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Shyama Prasad Raychaudhuri Oration Award

Impact of Viral Diseases on Global Aquaculture Production

Iddy Karunasagar

Nitte University, Mangalore, 575018

Aquaculture contributes to over half of global fish production. Since last three decades, global fish production by capture fisheries has been stagnating and the increase in fish production is coming only from aquaculture. But aquaculture sector has been facing serious problems due to diseases, particularly viral diseases. Today, there are number of vaccines available for many of the bacterial diseases affecting aquaculture, but there are very few vaccines for viral diseases. Shrimp account for the most international commodity and being invertebrates, crustaceans depend mostly on innate immune response. According to FAO data, 52.9% of global shrimp aquaculture production is contributed by one single species, Penaeus vannamei, which is also called Pacific white shrimp. Though this is an exotic species for Asian countries, they have shifted from the native species, Penaeus monodon, also called black tiger shrimp to P. vannamei. In the 1990’s, P. monodon used to be the dominant species cultured in Asia. But diseases caused by viruses like the white spot syndrome virus (WSSV) caused the shrimp aquaculture industry to almost collapse. This led to development of Specific Pathogen Free (SPF) broodstock of P. vannamei and this was the driving factor for shift in production from P. monodon to P. vannamei. But availability of SPF seed has not solved the disease problem in shrimp aquaculture industry. This is because WSSV has a very broad host range and can survive in a number of wild crustacean species and therefore present in the environment. My laboratory had the opportunity of working on the whole range of viral diseases affecting shrimp starting from early days of aquaculture in 1990’s and in this lecture, I will present our journey through this very complex issue that is impacting aquaculture.

Kameshwar Sahai Bhargava Oration Award

Experience in working with viral Hepatitis-E: A neglected Virus

R. K. Ratho

Dept of Virology, PGIMER, Chandigarh

Hepatitis E is an important public health concern and an estimated one-third of the world population has been infected with Hepatitis E virus (HEV). It is endemic in India and considered as an emerging disease in industrialized countries. HEV infection is usually an acute self-limiting disease, but recent reports have shown chronicity in solid organ transplant patients with extra-hepatic manifestations. Viral hepatitis leading to acute liver failure and high mortality (20-30%) is documented in pregnant women in developing countries including Indian subcontinents. Traditionally HEV Genotype 1 & 2 spread through feco-oral route whereas Genotype 3 & 4 is transmitted through zoonotic spread. Recently transmission through vertical, blood transfusion (Khuroo et al.), solid organ transplant (Kamar et al., 2008) and person to person contact have been reported. The observation by Debing et al. of ribavirin (RBV) resistant HEV isolates have added upon the existing problem. Hepatitis E has a mortality rate of 0.2–1% in the general population. Increased morbidity and mortality is observed in chronic liver disease patients super-infected with HEV (Hamid et al. 2002). In recent years, several cases of chronic HEV infection in transplant patients have been reported and related to genotype 3. HEV have also been associated with several non-hepatic manifestations i.e. acute pancreatitis, neurological syndrome and hematological manifestation (thrombocytopenia). The HEV related disease pathogenesis is still not fully understood. The pathogenesis of viral hepatitis due to Hepatitis E virus is complex in nature indicating an interplay of viral (genotype, inoculum dose) and host factors (stage of liver disease, pregnancy, distinct genetic polymorphisms). Studies with viral load estimation in Hepatitis E patients have pointed towards immune mediated as one of the modality of disease progression to ALF. The innate immune system plays an important role in protection against viral infection. One such important aspect is the recognition of the viral agent by Toll like receptors (TLRs) more so with significant TLR3 up-regulation in patients with acute viral Hepatitis (AVH) as compared to those with acute liver failure (Majumdar et al., 2015). Studies reveal the pathogenetic processes of ALF looking into TLR 3 and High mobility group box 1 protein (HMGB1). HMGB1 level estimation in serum have shown as a bad prognostic marker of ALF patients. TLR3 and IFN-γ were found to play an important role in HEV disease pathogenesis. Patients capable of expressing high levels of TLR 3 and robust IFN-γ response are able to limit the disease and recover uneventfully; possibly restrict in progressing to ALF. Alteration in NK cells phenotype and function in acute hepatitis E (Srivastava et al.) and HEV ORF3 as viroporins provide insight to the newer mechanism of pathogenesis. Up-regulated NLRP3
Pratip Shil

Climate Influence on Dengue in the Pune Urban Zone, India: Developing Early Warning Systems

Pratip Shil

1Scientist E, ICMR—National Institute of Virology

Introduction: Climate change is a reality. Evidence of changing SW Monsoon patterns over India. Rapid urbanization & changing human life style, India is facing an ever-increasing burden of Dengue. The geographic and climatic diversity of India necessitates regional level studies.

Objective: Develop mathematical models that explain the relationship between meteorological factors and disease, that can be considered as Early Warning System.

Methods: Procurement and analyses of meteorological data. Procurement and processing of epidemiological data. Development of time-series forecast models.

Results: significant in terms of Vector biology. Similar trends observed in Tropical countries. Aedes abundance, survival & vector competence to Dengue.

Conclusion: Our model provided fairly efficient forecast. This can be used as ahead-of-season projection (Early warning system).

Impact: Ahead-of-season projection will help Public Health authorities to plan control measures.

Telmisartan restricts Chikungunya virus infection in vitro and in vivo through the AT1/PPAR-γ/ MAPKs pathways

Saikat De

CSIR-Senior Research Fellow, Pt. Dr. Soma Chattopadhyay, Scientist-F, Molecular Virology Lab, Institute of Life Sciences (ILS), Bhubaneswar, Odisha, India

Introduction: Drug repurposing is a strategy to find new clinical applications of the existing drugs with minimum cost, time and risk. In silico analysis showed affinity of Telmisartan (TM) towards CHIKV proteins. Ang-II inhibitors clinically correlated to Influenza, Dengue virus (DENV), Coxsackie virus, Ebola virus, Western Equine Encephalitis virus and Sindbis virus. CHIKV also affects CNS and AT1 blockers such as TM, that can cross the blood-brain barrier. CHIKV-induced inflammation is one of the major causes CHIKV pathogenesis. TM PPAR-γ agonist and AT1 antagonist Angiotensin II (Ang II) mediated activation of AT1 receptor in the Renin-Angiotensin System (RAS) is a primary mediator of oxidative stress and inflammation. Peroxisome proliferator-activated receptors gamma (PPARγ) play important roles in antagonizing core inflammatory pathways.

Result: TM inhibits CHIKV infection efficiently. TM reduces the viral RNA and protein levels, TM interferes in the early and late inflammatory pathways.

Conclusion: Telmisartan restricts Chikungunya virus infection in vitro and in vivo through the AT1/PPAR-γ/MAPKs pathways.
and interfere with measures taken to control the spread of the virus. Until now, there is inadequate information about the characteristics of the asymptomatic and symptomatic patients. The association between SARS-CoV-2 viral load, cytokines and risk of disease progression remains mostly vague in COVID-19 in Indian scenario. All SARS CoV-2 positive patients were admitted to a selected hospital in Bhubaneswar, India. All patients were confirmed to be infected with SARS-CoV-2 by RT-PCR. Level of cytokines and circulating antibodies in plasma were assessed by Bioplex and isotyping respectively. One virus was isolated from symptomatic patient and whole genome sequencing was performed with four SARS CoV-2 sequences. Patients or their families were directly contacted to discover accurate patient details such as symptoms. Six asymptomatic and six symptomatic patients were admitted to hospital in Odisha, India. The viral load was higher in plasma and serum samples of symptomatic patients than asymptomatic cases. Three symptomatic patients were deceased. The patients having low Ct values in plasma samples had developed sufficient amount of antibody units. Virus has been isolated from the OP swabs of symptomatic patient with a very high viral load > 10^6 copies per mL. Level of 7 cytokines (IL-6, IL-1α, IP-10, IL-8, IL-10, IFN-α2, IL-15) were highly elevated in symptomatic patients whereas 3 cytokines (soluble CD40L, GRO and MDC) level were higher in asymptomatic patients. IgE, IgG2, IgG3 and IgG1 were significantly higher in symptomatic patients than in asymptomatic patients. Whole genome analysis showed that among four, two (139 and 164) of the strains belonged to 20A clade whereas the rest two (132), (165) belonged to 19B and 20B clade respectively with multiple mutations in Orf1ab, spike, Orf3a, Orf8 and nucleocapsid genes. These data suggests that higher viral load in plasma leads to generation of more antibodies. Cytokine storm and higher levels of CRP and ferritin are linked with symptomatic disease progression. Therefore, its role in disease pathogenesis should be further explored.

Integrated Proteomic and Metabolomic Reveal Dysregulation of Energy Pathways by Mutant Hepatitis E Virus In-Vitro

Shaheen Khan1, Yashwant Kumar2, Naga Suresh Veerapu1

1Virology Section, Department of Life Science, Shiv Nadar University, Dadri, India-201314; 2THSTI, 3rd Milestone Faridabad, Haryana, India – 121001

Hepatitis E virus (HEV) circulates as an ensemble of closely related but genetically distinct population of viruses in host organism is collectively termed as quasi species. We developed a method that couples an error-prone PCR (ep-PCR) and RNA synthesize to HEV populations1, which showed transient replication in cell culture (Agarwal et al., 2017). In this study, we took advantage of this approach to synthesize quasispecies like HEV populations. Here we attempted to study proteome and metabolome changes and integrated the data to understand differential interaction of mutant HEV and clonal HEV with host. Cells transfected with mutant HEVRNA showed increased glucose consumption compared to mock and clonal HEV over a period of 72 h. In the presence of cytochalasin B, a lGAT1 mediated glucose transport inhibitor, the glucose consumption by the transfected cells decreased significantly, which lead to tenfold decrease in virus titer at 48 h.p.t. Next we performed proteomic and metabolomics analysis on mock, clonal and mutant HEV-transfected cells. Our proteomic data suggested that mutant HEV cause enhanced expression of interferon stimulating genes (ISGs), such as IFIT1, DDX58, ISG15, MX and STAT3 at > 2.0-fold compared to clonal HEV, pointing that HEV-population is able to establish replication in cells as these ISG genes are widely used as surrogate marker for the viral infection. The expression of IFIT1 was also validated using western blot with HCV JFH1 strain as a positive marker for viral infection. In addition to ISGs, > twofold increase in Cytochrome C (CYC), which represents a biomarker for the activation of mitochondrial apoptotic pathway in cells was observed in cells carrying mutant HEV. Proteomic data suggested that HEV-ML perturb energy pathways, including glycolysis by altering the expression of glycolytic enzymes such as PGAM1, ENO1 and HK2, which is the first-rate limiting enzyme in the glycolysis to meet the high energy demands by the replicating virus. In addition to proteomics, metabolomics was also performed on the same cells, which also corroborated with the proteomic data and showed upregulation of metabolites involved in glycolysis, TCA cycle, fatty acid elongation and amino acid metabolism such as aspartate, succinate, acetyl CoA Malonyl CoA etc. Our study found novel association between host and virus factors and target relevant biomarkers and build elaborate markers of hepatitis E disease pathophysiology.

Potential therapeutics candidate targeting oxidative stress induced mechanism against Japanese encephalitis virus infection

Anjali Gupta1, Sinthiya Gawandi2, Vandana1, Inderjeet Yadav4, Hari Mohan3, Vidya G Desai1*, Sachin Kumar1*

Japanese encephalitis virus (JEV) is the leading causative agent of encephalitis and associated mortality among children. It modulates host cell machinery to its advantage, thereby increasing reactive oxygen species (ROS) and causing oxidative damage. In this study, we have analyzed new series of dinitroaryl substituted derivatives (1a-1f), containing pyrazole moiety and explored its potential anti-JEV activity. Out of six synthesized compounds, only two compounds 1b and 1f were selected based on minimal cytotoxicity (IC50 value). In Neuro2a cells, 1b and 1f resulted in more than 70% and 90% reduction of JEV at mRNA level respectively. In animal study, mice treated with compound 1b or 1f intraperitoneally, resulted in up to 41% and 70% reduction of JEV mRNA in spleen and 33% to 43% in brain tissue without any noticeable toxicity at a dose of 100 mg/kg/day till 96th hr. Both the compounds significantly upregulated the SQSTM1 protein involved in activating the NRF2-KEAP1 anti-oxidative pathway compared to the untreated control, 1b and 1f suppressed ROS up to 27% and 32% respectively by targeting endogenous anti-oxidative enzymes, thereby preventing it from being modulated by viral machinery. Hence, these compounds may manifest broad-spectrum antiviral effect against other flaviviruses in path towards the development of therapeutics.

Role of interleukin 6, c-reactive protein and ferritin levels in early prediction of dengue severity

Dr. Prasanna Gonti1, Dr. Rajeshwar rao2, Dr Nagamani K3

Introduction: Global prevalence of dengue is increasing with nearly 2.5 billion people at risk and 50 million new infections per year. India alone had 41 cases per million population in 2012 when the world had 10 cases per million population. The clinical presentation varies from mild/moderate dengue fever to severe dengue with significant mortality rate of around 5 to 30%. The main pathophysisology behind Severe dengue is considered to be the cytokine storm and release of abundant acute phase reactants. As most of the deaths are in the severe dengue patients there is Need for tests to predict Severe Dengue early in acute phase for the ease of monitoring high risk cases so as to decrease mortality. The present study was conducted with an aim to evaluate the role of INTERLEUKIN-6, FERRITIN, CRP levels in early prediction of dengue severity and also to study the clinical and epidemiological profile of dengue patients. Conclusion: Therefore, it is recommended that patients with levels of IL6 > 99 pg/mlCRP > 12 mg/L, and Ferritin > 810 ng/ml, are to be monitored.
Modulation of Autophagy and mTOR signalling pathway by Respiratory Syncytial Virus (RSV) in children suffering from acute lower respiratory infections

Sarjana Shuchi1, R K Ratho1,*, Gursimran Kaur Mohi1, Suresh Kumar2, Subhabrata Sarkar2, Isheta Jangra1

Introduction: Respiratory Syncytial Virus (RSV) is the most important cause of acute lower respiratory infections (ALRI) in children less than 5 years of age. The virus exists in nature as subtypes A and B which further have multiple genotypes. Autophagy and mTOR signalling pathways are present in host cell which either help in viral propagation or inhibit its growth.

Aims and objectives: To study the molecular phylogenesis of the circulating strains of RSV and to elucidate the gene expression of autophagy and mTOR signalling pathways in children with acute lower respiratory infection.

Method: Total of 145 nasopharyngeal aspirate (NPA) samples from children suffering from acute lower respiratory infection between 2 months to 1 year of age were subjected for the detection of RSV by conventional PCR. Representative RSV positive strains were subjected for sequencing. The NPA samples were centrifuged. The cellular RNA was extracted from the pellet of 25 RSV positive cases and 10 control subjects using TRIZOL reagent and were reverse transcribed for the semi-quantitative gene expression of mTOR signalling pathway and autophagy pathway genes. BECN1, NPC1, GABA RAP, ATG3 and ATG5 genes of autophagy and PDPK1, AKT1, mTOR, RICTOR, and TSC1 genes of mTOR signalling pathway were subjected for SYBR green real-time PCR.

Isolation, purification & characterisation of Bacteriophages specific to mycobacterial spp.

Pulkit Singh, Ritu Arora, Amit Singh, Sripad A. Patil, Urmi Bajpai

Prevalence of Drug Resistant Tuberculosis in India: India had an estimated 130,000 drug resistant TB cases in 2018. Prevalence of MDR was 3.5%, among new and 26.7% among previously treated TB patients in India. The gross number of TB cases continued to increase during the pre-pandemic era. Characterization of Bacteriophages High titre phage preparation and purification Isolation of phage genomic DNA using the Phenol-chloroform-isooamyl alcohol (PCI) method. Determination of methylation status of phage genome TEM analysis of selected phages to determine their capsid and tail dimensions. Cluster classification of mycobacteriophages based on a conserved region in Tape Measure Protein in phages.

Findings: Isolation & purification of mycobacteriophages: 20Plaque morphologies observed: clear, turbid, bulleye. Plaque size range: 1.2-5.75 mm Cluster classification using primers specific to cluster A (A1, A2, A3); ii) cluster B (B1) and cluster G: K- B1 cluster phages, 1 G phage; for the remaining cluster not assigned yet. A Mtb-infesting phage (W2) discovered in this study. Mtb phages are infrequently found according to the virus-host database.

