The details of the excellence in research work

Dr. Pragya Dhruv Yadav is Scientist F & Head, Biosafety level-4 laboratory, Asia's first state-of-the-art-facility to handle high risk pathogens at ICMR-National Institute of Virology. She has been working in the field of public health, since last two decades. Her expertise in viral diseases, outbreak investigation and diagnosis of unknown high-risk viruses are of immense importance and guide the public health policy for containment and treatment to limit the damage caused by the outbreaks. She has contributed significantly for high risk of Zoonotic pathogens such as Crimean Congo hemorrhagic fever (CCHF), Nipah (NiV) Kyasanur forest diseases virus (KFDV) and mpox; preparedness for Ebola and Yellow fever threat leading to improvised national public health surveillance policy for interventions and management.

A. Impact in Public health

i. Crimean Congo hemorrhagic fever (CCHF)

Crimean Congo hemorrhagic fever (CCHF) was first detected in India during a nosocomial outbreak in Ahmadabad district, Gujarat, in 2011, which caused deaths of three medical professionals, while treating affected patients. CCHF is a deadly viral hemorrhagic fever, *Ixodid* ticks found on the body of domestic animals and humans get this infection by bite of an infected tick and then human-to-human transmission occurs due to contact with infected blood or body fluids. Dr. Yadav's work showed the risk of CCHFV infection to livestock, animal husbandry, meat industry, slaughterhouses workers and veterinarians. Once index case reaches to the hospital setting, secondary cases due to human-to-human transmission occur and often some of the close contacts, hospital staff, including doctors and nurses succumb to the infection. Fatality up to 80% has been recorded earlier. Her work also demonstrated how; hospital acquired (nosocomial) infections also contribute to the spread of the virus. It led to the discovery, causation prevention and management of CCHF in India that helped improvising national public health surveillance policy for interventions and management and understanding transmission dynamics of highly infectious and pathogenic CCHF viral infections.

Her continuous supervision for diagnostic support to the Gujarat State contributed following way to draw the attention of health authorities, which helped in further preventing the spread of infections in rural and hospital settings and subsequently saved many lives:

- This proactive work has helped in discovering this deadly disease in Gujarat, thus could provide diagnosis within 15 hours.

- This has led to investigation of more than 45outbreaks in last 10 years in Gujarat and Rajasthan State.
- Developed technology for detection of CCHF human IgM and IgG antibody and Cattle and Sheep/Goat IgG antibody detection ELISA, CCHF Sheep and Goat IgG detection ELISA kit and CCHF Cattle IgG detection ELISA kit technology has been transferred to Cadilla healthcare Pvt Ltd.
- Trained Medical college staff for laboratory diagnosis and biosafety issue related to CCHF.
- Prepared documents/policy guidelines for Gujarat State to make them understand the gravity of situation.
- This led to collaborative efforts as joint activities on the investigations of this highly infectious diseases and state is on high alert for CCHF.
- Detected imported case of CCHF from Oman and informed the health Ministry and state level officials for necessary preventive measures.
- Nationwide serosurvey in domestic animals showed the prevalence of this virus throughout the country that emphasized; the need for indigenous diagnosis tests and the inclusion of this disease in our National "Infectious Disease Surveillance Program" to prevent the loss of human lives.
- **The studies performed have strengthened our Public health response system and a coordination was established among ICMR-NIV, IDSP, hospitals in Gujarat and Rajasthan, ERR, Govt. of India to track the contact cases of CCHF positive case. This has also improved method for collection and screening of ticks and animal samples from the affected area which ultimately could change the policy, fast reporting, barrier nursing and has helped in saving human lives.**

ii. <u>Application of Kyasanur forest disease (KFD) genomic data and molecular clock study to develop molecular and serological diagnostic assay for outbreak investigation and confirmation.</u>

- KFD is maintained in tick vectors (mainly *Haemaphysalis spinigera*) and various species of rodents, birds, and primates, and causes humans infections, which results in a severe febrile illness and high morbidity. Her work drew the attention of health authorities, which helped in further preventing the spread of infections:
- Due to unavailability of appropriate diagnostic assay, case management was a major challenge. She developed a real-time RT-PCR, RT-PCR, IgM, and IgG ELISA to quickly detect KFD infection in humans.
- Molecular clock analysis showed the ancestry of KFDV in India is 65 years ago and revealed that the current vaccine strain will still be useful today.

- She had trained State health laboratory technical staffs to use PCR and ELISA techniques for performing diagnosis of KFD.
- Played key role in formulating MoU to transfer this technology to State health laboratory, Karnataka for KFD case management. Now, the trained laboratories are self-sufficient for performing this assay and providing diagnostic services to Southern region.
- Continuous supervision for KFD diagnosis in BSL-4 laboratory has resulted in discovering its spread in Thirthahalli, Chamarajanagar district, Karnataka as well as adjoining Kerala, Tamil Nadu, Goa State and also in Maharashtra state.
- This had public health impact thus ICMR has formed a KFD task force for the detection of KFDV in newer areas to understand more about this disease.
- Diagnostic services provided to the government of Maharashtra in identifying KFD cases and outbreak during year 2016-17 and follow-up study of KFD cases revealed that virus specific IgG antibody exist for more than a year.
- She has developed indigenous ELISA kits that are commercialized on 29-11-2017 for marketing by Cadila healthcare Pvt Ltd., in India and at International Market.

