur P, Ravi D, Shiju S, Lobo E, Satapathy A, Alahari L, Dinesh P, Thakar V, Desai A, Rangaiah A, Munivenkatappa A, S K, Basawarajappa SG, Sreedhara H, KC S, B AK, Umar N, BA M, Vasanthapuram R. The burden of active infection and anti-SARS-CoV-2 IgG antibodies in the general population: Results from a statewide sentinel-based population survey in Karnataka, India, Int J Infect Dis. 2021 May 21;108:27-36. doi: 10.1016/j.ijid.2021.05.043.	12.074
9	Balasubramanian R, Nadh VA, Sahina S. Ecology of breeding habitats of mosquito population and screening for virus of Japanese encephalitis and West Nile in the coastal area of Kerala, India. J Vector Borne Dis. 2021 Jul-Sept;58(3);232-9. DOI: 10.4103/0972-9062.318307.	0.735
10	Balasubramanian R, Sahina S. The effect of flood on seasonal dynamics of Haemaphysalis (Acari: Ixodidae) tick vectors in Western Ghats forest area of Kerala, South India. J Environ Biol. 2022 Jan;43(1):66-72. DOI:10.22438/eb/43/1/MRN-1942.	NA
11	Balasubramanian R, Yadav PD, Sahina S, Nadh VA. The species distribution of ticks & the prevalence of Kyasanur forest disease virus in questing nymphal ticks from Western Ghats of Kerala, South India. Indian J Med Res. 2021 May;154(5):743-9. doi: 10.4103/ijmr.IJMR_234_19.	5.274
12	Behera SP, Kumar N, Singh R, Deval H, Zaman K, Misra B, Pandey A, Kant R, Kavathekar A, Kumar S, Nuthakki MR, Bondre VP. Molecular Detection and Genetic Characterization of Orientia tsutsugamushi from Hospitalized Acute Encephalitis Syndrome Cases During Two Consecutive Outbreaks in Eastern Uttar Pradesh, India. Vector Borne Zoonotic Dis. 2021 Oct;21(10):747-52.doi: 10.1089/vbz.2021.0003.	2.523
13	Bhardwaj SD, Choudhary ML, Gurav YK, Abraham P, Potdar VA, NIC Team. Epidemiological characterization of COVID-19 – Pune, 2020-2021. Indian J Med Res. May&Jun;153(5&6):542-545. doi: 10.4103/ijmr.IJMR_442_21.	5.274
14	Bhatia R, Abraham P. COVID-19 vaccines & pandemic. Indian J Med Res. 2021 May&Jun;153(5&6):517-21. doi: 10.4103/ijmr.ijmr_1821_21.	5.274
15	Bhattad DR, Kamble MB, Vaidya SR. Genome Sequences of Measles Virus D4 and D8 Genotypes from India. Microbiol Resour Announc. 2021 Jul 15;10(28):e0053821. doi: 10.1128/MRA.00538-21.	NA
16	Burney P, Patel J, Minelli C, Sapkal GN et al. BOLD Collaborative Research Group. Prevalence and Population-Attributable Risk for Chronic Airflow Obstruction in a Large Multinational Study. Am J Respir Crit Care Med. 2021 Jun 1;203(11):1353-1365. doi: 10.1164/rccm.202005-1990OC.	30.52
17	Cherian S, Potdar V, Jadhav S, Yadav P, Gupta N, Das M, Rakshit P, Singh S, Abraham P, Panda, S. SARS-CoV-2 Spike Mutations, L452R, T478K, E484Q and P681R, in the Second Wave of COVID-19 in Maharashtra, India. Microorganisms. 2021 Jul;9(7):1542. doi.org/10.3390/microorganisms9071542.	4.926

