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Medical Virology

Lead Lectures and Oral Presentations

MVO-1 In Vitro Inhibition of Entero Virus 70 Infection by siRNAs
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Epidemics of acute hemorrhagic Conjunctivitis caused by Entero Viruses 70 is a common problem in Northern India. The infection causes significant morbidity and have several complications. RNAi using siRNAs has good potential in this condition. We studied in vitro inhibition of EV 70 in tissue culture using siRNA against 4 region of viral genome. shRNAs against the 4 genomic regions of EV 70 were designed, synthesized and cloned in p Silencer vector. Conversion to siRNA in vivo in Hep-2 cells were confirmed by northern blotting using specific probes. Inhibition of EV 70 cytopathic effect (CPE) TCID50 in Hep 2 cells at were tested using these 4 siRNAs. Northern blotting confirmed that all the 4 shRNAs were converted to siRNAs in Hep2 cells. Three of the 4 siRNAs developed, inhibited CPE in EV 70 infected Hep2 cells. Three of the siRNAs did inhibit EV 70 replication in in vitro tissue culture system. If the siRNAs are found to be inhibiting viral replication in other assays and in vivo, they will be useful in acute hemorrhagic conjunctivitis caused by EV 70.

MVO-2 Bionomics of Vectors and Their Control
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Malaria, Dengue/Dengue Hemorrhagic fever, Chikungunya and JE are among other Vector Borne Diseases, continue to be of major public health importance in India. Needless to say, prevention and control of Vector Borne Diseases is closely linked to ecological factor and more importantly the bionomics of the vectors. Everything matters while planning the strategy for Prevention and control of Vector Borne Diseases. May it be the breeding habit and habitat, flight range, biting time, preference and survival of the vectors in extreme conditions. Ecology is changing fastly. This is affecting the vectors in their behaviors and mode of transmission. Global warming is another factor which is not only changing the ecology but also influencing the behavior of the mosquitoes responsible for spreading the diseases. In Union Territory Chandigarh the vector control methods range from environmental manipulation, Implementation of civic bylaws strong community participation and intensified Behavior Change and Communication activities. The integrated approach for Prevention and Control of Vector Borne Diseases has resulted into considerable decline in the Vector Borne Diseases in Chandigarh.

MVO-3 Epidemic of Hand, Foot and Mouth Disease in India, 2009–2010: Association of Multiple Enterovirus Serotypes
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Hand, Foot and Mouth disease (HFMD) is a childhood viral infection. The illness is manifested with fever followed by pharyngitis, mouth ulcers and rashes on hands and feet. Viral transmission occurs through direct contact with saliva, feces, respiratory droplets and vesicular fluid of an infected person. Outbreaks of HFMD are known to be caused mainly due to coxsackie virus-A16 (CA-16) and Enterovirus 71 (EV-71). Other EV types, CA-1, CA-6, CA-10, Echo-4 have also been found associated in sporadic cases. During June–October, 2009 and January–February 2010, suspected outbreaks of HFMD have been reported from Kerala, Tamil Nadu of southern India, West Bengal and Orissa states of eastern India. Investigation was carried out to establish viral etiological pathogens associated during the epidemic/using molecular approach. A total of 55 HFMD cases were investigated comprises of 31 from central Kerala, 9 from Tamil Nadu, 8 of West Bengal, 7 of Orissa states from India. Age distribution of HFMD patients ranged from 4 months to 7 years. Clinical features of the
patients showed rashes on face, hand and foot. EV detection carried out in vesicular fluid, stool, serum and throat swab specimens by RT-PCR of 5’NCR gene. Serotyping was carried out by using RT-PCR of viral protein of VP1/2A junction region followed by sequencing and phylogenetic analysis using neighbor-joining-algorithm and Kimura-2 parameter model of MEGA-4 software. Overall EV positivity detected in HFMD patients from Kerala, Tamil Nadu, West Bengal and Orissa states was found to be 51.6%, 66.6%, 62.5% and 71.4% respectively. Typing of VP1 gene sequences indicated presence of CA-6, EV-71, Echo-9 strains in Kerala and CA-16 in West Bengal, Orissa and Tamil Nadu. Phylogenetic analysis indicated CA-6, EV-71, Echo-9 strains showed 94.8–95.7% and 95–94.4% homology with Japanese, Australian and French strains. However, CA-16 strains were closer to Malaysian strains with 91.2–95.6% nucleotide homology. The present study documents the association of multiple types of EV’s i.e., CA-6, EV-71, Echo-9 and CA-16 strains contributing as prime viral pathogens in HFMD epidemics in the reported regions with new emergence of CA-6 circulating strain in Kerala, India.

MVO-4 Investigation of Hepatitis E Outbreak in Tasgaon, Western Maharashtra

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A large waterborne outbreak of viral hepatitis occurred in Tasgaon Town (Maharashtra) between 24 May and 27 June 2009. Clinicohistorical investigations were done along with sanitary survey. Investigations were undertaken between 23rd June 2009 and 8th September 2010. Sera were collected from 162 suspected hepatitis cases and there contacts and tested for anti HEV IgM/IgG antibodies (ELISA) and liver enzymes like Alanine Aminotransferase (ALT). Anti HEV IgM antibodies were detected in 45.7% (74/162), of cases suspected cases. The overall attack rate was 0.7%. Male to female ratio was 2:1. Majority (60.4%) of the cases were in the age group 20–40 years and recovered without any clinical complications. Weekly distribution of cases showed that the majority (79.4%, 116/146) cases occurred between 2nd and 3rd week of June. Dark urine (97.5%), jaundice (93.5%), fatigue (35.9%), abdominal pain (32.6%), anorexia (29.4%), vomiting (26.5%), fever (22.8%), giddiness (14.3%), diarrhoea (12.6%) and arthralgia (3.7%) were the prominent symptoms. Sera collected from 73 antenatal cases (ANCs) showed anti HEV IgM antibody in 3. Affected pregnant women had a normal outcome. A death of 32 year, male hepatitis E case was reported during the outbreak period that had cirrhosis of liver with oesophageal varices. Sanitary survey revealed that water pipelines were laid down in close proximity of sewerage system, and water posts were without tap. These are the likely sources of faecal contamination of water supplies. Among 17 water samples collected from various places, 5 were found to be unfit for drinking based on the routine bacteriological tests conducted at State Public Health Laboratory, Pune. No case occurred after the pipelines were repaired. This typical outbreak of hepatitis E re-emphasizes need for proper water supply/sewage disposal pipelines and adequate maintenance measures.

MVO-5 Real Time PCR in Diagnosis of Dengue

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Dengue and Dengue haemorrhagic fever are amongst the most important challenges in tropical diseases due to their expanding geographic distribution, increasing outbreak frequency, hyperendemicity and evolution of virulence. The global prevalence of dengue has grown dramatically in recent decades. WHO estimates 50–100 million cases of dengue virus infections worldwide every year resulting in 250,000 to 500,000 cases of DHF and 24,000 deaths each year. Public health laboratories require rapid diagnosis of dengue outbreaks for application of measures such as vector control. Laboratory diagnosis of dengue virus infection can be made by the detection of specific virus, viral antigen, genomic sequence and/or antibodies. Currently 3 basic methods used by laboratories for diagnosis of dengue virus infection are virus isolation and characterisation, detection of genomic sequence by nucleic acid amplification technology assay and detection of dengue virus specific antibodies/antigen. Molecular diagnosis based on reverse transcription (RT)-PCR s.a. one step or nested PCR, nucleic acid sequence based amplification (NASBA), or real time RT-PCR, has gradually replaced the virus isolation method as the new standard for the detection of dengue virus in acute phase serum samples. Several PCR protocols for detection have been described that vary in the extraction method, genomic location of primers, specificity, sensitivity and the methods to determine the products and the serotype. PCR-based dengue tests, due to the specificity of amplification, enable a definitive diagnosis and serotyping of the virus. In addition DNA sequencing of the amplification product enables the virus to be genotyped, providing important information on the sources of infection. More recently tests have incorporated fluorogenic probe, so called Taq Man technology for the specific Real Time detection of dengue 1–4 amplicons. Product is detected by a specific oligodeoxy nucleotide probe that is labelled with 6 carboxy-fluorescein (FAM). This technology offers the advantage of being both rapid and potentially quantitative. Second, the detection of product by hybridisation of fluorochrome labelled probes increases specificity. Third, as the product is detected without the need to open the reaction tube, the risk of contamination by product carry over is minimised. The advantages of speed, contamination minimisation and reduced turn around time justify application of this assay over the currently used nested PCR assay. During the period January 2007 to October 2009, Molecular laboratory received 900 samples from patients presenting with acute onset fever for Dengue RT-PCR. 195 (21.6%) samples were tested positive by this method. The disease peaks in the monsoon season with a percentage of 17.5%. Rapid tests, IgM and IgG capture ELISA are popularly used tests for diagnosis of dengue infection. Its utility is limited for diagnosing dengue in convalescence (8–14 days). Specificity is also compromised due to infections with flaviviruses: Japanese encephalitis and Chikungunya. Dengue NS1 Ag ELISA with its cost effectiveness, specificity and sensitivity should be considered as the test of choice for diagnosing Dengue in the acute phase of illness in the developing countries. Molecular diagnosis enables confirmatory diagnosis of Dengue in the acute phase of the illness and is suitable for further typing methods.

MVO-6 Emerging Regulatory Issues in Development and Commercialization of Recombinant Viral Vaccines

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Vaccine development in India, though slow to start, has progressed by leaps and bounds in the past 60 years. It was dependent on imported vaccines but now it is not only self-sufficient in the production of vaccines conforming to international standards with major supplier of the same to UNICEF. The role of Drug authorities is to enhance the public health by assuring the availability of safe and effective
vaccines, allergenic extracts, and other related products. Vaccine development is tightly regulated by a hierarchy of regulatory bodies. Guidelines provided by the Indian Council of Medical Research (ICMR) set the rules of conduct for clinical trials from Phase I to IV studies as well as studies on combination vaccines. These guidelines address ethical issues that arise during a vaccine study. A network of Adverse Drug Reaction (ADR) monitoring centers along with the Adverse Events Following Immunization (AEFI) monitoring program provide the machinery for vaccine pharmacovigilance. Genetic modifications have been developed to develop effective and cheaper vaccines by the use of recombinant technology. To ensure safety of consumers, producers, experimental animals and environment, Governments all over the world are following regulatory mechanisms and guidelines for genetically modified products. As with other industrializing countries undergoing rapid shifts, India clearly recognizes the need to restructure its regulatory system so that its biopharmaceutical industry can compete in international markets. Genetic Engineering Approval Council (GEAC), Recombinant DNA Advisory Committee (RDAC), Review Committee on Genetic Manipulation (RCGM), Institutional Biosafety Committees (IBSC) are responsible for development, commitment for parameters and commercialization of recombinant vaccines. To centralize and coordinate the whole system, Government has taken to form two agencies to regulate the regulation laws to develop recombinant pharmaceuticals products including vaccines. The first is the creation of the National Biotechnology Regulatory Authority (NBRA), under the Department of Biotechnology (DBT), as part of India’s long-term biotech sector development strategy. The second major initiative will affect the entire Indian pharmaceutical industry. This is the replacement of most state, district, and central drug regulatory agencies with a single, central, FDA-style agency, the Central Drug Authority (CDA). The CDA is expected to have separate, semi-autonomous departments for regulation, enforcement, legal, and consumer affairs; biotechnology products; pharmacovigilance and drugs safety; medical devices and diagnostics; imports; quality control; and traditional Indian medicines. It will set up offices throughout India and will be paid for inspection, registration, and license fees. Its enforcement powers will be strengthened by a new law increasing the criminal penalties for illegal clinical trials. In the manufacturing area, though, the country has been tightening the rules and enforcement. An amendment to the regulations, “Schedule M” of the Drug and Cosmetics Act, now specifies the Good Manufacturing Practice (GMP) requirements for factory premises and materials. These requirements were modeled after US FDA regulations, to improve regulatory coordination between Indian and US regulators. India has realized the importance of regulations in pharmaceutical specially in vaccine field but it will take several years to implementation of these. India has coordinated some of its regulatory functions with Western organizations. The US Pharmacopoeia established an office in Hyderabad in 2007. A representative of the Indian pharmaceutical lobby also recently has expressed openness to an expansion of the FDA’s oversight of Indian manufacturing. As India expands its global drug and biologicals production, US and Europe, as the world’s largest drug importers, will likely expand their regulatory support in the development of the country’s regulatory systems.

**MVO-7 Monoclonal Antibody Based Antigen Capture Immunoassay for Detection of Japanese encephalitis Virus Specific Circulating Nonstructural Protein (NS1); Implications for Early Diagnosis**

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Rapid diagnosis of Japanese encephalitis virus (JEV) infections is important for timely clinical management and epidemiological control in areas where multiple flaviviruses are endemic. However, the speed and accuracy of diagnosis must be balanced against test cost and availability, especially in developing countries. An antigen capture enzyme-linked immunosorbent assay (ELISA) for detection of circulating JEV specific nonstructural protein 1 (NS1) was developed by using monoclonal antibodies (MAbs) specific to recombinant (NS1). The applicability of this JEV NS1 antigen capture ELISA for early clinical diagnosis was evaluated with 200 acute phase serum/cerebrospinal fluid (CSF) specimens collected from different epidemics during 2007–2009. JEV NS1 antigen was detected in circulation from day 1 to 18. The sensitivity and specificity of JEV NS1 detection in serum/CSF specimens with reference to reverse transcriptase PCR was 82%, and 98.9%, respectively. No cross-reactions with any of the other closely related members of the genus *Flaviviruses* (Dengue, West Nile, Yellow fever and Saint Louis encephalitis (SLE) viruses) were observed when tested with either clinical specimens or virus cultures. These findings suggested that the reported JEV specific MAbs-based NS1 antigen capture ELISA will be a rapid and reliable tool for early confirmatory diagnosis as well as surveillance JE infections in developing countries.

**MVO-8 Rapid and Real-Time Detection of Swine Flu H1N1 Virus by Novel Reverse Transcription Loop Mediated Isothermal Gene Amplification (RTLAMP) Assay**

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The recent emergence of a novel human influenza A virus (H1N1) poses a serious global health threat. The H1N1 virus has caused a considerable number of deaths within a short duration since its emergence. A two-step single tube accelerated rapid real-time and quantitative Swine flu virus specific H1 RTLAMP assay is reported by targeting the H1 gene of the novel H1N1 hybrid virus. The feasibility of Swine flu H1 RTLAMP for clinical diagnosis was validated with a panel of 239 suspected throat wash samples comprising 116 confirmed positive and 123 confirmed negative cases of ongoing epidemic. The comparative evaluation of H1 specific RTLAMP assay with real-time RT-PCR demonstrated exceptionally higher sensitivity by picking up all the 116 H1N1 positive and 36 additional positive cases amongst the negatives that were sequence confirmed as H1N1. None of the Real-Time RTPCR positive samples were missed by RTLAMP system. The comparative study revealed that RTLAMP was 100-fold more sensitive than RTPCR with a detection limit of 1 copy number. These findings suggested that RTLAMP assay is a valuable tool for rapid, real-time detection as well as quantification of H1N1 virus in acute phase throat swab samples without requiring any sophisticated equipments.

**MVO-9 Management and Treatment of Herpes Simplex Keratitis (HSK)**

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Herpes simplex viral (HSV) keratitis is a common and serious external ocular infection leading to unilateral blindness primarily...
because of its recurrent nature. Despite considerable progress in understanding of the virus at cellular and molecular levels, the proper management of the disease in its different stages is still a dilemma particularly whether to use antiviral or steroids or both. The risk of using steroids with its attendant complications has to be weighed against the risk of progression of the disease if avoiding the use of steroids. This dilemma can be reduced to a considerable extent if basic principles of virology and pathogenesis are kept in mind. This article reviews current concepts of virological and clinical aspects of HSV keratitis to enable a broad understanding of the disease process. It is recognized several influential host factors including the fact that HSK is more common in men than women. It is observed that the ability of HSV to establish latent infection in sensory neurons and possibly cornea, but have as yet been unable to use this knowledge to prevent the disease limitations. Acknowledging limitations may further stimulate application of laboratory knowledge in coping with HSK which constitutes to present major challenge in terms of management.

MVO-10 Study on Effect of Human βHsp90 in Immunity of HCV Core Protein and HBV HbsAg
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There are more than 500 million individuals with hepatitis B and C in the world. In spite of vaccination in the different areas there are several reports about patients who got vaccine before. Also there is not efficient vaccine against of hepatitis C and one of the important problems in vaccine project is development of effective and suitable adjuvant in human vaccines. At present research we applied human βHsp90 protein as adjuvant and chaperon. This protein injected to BalbC mice as adjuvant together with recombinant proteins of HCV core and HBV HbsAg. Then humoral and Cellular immune systems of the mice were studied. Core and HbsAg genes were cloned into pETDuet-1 vector and thermal vector of pGFP-2 was used for human heat shock protein 90 expressions. The different combination of these three proteins was injected to mice and we evaluated the total IgG and IgG2a of mice sera after a week. Two weeks after booster injection, we studied the proliferation and cytokine secretion of spleen, inguinal and popliteal lymph nodes lymphocytes in vitro and ex vivo conditions. So the Core/HbsAg + hsp and Core + HbsAg + hsp complexes induced total IgG and IgG2a secretion. The spleen lymphocytes proliferation were increased equal to serum IgG2a level that was constant in second time bleeding with significant different to complexes with freund’s adjuvant. At first IL-4 and IL-5 cytokines were increased and then decrease of IL-4 meaned no hypersensitivity. The chaperon effect of Hsp90 on structure of core and HbsAg proteins was studied by CD and fluorometer. It could fold the proteins after heating and unfolding.

MVO-12 Novel Influenza A (H1N1) Pandemic: Present Scenario and Future Perspective
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A cyclone of viral infection, Mexico being the epicenter, grabbed the entire globe. World health authorities were on their toes to control the situation. Emergency health policies were formulated but everything but all the efforts were in vain. Within a short span of two and a half months it culminated the pandemic alert level of phase 6 declared by World Health Organization on June 11, 2009. The viral infection spread its wings at every corner of the world. The Indian subcontinent was also not spared by this virus, Pune being the leading affected zone under the risk of progression of the disease if avoiding the use of steroids. This dilemma can be reduced to a considerable extent if basic principles of virology and pathogenesis are kept in mind. The initial capture of HbsAg by the MAb could orientate it such that the same antibody could bind to it as a detection antibody after labeling with out steric hindrance. The development of an immuno-capture ELISA (IC-ELISA) to measure the HbsAg content using a monoclonal antibody (MAb) specific to determinant “a” of HbsAg in the experimental vaccine formulations is being discussed. Murine MAbbs developed against HbsAg, subtype adw2 were found to cross-react with the other subtypes viz. ad and ay too. The MAbbs have been characterized following which, one MAb HBs06 was chosen for developing IC-ELISA format for the quantification of the HbsAg in the final Algel adsorbed vaccines. The unadsorbed HbsAg was used to establish the standard curve of HbsAg/a. The ELISA had a sensitivity of 10 ng/ml of HbsAg. The recovery rate of HbsAg/a was found to be around 70% in the vaccines treated to desorb the antigen from Algel. Twenty seven experimental batches of monovalent Hepatitis B vaccines were analyzed for the HbsAg content, both by IC-ELISA and a commercial kit (AxSym kit, Abbott Laboratories, USA). The statistical analysis of IC-ELISA results indicated that an experimental equation f(x) = 0.0062(x) + 0.184, could precisely estimate the amount of HbsAg in the adsorbed vaccines. The amounts of HbsAg recovered from the adsorbed vaccines as estimated by the IC-ELISA format had a good correlation with the estimates derived from a commercial kit, which is being used by several vaccine manufacturers in India for the quality control of vaccine antigen. The varying amounts of vaccine antigens that could be recovered seemed to depend on the quality of the HbsAg and the methods of HbsAg adsorption to the alum gel during vaccine manufacture.

MVO-11 Immuno-Capture ELISA for Quality Assessment of Recombinant Hepatitis B Vaccine
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Hepatitis B virus (HBV) infection is vaccine preventable global public health problem. All commercially available vaccines contain one or more of the recombinant Hepatitis B envelope protein or surface antigen (HbsAg). Measurement of antigen responsible for immunogenicity of vaccine is central to quality assessment. The problems associated with the use of a polyclonal antibody in an assay with regard to its poorly defined nature and batch-to-batch variation has been mitigated by the use of MAbbs as described in this paper. The initial capture of HbsAg by the MAb could orientate it such that the same antibody could bind to it as a detection antibody after labeling with out steric hindrance. The development of an immuno-capture ELISA (IC-ELISA) to measure the HbsAg content using a monoclonal antibody (MAb) specific to determinant “a” of HbsAg in the experimental vaccine formulations is being discussed. Murine MAbbs developed against HbsAg, subtype adw2 were found to cross-react with the other subtypes viz. ad and ay too. The MAbbs have been characterized following which, one MAb HBs06 was chosen for developing IC-ELISA format for the quantification of the HbsAg in the final Algel adsorbed vaccines. The unadsorbed HbsAg was used to establish the standard curve of HbsAg/a. The ELISA had a sensitivity of 10 ng/ml of HbsAg. The recovery rate of HbsAg/a was found to be around 70% in the vaccines treated to desorb the antigen from Algel. Twenty seven experimental batches of monovalent Hepatitis B vaccines were analyzed for the HbsAg content, both by IC-ELISA and a commercial kit (AxSym kit, Abbott Laboratories, USA). The statistical analysis of IC-ELISA results indicated that an experimental equation f(x) = 0.0062(x) + 0.184, could precisely estimate the amount of HbsAg in the adsorbed vaccines. The amounts of HbsAg recovered from the adsorbed vaccines as estimated by the IC-ELISA format had a good correlation with the estimates derived from a commercial kit, which is being used by several vaccine manufacturers in India for the quality control of vaccine antigen. The varying amounts of vaccine antigens that could be recovered seemed to depend on the quality of the HbsAg and the methods of HbsAg adsorption to the alum gel during vaccine manufacture.
epidemiology of the spread of H1N1 virus. Children of school going age have become victim of this deadly virus as evident from the reporting data generated in the past few weeks. The mortality rate has also been slightly increased. The disease spread in wave pattern and presently the world is passing through the second wave of pandemic with more severity in young and otherwise healthy people with a predilection for lungs leading to viral pneumonia and respiratory failure. Now the pandemic gained hold in the developing world affecting more severely as millions of people live under deprived conditions having multiple health problems, with little access to basic health care. Current data about the pandemic from developed counties need to be very closely watched in relation to Shift in virus sub type, Shift of the highest death rate to youngsters, Successive pandemic waves, Higher transmissibility than seasonal Influenza, and Demographic differences etc. Presently the world appears to be better prepared. Vaccine is available in market in many countries. Even vaccine trials are actively going on in Indian population. Effective antivirals are available. Although till now H1N1 diagnostic centers worked with CDC/WHO recommended H1N1 specific primer, probes with Taqman chemistry by Real Time PCR, efforts on the development of indigenous diagnostics, Vaccines and chemoprophylaxis is going on to have a better combat against this highly infectious virus.

MVO-13 Continued Circulation of A226V Mutant Chikungunya Virus in Coastal Karnataka During 2008 Outbreak

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Chikungunya has resurfaced in the form of unprecedented explosive epidemic in 2006 after a long gap in India affecting 1.39 million of persons. The disease continued for the next two consecutive years affecting 59,535 and 64,548 persons during 2007 and 2008 respectively. The 2008 outbreak being the second largest among these three consecutive years, the information on the etiology and the mutations involved are useful for further control measures. Among the 2008 outbreaks, the Coastal Karnataka accounts for the 41,227 persons. An in-depth investigation of Chikungunya epidemic of Coastal Karnataka, India, 2008 by serology, virus isolation, RT-PCR and genome sequencing revealed the presence and continued circulation of A226V mutant ECSA (East central south African) genotype of Chikungunya virus. The appearance of this mutant virus was found to be associated with higher prevalence of vector Aedes albopictus and the geographical proximity of coastal Karnataka with the adjoining Kerala state. This is the first report of regarding the appearance of the novel mutant virus in coastal Karnataka, during 2008.

MVO-14 Diagnosis of Rota Viral Diarrhoea in Children: A Comparison of PAGE with ELISA

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Viral gastroenteritis occurs in two distinct epidemiological settings namely endemic (Group A rotavirus, Adenoviruses {serotypes – 31,40,41}Astroviruses, etc.) and epidemic (small round structured viruses {SRVs}, rotavirus group B and C). It has been reported that rotavirus accounts for 20–30% of acute diarrhea in children attending hospitals in India. Isolation of Rota virus has a sensitivity of about 500 infectious particles/ml. This level of sensitivity is reached by ELISA with much less labour. The laboratory diagnosis of rotavirus infection is done mainly by ELISA, which require expensive imported commercial kits and reagents as also expensive instruments. Hence, not many laboratories are able to diagnose rotavirus infection. In view of this we undertook to evaluate the reliability of the Polyacrylamide gel electrophoresis (PAGE) technique as developed by Herring et al. using the current study 51 out of 200 samples (25.5%) were positive for rotavirus infection by either PAGE or ELISA methods. The available data highlights the importance of rotavirus as a cause of diarrhea in children, which is severe enough to deserve specialized care. The observed proportion of 25.5% of all diarrhea cases being associated with rotavirus falls within the range of values reported by other workers. The reported positivity varies from 10.5 to 70.7%. In our study a complete concordance of ELISA and PAGE results were observed in 194 (97%) of the 200 tested specimens. This finding closely correlates with the findings of other authors who found a 96.7–97.14% concordance results between ELISA and PAGE methods. Some authors found RNA-PAGE method that is as sensitive and rapid as ELISA for detecting rotavirus In stool samples of cases of diarrhea and some others proposed ELISA is more sensitive than PAGE method fond to be 100% specific. The remaining 6 (3%) samples showed conflicting results. In a lone sample in which the OD value of ELISA test was 0.195, this value was almost at the cutoff level, the possibility of this sample being positive by ELISA test is doubtful. Negative result of the same sample in PAGE method is difficult to explain, the possibility of presence of lot of empty virus particles or due to low concentration of viral RNA in the fecal specimen and insufficient extraction of viral RNA could be possible. On the other hand, 5 of the samples which gave positive results by PAGE method were negative by ELISA test. These 5 samples had a typical 4-2-3-2 RNA pattern. The reason for their being ELISA negative thus remains unexplained, however blocking factors or the presence of inhibitory substance in stools might have been responsible. The samples containing predominantly complete particles can also give false negative results. Since, the group antigen is not exposed. Earlier studies have also reported PAGE to be the most sensitive technique although some are of view that it is laborious procedure. How ever, the PAGE system used in this study was very simple to perform and the results were available on the same day. The main requirement was of trained personnel and proper standardization of the technique. Most reports states that the greatest advantage of PAGE and silver stain method are its lack of ambiguity and the fact that it provides information about viral electropherotypes. The modified PAGE system was thus found to be reliable, simple and rapid, no expensive reagents were required. Locally available reagents from HI media were used. The cost of the chemical for PAGE per specimen was Rs. 24 approximately as compared to Rs. 110 per test by confirmatory ELISA. A locally produced slab gel electrophoresis system with power pack was the only equipment required. This method could be used for the routine diagnosis of rotavirus infection in the laboratory.