iii. <u>Outbreak investigations and surveillance of Nipah in India</u>

- Laboratory findings lead to understand that *Pteropus medius (formerly Pteropus giganteus)* bats are involved in Nipah virus transmission in India, which has caused two deadly outbreaks in India [2001 and 2007] in West Bengal.
- Bat survey study in Assam and West Bengal revealed the presence of Nipah virus in Dhubri district in human and bats and warned us to prepare on dealing such outbreak of Nipah in future.
- Her expertise helped in identifying Nipah outbreak of Kerala [2018 and 2019] in Kozhikode and Ernakulam in human cases. Complete genome study revealed its closeness to Bangladesh strain and its link to the outbreak of 2019.
- Development of diagnostic assay (IgM, IgG and point of care assay) was successful which helped in timey diagnosis of Nipah outbreak in Kerala in year 2018 and 2019.
- To identify the hotspot of the Nipah virus a countrywide survey is ongoing.
- Active collaboration was made with IEDCR, Dhaka, and Bangladesh to perform diagnostic kit validation and planning for clinical trial during outbreak.
- The quick set up of field laboratory has helped timely diagnosis and biosafety practices have helped to contain the outbreak of Nipah virus in Kozhikode, Kerala during September 2021.

iv. Ebola Virus preparedness

- Ebola threat griped India and ICMR-NIV, Pune has taken the lead to trained 13 laboratories for Ebola diagnosis in India. Under her supervision diagnosis was provided [24x7] on suspected Ebola cases. One Ebola recovered case [resident of India, returned from affected country] was followed for persistence of virus in semen and data showed persistence of viral RNA for 165 days. Together with this findings and CDC, USA data on recovered Ebola patients, WHO revised advisory and instead of 21 days set 42 days set point without cases for declaring any area free of Ebola outbreak.
- This was the first study, which showed long time presence of Ebola viral RNA in semen. Persistence of virus in semen gave new light on Ebola research.

v. Zika Virus preparedness

- In view of WHO declaring Zika viral disease a global public health emergency during 1 February 2016, nation preparedness for Zika virus was taken up on priority. Shortly BSL-4 laboratory has developed diagnostic RT-PCR and real time-RT-PCR assays and generated cloned positive controls.
- Provided reagents and training to 56 VRDL laboratories across the country as part of preparedness in case of Zika virus outbreak. Being WHO-CC diagnostic reagents and positive control was shared with WHO-SEARO countries.
- With all this preparedness first three cases of Zika were detected from Gujarat state in Fever surveillance and Antenatal clinic (ANC) surveillance. The Zika sequences from India showed highest homology with Malaysian strains.
- India has experienced outbreaks of ZIKV disease in two states: Rajasthan (September–October 2018) and Madhya Pradesh (October–November 2018). In the 2018 outbreaks of Rajasthan and Madhya Pradesh, a total of 159 and 127 ZIKV positive cases with 63 and 42 infected pregnant women were detected, respectively. Since cases from Jaipur, Rajasthan were reported from a restricted geographic location, a containment strategy with targeted interventions for vector control and case detection were implemented and worked excellently.
- The Gujarat strain is of old Asian lineage and is closely related to Malaysia 1966 strain isolated from *Aedes* mosquito, whereas the Rajasthan strains, though cluster separately, are more phylogenetically related to the ZIKV strains which have caused recent outbreaks in Brazil, USA, Guatemala, etc. The evidence indicates the circulation of two distinct ZIKV strains in India.
- The preparedness and availability of standardized and validated diagnostic methods has helped in timely detection of ZVD cases amongst healthcare workers in a private

hospital of Thiruvananthapuram district, Kerala state, India and a one case from Pune, Maharashtra, and outbreak in Uttar Pradesh during 2021.

vi. Yellow Fever preparedness

- Yellow fever emergency and alert by the ministry of health demanded the preparedness for laboratory diagnosis of Yellow fever. We prepared reagents of RT-PCR and positive control, and training was organized for National Centre for Disease Control (NCDC) and Virus Research Diagnostic Laboratory (VRDL).

vii. Biosafety preparedness

- I was involved in conducting Biorisk management training programs for SEAR countries and for laboratories, medical colleges, and hospitals in India. A number of training and workshop conducted in last ten years and that helped in rapid rollout of VRDL network facilities during the pandemic.

viii. Preparedness and surveillance of the mpox virus in India

- The World Health Organization (WHO) declared the current multi-country mpox virus (mpoxv) outbreak as a public health emergency of international concern (PHEIC) on July 23, 2022.
- On May 2022, National Guidelines on mpoxv was published under the aegis of MoHFW, ICMR-NIV Pune, NCDC
- Guidelines for sample collection and shipment were developed and circulated to WHO SEAR countries.
- For capacity building of testing for mpox at Maximum containment laboratory specific primer and probes were procured for detection of non-variola orthopox and mpox by real time PCR.
- Real time PCR for orthopox was validated using mpoxv DNA as positive control.
- ICMR-NIV, Pune also extended the support to SAER member countries and preparations are made supply the reagents to 7 SEAR countries.
- The training on mpoxv diagnosis was provided to 15 VRDLs across the country. Each laboratory was also supplied with reagents for testing.
- The clinical specimens of suspected mpox cases were referred for testing at ICMR-NIV Pune from different regions of the country. Out of all the referred cases, 27 cases were found to be positive for mpox from Kerala (n=12) and New Delhi (n=15).