18	Deoshatwar A, Salve D, Gopalkrishna V, Kumar A, Barve U, Joshi M, Katendra S, Dhembre V, Maheshwari S, Viswanathan R. Evidence-Based Health Behavior Interventions for Cholera: Lessons from an Outbreak Investigation in India. Am J Trop Med Hyg. 2021 Oct 25;106(1):229-32. doi: 10.4269/ajtmh.21-0625.	3.707
19	Deshpande GR, Kaduskar O, Deshpande K, Bhatt V, Yadav P, Gurav Y, Potdar V, Khutwad K, Vidhate S, Salunke A, Patil C, Shingade S, Jarande K, Tilekar B, Salvi P, Patsuthe S, Dange V, Kumar S, Gurav S, Chate S, Abraham P, Sapkal G. Longitudinal clinico-serological analysis of anti-nucleocapsid and anti-receptor binding domain of spike protein antibodies against SARS-CoV-2. Int J Infect Dis. 2021 Nov;112:103-110. doi: 10.1016/j.ijid.2021.09.024.	12.074
20	Deshpande K, Pt U, Kaduskar O, Vijay N, Rakhe A, Vidhate S, Khutwad K, Deshpande GR, Tilekar B, Saka S, Gadekar K, Patil R, Yadav P, Potdar V, Gurav Y, Gupta P, Kaur H, Narayan J, Sapkal G, Abraham P. Performance assessment of seven SARS-CoV-2 IgG enzyme linked immunosorbent assays. J Med Virol. 2021 Dec;93(12):6696-6702. doi: 10.1002/jmv.27251.	20.693
21	Deval H, Alagarasu K, Srivastava N, Bachal R, Mittal M, Agrawal A, Bote M, Gondhalekar A, Bondre VP, Kant R. Association of single nucleotide polymorphisms in the CD209, MMP9, TNFA and IFNG genes with susceptibility to Japanese encephalitis in children from North India. Gene. 2022 Jan 15;808:145962. doi: 10.1016/j.gene.2021.145962.	4.141
22	Deval H, Behera SP, Aggarwal A, Singh R, Misra B, Janardan V, Patil G, Sah K, Kumar N, Singh R, Bondre VP. Genetic characterization of dengue virus serotype 2 isolated from dengue fever outbreaks in the eastern Uttar Pradesh and western Bihar, India. J Med Virol. 2021 Jun;93(6):3322-9. doi: 10.1002/jmv.26239.	20.693
23	Deval H, Nyayanit DA, Mishra SK, Yadav PD, Zaman K, Shankar P, Misra BR, Behera SP, Kumar N, Kumar A, Bhardwaj P, Dwivedi GR, Singh R, Shete AM, Pandit P, Pandey AK, Yadav GK, Gupta S, Kumar M, Kavathekar A, Singh RS, Prajapati S, Kant R. Genome Sequencing Reveals a Mixed Picture of SARS-CoV-2 Variant of Concern Circulation in Eastern Uttar Pradesh, India. Front Med (Lausanne). 2022 Jan 7;8:781287. doi: 10.3389/fmed.2021.781287.	5.058
24	Dhar MS, Marwal R, Vs R, Ponnusamy K, Jolly B, Bhoyar RC, Sardana V, Naushin S, Rophina M, Mellan TA, Mishra S, Whittaker C, Fatihi S, Datta M, Singh P, Sharma U, Ujjainiya R, Bhatheja N, Divakar MK, Singh MK, Imran M, Senthivel V, Maurya R, Jha N, Mehta P, A V, Sharma P, Vr A, Chaudhary U, Soni N, Thukral L, Flaxman S, Bhatt S, Pandey R, Dash D, Faruq M, Lall H, Gogia H, Madan P, Kulkarni S, Chauhan H, Sengupta S, Kabra S; Indian SARS-CoV-2 Genomics Consortium (INSACOG)‡, Gupta RK, Singh SK, Agrawal A, Rakshit P, Nandicoori V, Tallapaka KB, Sowpati DT, Thangaraj K, Bashyam MD, Dalal A, Sivasubbu S, Scaria V, Parida A, Raghav SK, Prasad P, Sarin A, Mayor S, Ramakrishnan U, Palakodeti D, Seshasayee ASN, Bhat M, Shouche Y, Pillai A, Dikid T, Das S, Maitra A, Chinnaswamy S, Biswas NK, Desai AS, Pattabiraman C, Manjunatha MV, Mani RS, Arunachal Udupi G, Abraham P, Atul PV, Cherian SS. Genomic characterization and epidemiology of an emerging SARS-CoV-2 variant in Delhi, India. Science. 2021 Oct 14:eabj9932. doi: 10.1126/science.abj9932. Epub ahead of print.	63.714