Future experiments: Functional annotation of whole genome of W2 mycobacteriophage and identification of genes encoding endolysins. Cloning, expression and purification of lysins as recombinant proteins. Anti-mycobacterial assessment of purified lysins. One-step growth curve and pH/temperature stability of W2.
Adeno associated virus-2 (AAV-2) vector mediated expression of SARS-CoV-2 proteins to study virus induced immunomodulation and host immune response

Rutumbara Dash, Neeti Nadda, Sonu Kumar, Anzar Ashraf, Anoop Saraya, Shalimar, Baibaswata Nayak*

COVID-19 is currently a global pandemic caused by a novel coronavirus (SARS-CoV-2) and accounts for major cause of morbidity and mortality. Seemingly finding have shown that SARS-CoV-2 can evade the host immune system by altering the host transcriptional programme. Immuno-modulation by viral proteins depended upon their mechanisms of action such as enzymatic activity (methyl transferase, proteolytic, De-ubiquitinating, RNAs/DNAse activity); interaction with host protein, DNA/RNA and membrane modifications. Objective: Cloning of SARS-CoV2 gene in AAV-2 vector and helper free production of Adeno associated virus -2 expressing SARS-CoV2 protein for studying immunomodulation. Methodology: Synthetic constructs in cloned plasmid vector were obtained from Addgene as per MTA. This includes The plasmids SARS-CoV-2 NSP1,NSP3, NSP5, ORF3A,ORF3B, E, M, ORF6 and Spike -RBD region. Primer Designing, PCR amplification and Subcloning of SARS-CoV2 genes in the pAAV-CMV Vector: We have designed primers flanking start and stop codon with RE sites for subcloning in pAAV-CMV vector. The PCR product was subcloned in MCS site (Xbal/SpeI) within AAV-2 ITR. The GOI size was limited to <2.5 kb which is size limit for encapsulation in AAV particles. Results: PCR amplification of reporter and SARS-CoV2 genes. Subcloning in pAAV-vector. Result: AAV-2 rescue by co-transfection experiment, Co-Transfection and reporter gene expression, AAV-2 rescue expressing gene of interest and virus titration by Realtime PCR. Conclusion: We have successfully recovered AAV-2 virus expressing SARS-CoV2 protein, we are exploring the interplay between SARS-CoV2 structural and non structural proteome and host immune innate signaling with respect to the immune response.

RAS at Y93H in NS5A region: a main cause of drug resistance in Hepatitis C Virus

Sangeetha K, Mini P Singh*, Poonam Kanta, Kapil Goyal, Nisha Rani, Shashank Singh, Ajay Duseja, Thungapathra M, RK Rathe

Introduction: Global burden of Hepatitis C virus—58 million world population and about 1.5 million new people are infected each year. HCV has now become eradicable disease according to WHO, due the presence of direct acting antivirals (DAAs). Sustained Virologic response (SVR) increased to 95% and more. In spite of the high SVR rates, there are a small group of patients who fail the treatment even with current regimens of DAAs.

Objectives: To screen the HCV genotype 1 and 3 patients for the presence of RAS in the NS5A region. To carry out in silico modeling, molecular docking and molecular dynamics analysis of NS5A region with NS5A inhibitors in order to understand the interaction patterns.

Results: Overall: Genotype 3 (71.3%) more prevalent than genotype 1(28.7%) Chronic Kidney Disease (CKD) patients: Genotype 1 more prevalent (77.1%) than genotype 3 (22.9%). Conclusion: RAS Y93H play a pivotal role in predicting the response to treatment in the HCV infected individual. Hence, Y93H RAS should be monitored and optimal treatment should be given to prevent relapse. Also, AASLD guidelines showing the requirement of RAS testing—NVHC could include this in the Indian policy.

A natural broad-spectrum inhibitor of enveloped virus entry, effective against Sars-CoV-2 and Influenza A virus in preclinical animal models

Rohan Narayan, Mansi Sharma, Rajesh Yadav, Abhiijith Biji, Oyahida Khatun, Raju Rajmani, Pallavi Raj Sharma, Sharumathi Jeyasankar, Priya Rani, C. Durga Rao, Vijaya, Sutebidandanandam, Saumitra Das, Rachit Agarwal, Shashank Tripathi

The ongoing SARS CoV-2 COVID-19 pandemic has caused over 472 million infections and > 6 million fatalities globally. Influenza A Virus causes yearly seasonal outbreaks, epidemics, and occasionally even global pandemics. This calls for an urgent need for effective antivirals. Viruses tested in this study- SARS-CoV-2, AAV-6, IAV, Ad5, ZIKV, JEV, DENV, CVB3, HPIV-3, RV, HSV-1. PA exhibits broad-spectrum activity against enveloped viruses PA does not inhibit non-enveloped viruses PA targets viral membrane and inhibits viral-cellular membrane fusion PA restricts iav and sars-cov-2 in preclinical animal models. PA inhibits enveloped virus entry by compromising viral membrane integrity and inhibiting virus-cellular membrane fusion. PA showed promising antiviral efficacy against SARS-CoV-2 and IAV, especially as an oral prophylactic. Overall, our data establish PA as a broad-spectrum antiviral, with promising preclinical efficacy against pandemic viruses SARS-CoV-2 and IAV.

Poster Presentations

On the explanatory role of Human Feces in the Transmission of COVID-19: Evidences from India

Mallika Lavania1, V Potdar 2, M Joshi1, SM Jadhav3, M Shinde1, N Chavan1, Atanu Basu 4, Sharda Prasad4

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. SARS-CoV-2 infected cases diagnosis is based on the count of real-time reverse transcription-polymerase chain reaction (RT-PCR). In this study, we aimed to investigate SARS-CoV-2 presence and shedding in the stool samples of COVID-19 patients. We hundred and eighty four fecal specimens were collected from four different hospitals in Pune and detected the nucleic acid of SARS-CoV-2 using real-time RT-PCR. Among them, 15.4% had gastrointestinal symptoms. Whole genome sequencing (WGS) of SARS-CoV-2 from 55 stool samples of patients was performed to analyze mutational variations. Along with this TEM analysis was done on ten stool specimens from SARS-CoV-2 positive patients. In all, 173 of 280 (61.7%) stool samples were positive by Real time RT-PCR for SARS-CoV-2. We observed the shedding of virus until 55 days in a small cohort of 35 patients. WGS analysis showed the variants Alpha and Beta were highly prevalent in the first wave of COVID 19 infection while in the second wave, the infection by B.1.617.2 (Delta) and B.1.617.3 responsible for spreading the infection. TEM imaging of clarified and negatively stained specimens from SARS-CoV-2 infected individuals, showed the presence of distinct Coronavirus particles in two specimens. In the context of the COVID-19 pandemic, environmental surveillance for the detection of SARS-CoV-2 has become increasingly important. Findings from this study not only highlight the presence of SARS-CoV-2 in feces, but also led to speculation that it could be transmit via the fecal-oral route.
Investigation of Dengue outbreak among nursing students of the tertiary care hospital, North India

Pryanka Thakur1, Radha Kanta Ratho1, Rishi Chetanya1, Ishani Bora1, Gursimran Kaur Moli1, Karobi Das2

Department of Virology1, and National Institute of Nursing Education2, Postgraduate Institute of Medical Education and Research (PGIMER), Sec-12, Pin-160012, Chandigarh, India

*Corresponding Author: Radha Kanta Ratho: rathopgi@yahoo.com

Dengue virus (DENV) is an important mosquito borne arbovirus belonging to the family Flaviviridae. It has spread to most of the tropical and subtropical areas creating significant disease burden and economic loss in south East-Asia including India sub-continent. In 2021, India reported 123,106 dengue cases with 90 deaths (nvbdcp.gov.in), with North-India being endemic to dengue infection. In October 2021, we investigated a dengue outbreak within campus among nursing students of the tertiary care hospital. Twenty individuals with acute febrile illness i.e. fever ≤ 7 days of onset from the nursing hostel of PGIMER, were enrolled during the winter months of 2021 in the study. The blood samples were collected after obtaining written informed consent and were subjected for dengue NS1 antigen and dengue IgM by NS1 antigen ELISA and IgM-µ captured ELISA kits respectively. Samples positive for NS1 antigen were further subjected for the presence of viral RNA by nested reverse transcriptase polymerase chain reaction (RT-PCR). Serotyping was performed by multiplex PCR to identify the circulating serotypes. Among the twenty individuals presented with AFL, only 9 (45%) were positive for dengue NS1 antigen by ELISA. Of them, dengue RNA by RT-PCR was positive in 6 patients. Additional two individuals were positive for dengue IgM. Mean Age of patients was 22.45 ± 6.54 years. Among the 11 dengue positive patients, fever was the presenting symptom in 7 (63.6%), abdominal pain in 2 (18.1%), vomiting in 3 (27.2%), chills in 4 (36.3%) and joint pain in 7 (63.6%), with mean duration of fever as 3.18 ± 1.0 days. The platelet counts for all the 11 cases were within normal range. The total leucocyte count (TLC) in 4 patients were below reference range. The total leucocyte count (TLC) in 4 patients were below reference range. Further study of dengue virus as an aetiology for current outbreak with dengue serotype 2 circulating among the nursing students. Effective vaccine development would remain the strategic measure towards dengue control.

Serore prevalence of Hepatitis B virus and Hepatitis C virus co-infection among human immunodeficiency virus-infected patients: A cross-sectional observational study.

Dr. Mahesh Babu M1, Dr. K. Vishnuvardhana Rao2, Dr. M.S.S. Pradeep3

Introduction: Co-infection with hepatitis B & hepatitis C viruses in HIV positive patients is a most challenging health concern as these may lead to non-AIDS related mortality and morbidity conditions i.e. cirrhosis or hepatocellular carcinoma. We undertook the study to know the seroprevalence of HBV & HCV co-infection in HIV positive individuals.

Materials & Methods: A total of 103 HIV seropositive patients who were included in the study tested positive at ICTC as per NACO guidelines using 3 rapid test kits and they were further screened for the presence of HBV(HBsAg) & HCV(IgG) using rapid diagnostic kits.

Results: Out of 103 HIV seropositive patients tested, 62 were males & 41 females. In these 4.85% were HBsAg positive & 1.94% were HCV positive and none of the samples were positive for both HBV & HCV. Most of the patients who were positive for HIV, HBV or HCV belong to the age group of 30-39 years.

Conclusion: As co-infection with HBV & HCV in HIV positive individuals is related to early mortality with liver damage, routine screening of HBV & HCV in HIV infected persons along with health education & counselling for behavioural change is advised.

Detection Of SARS-CoV-2 IgG Antibody in Individuals Following Infection and Vaccination

Vedpathak Manoj1, Shinde Deepak1, Agarwal Sonal2, Bagewadi Prathamsh3

1Assistant professor, Dept. of Microbiology, Dr. V. M. GMC, Solapur
2Research Scientist (Medical), VRDL, Dr. V. M. GMC, Solapur
3Research Scientist (Non-Medical), VRDL, Dr. V. M. GMC, Solapur

Introduction: To contain the SARS-CoV-2 infection, number of measures including vaccination had taken to reduce virus transmission and mortality.1 Protection from viral infection is mainly achieved by virus neutralizing antibodies which are produced after infection or vaccination.2 But to what degree these can induces the production of neutralizing antibodies is poorly understood. Hence this study aims to determine development of SARS-CoV-2 IgG antibody against spike protein after infection and vaccination.

Materials and Method: A prospective study was conducted from July 2021 to December 2021 at tertiary care hospital, Solapur (Maharashtra, India) and approved by Institutional Ethical Committee: A total of 150 participants above 18 years of age were enrolled and assigned to 3 groups based on evidence of previous SARS-CoV-2 infection and status of vaccination as follows: Group A (n = 50) includes unvaccinated people who had SARS-CoV-2 infection 21 days before collection of sample; Group B (n = 50) includes those who had only first dose of Covid19 vaccine 21 days before collection of sample but not infected with SARS-CoV-2 till date; Group C (n = 50) includes those who had second dose of Covid-19 vaccine 21 days before collection of sample but not infected with SARS-CoV-2 till date; Those who had SARS-CoV-2 infection after vaccination and vice versa were not considered for this study to discriminate whether the antibodies were due to the natural infection or vaccination; Demographic data including age and sex were collected for statistical analysis; Written informed consent was obtained from all participants. From each participant 3 cc of blood was collected in vacuum container by phlebotomy under all aseptic precautions, then serum was separated from each participant 3 cc of blood was collected in vacuum container by phlebotomy under all aseptic precautions, then serum was separated after centrifugation and stored in screw cap cryovial at -80°C until tested by enzyme linked immunosorbsent assay (ELISA); Enzyme linked immunosorbsent assay (ELISA) were performed using ErbaLisa COVID-19 IgG kit having the sensitivity of 98.3% and the specificity of 98.1% which detects IgG present in the human serum against spike protein. Test was performed as per manufacturer’s instructions.

Results and Discussion: To access the development of antibodies, serological tests are useful that can also estimate the extent of immunity in the community. 3,4; this study shows, overall ~ 89.33% of people from community had developed immunity against SARS-CoV-2; In this study 88% participants from Group A, 84% from Group B and 96% from Group C were positive for SARS-CoV-2 IgG antibodies; Similarly, Lustig Y. et. al. (2021) 5 showed that 85-90% of infected individuals had antibodies while neutralizing and IgG antibodies in 96.5% and 99.9% of vaccinated participants; Instead of having detectable SARS-CoV-2 IgG antibodies in significant number of participants from all groups, there was no statistically significant difference noted. So, why to get vaccinated even after having antibodies from natural infection; The vaccine mounts a significantly higher immune response in infected as well as uninfected participants, but the level of antibodies were enormous in previously exposed people. 5,6; This unique properties of the vaccine responses may be
Molecular Characterisation of Human rhinovirus strains isolated from children with Acute Respiratory Tract Infections

Ishani Bora

Departments of Virology* and Paediatrics2, Post Graduate Institute of Medical Education and Research (PGIMER), Sec-12, Pin-160012, Chandigarh, India; Corresponding Author: Ishani Bora: ishambora16@gmail.com

Acute respiratory tract infections (ARTI) are one of the leading causes of morbidity and mortality amongst infants and children under 5 years of age contributing to 18% of all deaths, followed by diarrheal illnesses (15%) and malaria (11%). Amongst the various pathogens associated with ARTI in children, viruses contribute between 50-90%. Apart from the viruses—Influenza virus and human respiratory syncytial virus, human rhinovirus (HRV) also causes ARTI. Though mostly known for causing common cold, HRV is also known for causing lower respiratory tract infection (LRTI). Nasopharyngeal swab specimen of 154 paediatric patients of age group ≤5 years presented with ARTI were screened for HRV by Real Time PCR. Representative HRV positive samples were sequenced and phylogenetic analysis were done by aligning with global representative sequences. Out of 154 samples, fifty three (34.41%) were found to be HRV positive by real time PCR. Children in the sub-group of >1 month to 1 year age, who are positive for HRV was associated with the risk of SARI (OR, 95%, CI: 0.14, 0.04-0.47). Thirteen representative samples were sequenced. 7 were HRV-C, 5 were HRV-A and 1 was HRV-B. For phylogenetic analysis, they were aligned with forty-one global representative strains and evolutionary distances were computed using the Maximum Composite Likelihood method. The more common strains was HRV-C followed by HRV-A in the cohort study. Further surveillance and sequencing of more HRV strains round the year is required to understand any seasonal predominance. Whole-genome sequencing of HRV strains might help in providing further clarity and knowledge into the differences observed in clinical symptoms as well as outcomes.

Development of anti-SARS-CoV-2 mouse monoclonal antibodies of diagnostic therapeutic potential

Gururaj Rao Deshpande, Shankar Vidhate, Kirtee khutwad, Rashmi Gunjikar, Pragya Yadav, Gajanana Sapkal*

Presenting Author-Dr. Gururaj Rao Deshpande

Control and containment strategies of SARS-CoV-2 pandemic included; rapid development of efficient molecular, serological diagnostic reagents and preventive as well as therapeutic agents globally. The receptor-binding domain (RBD) of spike protein induces diverse neutralizing antibodies and nucleocapsid protein is critical for viral replication and viral assembly of SARS-CoV-2 and therefore both are key targets for immune protection and diagnosis. Hence, we developed and characterized monoclonal antibodies against RBD (spike protein) and N proteins of SARS-CoV-2. The recombinant RBD, N proteins and whole inactivated SARS CoV-2 virus were used to immunize the mice in different groups. A panel of hybridoma were generated and characterized in ELISA, immunofluorescence assays and Western blots. Additionally, neutralization potential of mAbs were determined by SARS CoV-2 live virus neutralizing assays. Among the 35 hybridomas generated, four were found to specific to SARB domain and seven were reactive exclusively to nucleocapsid protein of SARS CoV-2 virus. SRBD specific mAbs were found to be neutralizing B.1, Delta and Omicron variants of concern with different potential.