a. **Genome characterization of mpox cases detected in India: Identification of three sub clusters among A. 2 lineage**

We analyzed the complete genomes sequences of Monkeypox cases from Kerala (n=5 travelled from UAE) and Delhi (n=5 no travel history), India confirmed during July to August 2022. All the retrieved mpoxv sequences from India covering 90 to 99% genome belong to A.2 lineage of clade IIb. The A.2 mpoxv lineage divided in three sub clusters; first cluster Kerala n=5, Delhi n=2 aligned with the USA-2022 ON674051.1; while second of Delhi n=3 aligned with USA-2022 ON675438.1 and third consists of the UK, USA, and Thailand. Recent update in mpoxv lineage designated all the five sequences from Kerala as A.2.1. In addition to known 16 single nucleotide polymorphisms (SNPs) along with 13 APOBEC3 cytosine deaminase activity determined specific lineage defining mutations in A.2 lineage, 25 additional APOBEC3 mutations were found in 10 reported sequences. The study emphasizes need of enhancing genomic surveillance to understand the mutation and its linkage.

b. **A fatal case of mpox virus infection from Kerala India 2022**

We reported the first fatal case of mpox virus infection imported from UAE to Kerala, India in July 2022 in an apparently immunocompetent male with a single episode of acute onset generalized tonic-clonic seizures. The next generation sequencing on OPS/NPS specimen could retrieve 92.76% of the mpoxv genome and belonged to A.2 lineage of clade IIb.

c. **Clinical presentation, viral kinetics, and management of human mpox cases from New Delhi, India**

We studied the clinic-demographic characteristics, virological follow-up, and management of five confirmed mpox cases from New Delhi, India without any international travel history. A higher viral load was detected in lesion fluid (POD 9), followed by lesion roof (POD 9), urine (POD 5), lesion base (POD 5), and OPS/NPS (POD 5). The mpox virus DNA was detected in clinical samples from 5th to 24th POD. These mpox cases without international travel history suggest the underdiagnosed mpox infection in the community, emphasizing the need for active surveillance of mpoxv in the high-risk population.

d. **Persistence of infectious mpox virus on the surfaces of isolation ward in a hospital setting, India**

We report persistence of mpox virus on inanimate surfaces in mpox isolation ward. The persistence of viral DNA was observed in clinical specimens till 23rd day post illness. DNA was also found from bedside, common places and in patient care activities.

Infectious virus was isolated from surfaces of bed, linen, bed rails and floor. The findings emphasize the need for disinfection of mpox isolation ward twice a day.

B. Impact in Basic Research

She also discovered and characterized novel viruses new to science, e.g., Malsoor, Oya virus [Bunyavirus] Kundal, Karyana, Kammnapattai, Wad Medani [*Reoviridae*] etc. She has characterized many viruses first time from India, eg. Umbre, Chittor, Ganjam, Quaranfill, Tioman, EV-68, Coxie -10, Coxie -6 viruses, etc. All these viruses' data have been published, and diagnostic assay [PCR and ELISA] was developed. Her work on the molecular characterization of Bunyaviruses isolated from India has provided the genomic data, which was useful in designing primers, and standardizing PCR based diagnostic assays.

i. Identified complete genome of **Umbre, Ingwavuma, Nairobi sheep diseases virus (Ganjam /NSDV) and Chittoor virus**. Data showed that **Ganjam virus** is Asian variant of NSDV, an important cause of livestock death in South Africa. Recently another *Phlebovirus* isolated from ticks from KFD affected area named as Kaisodi virus was sequenced. A diagnostic assay has been made for future preparedness and that can be used in future for dealing with the outbreaks.
ii. **Thottapalayam** virus, isolated from shrew was characterized and found distinct from the other known Hantaviruses.
iii. **Proactive survey/work on the Novel virus discovery from Bats [reservoir of viral diseases] in India** has helped to determine the presence of highly infectious diseases like Ebola Reston, Marburg, Nipah and other possible viruses responsible for causing fatal diseases in human.
    - Isolation of novel **Adenovirus from *Rousettus leschenaultii*** species of Bats and discovery of Malsoor virus, a novel ***Phlebovirus*** from ***Rousettus leschenaultii*** bats that has close similarity with deadly viruses i.e., Heartland and Severe fever with thrombocytopenia syndrome.
    - The work further helped in developing novel **cell line from *Pipistrellus ceylonicus*** bat, which would be useful in future for virus isolation studies specially the bat derived pathogen.
    - Novel Equine encephalosis virus, a Reovirus from horses was identified for the first time in India and notified to Ministry of Health and Animal husbandry, New Delhi for its continuous surveillance.