MVO-15 Development and Evaluation of a Quantitative Competitive Reverse Transcription Polymerase Chain Reaction (QCR-RT-PCR) for Detection and Quantitation of Chikungunya Virus

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Chikungunya is one of the most important emerging arboviral infections of public health significance. Due to lack of a licensed
vaccine, rapid diagnosis plays an important role in early management of patients. In this study a QC-RT-PCR assay was developed to quantify Chikungunya virus RNA by targeting the conserved region of E1 gene. A competitor molecule containing an internal insertion was generated, that provided a stringent control of the quantification process. The introduction of 10-fold serially diluted competitor in each reaction was further used to determine sensitivity. The applicability of this assay for quantification of Chikungunya virus RNA was evaluated with human clinical samples and the results were compared with Real-time quantitative RT-PCR. The sensitivity of this assay was estimated to be 100 RNA copies per reaction with a dynamic detection range of $10^2$ to $10^{10}$ copies. Specificity was confirmed using closely related alpha and flaviviruses. The comparison of QC-RT-PCR result with real-time RT-PCR revealed 100% concordance. These findings demonstrated that the reported assay is convenient, sensitive and accurate method and has the potential usefulness for clinical diagnosis due to simultaneous detection and quantification of Chikungunya virus in acute-phase serum samples.

MVO-16 MMR Revisited: Need for a Booster
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In India, measles vaccine was introduced as part of expanded programme of immunization in 1985. Measles, mumps and rubella (MMR) vaccine is still not part of the national immunization schedule of India. The Indian association of paediatrics (IAP) recommends measles vaccine at 9 months of age and MMR vaccine at 15–18 months. However, in a recent policy update, IAP committee on immunisation opined that there is a need for a second dose of MMR vaccine for providing adequate immunity against MMR. The aim of the present study was to assess the extent of sero-protection against MMR at 4–6 years of age in children who have received one dose of MMR between 12 and 24 months of age. An attempt has also been made to assess the sero-response to the second dose of MMR vaccine in 4–6 years old children. A total of 106 consecutive children between the ages of 4–6 years who had received MMR vaccine between 12 and 24 months of age and attending the immunization clinic of GTB hospital, Delhi were enrolled. The vaccination status, anthropometry and physical examination findings were recorded. Three ml of venous sample was again withdrawn for estimation of post vaccination antibody titre. It was observed that 20.39%, 87.38% and 75.73% children were seroprotected for MMR respectively after 2.5–4.5 year of receiving first dose of MMR vaccine. Seroprotection rose to 72.62%, 100% and 100% for MMR respectively after 4–6 weeks of receiving second dose of MMR vaccine. Geometric mean concentration of antibody also rose significantly in all three diseases. In view of low seroprevalence of MMR and hence high susceptibility to infection at 4–6 years of age, who have already received MMR vaccine, there is need to boost the immune responses against these three diseases by giving a second dose of MMR vaccine.

MVO-17 Spectrum of Viruses Causing Sexually Transmitted Infections (STIs)
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Baseline information on the epidemiology of viral agents causing STIs and types of risk behaviour of affected persons are essential for any meaningful targeted intervention. The present study documents the pattern of viral STIs in patients attending a tertiary care hospital, correlating the syndromic approach and the laboratory investigations to determine the aetiology. Three hundred consecutive patients attending the STI clinic were diagnosed and categorized according to the syndromic approach of the WHO along with detailed history and demographic data. Majority of the patients were men (53.12%) with a mean age of 24 years. Men received education up to middle school. Half of the female subjects were illiterate. Sixty percent of the patients were married and among these, 19% were regular condom users. First sexual contact at or before 18 years of age was more in men (31% vs. 22.22% in women). Promiscuity was more among male patients who had contact with CSW. Genital Herpes was the commonest viral STI (86/300) followed by genital wart (60/300). Concomitant infection with more than one virus was seen in 35% of patients. HIV was prevalent in 10.3% of STI patients. Hepatitis B, Hepatitis C, Herpes simplex type 1 and Molluscum contagiosum were the other viral agents seen in STI clinic attendees at our centre.

MVO-18 Dengue: An Emerging Virus Disease in India
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Dengue is a life threatening disease in humans caused by Dengue Virus which is a enveloped RNA Virus and it belongs to flavivirus group as one of the Mosquito born virus group as one of the Mosquito born virus disease in India. This disease sometime occurs as epidemics and included in the list of Pyrexia of unknown origin (P.U.O.). This disease currently Prevalent in more than 100 countries world wide and annually 50–100 million people are infected with Dengue virus among which 2.5–5 lakhs cases were Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) which are serious forms of Dengue virus infection and due to this condition 25,000 deaths might occur annually World Wide and approximately 3 million children were hospitalized for the fast 3 decades. This disease is characterized by sudden onset of high fever with sever headache, pain in the back and limbs, lymphadenopathy maculo-papular rash over the skin and retro-bulbar pain. Early Diagnosis can be established with simple and rapid IgG/IgM antibodies detection in the blood samples of the patients based on the Bi-directional immunoassay system for its management and control to reduce morbidity and mortality. Details will be presented.

MVO-19 Investigation of a Chikungunya-like Outbreak in Tirunelveli District, Tamil Nadu, India
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An outbreak of Chikungunya-like illness was reported from certain districts of Tamil Nadu during the last quarter of 2009 with high morbidity. An investigation was carried out at two badly affected places in Tirunelveli district i.e. Melappalayam and Kadainallur to determine the etiological agent. Two hundred twenty seven serum samples were collected from the affected areas with the help of local medical practitioners and screened for CHIKV and dengue IgM.
MVO-20 Investigation of a Suspected Viral Fever Outbreak in Kozhikode District, Kerala, India

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An outbreak of suspected viral fever was reported from certain villages of Kozhikode district, Kerala state in June–July 2009. Preliminary investigations by NIV team demonstrated Chikungunya (CHIK) virus as the main etiological agent with very high incidence in Balussery, Kuttiady, Ulliyeri and Valayam. Fever surveys in 9 rural localities indicated attack rate of 62.5% for CHIK. Attack rates ranged between 20 and 79%, highest in Balussery and Panangad and lowest in Kodenchery. Among the 106 sera samples tested for CHIKV IgM antibodies, 38 (35.8%) were found positive. Entomological surveys revealed the presence of both Aedes aegypti and Ae. albopictus throughout the district. Ae. aegypti was the predominant species encountered in the municipal areas. Single larva survey demonstrated high BI (>50) in four out of nine villages surveyed and BI in general ranged between 12 and 130. The House index and Container index ranged between 12–60 and 1–16 respectively. The investigators also found a few confirmed cases of leptospirosis (n = 7) during the investigation. The high percentage of CHIKV IgM positivity, the clinical symptoms, and the presence of vector mosquitoes has clearly attributed the outbreak to Chikungunya.

MVO-21 Infection Control Measures to Reduce Hcv Seroconversion Among Adult Dialysis Patients in a Tertiary Care Hospital

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Hepatitis C virus (HCV) infection in hemodialysis (HD) is a significant problem associated with high rates of morbidity and mortality. The infection is not only due to the past blood transfusions, but also to nosocomial transmission which is preventable. Molecular biology techniques have provided evidence of nosocomial transmission of HCV within individual HD units. We retrospectively measured the HCV prevalence and seroconversion rates across 2 consecutive years (2007 (17.4%) and 2008 (17.9%)) and prospectively documented a fourfold reduction (4.2%) in these rates among the adult hemodialysis patients in the 3rd consecutive year (2009). Use of dedicated machines, single use of the dialysis tubes, isolation of HCV positive patients, along with strict enforcement of universal precautions were the major factors responsible for the decrease in the incidence in the last year. The observable reduction of seroconversion rates in our study, underlines the importance of enhanced surveillance and infection control procedures in haemodialysis units and suggests opportunities for improved HCV outcomes in these immunocompromised patients.

MVO-22 Pathology of Pandemic (H1N1) 2009

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Pandemic (H1N1) 2009 have emerged in early 2009 and still ongoing worldwide in more than 209 countries and territories where laboratory confirmed cases and deaths have been reported. Until the present there are only few reports describing the pathology of pandemic H1N1 2009. In humans, it has been shown that the main pathological changes associated with 2009 H1N1 infection are localized to the respiratory system where necrotizing bronchiolitis, tracheobronchitis, and diffuse alveolar damage, with variable degrees of pulmonary hemorrhage were observed in fatal cases. Immunohistochemistry revealed presence of viral antigen most commonly in the epithelium of the tracheobronchial tree but also alveolar epithelial cells and macrophages. Bacterial coinfection was also evident similar to past pandemics. Electron microscopy showed presence of virus particle in type II pneumocyte. There was evidence of ongoing pulmonary aberrant immune response in H1N1 infection. The pathological changes in organs other than lungs were mainly due to multiple organ failure. However, sound evidence for replication of influenza virus in extra respiratory tissues is still missing. In animal experiments, early lesions in the lungs included features of bronchopneumonia, mild to moderate necrotizing bronchiolitis, areas of acute alveolar edema, hemorrhage, and neutrophil predominant inflammatory infiltrate. Here we present the pathological findings in post-mortem tissues from fatal cases of pandemic H1N1 studied by light and electron microscopy. All patients tested positive for pandemic H1N1 infection by rRT-PCR on nasopharyngeal swab specimens (as per CDC guidelines). Diffuse alveolar damage was identified as a main histopathological feature in lung specimens. Other morphological changes noted were hyaline membrane formation, intra-alveolar edema, hemorrhage, inflammation, cytopathic effect, hyperplasia of type II pneumocyte, mild to moderate necrotizing bronchiolitis, areas of acute alveolar edema, hemorrhage, and neutrophil predominant inflammatory infiltrate. Here we present the pathological findings in post-mortem tissues from fatal cases of pandemic H1N1 studied by light and electron microscopy. All patients tested positive for pandemic H1N1 infection by rRT-PCR on nasopharyngeal swab specimens (as per CDC guidelines). Diffuse alveolar damage was identified as a main histopathological feature in lung specimens. Other morphological changes noted were hyaline membrane formation, intra-alveolar edema, hemorrhage, inflammation, cytopathic effect, hyperplasia of type II pneumocyte, desquamation and necrosis of bronchiolar epithelium, epithelial synecytium formation, squamous metaplasia, intraalveolar hemosiderin laden and foamy macrophages, and fibrosis. Ultrastructural findings were consistent with these observations. In addition, it also revealed presence of Orthomyxovirus-like particle in type II pneumocyte. Liver histology revealed areas of spotty or focal necrosis. The prominent pathological process of pandemic H1N1 2009 infection is diffuse alveolar damage and histopathological changes correspond with the time sequence of the disease. Further understanding of the pathology of human pandemic (H1N1) 2009 virus infection only can be achieved by directed and careful pathological analysis of additional influenza cases.

MVO-23 Emerging and Re-Emerging Human Viral Diseases in South East Asia

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Many new animal and human viruses are emerging most frequently at the rate of 2–3 viruses every 2 years, for the last 3 decades in many parts of the world. Most of the new viral agents are of RNA types, due to its rapid multiplication and frequent mutation than the DNA viruses. One-third of the viruses are of animal origin and more than 50% of them produce serious types of human diseases, including increased mortality rates. From the year 1967 onwards, when Marburg viral disease emerged in Germany among Veterinary
surgeons performing autopsy on a African green monkey, many exotic viral diseases are being discovered every 2–3 years in some parts of the world and most of them emerged in the African continent and in South East Asian regions. In South East Asia more than 10 new viral diseases have been reported for the last two decades. Many are discovered in Malaysia and in Australian continent. The important viruses emerged are SARS (Corona), Avian Flu (H5N1), Nipah, Hendra, Menangle, Bat Lyssa, Burmah Forest, Rose river, Tiomien and lately the Swine Flu (H1N1). More than 3 viruses are discovered in fruit or insect eating bats in Malaysia and in Australia. Some of the viral diseases are also re-emerge periodically in many countries in S E Asia—the main agents are J E, Dengue, Chikungunya, West Nile and Nipah viruses. The reasons for emergence of new viruses are attributed to many geographical and socio-economic and developmental factors. Some of them are Global warming, migration of birds/animals, increased global travel/tourism, deforestation, dam building, earth-quakes, floods, creating high ways through dense forests, establishing residential building in the forest, poor hygiene, changes in socio-economic conditions and sexual behavior. By these some of the viruses restricted to certain areas are disturbed and get disseminated to new regions and also jumps to newer hosts. Thus certain viruses attain new virulence or some hybrid viruses are created by spontaneous and induced mutations. Unexpected viral diseases lead to increased morbidity and mortality. Unless they are diagnosed in time, they may spread to many more people or to many other neighboring regions. Hence we should establish high standard diagnostic laboratories to identify the newly emerged or the re-emerged viruses. Besides, Virologists have to be trained in advanced diagnostic molecular techniques, facilities to be created for rapid communications and sufficient funds to be provided for meeting various needs in early diagnosis, successful management and control of both emerging and re-emerging viral diseases.

MVO-24 Study of Coxsackievirus Adenovirus Receptor (CAR) Expression in Myocardial Tissues of Dilated Cardiomyopathy Cases

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Myocarditis and dilated cardiomyopathy (DCM) are common causes of morbidity and mortality both in children and adults. The most common viruses involved in myocarditis are coxsackievirus B or adenovirus. Recently, the coxsackievirus and adenovirus receptor (CAR), a common receptor for coxsackieviruses B3, B4 and adenoviruses 2, 5 has been identified. Increased expression of CAR has been reported in patients with DCM suggesting utilization of CAR by these viruses for cell entry. The present study was designed to study the expression of CAR in myocardial tissue of patients with DCM. Formalin fixed myocardial tissues were obtained from autopsy cases. A total of 26 cases of DCM and 20 cases of controls which included non-cardiac (Group-A) and cardiac disease other than DCM (Group-B) were included in the study. Expression of CAR was studied by immunohistochemical staining of myocardial tissue using CAR specific rabbit polyclonal antibody and biotin conjugated secondary antibody. The tissue sections were considered positive when >25% of the cell showed brown color staining by immunohistochemistry (IHC). The CAR positivity in DCM cases was found to be 96% (25/26) as compared to 30% in control group A and 40% in control group B respectively. The CAR positivity was significantly higher in the test group as compared to both the control groups. Further CAR positivity in all the cellular types (myocytes, endothelial cells and interstitial cells) was found significantly higher in test group as compared to both the control groups. The expression of CAR was significantly higher in myocytes as compared to both endothelial and interstitial cells in all the groups. However, no significant difference was observed in CAR positivity between endothelial and interstitial cells. The present study highlights the increased expression of CAR in DCM cases with further significance of CAR expression in myocytes and endothelial cells. This may help further in understanding the tropism of viruses or cellular susceptibility, which in turn will help in appropriate diagnostic and therapeutic approach in management of viral myocarditis and DCM cases.

MVO-25 Dual Infection Studies on Aedes albopictus Mosquito with Respect to Dengue and Chikungunya Viruses

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A dual infection study with dengue and chikungunya viruses was carried out in Aedes albopictus mosquito. The susceptibility for both viruses was checked by intrathoracic inoculation and membrane feeding routes and the indirect fluorescent antibody test. The mosquitoes were singly infected at the initial stages and were later on super infected with the respective viruses. In some experiments simultaneous or mixed infection by both the viruses was also carried out. The detection of virus in singly and dually infected mosquito was checked at periodic intervals during the extrinsic incubation period. The viruses used during infection were of comparable titres to give equal chance of multiplication in mosquito. The results showed that Aedes albopictus was susceptible for dengue and chikungunya virus by different infection routes. The respective viruses multiplied at similar rates in singly and dually infected mosquitoes on different post infection days. Simultaneous infection and multiplication of dengue and chikungunya viruses was detected in dually infected mosquito by both routes of infection.

MVO-26 Bio Safety Measures and Dietary Management in Food Borne Viral Diseases: A Review

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Food Security and Safety vary widely around the world, and reaching these goals is one of the major challenges, raising public concern for the wellbeing of mankind, in particular. Industrialized production and processing as well as improper environmental protection have clearly shown severe limitations such as worldwide contamination of the food chain and water. Contaminated water and food during the processes of production, processing and handling are essentially responsible for food and water borne viral infections/diseases. The cases of viral food borne outbreaks are on the rise, creating a threat to human health. Recent researches indicate that epidemiological studies are meager to focus the frequently contaminated foods and food borne viral diseases. Current paper projects the etiology of select food borne viral diseases, probable reasons for non availability of appropriate methods to detect the viruses responsible for the diseases, routes of water and food borne transmission of enteric viral infections, currently available methods of detection of select viruses and bio safety measures to prevent food borne viral infections. Dietary/Nutritional Management in food borne viral diseases is crucial to control weakness and gastro enteric intolerance due to disease condition and antibiotic therapy. It will principally improve food intake, resulting in better nutritional status leading to optimum immune response.
MVO-27 Ethnomedicine for Jaundice (Viral Hepatitis) from Rayalaseema Region of Andhra Pradesh

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From time immemorial, the Rayalaseema region is inhabited by a large number of tribes like Yanadis, Chenchus, Irulas, Yerukulas, Sugalis, Koyas, Kattunayaka and Gadabas, who possessed good knowledge of home remedies. By enumeration studies it is known that this region is a rich source of medicinal plants and ethno-botanically this region remains under explored. Viral Hepatitis was known to mankind as Kamala (Jaundice) for more than 2,500 years ago as can be seen from medical texts such as Charaka and Sushruta Samhita (400 B.C.). Jaundice is a symptom rather than a disease and is characterised by yellowness of the skin and white of the eyes. Itching is often associated with the symptom. Causes of jaundice are varied, excess of bile from the liver or any damage to the liver which affects the normal excretion of bile pigments may be responsible. The plants and minerals have been utilized as a sources of remedy for jaundice and other human ailments from ancient times. Many herbalists treated jaundice with indigenous herbs which were free from side effect and kept this information as patent medicine. The present paper deals with the ethno-medicinal uses of 37 antiviral hepatitic plant species occurring in Rayalaseema Region of Andhra Pradesh. The paper reveals the methods of preparation and dose of administration of crude drugs, as suggested by tribal and non-tribal herbalists, from the plants which have been found new in comparison with the important published literature on the medicinal and economic plants of the country.

MVO-28 Food Borne Viral Diseases and Their Medical Management

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Food borne viruses are mainly belong to rotaviruses, enteropathogenic viruses, astroviruses, adenoviruses and caliciviruses, causes acute gastroenteritis (AG) which is an important health problem. The frequency of rotavirus as a cause of sporadic cases of AG ranges between 17.3% and 37.4%. Astroviruses cause AG, with a frequency ranging between 2 and 26%. Outbreaks have been described in schools and kindergartens, but also in adults and the elderly. The frequency of identification of adenoviruses 40 and 41 as causes of sporadic AG in non-immuno suppressed children ranges between 0.7% and 31.5%, although there is probably underreporting because the sensitivity of conventional techniques is low. Caliciviruses are separated phylogenetically into two genera: Norovirus and Sapovirus. Norovirus is frequently associated with food- and water-borne outbreaks of AG. It is estimated that 40% of cases of AG due to norovirus are food borne. In Sweden and some regions of the United States, norovirus is the first cause of outbreaks of food borne diseases. Sapovirus outbreaks due to person-to-person and food borne transmission affecting both children and adults have recently been reported in countries such as Canada and Japan. It has been predicted that the importance of diarrhoeal disease, mainly due to contaminated food and water, as a cause of death will decline worldwide. Evidence for such a downward trend is limited. This prediction presumes that improvements in the production and retail of microbiologically safe food will be sustained in the developed world and, moreover, will be rolled out to those countries of the developing world increasingly producing food for a global market. Sustaining food safety standards will depend on constant vigilance maintained by monitoring and surveillance but, with the rising importance of other food-related issues, such as food security, obesity and climate change, competition for resources in the future to enable this may be fierce. In addition the pathogen populations relevant to food safety are not static. Food is an excellent vehicle by which many pathogens (bacteria, viruses/prions and parasites) can reach an appropriate colonization site in a new host. Although food production practices change, the well-recognized food-borne pathogens, such as Salmonella spp. and Escherichia coli, seem able to evolve to exploit novel opportunities, for example fresh produce and even generate new public health challenges, for example antimicrobial resistance. In addition, previously unknown food-borne pathogens, many of which are zoonotic, are constantly emerging. Awareness and surveillance of viral food-borne pathogens is generally poor but emphasis is placed on Norovirus, Hepatitis A, rotaviruses and newly emerging viruses such as SARS. It is clear that one overall challenge is the generation and maintenance of constructive dialogue and collaboration between public health, veterinary and food safety experts, bringing together multidisciplinary skills and multi-pathogen expertise. Such collaboration is essential to monitor changing trends in the well-recognized diseases and detect emerging pathogens. It is also necessary to understand the multiple interactions between these pathogens and their environments during transmission along the food chain in order to develop effective prevention and control strategies.

MVO-29 Oncolytic Virus

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The first oncolytic virus to be approved by a regulatory agency was a genetically modified adenovirus named H101 by Shanghai Sunway Biotech. It gained regulatory approval in 2005 from China’s State Food and Drug Administration (SFDA) for the treatment of head and neck cancer. Virus gene therapy has never been used successfully against cancer, mainly due to poor transduction of cells. This problem is solved by oncolytic viruses. The use of viral agents to treat cancer is now a real possibility, and several very promising advances have been made, e.g. ONYX-O15 AND MV-ERV. The most advance oncolytic virus, based on herpes simplex is OncoVEX GM-CSF which is in Phase 3 clinical trails in melanoma and head and neck cancer. Oncolytic viruses have the potential to solve the problem of selectively killing cancer cells. However, altering the host range or tissue specificity of any virus has significant safety implications. In vitro characterization on a variety of human tumor cells of different tissue origins showed that the plaques from this virus (Fu-10) are phenotypically unique and are significantly larger than those from the parental G207 virus, a well-characterized oncolytic HSV lacking fusogenic function. Furthermore, the syncytial formation caused by this virus depended on HSV replication, indicating that cell membrane fusion will only occur in dividing cells (such as tumor cells) where the virus can undergo a full infection cycle but not in normal cells where the viral replication is restricted. The virus should be able to tolerate storage and production at high titres. There are two main approaches for generating tumour selectivity: transductional and non-transductional targeting. Transductional targeting involves modifying the specificity of viral coat protein, thus increasing entry into target cells while reducing entry to non-target cells. Non-transductional targeting involves altering the genome of the virus. Transductional approach to tumour selectivity has mainly focused on adenoviruses, although it is entirely viable with other viruses. However, it should be
recognized that increasing the host tissue range of a virus has serious safety implications. The most commonly used group of adenoviruses is serotype 5 (Ad5), whose binding to host cells is initiated by interactions between the cellular coxsackievirus and adenovirus receptor (CAR), and the knob domain of the adenovirus coat protein trimer. Bi-specific adapter molecules can be administered along with the virus to redirect viral coat protein tropism. These molecules are fusion proteins that are made up of an antibody raised against the knob domain of the adenovirus coat protein, fused to a natural ligand for a cell-surface receptor. This method involves genetically modifying the fiber knob domain of the viral coat protein to alter its specificity. Wickham et al. (2003) added short peptides to the C-terminal end of coat protein, which successfully alter viral tropism. Transcriptional targeting places an essential viral gene under the control of a tumour-specific promoter, meaning the gene is only expressed in cell types where all the transcription factors required for promoter function are active. Cancer cells and virus-affected cells have similar alterations in their cell signaling pathways, particularly those that govern progression through the cell cycle. Double targeting with both transcriptional and non-transcriptional targeting methods is more effective than any one form of targeting alone. Viruses can be used as vectors for delivery of suicide genes, encoding enzymes that can metabolise a separately administered non-toxic pro-drug into a potent cytotoxin, which can diffuse to and kill neighboring cells. Angiogenesis (blood vessel formation) is an essential part of the formation of large tumour masses. Angiogenesis can be inhibited by the expression of several genes, which can be delivered to cancer cells in viral vectors, resulting in suppression of angiogenesis, and oxygen starvation in the tumour. Cytotoxic T-cell responses directed against virus-infected cells have been identified as an important factor in tumour necrosis. However, since viruses are normal human pathogens, they induce an immune response, which reduces the effectiveness of viruses. VSV infects insects as well as live stock, where it causes a relatively localized and non-fatal illness. These (interferons) molecules activate genetic anti viral defense programs that protect cells from infection and prevent spread of the virus. Poliovirus is a natural neuropathogen, making it the obvious choice for selective replication in tumours derived from neuronal cells. Gromeier et al. (2000) replaced the normal poliovirus IRES with a rhinovirus IRES, altering tissue specificity. In science fiction, the concept of an oncolytic virus was first introduced to the public in Jack Williamson's novel *Dragon's Island*, published in 1951, although Williamson's imaginary virus was based on a bacteriophage rather than a mammalian virus. Dragon's island is also known for being the source of the term "genetic engineering".