In conclusion, the panels of mAbs showed significant and specific reactivity against SARS CoV-2 proteins. Therefore these mAbs can be useful in increasing specificity and sensitivity of serodiagnostics assays. Additionally, the neutralizing potential of these mAbs indicating considerable value in efficient therapeutics applications.

Utility of CDC DENV1-4 Real Time PCR assay and Trioplex RT PCR assay for the diagnosis of Dengue in patients with acute febrile illness

Subhabrata Sarkar1, Parakriti Gupta2, Gajanan Sapkal3, Ishani Bora1, Shveta Shethi2, Kanavalpreat Kaur4, RK Ratho1*

Introduction: Dengue is one of the most common viral infections which can be diagnosed by molecular assays during the acute phase of infections. For molecular diagnosis, conventional RT-PCR using hemi-nested PCR by Lanciotti et al. is followed worldwide. CDC DENV-1–4 Real-Time RT-PCR assay and CDC triplex PCR assay are the other recent alternative molecular diagnostic modalities based on the one step Real Time PCR platform.

Methodology: The study evaluated performance of CDC DENV-1–4 Real-Time Assay, Trioplex RT-PCR and heminested conventional RT-PCR assay in the diagnosis of DENV in acute phase of illness and determined the clinical sensitivity, specificity, accuracy in relation with composite reference standard as well as inter-assay comparison.

Results and discussion: Among the 107 study samples, 88 samples were reference positive i.e. positive by one or more serological assays: a) Only NS1 antigen (Ag) ELISA positive (n = 80) b) both NS1 Ag ELISA and dengue IgM ELISA positive (n = 5) c) dengue IgM ELISA positive and NS1 Ag negative (n = 3). Samples negative for both NS1 Ag ELISA and Dengue IgM ELISA (n = 19) were taken as composite reference test negative. Area under the curve of ROC for CDC DENV-1–4 RT-PCR Assay, Trioplex RT-PCR assay and conventional multiplex RT-PCR were 0.841, 0.773 and 0.693 respectively. Moderate agreement was observed among CDC DENV-1–4 rRT-PCR assay and conventional multiplex RT-PCR (kappa, 0.49), with 73.83% concordance. Trioplex RT-PCR assay and conventional multiplex RT-PCR were 81.3% concordant and displayed substantial agreement (kappa, 0.61). Maximum concordance values of 86.9% were recorded among CDC DENV-1–4 rRT-PCR assay and Trioplex assay with kappa value of 0.74, suggestive of substantial agreement. All the three molecular assays had 100% specificity. CDC DENV-1–4 rRT-PCR assay was noted to be the most sensitive assay (68.18%) [p < 0.001]. The sensitivity of 54.55% was documented in case of CDC Trioplex PCR (48/88) [p < 0.05] and the least was noted for conventional multiplex RT-PCR (38.64%, (34/88)). CDC DENV-1–4 rRT-PCR assay exhibited highest sensitivity of 82.9% and concordance of 84.4% for samples collected within first 3 days of illness, in comparison with composite reference test, followed by CDC Trioplex RT-PCR assay, which had sensitivity of 73.17% and specificity of 82.9%.
Investigating natural inhibitors against HCV Entry—an in silico study

Maitri Singh*, MD Irfan, Suman Nandy, Aparna Mukhopadhyay

Hepatitis C virus (HCV) is a blood-borne disease that progresses slowly. It is the most common cause of several liver related diseases such as fibrosis, cirrhosis, steatosis, which left untreated can severely damage liver and lead to the development of hepatocellular carcinoma (HCC). Although there are several drugs available for treatment for more than 25 years high, capacity of mutation makes few drug pangenotypic. Furthermore, all drugs come with side effects and are very expensive. Till date HCV has 8 identified genotypes of which, genotype 1a is mostly prevalent and extensively studied. Tetraspanin protein CD81 is a potential therapeutic target as it is one of the key receptors mediating HCV entry by interacting with HCV envelope glycoprotein E2. This study is aimed to investigate effective natural entry inhibitors against CD81 and HCV genotype 1a. Literature survey revealed a list of 83 compounds which have been reported to restrict entry of HCV. The structure of these inhibitors were either generated using Marvin’s sketch tool or downloaded from PubChem. Docking studies were performed using these inhibitors against CD81 and HCV E2 1a by using AutoDock tool, Galaxy web, PatchDock and SwissDock servers. Potent inhibitors were selected based on their Pose score and binding affinity against their receptors. Non bonded interaction actions such as hydrogen bonds, hydrophobic interaction were calculated between inhibitors and the proteins CD81 and HCV E2 1a. Inhibitor Eugenin gave the best result against CD81 whereas Oleanolic acid derivative 70 was best against HCV E2 1a among the other inhibitors. Furtherwork is ongoing to identify the exact interaction residues and to find compounds that could inhibit E2 of other genotypes as well. Inhibitor studies will also guide us to generate potential preventives with pangenotypic activity and less side effects.

An Immunological Approach Towards DRDE-06 and S 27 Strains of Chikungunya Virus

Chandan Mahish1, Tapas Kumar Nayak2, Puspen Mondol1, Priyanshu Singh1, Soma Chattopadhyay3, Subhasis Chattopadhyay1

1National Institute of Science Education and Research, An OCC of Homi Bhabha National Institute, Bhubaneswar, 752050, India; 2Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA; 3Institute of life sciences, NALCO Square, Bhubaneswar, 751023, India

Chikungunya virus (CHIKV), an Alphavirus within family Togaviridae, is known for its recent endemic nature throughout the globe since its first identification in Tanzania, Africa. Long before its discovery, CHIKV was often inappropriately counted as Dengue due to similar pathophysiological manifestations like body ache, high fever, nausea, vomiting due to generation of rapid proinflammatory responses in host. Although CHIKV infection is self-limiting and has low mortality rate, reports suggest that maternal-fatal transmission may lead to a high possibility for neurological and developmental complexities. Our aim of the study is to comparatively analyse the immune response of two pathogenic strains of CHIKV namely DRDE-06 and S 27 towards disease progression in vivo. For the same, we have injected the CHIKV strains into C57BL/6 mice and harvested serum sample for quantitative real time PCR analysis for determining the copy number of E1 gene of CHIKV. Also, we have used the serum for determining the proinflammatory cytokines as a functional response of CHIKV infection via ELISA multiplexing. We have isolated the quadriceps muscle of uninfected and infected mice and prepared single cell suspension for comparative Flow Cytometry based analysis of muscle monocytes and muscle T cells. We have also analysed splenocytes from all mice groups in Flow cytometry to investigate comparative T cell activation profile. Our observations lead towards the conclusion that DRDE-06 (D-06) strain of CHIKV shows more virulence in comparison to S 27 strain of CHIKV under similar experimental conditions.

Network pharmacology based approach to identify novel inhibitors against Japanese encephalitis virus

Vimal K Maurya4, Swatantra Kumar, Shailendra K Saxena

Centre for Advanced Research (CFAR)-Faculty of Medicine, King George’s Medical University (KGMU), Lucknow 226003, India.

E-mail: shailen@kgmcindia.edu

Background: Japanese encephalitis virus (JEV) is a mosquito-borne neurotropic flavivirus and is the foremost cause of viral encephalitis in humans for which no specific antiviral drugs are available. Alkaloids present in Atropa belladonna exhibits neuroprotective and anti-inflammatory activity. Therefore, the present study was planned to identify novel antiviral agents derived from belladonna against JEV infection.

Methods: Network pharmacology approach has been used to identify the novel inhibitors present in belladonna. Further, the drug-likeness parameters, pharmacokinetics, and toxicity parameters were also determined. At last, the antiviral activity of belladonna was confirmed in the cell line model using cytopathic effect (CPE) inhibition assay, Western blotting, and Immunofluorescence on BHK-21 cells.

Results: We have found that belladonna inhibited virus-induced CPE in a dose-dependent manner with increase in cell viability when treated with different concentration of belladonna extract where 2.5 μg/ml concentration of the drug was found increase the cell viability by 1.67 fold (p = 0.0003) during JEV infection. However, as we have increased the concentration the viability of cells were reduced. Moreover, we have found that belladonna alkaloids primarily targets JEV helicase protein marked by significant reduction in JEV copy number and NS3 protein expression. Importantly, drug-likeness parameters, pharmacokinetics, and toxicity studies reveal that except belladonnine, all alkaloids represent good BBB permeability, intestinal absorption with no reported toxicity.

Conclusions: The results from this study confirmed that belladonna may be used as a therapeutic agent for restricting JEV infection via inhibition of key viral proteins.

Plant Virology

Threats and options of emerging transboundary disease in Sub-Saharan Africa – Maize Lethal Necrosis

Suresh L.M., Yoseph Beyene, Manje Gowda, Michael Olsen, Dan Makumbi, Walter Chivasa, and B.M. Prasanna

International Maize and Wheat Improvement Center (CIMMYT), ICRAF Campus, UN Avenue, Gigiri, PO Box 1041-00621, Nairobi, Kenya
Maize (Zea mays L.) is the most important cereal crop in sub-Saharan Africa (SSA), covering over 35 million ha, largely in smallholder farming systems that produce over 70 million metric tons (MMT) of grain. Environmental conditions prevalent in the different agro-ecological zones of Sub-Saharan Africa are very conducive to the growth and spread of pathogens. Maize production in sub-Saharan Africa is affected by a wide array of diseases. Many fungal and viral diseases have been affecting the maize crop and its productivity. Diseases often reduce production and cause up to 100% yield loss under severe epidemics depending on environmental conditions. There are various threats due to this transboundary Maize Lethal Necrosis (MLN) disease in maize on the food security, livelihood, and income of several million smallholder farmers in the SSA region. MLN disease first appeared in Kenya in 2011 and became a major threat to maize production in eastern Africa in subsequent years. In eastern Africa, MLN is caused mainly by the synergistic interaction between two viruses, Maize Chlorotic Mottle Virus (MCMV) and Sugarcane Mosaic Virus (SCMV). MLN can cause up to 100% yield loss in susceptible maize varieties. The disease poses a complex challenge as the MLN-causing viruses are transmitted by insect vectors, and also through contamination of the seed, especially by MCMV. It was very critical to have various options to tackle these threats. CIMMYT implemented a multipronged strategy as an option in partnership with several international and national partners to tackle the MLN challenge. These efforts included: a) establishing a state-of-the-art MLN Screening Facility in partnership with Kenya Agriculture and Livestock Research Organization (KALRO) in Naivasha for identifying sources of resistance to MLN, MCMV and SCMV under artificial inoculation; b) accelerated breeding and deployment of MLN-tolerant/resistant maize varieties with other relevant traits preferred by African smallholders; c) optimizing MLN diagnostic protocols; d) strengthening capacities of national plant protection organizations (NPPOs) across sub-Saharan Africa on MLN diagnostics, monitoring and surveillance system; d) creating awareness among the maize seed sector institutions on SOPs for producing and exchanging MLN-free commercial seed; e) disseminating information on farming practices for minimizing MLN incidence; e) establishing an MLN Phystosanitary Community of Practice involving various stakeholders, including national plant protection organizations (NPPOs), seed companies, regional/sub-regional organizations, etc.; and f) probing the epidemiology of the disease, especially the factors underlying seed contamination by MCMV. These comprehensive efforts have led not only in preventing the further spread of MLN into other major maize-growing countries in sub-Saharan Africa, especially southern and Western Africa, but also minimized the incidence of the disease in the MLN-endemic countries in eastern Africa.

miRNA-induced Gene Silencing (MIGS) for control of multiple pathogens

Basavaprabhu L. Patil
ICAR-Indian Institute of Horticultural Research, Bengaluru-560089, India
E-mail: basavaprabhu.patil@icar.gov.in, blpatil2046@gmail.com

Gene silencing is one of the most important mechanisms of gene regulation in plants, which is mediated by 19–24 nt sized small RNAs. In plants, the small RNAs can be grouped into different classes, microRNAs (miRNAs), small interfering RNAs (siRNAs), trans-acting siRNAs (tasiRNAs) etc. Transgenically induced RNA interference (RNAi), has been employed to control diverse plant viruses (Patil et al., 2011, Mol. Plant Pathol. doi: 10.1111/j.1364-3703.2010.00650.x.). The tasiRNAs are recently identified class of small RNAs, which are derived from TAS gene-derived transcripts after being acted upon by a miRNA. The miRNA173 directs the cleavage of TAS1 & TAS2 leading to the generation of tasiRNAs from the sequences located downstream of miRNA173 recognition site. The cleavage mediated by miR173 is sufficient to initiate transitivity, and targeting of a given gene by miR173 results in the production of secondary siRNAs originating from the target nucleotide sequence. The above studies lead to the emergence of a gene regulation technique termed as “miRNA-Induced Gene Silencing” (MIGS), that is essentially based on the unique feature of the miR173 to trigger the generation of secondary siRNAs (tasiRNAs) from its target sequences. MIGS can also be used to simultaneously silence multiple genes by fusing multiple MIGS modules (miR173 target site plus sequence of interest) to generate a single MIGS construct, which subsequently can be cloned into a binary vector for plant transformation. This fusion MIGS construct is capable of simultaneously silencing different genes with same efficiency. In collaboration with ICAR-IARI (India), Chennveda (India) and UC Davis (USA), we have explored the utility of MIGS technology for the combinatorial management of cotton leaf curl disease, whitefly and root knot nematode. The study demonstrated successful validation of the MIGS approach in the model host plant Nicotiana benthamiana (Hada et al., 2021, Pest Management Science, doi: 10.1002/ps.6384) and also in cotton.

Coat protein-mediated resistance to cucumber mosaic virus in black pepper

K.A. Revathy, M.V. Jiby, and A.I. Bhat*
Division of Crop Protection, ICAR-Indian Institute of Spices Research, Kozhikode 673012, Kerala
*Email: lshwarabhat.a@icar.gov.in, abib65@yahoo.co.in

Cucumber mosaic virus (CMV) infects a large number of plant species including Piper nigrum L. and related species. As natural resistance to CMV is absent in Piper spp, the study was undertaken to produce transgenic P. nigrum plants harboring the complete coat protein (CP) gene of CMV via Agrobacterium–mediated transformation and their evaluation for resistance against the virus. Among one hundred and nine hardened transformed plantlets, eight revealed the presence of the transgene in PCR. The production of transcript in these plants was assessed by reverse transcription-polymerase chain reaction (RT–PCR) and buildup of CMV CP by direct antigen coated-enzyme linked immunosorbent assay (DAC–ELISA). Screening of all eight transgenic lines against CMV through cleft grafting revealed that all lines except one were symptomless or showed mild or moderate symptoms. The transgenic line with the highest resistance was vegetatively propagated and integration of transgene in these clones was validated by Southern hybridization. The presence of transcript in clones was affirmed by northern blotting and western blotting ratified translation of transgene. Further, relative expression studies proved manifold expression of transgene compared to actin gene as analyzed by RT–qPCR. These studies validate the stable integration and expression of transgene which might be inhibiting the movement of virus to the scions in graft inoculated plants. This paves the way to the production of transgenic CMV resistant P. nigrum using CP and other desirable genes, the only effective method to combat CMV attack in the crop.

Thrips transmission of tospoviruses: infection, determinants, and pathogenic effects

Amalendu Ghosh
Advanced Centre for Plant Virology, Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi 110012;*Email: amal4ento@gmail.com
The present understanding of thrips-tospovirus relationship is largely based on the Franktiella occidentalis (Thysanoptera: Thripidae) and tomato spotted wilt virus (TSWV). Less is known about the relationship of Thrips palmi with other predominant tospoviruses. T. palmi is one of the key agricultural pests and predominant tospovirus vectors in Asia. It feeds on more than 200 plant species belonging to family Cucurbitaceae, Solanaceae, Fabaceae, and Asteraceae. The distribution of T. palmi was thought to be restricted to southern Asia, but it has spread throughout Asia in recent decades. T. palmi has widely invaded the Pacific, Florida, the Caribbean, South America, Africa, and Australia. To date, seven tospoviruses (family Tospoviridae, order Bunyavirales) viz. groundnut bud necrosis virus (GBNV), melon yellow spot virus (MYSV), calla lily chlorotic spot virus (CCSV), watermelon silver mottle virus (WSMV), watermelon bud necrosis virus (WBNV), tomato necrotic ringspot virus (TNRV), and capsicum chlorosis virus (CaCV) are known to be transmitted by T. palmi. T. palmi acquires the tospoviruses at the early larval instar and can only transmit the virus during the adult stage. The anterior midgut is the first to be infected with tospovirus (WBNV) in the first instar larvae. The midgut of T. palmi is connected to the principal salivary glands (PSG) via ligaments and the tubular salivary glands (TSG). The infection progresses to the PSG primarily through the connecting ligaments during early larval instars. Maximum virus-specific signal in the anterior midgut and PSG indicates the primary sites for tospovirus replication. Tospovirus infection is retained in the longitudinal and visceral circular muscles of the gut in the adult stage. No infection in epithelial cells is visible in adults. GBNV-nucleoprotein (N) localizes in the nucleus of T. palmi cells in primary cell culture. It has been predicted that virus particles probably enter vector cells by clathrin-mediated endocytosis. In response, a total of 2,365 (1384 up- and 981 downregulated) genes of T. palmi are differentially expressed. A group of genes associated with host innate immune and stress response and endocytotic pathways is upregulated in viruliferous T. palmi. Silencing of T. palmi UHRF1-binding protein 1-like (UHRF1BP1L) associated with endocytosis and phosphorylformylglycinamidine synthase (PFAS) associated with host immune response using chemically modified antisense oligos induce regulation in virus titer, morpho-deformities, and knockdown in T. palmi. The survivability and oviposition potential of T. palmi decrease post tospovirus exposure. Downregulations of larval cuticle protein A2-like, pupal cuticle protein C1B-like, endocuticle structural glycoprotein SgAbd-2-like are accompanied by delayed larval and pupal development in viruliferous T. palmi. The virus exposure favors a female-biased ratio in the experimental population.