C. Impact in Biocontainment Facility Creation

The construction of Biosafety level-4 (BSL-4) laboratory [the flagship project of ICMR] was over by the year 2011. Being a core scientific personal involved right from the beginning of setting up of this lab into the operation. As In-charge, I look after the operation and running of BSL-4 laboratory. The facility is operational, and training of BSL-4 laboratory work practices was provided to staff for the handling of deadly pathogens. Many outbreaks/sporadic cases were investigated and confirmed of high-risk pathogens i.e., CCHF, Nipah, Ebola, mpox and genome was sequenced and published.

- Several molecular and serological assays were standardized and made available not only to virus research diagnostic laboratory (VRDL) labs of Department of Health and Research, Govt. of India but also to World Health Organization member state of Southeastern region which has helped in performing diagnosis of these pathogens.
- Disseminating the knowledge towards current diagnostic methods available for re-emerging and newly emerging diseases under teaching and training program for biosafety, BSL-3, and BSL-4 Laboratory work practices for handling of deadly pathogens.
- During H1N1 pandemic, I served as one of resource person to provide training to different centers of country to establish diagnostic services and provided 24X7 hour a day services to nation During Ebola outbreak in Africa, she served the nation by providing quick diagnosis on suspected samples of traveling patients and suspected of Ebola case.
- Her dedicated efforts in the identification of novel viruses, development of diagnostic tools, strengthening nationwide laboratory capacities to cope up with public health emergencies have helped in building a strong public health system across the country in combating many virus threats. She proved her potential as dedicated virologist during SARS-CoV-2 pandemic with her efforts on basic, applied, and translational research. Altogether, all these efforts have been useful in strengthening the public health system of the country which responded quickly to fight against the virus threats in the past and future outbreaks.
- Countries first H5N1 human case samples was confirmed by NGS and virus isolation and reported to MoH.

D. Impact in SARS-CoV-2 Pandemic

The ongoing pandemic of Coronavirus disease-19 (COVID-19) has created the most dreadful situation in the affected countries across the globe. Dr. Pragya D Yadav has made significant contribution in these areas and some of them have been cited here.

1. **Identification of first three COVID-19 cases and sequencing of two SARS-CoV-2 viruses from India.**
   In the light of the SARS-CoV-2 outbreak in Wuhan, China in December 2019 and importation of cases, many suspected cases were screened as per define travel history of Wuhan or contact with SARS-CoV-2. Throat swab samples collected from the total of 88 suspected cases were tested using the WHO recommended protocol and three of them were found to be positive. These positive samples were identified from Kerala state having a travel history from Wuhan, China. These samples were sequenced using the Next generation sequencing (NGS) to retrieve the complete genomic sequences. The sequences of the Indian SARS-CoV-2 strains using the different sequences for the GISAID indicated a globally emerging heterogeneity. This study also designed the *in-silico* model for the spike gene region and predicted the B-cell and cytotoxic T-cell epitopes in the spike protein. These epitopes can be considered suitable for the vaccine design and diagnostic analysis in the future.

2. **Laboratory Diagnosis**: During this ongoing pandemic of COVID-19, in early phase, she coordinated the response in setting up the diagnosis and laboratories, collection of samples and diagnostic provision to stranded citizens of India in Iran.

3. **Genomic analysis of SARS-CoV-2 strains among individuals returning to India from Italy, Iran, China.**
   To understand the variation in the genetic sequences of the virus, sequences used in the study were retrieved from the throat/nasal swab samples belonging to Italian tourist, their Indian contact cases, Indian nationals in the Iran and the first two SARS-Cov-2 sequences from Wuhan, China. SARS-CoV-2 sequences were retrieved using the NGS method and 563 sequences were downloaded from GISAID. These sequences were aligned, and a phylogenetic tree was generated. An overall divergence of 0.03% was observed in the sequences used in the study. Further the phylogenetic tree demonstrated the presence of ‘G’ and ‘S’ clade sequences in the retrieved set. Nucleotide mutations were compared using

the Wuhan reference strain. It was observed that the nucleotide variation observed in the sequences linked to the specific regions of their origin.

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

   Phylogenetic analysisof the SARS CoV-2 genomes of strains circulating across the country: From January 2020 to March 2021, a pool of positive COVID-19 samples is being sequenced and virus mutations are monitored in each state of country which helped in monitoring the variant in Indian population.

5. **Detection of coronavirus in *Pteropus* and *Rousettus* species of bats from different states of India**

   Bats are the natural reservoirs for some potential human pathogens. An association of Nipah virus with *Pteropus medius* has been known and reported earlier. The recent COVID pandemic, caused by the severe acute respiratory syndrome coronavirus-2(SARS-CoV-2) is hypothesized to be originated from bat. This study was conducted to assess the presence of coronavirus in two species of bat *P. medius* and *Rousettus* species from representative states of India that were collected form 2018-2019. A single step PCR designed for the *RdRp* gene was used to identify the bat-coronavirus (Bt-CoV) in bats. The samples that tested positive were sequenced. Partial RdRp genes were retrieved from three *Rousettus* and eight *Pteropus* bat specimens. Distinct sub-clustering was observed for the Bt-CoV sequences which overall were based on the type of host from which the sequences were retrieved. All the sequences of the Bt-CoV grouped in the Lineage-D of the betacoronavirus genus. The study could retrieve ~94.3% of two different Bt-CoVs from the *Rousettus* samples. This study presents the need of increased screening of the bats to identify the presence of other novel viruses apart from the existing known ones. This would help in proactive surveillances of the potential emerging pathogens.