**Veterinary Virolology**

**Lead Lectures and Oral Presentations**

**VVO-1 Inhibition of Rabies Virus Multiplication by Small Interfering RNAs (siRNA) Targeting Rabies Virus Polymerase (L) Gene Delivered Through Adenoviral Vector**

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Rabies is one of the oldest and most fatal viral diseases still having considerable public health importance. Clinical rabies is incurable and there is a need to develop effective anti-rabies therapeutic. Small interfering RNAs (siRNAs) targeting indispensible viral genes are considered to be effective means of suppressing virus multiplication in vitro and in vivo. In the present study we investigated the effect of siRNAs targeting rabies virus polymerase (L) gene on virus multiplication in vitro and in vivo. The siRNA sequences along with loop sequences were cloned into adenoviral transfer vector and cotransfected with adenovirus backbone plasmid into HEK-293 cells for homologous recombination to produce recombinant adenovirus encoding small hairpin RNAs (shRNAs) targeting rabies virus L gene (rAdV-L). To analyse the effectiveness of these siRNAs targeting rabies virus L gene, the BHK-21 cells expressing siRNAs in shRNA form were produced by transduction of cells with rAdV-L. The transduced BHK-21 cells expressing siRNAs were infected with rabies virus PV-11 strain. There was reduction in rabies virus multiplication as analysed by reduction in fluorescent foci forming unit (ffu) count by 51.85% (70 ffu in BHK-21 cells expressing siRNA-L compared to 135 ffu in BHK-21 cells expressing negative siRNA). The expression of L gene mRNA was reduced by 16.11-fold in rabies virus infected rAdV-L transduced cells compared to rAdV-Neg transduced cells (Negative control) as detected using real-time PCR. After analyzing the effectiveness of rAdV-L in vitro, its effectiveness was also evaluated in vivo in mice after virulent rabies challenge. The mice were inoculated with 10^7 plaque forming units (PFU) of rAdV-L in masseter muscle (i/m route) and challenged with 15 LD50 rabies virus challenge virus standard (CVS) strain. The results indicated 50% protection with improved median survival from 7 to 11 days compared with group of mice treated with rAdV-Neg. The results of this study indicated that siRNAs targeting rabies virus polymerase (L) gene delivered through adenoviral vector inhibited rabies virus multiplication in vitro and in vivo.

**VVO-2 Highly Versatile Baculovirus Expression System for Making Virus Like Particles (VLPs) Vaccine: An Example Of Bluetongue Virus Subunit Vaccine**

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We have developed a new strategy for constructing *Autographa californica* multiple nuclear polyhedrosis (ACMNPV) baculovirus genome that produce Virus like particles (VLPs) of Bluetongue virus (BTV) in the infected insect cells. A recombinant baculovirus genome carrying four BTV structural genes (VP2, VP3, VP5 and VP7) at different loci was produced. The BTV–VLPs representing BTV-1, 2 and 4 were successfully produced and purified from the infected Spodoptera frugiperda (SF-9) cells using these recombinant baculovirus. The morphology of the VLPs was validated by electron microscopy in comparison to the authentic BT virions. The VLPs produced here were stable and were highly immunogenic with intact outer layer which is rapidly lost during normal infection of BTV. These BTV–VLPs elicited long lasting protective immunity in vaccinated sheep against virulent virus challenge. With the use of BTV–VLPs it was also possible to differentiate the infected and vaccinated animals (DIVA). VLP-based BTV vaccine has potential advantages with regard to controlling the spread of BTV with multiple serotypes. It is possible to produce milligram quantities of correctly folded and processed protein complexes using this Baculovirus expression system and hence it is a more promising system for producing new generation vaccines like VLP subunit vaccine against any viral diseases in large scale.
**VVO-3 Molecular Characterization of Camelpox Virus from Outbreaks in Rajasthan in 2008–2009**

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The camelpox virus (CMLV), the etiological agent of camelpox belongs to the family Poxviridae, subfamily Chordopoxvirinae, under the genus *Orthopoxvirus*. The disease primarily causes skin eruptions in addition to severe generalized exanthema affecting mostly young camels of 2–3 years of age. The CMLV outbreaks occur frequently in the North-Central regions of the country. This report describes outbreaks of CMLV in camels housed in Border Security Force (BSF) camps at Jaisalmer and Barmer districts of Rajasthan and their molecular characterization. The first camelpox outbreak was recorded during the second week of December 2008 in Sector Head Quarter (SHQ) of Border Security Force (BSF), Bikaner followed by subsequent outbreaks of the disease in Jaisalmer and Barmer districts of Rajasthan during April–May 2009. The possible cause of spread of the disease could be attributed to the hordes of infected camels in surrounding villages. Biological samples (blood, scabs and swabs) were collected from infected animals for identification and characterization of the etiological agent. The disease was confirmed on the basis of isolation of the virus in vero cell line, detection of the CMLV antibodies by serum neutralization test and amplification of CMLV specific 243 bp fragment of C18L gene by conventional as well as real-time PCR. Three full-length genes (A27L, H3L and D8L) and one partial gene (C18L) of the isolated viruses were PCR amplified using published primers. The amplicons were cloned and sequenced. The sequences were submitted to GenBank. The ORFs (A27L-333 bp, H3L-975 bp, and D8L-915 bp) were aligned with *Orthopoxvirus* sequences available in the database using NCBI BLAST. CMLV isolates shared 97.7–100% sequence identity among themselves at the nucleotide and amino acid level. Furthermore, the phylogenetic analysis based on the amino acid sequences of all the three genes, followed a similar branching pattern with CMLV isolates clustering closely with variola virus (VARV) and vaccinia virus (VACV). The close relatedness between the viruses triggers concerns over the possibility of conversion of CMLV, which could result in the development of a new human pathogen.

**VVO-4 Protective Immune Response Against PPR, Goat Pox and ORF Viruses in Goats Vaccinated with Triple Vaccine Containing the Thermo Stable PPR, Highly Attenuated Goatpox and Attenuated ORF Viruses**

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*Peste des petits ruminants* (PPR), goatpox and ORF are OIE notifiable diseases of small ruminants especially goat and sheep. These diseases are economically important, in enzootic countries like India and cause significant loss and are major constraints in the productivity. Considering the geographical distribution of PPR, goat pox and ORF infections and prevalence of mixed infection, in the present study, safety and potency of the experimental triple vaccine comprising attenuated strains of thermostable-PPR virus (PPRV Jhansi, P-50) grown at 40°C, high passaged goat poxvirus (GTPV Uttarkashi, P100) and attenuated ORF virus (ORFV Mukteswar, P51) was evaluated in sub-Himalayan local hill goats. Goats simultaneously immunized with 1 ml of vaccine consisting of either 10^3 TCID50 or 10^5 TCID50 of each of PPRV, GTPV and ORFV were monitored for clinical and serological responses for a period of 3–4 weeks post-immunization (pi) and post challenge (pc). Specific immune responses i.e., antibodies directed to PPRV, GTPV and ORFV could be demonstrated by PPR competitive ELISA kit and capripox indirect ELISA, SNT, respectively following immunization. All the immunized animals resisted infections when challenged with virulent strains of either GTPV or PPRV or ORFV on day 28 dpi, while in contact control animals developed characteristic signs of respective disease. Further, PPR viral antigen could be detected by using PPR sandwich ELISA kit in the excretions (nasal, ocular and oral swab materials) of unvaccinated control animals after challenge but not from any of the immunized goats. Triple vaccine was found safe at dose as higher as 10^5 TCID50 and induced protective immune response even at lower dose (10^2 TCID50) in goats, which was evident from sero-conversion as well as challenge studies. The study indicated that these viruses are compatible and did not interfere with each other in eliciting immune response, paving the feasibility of use of this triple vaccine in combating these infections simultaneously.

**VVO-5 Occurrence of Classical Swine Fever in Pigs in Assam During 2005–2009**

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Classical swine fever (CSF) is enzootic in Assam and other North Eastern States of India. During April 2005 to December 2009, 2215 tissue samples from 1408 pigs slaughtered for human consumption and 172 samples from pigs having the symptoms and lesions of CSF from different districts of Assam were examined for the presence of CSF virus by sandwich ELISA. Percentage of the tissue samples positive for CSF virus from the slaughtered and diseased pigs were 24.51 and 88.95 respectively. The highest number of slaughtered pigs from Sonitpur district followed by Baksa and Morigaon districts were positive for CSF virus antigen. Presence of the viral antigen was detected in kidney, spleen, tonsil, lymphnode and intestine of the slaughtered pigs. Pigs slaughtered in different months were also found positive for the viral antigen. All the ELISA positive samples were negative for bovine viral diarrhea virus antigen in the commercially available ELISA kit. Isolation of CSF virus could be made in PK-15 cell line from the samples selected at random from the different districts and phenotypic characterization of the isolated virus was done by immunoperoxidase test using CSF virus specific monoclonal antibodies against E2 protein. The year wise occurrence of CSF and a CSF outbreak that occurred in organized pig herds during 2009 have been described. The study showed the increasing incidence of CSF in pigs in Assam and appropriate control and preventive measures for the disease are essential for the growth of pig husbandry sector in Assam in particular and the country as a whole.

**VVO-6 Genetic Characterization and Evolutionary Lineage Analysis of Toll-like Receptor 7 of Bos grunniens (Yak) from Himalayan Region of India**

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Toll-like receptors (TLRs), primary sensors of microbial origin, play a crucial role in the innate immunity. Till now 13 mammalian TLRs have been identified, while there is no information available on TLRs of Yak. This study is part of World Bank Funded-ICAR project. Yak, named *Bos grunniens* for its distinctive vocalization and relationship with cattle, is natural habitat of extremely cold environment. When these animals comes to a lower altitude grazing land, adjacent to villages, become susceptible to the diseases of cattle, buffalo etc. Thus, present study was undertaken to with genetic characterization and evolutionary lineage analysis of Yak TLRs. We worked on TLR7 gene, which plays an important role in recognition of ssRNA viruses. Total RNA was extracted from mitogen stimulated PBMCs of Yak. The RT-PCR conditions were standardized for full length amplification of TLR gene 7 using specific self designed primers. The expected amplicon of 3559bps was obtained. It was cloned in pGEM-T-Easy vector followed by transformation in *E. coli* Top10 strain. The recombinant clones were screened, picked up for plasmid isolation and final release of TLR7 was confirmed by restriction digestion. The cloned TLR7 product was sequenced and analyzed for the nucleotide and deduced amino acid sequences, and 3D structure analysis. The results revealed that Yak shows more than 98% sequence homology with other *Bos indicus* breeds and *Bos taurus* breeds. However, identity was less than 88% with other animal species (equine, murine, feline, canine etc.). The evolutionary lineage findings cluster Yak more closely with bovine species. Point mutations revealed changes at 25 nucleotide positions with corresponding amino acid change at 15 positions. SMART analysis of Yak protein domain architecture revealed Toll-Interleukin 1 receptor (TIR), Leucine rich repeats (LRR) and signal peptide region. The variations in Yak mainly lie in the LRR region. Homology modeling revealed horse shoe shaped structure with 5 alpha helix. The additional alpha helix present in Bos indicus was not detected in Yak. The present study shows existence of genetic variability in TLR7 gene of Yak, in particular the LRR region, which plays an important role in the pathogen recognition and the evolutionary lineage analyses shows its closeness with other bovine species.

**VVO-7 Emerging Viral Diseases of Fish and Shrimp**

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In this new millennium, aquatic animal health management strategies in Asia expanded and adjusted to the current disease problems faced by the aquaculture sector. This presentation will briefly discuss some of the most serious trans-boundary pathogens affecting Asian aquaculture including a newly emerging disease and highlight recent regional and national efforts on responsible health management for mitigating the risks associated with aquatic animal movement. A regional approach is fundamental since many countries share common social, economic, industrial, environmental, biological and geographical characteristics. Capacity and awareness building on aquatic animal epidemiology, science-based risk analysis for aquatic animal transfers, surveillance and disease reporting, disease zoning and establishment of aquatic animal health information systems to support development of national disease control programs and emergency response to disease outbreaks are needed. Molecular diagnostics with emphasis towards standardization and harmonization, inter-calibration exercises and quality assurance in laboratories, accreditation program and utilization of regional resource centres on aquatic animal health will also be needed. Whilst most of these strategies are directed in support of government policies, implementation will require pro-active involvement, effective cooperation and strategic networking between governments, farmers, researchers, scientists, development and aid agencies, and relevant private sector stakeholders at all levels. Their contributions are essential to the health management process. Generally, aquaculture plays an important role in economy as harvests from natural waters have declined or, at best, remained static in most countries. Fish and shrimp, the main aquaculture product sources, have gained the most attention. Many factors can cause losses in yields of fish products and infectious disease in fish and shrimp is the biggest threat to the fishery industry. Shrimp and fish aquaculture has grown rapidly over several decades to become a major global industry that serves the increasing consumer demand for seafood and has contributed significantly to socio-economic development in many poor coastal communities. However, the ecological disturbances and changes in patterns of trade associated with the development of shrimp and fish farming have presented many of the pre-conditions for the emergence and spread of disease. Shrimp and fish are displaced from their natural environments, provided artificial or alternative feeds, stocked in high density, exposed to stress through changes in water quality and are transported nationally and internationally, either live or as frozen product. These practices have provided opportunities for increased pathogenicity of existing infections, exposure to new pathogens, and the rapid transmission and trans boundary spread of disease. Not surprisingly, a succession of new viral diseases has devastated the production and livelihoods of farmers and their sustaining communities. This review examines the major viral pathogens of farmed shrimp and fish, the likely reasons for their emergence and spread, and the consequences for the structure and operation of the shrimp farming industry. In addition, this review discusses the health management strategies that have been introduced to combat the major pathogens and the reasons that disease continues to have an impact, particularly on poor, small-holder farmers in Asia. Among various causative agents of fish and shrimp diseases such as bacteria, fungi, parasites and so on, viruses are one of the most destructive pathogens. Until now, approximately 60 different viruses have been detected in fish. Some of them are namely infectious pancreatic necrosis virus (IPNV), channel catfish virus disease (CCVD), infectious hematopoietic necrosis (IHN), infectious salmon anemia (ISA) and lymphocystis. Among various causative agents of fish and shrimp diseases such as bacteria, fungi, parasites and so on, viruses are one of the most destructive pathogens. Until now, approximately 60 different viruses have been detected in fish. Some of them are namely infectious pancreatic necrosis virus (IPNV), channel catfish virus disease (CCVD), infectious hematopoietic necrosis (IHN), infectious salmon anemia (ISA) and lymphocystis.

**VVO-8 Cloning and Sequence Analysis of Haemagglutinin gene of Indian Camelpox Virus (CMLV) Isolates**

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Camelpox is a wide-spread infectious viral disease of camels. Camelpox is caused by Orthopoxvirus cameli virus which belongs to genus Orthopoxvirus within family Poxviridae. Based on sequence analysis, it has been determined that the camelpox virus is the most closely related to variola virus, the aetiological agent for small pox. In January 2007, a sporadic outbreak of camelpox occurred in camel herds of Rajasthan. Since then, the incident of camelpox is continuing among the dromedarian camels. Sequence analysis of the most important viral genes will be useful for the molecular epidemiological studies. The Haemagglutinin (HA) gene encodes a glycoprotein that
can be detected on the infected cell plasma membrane and on the tegument covering the outer membrane of extracellular enveloped virions. The Haemagglutinin protein is synthesized late in infection by orthopoxviruses, and no other poxvirus genus encodes such a gene, which makes the presence of antibodies against the HA protein. Keeping this in view, the HA gene of the camelpox virus isolates was amplified from the infected scab materials by PCR and subsequently the amplicon was cloned into pET32-(a) vector for sequence analysis. The sequence analysis revealed that 100% nucleotide and amino acid identity of Indian CMLV with that of Kazakhstan.

VVO-9 Phylogenetic Analysis of Bluetongue Virus Based on the Segment 10 as a Potential Tool to Reveal Virus Movement
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Bluetongue is a non-contagious arthropod-borne disease of domestic and wild animals, and is caused by bluetongue virus (BTV), a member of the Orbivirus genus of the family Reoviridae. The virus causes mild to severe disease in sheep, and can be carried and spread by other animals like cattle, goats and wild ruminants. The virus is transmitted by midges belonging to Culicoides spp. Worldwide, at least 24 serotypes of BTV have been identified based on serum neutralization test. The genome of BTV comprises of ten double stranded RNA segments, each coding for one protein. The segments of different serotypes as well as strains of the virus can independently re assort in host cells, giving rise to new strains. BTV isolates from the same geographic region have been termed as 'topotypes' and initial observation on segment 3 nucleotide sequences identified a correlation between topotypes and genetic information. Later topotyping was proposed based on segment 10, on the premise that the encoding protein NS3, which is involved in virus egress from insect cells, would lead to evolutionary fitness in parallel with the geographic distribution of the different Culicoides species. Further studies attempted to extend this to nucleotide sequence homology in segments 7 and 10, but failed to identify clear cut correlations or any evidence for positive selection. For example, South African isolates were found not to cluster into separate African lineage. In this study, we carried out a more extensive analysis of segment 10 sequences. Our analysis showed no segregation of isolates into topographically distinct groups. Instead we observed topological clustering of the clades, and we attribute this to genetic bottlenecks resulting in genetic drift and founder effect leading to homogenous gene pool in a geographical area. We hypothesize that when a new virus enters a geographical area where local BTV strains are already circulating, the new genes/segments would enter into a bigger gene pool. Consequently, the newer incursions into a heavily endemic area tend to get diluted and disappear from the population because the rate of drift is inversely proportional to the population size, unless they are positively selected. Use of live attenuated vaccine in Israel, Europe, South Africa and USA also led to more homogenous population similar to the vaccine strains due to continuous infusion of the vaccine type genes into the gene pool. We conclude that restriction of specific strains to certain geographical areas could generate uniquely imprinted genotypes which would not only indicate origin but also predict movement of viral strains to new areas.

VVO-10 Viral Diseases of Zoonotic Importance: Indian Context
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Zoonoses are generally defined as animal diseases that are transmissible to humans. They continue to represent an important health hazard in most parts of the world, where they cause considerable expenditure and losses for the health and agricultural sectors. The emergence of these zoonotic diseases are very distinct, hence their prevention and control will require unique strategies, apart from traditional approaches. Such strategies require rebuilding a cadre of trained professionals of several medical and biologic sciences. The article discusses virus infections that have significant zoonotic implications for India.

VVO-11 Buffalopox Zoonotic Outbreak Investigations from Western Maharashtra (2009)
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Buffalopox is a contagious viral disease affecting milch buffaloes and rarely, cows, with a morbidity rate up to 80% in the afflicted herd. Although the disease is not responsible for high mortality, it adversely affects the productivity of the animals, resulting in large economic losses. Furthermore, the disease has zoonotic implications, as outbreaks are frequently associated with human infections, particularly in the milkers. The causative agent, buffalopox virus (BPXV), is closely related to vaccinia virus. The outbreaks of febrile rash illness among humans and buffaloes were investigated in the villages of districts Solapur and Kolhapur of Western Maharashtra. Clinico-epidemiological investigations of humans and buffaloes were carried out and representative clinical samples were collected respectively. The samples include vesicular fluid, scab, and blood. Laboratory investigations for Buffalo-Pox virus (BPXV) was done by PCR on blood samples, scabs and vesicular fluid. In vitro virus isolation attempts were carried out by using Vero E-6 cells. Negative staining electron microscopy was also employed for detection of virus particles. A total of 166 human cases with pox lesions on hand and other body parts from village Kasegaon, District-Solapur and 185 cases from 20 different villages of Kolhapur district were reported. Besides pox lesions patients were having fever, malaise, pain at site of lesion and axillary and inguinal lymphadenopathy. In Kasegaon village, attack rate in human cases was 6.6% and in buffaloes 41.9% (231/551). Whereas in Kolhapur area attack rate in buffaloes was 11.75% (2633/22398). BPXV was confirmed in blood, vesicular fluid and scab specimens from human cases and scab specimen from buffalo by polymerase chain reaction (PCR) method. The BPXV was also isolated from 3 different clinical specimens and further identified by PCR and electron microscopy. Clinical manifestation of the disease in buffaloes from Solapur district was as reported earlier like common pox lesions on teats and udders whereas the buffaloes from Kolhapur district had lesions on hairless parts of ears and on the eyelids with purulent discharge. BPXV from human and buffalo cases showed similarity.

VVO-12 Modern Approaches for Vaccine Development Using Foot and Mouth Disease as Model System
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Vaccines have been made against several diseases and used for controlling the afflictions. However a few of them were not effective for successfully controlling the disease. The reasons for the failure are...
many, the major being, either the pathogen is not completely cleared from the vaccinated animal or it reemerges after changing its antigenic structure, thus making the vaccination programme less effective. In addition to this, emergences of newer diseases such as HIV the development of suitable vaccines have become a challenging task. This is especially true in the case of viral diseases. These challenges have warned the researchers “that protection by vaccination is not that simple and strait forward approach”, and lot need to be understood in terms of host virus interaction and role of environment in perpetuating the disease. So the immediate step that was considered was the environmental safety by way using non infectious materials as vaccines. With the understanding that has been developed in molecular immunology and molecular biology and with the availability of molecular tools that have been developed through recombinant DNA technology the field of vaccinology has changed dramatically to emerge as modern vaccinology. This presentation deals with the modern approaches that are being used to produce effective vaccines in the case of foot and mouth disease of cloven footed animals. The similar approach may be worked out for other viral diseases also. Despite the availability of an inactivated vaccine that is noted to provide solid immunity against the disease over a short period of time, the search for an ideal vaccine, the criteria for which are; safety of the vaccine for environment, easy in its preparation, does not require a cold chain for its storage, provides longer lasting immunity, economically viable and may be able to clear the virus in case of persistent infection is on. The advent of recombinant DNA technology together with the information available on the molecular biology of viruses has enabled to design the development of newer vaccines that can induce strong cellular and humoral responses. The underlying principal in the present vaccine development strategy world over is the virus antigen gene has to be expressed in the tissue and the vaccine backbone has to trigger the immune system for eliciting desired immune response. Bangalore Campus of IVRI has been vigorously pursuing research to develop ideal vaccines for foot and mouth disease keeping above principal in mind to achieve the previously mentioned criteria. The approaches selected are to see that the virus antigen/s replicate transiently in the host. The self replicating vaccines that have been developed are pox virus vectored vaccines, alpha virus replicase based vaccines and FMDV vectored vaccines. The approach and the result obtained so far will be discussed.

VVO-13 Silkworm Viral Diseases and Their Management
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Silkworm, Bombyx mori is affected with various diseases caused by viruses viz., nucleopolyhedrosis (BmNPV), densovirus (BmDNV) and infectious flacherie (BmIFV). Silkworm viral diseases form major constraints for the silk cocoon production in all the sericultural countries. The losses due to silkworm diseases is estimated about 20–40% and among them viral diseases are most common. In sericulture, prophylactic measures play a vital role in the management of silkworm diseases. These include disinfection of silkworm rearing house and appliances, rearing area, rearing surroundings, silkworm egg and body, and rearing bed disinfection associated with maintenance of general hygiene and personnel hygiene. All these activities are generally carried out as rituals by using general disinfectants often with partial success. Recent trends in complete management of silkworm diseases include development of silkworm hybrids evolved from disease resistant/tolerant breeds, effective eco- and user-friendly disinfectants, anti-microbial feed-supplements and use of transgenic silkworms. Biotechnological breakthrough in this regard is through RNA interference (RNAi) approach involving dsRNA mediated nuclear polyhedrosis management and this is presently pursued by APSSRDI, Hindupur in collaboration with Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad.

VVO-14 Analysis of White Spot Syndrome Virus for Possible Genomic Link to Virulence
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White spot syndrome viral infection still continues unabated in the shrimp farms of India. Despite the various management measures, there are incidences of large scale infection causing huge economic losses to the farmers. We have investigated the infected and uninfected shrimp samples for the prevalence of different genogroups of white spot syndrome virus (WSSV) isolates and specific genes for identifying the possible genomic link to virulence of the isolates. Of the 193 samples analysed, 83 were found to be positive for the presence of the virus either in first step (48) or in nested PCR (35). VNTR analysis of ORF94, ORF75 and ORF125 revealed that the strains are genetically different. Multiplex PCR designed to identify the strains based on ORF94, ORF125 and ORF14/15 differentiated genogroups by characteristic fingerprint patterns. Pathogenicity study conducted using Penaeus monodon juveniles with 25 WSSV isolates showed the presence of virulent and low virulent isolates with mortality ranging from 0 to 100%. Investigations on six functional ORFs in these isolates indicated that the virulence could probably be linked to ORF112, ORF113 and ORF115, which are reported to be the class I cytokine receptor, anti apoptosis and transcriptional regulator genes respectively involved in WSSV multiplication.

VVO-15 Replication Competent Mini-Genome Construct of Foot and Mouth Disease Virus (FMDV) Asia I as a Vector for Vaccine Development
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Viral vectors carrying foreign genes as delivery system are gaining importance for development of safe and efficacious vaccines. Their importance lies in the broad host range of the virus and the safety and replicating efficiency as a vector. Pox viruses and Adenoviruses have been successfully used to deliver foreign antigens. However, these vectors have the disadvantage of eliciting antibodies against other virus specific proteins which make them less useful for repeated vaccination. Here we report the development of FMDV as a viral vector. Since the viral genome is small, virus specific proteins are limited. In addition, replication competent nucleic acid ensures that viral infection associated host antibodies have less effect on replication. We have used the genome of Asia I serotype as the backbone. cDNA fragments corresponding to the complete RNA genome were generated through RT-PCR from plaque purified virus, cloned separately in plasmid vector and sequenced. Based on the sequence reports primers were designed and the vector was constructed by linking the left and right half of the genome without the capsid protein genes. Internal BamHI site was created to insert gene of interest. Replication
of the vector was confirmed by the production of synthetic Asia I virus using structural protein genes (P12A) of Asia I. This vector can act as a Self-Replicating RNA Vaccine with desired gene insertion. The developed FMDV based viral vector may be used for the production of chimeric viruses and dual vaccines.