Roles of coding and non-coding regions of betasatellite DNA in pathogenesis

Supriya Chakraborty

Molecular Virology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi -110 067; supriyahakrasls@yahoo.com

Satellites are additional viral components that can influence pathogenesis of helper viruses. Ability of satellites to infect wide range of organisms along with their helper viruses necessitate logical attempts to understand their biology. Majority of the satellites influence pathogenesis of helper viruses through regulating the interaction between helper viruses and their hosts. However, mechanism that regulates the trilateral interaction among hosts, satellites, and helper viruses primarily remains unanswered. Geminiviruses, the largest group of plant viruses, consists of ssDNA genome and are known to be associated with single stranded satellite DNAs such as alphasatellite, betasatellite and deltasatellite. Among these DNA satellites, betasatellite has emerged as a serious threat to crop ecosystem of the tropical and sub-tropical regions worldwide. Betasatellites are primarily associated with the majority of monopartite begomoviruses. Their genome contains four common structural features - an A-rich region, a satellite conserved region (SCR) containing a hairpin loop structure with the nonanucleotide TAATTATTAC, and two ORFs, B1 and B3 in the complementary sense strand and B1V in the virion sense strand. We have identified role of the non-coding region (NCR) in regulating betasatellite pathogenesis in plants. Furthermore, our findings suggest that betasatellite-encoded B1 protein damages ultrastructure of chloroplasts, modulates symptom development, accomplishes counter-defense and regulates viral titre. A growing body of research suggests that betasatellite encoded B1V and B3 proteins can be recognized by host defense to prevent the virus spread and therefore, can be potential targets to control the begomoviral infections.

Impact of Climate change on emergence of plant viruses

M. Krishna Reddy

Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bengalore-560089, India; Email: KrishanaReddy.M@icar.gov.in, mkrreddy60@gmail.com

Plant viruses cause considerable economic losses and are a threat for sustainable agriculture. The frequent emergence of new viral diseases is mainly due to international trade, climate change, and the ability of viruses for rapid evolution. Changes in global climate driven by increasing concentrations of atmospheric carbon dioxide and temperature will have significant consequences for future food production, quality, distribution and security. In addition, changing climate conditions can contribute to a successful spread of newly introduced viruses or their vectors and establishment of these organisms in areas that were previously unfavourable. Major contributory factors for the emergence and spread of new viruses include evolution of variants of viruses, changes in vector biology, changes in the cropping systems, introduction of new crops, the movement of infected planting materials/seed. The most important viruses of Apids, thrips and whitefly associated viruses, which have emerged during the last two decades or so belong to Begomo, Cucumo, Crini, Polero, Poty and Tospoviruses such as Cucumber mosaic virus, Chilli vein mottle virus, Chilli leaf curl virus, Okra Cucurbit aphidborne yellows virus, Elation leaf curl virus, Pepper vein yellow virus, Tomato leaf curl New Delhi virus, Tomato chlorosis virus, Capsicum chlorosis virus, Groundnut bud necrosis virus, watermelon bud necrosis virus and Zucchini yellow mosaic virus. emerged in vegetable crops which are seriously impacted the yields of vegetable crops in India. Climate change is expected to influence life history and trophic interactions among plants, whiteflies and their natural enemies. changes in temperature and humidity and other events associated with climate change influence the reservoirs of viral infections, their transmission by insects and other intermediates, their survival outside the host as well the success of infection in plants. Several reports have shown that increases in environmental temperature can enhance the cell-to-cell and systemic propagation of viruses within their infected hosts. These observations suggest that earlier and longer periods of warmer weather may cause important changes in the interaction between viruses, vectors and their host’s plants, thus posing risks of new viral diseases and outbreaks in Crop plants. As viruses target plasmodesmata (PD) for cell-to-cell spread, these cell wall pores may play yet unknown roles in the temperature-sensitive regulation of intercellular communication and virus infection. Understanding the temperature-sensitive mechanisms in plant-virus interactions will provide important knowledge for protecting crops against diseases in a warmer climate. Greater efforts are required to
improve understanding of the complex effects of climate change on multi-species and multitrophic interactions in the agro-ecological systems inhabited by insect vectors, and to use this new knowledge to develop robust and climate-smart management strategies.

CRISPR-Cas9 system to mitigate plant DNA viruses
Anirban Roy, Sunil Kumar Mukherjee
Plant Pathology Division, IARI, New Delhi 110012

CRISPR-Cas has brought revolution in Biology. Initially it was conceived that the spacers of CRISPRs impart bacterial immunity against bacteriophages and other extra chromosomal elements. When the mechanism of action of CRISPR system gradually emerged, investigators introduced such system in eukaryotic cells. Due to the prevalence of repair machinery, introduction of such systems resulted in site-specific mutation or insertion of large chunk of DNA at the specified site. Thus both inactivation and reactivation of genes were made possible. Such observations opened a plethora of biotechnological opportunities in medical and agricultural fields. Here we would show how Plant DNA viruses could be removed from plants using CRISPR-Cas9 systems as we are interested in virus-free foods. The DNA genomes of plant viruses can be directly targeted for inactivation and this principle has been demonstrated for Geminiviruses, Nanoviruses, Cauliflower Mosaic Viruses, Banana streak and some unknown mechanism. However, there has not been any direct demonstration of the mechanism by which the rice viruses interact with the host RNAl pathway components. RNAi components in rice include 19 AGOs, 8 DCLs and 5 RdRps, which have unclear functions in viral defense providing resistance and are probable subsequent targets of repression by virus as its counter strategy. The present work involves the selection of candidate RNAi genes after the expression analysis of the RNAi genes upon tungro virus infection and correlation of the virus titer with the RNAi gene expression. This was followed by cloning of selected RNAi genes in VIGS vector for their transient silencing in rice. The RNAi gene silenced rice plants were assessed for their susceptibility against virus. On an average around 50% silencing was observed and a negative correlation was found between the RNAi gene silenced plants and their viral titers.

Isolation and characterization of novel lytic bacteriophages consortia against ESBL and AmpC producing aquatic strains of Aeromonas hydrophila
Alka Nokhwal1, Ravikant1, Taruna Anand2* and R.K. Vaid2

Aeromonas hydrophila is a zoonotic pathogen displaying resistance to multiple antimicrobials such as Extended spectrum β-lactamases and AmpC β-lactamases. Although popularly known as a fish pathogen, it also causes morbidity in humans and other animals, thus being of huge economic importance. Their ability to carry multidrug resistance genes and form biofilms indicates that they are not easily managed through conventional techniques and urge the need to look for other disinfection strategies. Bacteriophages, owing to their numerous advantages and natural abundance, offer a potential alternative approach for controlling their pathogenicity. In this study, we isolated and characterized five phages from pond water which showed lytic activity against A. hydrophila strains. One step growth curve of phages revealed that the latent periods ranged from 30-60 min and burst sizes of one lytic cycle ranged from 34-101 PFU per infected cell. Observation of the phages by transmission electron microscopy indicated that phages belong to Myoviridae and Siphoviridae families. Further characterization studies involved phage stability over different temperatures, pH and organic solvents, SDS-PAGE analysis, PCR amplification of important genes from phage genome using different phage primers and host range determination by spot tests. Here, we aim to develop candidate biocontrol agents for A. hydrophila. The antimicrobial phage therapy can be extended for its protective effects in clinical and environmental settings. Keywords: Zoonosis, antimicrobial resistance, pathogenicity, phage therapy, host range

Investigating the antiviral role of RNAi components in rice (Oryza sativa L.) against Rice tungro bacilliform virus
Gaurav Kumar 1, Indranil Dasgupta1

Rice tungro is among the most damaging viral disease of rice resulting from infection by two viruses: Rice Tungro Bacilliform Virus (RTBV) and Rice Tungro Spherical Virus (RTSV) through the green leafhopper (Nephotettix virescens) vector. RNA silencing is one of the major strategies of the antiviral immune system in plants involving a coordinated action of Dicer-like proteins (DCLs), Argonautes (AGO) and RNA-dependent RNA polymerases (RdRPs). Recent reports claim that there exists a viral counter strategy involving repression of transcripts of RNAi pathway components upon virus infection either by modulation of associated miRNAs or through some unknown mechanism. However, there has not been any direct demonstration of the mechanism by which the rice viruses interact with the host RNAi pathway components. RNAi components in rice include 19 AGOs, 8 DCLs and 5 RdRps, which have unclear functions in viral defense providing resistance and are probable subsequent targets of repression by virus as its counter strategy. The present work involves the selection of candidate RNAi genes after the expression analysis of the RNAi genes upon tungro virus infection and correlation of the virus titer with the RNAi gene expression. This was followed by cloning of selected RNAi genes in VIGS vector for their transient silencing in rice. The RNAi gene silenced rice plants were assessed for their susceptibility against virus. On an average around 50% silencing was observed and a negative correlation was found between the RNAi gene silenced plants and their viral titers.

Insights into the host protein interactions with Transcriptional Activator Protein (TrAP) of Chilli leaf curl Ahmedabad Virus: An In-silico approach
Gnanaprakash Jeyaraj and Swapna Geetanjali A*

For the successful infection and ensuring spread in the host, the viral proteins need a strong interaction with their host proteins. An In-silico approach was brought up to reveal this interaction between the AC2/Transcriptional Activator Protein (TrAP) of Chilli leaf curl Ahmedabad Virus (CHLCAV), family Geminiviridae and known crystal structures of the proteins belonging to the Solanaceae family, which is known as its natural host. TrAP plays a role in the promoter activity of viral genes and has a known silencing suppressor activity in the host. The 3D structure of TrAP was modelled using Modeller 9.25, and Molecular dynamics simulation for the modeled structure was performed using GROMACS. For this protein-protein interaction (PPI) study, the host proteins’ crystal structures were downloaded from Protein Data Bank (PDB). Protein-Protein docking between these TrAP and host proteins was done using the cluspro webserver. The protein complexes were ranked based on the docking scores and interactions. The docking scores observed the strongest between Patain-17 and TrAP, and the weakest were identified between floral defensin-like protein 1 and TrAP. To validate the stability of the protein complex, a total of 20 ns Molecular Dynamic simulation was performed. Amino acid residues that are important for playing a role in the interaction of the domains may have an authorised role in pathogenicity and the viral life cycle. This study points out the interaction between the host proteins and TrAP of CHLCAV. However, more research in the form of transient expression analysis is required to confirm the nature of these interactions and gain a better understanding of the direct interactions occurring between these complexes.
Roles of coding and non-coding regions of beta satellite DNA in pathogenesis

Supriya Chakraborty

Satellites are additional viral components that can influence pathogenesis of helper viruses. Ability of satellites to infect wide range of organisms along with their helper viruses necessitate logical attempts to understand their biology. Majority of the satellites influence pathogenesis of helper viruses through regulating the interaction between helper viruses and their hosts. However, mechanism that regulates the trilateral interaction among hosts, satellites, and helper viruses primarily remains unanswered. Geminiviruses, the largest group of plant viruses, consists of ssDNA genome and are known to be associated with single stranded satellite DNAs such as alphasatellite, betasatellite and deltasatellite. Among these DNA satellites, betasatellite has emerged as a serious threat to crop ecosystem of the tropical and sub-tropical regions worldwide. Betasatellites are primarily associated with the majority of monopartite begomoviruses. Their genome contains four common structural features—an A-rich region, a satellite conserved region (SCR) containing a hairpin loop structure with the nonanucleotide TAATATTAC, and two ORFs, βC1 in the complementary sense strand and βV1 in the virion sense strand. We have identified role of the non-coding region (NCR) in regulating betasatellite pathogenesis in plants. Furthermore, our findings suggest that betasatellite-encoded βC1 protein damages ultrastructure of chloroplasts, modulates symptom development, accomplishes counter-defense and regulates viral titre. A growing body of research suggests that betasatellite encoded βV1 and βC1 proteins can be recognized by host defense to prevent the virus spread and therefore, can be potential targets to control the begomoviral infections.

Expression of LwaCas13a protein for the application in diagnostic and therapeutics

Taruna Gupta1, Ashirv Chivarstava1,2,* Narayan Rishi1

CRISPR/Cas13 has demonstrated unique and broad utility in RNA editing, nucleic acid detection, and disease diagnosis. CRISPR/Cas nucleic-acid-based nucleic acid detection and therapeutics has exposed great potential for the development of next-generation molecular technology due to its high reliability. The CRISPR-Cas system has recently emerged as a versatile tool for medical research for gene editing, epigenetic control, and disease diagnosis by using several Cas nucleases, such as Cas9, Cas12a, Cas12b, and Cas13a. The use of CRISPR-Cas13-based detection of viral infection may results in the development of rapid, affordable, and multiplexed point-of-care diagnostic system. Since, RNA viruses are prone to mutation and cause emergence of new strains, therefore, the chances of false positive results are associated with the currently adopted the screening and diagnosis technique of viral infection with quantitative RT-PCR (qRT-PCR)-based kits. Cas13a from Leptotrichia wadei (Lwa-Cas13a) as the most effective Cas enzyme as it can be heterologously expressed in mammalian and plant cells for targeted knockdown of either reporter or endogenous transcripts and are being used for both DNA and RNA viruses. Advancement in the current researches on CRISPR-Cas13a technique shows its potential to become the next-generation diagnostic tool for early, rapid, and reliable nucleic acid-based diagnostics and furthermore its potential as therapeutics. Here, we have discussed the bacterial expression of LwaCas13a for which the cas13a was amplified from vector procured from add gene, further cloned in bacterial expression vector pET28a for induction and expression of Cas13a. Purification of expressed protein was done by Ni-NTA column based on standardised conditions which was confirmed by western blotting using anti-His antibody and also the current use of in vitro RNA target activity of Cas13a as a customizable tools for detecting and depleting RNAs of interest.

Screening of multiple begomoviruses causing Chilli leaf curl disease in ten major chilli growing areas of Tamil Nadu

Jayanthi P1, Pradeep Kumar2, Anirban Roy2, Bikash Mandal2, Swapna Geetanjali A*3

Begomoviruses (Geminiviridae), transmitted by whiteflies, constitute one of the most important groups of plant viruses posing a severe threat to economically important crops. Leaf curl disease has become a severe problem in several Indian states. Chilli is a dicotyledonous flowering plant that belongs to the family Solanaceae. Chilli samples were collected from several places across Tamil Nadu. Multiple begomoviruses were explored using begomovirus species-specific Primer in the key commercial chilli production locations in the ten districts of Tamil Nadu (Krishnagiri, Dharmapuri, Kanchipuram, Thiruvannamalai, Thiruvallur, Salem, Vellore, Thoothukudi, Virudhunagar, and Tirunelveli). During the years 2018-2021, a significant incidence of leaf curl disease was detected in these places, with symptoms including yellowing, mottling, curled leaves, profuse branching, and plant stunting. Six hundred and seventy-three random samples were collected from all the 10 districts. Total genomic DNA was extracted from these samples and analysed by PCR using species-specific primers ToLCBaV, ToLCGuV, ToLCjV, ToLCPaV, ToLCNDV, & ChiLCV, as well as universal primers for begomovirus DNA A and beta satellite (CLB F/CLB R). The PCR results revealed that all six begomoviruses were present in the Thiruvannamalai district and five begomoviruses were present in the Krishnagiri district. Among the six viruses, ChiLCV is the most prevalent virus discovered in all ten districts (45.7 per cent). ToLCjV and ToLCPaV, on the other hand, were found in a few samples (1.5-3.6 per cent). Only 17% of the samples tested positive for beta satellite. In the key chilli-growing districts of Tamilnadu, at least six distinct begomoviruses were found, with ChiLCV being the most common begomovirus associated with leaf curl disease.