6. **First SARS-CoV-2 isolation and propagation**

   Her efforts and perseverance resulted in the isolation of SARS-CoV-2, which was a breakthrough. The virus was successfully isolated from referred clinical specimens using Vero CCL-81 cells in the BSL-4 facility of institute. The virus isolates were confirmed using real time RT-PCR and further characterized using electron microscopy and next-generation sequencing (NGS). The virus kinetics, morphology at different temperature and pathogenicity were studied in the Syrian hamster animal model.

7. **Neutralization assay development** for screening of animal's and human serum samples to understand the impact of vaccine for further research and titer of antibody in samples.

8. **COVID KAVACH ELISA**: This early isolation of the virus and subsequent work in developing the indigenous IgG antibody ELISA COVID KAVACH test against SARS-CoV-2 was a major contribution in the domain of public health and the fight against COVID-19. The kit is developed indigenously for the diagnosis of human samples which is **1) Cost-effective, 2) Sensitive, 3) Rapid, and 4) user- friendly**, many samples can be tested at any level of clinical setting, public health centers, and hospitals. This indigenously developed ELISA has a gamma inactivated SARS-CoV-2 virus antigen, which detects IgG antibody in serum/plasma samples in contact tracing, sero-epidemiological studies, and future vaccine evaluation studies. Due to the inactivated nature of the antigen, this kit can be used in most laboratory settings across the country. This information will be helpful particularly for instituting preventive measures in the affected area. In this assay, patient serum/plasma samples can be heat-inactivated and used for the assay. **The technology was transferred** to seven Indian companies. She is supplying antigens to these companies to make kits for performing different studies and testing IgG antibodies form the patients.

9. **Establishment of Antiviral testing platform**: She has established and standardized the testing of antiviral drugs against SARS-CoV-2 and screened more than 60-compounds of Indian pharmaceutical companies and these compounds are in various stages of preclinical evaluations.

10. **Susceptibility study in animal models:** Studied SARS CoV-2-virus propagation, ability of infant mice models like CD (by intranasal, intracerebral, and intraperitoneal route), AG9 (by intranasal and intraperitoneal route) and C57 BL/6 (intracerebral route). None of the models supported virus propagation. Susceptibility of various rodent models like adult BALB/c mice, adult C57BL/6, infant CD mice, infant AG9 mice and hamsters to SARS CoV-2 was studied in the BSL-4 laboratory.

11. **Development of equine antisera against SARS-CoV-2:** The pandemic of COVID -19 caused by SARS-CoV-2 had a humongous impact on the mankind with over a million people succumbing to it worldwide. Although there are few drugs approved for the treatment, there is not yet a safe and effective vaccine available for COVID-19. Also, the passive immunization therapy with convalescent plasma has been an effective treatment option for other viral disease has limitation of availability. The prior use of immunoglobulins generated in animals has proven to be effective in several viral and bacterial diseases. She has collaborated with eBiologcals, Hyderabad and developed,

evaluated equine hyper immune globulin raised against inactivated SARS-CoV-2 virus. Post immunization neutralization titres of the equines demonstrated high neutralizing antibodies. The quality control assessments of the different batches proved to have consistent NAb titres. The study provides evidence for the potential of generating highly purified F(ab')2 from equines against SARS-CoV-2 that can demonstrate consistent and high neutralization activity. Further, *in-vivo* testing for efficacy of this indigenously developed, cost effective product will pave the way to clinical evaluation. Working further towards the goal of self-reliant India, she has developed hyperimmune anti equine serum in collaboration with Indian firm, which is at the stage of animal studies and will be used as a therapeutic agent against COVID-19.

12. **The national serosurvey of SARS-CoV-2, May-June 2020:** She was part of a national serosurvey, conducted to estimate the seroprevalence of SARS-CoV-2 infection among adult population of India. From May 11 to June 4, 2020, a randomly sampled, community-based survey was conducted in 700 villages/wards, selected from the 70 districts of the 21 States of India, categorized into four strata based on the incidence of reported COVID-19 cases. Serum samples were tested for IgG antibodies using COVID Kavach ELISA kit. A cumulative 6,468,388 adult infections (95% CI: 3,829,029-11,199,423) were estimated in India by the early May. Seroprevalence of SARS-CoV-2 was low among the adult population in India around the beginning of May 2020. Further national and local serosurveys are recommended to better inform the public health strategy for containment and mitigation of the epidemic in various parts of the country.

13. **Providing virus for vaccine development and R&D**: The live virus was provided to Cadilla health care and BBIL, Hyderabad for conducting vaccine studies. Similarly, inactivated virus was provided to CCMB, Hyderabad and ICGEB, Delhi in early phase.

    Preclinical studies to determine immunogenicity and challenge studies for vaccine efficacy in collaboration with industries.