VVO-16 Bluetongue in Indian Native Sheep

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Bluetongue (BT) has become one of the most important sheep diseases of southern India. The disease once confined to the exotic sheep viz Southdown, Rambouillet, Russian Merino and Corriedale, now become established in native sheep of South India causing severe outbreaks in the region. The disease is being reported annually from the south Indian states Andhra Pradesh, Karnataka, Tamil Nadu and Maharashtra, and is causing socioeconomic problems among the sheep farmers. However, the disease has not yet been reported from the North Indian states since few years. Bluetongue outbreaks in native sheep were initially reported in 1981 in Karnataka and in adjoining regions of Maharashtra and Andhra Pradesh with mortality rates ranging from 2% to 50%. Morbidity was as high as 80%. Later, in 1983, BT outbreaks were reported all over Andhra Pradesh with a case fatality rate of 21.9%. From 1985 onwards the outbreaks were noticed regularly in Andhra Pradesh with case fatality rates ranging from 2.37% to 38.14%. A cyclical pattern of the disease was observed with variations in severity of infection. Similar pattern of incidence was also observed in other south Indian states viz. Maharashtra, Tamil Nadu and Karnataka. The disease appears to be more severe in rural flocks than organized farms. Our investigations revealed the Morbidity, mortality and case fatality rates among rural and organised farms as 9.34%, 2.69%, 28.84% and 6.22%, 0.47%, 7.63% respectively. Higher morbidity and mortality in rural areas may be due to stress factors like poor nutrition, parasitic burden, fatigue due to long walks and non availability of veterinary aid. Kulkarni et al. 1992 also reported the severe BT outbreaks in rural areas of Maharashtra with overall morbidity, mortality and case fatality of 32%, 8% and 25% respectively. All the south Indian sheep breeds were found to be susceptible and clinical form of the disease is evident in all of them though Saravanabava (1992) reported variations in susceptibility among the indigenous sheep. Trichy black and Ramnad white sheep were found to be more susceptible than the Vambur and Mecheri sheep of Tamil Nadu. Prevalence of bluetongue in sheep, goat and cattle appears to be high in the region. Serological surveys conducted in Andhra pradesh during 1991 revealed the prevalence of BTV antibodies in sheep (47.5%) goats (43.56%) cattle (33%) and buffaloes (20%). Similar high prevalence of BTV antibodies in sheep and goats were also reported from the other states in the region. Clinical disease has not been recorded in Kerala though BTV antibodies were recorded in sheep (13.76%) and goats (7.10%) (Ravi Sankar 2003). Culicoides are the known biological vectors of BTV. All the culicoides species are not capable of transmitting the BTV. The occurrence of the disease is related to the presence of the competent vectors in the area. Jain et al. (1988) established the involvement of the culicoides in transmitting the BTV by isolating the virus from culicoides at Haryana, the North Indian state. C. imicola and C. oxystoma were found to be prevalent in Andhra Pradesh and Tamil Nadu. Narladenkar et al.(1993) reported the presence of C. schultzei, C. perigrinus and C. octoni in Marathwada region of Maharashtra. Culicoid vectors are significantly affected by the climate and annual variations in the climate reflects the outcome of the disease. The monsoon season (June to Dec) with the temperature ranging from 21.2 to 35.6°C appears to be favourable period for the multiplication of culicoides. The maximum No of outbreaks were recorded during the North East monsoon period (Oct-Dec) followed by South West monsoon period (June to Sep) in the region. However, details on the distribution of the competent vectors, feeding habits and their dynamics in the region is lacking Multiple BTV serotypes were found to be circulating in the region.(Kulkarni and Kulkarni 1984; Janakiraman etal. 1991; Mehrotra et al. 1996) A total of 10 serotypes viz. 1–4, 8, 9, 15, 16, 18 and 23 were identified based on the virus isolations. Sreenivasulu et al. 1999 isolated BTV serotype 2 from an outbreak of BT in native sheep of Andhra Pradesh. BTV serotype 9, 15 and 21 were also isolated from the outbreaks occurred in Andhra Pradesh. Some of the isolates need to be serotyped. Deshmukh and Gujar (1999) isolated BTV type 1 from Maharashtra. Following is the summary of the distribution of BTV serotypes in this region. Clinical picture of BT in native sheep appears to be slightly different, the major difference being that swelling of lips and face was less conspicuous. Mucocutaneous borders appeared to be very sensitive to touch and bleed easily upon handling. The classical signs of cyanosis of tongue and reddening of coronary band are not the common features of the disease in native sheep. The disease was also confirmed by the virus isolation and identification. Clinical disease has not been reported in cattle, buffaloes and goats in spite of high seroprevalence. In conclusion BT is established in native sheep and causes severe economic losses to the farmers. The disease is concentrated in the southern peninsula of the country. The disease is seasonal and is associated with the rain fall. Multiple serotypes appear to be circulating in this region. The BTV serotypes were of virulent in nature as evident by severe outbreaks.

VVO-17 Management of Viral Diseases in Shrimp Culture at East Coast of Andhra Pradesh a Review

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In less than Three decades, the Penaeid shrimp culture industries of the world developed from their experimental beginnings into major industries providing hundreds of thousands of jobs, billions of U.S. dollars in revenue, and augmentation of the world’s food supply with a high value crop. Concomitant with the growth of the shrimp culture industry has been the recognition of the ever increasing importance of disease, especially those caused by infectious agents. In India viral diseases have become an important limiting factor for growth of shrimp aquaculture industry. Although more than 30 different viral pathogens have been identified in different species of shrimp world wide, only a few viruses have identified which are causing disease problems in cultured Tiger shrimps in India, East Coast of Andhra Pradesh, in particular. Diagnostic methods for these pathogens include the traditional methods of morphological pathology (direct light microscopy, histopathology, and Transmission Electron Microscopy), enhancement and bioassay methods, traditional microbiology, and the application of serological methods. While tissue culture is considered to be a standard tool in medical and veterinary diagnostic labs, it has never been developed as a useable, routine diagnostic tool for shrimp pathogens. The need for rapid, sensitive diagnostic methods led to the application of modern biotechnology to Penaeid shrimp disease. The industry now has modern diagnostic genomic probes with nonradioactive labels for viral pathogens like Infectious hypodermal and hematopoietic Necrosis (IHHNV), Hepatopancreatic Creptic Virus (HPV), Taura Syndrome Virus (TSV), White Spot Syndrome Virus (WSSV), Monodon Baculo Virus (MBV), and BP. Highly sensitive detection methods for some pathogens that employ DNA amplification methods based on the polymerase chain reaction (PCR) now exist, and more
PCR methods are being developed for additional agents. These advanced molecular methods promise to provide badly needed diagnostic and research tools to an industry reeling from catastrophic epizootics and which must become poised to go on with the next phase of its development as an industry that must be better able to understand and manage disease. Within this field, shrimp immunology is a key element in establishing strategies for the control of diseases in shrimp aquaculture. Research needs to be directed towards the development of assays to evaluate and monitor the immune state of shrimp. The establishment of regular immune checkups will permit the detection of shrimp immunodeficiencies but also to help monitor and improve environment quality. For this, immune effectors must be first identified and characterised. In the end, however, the assumption may be made that the sustainability of aquaculture will depend on the selection of disease-resistant shrimp, i.e. to develop research in immunology and genomics at the same time. The development of strategies for prophylaxis and control of shrimp diseases could be aided by the establishment of a collaborative network to contribute to progress in basic knowledge of penaeid immunity. However, to improve efficiency, it appears essential also to open this network to complementary research areas related to shrimp pathology, physiology, genetics and environment.

VVO-18 Studies on Antigenic Relationship Between BTV-2, -9 and -15
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Bluetongue is an important viral disease of sheep causing severe economic losses to the farmers. Lack of effective vaccine is the major impediments in controlling the disease. Multiple serotypes were found to be circulating in the state. Attempts are being made to develop the vaccine employing the available serotypes to control the disease. Hence, it is essential to identify the antigenic relationship among the serotypes to identify the candidate vaccine strains to be incorporated in the preparation of vaccine. Reciprocal cross neutralization test was employed to find out the R% values between BTV-2, -9 and -15 which indicated the extent of antigenic relationship between the serotypes. R% value between BTV-2 and BTV-9 was recorded as 2.8 R% value of 3.53 and 2.8 were observed between BTV-2 and -15 and BTV-9 and -15 respectively. The R% values recorded in the present study revealed a weak antigenic relationship between the BTV serotypes. The extent of antigenic relationship between the BTV serotypes was also determined by multiple sequence alignment of the nucleotide and amino acid sequences of the reference BTV serotypes 2, 9 and 15. The sequence analysis of the VP2 gene revealed a homology of 47–53% and 29–41% at the nucleotide and amino acid levels respectively. R% values obtained using reciprocal cross neutralization test with the BTV-2, 9 and 15 serotypes isolated in native sheep of Andhra Pradesh and the genomic analysis of the reference serotypes of BTV-2, 9 and 15 revealed very weak antigenic relationship and were highly divergent.

Plant Virology

Lead Lectures and Oral Presentations

PVO-1 The Rise and Fall of a Nobel Prize Virologist: From 2-4D to Slow Viruses
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In 1939 a 16-year old high school student was hired as a summer helper at the Boyce Thompson Institute in Yonkers, New York. Working for 8 weeks, the brilliant student synthesized 1 g of 2-4D and left. No record of his name was kept and he was forgotten. When the synthesized compound was applied to broad-leafed plants by the plant physiologist Percy Zimmerman, the plants grew so rapidly that they collapsed and died. The first discovered weed killer was patented and the license sold to the Dupont Company. I tried to find out how Zimmerman made his discovery and it took me more than 30 years before I unveiled the mystery. When I met the real discoverer, he was already a famous virologist, D. Carleton Gajdusek, head of the Communicative Disorders, National Institutes of Health, in Bethesda, MD. He completely forgot how his scientific career started and when I reminded him about 2-4D, he requested that I send him the names of those who, in 1939, worked in Yonkers, and helped him in his work. When Gajdusek received the Nobel Prize in 1976, he described in detail his work at the Boyce Thompson Institute, which he had forgotten until I reminded him about it. We became close friends and he gave me 9 unpublished, mimeographed volumes, of his work in New Guinea and Micronesia. There, he described in detail that Kuru disease, transmitted by cannibalism in 3 villages in the highlands of New Guinea, was caused by an infectious agent, which Gajdusek called a “slow virus”. Today we know that Kuru, scrapie of sheep, and the “mad cow disease” are caused by prions. For the work on Kuru, Gajdusek was awarded the Nobel Prize in Medicine and Physiology in 1976. During many years of study on Pacific islands, Gajdusek adopted more than 50 children, whom he brought to the United States. He paid for their schools and college education from his Nobel award and his salary. One of his adopted sons accused him of child abuse. Convicted, he spent a year in jail, before leaving the United States for Europe. There he was accepted everywhere with open arms. He died in Norway in 2008.

PVO-2 A Future Scope for the Management of Viral Diseases of Crops by Systemic Resistance Inducers from Higher Plants
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Plant viruses are estimated to cause economic losses world wide of about $15 billion per annum. The solution for the management of viral diseases of crops lies in the strategies of the integration of several methods viz. chemical, cultural, varietal and botanical, to control both viruses and their vectors. We have reported that a large number of antiviral substances from plants of different families induced systemic resistance in plants against virus infections and protect the crops against infection and spread of virus under field conditions. We for the first time reported that the proteinaceous or and glycoproteinaceous substances isolated from roots of Boerhaavia diffusa, leaves of Azadirachta indica, Clerodendrum aculeatum and a few others have protected the infection of phytopathogenic viruses in many crop plants. Boerhaavia diffusa glycoprotein creates an antiviral state in the host plant that persists for up to several days. If this substance applied on a frequent basis, could maintain a highly non-specific antiviral state and protects the crop against infection. These antiviral substances are of non chemical nature, non hazardous, easily biodegradable, did not leave any residual effect and eco friendly. Besides, their virus inhibitory effect against plant viruses we have demonstrated that these substances could prevent the infection of animal viruses also (Semiliki forest virus and Mouse sarcoma virus).
PVO-3 Perspectives on Engendering Transgenic Resistance Against Viral Diseases in Horticultural Crops

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Diseases especially those by viral pathogens cause greater economic losses in most horticultural crop species throughout the world as compared to agricultural crops. Non-genetic methods of management of these diseases include quarantine measures, eradication of infected plants and weed hosts, crop rotation, use of certified virus-free seed or planting stock and use of pesticides to control insect vector populations implicated in transmission of viruses. However, none of these measures is likely to provide an enduring solution against these diseases especially those caused by viruses due sometimes to the huge expenditure involved, but mostly to the questionable effectiveness and reliability of those methods. As key control pesticides are getting increasingly abandoned, development of alternative methods to control diseases has been a felt-need in the recent past. Though breeding for disease resistance generally provides a reliable security in a long run, introgression of host plant resistance did not materialise in most important crops. Non-availability of an appropriate source of resistance in inter-ferile relatives, linkage to undesirable traits, or often times polygenic nature of such sources of resistance are the stumbling blocks in breeding programs. The limitations of conventional breeding and routine cultural practices prompted the need for the development of other approaches of virus control that could be fully incorporated into traditional methods. In this perspective, the concept of pathogen-derived resistance offers an attractive strategy to evolve newer methods of virus management, by transforming crop plants with nucleotide sequences derived from the pathogen’s genome. An increasing number of molecular characterisation of plant virus genomes and the stable transformation of a number of horticultural crop species have in fact opened an avenue for molecular breeding against virus pathogens. Successful field-testing of genetically modified crop cultivars renders proof of their supremacy over existing cultivars. It also contributes to demonstrate their capability with regard to environmental safety with a view to winning over public concern and scepticism. In general, the eventual commercialisation transgenic lines expressing virus resistance will rely upon a host of factors including their field performance, genetic stability, public acceptance and the resolution of environmental concerns and patent related issues. As such, elaborate field trials and allied studies are now required to adapt genetically engineered horticultural crops expressing virus resistance for their implementation into practical agriculture. A few examples from current research at TNAU, in India or elsewhere will be discussed in this presentation.

PVO-4 Advances in Virus Diagnostics for Management of Viral Diseases in Horticultural Crops

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In recent times there has been greater emphasis on vegetatively propagated crops in India to help diversify the Indian agriculture. Fruit, flower, spice and plantation crops are important vegetatively propagated horticultural crops, which have become a driving force for economic development in several parts of India. However, most of the vegetatively propagated crops are threatened by biotic stress caused by plant pathogens in general and plant viruses in particular. Plant viruses produce specific and non specific symptoms and in some cases no symptoms are produced. Correct identification and diagnosis of viral diseases is first step in the management of any disease including viral diseases. There have been two major breakthroughs in virus diagnostics during last four decades. The first one was serological assay using monoclonal or polyclonal antibodies in enzyme linked immunosorbent assay (ELISA) and the other one was the use of in vitro amplification of DNA in polymerase chain reaction (PCR). A significant development in serological assays has been its simplification in form of user’s friendly quick strip/dip stick method. The one-step lateral-flow (LF) tests have been developed for the on-site detection and identification of several plant viruses. Rapid advancement in virus genome characterization has led to the development of novel approaches of nucleic acid based diagnostics which include conventional PCR, real time PCR, multiplex PCR, micro/macro arrays and bioschips. PCR protocols already exist for many plant viruses of citrus, banana, apple, papaya, vegetables, ornamental and spice crops. A further advancement has led to development of real-time PCR assay which is relatively easy but requires training for diagnosticians. In real-time PCR assays, results can be available within 20 min. The nucleic acid template preparation in PCR has been simplified. Membrane based DNA template protocol and co-isolation of nucleic acid template preparation are novel approaches in PCR detection of virus and virus like pathogens. Since many of the horticultural crops are often infected by more than one virus, their individual detection by PCR is not only expensive but also time consuming. Therefore, multiplex PCR has been developed where in genome of more than one virus could be amplified and detected in the same reaction mixture. Development of nucleic acid based chip is now one of the fastest and recent growing areas in the field of pathogen detection. These nucleic acid based chips have been named as DNA/RNA chips, Biochips, Genechips, Biosensors or DNA arrays. When it comes to applications of microarray technology for plant viruses, it is not too difficult to see the value of a method that could potentially detect a whole range of viruses using a single test. However, microarrays are unlikely to become the only method in use in a diagnostic laboratory.

PVO-5 Elucidation of Etiology of Foorkey Disease of Large Cardamom

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Large Cardamom (Amomum subulatum), family Zingiberaceae is an important cash crops cultivated in the sub-Himalayan mountains of Sikkim and Darjeeling District of West Bengal. The large cardamom is a perennial crop that produces large dark brown/red coloured aromatic capsules at the base near rhizome. India is the largest producer and exporter of large cardamom. The crop is known to be affected by foorkey disease characterized by proliferation of excessive dwarf tillers, which leads to a total loss of yield. Large Cardamom is extensively propagated through sucker, which facilitates spread of the disease. Foorkey is considered one of the major constraints of large cardamom cultivation in India. The disease was known since 1936; however, its etiology remained unaddressed for a long time. In 1964–1968, foorkey was considered to be a viral disease on the basis of transmission by aphid, Pentalonia nigronervosa and Myzomyzus kalimpongensis. Association of specific virus was obscured largely due to lack of visualization of the viroids in electron microscope. In 2004, association of a nanovirus was evident based on the similarity (83%) of replication associated protein gene of bunchy top virus (BBTV). Nanoviruses being multi component ssDNA viruses, polymerase chain reaction (PCR) and rolling circle amplification (RCA) were employed to clone and characterize the
unknown DNA component associated with the foorkay disease. A total of thirteen full-length clones obtained by both PCR and RCA approaches were sequenced, which revealed six novel full-length DNA components encoding putative master-Rep (M-Rep), satellite-Rep (sRep) and coat protein (CP), three DNAs of unknown function (FU1, FU2 and FU3) and a partial DNA component encoding putative nuclear shuttle protein (NSP). All the circular DNA components contained 1079–1134 nucleotides and a potential stem-loop structure. The Rep, sRep and CP components contained one major open reading frame (ORF) on the virus sense strand, whereas, three full-length U1, U2 and U3 DNAs contained no major ORF. Except the sRep, all the DNA components contained strikingly similar stem and loop structure. Rep, sRep and CP components shared maximum sequence similarities (58.7–78.3%) and phylogenetic affinity with that of BBTV and *Abaaca bunchy top virus* in the genus *Babavirus* and a distant relationship with the members in the genus *Nanovirus* under family *Nanoviridae*. Molecular detection of the virus in large cardamom plants was achieved based on PCR using DNA component-specific primers and dot blot hybridization. The study elucidated the etiology of foorkay disease and provided evidence of a new member, Large cardamom bushy dwarf virus under the genus *Babavirus* and family *Nanoviridae*.

### PVO-6 Transmission of Virus Causing Mosaic in Soybean

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To study the transmission of virus causing mosaic symptoms on soybean various transmission studies were carried out. The leaves showing mosaic symptoms from naturally infected soybean plants from field were used as primary inoculums for these studies. The sap inoculation studies indicated that the virus causing mosaic in soybean was transmissible by mechanical means from soybean to soybean and other hosts. In aphid transmission studies it was revealed that the two aphid species viz. *Aphis gossypii* and *Myzus persicae* were found to transmit the virus in nonpersistent manner from soybean to soybean. *Myzus persicae* was found to be more efficient (70%) than *Aphis gossypii* (65%) in transmitting the virus. The virus is found seed borne in soybean to the extent of 30%. The virus produced systemic symptoms on soybean. Systemic symptoms included dark green vein banding with interveinal chlorosis, leaf curling and mosaic with blistering. The systemic symptoms produced by virus upon mechanical inoculation, aphid transmission and seed transmission on soybean were similar to those produced by plants infected naturally. This virus causing mosaic symptoms on soybean was not transmitted by dodder.

### PVO-7 Managing the Risk of Introducing Seed-Transmitted Viruses Associated with Import of Wheat Under WTO Regime

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Wheat (*Triticum aestivum* L.), an important cereal crop grown widely in India is susceptible to 20 viruses. A preliminary risk analysis revealed that *Barley stripe mosaic virus* (BSMV), *High plains virus* and *Wheat streak mosaic virus* (WSMV) are not reported from India and can be introduced through import of seeds. This calls for stringent quarantine processing of imports. As per the Plant Quarantine (Regulation of Import into India) Order, 2003, the National Bureau of Plant Genetic Resources (NBPGR) is empowered for quarantine processing of germplasm including transgenic planting material imported for research purposes into the country. During the last two decades, a total of 49,923 samples of wheat including transgenics were imported from CIMMYT (Mexico), ICARDA (Syria) and many other countries. These were grown in post-entry quarantine nursery each year at NBPGR, New Delhi and the transgenic samples were grown in National Containment Facility of level-4 (CL-4) since its inception to ensure that no viable biological material/pollen/pathogen enters or leaves the facility during quarantine processing of transgenics. In addition, post-entry quarantine inspections of the transgenic wheat grown by indenters are also undertaken by NBPGR quarantine scientists. The plants of the accessions showing virus-like symptoms were further subjected to a combination of more than one virus detection techniques viz., infectivity test under controlled conditions, electron microscopy, enzyme-linked immunosorbent assay and dot immunobinding assay. BSMV and WSMV, which are economically important and yet not reported from India were intercepted in imports from USA. Also, *Maize dwarf mosaic virus* not reported on wheat in India was intercepted and vectors of MDMV are present in India. The infected plants were uprooted and incinerated. The harvest from only virus-free plants was released to the indenters. The present findings highlight the importance of risk analysis and quarantine processing in minimizing/eliminating the risk of introducing destructive exotic viruses/their strains, along with imported planting material. Under the WTO regime the Sanitary and Phytosanitary Agreement stipulates internationally accepted standards for quarantine and phytosanitation to be adopted for global movement of agricultural commodities for trade.

### PVO-8 Virus-Induced Gene Silencing: Using a Modified Vector Derived from Rice tungro bacilliform virus for Gene Silencing in Rice

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Virus-Induced Gene Silencing (VIGS) is a technique in which viral genomes are used, usually after appropriate modifications, for transient gene silencing in plants. The mechanism behind VIGS is the phenomenon called RNA-interference (RNAi), which is widespread in many organisms and is believed to be form of inherent defence system against intracellular pathogens, such as viruses and transposons. Double-stranded RNA or RNA containing strong secondary structures, commonly produced during viral infections, are believed to cause triggering of RNAi, which employs a battery of proteins and nucleoprotein complexes to identify and degrade specific viral transcripts. In VIGS, viral genomes not causing severe symptoms, but which can accumulate and spread efficiently in the host plant are used as vectors in which a host gene is cloned and introduced into the plant. Upon replication, the viral vector triggers RNAi response in the host plant, which also targets the host gene, leading to its silencing and subsequently, the silenced phenotype revealing gene function in vivo. VIGS has been used extensively to study gene functions in dicot plants, such as tobacco, tomato, pea, soybean, etc., using vectors derived from *Tobacco mosaic virus*, *Potato virus X*, *Tobacco rattle virus* and others. For monocot plants, such as barley, maize and rice, there are only two VIGS vectors described; one from *Barley stripe mosaic virus* and another from *Brome mosaic virus*. To develop a versatile VIGS vector for rice, *Rice tungro bacilliform virus* (RTBV), a member of Family Caulimoviridae, Genus Tungroviroid, a virus containing double-stranded circular DNA, was modified by deleting genes not essential for replication and spread of the cloned viral DNA. Upon introduction to rice plants through agroinoculation, the cloned DNA accumulated within 2 weeks, but did not cause symptoms of the
PVO-9 Reference Genes as Internal Control for Studying Barley yellow dwarf virus Infections in Cereals by Quantitative Real-time PCR

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Reference genes are commonly used as an/the endogenous normalisation measure for the relative quantification of target genes. The expression (characteristics) of seven potential reference genes was evaluated in tissues of 180 healthy, physiologically stressed and Barley yellow dwarf virus (BYDV) infected cereal plants. These genes were tested by RT-qPCR and ranked according to the stability of their expression (characteristics) using three different methods (Two-way ANOVA, GeNorm and NormFinder tools). In most cases, the expression (characteristics) of all genes did not depend on the abiotic stress conditions or on the virus infections. All the genes showed significant differences in expression (characteristics) among plant species. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Beta-tubulin (TUBB) and 18S ribosomal RNA (18S rRNA) always ranked as the three most stable genes. On the other hand, Elongation factor-1 alpha (EF1A), Eukaryotic initiation factor 4a (EIF4A4), and 28S ribosomal RNA (28S rRNA) for barley and oat samples; and Beta-tubulin (TUBB) for wheat samples were consistently ranked as the less reliable controls. The BYDV titre was determined in two oat varieties by RT-qPCR through three different quantification approaches. Statistically, there were no significant differences between the absolute and the relative quantification, or between quantification using GAPDH + TUBB + TUBA +18S rRNA and EF1A + EIF4A + 28S rRNA. The geometric average of GAPDH, 18S rRNA, TUBA and TUBB is suitable for normalisation of BYDV quantification in barley and oat tissues. For wheat samples, a combination of GAPDH, 18S rRNA, TUBB, EIF4A and EIF1A is recommended.

PVO-10 Plant Virus Diseases of Quarantine Significance

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Large scale production and import of propagative material poses potential risk of introducing several destructive pathogens particularly viruses and Mycoplasma like organisms in our country. This demands adequate quarantine safe guards such as growing them under approved post entry quarantine facility for specific period so as to facilitate virus detection, thereby curtailing risk. When such facilities are coupled with propagation by tissue culture will ensure virus free propagative plant material. The requirement of nationwide network of post entry quarantine facility working in close collaboration with crop institutions are very much emphasized for considering import of high risk plant genera for agriculture development. Present paper discusses about virus disease of quarantine importance affecting ornamental and fruit plants such as Chrysanthimum, Dahlia, Dianthus, Rosabengalensis, Cattleya, Cymbidium, Dendrobium, Lilium, Citrus, Vitis etc. The paper also discusses on immunodiagnostic methods of detection and methods of obtaining virus free propagative material.