Plant-based generation of Virus like Particles and Expression Platforms for the production of Vaccines, Biosimilars and Therapeutics to address Global Health Security and Inequities

Sunil Kumar Srivastava

Medical Microbiology & Immunology Section, Department of Microbiology, Swami Shraddhanand College, University of Delhi, Delhi, India

Plant virus expression systems in conjunction with Agrobacterium transfer have been successfully deployed as an effective production platform of vaccines for infectious diseases, biosimilars like monoclonal antibodies, different types of therapeutics, and pharmaceuticals. The use of VLPs for SARS CoV2 spike and VLPs of Influenza hemagglutinin have been adopted by some countries in their vaccination programmes. The cocktail of monoclonal antibodies against Ebola virus produced by using a plant virus platform, was successfully used to cure Ebola patients during the 2014-16 epidemic in West Africa. This has a great potential to be widely used in middle and low-income countries where there is an inequity for production, lack of supply chain, and inadequate modern transportation facilities. This has been widely witnessed across several low- and middle-income countries during the ongoing COVID-19 pandemic. This technology is not only highly cost-effective but can also be scaled up rapidly to produce a very large quantity of good-quality vaccines, therapeutics, etc. with a short turnaround. This can effectively address threats to Global Health Security pertaining to pandemic preparedness and response posed by epidemics of emerging and re-emerging diseases. Moreover, due to its ease of adoption, the technology lends itself to broader applicability in the ‘One Health’ approach. This
technology has also shown great potential to address some of the world’s most growing health concerns, including both communicable and non-communicable diseases like cancer. In a country like India, which has a heavy burden of both kinds of diseases, this technology can prove beneficial; the government should invest more in such technologies. In this presentation, I will detail the various steps used in this technology and elaborate on its utility for both prevention and control of COVID-19, Influenza, and Ebola. A comparative study made with other production platforms will also be discussed.

Poster Presentations

Serodiagnosis of tobacco streak virus in okra

Arulsia1, R. Kannan1, N. Rajinimala1 and N. Balakrishnan2
1Department of Plant Pathology, 2Department of Agricultural Entomology, TNAU, Coimbatore-3

A new streak virus is prevalent in almost all okra growing regions of southern districts in Tamil Nadu, but at a low to moderate severity. Symptoms of this virus in okra include chlorotic spots with mild mosaic, veinal browning, chlorotic leaf blots, distortion of leaves and malformed fruits. In this study, the natural occurrence of the new virus on okra was observed and its sap transmission properties were documented on the local lesion host and weed plants and the confirmation of virus was done through ELISA analysis. Okra plants showing the typical symptoms were collected from different locations. The virus inoculum was prepared in 0.1 M sodium phosphate buffer (pH 7.0) and inoculated into test plants (i.e., cowpea & okra). Inoculated plants were observed 3-4 days after inoculation (DAI) for the development of symptoms. Mechanical sap inoculation was used to test the host range on weed species belonging to different families. DAS-ELISA was performed with TSV specific antiserum for okra samples and artificially inoculated weed samples. Symptoms like chlorotic spots, necrotic lesions, light and dark green patches were observed on the local lesion host (cowpea). DAS-ELISA results revealed that the samples with typical viral symptoms had a strong positive reaction, with absorbance values nearly double times higher than the apparently healthy samples. Out of 20 samples tested, 15 samples reacted positively with polyclonal antiserum to TSV by DAS-ELISA. The absorbance values (A405 nm) ranged from 0.60—0.97 in weed plants. The absorbance values (A405 nm) of bhendi field samples ranged 0.834—1.086. TSV suspected symptomatic bhendi samples were sap transmitted to the cowpea seedlings (CO7) and DAS-ELISA results confirmed by western blotting using anti-His antibody and also the expression of Cas13a. Purification of expressed protein was done by Ni-NTA column based on standardised conditions which was confirmed by western blotting using anti-His antibody and also the current use of in vitro RNA target activity of Cas13a as a customizable tools for detecting and depleting RNAs of interest.

Development of a CRISPR/Cas 12a based detection tool for begomovirus in India

Tripti Singhal1, Jitendra Kumar2, Ashish Srivastava1,3, Narayan Rishi1
1Amity Institute of Virology & Immunology, Amity University Uttar Pradesh, Sector 125, Noida; 2Department of Agronomy and Plant Genetics, University of Minnesota, 1551 Lindig Street St. Paul, MN 55108; 3Department of Entomology & Plant Pathology, University of Arkansas system, Fayetteville, USA 72701; Email ID for correspondence – singhaltripiti82@gmail.com

In the last few decades there have been pandemics and epidemics of plant viruses observed which have a global economic impact of more than $30 billion USD annually (Jones, 2021). In South Asian countries, several plant viruses are reported to cause serious economic losses and among them whitefly (Bemisia tabaci) transmitted begomovirus are said to be highly contagious in nature and their effects are often more drastic. Ageratum enation virus (AEV) and Mungbean yellow mosaic India virus (MYMIV) are the major constraint for many annual crops and legumes including soybean in northern India. Earlier the infection of AEV and MYMIV was limited to certain host species, however, the recent reports on tomato, amaranthus, opium poppy and some weeds species were observed. Plant viruses spread through various means, from mechanically to the insect vectors and act as obligate parasites, therefore, are extremely challenging to eradicate. Geminivirus is an important class of virus which may have been reported extensively in the last two decades on several new hosts. They majorly infect dicot annual crops and perennial shrubs, therefore, essentially required to detect them on field and dispose to check their vector transmission to healthy crops. In this study, we have chosen two very important begomoviruses, AEV and MYMIV which infect a wide range of crops. Here, we have utilized the binding and cleaving ability of LbaCas12a protein with the target to detect the

Expression of LwaCas13a protein for the application in diagnostic and therapeutics

Taruna Gupta1, Ashish Srivastava1,2*, Narayan Rishi1
1Amity Institute of Virology & Immunology, Amity University Uttar Pradesh, Noida; 2Department of Entomology and Plant Pathology, University of Arkansas System, Fayetteville, Arkansas 72701; Corresponding author Email ID: asrivastava10@amity.edu

CRISPR/Cas13 has demonstrated unique and broad utility in RNA editing, nucleic acid detection, and disease diagnosis. CRISPR/Cas nuclease-based nucleic acid detection and therapeutics has exposed great potential for the development of next-generation molecular technology due to its high reliability. The CRISPR-Cas system has recently emerged as a versatile tool for medical research for gene editing, epigenetic control, and disease diagnosis by using several Cas nucleases, such as Cas9, Cas12a, Cas12b, and Cas13a. The use of CRISPR-Cas13-based detection of viral infection may result in the development of a rapid, affordable, and multiplexed point-of-care diagnostic system. Since, RNA viruses are prone to mutation and cause emergence of new strains, therefore, the chances of false positive results are associated with the currently adopted screening and diagnosis technique of viral infection with quantitative RT-PCR (qRT-PCR)-based kits. Cas13a from Leptotrichia wadei (Lwa-Cas13a) as the most effective Cas enzyme as it can be heterologously expressed in mammalian and plant cells for targeted knockdown of either reporter or endogenous transcripts and are being used for both DNA and RNA viruses. Advancement in the current research on CRISPR-Cas13a technique shows its potential to become the next-generation diagnostic tool for early, rapid, and reliable nucleic acid-based diagnostics and furthermore its potential as therapeutics. In this study we have discussed the bacterial expression of LwaCas13a for which the cas13a was amplified from a vector procured from addgene, further cloned in bacterial expression vector pET28a for induction and expression of Cas13a. Purification of expressed protein was done by Ni-NTA column based on standardised conditions which was confirmed by western blotting using anti-His antibody and also the current use of in vitro RNA target activity of Cas13a as a customizable tools for detecting and depleting RNAs of interest.
virus infection in the field. We proposed here a new Collateral Cleavage Independent CRISPR/ Cas12a based detection system (CCICRISPR) for plant viruses.

Inhibition of potato leafroll virus multiplication and systemic translocation by siRNA constructs against putative ATPase fold of movement protein

Tushar Ranjan

Department of molecular Biology and Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur-813210 (Bihar)

Viruses cause many severe plant diseases, resulting in immense losses of crop yield worldwide. Therefore, developing novel approaches to control plant viruses is crucial to meet the demands of a growing world population. Recently, RNA interference (RNAi) has been widely used to develop virus-resistant plants. Once genome replication and assembly of virion particles is completed inside the host plant, mature virions or sometimes naked viral genomes spread cell-to-cell through plasmodesmata by interacting with the virus-encoded movement protein (MP). We used the RNAi approach to suppress MP gene expression, which in turn prevented potato leafroll virus (PLRV) systemic infection in Solanum tuberosum cv. Khufri Ashoka. Potato plants agroinfiltrated with MP siRNA constructs exhibited no rolling symptoms upon PLRV infection, indicating that the silencing of MP gene expression is an efficient method for generating PLRV-resistant potato plants. Further, we identified novel ATPase motifs in MP that may be involved in DNA binding and translocation through plasmodesmata. We also showed that the ATPase activity of MP was stimulated in the presence of DNA/RNA. Overall, our findings provide a robust technology to generate PLRV-resistant potato plants, which can be extended to other species. Moreover, this approach also contributes to the study of genome translocation mechanisms of plant viruses.

Veterinary Virology

Crimean-Congo Hemorrhagic Fever: Current status

Divakar Hemadri

ICAR-NIVEDI, PB No. 6450, Yelahanka, Bengaluru-64

Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic viral disease, which in humans is characterized by acute hemorrhagic illness, leading to hypovolemic shock and death in extreme cases. The disease is primarily transmitted through the bite of an infected tick; while, nosocomial transmission has been reported. Clinical disease is not seen in animals, however, they are a major threat to humans especially to those in high-risk occupations such as animal husbandry, butchering and slaughterhouse works, and veterinary practice. The causative agent of the disease, Crimean-Congo hemorrhagic fever virus (CCHFV) belongs to the genus Nairovirus of the family Nairoviridae and the order, Bunyavirales.

The disease is endemic in many regions of Africa, Asia, Eastern Europe, and the Middle East. CCHFV has the greatest geographic range of any tick-borne virus and has been reported from more than 30 countries across four regions: Africa (Democratic Republic of Congo, Uganda, Mauritania, Nigeria, South Africa, Senegal, Sudan), Asia (China, Kazakhstan, Tajikistan, Uzbekistan, Afghanistan, Pakistan, India), Europe (Russia, Bulgaria, Kosovo, Turkey, Greece, Spain), and the Middle East (Iraq, Iran, Kuwait, Saudi Arabia, Oman, United Arab Emirates (UAE)). It can be stated that the geographic distribution of CCHF coincides with that of ixodid ticks, particularly those of the genus Hyalomma. In Europe, Hyalomma marginatum is the main CCHFV vector, while Hyalomma asiaticum appears to be the principal vector in Asia. CCHFV is linked to Hyalomma anatolium ticks in India. Hyalomma ticks are found regions with dry and arid climates, and the abundant small and large mammals, which support haematophagy and the different stages of their life-cycle. In ticks, both transovarial and transstadial transmission have been reported. Thus the virus circulates in nature through an enzootic tick-vertebrate-tick cycle and many species of domestic and wild animals, pick the infection by tick bites and become asymptomatic carriers of CCHFV in an enzootic transmission cycle transmitting the disease to humans. Therefore, the majority of CCHF cases have been reported amongst people involved in livestock-related work, such as agricultural/farming, grazing animals, milking, slaughtering and veterinarians. Nevertheless human to human transmission do occur, particularly in those who are associated with providing health care to affected patients. The disease in humans is characterized by fever, myalgia, diarrhea and vomiting during the prodromal phase, progresses to ecchymoses, petechial rash, bleeding from mucosal/ punctured sites, coagulation disorders, fatal hemorrhagic manifestations including disseminated intravascular coagulation and death. In contrast to humans, animals do not show signs of illness. The paper describes about the current status of prevalence in India and elsewhere, phylogeny, diagnosis, gaps in knowledge.

Comparative diagnostic efficacy of Avidin-Biotin recombinant nucleoprotein competitive ELISA for serosurveillance and monitoring of peste des petits ruminants in sheep and goats

V. Balamurugan, Prajakta P. Bokade, K.Vinod Kumar, S. SowjanyaKumari, M. Nagalingam, D. Hemadri and B. R. Shome

Email ID for Correspondence: balavirol@gmail.com, b.vinayagamurthy@icar.gov.in

Indian Council of Agricultural Research -National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Yelahanka, Bengaluru, Karnataka, India

In this study extensive evaluation of Avidin-Biotin recombinant nucleoprotein competitive ELISA (ABrC-ELISA) was carried out by using large-scale sera as mass screening to make use of this assay for serosurveillance and sero monitoring of PPR in sheep and goats to increase its diagnostic efficacy value and strengthen findings associated with the assay. The recombinant PPRV nucleoprotein was over-expressed in E. coli, Ni-NTA affinity-purified, and characterized and used as coating diagnostic antigen in ABrC-ELISA and evaluated using the field sera from animals. On evaluation of diagnostic performance or efficacy of this assay using the pre-vaccinated and post-vaccinated sera of sheep and goats (n = 1437), the ABrC-ELISA showed the relative diagnostic sensitivity (DSn) of 87.2% (95% CI: 84.1-90%) and diagnostic specificity (DSP) of 92.0% (95% CI: 90-93.7%), against existing indigenous H protein-specific PPR competitive ELISA kit with an accuracy of 90.1% (95% CI: 88.5-91.7%) and diagnostic specificity (DSp) of 92.0% (95% CI: 84.1-90.7%), against existing indigenous H protein-specific PPR competitive ELISA kit with an accuracy of 90.1% (95% CI: 88.5-91.7%) and good or substantial agreement of Cohen’s Kappa value of 0.79 ± 0.017 SE (95% CI: 0.76 to 0.82). These findings suggest that the ABrC-ELISA has potential as an alternative assay for the detection of the PPRV nucleoprotein antibodies since it compared well with the live attenuated antigen-based competitive ELISA kit. The evaluated ABrC-ELISA is an alternative diagnostic tool of rapid, sensitive, and specific which can be used extensively under field conditions for the detection of the PPRV antibodies, for sero surveillance, and sero monitoring of PPR in sheep and goats at the eradication/post-eradication phase in the disease-controlled countries or PPR non-enzootic countries.
Non-correlation of hi titre with protection in a canine Parvoviral outbreak from an organized kennel in North India

Lt Col Mitesh Mittal1, Maj Manik Sharma2 and Col Ashish Tiwari3

Canine parvovirus (CPV-2) is commonly implicated as the leading cause of severe haemorrhagic gastroenteritis and fatalities especially in young puppies. Modified live attenuated vaccine (MLV) remains the most effective method for controlling canine parvoviral enteritis in dogs worldwide. Primary vaccination is generally given to pups at the age of 6 weeks, followed by two booster doses at 10 weeks and 14 weeks age, respectively. The post-vaccination antibody titre is measured by Haemagglutination Inhibition (HI) with a titre of 1:80 considered as protective. In this study, there was a severe canine parvoviral outbreak in vaccinated pups from an organized kennel in North India in 2021. The pups received MLV at 6 weeks, and post-vaccination antibody titre was estimated by Immuno-Comb Canine Vaccin-Check® Kit IgG Antibody Test Kit (Biogal Galea Laboratories, Israel). The HI titre as measured by the kit in the pups ranged from 1:320 to 1:640 indicating that they were having a high HI titre. Despite having a very high HI titre, there was a severe parvoviral enteritis outbreak among the vaccinated pups with mortality in some pups. The possible explanation for such an outbreak in vaccinated pups may be due to circulation of CPV-2 antigenic variant(s), which differ from the strain present in the vaccine formulation. The findings indicate that the vaccine induced antibody is not cross-protective against newer CPV-2 antigenic variants. Future studies are underway to develop a molecular based DIVA (Differentiation of Vaccinated and Infected Animals) test to rapidly distinguish field strains from vaccine strains and to identify CPV-2 antigenic variant(s) that caused the fatal outbreak.