25	Ella R, Reddy S, Blackwelder W, Potdar V, Yadav P, Sarangi V, Aileni VK, Kanungo S, Rai S, Reddy P, Verma S, Singh C, Redkar S, Mohapatra S, Pandey A, Ranganadin P, Gumashta R, Multani M, Mohammad S, Bhatt P, Kumari L, Sapkal G, Gupta N, Abraham P, Panda S, Prasad S, Bhargava B, Ella K, Vadrevu KM; COVAXIN Study Group. Efficacy, safety, and lot-to-lot immunogenicity of an inactivated SARS-CoV-2 vaccine (BBV152): interim results of a randomised, double-blind, controlled, phase 3 trial. Lancet. 2021 Dec 11;398(10317):2173-84. doi: 10.1016/S0140-6736(21)02000-6.	202.731
26	Ella R, Reddy S, Jogdand H, Sarangi V, Ganneru B, Prasad S, Das D, Raju D, Praturi U, Sapkal G, Yadav P, Reddy P, Verma S, Singh C, Redkar SV, Gillurkar CS, Kushwaha JS, Mohapatra S, Bhate A, Rai S, Panda S, Abraham P, Gupta N, Ella K, Bhargava B, Vadrevu KM. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial. Lancet Infect Dis. 2021 Jul;21(7):950-61. doi: 10.1016/S1473-3099(21)00070-0.	71.421
27	Ella R, Vadrevu KM, Jogdand H, Sapkal GN et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial [published correction appears in Lancet Infect Dis. 2021 Apr;21(4):e81]. Lancet Infect Dis. 2021;21(5):637-646. doi:10.1016/S1473-3099(20)30942-7	71.421
28	Ganneru B, Jogdand H, Daram VK, Das D, Molugu NR, Prasad SD, Kannappa SV, Ella KM, Ravikrishnan R, Awasthi A, Jose J, Rao P, Kumar D, Ella R, Abraham P, Yadav P, Sapkal GN, Shete A, Desphande GR, Mohandas S, Basu A, Gupta N, Vadrevu KM. Th1 Skewed immune response of Whole Virion Inactivated SARS CoV 2 Vaccine and its safety evaluation. iScience. 2021 Apr 23;24(4):102298. doi: 10.1016/j.isci.2021.102298.	6.107
29	George S. Detection of Rubella Virus by Tri-Primer RT-PCR Assay and Genotyping by Fragment RT-PCR. Methods Mol Biol. 2022;2392:53-64. doi: 10.1007/978-1-0716-1799-1_4.	NA
30	Gokhale M, Cherian S, Abraham P. Kalyan Banerjee (1937-2021). Indian J Med Res. 2021 Apr;153(4):510-1. doi: 10.4103/ijmr.ijmr_1384_21.	5.274
31	Gokhale MD, Sreelekshmy M, Sudeep AB, Shete A, Jain R, Yadav PD, Mathapati B, Mourya DT. Detection of possible Nipah virus infection in Rousettus leschenaultii and Pipistrellus Pipistrellus bats in Maharashtra, India. J Infect Public Health. 2021 Jun 11;14(8):1010-2. doi: 10.1016/j.jiph.2021.05.001.	7.537
32	Gopalan RB, Babu BV, Sugunan AP, Murali A, Ma MS, Balasubramanian R, Philip S. Community engagement to control dengue and other vector-borne diseases in Alappuzha municipality, Kerala, India. Pathog Glob Health. 2021 Jun;115(4):258-66. doi: 10.1080/20477724.2021.1890886.	3.735
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List of Patents applied

From 1st April 2021 to 31st March 2022

1. Title of patent "Development of HEK293 knock-out cell line of EV-A71 receptors SCARB2 and PSGL1 genes".

   Inventors: Dr. Shyam Sundar Nandi (PI), Dr. Upendra Lambe, Ms. Sonali Ankush Sawant, Dr. Jagadish Deshpande. Indian Patent Application No: 202111048030, September, 2021

2. Title of patent "Instrument free nucleic acid extraction method".

   Inventors: Dr. Shyam Sundar Nandi (PI), Dr. Upendra Lambe, Ms. Sonali Ankush Sawant, Dr. Jagadish Deshpande. Patent application accepted by IPR Cell, ICMR HQ for submission to Indian Patent Office, August 2021. Indian Patent Application No: 202111043228.

3. Title of patent "Development of screening assays for detection of epidemiologically important SARS-CoV-2 variants".

   Inventors: Dr. Shyam Sundar Nandi (PI), Dr. Upendra Lambe, Ms. Sonali Ankush Sawant, Dr. Jagadish Deshpande. Indian Patent Application No: 202111032470, July 19, 2021.

4. Title of the patent "RT-LAMP Assay for detection of human β -Actin housekeeping gene."

   Inventors: Dr. Shyam Sundar Nandi (PI), Upendra Lambe, Sonali Ankush Sawant, Trupti Gohil, Dr. Jagadish Mohan Deshpande. Indian Patent Application Number: 202111012867, March 2021. International PCT application number PCT/IN2022/050183, 30th May 2022.

5. Title of the patent "Rapid RT-LAMP assay for detection of SARS-CoV-2"

   Inventors: Dr. Shyam Sundar Nandi (PI), Upendra Lambe, Sonali Ankush Sawant, Trupti Gohil, Dr. Jagadish Deshpande. Indian Patent Application Number: 202011023573, June 2020. PCT Application number: PCT/IN2021/050549, June 2021

6. Multiplex single tube Real Time RT PCR assay for detection of SARS CoV-2.

   Inventors: Dr. Varsha Potdar, Sheetal Jadhav, Veena Vipat , ML Choudhary. Indian patent application: 202111015708 April 2021.