PVO-11 Molecular Mapping of Genes Conferring Resistance to Rice Tungro Virus Disease

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Rice tungro virus disease (RTV) is a devastating viral disease of rice crop. Rice tungro is caused by the joint infection of two unrelated viruses, Rice tungro bacilliform virus (RTBV), containing double-stranded DNA and Rice tungro spherical virus (RTSV), containing single-stranded RNA. RTBV and RTSV, also known as the ‘Tungro virus complex’ are transmitted exclusively by the green leafhopper (GLH), Nephotettix virescens. Rice tungro occurs as epidemics in regular cycles and has been reported in the last 50 years from all the major rice growing regions of India, especially prevalent in the southern and eastern states. Development of the durable resistant varieties to tungro is crucial for the management of the disease. Molecular breeding, involving the use of DNA markers linked to the resistant gene(s) for selection, can overcome the difficulties encountered in conventional resistant breeding programs. For successful marker-assisted selection (MAS), the identification of closely linked markers through the process of gene tagging and mapping is a prerequisite. Attempts have been initiated for identification of tungro resistance genes through molecular mapping and their introgression into the target varieties using marker-assisted selection at DRR, Hyderabad. The inheritance of resistance to rice tungro virus disease was studied in seven resistant rice cultivars with field evaluation at hot spot locations. The microsatellite markers linked to rice tungro resistance in Utri Merah was studied and found that resistance genes were linked to RM 336 on chromosome 7. Through molecular mapping two QTL were identified controlling RTV resistance on chromosomes 7 and 2 in ‘Utri Rajapen’ explaining 40.8% and 21.6% of the phenotypic variance. In variety ‘Vikramarya’, another two QTL for RTV resistance were detected on chromosomes 7 and 1 explaining 18.7% and 16.4% of the phenotypic variance. The closely linked markers identified in this study flanking the gene of interest through mapping will improve the efficiency and precision of introgression programs in marker assisted breeding for RTV resistance. Functional characterization of these QTL for RTV resistance is under progress.

PVO-12 Evaluating Virus Resistance Constructs Against RNA and DNA Viruses Infecting Cassava

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There is only a limited pool of natural virus resistance in Cassava against cassava mosaic geminiviruses and cassava brown streak Ipomovirus hence the development of transgenic resistance in this significant crop might present an option. RNA mediated resistance through the expression of inverted-repeat dsRNA sequences derived from the virus genome and the modification of plant microRNA to
produce antiviral artificial microRNA are strategies that have recently been proven very effective for induction of virus resistance (immunity) against a number of RNA viruses. Results from RNA interference strategies against geminiviruses never resulted in immunity of transgenes. However, it suggest that viral mRNA are targets of RNA silencing and that the success of the strategy depends on the relevance of the target gene in the systemic spread of the virus. We have generated a number of RNA silencing constructs to induce resistance against CBSV and the Indian cassava mosaic viruses ICMV and SLCMV. Due to the serious problems inherent with transformation of cassava and subsequent resistance screening, these constructs were tested for efficiency either by transient—or by transgenic expression in *N. benthamiana*. Complete immunity was reached in transgenic *N. benthamiana* against CBSV using inverted repeat or amiRNA constructs. Using different species of CBSV for resistance screening, immunity was broken, to show the minimum context for broad spectrum resistance. Similarly, highly specific resistance was reached in expression of amiRNA. In contrast, virus resistance against ICMV/SLCMV using single amiRNA constructs was not successful. Results from the experiments to generate virus resistance against CBSV and ICMV/SLCMV will be shown; methods to evaluate efficacy of RNAi gene constructs by transient gene expression in *N. benthamiana* and strategies to develop efficient resistance against RNA and DNA viruses in cassava will be discussed.

**PVO-13 PCR Based Detection of a Virus Causing Leaf Curl Disease on *W. somnifera* in District Aligarh (UP)**

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*Withania somnifera* considered as Indian Ginseng grows prolifically in India, Srilanka and Bangladesh. The plant has tremendous medicinal values used as aphrodisiacs, diuretics and for increase health and longevity. A virus causing leaf curl on *Withania somnifera* in the campus of Aligarh Muslim University, was identified as an isolate of begomovirus on the basis of host range, symptomatology, transmission electron microscopy and PCR. Whitely transmission test was attempted and the disease could be successfully transmitted from *W. somnifera* to *W. somnifera*, *Petunia hybrida*, *Parthenium hysterophorus* and *Vigna unguiculata*. The inoculated *W. somnifera* produced systemic symptoms of leaf curling, vein thickening and vein clearing. Twinned, icosaedral particles were observed by TEM. Positive amplification at ~ 800 bp obtained through PCR, was cloned into a pGEM-T easy cloning vector (Promega, USA). Further analysis of the data to find out the identities and phylogenetic relationship of the virus isolate is under study.

**PVO-14 Virus Indexing of In Vitro Conserved *Allium sativum* L. Germplasm Against Six Viruses**

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The NBPGR, New Delhi has the mandate of plant genetic resources management in India. More than 150 indigenously collected garlic (*Allium sativum* L.) germplasm accessions are being conserved in vitro at National Gene Bank. Out of these, 117 accessions were indexed during 2005–2009 for presence of six associated Allium viruses viz., *Carnation latent virus* (CLV), *Garlic common latent virus* (GCLV), *Leek yellow stripe virus* (LYSV), *Onion yellow dwarf virus* (OYDV), *Shallot latent virus* (SLV) and *Shallot yellow stripe virus* (SYSV) for which commercial antisera kits are available. Leaf samples of all the accessions were taken for indexing from in vitro shoots cultures. Cultures were raised from shoot buds isolated from clones and tested by DAS–ELISA as per the protocol of the kits used and electron microscopy. Results indicate presence of Allium viruses in different accessions as follows: CLV is present in 36, GCLV in 40, LYSV 15, OYDV in 42, SLV 4 and SYSV in 19 accessions. Flexuous rod particles belonging to *Carlavirus, Potyvirus* and *Closterovirus* groups were detected in infected samples by leaf-dip method under electron microscope. A total of 29 accessions were found to be free from any of the six viruses tested by ELISA that can be used as mother plants. The aim of the present investigations was to generate passport data on biotic stress for the accessions indexed and subsequently to be conserved, in vitro.

**PVO-15 First Record of Zucchini yellow mosaic virus and Tomato yellow leaf curl virus on Bitter Gourd (*Momordica charantia L.*) in Northern India**

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Bitter gourd (*Momordica charantia L.*) which is also called bitter melon, balsam apple and balsam pear belongs to family cucurbiteae. It is an important traditional vegetable of nutritive and medicinal value that is cultivated in tropical and sub-tropical Asia, but is considered as a weed host reservoir for viruses in Jamaica. Viral disease-like symptoms were observed occurring naturally on the crops of bitter gourd grown in the fields of Northern India during 2007–2009. An incidence of 78.5% of diseased plants was recorded which showed chlorotic spots and mosaic ranging from mild motting to green blisters along with leaf smalling, leaf and fruit deformations, bud necrosis and stunted growth whereas 20.2% plants exhibited leaf curling alone or in combination with mosaic-type disease. A reduction of 34.5% in fruit yield was recorded in mosaic-like disease which could be attributed to lesser fruit setting due to bud necrosis, smaller fruit size and stunted plant growth. Such plants produced deformed, notched, irregularly shaped fruits wherein pre-mature yellowing and necrosis on the anterior and posteriors ends made 22.4% fruits unfit for marketability. The dwindling yield and production of unmarketable fruits posed a major constraint for profitable cultivation of this economically important crop, thus warranting for studies on etiology and management of these diseases. The mosaic-like disease was transmitted to healthy seedlings of bitter gourd at 2-leaves stage by sap inoculation as well as by aphid viz., *Myzus persicae* Sulz. and *Aphis gossypii* Glov. The leaf curl-like disease could neither be transmitted mechanically nor through aphid vectors but was successfully transmitted by viruliferous whitefly *Bemisia tabaci* Gen. ELISA test was positive with IgG of *Zucchini yellow mosaic virus* (ZYMV) in leaf samples with mosaic-like disease symptoms but negative in samples showing only leaf curl-like symptoms. A positive reaction with tomato yellow leaf curl (*TYLCV*) IgG was obtained with leaf samples taken from plants showing leaf curl-like symptoms alone or in combination with mosaic-like disease. In an attempt for the management of these diseases, 19 genotypes were screened during 2008 and 2009 against these viruses in the field and glasshouse. The results recorded on appearance of symptoms and ELISA showed that none of the screened genotypes were free from both the viral diseases whereas Pusa Do Mausmi, HKH-96, IC 68255 and IC 62837 were quite promising since these showed mild mosaic only and were...
resistant to leaf curl disease till the end of the crop. The present studies on the basis of symptomatology, transmission and ELISA confirmed the association of zucchini yellow mosaic potyvirus and tomato yellow leaf curl begomovirus, the two hitherto new diseases not reported earlier from these north Indian states. However, this is the first report of natural occurrence of ZYMV and TYLCV on bitter gourd from India.

PVO-16 Molecular Identification of Cucumber mosaic virus and Tomato aspermy virus Isolates from Chrysanthemum and Their Possible Management

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Chrysanthemum (Chrysanthemum morifolium) is a popular ornamental plant grown worldwide for beautiful bright blooms of various shape, size, color and long vase life. It has been considered among five commercially important floricultural crops. C. morifolium has been reported as susceptible to Cucumber mosaic virus (CMV), Tomato aspermy virus (TAV), Chrysanthemum virus B (CVB) which caused drastic reduction in yield as well as in market quality of blooms and ultimately affects the floriculture industry of the country. Since chrysanthemum is being propagated by cuttings and the mother stock once infected, act as a source for disease spread in successive generations, therefore, molecular identification and their disease management seems to be essential. The leaf samples from naturally infected chrysanthemum plants exhibiting various symptoms: yellow mosaic, marginal yellowing, vein yellowing, vein banding on leaves, stunting of whole plant and flower deformations were collected from eighteen locations in India during the survey from 2005 to 2008. Infection of CMV and TAV was detected by ELISA and RT-PCR using their specific antiserum and primers, respectively, which indicated the distribution of both the virus in India. The complete RNA3 genome of CMV and TAV isolates were amplified by RT-PCR employing their specific primers, cloned in pGEM-T easy vector and sequenced. The consensus sequence data without ambiguities of both the viruses were deposited to GenBank database (CMV: EF153733 and TAV: EU163411). The CMV isolate under study (EF153733) showed high (99%) nucleotide sequence identities and closest phylogenetic relationships with several strains of CMV of subgroup IB. The analyses of TAV isolate under study (EU163411) revealed high 99–98% nucleotide identities close relationships with V-TAV strain (from Australia). Based on molecular analyses, virus isolates from chrysanthemum were identified as Indian strain of CMV and TAV. Elimination of CMV and TAV was achieved by culturing 0.3 mm long shoot meristem of infected Chrysanthemum cv. Pooja plants on MS medium supplemented with 3.0 mg/l BAP and 0.5 mg/l NAA. The regenerated plants were indexed by DAC-ELISA and confirmed by RT-PCR. A total of 78.1% CMV and TAV-free shootlets were obtained from the regenerated shoot meristem as indexed by DAC-ELISA, of which only 65.6% were found truly virus-free when confirmed by RT-PCR. Out of our efforts total 21 CMV and TAV-free plants could be established in glasshouse which showed better growth and flowering. An another cultivar of Chrysanthemum cv. Kundan was transformed using an expression cassette harboring the coat protein gene of CMV under the control of CaMV 35S constitutive promoter and nos terminator in pROK2 binary vector through Agrobacterium tumefaciens (strain LBA 4404). Transgenic plants were successfully obtained which showed successful integration of CMV-CP transgene as were confirmed by Southern blot analysis for presence of CP gene and copy number. Successful transcription and translation of CP gene was also confirmed by northern blot analysis and western blot immunoassays. The transgenic lines were evaluated by challenge inoculations under glass house conditions which did not show any local or systemic symptoms conferring the resistance against CMV infection. The standardized protocol would help to eliminate the other virus infection from other elite cultivars of chrysanthemum. Development of sensitive diagnostic protocol of RT-PCR using CAV and TAV specific primers would help for indexing of chrysanthemum germplasm bank as well as in search of alternate hosts/vector and search of CMV, TAV-free mother culture for mass propagation.

PVO-17 Development of Virus Resistant Citrus and Rice Through RNA Interference

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Citrus is one of the most important commercial and nutritional fruit crops in the world, hence it needs to be improved to support diverse needs of world population. Genetic manipulation through conventional techniques in Citrus is constrained by various biological limitations comprising long juvenile period, high heterozygosity, sexual incompatibility, nucellar polyembryony and large plant size which hinder cultivar improvement. Further, Citrus spp. are very susceptible to different kinds of insects, pests and diseases. One of the most devastating diseases of citrus is the ‘Tristeza’ disease, caused by a single-stranded citrus tristeza virus (CTV), which causes phenomenal economic damage to citrus industry. Therefore, the main objective of our study is to develop transgenic Citrus sinensis (Sweet orange) plants resistant to CTV. As a prelude to this investigation, initially studies were carried out to optimize protocols for efficient plant regeneration and Agrobacterium-mediated transformation for Nagpur sweet orange, which is a popular and elite citrus cultivar in India. Organogenesis was induced in etiolated epicotyl explants of one-month-old axenically raised polyembryonic seedlings by culturing them in MT medium supplemented with 30 g/l sucrose with varying concentrations of plant hormones. It was found that BAP at 1 mg/l without auxin was best for efficient shoot regeneration in Citrus using epicotyl explants. A 100% regeneration frequency was obtained and multiple shoot formation was obtained from both the cut ends of all the explants. An average of 8.24 well-differentiated shoots per explant were obtained, all of which rooted normally under the influence of 1 mg/l IBA. This improved regeneration protocol was utilized in standardizing Agrobacterium-mediated transformation of citrus using A. tumefaciens strain EHA 105, containing binary plasmid pCAMBIA 2301 that harbors GUS reporter gene and NPT-II plant selection marker gene. One-month-old epicotyl explants infected with over-night grown Agrobacterium (A. tumefaciens strain EHA105) harboring binary plasmid pCAMBIA 2301 that harbors GUS reporter gene and NPT-II plant selection marker gene. One-month-old epicotyl explants infected with over-night grown Agrobacterium (A. tumefaciens strain EHA105) harboring binary plasmid pCAMBIA 2301 that harbors GUS reporter gene and NPT-II plant selection marker gene. One-month-old epicotyl explants infected with over-night grown Agrobacterium (A. tumefaciens strain EHA105) harboring binary plasmid pCAMBIA 2301 that harbors GUS reporter gene and NPT-II plant selection marker gene. One-month-old epicotyl explants infected with over-night grown Agrobacterium (A. tumefaciens strain EHA105) harboring binary plasmid pCAMBIA 2301 that harbors GUS reporter gene and NPT-II plant selection marker gene. One-month-old epicotyl explants infected with over-night grown Agrobacterium (A. tumefaciens strain EHA105) harboring binary plasmid pCAMBIA 2301 that harbors GUS reporter gene and NPT-II plant selection marker gene. One-month-old epicotyl explants infected with over-night grown Agrobacterium (A. tumefaciens strain EHA105) harboring binary plasmid pCAMBIA 2301 that harbors GUS reporter gene and NPT-II plant selection marker gene. One-month-old epicotyl explants infected with over-night grown Agrobacterium (A. tumefaciens strain EHA105) harboring binary plasmid pCAMBIA 2301 that harbors GUS reporter gene and NPT-II plant selection marker gene. One-month-old epicotyl explants infected with over-night grown Agrobacterium (A. tumefaciens strain EHA105) harboring binary plasmid pCAMBIA 2301 that harbors GUS reporter gene and NPT-II plant selection marker gene.
control of maize ubiquitin promoter and ORF IV genes as well as dsRNA construct with ORF-IV gene of ease. The indica rice variety, Pusa Basmati 1 has been transformed via Agrobacterium-mediated transformation with the coat protein and ORF IV genes as well as dsRNA construct with ORF-IV gene of RTBV using pCAMBIA 1380 as the plant binary vector under the control of maize ubiquitin promoter and hpt as plant selection marker. PCR and Southern analysis has confirmed the integration of the transgene. The T1 and T2 transgenic plants were challenged by the virus to check for the resistance and some of the transgenic lines have exhibited moderate resistance against tungro disease.

PVO-18 Current Status of Genetic Diversity of Sugarcane yellow leaf virus

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Yellow leaf disease caused by Sugarcane yellow leaf virus (SCYLV) is the most studied disease of sugarcane in current years. The genome of SCYLV have been fully sequenced and characterized, and the virus was recently assigned to the genus Polerovirus of the family Luteo-viridae. Molecular and immunological assays were developed to diagnose the virus in symptomatic and asymptomatic plants. Saccharum species are the only known natural hosts of SCYLV. Diversity studies showed that SCYLV is a variable virus, and several genotypes of this pathogen have been described. SCYLV was found to be widespread in most sugarcane producing countries. The phylogenetic analyses of the entire genome of SCYLV revealed the occurrence of three genotypes of SCYLV (BRA, PER and REU) based on the geographical location where it was first detected; Brazil, Peru and Reunion, respectively. Additionally, a virus isolate from Cuba showed only 77–80% amino acid sequence identities in ORF1 with isolates of genotypes BRA, PER and REU, which suggest that the Cuban isolate represent a new genotype (CUB). Comparison of partial sequences encoding for ORF1 and 2 revealed that YLD in sugarcane in India is caused by at least three genotypes, viz., CUB, IND and BRA-PER, of which a majority of the samples were found infected with Cuban genotype (CUB) and lesser by IND and BRA-PER genotypes. A recent study in India revealed that although high level of homogeneity was observed within Indian isolates and CUB isolate, but there is great variability existed between Indian isolate and the rest of the SCYLV world isolates from different geographical sites of origin. This scenario suggests the existence of five SCYLV genotypes (CUB, BRA, PER, REU and IND). Of them, probably the genotype IND might be found only in India besides CUB and BRA-PER. The genotype IND was identified as a new genotype and this was found to have significant variation with the reported genotypes.

PVO-19 Begomovirus, Tomato leaf curl virus, Newly Reported in Spearmint

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Spearmint (Mentha spicata cv. Viridis; family Lamiaceae), an important essential oil-bearing crop, is cultivated on 0.15 million ha land of India. Approximately 80% of its planting consists of identical genetic material released by CIMAP, Lucknow, India. In a field of M. spicata cv. Viridis at CIMAP, Lucknow, and in adjoining areas the occurrence of a begomovirus-caused disease was for the first time observed during May and June 2006. Typical symptoms were foliar rugosity, light yellow mosaic, upward curling, crinkling and retarded growth leading to drastic reduction in the herb’s yield. In 2007, the disease incidence was recorded to be in the range of 50–60%. The disease also appeared in the Tarai region of Uttaranchal province. Infected plants were used for electron microscopy, whitefly transmission tests and polymerase chain reaction (PCR)-mediated begomovirus-specific diagnostics, but neither virus particles (in leaf dip), nor cytopathic effects or inclusion bodies were detected. However, from the naturally infected plants, the disease was transmitted to healthy seedlings of spearmint by whiteflies (Bemisia tabaci), but not by aphids or mechanical inoculation. Total DNA was extracted from Mentha samples with and without symptoms from different locations. The presence of a begomovirus was detected by PCR using begomovirus coat protein gene-specific primers (forward 5'-ATGCCGAACGCACCCAG-3' and reverse 5'-TTAATTSCGAC CGAATCAT-3'). A product 771 bp in size was amplified from samples with symptoms but not from symptomless plants. Amplicons were cloned into PCR-TOPO TA cloning vector V2.0 (Invitrogen) and selected clones sequenced in both orientations and data submitted to GenBank (Accession. No. EU263016). Sequence analysis showed the highest levels of sequence identity (93%) with the begomovirus Tomato leaf curl Pakistan virus (DQ116684). This is the first report of a begomovirus associated with an economically relevant disease of spearmint in India.

PVO-20 Molecular Characterisation of Coat Protein Gene of Tobacco leaf curl virus isolates of Andhra Pradesh

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Tobacco leaf curl disease is caused by geminivirus and the virus is transmitted through whitefly. Different types of viruses cause different symptoms. With mild types, the leaves show slight wrinkles and drooping. In case of severe types, the leaves and stem curl and leaves become thick and shrunkled and unfit for curing. No source of resistance to this disease is available either in the germplasm or in the wild Nicotiana species. Expression of truncated defective transdominant viral coat protein gene has proved more promising in developing resistance to begomoviruses. Hence, in our study, an attempt has been made to study the coat protein gene (cp) of Tobacco leaf curl virus isolates of tobacco growing regions of Nellore and Khammam districts. Such studies will be helpful in developing tobacco transgenics expressing defective transdominant viral coat protein that confers resistance to leaf curl virus prevalent in these regions. Initially, PCR primers specific to coat protein gene (cp) of Tobacco leaf curl virus were designed based on the sequences of Tobacco leaf curl virus-Karnataka1 (TbLCV-Kar1) and Tobacco leaf curl virus-Karnataka2 (TbLCV-Kar2) genes available in the NCBI data base. The designed primers were used to specifically amplify a part of CP gene from the total DNA isolated from the tobacco plants, collected from Nellore and Khammam districts showing leaf curl symptoms. This confirmed the presence of leaf curl virus in plants showing leaf curl symptoms. The amplified DNA was eluted, purified and sequenced (NCBI GenBank accession No. EU 518373-77). Using the NCBI and BLAST search tools in the NCBI website, the alignments of eluted DNA sequence with sequences in the NCBI Database was determined. Most of the cases, sequences aligned with leaf curl virus sequences of
croton, tobacco, tomato, mesta, calendula etc. Among the isolates, depending on the sequence, 78–100% similarities were observed. The eluted fragment of DNA sample was also cloned into JM109 E. coli strain having pUC18 plasmid. The transformants were identified in the presence of X-Gal and IPTG on Luria–Bertani plates containing Ampicillin. The transformed colonies appeared as white and non-transformants appeared as blue.

PVO-21 Pepper (Sweet and Hot) Viruses: Tools and Techniques for Detection, Identification and Management
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Pepper (Capsicum annuum Linn.) is one of the most important vegetables/spices cultivated in a variety of soils and climates throughout the world. India has its first position in cultivation and export of this commodity in the world. The area under cultivation is about 9.565 million ha with the production of 9.455 million tones per annum. Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu, Uttar Pradesh and Bihar are having largest areas under cultivation of this crop in India. Unfortunately, this crop is highly vulnerable to a number of viruses resulting in tremendous losses in yield and quality of fruits. So far, more than 82 viruses have been reported from different parts of the world, infecting naturally as well as artificially. They belong to various genera (viz., Potyvirus, Cucumovirus, Carlaviruses, Potexvirus, Tobravirus, Luteovirus, Tombusvirus, Fabavirus, Nepovirus, Tymovirus, Alfamovirus, Ibarvirus, Cryptovirus, Necrovirus and satellite virus, Gemini virus, Tospovirus, Rehabsovirus andCornovirus). There are reports of some uncharacterized viruses which are: Chilli mosaic virus, Puerto Rican pepper mosaic virus, Aster ringspot virus, Luceoma mosaic virus, Chilli puckering virus, Brinjal mosaic virus, Bean distortion dwarf virus (BDDV), Sweet potato mosaic virus (SPMV), Plantago mosaic virus (PMV), Potato spindle tuber virus, Lettuce big vein virus (LeBVV), Green vein banding virus, Launaea mosaic virus, Marigold mottle virus (MMV) and Pepper yellow vein virus (PYVV). Infections by these viruses manifest in various types of symptoms (viz., mosaic, mottling, vein-clearing, vein-bandings, interveinal chlorosis, flecks, streaks, ring spotting, distortion, browning, necrosis, leaf narrowing, netting, blistering and shoe-string in leaves) which serve as a key during the survey for recognizing the diseased plants. Streaks (chlorotic and necrotic) have also been observed on stem and fruits of infected plants. In some cases fruits exhibit mosaic, mottling, rings and tumorous swellings which make them unfit for marketing. Natural spread of these viruses takes place by various vectors viz., Aphids, whiteflies, thrips, leaffoppers, beetles, fungis and nematodes. In India, only 19 viruses have been reported so far and about 13 of them have been characterized. Hundred percent yield loss has been reported in case where infection occurred at early growth stage. The identification and diagnosis of these viruses can be done by various methods viz., biological indicators, chemicals, physical properties, serology (ELISA, DIBA andISEM), electron microscopy of the virus particles, presence of inclusion bodies and molecular methods (PCR, RT-PCR, Nested-PCR, RFLP, nucleic acid probes and gene sequencing). Epidemiological studies play a pivotal role in the management of virus diseases. In this context, the virus-vector relationship, role of weed hosts, role of alternate and parallel hosts and weather parameters are highly important. The virus cannot be controlled completely; nevertheless, they can be managed by following a combined approach of management practices like, cultural or agronomic practices, chemical and biological control of insect-vectors, regulatory methods and biotechnological approaches.