High prevalence of anti-SARS-CoV-2 antibodies in canines in India

Nitin Virmani1*, B.C. Bera1, Taruna Anand1, Rachki1, Dinesh Saini1 and B.N. Tripathi2

The current pandemic of COVID19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was a worst catastrophe in the modern world after the Spanish flu of 1918. The severe acute respiratory syndrome coronavirus (SARS-CoV) of 2002 and Middle East respiratory syndrome coronavirus (MERS-CoV) of 2012 were relatively less fatal outbreaks when we compare them with the SARS-CoV2 of 2019. There have been many reports of detection of SARS-CoV-2 antibodies/ virus in animals during the current COVID-19 pandemic. Reports of infection in animals such as tigers and lions in India and USA, cats in USA and Belgium, dogs in Hong Kong and many other countries, minks on Netherlands indicate the threat the virus can cause for animal health due to reverse zoonosis and also the need to prevent the danger of various feline or canine species becoming reservoir hosts leading to zoonotic threat. Thus, there was an urgent need to screen our animal population for the SARS-CoV-2 and to develop specific and sensitive diagnostics for it. In this process, we have developed a recombinant nucleocapsid protein based indirect ELISA for detecting antibodies against the SARS-CoV-2 virus in canines. For development of the assay, the codon optimized recombinant N protein of SARS-CoV-2 was cloned into prokaryotic vector and a His-tagged recombinant NP protein was expressed and purified. The rNP showed reactivity with the antibody against SARS-CoV-2 by western blotting. The test was initially standardized on human serum samples from known history which tested positive by qRT-PCR assay. For negative samples precovid serum samples were utilized. Later the samples from canines were screened and for ruling out any cross reactivity samples from canines from precovid period were also screened. None of the precovid samples (n = 30) showed any titres for the SARS-CoV-2 antibodies. The serum samples (n = 423) tested from canines collected in 2021 from Delhi, Gurugram, hisar and Bengaluru showed a positivity of 32.22% at the rpp value of 25%. The assay has been validated with gold test i.e. serum neutralization assay and has been found to be 95.66% sensitive and 89.76% specific. The study underscores the need for surveillance of SARS-CoV-2 in animal species and the iELISA tool developed by us is a highly sensitive and specific assay for screening of antibodies in canines for seromonitoring as well as for investigation of outbreaks.

One Health Approach to Prevent Future Pandemics

Siddharth Srivastava1, Sunil Kumar Srivastava2 and Nitin Chauhan2

1The George Institute for Global Health India, New Delhi, India; 2Department of Microbiology, Swami Shraddhanand College, University of Delhi, Delhi, India

In the last five decades or so, the world has witnessed recurring outbreaks of emerging and re-emerging zoonotic infectious diseases, causing epidemics and pandemics from time to time. The principal drivers of their emergence are associated with changes in ecosystems hastened by climate change1 and coupled with human activities like excessive land use, intensification of agriculture, urbanization, international travel, and trade. To prevent such global health security threats, there is a dire need to focus on “One Health” which emphasizes on the recognition of the fact that the health of humans, animals, plants, and the environment is fundamentally intertwined and interrelated with each other. According to CDC, One Health is a collaborative, multisectoral, and transdisciplinary approach—working at the local, regional, national, and global levels—with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment. Ecosystem alterations and crowded places like wet markets bring people plus domestic animals in close contact with wildlife resulting in a species spillover as seen in AIDS, Rabies, Flu (avian & swine), Ebola, Nipah, Hendra, Japanese Encephalitis, Zika, SARS, MERS, and COVID-19 which assumed epidemic and pandemic proportions. One Health policy, is therefore, a crucial component to help countries for preparing, recognizing, coping with and managing the adverse impacts of zoonosis in human health. Modern tools like metagenomics, whole-genome and exome sequencing using the NGS platform can help in genome surveillance for tracking mutations of increased virulence and transmission, the emergence of new strains, and tracing viral reservoirs. Also, the pandemic preparedness has to be updated from time to time—like new vaccine platforms, new drug discovery, etc. The present pandemic has exposed the weaknesses of public health systems, around the world which therefore needs to be strengthened at all levels—local, national and global. In this presentation, the case studies of Ebola, Zika2, and COVID-19 outbreaks will be discussed and lessons learnt from the One Health perspective analyzed.

Recombinant neurovirulent Equine herpesvirus-1 generated from non-neurogenic strain by inserting point mutation (A2254G) in DNA polymerase gene

Bera B. C.1, Virmani Nitin1, Anand Taruna1, Pavulraj S1, Rekha Sansanwal1, Singh R. K2 and Tripathi B. N.3

Equine herpesvirus myeloencephalopathy (EHM) is a neurologic disease of horses caused by Equine herpesvirus-1 (EHV-1) infection leading to damage of blood vessels in brain and spinal cord.
Neurological form of the disease although unexplored in India is one of the most burning problems globally. The SNP (N752D) in DNA polymerase protein of EHV1 is associated with neuropathogenic potential. Here we report the generation of recombinant neurogenic EHV1 (Tohana strain) isolated from abortion cases by inserting SNP (A2254G) in DNA pol gene (ORF30) employing BAC mediated Enpassant mutagenesis strategy. Transfer cassette having a 50 bp homologous region of ORF30 with SNP (A2254G) and kanamycin-resistance (kanR) sequence was constructed and incorporated into EHV1- BAC clone in E. coli by electroporation. Subsequently, Kanamycin gene removed and incorporated point mutation. Recombinant clones were screened by replica plating, confirmed by amplification of ORF30 gene and sequencing. Sequence data revealed the presence of nucleotide G at position 2254 in ORF30. EHV1-BAC (A/G) plasmid DNA was purified from recombinant E. coli and purified plasmid was transfected into RK13 cell cultures at 24 h after transfection. Larger CPE was found at 48 h and complete CPE observed at 72 h after transfection. The plaque size of the neurogenic EHV-1 was larger compared to wild virus. The in-vitro growth kinetics was studied in RK13 cell cultures at 24 h after transfection. The plaque size of the neurogenic isolate from wild EHV-1. The in-vitro growth kinetics was studied in RK13 cell cultures at 24 h after transfection. Larger CPE was found at 48 h and complete CPE observed at 72 h after transfection. The plaque size of the neurogenic isolate from wild EHV-1 was larger compared to wild EHV-1. The in-vitro growth kinetics was studied. The generated neurogenic isolate from non-neurogenic EHV-1 will serve as resource for studying the disease pathogenesis and development of live vaccine candidates for preparedness to control the neurological disease.

First report on complete genome analysis of African swine fever virus of Indian origin

Dhanapal Senthilkumar1*, Katherukamem Rajukumar1, Govindaraju Venkatesh1, Fateh Singh1, Chakradhar Tosh1, Subbiah Kombiah1, Chandan K. Dubey1, Amitav Chakravarty2, Nagendra N. Barman3, Vijendra Pal Singh1

1ICAR-National Institute of High Security Animal Diseases, Bhopal, India; 2North Eastern Regional Disease Diagnostic Laboratory, Guwahati, India; 3College of Veterinary Science, Assam Agricultural University, Guwahati, India; Email: senetpath@gmail.com

African Swine Fever (ASF) is a highly contagious haemorrhagic viral disease affecting pigs and wild boars with case fatality rate of up to 100%. It is caused by a unique DNA virus belonging to the family Asfarviridae, genus Asfivirus. The complete genome of ASF viruses isolated during the first outbreaks in Arunachal Pradesh and Assam states of India were analyzed. The size of assembled complete genome of ASFV isolate from Assam and Arunachal Pradesh was 190517 bp and 190572 bp, respectively. The average coverage depth of the assembled sequences of the two ASFV isolates were 801 x and 978x. Both sequences of Indian ASFVs had insertion of one extra TRS (TATATAGGAA) in the intergenic region between I73R and I329L and a few unique mutations were observed in MGF 369-11L, MGF 505-4R, K205R and B263R genes. Complete genome based phylogenetic analysis of Indian isolates with 33 additional ASFV sequences retrieved from GenBank showed their clustering under clade 2.2.2 with other p72-genotype-II ASFV reported from Georgia, Tanzania, China, Vietnam, Poland, Ukraine, East Timor etc. between 2007 and 2020. Phylogenetically, concatenated nucleotide sequence of 14 open reading frames (ORF) with single nucleotide polymorphism of Indian isolates grouped separately with other Asian ASFVs. Thus, our results showed the significance of the 14 ORFs in understanding the evolution of ASFV in Asian countries and their divergence from prototype Georgia/2007 ASFV.

Characterization of accessory viral protein, W, of Newcastle Disease Virus

B Nagaraj Nayak, Kalaimagal Rajagopal, Revathi Shumugasundaram, Pachinella Lakshmana Rao, Saraswathy Vaidyanathan and Madhuri Subbiah

RNA editing is one of the important overprinting mechanisms seen in the viruses belonging to the family Paramyxoviridae. It helps the viruses to use their compact genome effectively. RNA editing of phosphoprotein (P) gene in the avian paramyxovirus, Newcastle disease virus (NDV), results in two additional proteins, V and W, which share a common N-terminus with P protein. While the V protein is a well known IFN antagonist, the function of W protein is largely unexplored. Percentage mRNA obtained from our wet lab work and sequencing showed that W mRNA of account for 7%—9% of the total P gene products and it harbour nuclear localization signal (NLS) in the unique C-termius, suggesting that W protein may have an undetermined function in the nucleus of the host cell. To gain insights into the function of W protein, we studied the cellular compartmentalization of W protein of NDV strain Komarov (NDV strain K) and found that W protein gets localized in the nucleus. Our studies showed that mutation of the putative NLS resulted in the redistribution of W protein to cytoplasm. Treatment of W plasmid transfected cells with ivermectin (inhibitor of importin-β pathway), confirmed the role of importin-αβ pathway in W protein nuclear localization. Further, to understand the possible role of W protein in viral replication, we studied the viral growth kinetics of NDV strain K in W protein overexpressing cells and found that neither the over expression nor the localization of W protein influenced the viral replication. However, the possible role of W protein in virus host interaction cannot be overlooked.

Role of Non-structural Proteins PPRV C and PPRV V in the Molecular Pathogenesis of Peste des petits ruminants virus (PPRV)

Juhi Jain, Prof. Rajeev Kaul, Department of Microbiology, University of Delhi South Campus

Department of Microbiology, University of Delhi South campus, Delhi

Email ID for Correspondence: jjain3101@gmail.com, rkaul@south.du.ac.in

PPRV (Peste des petits ruminant virus), is a Morbillivirus belongs to the family Paramyxoviridae. It is non-segmented, negative sense single-stranded RNA virus which causes acute, highly contagious and an economically important disease PPR (Peste des petits ruminant) of small ruminants, also known as Pseudorinderpest, Goat plaque, Ovine rinderpest, Kata with 90% morbidity and mortality rate. The susceptible hosts for the virus include sheep and goat, however causes subclinical infection in cattle as well. The virus spreads via respiratory route. PPR is an OIE (Office International des Epizooties) listed disease which is aimed to be eradicated by 2030. PPRV is antigenically related to globally eradicated Rinderpest virus. It is well known to prompt immune-suppression which results in an increased susceptibility to opportunistic infections that can lead to life-threatening complications such as pneumonia. The mechanism behind this is poorly understood. Here, our RT-qPCR data showed the abrupt rise in the P/V/C transcript at 72 h when Vero h Slam (VHS) infected with 1 MOI Sungri/96 vaccine strain. Also, the non-structural proteins PPRV C & PPRV V have previously been shown to inhibit the interferon induction which plays an important role in innate immunity, hence making them suitable candidates for this study. The ISRE (Interferon Stimulating Response Element) based luciferase reporter assay has shown strong downregulation via PPRV C & PPRV V genes. The
Foot-and-mouth disease is a highly contagious viral disease that affects cattle, buffalo, pig, sheep, goat and other cloven-hoofed animals. The present study reports the prevalence of FMD virus-nonstructural protein antibodies (NSP-Abs), which is an underlying indicator of past/recent FMDV infection in small ruminants and pigs randomly sampled in Haryana state during 2019 and 2020 by the Department of Animal Husbandry and Dairying, Government of Haryana. The state of Haryana has shown relatively low NSP-Ab prevalence in small ruminants and pigs sampled during 2019 and 2020 by the Department of Animal Husbandry and Dairying, Government of Haryana. The state of Haryana has shown relatively low NSP-Ab prevalence in small ruminants and pigs sampled during 2019 and 2020 by the Department of Animal Husbandry and Dairying, Government of Haryana.

**Phylogenetic and structural analysis of p72 (B646L) gene of African swine fever virus**

Anuradha Sharma, Nisha Singh, Sachin Kumar, NN Barman, and Yashpal Singh Malik

College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab; 2Indian Institute of Technology, Guwahati, Assam, India; 3College of Veterinary Science, Assam Agricultural University, Guwahati, Assam, India

Presenting author: s.anuradha21@ymail.com; *Corresponding Email: malikyps@gmail.com

African swine fever is a deadly hemorrhagic disease associated with high morbidity and mortality in pigs. The causative agent is a large DNA virus known as African Swine Fever Virus (ASFV) with a genome size of 170-194kbp. The major capsid protein p72 is a dominant structural component constituting 30-33% mass of the virion, thus acting as one of the important targets for diagnostics and vaccine development. In the present study, we analyzed the evolutionary and phylogenetic relationship between Indian isolates of ASFV p72 (B646L) gene based on 44 diverse p72 strains derived from different parts of the world. Of them, four ASFV p72 strains were of Indian origin. Furthermore, performed protein sequence analysis, identified conserved domains motifs, protein structure and active sites prediction. The phylogenetic analysis suggested that out of the four Indian isolates, the two isolates MT612961 and MT612962 share sequence identity with isolates from China, Vietnam and Hungary, whereas the other two sequences have acquired mutations and evolved independently. The sequence and structure analysis depicted that the average molecular weight of p72 ~ 16558.93kDa and it is rich in serine (9.0%), both valine and arginine (8.9%) and isoleucine (8.3%) and less amount of tryptophan (1.1%), glutamine (1.95%) and methionine (2.15%). We identified a total 10 conserved motifs across 44 different isolates of p72, of them 5 motifs are found to be more profuse and present in most of the isolates of p72. Sequence and structure analysis revealed that the two isolates of Indian p72, MT612961 and MT612963 are related to China MW521382, MT612963 and MT612964 are independently evolved. It was concluded from the current findings that the ancestors of the Indian isolates could be originated or derived from China, Vietnam or Hungary, however, ASFV in India is progressive and has already undergone mutations. Identification of newly evolved ASFV variants and corresponding protein structure could help in developing suitable indigenous diagnostics and treatment strategies.
Countrywide survey of porcine reproductive and respiratory syndrome indicates widespread seroprevalence in India

Divakar Hemadri, Jagdish Hiremath, Kuralayana Prataputthamapra Suresh, Jayasankar Jayaraman, Sharanagouda S. Patil, Parimal Roy, Rajangam Sridevi

1ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), PB No. 6450, Yelahanka, Bengaluru-560064, India; 2ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Post Box No. 1603, Eronakulam North P.O., Kochi-682 018, India

Email for correspondence: divakar.hemadri@icar.gov.in

Porcine reproductive and respiratory syndrome (PRRS) is an important emerging disease in India, but not much is known about its prevalence in various states. This study was conducted to estimate the prevalence of PRRS and understand the distribution of the disease. A total of 6089 pig-serum samples from 27 states/union territories of India were screened for PRRS virus specific antibodies using a commercial ELISA kit (PrioCHECK® PRRS virus antibody porcine kit, Thermofischer, USA). Of these samples, 1346 were found positive, indicating an overall prevalence of 17.8%. Samples from all states except Himachal Pradesh, Kerala, Madhya Pradesh, Puducherry, Punjab and Sikkim were positive. The prevalence was slightly higher in the north-easter region and younger (26.2%) age groups. The spatial analysis indicated pockets of high seroprevalence in Nagaland and Manipur states, bordering Myanmar. Prevailing husbandry practices and cross border trafficking of animals could be reasons for higher seroprevalence in the said states. This is the first study conducted on a national scale that provides an insight into the widespread prevalence of PRRS in the country. Findings from the study would be helpful for making policy decisions for controlling the disease.

Poster Presentations

Molecular characterization of elephant endotheliotropic herpesvirus detected in Asian elephant of Assam, India

IVS Best Poster Award in Medical Virology

Disease dynamics of the novel SARS-CoV-2 Omicron Variant of Concern of B.1.1.529 lineage

Sanjaya Ansari, Swatantra Kumar, Shailendra K Saxena

Centre for Advanced Research (CFAR), Faculty of Medicine, King George’s Medical University (KGMU), Lucknow 226003, India.

shailen@kgmcindia.edu

Ovel Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) variant, Omicron (PANGO lineage B.1.1.529) being reported from all around the world. WHO has categorized Omicron as a Variant of Concern (VOC) considering its higher transmissibility and infectivity, vaccine breakthrough cases. Therefore, this study was planned to investigate the transmission dynamics and mutational prevalence of the novel SARS-CoV-2 Omicron variant. To identify the global prevalence of Omicron SARS-CoV-2, we have used the phylogenetic assignment of named global outbreak (PANGO) lineage based on the Nextstrain. Divergence of Omicron was determined based on the S1 gene mutations among the predominant variant of SARS-CoV-2. Furthermore, we identify the mutational hotspots and analyzed the transmission dynamics of Omicron. Our phylogenetic analysis suggests that Omicron (BA.1) was clustered distinctively from the other VOCs producing a monophyletic clade. In the spike glycoprotein, which may alter transmissibility, infectivity, neutralizing antibody escape, and vaccine breakthrough cases of COVID-19. Our analysis suggests that the prevalence of the mutation in RBD as K417N, N440K, and G446S is 22.53%, 25.08%, and 25.73%. The transmission dynamics analysis suggests that the Omicron was first identified in South Africa and then it was reported in the United Kingdom followed by the United States and Australia. The findings of this study will be utilized for a better understanding of global transmissibility, pathogenesis, and the development of effective preventive and therapeutics.