14. **COVAXIN an indigenous vaccine development**: The isolation of SARS-CoV-2 and further genome sequencing of the virus helped in identifying the strain of the virus, which has been used to develop the inactivated whole virion vaccine COVAXIN in collaboration with Bharat Biotech International Limited (BBIL). ICMR-NIV, Pune signed an MoU and Material Transfer Agreement (MTA) with BBIL, Hyderabad for the development of whole-virus inactivated vaccine for COVID-19.
    i. At BBIL, the genetic stability and adaptability of the SARS-CoV-2 strain to grow at higher titer was evaluated using Vero CCL-81 cells. Subsequently, the virus was

inactivated and purified, and vaccine candidate formulations were prepared. Toxicology, immunogenicity, and protective efficacy were studied in mice, rats, and rabbits in collaboration with BBIL and ICMR-NIV. The results supported initiation of Phase I/II clinical trials, to assess the safety and optimal dose of the vaccine candidate.

ii. **Preclinical study in hamster model:** In parallel, the preclinical studies in Syrian hamsters at in the BSL-4 facility of ICMR-NIV, Pune were undertaken. The results demonstrated remarkable immunogenicity and protective efficacy.

iii. **Preclinical study in Rhesus macaque model:** non-human primate studies were performed in the BSL-4 facility to demonstrate the safety and efficacy of this indigenous vaccine using live SARS CoV-2. The vaccine demonstrated effective results in macaques.

iv. **Phase 1, 2 and 3 trials:** These studies established the safety and efficacy of this indigenous vaccine and are presently in phase-3 clinical trials. The development and demonstrating the efficacy of this indigenous vaccine in such a short period is one of the major achievements for the country towards self-reliant India, public health, and the war against COVID-19.

v. As a part of the regulatory guidelines, all the data were submitted to the DCGI and CDSCO. On January 2, 2021, DCGI recommended the grant of permission for restricted emergency use of COVAXIN™ after the meeting of Subject Expert Committee of CDSCO.

15. **Evaluation of Protective Efficacy of ZyCoV-D DNA Vaccine against SARS-CoV-2 Virus Challenge in Rhesus Macaques Serum**: The ZyCoV-D DNA Vaccine candidate of Cadila Healthcare Ltd., comprises of a DNA plasmid vector carrying the gene encoding the spike protein (S) of the SARS-CoV-2 virus. The S protein of the virus includes the receptor binding domain (RBD), responsible for binding to the human angiotensin converting enzyme (ACE)-2 receptor, which mediates the entry of virus inside the cell. This study was conducted to understand immunological response by evaluating level of antibody in Rhesus monkeys. These primates were immunized with the vaccine at the Zydus Primate Centre and were then subjected to SARS-CoV-2 viral challenge to assess the protective efficacy against SARS-CoV-2 infection at ICMR-NIV, Pune. The immunized animals demonstrated immunogenicity and protective efficacy of the vaccine candidate. The clinical trials demonstrated the efficacy of 66.6% against symptomatic COVID-19 and 100% against moderate or severe disease in its interim analysis. On 1 July 2021, Cadila Healthcare applied to the Drugs Controller General of India (DCGI), seeking approval for Restricted Use in Emergency Situation for the vaccine., The Subject Expert Committee of the Central

Drugs Standard Control Organization (CDSCO) recommended that the DCGI grant the approval, which the DCGI granted the approval on 20 August 2021.

16. **Comparison of the immunogenicity & protective efficacy of various SARS-CoV-2 vaccine candidates in non-human primates:** The aim of this systematic review was to compare immunogenicity and protective efficacy of various SARS-CoV-2 vaccine candidates tested in non-human primate (NHP) models. Our findings highlighted onset of quick immunogenicity and protective efficacy of mRNA-1273, followed by Ad26.CoV2. S, NVX-CoV2373, BNT162b2, RBD and BBV152 vaccine candidates in preclinical trials as compared to the others. NHP data also showed correlation with clinical trial data available for a few vaccines. Preclinical trials of COVID-19 vaccine candidates in NHPs yielded promising results, with some candidates faring better than others.

17. **Convalescent plasma in the management of moderate covid-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID Trial):** From ICMR-NIV, Pune I was involved in Clinical Trial Registry of India CTRI/2020/04/024775 of the effectiveness of using convalescent plasma to treat moderate corona virus disease 2019 (covid-19) in adults in India. 464 adults (≥18 years) admitted to hospital (screened 22 April to 14 July 2020) with confirmed moderate covid-19. 235 were assigned to convalescent plasma with best standard of care (intervention arm) and 229 to best standard of care only (control arm). Participants in the intervention arm received two doses of 200 mL convalescent plasma, transfused 24 hours apart. The presence and levels of neutralising antibodies were not measured a priority stored samples were assayed at the end of the study. Progression to severe disease or all-cause mortality at 28 days after enrolment occurred in 44 (19%) participants in the intervention arm and 41 (18%) in the control arm (risk difference 0.008 (95% confidence interval −0.062 to 0.078); risk ratio 1.04, 95% confidence interval 0.71 to 1.54). Convalescent plasma was not associated with a reduction in progression to severe covid-19 or all-cause mortality. This trial has high generalist ability and approximates convalescent plasma use in real life settings with limited laboratory capacity.