PVO-22 Insight into Viral Cell to Cell Movement: Molecular Characterization of Movement Protein of Seshbania mosaic virus
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The cell-to-cell movement of plant viruses is one of the most fascinating phenomena in their evolution. To establish a systemic infection, the virus has to spread from cell to cell, negotiating the cell wall barrier and move along the network of plant vasculatures. For this purpose the viruses use a class of proteins designated as Movement Proteins (MP). These proteins are poorly conserved and their biochemical properties are not well understood. Seshbania mosaic virus (SeMV) a member of the genus Sobemoviruses infects Seshbania grandiflora belonging to Fabaceae family and is native to Tirupati, Andhra Pradesh, India. It is a single-stranded positive sense RNA virus with a genome length of 4149 nucleotides. The genome encodes four potential overlapping open reading frames (ORFs). ORF-1 encodes an 18.4 kDa putative MP. In silico analysis revealed that the protein has very limited sequence similarity with other known MPs and is predominantly z-helical. The SeMV MP gene was cloned in pRSET C vector and over-expressed in E. coli. MPs interact with viral genome and assist in their transport to neighbouring cells. Therefore, nucleic acid binding studies were carried out with the recombinant SeMV MP. The results suggest that, MP binds specifically to genomic RNA and forms large complexes in vitro, unlike other MPs which can bind nucleic acids non-specifically. It was observed that SeMV MP interacts with native virus in a concentration and pH dependent manner. Deletion studies of the MP were undertaken to delineate the domains which may be involved in the above mentioned interactions. It was observed that N terminal 49 amino acids were essential for interaction with NV. In order to identify other interacting partners of SeMV MP, all the genes encoded by SeMV were expressed using yeast 2 hybrid system. When scored for the activation of reporter genes, it was observed that in yeast also, MP interacted with CP via the N terminus. Interestingly, it was observed that MP could also interact with Viral Protein genome linked (VPg) and 10 kDa Protein, P10 encoded by the ORF 2a of SeMV. None of the other proteins of SeMV interacted with MP. VPg is covalently linked to the 5’ end of the genomic RNA and the specificity demonstrated by MP with respect to genomic RNA recognition in vitro could be due to the interaction with VPg. To test this possibility, genomic RNA was treated with pronase and in vitro nucleic acid binding studies were carried out with recombinant MP. The results suggested that MP can only recognize VPg linked genomic RNA. The other protein P10 which interacted with MP has been shown to be an NTPase recently. This suggests that probably P10 helps MP in translocation across the plasmodesmata with the cargo of genomic RNA, by supplying energy necessary for this active process. P10 and VPg interaction with MP was abrogated by deletion of 49 amino acids from the N terminus of MP, although in the case of VPg interaction was lost also with deletion of C terminal 19 amino acids. These observations enable us to propose a model of SeMV movement, which is distinct from those suggested for other MPs.

PVO-23 Assessment of Losses and Management of Viral Diseases of Horticultural Crops
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Horticultural crops like temperate, tropical and sub-tropical fruits, tuber crops, vegetables, ornamental and medicinal plants are store house for numerous diseases caused by viruses, viroids and phytoplasmas.
Economic losses worth millions of rupees are caused due to these diseases annually. Virus diseases are frequently less conspicuous than those caused by other plant pathogens and last for much longer. This is especially true for perennial crops and those that are vegetatively propagated. One further problem with attending to assess losses due to various diseases on a global basis is that what most of the data are from small comparative trials rather than wide scale comprehensive surveys, even the small trials do not necessarily give data that can be used for more global estimates of losses. This is for several reasons, including: (1) variation in losses by a particular crop from year to year; (2) variation from region to region and climatic zone to climatic zone; (3) differences in loss assessment methodologies; (4) identification of the viral etiology of the disease; 5 variation in the definition of the term ‘losses’ and (6) compilation with other loss factors. There are several factors which affect crop losses e.g. Variety/cultivar, Type of pathogen, Types of strains involved, Genetic variability among pathogens, Incidence and intensity of the disease, Vector population, Types of vectors (Arthropods, mites, nematodes, fungi, etc.), Virus/plasmodia–vector relationship, Type of infection (Single/multiple), Physiological, anatomical and metabolic changes, Environmental factors (rain fall, photo relationship, Type of infection (Single/multiple), Physiological, anatomical and metabolic changes, Environmental factors (rain fall, photo relationship, Type of infection (Single/multiple), Physiological, anatomical and metabolic changes, Environmental factors (rain fall, photo relationship, Type of infection (Single/multiple), Physiological, anatomical and metabolic changes, Environmental factors (rain fall, photo relationship). Epidemiology, Cultural practices, Time of infection vis-à-vis age of the plant. Direct losses are caused in terms of reduction in growth and vigour, reduction in yield (quantitative loss) and reduced income. Indirect losses are caused in terms of marketability (qualitative loss i.e. size, shape, etc.; reduced export potential), connoisseurs’ acceptability (visual attraction) nutritional value, reduced photosynthesis, reduction in quality of the seed/planting material, reduced life span of the plant (Degeneration), reduced keeping quality and reduced consumer appeal (grading, taste, texture, composition), danger of infection to the next crop, costs of attempting to maintain crop health., cultural hygiene on farm including vector control, production of disease-free propagation material, eradication programme, breeding for resistance, research, extension and education. In order to find out suitable management strategy of the diseases, it is very important to identify, diagnose and detect the disease causing agents. The important diagnostic techniques include: symptomatology, mode of transmission, serology, electron microscopy and molecular techniques. The serological methods include: Enzyme-linked Immunosorbert Assay (ELISA), immunofluorescence assay, ISEM (Immunosorbert Electron Microscopy), DIBA (Dot Immunobinding Assay) and Western blotting. Molecular detection includes: Nucleic acid hybridization, Southern blot, Northern blot, Polymerase Chain Reaction (PCR), Reverse transcription-PCR (RT-PCR), Immunocapture RT-PCR, Real time PCR, Nested PCR, Multiplex PCR, Heteroduplex mobility assay, Macro and micro array analysis, Restriction Fragment Length Polymorphism (RFLP) and sequencing. The viral diseases can be managed by: phytosanitization, forecasting, quarantine, certification, notification, and hygiene. Removal or avoidance include: sources of infection in or around the crop, roguing and eradication scheme, virus-free seed, Virus-free vegetative stock, propagation and maintenance of virus-free stock, cultural practices and transgenic resistance. Genetic engineering provides a novel approach to develop virus-resistant plants and to increase the potential to implement control strategies that are effective and eco-friendly.

PVO-24 Surveying of Leaf Curl Disease and Detection of Begomovirus by PCR in Chilli Under Hot Arid Region of Rajasthan

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Chilli is the major vegetable and spice crop grown in Thar Desert areas of Rajasthan. Leaf curl disease (ChLCD) is one of the major constraints in chilli cultivation faced by farmers and cause yield loss up to 100%. A survey was conducted in major chilli growing areas of Thar Desert; Bikaner, Nagaur, Jodhpur and Jalore districts of Rajasthan during November, 2009 to understand the present status of leaf curl disease in Chilli. Among the four district surveyed for ChLCD, the disease incidence was recorded maximum (up to 98%) in Jodhpur district followed by Jalore district (up to 88%). No relation was found between the disease incidence and varieties. The major varieties grown in these area are; Mehsana, RCH (Mandoria), Haripur Raipur, Mathania and local cultivars. The number of whitefly was also counted in top, middle and bottom leaf of chilli grown in these areas. The average number of whitefly per plant ranged from 0.0 to 4.0. More number of whitefly (4.0) was recorded in Jodhpur district and lowest (1.8) in Jalore district. Total DNA was extracted from three leaf curl infected samples from each district and tested for the presence of begomovirus using coat protein (CP) and DNA-β specific primers. All the samples were positive for CP and DNA-β amplifications by PCR. The cloning and sequencing of selected CP gene and DNA-β fragments are in progress. The preliminary investigations shows that the leaf curl disease of chilli is widespread in the arid region of Rajasthan and may be caused by begomovirus associated with satellite DNA-β.

PVO-25 Plant Viruses as Epitope Presentation Systems

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The usefulness of viral capsids as carriers for small antigenic peptides has been illustrated for several plant viruses (for a review, see Koprowski and Yusibov 2001) and vaccines based on chimeric virosomes have been shown to be highly effective. Specifically, McLain et al. (1996) have demonstrated the stimulation of neutralizing antibodies to human immunodeficiency virus type 1 in three strains of mice immunized with a 22 amino acid peptide of gp41 expressed on the surface of a plant virus, Cowpea mosaic virus. Marusic et al. (2001) have expressed a neutralizing epitope (highly conserved ELDKWA) from HIV-1 gp41 as an N-terminal translational fusion with the Potato virus X (PVX) coat protein. The resulting chimeric virus particles, purified and used to immunize mice intraperitoneally or intranasally, were able to elicit high levels of HIV-1-specific immunoglobulin G (IgG) and IgA antibodies. As far as potyvirus-based vectors for the expression of heterologous peptides and proteins are concerned, Plasmopox potyvirus (Fernandez et al. 2002) and Zucchini yellow mosaic virus (Atari et al. 2002) are the two potyviruses that have been engineered to express heterologous epitopes on the surface of their coat proteins. In India, Dr. Vrati’s group (Saini and Vrati 2003) at National Institute of Immunology has expressed Japanese encephalitis virus epitope on the CP of Johnsomgrass mosaic viral, which is a potyvirus and has shown that the chimeric virus particles induce neutralizing antibodies and protect mice against the virus challenge. In our lab we have expressed epitopes from HIV-1 on the surface of the coat protein of Cardamom mosaic virus, which belongs to the genus Macluravirus of family Potyviridae. Results of these studies will be presented.

PVO-26 Detection and Characterization of Papaya ringspot virus-W Isolate Infecting Bottle Gourd (Lagenaria siceraria) from Tamil Nadu

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Bottle gourd, *Lagenaria siceraria* is a cucurbitaceous vegetable commonly grown in southern part of India. During 2008–2009, symptoms of viral infection were observed in bottle gourd raised at the orchard of Tamil Nadu Agricultural University, Coimbatore. The disease incidence ranged from 30 to 40%. The leaves of affected plants exhibited mosaic, leaf distortion, mottling, leaf size reduction and vein thickening. The infected field samples were analyzed for the presence of virus by mechanical inoculation. Typical symptoms as observed in field were noticed after 13–15 days of inoculation on bottle gourd. The virus induced chlorotic local lesions on *Chenopodium amaranticolor* and systemic symptoms on *Lagenaria siceraria*, *Trichosanthes cucumerina*, *Luffa acutangula*, *Cucumis sativus*, *C. melo* and *Cucurbita moschata* while no symptom was observed on *Carica papaya*, *Nicotiana glutinosa*, *N. benthamiana*, *N. tabacum* (White burley and Samsam), *Datura stramonium*, *Haliathus annuus*, *Amaranthus viridis*, *Trianthema portulacastrum*, *Portulaca oleracea*, *C. album* and fabaceous hosts. The virus caused 32.1% and 57.1% reduction in plant height over healthy, while the number of leaves got reduced to 23.1% and 24.3% after 15 and 30 days of inoculation respectively. A positive reaction was observed with the antisera to *Papaya ringspot virus* (PRSV) in DAS-ELISA and not with that of *Cucumber mosaic virus*. Electron micrograph from the sap inoculated bottle gourd leaves revealed the presence of flexuous rod shaped particles measuring 750 × 12 nm. Extracted sap from the leaves of bottle gourd was infective to the dilution of 10^−3 and found to be thermally inactive above 55°C temp. The virus sap was infective up to 30 h when at room temperature (30 ± 2). Further, the virus was confirmed as PRSV by RT-PCR based on the 650 bp product amplified from the N-terminal region of the coat protein gene. From these biological, physical morphological, serological and molecular studies the virus isolate infecting bottle gourd was confirmed as PRSV-W.

**PVO-27 Natural Occurrence of Papaya ring spot virus-W in Cucurbits in Gorakhpur Region (UP)**

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A survey of cucurbits (*Cucumis melo, C. sativus, Cucurbita maxima, Luffa acutangula and L. cylindrica*) cultivated in and around Gorakhpur district (UP) was conducted during 2008 for the presence of virus diseases. Disease symptoms observed were: mosaic mottling, vein banding, distortion and curling of leaves. The disease incidence ranged from 15 to 30%. A total of 50 samples were collected to carry out analysis for the presence of virus (es) using host range, ELISA test and Electron microscopic studies. On mechanical sap inoculation, the virus isolate produced local lesions on *Chenopodium amaranticolor*, and systemic symptoms on *Cucumis melo*, *C. sativus*, *Cucurbita maxima*, *C. pepo*, *L. acutangula* and *L. cylindrica*, but it could not infect to *Nicotiana* spp. and *Carica papaya* (Cv. Pusa Dwarf). In DAC–ELISA test, the samples collected from *Cucumis melo, C. maxima*, and *L. acutangula*, reacted positively with the polyclonal antiserum of *Papaya ring spot virus-W* (PRSV-W) and negatively with the polyclonal antiserum of *Zucchini yellow mosaic virus* (ZYMV) (Agdia, USA). Electron microscopic examination of leaf dip preparations made from the same samples revealed the presence of 750 nm long, flexuous-filamentous virus particles. The aim behind this study was to explore information about the presence of potyviruses in cucurbits in the Gorakhpur region of Eastern Uttar Pradesh.

**PVO-28 Whitely Detection, Transmission and Diversity of Satellite DNAβ Associated with Bhendi yellow vein mosaic virus Infecting Okra in India**

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_Bhendi yellow vein mosaic* (BYVMV) belong genus Begomovirus, family Geminiviridae, single stranded DNA virus, which is readily transmitted two insect biotype vectors (both indigenous and b-bio-type) of whitefly (*Bemisia tabaci*) in nature. However, the identification of these two biotypes by visual observation is very difficult because they are morphologically similar. Hence by using silver leaf bioassay and SCAR markers analysis can distinguish quickly and reliably biotype B of the whitefly, *Bemisia tabaci* (Gennadius) from that indigenous biotypes. After differentiation the whitely used for study virus–vector relationship of the BYVMV, it was found that the biotype B of whitely more efficient transmitting the BYVMV than the indigenous vector whitely *Bemisia tabaci*. Further, a virus causing symptoms of yellow vein, vein clearing complete yellowing, leaf curl and enation of bhendi was isolated from different geographical region of India, were cloned and sequenced. Analysis of complete nucleotide sequence of 37 DNAβ molecules of the present isolates causing yellow vein disease and enation was ranged from 1324 to 1403 nt and are cluster into six major groups of each associated with a different begomovirus species with the nucleotide identity ranged from 60 to 99%. All 37 DNAβ molecules having a highly conserved a single open reading frame, an adenine-rich region, and the satellite conserved region (SCR). The SCR contains a potential hairpin structure with the loop sequence TAA-TATTAC, similar to the origins of replication of geminiviruses.

**PVO-29 Evaluation of Resistance Inducing Substances for Management of Bittergourd Mosaic**

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Bittergourd (*Momordica charantia*) is an important vegetable crop of Kerala. The crop is affected by several diseases of which Mosaic is a prominent one. A field experiment was conducted to evaluate the efficacy of potentised inducing substances (RIS) viz., mosaic affected bittergourd plant tissue, ash of mosaic affected bittergourd plant tissue, plumago and salicylic acid for control of bittergourd mosaic in March 2008. RIS were applied as drench and foliar spray at three potency levels twice, before flowering of the crop. The experimental crop was grown as per the package of practice recommendations in split plot design with five replications per treatment. The disease incidence, disease severity and yield of the crop were recorded. The result of the experiment shows that spraying was more effective than drenching of treatments for reducing mosaic incidence and severity. Among treatments, infected plant extract at 1 × potency was the most effective one for reducing mosaic incidence and it showed the maximum incubation period and minimum disease severity. The spray application of treatments produced significantly higher yield than drenching. Among the treatments, ash of infected plant at 1 × and 30 × potency and infected plant extract at 6 × potency were on par and produced comparatively higher yield.
PVO-30 Viral Diseases of Edible Aroids—Diagnosis and Identification of the Virus

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Elephant foot yam (Amorphophallus paeoniifolius), colocasia (Colocasia esculenta) and tannia (Xanthosoma sagittifolium) are the major edible aroids cultivated in India. The elephant foot yam cultivation is gaining importance due to its high production potential, nutritional and medicinal values and good economic returns. All these aroids are vegetatively propagated and viral diseases are spreading through planting materials. CTCRI has the mandate of producing healthy planting materials of these edible aroids. Accurate diagnosis and identification of the virus is essential for production of healthy planting material and effective management of the disease. Though occurrences of viral diseases on edible aroids in India were known in 1960s, not much attention was given for detection and identification of the virus involved. In case of elephant foot yam 5–30% mosaic 1960s, not much attention was given for detection and identification of the virus involved. In case of elephant foot yam 5–30% mosaic

PVO-31 Sugarcane yellow leaf virus (SCYLV) Causing Yellow Leaf Disease in Sugarcane: Genetic Diversity, Impact on Sugarcane and Management

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Yellow leaf disease (YLD) caused by Sugarcane yellow leaf virus (SCYLV) is a recently recorded disease in India and is found wide spread throughout country. In popular varieties, the disease incidence varied from 0 to 75.0% and attained epidemic levels under field conditions. Detailed studies on the impact of YLD on sugarcane revealed that the virus infection significantly reduces various cane growth parameters, cane yield and juice quality. Sequence comparisons of the coat protein (CP) and movement protein (MP) of 22 SCYLV isolates from India and database sequences showed a significant variation between Indian isolates and the database sequences both at nt and aa level in the CP/MP coding regions. The significant variation in our isolates with the database isolates, even in the least variable region of the SCYLV genome showed that the population existing in India is different from rest of the world. Further, comparison of partial sequences encoding for ORF 1 and 2 revealed that

PVO-32 Mycoviruses: Biocontrol of Fungal Phytopathogens

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Mycoviruses are viruses that infect fungi. They have been identified in all major fungal families. In the present scenario, mycoviruses are the important means of biocontrol of plant fungal pathogens. Most identified fungal viruses have double stranded RNA genomes, often with more than one dsRNA present per virus particle, and have been spherical in shape. These viruses are mostly vesicle bound, as other viruses have protein coatings. To be a true mycovirus, they must demonstrate an ability to be transmitted—in other words be able to infect other healthy fungi through anastomosis and spores. Mycoviruses lead ‘secret lives’, reduce the ability of their fungal hosts to cause disease in plants. This property, known as hypovirulence (Hypovirulence is a term used to describe reduced virulence found in strains of pathogens), this phenomenon was first observed in Cryphonectria (Endothia) parasitica (chestnut blight fungus) on European Castanea sativa in Italy, where naturally occurring hypovirulent strains were able to reduce the effect of virulent ones. These slower growing hypovirulent strains of C. parasitica contain a single cytoplasmic element of double-stranded RNA (ds RNA) similar to that found in mycoviruses that was transmitted by anastomosis in compatible strains through natural virulent populations of C. parasitica. Hypovirulence has also been reported in many other fungal plant pathogens, including Rhizoctonia solani, Gaeumannomyces graminis var. tritici, Ophiostoma ulmi, Sclerotinia homoeocarpa, Diaporthe ambigua Alternaria alternata, and Fusarium sp. etc. Hypovirulence has attracted attention owing to the importance of fungal diseases in agriculture and the limited strategies that are available for the control of these diseases. It reduces the use of toxic fungicides which also affect the plant growth. The symptoms resulted by the mycoviruses are reduction in growth, reduction in pigmentation and sporulation, excessive sectoring and aerial mycelial collapse. These are the consequences of alteration in complex physiological and biochemical processes involving interaction between host and virus.

PVO-33 How Diverse are the Tospoviruses in India?

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Of 20 tospoviruses negatively impacting the cultivation of several field and horticultural crops world wide, five have been recorded from India: Capsicum chlorosis virus (CaCV) on capsicum, Groundnut bud necrosis virus (GBNV) on groundnut, Iris yellow spot virus (IYSV)
PVO-34 Studies on Developing Yellow Mosaic Virus Resistance Using a Pathogen-Derived Gene (Rep) and a Nonviral ssDNA-Binding Protein Gene

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Yellow Mosaic disease (YMD) is a serious geminiviral disease in South and South-east Asia caused by yellow mosaic virus (YMV) in Vigna mungo and V. radiata. YMV belongs to begomoviruses which infect dicotyledonous plants, transmitted by white flies and mostly bipartite i.e. genome is divided into two components, DNA ‘A’ and DNA ‘B’. YMD causes 85–100% yield loss when infected at seedling stage. DNA ‘A’ encodes for proteins required for viral replication and encapsidation. Among them ‘Rep’ is the only virally encoded protein for indispensable for viral replication. DNA ‘B’ encodes for proteins required for movement and systemic spread. Pathogen-derived resistance (PDR) is genetically engineered resistance achieved by expressing a part of viral genome or virally associated sequences in transgenic plants. One attractive approach of PDR is expressing Rep gene in sense and antisense orientations in host plants to block viral replication. A major constraint in genetic engineering of V. mungo is the difficulty in regenerating transgenic plants from transformed tissues. Meanwhile we have generated transgenic tobacco plants (permissive host) constitutively expressing Rep-sense, Rep-antisense and nonviral ssDNA binding protein (Agrobacterium vir E2) by Agrobacterium-mediated transformation. Transgenic nature of plants was confirmed by Southern hybridization analysis, using respective probes. The tobacco leaf disc assay, a simple and rapid method to determine viral titre was used to evaluate the effect of the above genes on MYMV-Vig replication. Of the five vir E2 transgenics, two showed significant reduction in viral titre. In case of Rep-antisense tobacco lines two out of six showed reduction in viral DNA levels. In two Rep-sense transgenic lines, viral replication was blocked completely. Thus, Rep-sense gene of MYMV-Vig and virE2 of Agrobacterium emerge as two potential candidate genes for developing resistance against YMV. The knowledge of Rep-sense based resistance and the nonviral strategy deploying the ssDNA-binding protein can be used in future for the development of YMV resistance in V. mungo.

PVO-35 Distribution, Epidemiology, Molecular Variability and Host–Pathogen Interaction of Begomovirus Complexes Associated with Yellow Vein Mosaic Disease of Mesta Crop in India

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Yellow vein mosaic disease of mesta (Hibiscus spp.) poses a serious threat to the cultivation of this crop in India. All the eight cultivars tested were highly susceptible to the disease. The effect of the disease in terms of loss in fibre yield was greatest (around 70%) in plants that were inoculated at an early stage of growth. A regression approach was adopted to consider the relationship of whitely vector populations with weather conditions and disease spread which explained that different conducive weather factors facilitated the build up of whitely populations and contributed to the spread of the disease. The disease was found to be associated with two different whitely-transmitted monopartite begomoviruses, Mesta yellow vein mosaic virus and Mesta yellow vein mosaic Bahraich virus, together with two betasatellite species, Cotton leaf curl Multan betasatellite and Ludwigia leaf distortion betasatellite. These begomovirus complexes were detected in different combinations throughout the mesta growing regions of India. The begomovirus infection in susceptible H. cannabinus plants, results in elevated Nitric Oxide (NO) and reactive nitrogen species (RNS) production during early infection stage not only in infected leaf but also in root and shoot. Production of NO was further confirmed by oxxyhemoglobin assay. In addition evidence for protein tyrosine nitration during the early stage of viral infection clearly showed the involvement of nitrosative stress. This is the first evidence of nitrosative stress in plant in a compatible host–virus interaction.

PVO-36 Need for Database for Plant Virus, Phytoplasma and Viroid Diseases Research in India

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An increase in incidence of plant viruses and in turn the crop loss have prompted to take up the studies on identification, transmission, ecology and epidemiology to combat some of the devastating viral diseases in cereal and horticultural crops. In India Studies on Plant Virology was initiated as early as 1901 by late Prof. McCarthy, C.D. on Sandal spike disease and subsequently extensive research was carried on different viruses by late professors like E. J. Butler, L. C. Coleman, B. P. Pal, R. S. Vasudeva, H. S. Pruthi, S. P. Capoor, S. P. Raychaudhuri, K. Ramakrishna, G. S. Varma and so on. In India from 1920 to 1950, much of the plant virology work was carried out on aspects like host range, physical properties, vector transmission and so on due to limited laboratory facilities. During 1960–1970, besides virus identification, studies on virus vector relationship in different virus–host combinations were carried out. During 1970–1980 studies on various aspects of physiology of virus infected plants and indexing of certain fruit crop viruses was continued. Viruses diseases like tobacco mosaic, tomato leaf curl, yellow vein mosaic of okra, chilli leaf curl, rice tungro and viruses on fruit crops like citrus, banana, papaya and other small fruits, cardamom with kattae disease, wilt disease in coconut were carried out for which the etiological agents were identified just before 2 decades and number of aspects
were attempted. In the early and also in recent years, much of the work was carried out on whitely transmitted Yellow mosaic/leaf curl viruses of legume and solanaceous crops. In recent years Ilar (Tobacco streak virus) and Tospo viruses (Tomato spotted wilt and Groundnut bud necrosis virus) are extensively prevalent on vegetable, oilseed and ornamental plants besides having number of weed hosts and exhaustive data is available. Viroid and Cryptic viruses were also recorded on crops like citrus, apple and certain ornamental plants. The nature of some of the phytoplasma etiological agents were identified and in future number of variations in etiological agents will emerge.

In India the research work is carried out on plant viruses from 1903 on various aspects were published in more than 8652 research articles and in more than 100 journals. During March 2010 the hard copy of the “Compendium” on Plant virus, phytoplasma and viroid disease research in India is being released on the occasion of IVS Silver Jubilee celebration held at Tirupati. Very soon the plant virus database would be kept on the official network of Indian Virological Society and also on CERA so that it would be available for the present and future generations who have interest in plant virus research.