Apriya Bharali1*, Lukumoni Buragohain1, Nagendra Nath Barman1, Samshul Ali1, Ankita Choudhury1, Nipun Gogoi1

Asian elephants (Elephas maximus), particularly juveniles are very much prone to elephant endotheliotropic herpesvirus-hemorrhagic disease (EEHV-HD). The causative agent of EEHV-HD is elephant endotheliotropic herpesvirus (EEHV) which is a dsDNA virus belonging to the family Herpesviridae and genus Proboscivirus. Based on genetic analysis, EEHV has been classified into eight different types viz. EEHV1A, EEHV1B and EEHV2 – EEHV7. Elephant endotheliotropic herpesvirus infections are reported worldwide and several cases were reported from India including Assam. In this study, we have reported the incidence of haemorrhagic disease in an Asian elephant of Assam that was confirmed to be EEHV infection. A through autopsy was conducted and the representative tissue samples (heart, lung, kidney, mesentery, tongue and spleen) were used for detection of EEHV by PCR. Then, phylogenetic analysis was done in MEGAX software to reveal the types of EEHV. In autopsy, the striking gross morphological changes especially, paint brush haemorrhage on heart, hyperaemic and consolidated lung, ecchymotic haemorrhage on liver, splenomegaly and cyanotic tongue indicates EEHV infection. Subsequently, DNA was extracted from pooled tissue samples and PCR was conducted targeting U38 gene and U60 gene. The PCR was found to be positive for both the genes of EEHV. The PCR amplified products were subjected to sanger sequencing which was outsourced. The sequencing data were further analysed by using bioinformatics tool and both the amplified products were to confirmed to be EEHV1A having more than 99% nucleotide identity with other EEHV1A strains available in public domain. Further, phylogenetic analysis also revealed that the detected EEHV strain (EEHV/AS/02) belongs to EEHV1A. Based on the earlier EEHV reports from Assam and the findings of this study, it can be deduced that the most common circulating EEHV type in the elephant population of Assam is EEHV1A and most probably it might have become endemic in this state of India. Thus, the findings of the study will strengthen the molecular epidemiology of EEHV in India as well as it be will be an add-on to design indigenous diagnostics tool in future for the locally circulating EEHV strains in our country.

Spill over of African swine fever virus to the wilds of Assam, India: An emerging threat

African swine fever (ASF) is a highly transmissible haemorrhagic and fatal viral disease of wild boars and domestic pigs. It is caused by the African swine fever virus (ASFV), a complex, large, cytoplasmic,icosahedral dsDNA virus classified as the unique member of the family Asfarviridae. Since the first outbreak of ASF in China in August 2018, several hundred cases of ASF have been reported in other Asian countries. India recorded the first outbreak of ASF in its North-eastern region (NER) in the year 2020. The current study was undertaken to investigate the spill over of ASFV in the wild pigs of Northeast India, particularly of Assam. ASF suspected mortal tissue remains, and blood samples of wild boar collected from different locations of Assam and Arunachal Pradesh were sent to Advanced Animal Disease Diagnosis and Management Consortium (ADMaC) Laboratory, College of Veterinary Science (Guwahati, India). The
Development of avian influenza H9N2 virus vaccine for chickens

C. Tosh, Manoj Kumar, S. Nagarajan, S. Bhatia, H.V. Murugkar, V.P. Singh

ICAR—National Institute of High Security Animal Diseases, Anand Nagar, Bhopal – 462022, India

H9N2 subtype low pathogenic avian influenza (LPAI) virus infection in poultry leads to economic losses due to reduced egg production (up to 40%) and mortality (2-3% in growers and 10-30% in layers). Properly administered vaccine would protect chickens against clinical signs and mortality during the winter season (January to February) in different regions/zones. The vaccine shedding virus ranged between 2-3/8 in boosted and 2-5/8 birds in non-boosted groups. The vaccine would protect chickens against clinical signs and mortality for up to 6 months. No clinical signs were observed in birds inoculated with vaccine. The vaccine shedding virus was found to be safe and induced protective antibody titres which was maintained till 6 months and reduced virus shedding in chickens.

Current Scenario of Peste des petits ruminants Control and Eradication Strategic plan in India

V. Balamurugan, K Vinoth Kumar, G. Govindaraj, K.P. Suresh, B.R. Shome

Indian Council of Agricultural Research—National Institute of Veterinary Epidemiology and Disease informatics (ICAR-NIVEDI), Yelahanka, Bengaluru-560064, Karnataka, India.

Foot-and-mouth disease outbreak in an organized pig farm at Nongpiyuir, Meghalaya

M. Rout*, K. Bora, K. Pargai, L. Dkhar, H. Kylla, S. Subramaniam, J.K. Mohapatra and R.K. Singh

Foot and mouth disease (FMD) is a severe, highly contagious viral disease of livestock bearing a significant economic impact.
Foot-and-mouth disease in mithun and cattle of Arunachal Pradesh in India
M. Rout1, O. Tapir, P. Deka, J.P. Tripathy, P. Giri, S. Subramaniam, J.K. Mohapatra and R.P. Singh

The indigenous people of Arunachal Pradesh, one of the north-eastern states of India are proud of mithun as the state animal. Apart from being a matter of pride for the farm owners, it is the principal source of dairy products. With around 350 thousand mithun, Arunachal Pradesh has the highest population of mithun in India. In addition to cattle, buffalo, pig, goat and sheep, foot-and-mouth disease (FMD) virus causes severe disease in several semi-domesticated and wild cloven-hoofed animals. During August 2021, outbreaks of FMD were reported in mithun and cattle of Arunachal Pradesh. The affected cattle and mithun exhibited similar clinical symptoms of fever, inability to take feed, smacking of lips and salivation with lameness. Erosive lesions in tongue, dental pad, around the hoof and in inter-digital space suggestive of FMD were observed. The clinical samples (tongue epithelium/foot epithelium) collected from 18 mithuns and 5 cattle were processed and subjected to FMD virus serotype differentiating sandwich ELISA and multiplex RT-PCR for serotype confirmation, where samples were found positive for FMDV serotype O. In NSP serology, 32 (76.19%) serum samples were found positive for 3AB NSP antibodies of FMDV. Introduction of infected animals from outside along with improper biosecurity measures might have contributed to the outbreak in the farm. However, the role of ongoing FMD outbreaks in cattle in nearby areas as well as the access of farm attendants/animal care-takers commuting from those areas into the farms cannot be underemphasized.

Serological detection of SARS-CoV-2 neutralizing antibodies in bovine, canine and caprine species in India
Mousumi Bora1, Pensisng Pavan Kumar1, Manu M1, Prasanta Kumar M Mishra1, Rahul G Kadami1, Durvij Prasad Bora2, Nagendra Nath Barman2 and Ramadevi Nimmanapalli1

As Coronavirus disease (COVID-19) pandemics entered the third year, human infections have been observed at an intense level globally surpassing around 6 million deaths. Recent studies have established that the causative agent of COVID-19, SARS-CoV-2 infects a wide range of animal hosts including wild, captive and companion animals. Therefore, the carrier status and transmission of SARS-CoV-2 in domestic animals need to be studied due to its frequent mutations, species jumping and reverse-zoonoses prospects. In the present study, we have investigated the first serological survey of SARS-CoV-2 neutralizing antibodies in domestic animals in different states of India. Results are reported for 727 sera (578 bovine, 141 canine and 8 caprine) collected during the first and second wave of COVID-19 human infections. Among the tested sera samples, 36.88% of canines (52/141), 12.5% caprine (1/8) and 11.76% of bovine (68/578) were found to be positive for COVID-19 neutralizing antibodies by a double antigen sandwich ELISA. Our findings revealed that human-animal interactions might have resulted in COVID-19 transmission to animal populations. The present study also highlights the importance of longitudinal studies to monitor SARS-CoV-2 adaptation and evolution in animal population to predict the chances of future pandemics.

Monoclonal antibody (mAb) based Solid phase competition ELISA (SPCE) to measure anti-Foot and Mouth Disease (FMD) virus serotype A antibodies for vaccine potency testing
R. Hema Syee1*, V. Bhanuprakash, R.P. Tamil Selvan, Madhusudan Hosamani, Shanmugananthan1, K. Narayanan

Foot-and-mouth disease (FMD) is a highly contagious, transboundary viral disease caused by foot-and-mouth disease virus (FMDV). FMDV mostly affects even toed ungulates. FMDV A serotype is antigenically and genetically diverse compared to other FMDV serotypes. In India, to detect the antibody profile for vaccine potency testing, currently VNT, LPBE and polyclonal antibody (pAb) based SPCE were utilized. However, all these tests are besotted with several drawbacks. To overcome these drawbacks, two mAb based SPCEs were developed to detect anti-FMDV A antibodies and their diagnostic ability were compared with VNT and pAb based SPCE. A panel of 12 mAbs specific to FMDV A were characterized for their neutralization, serotype specificity, isotyping, blotting and immunofluorescence properties. Based on these properties, the mAbs designated as 6E8D11 (mAb#1) and 2C4G11 (mAb#2) were selected to develop two SPCEs. The optimum dilutions of each reagent in pAb, mAb#1 and mAb#2 based SPCEs were optimized by checkerboard titration. FMD positive (n = 223) and negative samples (n = 230) with unequivocal history were screened by VNT, pAb, mAb#1 and mAb#2 SPCEs independently to detect the anti-FMDV A antibodies. The study revealed pAb, mAb#1 and mAb#2 SPCEs had a relative sensitivity of 80.27, 86.10 and 86.10%; and specificity of 99.13, 99.13 and 99.57%, respectively. Further, the correlation, level of agreement and repeatability of the aforementioned assays were excellent. The analytical specificity of SPCE was assessed by using other diseases (n = 7) and FMDV serotypes O and Asial positive sera(n = 5); the results revealed mAb #2 SPCE is highly exclusive/inclusive. Comparison of different assays indicated that mAb #2 SPCE have a great potential in helping control and eradication of FMD in India, prospectively.
First report on complete genome analysis of African swine fever virus of Indian origin.

Dhanapal Senthilkumar1*, Katherukamem Rajukumar1, Govindarajulu Venkatesh1, Fatee Singh1, Chakradhar Tosh1, Subbiah Kombiah1, Chandan K. Dubey1, Amitav Chakravarty2, Nagendra N. Barman1, Vijendra Pal Singh1.

1ICAR-National Institute of High Security Animal Diseases, Bhopal, India; 2North Eastern Regional Disease Diagnostic Laboratory, Guwahati, India; 3College of Veterinary Science, Assam Agricultural University, Guwahati, India; * Presenting author- email: senvetpath@gmail.com

African Swine Fever (ASF) is a highly contagious haemorrhagic viral disease affecting pigs and wild boars with case fatality rate of up to 100%. It is caused by a unique DNA virus belonging to the family Asfivirus, genus Asfivirus. The complete genome of ASF viruses isolated during the first outbreaks in Arunachal Pradesh and Assam states of India were analyzed. The size of assembled complete genome of ASFV isolate from Assam and Arunachal Pradesh was 190517 bp and 190572 bp, respectively. The average coverage depth of the assembled sequences of the two ASFV isolates were 801 and 978x. Both sequences of Indian ASFVs had insertion of one extra TRS (TATATAGGAA) in the intergenic region between 173R and I329L and a few unique mutations were observed in MGF 369-11L, MGF 505-4R, K205R and B263R genes. Complete genome based phylogenetic analysis of Indian isolates with 33 additional ASFV sequences retrieved from GenBank showed their clustering under clade 2.2.2 with other p72-genotype-II ASFV reported from Georgia, Tanzania, China, Vietnam, Poland, Ukraine, East Timor leste etc. between 2007 and 2020. Phylogenetically, concatenated nucleotide sequence of 14 open reading frames (ORF) with single nucleotide polymorphism of Indian isolates grouped separately with other Asian ASFVs. Thus, our results showed the significance of the 14 ORFs in understanding the evolution of ASFV in Asian countries and their divergence from prototype Georgia/2007 ASFV.

IVS Young Scientist Award

Medical Virology

Mechanistic analysis of host-protein crosstalks during Japanese encephalitis virus mediated-neuropathogenesis and development of therapeutic and preventive interventions

Swatantra Kumar, Shailendra K Saxena

Centre for Advanced Research (CFAR), Faculty of Medicine, King George’s Medical University (KGMU), Lucknow 226003, India.

shailen@kgmcindia.edu

Abstract

Japanese encephalitis virus (JEV) is a major cause of viral encephalitis in South-East Asia and the western Pacific regions with no specific treatment available. The peripheral infection mostly gets cleared off due to activation of innate immune response, and therefore, the level of viremia is low in the blood. However, JEV infects the central nervous system (CNS) via breaching the blood–brain barrier (BBB) and infects primarily neurons and microglia cells. The helicase and protease domains of NS3 protein have been found to be one of the crucial factors that induce cellular apoptosis via inducing caspaseexpression. Microglia cells are known to act as the virus reservoir during JEV infection which gets activated and adopts the inflammatory state. However, the exact mechanism of neuropathogenesis is poorly understood. Therefore, in the present study we investigated the host-protein crosstalks during JEV induced microglia and neuronal cell models and designed antiviral therapeutics. We have found that JEV induces neuronal cell death via activation of the extrinsic pathway of apoptosis which was marked by overexpression of Caspase-3 and Caspase 8 protein expression. JEV infection was found to induce the expression of CX3CR1 receptors exclusively expressed on microglia cells that showed its activated stage which was further identified by level of TNF-α and IL-6. To further understand the mechanism of action, we performed RNA-Seq of microglia cells infected with JEV. Based on these data, we have designed a novel therapeutic strategy to target the NS3 protein of JE. Collectively, for the first time our data suggests that treatment with novel therapeutics reduces microglia cell activation and significantly reduces neuronal cell death.

Modulation of Autophagy and mTOR signalling pathway by Respiratory Syncytial Virus (RSV) in children suffering from acute lower respiratory tract infections

Authors name: Sarjana Shuchi1, R K Ratho1*, Gursimran Kaur Mohi1, Suresh Kumar2, Subhabrata Sarkar1, Isheeta Jangra.1

1Department of Virology, *Department of Paediatrics, Post Graduate Institute of Medical Education and Research, Chandigarh

*Corresponding author: rathopgi@yahoo.com.

Abstract

Respiratory Syncytial Virus (RSV) has become the most important cause of acute lower respiratory tract infections (ALRTI) in children less than 5 years of age. The virus exists in nature as subtypes A and B which further have multiple genotypes. Autophagy and mTOR signalling pathways are present in host cell which either help in viral propagation or inhibit its growth.

Aims and objectives: To study the molecular phylogenesis of the circulating strains of RSV and to elucidate the gene expression of
Autophagy and mTOR signalling pathways in children with acute lower respiratory infection.

Method: Total of 145 nasopharyngeal aspirate (NPA) samples from children suffering from ALRTI between 2 months to 1 year of age were subjected for the detection of RSV and Human Bocavirus (HBoV) DNA by conventional PCR. Representative RSV positive strains were subjected for sequencing. The NPA samples were centrifuged. The cellular RNA was extracted from the pellet of 25 RSV positive cases and 10 control subjects using TRIZOL reagent and were reverse transcribed for the semi-quantitative gene expression of mTOR signalling pathway and autophagy pathway genes. BECN1, NPC1, GABA RAP, ATG3 and ATG5 genes of autophagy and PDPK1, AKT1, mTOR, RICTOR, and TSC1 genes of mTOR signalling pathway were subjected for SYBR green real-time PCR.

Result: Of the 145 samples, 69 samples were RSV RNA positive and 2 samples were positive for HBoV DNA. The representative 30 RSV strains belonged to RSV A subtype and clustered with the novel ON1 genotype which has replaced the previously circulating NA1 genotype. No RSV B subtype was detected. For relative gene expression analysis, β-actin gene was used as internal control. There was significant upregulation in NPC1 and ATG3 autophagy genes while AKT1, mTOR and TSC1 genes of mTOR pathway were significantly downregulated in RSV positive patient except RICTOR gene which was significantly upregulated.