18. **Isolation and characterization of SARS-CoV-2 variants from international travelers and local community in India:** During this pandemic phase, the new genetic mutations acquired by the virus have led to new variants, indicating it to be still evolving. Numerous SARS-CoV-2 Variants of Concern (VOCs) and Variants of Interest (VOIs) i.e., Alpha, Beta, Zeta, Kappa, Delta, Delta derivatives and Omicron has been isolated from clinical specimens of international travelers and local community using cell culture or animal models The high rate of transmission of new variants has alerted the public health system

of all the countries. Dr. Yadav's lab has effectively isolated and characterized these variants in Maximum Containment Laboratory, ICMR-NIV, Pune which has helped to study the effectiveness of Covaxin and Covishield vaccines against these variants.

19. **Performance evaluation of Truenat™ Beta CoV & Truenat™ SARS-CoV-2 point-of-care assays for COVID-19:** The rapid diagnosis of coronavirus disease 2019 (COVID-19) is a significant step towards the containment of the virus. Rapid point-of-care (PoC) assays (Truenat Beta CoV and Truenat SARS-CoV-2 assays) for the diagnosis of COVID-19 have been developed which are expected to shorten the turnaround time of reporting of results and can be used for field investigations of COVID-19. The rapid PoC screening and confirmatory assays were prospectively validated under technical supervision of mine. Real-time reverse transcription-polymerase chain reaction (rRT-PCR) was considered as the reference standard against which the rapid assays were validated for all samples tested based on analytical sensitivity, precision/inter-machine variation, clinical sensitivity, and clinical specificity. Truenat Beta CoV and Truenat SARS-CoV-2 assays showed concordant results when compared with the reference standard rRT-PCR. These PoC assays exhibited 100 per cent sensitivity, specificity, positive predictive value, and negative predictive value. Truenat Beta CoV and Truenat SARS-CoV-2 assays showed concordance with the reference standard assay and may be recommended for screening and confirmation of SARS-CoV-2 in the field settings. These assays were used countrywide in many field labs for screening COVID-19 samples and helped in management of the patients.

20. **Inactivation efficacy of Truenat Swab Collection Media and Lysis buffer to use in BSL-2 setting for COVID-19 diagnosis:** Inactivation of SARS CoV-2 after treatment with Trueprep® AUTO lysis buffer was confirmed with conventional real-time RT-PCR, which was also evident by the absence of Ct value and cytopathic effect in Vero CCL-81 cells at the second passage.

21. **Efficacy of inactivation of Swab Collection Media and lysis buffer:** The experiments conducted as per reference of, concluded on virus spiked Swab Collection Media and lysis buffer showed that it can inactivate the virus / clinical samples containing viral particles with 100% efficiency as is evident as no cytopathic changes was observed in vitro cell culture and the negative results by SARS CoV-2 real-time RT-PCR.

22. **Neutralization potential of COVID-19 vaccines against SARS-CoV-2 Variants of Concern:** Ever since the emergence of first VOC Alpha and subsequent other VOCs, the public health experts have raised the alarm over the immune escape of these new variants. These variants have been a potential new threat to the currently available vaccines which effectively

evaded the immune response generated with natural infection or vaccination. Even with the aggressive vaccinations' campaigns in India, the country has observed the third wave of the pandemic. This suggests the waning immune response post vaccination or immune evasion with the Omicron variant. The susceptible immune escape of these variants was a serious concern which needed to be further explored. Considering this, she has studied the neutralization efficacy of Covishield and Covaxin against Alpha, Beta, Kappa, Delta, and Omicron variants and found to effectively neutralize these variants compared to B.1. Although, the immune response generated with Covishield and Covaxin partially rescue the protection against Beta, Delta, and Omicron, it could still effectively neutralize these VOCs.

Her contributions have helped in saving human lives and the saving economy in different outbreaks in India and set a true example for the country making self-reliance in science. I wish her best in her future endeavor. She published 165 papers in peer reviewed journals and answered many unknown facts of virus and disease, helped in strengthening the countries response and by developing vaccine made India self-resilient in this tough time. Only on COVID-19 within a year she has published total 41 papers in peer reviewed journals.

(b) Names of the industries in which the technology(ies) has (have) been used (give the production figures of products)

- ✓ After establishment of BSL-4 laboratory, efforts were made to develop and establish. serological and molecular assay for emerging and highly infectious diseases, which can be used for survey and diagnosis of KFD and CCHF virus at low cost in our country.