PVO-37 Virus Vector Relationship of Whitely Transmitted Gemini Viruses (WTGs) of Vegetables in Cassava Based Cropping System of Peninsular India

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Cassava (Manihot esculenta Crantz.) is the major tuber crop in Peninsular India, it is grown in an area of 2.4 lakh hectares with the annual production of 6.7 million tonnes both for direct consumption and the starch grain (sago) producing industries, mainly in the southern states of Tamil Nadu, Kerala and Andhra Pradesh (FAO 2005). In Tamil Nadu, cassava primarily produced for sago producing industries where it is considered as an industrial crop rather than food crop, so the resource rich farmers are cultivating the cassava as irrigated crop in their fertile land and the poor farmers are raising the crop under rainfed conditions. In south India in addition to cassava there is a practice of intercropping important vegetable crops like, tomato, brinjal, legumes and gourds in Cassava fields since all the above mentioned crops are short duration and are money spinners for the farmers. Unfortunately, the major production constraint in these vegetable crops including Cassava is the Geminiviruses belonging to the family of Geminivirus and Genus Begomovirus which are transmitted by dreaded whitefly Bemisia tabaci Genn. Several attempts are being made by many people to study the vector virus relationship of the whiteflies with that of the respective Gemini viruses associated in their respective crops and we have investigating on this aspect since 2000 and some of the results are discussed hereunder. From 2003 to 2005, the field study on cassava revealed that percentage of cutting borne infection of cassava mosaic virus (SLCMV) was 90.4 and whitely transmission to the tune of 9.53%. An overall mean of sixteen whiteflies per plant were recorded at the age of 3 months and after that, whitely count reduced. The disease was transmitted by the whitely Bemisia tabaci in a persistent manner. Under insect proof conditions, it was observed that cassava mosaic virus was transmitted by the whiteflies to the tune of 10% on cv. CO2 (raised from true seeds). Studies were undertaken to gather information on a new virus disease on bitter gourd (Momordica charantia) from 2000 to 2002. The disease was identified as a whitely transmitted gemini virus disease and designated as bitter gourd yellow mosaic virus (BGYMV) disease. BGYMV was transmitted to healthy bitter gourd plants by healthy whitely (B. tabaci Genn). Minimum of five whiteflies were required to transmit the virus. However, 100% transmission of BGYMV disease was obtained when 45 whiteflies were released per healthy plant. One hour of AAFP (Acquisition accession feeding period) and IAFP (inoculative accession feeding period) were required for the whiteflies to transmit the disease. Whitely transmission of BGYMV (by artificial means) was confirmed by PCR (using degenerate primer for gemini virus and specific primer for ICMV DNA-A) on plant species viz., Momordica charantia and Manihot esculenta and weed species viz., Acalypha indica, Malvastrum coromandelianum and Croton sparsiflorus. A behaviour study of the whitefly vector B. tabaci on Tomato leaf curl virus (TLCV) on tomato was conducted during 2001–2004. The tomato plants of 20–30 days old were found to be more susceptible to infection of Tomato leaf curl virus when inoculated with whitely vector (B. tabaci Genn.). Minimum of one whitely was required to transmit the virus The virus persisted in the vector for periods of up to 10 days but not throughout the life span of the insect. The virus could be acquired by the adult and larval stage of the insect, but not transmitted to the progeny. TLCV was transmitted to Lycopersicon esculentum Nicotiana benthamiana, N. glutinosa, N. tobaccom, Abelmoschus esculentus Solanum nigrum, Datura stramonium, D. metel, Parthenium hysterophorus and Euphorbia geniculata by whiteflies. The Tomato leaf curl virus (TLCV) antigen content detected by TAS-ELISA in whitely (B. tabaci) was more in the insects fed on the young leaf of older plant and with a longer acquisition access period of 12 h. The whitely-transmitted gemini-virus (TYLCV) was detected in viruliferous whitely by PCR using degenerate primer for geminivirus (Deng et al. 1995). The amplified product size of all the samples was 500 bp. Further work on the cross infectivity of Bemisia tabaci in various crops are in progress.

PVO-38 Detection of Endosymbionts from Different Population of Bemisia tabaci of Tamil Nadu

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Whitely, Bemisia tabaci (Gennandius) a polyphagous insect is the vector for more than 95% of the Geminiviruses in India. In peninsula India a number of diseases caused by the members of geminivirus which is vectored by whitely cause Economical losses to crop plants. This insect harbours many secondary symbionts viz., Rickettsia, Hamiltonella, Wolbachia, Arsenophonus, Cardinium and Fritschea which are used to differentiate the biotypes in whitely populations, to study the insecticide resistance, host range, virus transmission and speciation (Chiel et al. 2007). In an attempt to study the occurrence of these endosymbionts in the Indian Whitely, samples of naturally occurring whitelys of Cassava and Eggplant were collected from Tamil Nadu Agricultural University Farms, Coimbatore, India during September 2008 and analysed with bacterium (symbiont) specific primers at DSMZ GmbH Plant Virus Division, Braunschweig, Germany. The results suggest that among the symbionts tested in the whitelys, Arsenophonus sp. was alone present, particularly in eggplant whitely population. The partial sequence analysis of the 548 bp of the RNA2 gene carried out with Vector NTI (version 10.0) using blast algorithm with the help of the NCBI alignment revealed that the sequenced portion had only 64.8% homology with KY 264675, KY 264676 and KY 264677 sequences of the whitely reported earlier. This preliminary study indicate the possibility of variation prevailing among the whitely populations of various crops in India.
PVO-39 Standard of Scientific Research Publications
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In recent years there has been growing concern regarding the standard of scientific researches in India. The strengths, weaknesses, opportunities and Threats (SWOT) analysis on Indian scientific research reviewed the progress of science during the last six decades. Although the ‘strengths’ were highlighted in good measure, it was the list of ‘weaknesses’ that called for attention to upgrade the standard of research and ‘opportunities’ that provide scope for overall scientific growth. A comparison between India and other countries in terms of research papers published revealed that India’s contribution to science has come down enormously. What ails Indian Science? Should we compare the growth of Indian science with other developed countries? What criteria should be adopted to judge the quality and standard of scientific research? How to motivate the scientists to improve their scientific output? How do motivate the scientists to improve their scientific output? How do Indian journals perform in maintaining quality? This paper analyses critically the scientific journals around the world, based on the scores allotted by the National Academy of Agriculture sciences (NAAS) in 2003 and 2007 for 1460 and 1608 journals respectively. In general, the Indian journals performed poorly irrespective of the disciplines with only 25–30% in the high standard. The paper dealt with the reasons for low impact factor, the anomalies in the allotment of scores to wide spectrum of the journals and the disadvantages the scientists face with the scoring system. A case study was presented of an Institute with over 50 scientists whose publications were analyzed to discuss the merits and demerits of the system. The performance of the journals published by prestigious academics, societies and councils was also projected. The paper concluded with the need for enhancing the image of the country through research publications in high standard journals and the role of various scientific bodies with short and long term measures.

Medical Virology
Poster Session
MVP-1 Suitability of Tear for Diagnosis of Ocular Herpes Virus Infection
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Herpes Simplex Virus (HSV) keratitis is a leading cause of corneal blindness throughout the world. The infection can be diagnosed by clinical manifestations but in case of atypical ocular cases, laboratory diagnosis is more helpful in timely management of disease. Collection of corneal scrapings in all cases of stromal and epithelial keratitis may not be possible, but collecting tear fluid is a convenient procedure causing less discomfort to the patients. Therefore, the present study was intended to evaluate the suitability of tear specimens for detecting HSV by polymerase chain reaction (PCR) and immunofluorescence (IFA). Tear fluid and corneal scrapings were collected from 134 patients of suspected herpetic keratitis. HSV-1 antigen was detected by IFA using rabbit anti-HSV antibodies. PCR was performed to amplify 111 bp region of thymidine kinase (tk) coding gene and 144 bp region from DNA polymerase coding gene of HSV. Out of 134 patients HSV antigen was detected in 25 (18.65%) of corneal scrapings and 15 (11.19%) of tear specimens and in 12 (8.95%) patients from both the specimens. HSV gene could be amplified in 44 (32.83%) of corneal scrapings and 16 (11.94%) of tear fluids and in 13 (9.71%) patients from both the specimens. Although, corneal scraping seemed to be marginally superior material for detection of HSV, tear fluid may also serve as an appropriate alternative clinical specimen, due to ease of collection and least discomfort to the patients. In either cases PCR detected higher number of HSV cases than IFA. Therefore if and when feasible, both IFA and PCR should be used simultaneously on each specimen to obtain best results.

MVP-2 Noroviral Infection in Hospitalized Children with Acute Gastroenteritis in New Delhi, India
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Noroviruses (NoVs) are the leading cause of acute gastroenteritis in children. NoVs are responsible for >90% non bacterial acute gastroenteritis food borne outbreaks in US. These viruses are also a common cause of sporadic acute gastroenteritis in closed community outbreaks like day care centre, nursing homes, cruise ship and hostels etc. NoVs transmission occurs through contaminated food and water, by fecal-oral route. The present study was conducted to estimate the noroviral infection in children with acute gastroenteritis. From May 2008–April 2009, 127 stool specimens were collected from children <5 years of age, admitted with acute gastroenteritis in Oral Rehydration Unit, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi. RT-PCR was carried for samples using genogroup specific primers from RNA polymerase gene for GI and GII of NoVs. Sequencing and phylogenetic analysis was done on amplicons of GI and GII NoVs positive samples. Results: NoVs were detected in 29 (22.8%) of 127 samples tested. The majority of NoV GI detected in 19 (65%) followed by NoVGII 17 (53%). Mixed infection of NoVGI and NoVGII was seen in 7 (24.13%) samples. Phylogenetic analysis of partial nucleotide sequences from RdRP region of 22 strains of GI and GII NoVs showed a lot of genetic diversity. Phylogenetic analysis of GI showed, 5 strains clustered with GIH.4 One strain each clustered with GIH.1, GIH.2, GIH.3 and two strains clustered to GIH.6, whereas one strain is untyped. Sequence analysis for GI also revealed genetic diversity. Four strains of GI grouped in GIH.4 cluster. One each strain clustered with GIH.1, GIH.3b, and GIH.7 and found 4 novel GI strains clustered separately. In conclusion, varied genotypes of NoVs are circulating in New Delhi, India causing acute gastroenteritis in children. NoVs are emerging viral pathogen and NoV GI H.4 variants are found predominantly in pediatric acute gastroenteritis.

MVP-4 The Role of Serum Level and Polymorphism of IL-6 in Patients with Chronic Hepatitis C Virus Infection
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Cytokines play a key role in the regulation of immune responses. In hepatitis C virus infection (HCV), the production of inappropriate cytokine levels appears to contribute to viral persistence and to affect response to therapy. IL-6 is produced by a variety of cells including T cells, phagocytes and fibroblast. Cytokine genes are polymorphic at specific sites, and certain mutations located within coding/regulatory regions have been shown to affect the overall expression and
secretion of cytokines in patients with HCV infection. To correlate the serum levels and polymorphism of IL-6 gene in chronic hepatitis C patients and healthy controls. Forty patients positive for HCV RNA attending the Medicine out patient department and wards of Lok Nayak Hospital, New Delhi as well as forty healthy controls were enrolled for the study. The serum level of IL-6 was detected by ELISA. Genomic DNA was extracted from whole blood of HCV infected patients and healthy controls by using AccuPrep Genomic DNA Extraction Kit according to manufacturer’s instruction. The genotyping of IL-6 promoter (−174 variant) was carried out by PCR and direct sequencing using the method of Patricia Woo et al. 1998. The serum level of IL-6 was significantly down regulated in HCV infected chronic patients as compared to the healthy controls. Genotyping of −174 promoter variants of IL-6 was performed by PCR and direct sequencing. IL-6 Polymorphism in the G/G, G/C and C/C allele was non significant when compared to HCV patients and healthy controls. The IL-6 serum levels were significant among HCV infected patients when compared to healthy controls. The polymorphism in the promoter region of IL-6 (−174) was found non-significantly associated in HCV patients compared to healthy controls. In conclusion, the present study suggests that the host IL-6 polymorphism alone may not play a significant role in the outcome of HCV infection.

MVP-5 Circulation of Enteric Viruses in Hospitalized Patients

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Acute gastroenteritis (AGE) is a global health problem and has been associated with multiple etiological agents, which include bacteria, protozoa and viruses. Viral gastroenteritis is considered as the second most common illness in children after upper respiratory tract infection. Among enteric viruses, rota, noro, enteric adenov, astro and enterovirus are found to be associated with gastroenteritis. Although, association of enteric viruses has been established in children hospitalized for AGE no such data is available from hospitalized children other than enteric infections. To determine the prevalence of enteric viruses circulating in hospitalized children. Fecal samples, n = 292 (177 symptomatic and 115 asymptomatic for AGE) were collected from children <5 year of age from three different hospitals across the city of Pune from June 2008 to Feb. 2009. Detection of group A rotavirus was carried out by using antigen captured ELISA. RT-PCR and PCR was carried out for the detection of norovirus, enterovirus, astrovirus and enteric adenovirus detection by using primers targeted to RdRp gene, 5’NCR gene and conserved gene for serine protease and hexon gene respectively. Out of 177 fecal samples tested for enteric viruses in AGE cases, the prevalence of rota, entero, noro, enteric adenov and astrovirus were 33.3% (59), 14.7% (26), 6.2% (11), 2.8% (5) and 1.1% (2) respectively. However, the presence of these viruses in the asymptomatic cases (n = 115) was detected at 7.8% (9), 5.2% (6), 7.8% (9), 0.86% (1) and 1.7% (2) levels respectively. Mixed infections of enterovirus and rotavirus were found in both symptomatic 1.6% (3) and asymptomatic cases 0.8% (1). However, mixed infection of enterovirus with adenovirus were found only in asymptomatic cases 0.8% (1). No marked difference was observed in the seasonal pattern of all viruses in the patients with or without gastroenteritis. The findings of this study document highest circulation of rotaviruses in patients symptomatic and asymptomatic for AGE. The entero and noroviruses remain second most important enteric viruses in these patients.

MVP-6 Antigenic Characterization of Influenza A (H3N2) Strains from Human Ill Cases in Pune, India, 1990–2009

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Influenza in humans is a major public health concern and the understanding of its evolution in the light of its “antigenic drift” helps prediction of epidemics and update of yearly influenza vaccine. To antigenically characterize influenza A (H3N2) isolates and study antigenic drift during 1990 to 2009 in Pune city. Patients with Influenza like illness were identified using a strict case definition from dispensaries located in different areas in Pune and clinical samples (NS/TS) were collected after obtaining informed consent. These clinical samples were processed in vivo (in fertile eggs) and in vitro (in MDCK cell culture) for isolation. Samples showing embryonic death in ovo or CPE in tissue culture, were tested in HA test employing G.pig/Turkey RBC’s. Positive isolates were tested in HI test for typing and sub-typing of influenza virus strain, using WHO reference influenza immune sera. Current study shows the pattern of reference drift involved in influenza A (H3N2) isolate strains from 1990 to 2009 from Pune city. During 1990–2009, 107 influenza A (H3N2) strains were isolated in Pune. In the year 1991, A (H3N2) isolates identified were A/Beijing/353/89 like, during 1994–1995, influenza virus A (H3N2) was not detected in Pune. In 1996 reappeared and A/Johannsburg/33/94 type of influenza A (H3N2) virus were circulating in the community. From 1998 to 2000, A/Sydney/05/97 like virus was circulating. From 2002 to 2005, A/Panama/2007/99 was the circulating strain. In 2006-A/California/7/2004 and in 2007 and 2008-A/Wisconsin/67/2005 and further in 2009 to date A/Brisbane/10/2007 is circulating in the community. These isolates matched with H3N2 vaccine strains in the corresponding years.

MVP-7 Cloning and Sequence Analysis of Complete NSPI Gene of Chikungunya Virus Causing Epidemic in Andhra Pradesh, India 2009

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Chikungunya fever (CHIKV) is an acute arthropod borne viral illness reported from many parts of Africa and South East Asia. CHIKV has emerged in the form of unprecedented explosive epidemic in 2006 after a long gap of 32 years in India affecting 1.39 million persons in 15 states/union territories. The disease further declined to an estimated 59 thousand, 95 thousand and 62 thousand cases by the end of 2007, 2008 and 2009 respectively (NVBDCP 2009). CHIKV is an arbovirus belonging to the genus alpha virus, family Togaviridae and containing (+)ssRNA as the genetic material. The virus is primarily transmitted by the mosquito species Aedes aegypti and Aedes albopictus. The disease is usually characterized by abrupt onset of fever, nausea, vomiting, headache, arthralgia, myalgia, and rashes. The typical sign of the disease is the polyarthralgia which is very painful and persists for several months in some cases. Illness caused by CHIKV can be confused with diseases such as dengue or yellow fever. Hence laboratory confirmation of suspected cases is quite necessary to initiate mosquito control measures during an epidemic. In our present investigation a primer pair DVRChk-F/DVRChk-R which amplifies 330 bp fragment of E1 gene of CHIKV was used for screening acute phase samples as described by us earlier (Naresh Kumar et al. 2007). Out of 27 acute phase samples, 19 were found positive for CHIKV specific RNA and out of 17 convalescent phase samples 13 were positive for CHIKV IgM antibodies. We further designed a set of primers (CHIK-NSPIF/CHIK-NSPIR) to amplify the complete NSPI
Current use of both diagnostic assays namely NS1 antigen as well as IgM antibodies usually occur after fifth day of the infection. Consequently, NS1 antigen ELISA is a good and effective tool for detection of dengue infection before five days and obviates the need of costly reagents and expensive equipments. A total of 145 blood samples were collected from clinically suspected cases of Dengue fever hospitalized in the medicine ward of Lok Nayak Hospital, Delhi. The samples were tested for the presence of anti-dengue IgM antibody using MAC ELISA and dengue virus antigen was detected by using PanBio Dengue NS1 Antigen Capture ELISA, Assay. Of the total number of 145 patient’s samples tested, 88 (60.68%) were positive for either NS1 antigen or IgM antibody by MAC ELISA. Dengue NS1 antigen-capture ELISA could diagnose dengue infection in 58 samples (65.9%). In comparison, anti-dengue IgM antibodies were detected in only 53 (60.22%) samples. Overall, an additional 35 (39.7%) positive cases of Dengue could be detected when NS1 antigen assay was also used in the study. Highest NS1 antigen positivity was encountered among the samples collected on the 3rd day of fever whereas MAC ELISA for anti IgM antibody was positive after 4th day and gradually there was an increase in the positivity towards the convalescent phase of the disease. The results of this study indicate that NS1 antigen based ELISA test can be an useful tool to detect the dengue virus infection in patients during the early acute phase of disease since appearance of IgM antibodies usually occur after fifth day of the infection. Concurrent use of both diagnostic assays namely NS1 antigen as well as MAC ELISA will improve the overall detection of dengue infection. Early detection of acute dengue virus infection is crucial to provide timely information for the management of patients.

**MVP-9 Cytomegalovirus Infection in Patients with *Pneumocystis jirovecii* Pneumonia**

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Cytomegalovirus may replicate within the lungs both of recipients of transplants and in patients infected with the human immunodeficiency virus (HIV). The hypothesis is that a host damaging immune response might be provoked by cytomegalovirus infection and cause a severe pneumonia in recipients of allogeneic transplants and in individuals with condition/s leading to impairment of cellular immunity. Methods: A prospective was carried out in patients with a high clinical index of *Pneumocystis jirovecii* pneumonia for a period of 36 months. Relevant respiratory clinical samples were collected from infected patients with a special reference to clinical specimens such as broncho-alveolar lavage (BAL). Out of total 300 hundred samples studied, *Pneumocystis jirovecii* pneumonia was diagnosed in 50 patients using polymerase chain reaction (PCR) using three different target genes for molecular diagnosis of *Pneumocystis jirovecii* pneumonia study population. Concurrent cytomegalovirus infection was identified as the only infectious agent in five (10%) patients. Patients with dual infection (CMV-PjP) were more likely to be ventilated ($P < 0.05$) than those with PCP alone. Cytomegalovirus infection may occur in immunocompromised patients such as HIV infected patients and post-transplant (PRT) recipients. Although survival from PCP and CMV has improved over time, these infections are serious and are potentially fatal. Observations noted in our study support the hypothesis that concurrent cytomegalovirus infection may be attributing to some immunopathological condition/s and might help explain the worse prognosis of *P. jirovecii* pneumonia in patients co-infected with CMV and warrants further studies.

**MVP-10 Survey, Screening and Molecular Characterization of Chikungunya Virus Causing Focal Outbreaks in Kadapa District of Andhra Pradesh, India 2008–2009**

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Chikungunya virus (CHIKV), belonging to the genus Alphavirus, family *Togaviridae* is transmitted by *Aedes* mosquitoes and is usually characterized by fever, arthralgia, and myalgia. It was first reported from Makonde plateau, Tanzania during 1952–1953 and since then has been responsible for explosive epidemics in Africa, India and Southeast Asia. It is an enveloped virus containing positive sense single stranded RNA as its genetic material and is about 11,805 nucleotides in length. Andhra Pradesh was the first state to report CHIKV epidemic during the current outbreak in India and one of the worst affected state. In Andhra Pradesh 23 districts were affected and nearly 77,533 CHIKV cases were suspected by the end of 2006 (Chikungunya Facts, NVBDCP). During the months of March and April 2008, the Mobile Medical Unit Office, Kadapa district, Andhra Pradesh observed a huge influx of patients to the Primary Health Center (PHC) with crippling arthralgia and fever in Kokkaparappalli village. During our epidemiological surveys the patients with severe fever, joint pains, myalgia and headache characteristic of CHIKV infection were observed in this village. Similar fever cases with arthralgia were observed in different regions of Kadapa district. A survey was carried out form March 2008-April 2009; in different regions in Kadapa district and blood samples from patients exhibiting symptoms compatible with CHIKV infection were collected. RT-PCR and IgM strip analysis were further used for screening and confirmation of the causative agent. A primer pair DvRChIK-F/DvRChIK-R was used for screening of acute phase samples (Naresh Kumar et al. 2007). Out of 256 acute phase samples screened 140 were positive by RT-PCR and out of 173 convalescent phase samples 119 were positive for CHIKV specific IgM antibodies. Four of the CHIKV isolates were cloned, sequenced and submitted to the Genbank DNA database (GQ119362, GQ119363, GQ119364, and FJ225403). Comparative homology analysis of GQ119362 with other isolates revealed 99.23 ± 0.52 and 100% of homology at nucleotide and amino acid level with East/Central African isolates. The present study confirms continued CHIKV circulation during 2008–2009 in Kadapa district of Andhra Pradesh and the causative agent is identified to be of East/Central African origin. The study also urges the need for intensifying
the epidemiological and entomological surveillance to combat any such CHIKV outbreak in the near future.

**MVP-11 Surveillance of Enteroviruses in Acute Gastroenteritis Patients from Pune (Maharashtra), India**

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Acute Gastroenteritis (AGE) is one of the most common diseases in humans with a significant cause of morbidity and mortality reported in developed and developing countries. Though, rotavirus is the leading causative agent associated with severe diarrhoea among infants and young children. Recently, association of Calici, Astro, Adeno and some of the serotypes of Enterovirus have been reported in sporadic and outbreaks of gastroenteritis. Recently, few reports are available on association of certain serotypes of entero and echoviruses in sporadic and outbreak infections of gastroenteritis from other countries. The most common route of transmission of enterovirus is feco–oral route, but the transmission can be of food borne, water borne, person to person or by other unknown modes. In India, so far no such study is available on Enteroviruses associated in AGE among hospitalized children. The present study was conducted to know the prevalence and genogroups of EVS in sporadic infections of AGE in Pune, India. A total of 239 faecal specimens were collected during Jan 2006–Dec 2007 from children, ≤5 years of age hospitalized for AGE from Pune (Maharashtra), India. The severity of diarrhoea was assessed using the Vesikari Scoring system. RNA was extracted from 30% stool suspension by using QIAamp Viral RNA mini extraction kit, QIAgen. All specimens were tested for the presence of EV-RNA by RT-PCR using primers selected from the conserved gene of enterovirus (5’NCR). Sequencing and phylogenetic analysis were carried out by using MEGA-4 Neighbour-joining Algorithm and Kimura-2-Parameter to know the prevalent enterovirus genogroup(s). Of the 227 faecal specimens, 43 (18.50%) were tested positive for EV-RNA. Clinical severity score calculation showed that majority of the patients (64.28%) with enterovirus infection experienced severe disease while 33.33% and 4.76% of positive cases presented with moderate and very severe disease respectively. EV infections were found highest in the month of July. Sequence analysis (5’NCR) suggested the presence of enteroviruses with the highest homology to HEV-B (67.44%), followed by HEV-C (23.25%) and HEV-A (9.30%). The study indicates circulation of HEV-B in higher proportion in children with AGE in India.

**MVP-12 Regulatory Type CD4+ T cells in Chandipura Virus Infected Young Susceptible Mice**

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Chandipura virus is a member of Rhabdoviridae family associated with an encephalitic illness in human beings. First time it was identified in 1965 from blood of two patients from Chandipura village in Maharashtra, India. It was observed that children below 15 years of age were vulnerable but adults were refractory to the infection. Symptoms like high grade fever, vomiting, convulsions and grade IV coma were observed. Children died within 48 h of hospitalization. T regulatory cells are known to suppress the potential deleterious activation of T helper cells. This study was carried out to understand the role of T regulatory cells during infection in mice. Thirty-three days old suckling mice were inoculated with Chandipura Virus. CD4+ cells were purified from Spleen of infected as well as control mice every 24 h up to 96 h Post infection (PI). CD4+ cells were separated by magnetic column based cell sorting. The purity was checked by staining with anti mouse CD4-FITC conjugate and it was approximately 70%. The purified cells were phenotypically characterized by staining with anti mouse CD25-PE, CD127-PE-Cy7, CTLA-4-APC conjugates and analyzed by flow cytometer. The expression of FoxP3, TNF-α, IL-10 transcripts was quantitated by SYBER green based Real Time RT-PCR. Up regulation of CD25 and down regulation of CD127 and CTLA-4 was noticed at 72 and 96 h PI. At this PI hours, up regulation of FoxP3, TNF-α and IL-10 was also observed. This experiment suggests that regulatory phenotype of CD4+ cells appeared at 72 and 96hPI and might be involved in immune regulation.