Conclusion: On phylogenetic analysis, all 30 strains isolated in this study clustered with the novel ON1 genotype of RSV A with 72 bp duplication in their hyper-variable region of the G (Glycoprotein) gene. By upregulation of autophagy genes in one hand and suppressing the mTOR signalling genes on other hand, it is apparent that RSV facilitates its survival within the autophagosomes during infection. Thus, the findings have a clarity in understanding the immunopathogenesis of RSV in young children which frequently leads to pneumonia, further aggravating the severity and increased mortality.

Alteration in proteome of SH-SY5Y cells infected with Dengue virus serotypes

Amita Sharma, Ravi Vasanthapuram, Manjunatha MV, Anita Desai Department of Neurovirology, National Institute of Mental Health and Neuroscience (NIMHANS) Bangalore, India.

Background: The association of DENV-2 and DENV-3 with neurological manifestation has been reported frequently. However, understanding of dengue neuro-pathogenesis remains elusive. Proteomics has emerged as one of the widely applied advanced methods to study dynamics of host-virus interactions. In order to gain better understanding of the cross talk between dengue virus serotypes and neural cell proteins, this study was therefore designed to investigate the host cell proteome of DENV serotypes 1–4 infected human neuroblastoma (SHSY5Y) cells using TMT labelling coupled with high-resolution mass spectrometry.

Material/Methods: SH-SY5Y cells were mock-infected or infected with DENV serotypes (1-4). The presence of DENV antigen was detected in SH-SY5Y cells by Immunofluorescence assay (IFA) using DENV serotype specific monoclonal antibodies. Cells were harvested and normalized. The protein was digested enzymatically then peptides were further processed for TMT labelling. Fractionation was done by basic Reverse Phase Liquid Chromatography. Each TMT labelled fraction was analysed in three technical replicates on Orbitrap Fusion. The LCMS/MS data was searched against a combined protein database of Human RefSeq database and Dengue virus serotypes (1-4) (RefSeq, NCBI) using SEQUEST search engine through Proteome Discoverer (Version 2.1.0.81) software. Statistical, gene ontology and pathway analysis were performed on differentially abundant proteins using various bioinformatic tools and software.

Results: The mock infected and DENV infected SH-SY5Y cells were processed for comparative proteomic analysis. A total of 5219 proteins were identified, which were supported by 34,675 peptides. Of the 5051 cellular proteins quantified by TMT labelling, 425 proteins were differentially altered (1.5-fold) in DENV-1 infection, 397 proteins in DENV-2, 241 proteins in DENV-3 and 529 proteins in DENV-4 infection. The range of up regulated proteins was higher in DENV-2 and 3 infected SH-SY5Y cells as compared with DENV-1 and 4 infected cells. A total of 24.5% of proteins were common between the four serotypes of DENV. Of the biological processes identified by gene ontology, defence response to virus was commonly up regulated to cells infected with all four serotypes. This biological process was significantly enriched in DENV-2 and 3 (p 0.000) than in DENV-1 and 4 (p 0.002). Of the molecular functions identified, proteins involved in binding and integrin binding were common to cells infected with all four serotypes. A significant up-regulation of tubulin alpha-3C/D chain was observed in DENV-2, 3 infected neuronal cells as compared to DENV-1 and 4. STRING analysis revealed most common interacting proteins identified was related to immune response.

Conclusion: This study reports for the first time the differential alterations in the proteome of DENV infected human neuronal cells using TMT labelling. The results demonstrated over expression of key proteins identified as marker of disease severity were more evident in DENV-2 and DENV-3 infected cells as compared to DENV-1 and DENV-4. This finding probably explains the more frequent association of DENV-2 and DENV-3 infection.

Plant Virology

Defence arsenal of tomato against ToLCNDV: Autophagy in the action

Ashish Prasad, Manoj Prasad

National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi 110,067.

E-mail: ashishprasad0411@nipgr.ac.in, ashishprasad0411@gmail.com.

Viruses are acellular pathogens that cause several diseases in both plants and animals. They lack essential cellular features required for the central processes of genome replication, transcription and translation thus, are dependent on their hosts and hijack host cellular processes. Plant viruses are known to cause huge agro-economic losses throughout the world every year. Tomato leaf curl New Delhi virus (ToLCNDV) is a member of the Geminiviridae family. It is reported to cause crop loss up to 100% in severely infected fields. It is transmitted from one location to the other with the help of Bemisia tabaci. The role of autophagy in the growth, development and stress responses of plants is poorly understood. In this study, we have shown the antiviral role of selective autophagy during ToLCNDV infection. The viral Transcriptional activator protein (TrAP) interacts with the autophagy-related protein 8f (ATG8f) of Solanum lycopersicum. This leads to the targeting of TrAP to autophagosomes which later fuses with the vacuole where TrAP degradation takes place. Since TrAP is a nucleus localized protein, its export might be mediated by Exportin1 as suggested from our results. In summary, we have shown for the first time how selective autophagy acts as a defence mechanism against ToLCNDV in S. lycopersicum.
Geminivirus Pre-Coat Protein Regulates Symptom Expression And Recovery Through Dynamic Interplay With Host Rna Dependent Rna Polymerase 1 In Nicotiana Tabacum Cv. Xanthi

Ashish Kumar Singh, Saumik Basu, Nibirh Kumar Kushwaha and Supriya Chakraborty.

Affiliation: School of Life Sciences, Jawaharlal Nehru University, New Delhi Address: Molecular Virology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India -110,067; E-mail: aks.ibt@gmail.com.

Introduction: Plants have evolved several strategies to fight against viral pathogens. Transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS) are the predominant cellular defence mechanism acting against virus infection in plants. Viruses have also evolved to encode suppressor proteins to counteract host defence response. Virus-infected plants can occasionally overcome infection through a process termed recovery/remission. Leaf curl diseases caused by begomoviruses are the major threats for cultivation of solanaceous crops worldwide. Tomato leaf curl New Delhi virus (ToLCNDV) and Tomato leaf curl virus (ToLCV) are predominant begomoviruses causing huge crop loss of several vegetable crops in India. In the present study, differential pathogenesis of ToLCV and ToLCNDV was investigated on Nicotiana tabacum plants. Aim: In this study we aimed to elucidate the mechanism involved in the virus-specific symptom recovery on N. tabacum (cv. Xanthi) plants.

Materials and Methods: N. tabacum (cv. Xanthi), N. benthamiana and NtRDR1 overexpression line of N. benthamiana (NbNtRDR1) plants were inoculated with infectious constructs of ToLCV (AY190290 and AY190291), ToLCNDV (U15015 and U15017) and their mutants. Plants were inoculated through stem-prick method using Agrobacterium harbouring infectious clone of viral genome. Viral DNA were detected through Southern blotting and PCR, and quantified. RNA level was detected through quantitative real time PCR and northern blotting. Bisulfite sequencing was performed to investigate the level of viral DNA methylation in virus infected plants.

Results: Infectivity of ToLCV and ToLCNDV was investigated on Nicotiana benthamiana and N. tabacum plants. Both the viruses induced severe symptoms on N. benthamiana plants. However, N. tabacum (cv. Xanthi) plants inoculated with ToLCV exhibited symptom remission unlike ToLCNDV infection. Concurrent to symptom severity, accumulation of virus specific siRNAs enhanced up to two week post inoculation and subsequently declined in ToLCV infected N. tabacum plants. RNA-dependent RNA polymerase 1 (RDR1) is known to be one of the major players in biogenesis of virus-derived siRNAs and for defence against viruses. Our result revealed enhanced level of RDR1 in the recovered leaves of ToLCV infected N. tabacum plants. Further, NtRDR1 over-expression line of N. benthamiana (NbNtRDR1) also showed symptom remission at later stage of ToLCV infection. Enhanced level of methylation of ToLCGV promoter and increased siRNA accumulation was found in the recovered leaves of NbNtRDR1 transgenic plants. ToLCV infection on NbNtRDR1 resulted in symptom recovery similar to the tobacco plants. Furthermore, the pre-coat protein (AV2) mutants of both the viral strains led to symptom recovery on N. benthamiana. AV2 mutant of ToLCNDV failed to induce symptoms on NbNtRDR1 plants.

Conclusion: In conclusion, NtRDR1 promotes methylation of the ToLCV promoter, and contributes to the lower abundance of viral transcripts and enhances levels of siRNAs. AV2 encoded pre-coat protein is the pathogenicity determinant of both the virus and influence the RDR1 level for virus infection. ToLCV-AV2 is inefficient to block NtRDR1 resulted in the symptom recovery on tobacco whereas ToLCNDV-AV2 can block RDR1-mediated antiviral defence.

Veterinary Virology

Interaction of the host cellular protein NDUF4F4 with 2C protein of foot-and-mouth disease virus

Sonalka Mahajan1, Gaurav K. Sharma2, Kavita Bora3, Bramhadev Pattnaik.3

1Division of Biological Standardization, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, 243,122, India; 2Centre for Animal Disease Research and Diagnosis, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, 243,122, India; 3 ICAR-Project Directorate on Foot and Mouth Disease, Muktakeswar, Uttarakhand, 263,138, India *Corresponding Authors: Sonalka Mahajan; sonalkam1@gmail.com.

The 2C protein of foot-and-mouth disease virus (FMDV) is reported to play a critical role in the virus replication complex and modulating the host’s immune response. However, the underlying molecular intricacies of subversion of cellular machinery remains poorly understood thus, emphasizing the need to study 2C-host interactions. We identified the host proteins interacting with the 2C using yeast-two hybrid (Y2H) approach, which is one of the most recognized, high-throughput tools to study protein–protein interactions. The cDNA libraries are indispensable and critical tools for performing protein–protein interaction studies. A high quality Y2H cDNA library from the LFBB cell line was constructed and characterized. The total RNA was extracted from the LFBB cells and the switching mechanism at the 5’ end of RNA template (SMART) technique was employed for the cDNA synthesis. Subsequently, double stranded cDNA was amplified by long-distance PCR, purified and co-transformed with pGADT7-rec vector in yeast strain Y187. The quality parameters of the constructed library were evaluated and library was observed to be of high quality and suitable for screening. Further, the FMDV-2C bait was characterized for autoactivation, toxicity, and expression and was found to be suitable for mating with cDNA library. On preliminary screening a total of 32 interacting host proteins were identified which were reduced to 22 on subsequent confirmation with alternative yeast based assays. Amongst these, NMI/2C interaction has been reported earlier by Wang et al. (2012). We also reported the 2C/MARCH7, an E3 ubiquitin ligase interaction (Mahajan et al., 2021). Here we report the interaction between FMDV 2C and NDUF4F4, NADH:ubiquinone oxidoreductase complex assembly factor 4. NDUF4F4 is an assembly factor of mitochondrial respiratory chain complex I that associates with assembly intermediates of the Q-module. Previously, the mitochondrial respiratory chain has been shown to affect the propagation and pathogenesis of many viruses but no studies have investigated the role of the mitochondrial respiratory chain in the FMDV life cycle. After confirmation of FMDV 2C/NDUF4F4 interaction by yeast based assays, we also confirmed the interaction using mammalian two-hybrid system and immunofluorescence microscopy. This study leads to the identification of novel 2C/NDUF4F4 interaction which enhances our understanding of 2C-host interface though the precise role of NDUFAF4 in FMDV life cycle needs to be further elucidated.

Pathogenicity and immunogenicity of attenuated double deletion mutant (vTOHAR6/eE) of EHV1 vis a vis wild type virus in murine model

Stephanie S. Pradhan*, Venkataramireddy, B. B.C.Bera, Taruna Anand, Aashwina Madhwal, Rhushikesh Khetmalis, Rekha Sansanwal, K. Supriya, Pavulraj, S., B.N.Tripathi and Nitin Virmani.

ICAR-National Research Centre on Equines, Sirsa Raod, Hisar 125 001, Haryana Presenting author: steph josephite@yahoo.com.
Equine herpesvirus 1, an OIE notifiable disease of equines causes significant economic losses by inducing abortions in pregnant mares, respiratory disease, neurological disorders and early neonatal death in foals. It continues to be a challenge as it is capable of evading the immune responses of the host in many ways. Despite widespread vaccinations there has always been difficulty in eradicating EHV1 from a population because of latent infections. With the advent of recombinant technology, novel vaccination strategies entail the use of second generation vaccines that stimulates both humoral and cell mediated immune responses in a way analogous to naturally occurring disease. Recombinant technology has been employed to introduce some clear cut modifications in the genome of viruses for proper and stable attenuation in order to develop recombinant live vaccines. In this context, National Research Centre on Equines has developed a double deletion mutant of EHV1 through deletion of specific genes employing BAC technology. Hence, the present study developed a double deletion mutant of EHV1 through deletion of vaccines. In this context, National Research Centre on Equines has proper and stable attenuation in order to develop recombinant live vaccines. In this context, National Research Centre on Equines has developed a double deletion mutant of EHV1 through deletion of specific genes employing BAC technology. Hence, the present study was carried out to determine the pathogenicity and immunogenicity of double deletion mutant (vTOHIR6/gE) of EHV1 vis à vis wild type (vRaj) in BALB/c mice. For this 3–4 weeks old BALB/c mice were inoculated intranasally with vTOHIR6/gE and vRaj at a dose rate of 107 PFU/mouse followed by a booster on 14th day. Post inoculation, animals were sacrificed on 3, 17, 21 and 28 days to compare the pathology between groups. Infected mice were also monitored daily for the clinical signs and body weight loss. From the experimental procedure, it was observed that mice infected with vRaj showed greatest degree of clinical signs and body weight loss (7-8dpi) as compared to vTOHIR6/gE (3dpi). Pathological lesions were mostly restricted to the lungs wherein the greatest degree of severity was observed at 3dpi in the wild virus group. Most common lesions observed were necrotizing bronchiitis, alveolitis and bronchiolitis accompanied with perivascular and peribronchiolar cuffing. This reduced pathology observed in the mutant group might be attributed to the novel combination of gene deletions wherein genes responsible for viral cellular spread (gE) and maturation and egress (IR6) were removed. Also, vTOHIR6/gE exhibited higher protective efficacy as it stimulated both the arms of adaptive immunity as evidenced by high neutralizing antibody titres and enhanced CD8 responses. Thus, the aforementioned two deletion mutant qualifies to be a good MLV vaccine candidate against EHV1.

The evolution and genetic characterization of low pathogenic avian influenza H9N2 viruses in India

Deeksha S. Tare (presenting author), Shailesh D. Pawar*, Sachin S. Keng, Sadhana S. Kode.

Affiliation: Indian Council of Medical Research-National Institute of Virology, 130/1, Microbial Containment Complex, Sus Road, Pashan, Pune 411,021, Maharashtra *Corresponding author.

Abstracts

Methods: A total of 907 Cloacal/tracheal swabs and poultry drinking water specimens were processed for virus isolation using embryonated chicken eggs, followed by hemagglutination assay. Virus identification was performed using the hemagglutination inhibition assay and RT-PCR. Sanger-based full genome sequencing was performed. Molecular and phylogenetic analyses were done using BioEdit (CDC, Atlanta) and MEGA (v6). Sequence similarity was determined using NCBI BLAST. Selection pressure analysis was performed using the Datamonkey webserver.

Results: A total of 30 LPAI-H9N2 viruses were isolated. Different genotypes of H9N2 viruses were revealed for viruses reported in the years 2004 to 2020, with the possible progenitors being (a) classical G1 lineage, (b) HPAI-H7N3 from Pakistan, (c) HPAI-H5N1 viruses from India; and (d) MidEast B sublineage, indicating reassortment between HPAI and 2 LPAI viruses. The M and NP genes of all the viruses belonged to the classical G1 lineage, PB2, HA and NA belonged to the MidEast B sublineage. The PB1, PA and NS genes showed different combinations of HPAI H7N3 and H5N1 as progenitors. The first human H9N2 from India, A/India/TCM2581/2019 showed a distinct genotype. Molecular analysis revealed several mammalian-associated substitutions in the hemagglutinin and internal genes of the contemporary viruses. The mammalian-associated substitution HA-Q226L was present in all the viruses reported after 2009. They also showed the tribasic cleavage site motif KSKR rather than the previously reported dibasic KSSR/RSRR. The neuraminidase protein of one isolate had a unique two-amino acid deletion in the stalk region. The polymerase genes of contemporary viruses possessed substitutions for enhanced replication/virulence, for example PB2-A588V, PB1-D622G, PA-K356R, showing a shift in mutation pattern. Selection pressure analysis revealed several sites under positive diversifying selection pressure, namely, HA (153, 168 and 198); NA (42, 381); NP (496, 497); and M2 (27-adamantane resistance). The site HA-198 is known for determining binding affinity and replication in mammalian cells.

Conclusion: The contemporary AI viruses reported from India show a gradual shift towards mammalian adaptation. In view of the recently reported human case of AI H9N2, there is an urgent need to carry out human-animal interface studies and active surveillance in the subcontinent.