1. ***Indigenous vaccine for COVID-19:***

   Development of Covaxin vaccine in collaboration with Bharat Biotech International Ltd. Hyderabad

   The COVID-19 pandemic in India started with detections of the first case and its genomic characterization from Kerala state and genomic sequences from COVID-19 positive cases that till August 2020 no variant of concerns (VOCs) was present in India. We made efforts to isolate SARS-COV-2 and characterized the prototype virus best suited for a vaccine candidate. In a collaborative effort with Bharat Biotech Pvt Ltd, Hyderabad, we have developed and assessed the immunogenicity of an inactivated SARS-CoV-2 vaccine formulated with Toll-like receptor 7/8 agonist molecule adsorbed to alum (Algel-IMDG) in rodents and mice models proving a good candidate with safety and immunogenicity. A further first study in India was conducted in a containment facility to understand the immunogenicity and protective efficacy in Rhesus macaques and hamster model for this vaccine. A wonderful safety, immunogenicity, and protection were observed in animal models. The vaccine candidate BBV152 was successfully evaluated in Phase I/II/III

(NCT04471519) clinical trials in India and this study substantiates the immunogenicity and protective efficacy of BBV152. The overall efficacy against symptomatic disease is 77·8% in Phase III clinical trial. Efficacy for severe symptomatic COVID-19 was 93.4% and against asymptomatic COVID-19 is 63·6% while against the Delta Variant of Concern (B.1.617.2), BBV152 conferred 65·2% protection Drug Controller Government of India granted Emergency Use Authorization in January 2021. This Indigenous vaccine enhanced the self-resilience of the country and was being used in the mass immunization program of not only India but in many other countries. With the emergence of VOCs, a huge effort was made to isolate these VOCs and used to understand their impact on vaccinated individuals. Continues efforts were made to understand the genomic evolution in infected cases throughout the country. Further sera of vaccinated individuals, COVID-19 recovered individuals and breakthrough cases were evaluated for their effects toward neutralization of B.1, Alpha, Beta, Delta, and Delta AY.1 variants. This vaccine showed excellent protection against these VOCs. All these efforts have made India to self-resilient and saved many lives from high-risk viral pathogens.

2. ***CCHF Sheep and Goat IgG ELISA & Anti-CCHF Bovine IgG ELISA*** *[ICMR-NIV &Zydus Cadila]*
3. ***Anti KFD Human IgM antibody ELISA*** *[ICMR-NIV &Zydus Cadila]*
4. ***Anti KFD human IgG antibody ELISA*** *[ICMR-NIV &Zydus Cadila]*
5. ***Anti-CCHF human IgG antibody ELISA*** *[ICMR-NIV &Zydus Cadila]*
6. ***Anti-CCHF Human IgM ELISA [****ICMR-NIV &Zydus Cadila]*
7. ***Anti-Nipah Human IgM antibody ELISA*** *[ICMR-NIV]*
8. ***Anti-Nipah Human IgG antibody ELISA*** *[ICMR-NIV]*
9. ***Anti-Nipah Bat IgG antibody ELISA*** *[ICMR-NIV]*
10. ***Anti-Nipah Swine IgG antibody ELISA*** *[ICMR-NIV]*
11. ***Point of care Nipah virus assay*** *[ICMR-NIV & MolBIo Pvt. Ltd]*
12. ***Point of Care KFD virus detection assay*** *[ICMR-NIV & MolBIo Pvt. Ltd]*
13. ***Anti-SARS Human IgG antibody ELISA (COVID KAVACH ELISA)*** *[ICMR-NIV &7 commercial firms]*

Relevance of these technologies for public health:

- Covaxin is the first indigenous COVID-19 vaccine which has been administered to millions of people in India. This has helped to curb the spread of SARS-CoV-2.
- CCHF is a Biosafety level 4 agent, which causes high mortality in humans. Currently there is no diagnostic ELISA kit available commercially for detection of anti-CCHF IgG antibodies in domestic animals. Workers involved with livestock sector who are in close proximity to

animals are at high risk [that includes a larger population from rural areas, since these animals are in close proximity with the human and get infected through the infected tick bites].

- The ELISAs can detect anti-CCHF IgG antibodies from sheep and goat samples and a separate kit has been developed to detect the IgG antibodies from Cattle samples. This information will be helpful to take up preventive measure in affected area. This kit can be used for the sero-survey studies to understand the prevalence of this disease in any areas to understand the exposure of risk or likely possibility of any infection happening to human population by CCHF virus. The kit developed revealed anti CCHF antibodies among livestock in 22 states and 1 union territory of India, indicating that this virus is widespread in this country. Anti-CCHF IgM and IgG ELISA assays helped in detecting human cases in Rajasthan and Gujarat State.
- KFDV was first recognized in 1956 in Shimoga, Karnataka state and now present in six states of country. KFD virus is a high-risk group of pathogens (BSL-4) for other countries and a risk group-3 pathogen for India. Currently there is no diagnostic ELISA kit available for detection of anti-KFD IgM antibodies. This is the first anti-KFD IgM antibody detection ELISA, it is cost effective for Indian setting. The kit is developed indigenously for diagnosis of human samples during acute phase of illness [4-5th POD onwards].
- Development of kit for diagnosis of KFD has eased task of investigating KFD outbreaks and helped in timely diagnosis of sporadic cases. Large number of KFD samples referred from various outbreak area are currently being screened using the indigenously developed anti KFD human IgM detection kit.
- Nipah tests (ELISA and Point of Care) were used in different outbreaks and early detection helped in containment and saving lives.
- COVID KAVACH ELISA was used for vaccine studies, screening of the human samples during pandemic.

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Place: Pune

Dr. Pragya D. Yadav