**MVP-13 Analysis of Binding Properties of VP2 Protein of Human Parvovirus B19 Through in Silico Molecular Docking**

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Human parvovirus B19, a member of the Parvoviridae family, is a pathogen associated with a wide variety of diseases. Most commonly, it causes childhood rash erythema infectiosum, but in some cases more serious symptoms such as persistent arthropathy, critical failures of red cell production causing transient aplastic crisis, this infection in pregnancy causes hydrops fetalis and myocarditis. Traditional immunosuppressive therapy being unsuccessful, anti-viral therapy might be worthy of consideration. Functional annotation would provide role of viral proteome in its survival and pathogenic mechanisms. SVMProt functional family annotations of VP2 protein had deciphered its zinc-binding, coat protein, outer membrane, chlorophyll biosynthesis, DNA repair and calcium-binding nature. VP2 protein is having a key role in viral assembly of B19 virus and being non-homologous to human proteome, it was identified as an attractive molecular target for structure based drug discovery. The VP2 protein crystal structure was energy minimized using CHARMM. A structure based virtual screening method was applied using LigandFit to identify potential inhibitors of VP2 protein from ChemBank database and ten potential Human parvovirus B19 VP2 inhibitors were proposed.

**MVP-14 Molecular Epidemiology of Dengue Virus in North India**

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More than one-third of world population in living under the threat of dengue viral infection which presents in the form of self limiting dengue fever (DF) to life threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The exact region of this wide range of pathogenesis is still not fully explained. Certain serotypes and genotypes have been implicated in severe form of Dengue viral infection in DHF and DSS. The present study is therefore planned to study the dengue serotypes and genotypes circulating in North India. Seventy-two acute phase serum samples collected from clinically suspected dengue patients during 2008 were included in the study. Dengue viral serotypes were determined by nested RT-PCR using Dengue consensus and all serotype specific primers. The RT-PCR positive samples were subjected to sequencing using ABI...
PRISM 310 genetic analyzer. The drafting of the sequences was performed using BioEdit software and were submitted in GenBank. For phylogenetic interpretation DENV representing the full extent of genetic diversity in DENV-1, DENV-2 and DENV-3 were collected from GenBank. Neighbor joining algorithm was implemented with bootstrap value of 10,000 replicates for phylogenetic inference using MEGA 4.0.2. The genomic region 123 to 644 (C-prM gene junction) of DENV were amplified directly from patient serum. Twelve of 72 samples were positive for dengue viral RNA. Of these 4 were Dengue type 1, 1 was Dengue type 2 and 7 were Dengue type 3. For molecular epidemiological survey and genotyping of the sequences more than 100 sequences from different geographical areas including sequences from previously reported north Indian isolates were compared with our present data set. The critical analysis of the sequences revealed: 4 Dengue Type 1 sequences were clustered within sub-type 2 of genotype III and all the 7 sequences of DENV-3 clustered along with genotype III. Thus, among the dengue types 1, 2 and 3 currently circulating in North India, Dengue type 3, genotype III, being the predominant one followed by, genotype III of Dengue type 1.

MVP-15 Identification of BA Genotype of Respiratory syncytial virus (RSV) in Nasopharyngeal Aspirates from Delhi
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Respiratory syncytial virus (RSV) is one of the major etiological agents of acute respiratory infection (ARI). RSV is classified into two major groups A and B. RSV G protein is the surface glycoprotein which is involved in attachment of virion to the host cell. G protein is the major neutralizing antigen and is considered as a major target for vaccine development. BA genotype of group B RSV with 60 bp duplication in G protein gene is reported from different parts of the world including India. Rapid global spread of the BA genotype suggests that they may have selective advantage over other genotypes. The present study is targeted to detect the BA genotype form Delhi. Nasopharyngeal aspirates (NPAs) from children with acute lower respiratory tract infection were tested for RSV by N gene PCR. N gene PCR positive NPAs were subjected to partial group B specific G gene PCR. Sequencing and phylogenetic analysis done for G gene PCR positive amplicons. Forty-two NPAs demonstrated positive for RSV by N gene PCR. By partial G gene PCR 39 of these samples were typed as RSV group B and 3 as RSV group A. Out of these 39 group B positives 25 were subjected for sequencing. Sequence analysis revealed 13 BA genotype positive sequences. Sequence analysis results will be presented.

MVO-16 Influenza Surveillance in Pune, India
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Influenza viruses, unlike other viruses for which vaccines have been developed, undergo rapid and unpredictable antigenic variation in the hemagglutinin (HA). Because of this antigenic variability and its consequences, regular surveillance is necessary. Virological surveillance of influenza in different areas of Pune during 1st June 2009 to 31st Dec 2009 was carried out. Indian Council of Medical Research in collaboration with CDC has established an influenza surveillance network in India. NIV Pune is one of the regional centers of this network. Using a case definition of Influenza like illness (ILI), patients were identified, from different dispensaries located in different areas of Pune. Respiratory samples (Nasal swab and Throat swab) were collected from these patients, for detection of influenza virus. Real time PCR was used for the primary screening of clinical samples. Positive samples from real time PCR are inoculated in Madin-Darby Canine Kidney cell line for virus isolation. These isolates were antigenically characterized by Hemagglutination inhibition test using WHO reference immune sera. From 1st June 2009 to 31st Dec 2009, 241 patients presented with symptoms of fever at the time of presentation (91.1%), cough (81.8%) nasal discharge (68.2%), chills and rigor (34.2%), body ache (27.4%), headache (27.4%), sore throat (19.3%) were screened for influenza. Male to Female ratio was 1:1.2. 24.2% of the patients were children below 5 years of age. 110 samples were positive for influenza by real time PCR, out of which 15 A (H1N1), 38 A (H3N2), 41A (H1N1) pdm2009, 4 Type B, 11 samples were untypable, one sample was positive for both A (H1N1) and A (H3N2). Real time PCR results showed 45.6% positivity during June to December. Out of these 61.8% was positive for seasonal Influenza and 37.2% was A (H1N1) pdm2009. From PCR positive samples 8 isolates of influenza virus were obtained, of which 2 isolates were A (H1N1) and 6 pdm A (H1N1). Influenza activity was observed during June to December 2009. Group A rotaviruses of the family Reo viridae, are regarded as a major cause of acute diarrhoea in the young ones of humans and animals. Sequence analysis of the outer capsid VP7 and VP4 genes have classified group A rotaviruses into 23 G and 31 P genotypes. In humans, G1–G4 and G9 are common G genotypes, whereas P[4] and P[8] are major P genotypes. Among the VP4 genotypes, P[8] accounts for 73.8% of global prevalence of human rotavirus infections, and hence, it is important as an effective vaccine candidate. Phylogenetic analysis of [P8] strains detected from different parts of the world have classified these strains into four lineages and six sublineages on the basis of diversity in amino acid sequences. To characterize and classify the human rotavirus strains of P[8] specificity, the studies were conducted for RV surveillance in children <5 year of age hospitalized for acute gastroenteritis in Pune, western India during the year 2006. A total of 27 RV strains showed the presence of G1P[8] specificity as characterized by multiplex PCR (Kang et al. 2009, JID, 200:S147–S153). The viral RNA was extracted from 30% fecal suspensions of 21 specimens. RT-PCR and nucleotide sequencing were carried out to obtain VP8* region of VP4 gene. Sequences were analyzed phylogenetically to classify the strains into lineages and sublineages. The phylogenetic analysis of VP4 genes of P[8] strains from western India were grouped with HUN9 like lineage. All strains clustered within three of the four distinct sublineages [I–IV] of the HUN9-like lineage. The sublineage II was predominant (102/21, 47.6%) followed by sublineages IV (2/21, 9.5%) and I (1/21, 4.8%). Interestingly, 8 specimens (38%) showed divergence with sublineages of HUN9 like lineage and grouped separately. The strains showed highest amino acid identity (95.8–99.2%) with strains of HUN9 like lineage. This report documents the co-circulation of genetically diverse human rotavirus strains of P[8] specificity in western India.
MVP-18 Genetic Variation of Human Influenza A (H1N1) Viruses in Pune, India

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Genetic analysis of influenza virus gene coding for the hemagglutinin (HA) protein helps to monitor the evolution of influenza viruses and to determine degree of relatedness between viruses isolated in same/different geographical areas during different years. National Institute of Virology is a National Influenza Center and carrying out influenza surveillance since 1976. Surveillance includes sample collection, virus isolation and preliminary antigenic characterization and genetic analysis. To identify variations in hemagglutinin genes from influenza A(H1N1) viruses isolated from Pune during 2005–2009, 46 Influenza A(H1N1) strains during 2005–2009 were isolated in Madin-Darby Canine Kidney (MDCK) cells and antigenically characterized by hemagglutination inhibition (HI) test. For genomic characterization 43 strains (13-2005, 5-2006, 22-2007, 3-2009) representative isolates of A(H1N1) were selected. Viral RNA was extracted followed by RT-PCR. Purified PCR product was used for sequencing of HA1 domain of HA gene. The comparison between recommended vaccine strains and circulating strains was analyzed by the construction of phylogenetic trees using MEGA 3.1 version. Based on nucleotide sequence alignment A(H1N1) strains were genetically similar to the recommended vaccine strains for the year 2005–2009. Out of 13 strains in 2005, 9 were form cluster with vaccine strain A(H1N1)/New Caledonia/20/99 for 2005–2006, remaining 4 strains were like A(H1N1)/Brisbane/59/2007 which was the vaccine strain for 2008–2009. All 5 strains during 2006 were form cluster with A(H1N1)/Brisbane/59/2007 though vaccine strain for 2006–2007 was A(H1N1)/New Caledonia/20/99. 22 strains from 2007 also clustered with A(H1N1)/Brisbane/59/2007, vaccine strain for 2007–2008 was A/Solomon Islands/3/2006. 3 strains from 2009 formed a cluster with A(H1N1)/Brisbane/59/2007 which was vaccine strain for 2008–2009 and 2009–2010. In 2005 and 2006 Pune strains preceded vaccine strains and at all other time points, Pune strains matched with the vaccine strains. Surveillance is necessary in India where emergence of new strain may be detected earlier. Early detection of drifted strain may be helpful in composition of vaccine strain.

MVP-19 Epidemiological Study and IgM Antibody Based Serological Diagnosis of Chikungunya Virus Infection in Patients During 2008–2009 in Cuddapah District of Andhra Pradesh

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Chikungunya disease (CHIK), a mosquito borne debilitating disease, is caused by chikungunya virus (CGV) an alphavirus belonging to the togaviridae family. India experienced massive outbreaks of chikungunya during 1960s and 1970s. The first CHIK outbreak occurred in Andhra Pradesh in 1965. CGV had almost disappeared from India after 1973 and since then, no case was reported till the end of 2005. In 2005, many suspected fever cases were reported from Coastal parts of Andhra Pradesh. The epidemic outbreak was continued and ongoing affecting the southern districts of Andhra Pradesh. With the approval of cuddapah district medical and health officer we carried out a preliminary epidemiological investigation and blood samples collection from CGV infection suspected patients during the months of July, 2008 and March, 2009. Approximately 200 samples were collected in 12 different localities of the district. The symptoms recorded were sudden high grade fever, arthralgia, myalgia, head ache, conjunctivitis and skin rash. High morbidity with severe arthralgia persisted for several months made the people mentally and physically weak. No genderwise difference was observed for any of the symptom. Although there is no specific treatment or vaccine available currently, the confirmative rapid diagnosis based on detection of viral nucleic acid or IgM antibodies in serum, an indication of recent infection, helps in epidemiological monitoring, symptomatic treatment of patients and determining prognosis. Serological detection of anti-CGV IgM antibodies was performed using rapid immuno-chromatographic assay (RICA) and IgM-antibody capture enzyme linked immunosorbant assay (MAC-ELISA). Eighty convalescent sera were tested by RICA and 60 of them were found positive for anti-CGV IgM antibodies. Twenty-five anti-CGV IgM antibody RICA positive sera were further assayed using MAC-ELISA. More sera from the patients are currently being tested to compare the sensitivity of these two serological assays in anti-CGV IgM antibody based early serological diagnosis of CGV infection and the findings will be presented.

MVP-20 Multiplex PCR for Diagnosis of Dengue and Chikungunya Viral Infections

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Members of Flavivirus genus pose public health problems around the world. Increasing cases of dengue (DEN) and chikungunya (CHIK) transmitted by common mosquito vector Aedes aegypti in the Indian subcontinent; Africa and Southeast Asia constitute the major geographical burden of arbovirus diseases. The similarities in clinical presentations of reemerging chikungunya virus with dengue virus have again compounded the problem of specific diagnosis. Thus the present study was designed to evaluate the utility of multiplex PCR (mPCR) for simultaneous and rapid detection of dengue and chikungunya viral infections. Seventy-two acute phase blood samples from clinically suspected dengue cases were subjected for dengue and chikungunya uniplex PCR using dengue genus specific primers and E gene specific primers for chikungunya virus as well as multiplex PCR was developed for simultaneous detection of dengue and chikungunya infection. Standard strains of dengue and chikungunya virus were used as controls. 13 of the 72 clinically suspected dengue samples were found to be positive for dengue viral RNA by dengue uniplex PCR as well as dengue chikungunya mPCR whereas none of the samples were found to be positive for chikungunya virus infection by both uniplex chikungunya PCR and dengue chikungunya mPCR. The result of dengue and chikungunya uniplex PCR was found to be 100% concordant with dengue chikungunya multiplex PCR. Dengue chikungunya multiplex PCR was found to be a potential rapid test to detect dengue and chikungunya viral infections simultaneously in clinical samples.

MVP-21 Comparison of HIV Seroprevalence in Blood Donors Using Fourth Generation Elisa Versus Third Generation Elisa Assay

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Transmission through blood and blood products can be reduced to a great extent by efficient and reliable testing of the blood. The newer fourth generation ELISA assays simultaneously detect antibodies
against HIV-1 and 2 and the presence of p24 antigen and thus shorten the window period to about 14 days, as compared to 22 days with third generation ELISA. To compare the HIV seroprevalence among blood donors using fourth generation ELISA (antigen–antibody) versus third generation ELISA (antibody) assay. This was a prospective study involving 5100 blood donors of which 3400 were voluntary donors (1700 being students and 1700 being non students) and 1700 were replacement donors. All the blood units were screened for HIV seroreactivity using third generation ELISA (Microlisa–HIV microwell ELISA kits by J. Mitra and Co. Pvt. Ltd.) and fourth generation ELISA (Eliscan HIV advance fourth generation ELISA kits by RFCL). Third generation kits approved by National AIDS Control Organization, Ministry of Health and Family Welfare, Government of India were used. Manufacturer’s instructions were strictly followed while performing each assay. The presence or absence of detectable HIV antigen or antibodies to HIV-1 and/or HIV-2 was determined by comparing the absorbance measured for each sample to the calculated cut-off value. All samples found positive or in grey zone with either with 3rd or 4th generation ELISA were further confirmed by Western Blot assay (by J. Mitra and Co. Pvt. Ltd.). HIV seroprevalence among blood donors as estimated with 3rd generation ELISA was 10/5100 i.e.; 1.96 per 1000 donations (or 0.196%). Among the subgroups, student voluntary donors had HIV seroprevalence of 1/1700 i.e.; 0.58/1000 donations (or 0.058%), nonstudent voluntary donors as 6/1700 i.e.; 3.52/1000 donations (or 0.352%) and replacement donors as 3/1700 i.e.; 1.76/1000 donations (or 0.176%). HIV seroprevalence as estimated with 4th generation ELISA was 18/5100 i.e.; 3.52 per 1000 donations (or 0.352%). Among the subgroups, student voluntary donors had seroprevalence of HIV of 3/1700 i.e.; 1.76/1000 donations (or 0.176%), nonstudent voluntary donors 7/1700 i.e.; 4.11/1000 donations (or 0.411%) and replacement donors 8/1700 i.e.; 4.70/1000 donations (or 0.47%). Fourth generation ELISA assay gave higher seroprevalence as compared to third generation ELISA assay (3.52/1000 donations Vs 1.96 per 1000 donations). Fourth generation ELISA gave a yield of 0.78 window period units per 1000 donations additional to those which tested positive by the current 3rd generation assays. Fourth generation ELISA detects a higher number of seroreactive samples which are in window period for 3rd generation ELISA and would be a better method for screening blood donors for HIV as compared to 3rd generation ELISA to improve blood safety. Blood transfusion services are already familiar with ELISA technology; hence the blood safety can be improved without need for further infrastructure development and staff training.

MVP-23 Potpourri of Sexually Transmitted Infection in a Female Sex Worker

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Sex workers are one of the core groups for transmission of STI/HIV and as a “bridge group” to the general population. Accordingly, highest priority is given to this group in targeted intervention for prevention of HIV/AIDS. Here we are describing one such female sex worker who was harbouring 5 concomitant STI including 4 viral STI. A 25 year old female sex worker was brought to the STI clinic of a tertiary care hospital by NGO with complaint of genital discharge for 3 days. On per speculum examination, cervix was slightly erythematous, tender with mucopurulent discharge. There was no vaginal discharge or ulcer in anogenital area. However, there was a wart at lateral wall of vagina. As per NACO syndromic management guideline, treatment was given for N. gonorrhoeae, C. trachomatis and HPV. Cervical swab was taken and subjected to various microbiological investigation for the detection of STI viz N. gonorrhoeae, C. trachomatis, T. pallidum, Candida spp., T. vaginalis, HSV-1, HSV-2, HIV, HBV, HCV, HPV and M. contagiosum. Saline wet mount showed pus cells, but no yeast cells or trophozoite of Trichomonas vaginalis. Gram stained smear showed more than four polymorphonuclear leucocytes in the absence of gram-negative intracellular diplococci and a presumptive diagnosis of non gonococcal urethritis was made. No organism was isolated on any culture media after appropriate incubation. Cervical swab was negative for antigen of C. trachomatis. Serum was tested positive for HBV, HCV, HSV-2 and T. pallidum though it was seronegative for HIV. In the present case, the female sex worker was harbouring four viral STI viz HSV-2, HBV, HCV and HPV alongwith T. pallidum. However clinically she was diagnosed and treated accurately only for genital wart while cervical discharge due to HSV-2 was misdiagnosed. It is necessary to try to test alternative approaches such as periodic presumptive therapy of viral STI, because this will not only boost up the efforts of STI control in the target group but also help in HIV control. Alternatively, regular clinical and laboratory screening for viral STI may be tried.

MVP-22 Salivary and Urinary Presence of Hepatitis A Virus RNA in Hepatitis A Patients

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Hepatitis A virus (HAV) is highly endemic in developing countries. It is the most common cause of infectious hepatitis especially where inadequate sanitary conditions prevail. The virus perpetuates from one susceptible individual to other and majority of the population acquires this infection in their childhood. HAV is transmitted largely by faecal–oral route. Although serum and stool samples are widely used for detection of HAV/HAV RNA other specimens have been rarely reported for this purpose. The present study was proposed to examine saliva and urine specimens of hepatitis A patients for the excretion HAV. Serum, urine, saliva and stool samples were collected from eighty-two pediatric cases (1.6–15 years) with suspected hepatitis during Oct 2007–Jan 2009. The patients were clinically examined for characteristic signs and symptoms and elevated serum alanine aminotransferase activity (ALT) and were then referred to the National Institute of Virology, Pune, for serological diagnosis of viral hepatitis A. Anti-HAV IgM capture ELISA and RT-PCR targeting partial RNA polymerase region (3D) was carried out on all freshly collected samples. PCR products of the specimens indicating amplification of HAV RNA were sequenced and compared using Basic Local Alignment Search Tool (BLAST). Of the 82 patients, 56 were tested seropositive for anti-HAV IgM and confirmed to have recent infection of hepatitis A. Seroreactivity was used as gold standard for the assay. ALT levels in these patients were in the range of 44–3600 IU/l. Nearly 96.4% and 87.5% were positive for salivary and urinary anti-HAV IgM antibodies respectively. HAV RNA was detected in 69.6% of the serum samples, 57.1% of the stool samples, 10.7% of the urine samples and 7.1% of the saliva samples respectively. All patients indicating urinary and salivary presence of HAV RNA were positive for viremia and fecal excretion. Phylogenetic analysis of partial RNA polymerase region showed close homology with sub genotype IIIA strains. Our study documents the presence of HAV RNA in urine and saliva samples of children with acute hepatitis A indicating possible risk of transmission of the infection through urine and saliva.

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Sex workers are one of the core group for transmission of STI/HIV and as a “bridge group” to the general population. Accordingly, highest priority is given to this group in targeted intervention for prevention of HIV/AIDS. Here we are describing one such female sex worker who was harbouring 5 concomitant STI including 4 viral STI. A 25 year old female sex worker was brought to the STI clinic of a tertiary care hospital by NGO with complaint of genital discharge for 3 days. On per speculum examination, cervix was slightly erythematous, tender with mucopurulent discharge. There was no vaginal discharge or ulcer in anogenital area. However, there was a wart at lateral wall of vagina. As per NACO syndromic management guideline, treatment was given for N. gonorrhoeae, C. trachomatis and HPV. Cervical swab was taken and subjected to various microbiological investigation for the detection of STI viz N. gonorrhoeae, C. trachomatis, T. pallidum, Candida spp., T. vaginalis, HSV-1, HSV-2, HIV, HBV, HCV, HPV and M. contagiosum. Saline wet mount showed pus cells, but no yeast cells or trophozoite of Trichomonas vaginalis. Gram stained smear showed more than four polymorphonuclear leucocytes in the absence of gram-negative intracellular diplococci and a presumptive diagnosis of non gonococcal urethritis was made. No organism was isolated on any culture media after appropriate incubation. Cervical swab was negative for antigen of C. trachomatis. Serum was tested positive for HBV, HCV, HSV-2 and T. pallidum though it was seronegative for HIV. In the present case, the female sex worker was harbouring four viral STI viz HSV-2, HBV, HCV and HPV alongwith T. pallidum. However clinically she was diagnosed and treated accurately only for genital wart while cervical discharge due to HSV-2 was misdiagnosed. It is necessary to try to test alternative approaches such as periodic presumptive therapy of viral STI, because this will not only boost up the efforts of STI control in the target group but also help in HIV control. Alternatively, regular clinical and laboratory screening for viral STI may be tried.
MVP-24 Molecular Characterization of Untypable Rotavirus Strains from Pune, Western India

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Group A rotaviruses are the most significant cause of acute gastroenteritis in pediatric population worldwide. Epidemiological surveillance plays an important role in tracking the changing pattern of rotavirus strains in circulation. The multiplex PCR based on the amplification of VP7 and VP4 genes enables identification of the rotavirus genotypes. High frequency of point mutations in genes/ genome of the virus alters the primer binding site (nucleotide sequences) which results into mismatches and failure in genotyping. Hence, many strains remain untyped. This report presents the characterization of untypable rotavirus strains from Pune, India. The objective of this study was to characterize the untypable strains of rotavirus using new primer sets and modified conditions. Stool specimens were collected during December 2005–June 2009 from children aged <5 years suffering from acute gastroenteritis. All of the specimens were tested for the presence of group A rotavirus by ELISA. RNA was extracted from the rotavirus positive fecal specimens. G and P genotypes were determined as described (Kang et al. 2009. Journal of Infectious Diseases 200:5147–5153). New sets of primers and the modified PCR strategies recommended by CDC, Atlanta, were again used for the typing of previously untyped rotavirus strains. Three hundred and ninety-six of 1050 (37.7%) specimens were typed for both G and P types respectively. The typing rate was increased by 12.87% (85.1%) and 8/396 (2.0%) remained untypable for G, P, and both respectively. With the new primer sets and PCR strategies 28/33 (84.8%), 18/396 (4.5%) and 8/396 (2.0%) remained untypable for G, P, and both respectively.

MVP-25 Studies on Co-Infection of Densonucleosis Virus and Chikungunya Virus in Aedes aegypti Mosquitoes

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Densonucleosis viruses (DNV) belong to Parvoviridae family. They are the etiological agents of insect’s disease known as densonucleosis, which leads to death or loss of vital functions of the infected insect. Densonucleosis virus of mosquitoes has generated lot of scientific interests because of its tremendous potential in biological control and its application as a transducing vector. Earlier, we have reported the isolation and characterization of a DNV from Aedes aegypti mosquitoes and its prevalence among different Ae. aegypti populations from India. There are reports suggesting that when Aedes albopictus mosquitoes co-infected with Dengue-2 and DNV, the multiplication of DEN-2 is suppressed. The present study focuses on the effect of co-infection of Ae. aegypti mosquitoes with DNV and Chikungunya virus (CHIK). The first instar mosquito larvae were infected with DNV and the emerging DNV infected females were then infected with CHIKV by oral feeding. Thus obtained CHIK infected female mosquitoes were analyzed by real time PCR for both DNV and CHIKV on alternate days post-infection, up to the 14th day. The data showed no significant difference in the multiplication of either of the viruses after co-infection. Results suggest that CHIKV neither stimulates the replication of DNV nor is its own replication suppressed due to co-infection. This study forms an initial step in understanding the role played by such endogenous viruses on the vector dynamics.

MVP-26 Role of Brain Infiltrating Lymphocytes in Chandipura Virus Pathogenesis in Mice

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Chandipura virus pathogenesis is manifested as encephalitis in young children with a very high mortality rate. This damage could be due to direct replication of the virus in brain parenchymal tissue or immune system mediated. This study aims at elucidating the role of brain infiltrating lymphocytes in pathogenesis using mice as the model system. Mice were inoculated intracerebrally with the virus and the perfused brain tissue was used to isolate the lymphocytes. Control mice were inoculated with an equal amount of media. In order to standardize the procedure for isolation of lymphocytes from brain tissue, splenocytes were processed to isolate the lymphocytes using Histopaque density gradient method. Methods to isolate lymphocytes from brain tissue as described by earlier workers were tested for the ease and efficiency of procedure using known suspension of lymphocytes from spleen. Percoll density gradient method provided optimum yield of lymphocytes with an ease of handling. In this, brain cell suspension used to prepare 30% Percoll was layered over 70% Percoll prepared using media in 1:2 ratio. Density gradient centrifugation is carried out at 900×g for 20 min at 15°C to obtain lymphocyte layer at the interface. Leishman staining was performed to analyze the morphological characteristics of isolated lymphocytes. Normal lymphocytes showed dark blue stained nucleus. Some bigger sized cells with diffused nucleus characteristic of atypical lymphocytes were observed and some of the cells were surrounded by hair like structures. Phenotypic characterization was carried out using flow cytometry. The presence of CD4+, CD8+ and CD19+ cells was observed. The percentages of CD4+, CD4+ and CD19+ cells was found to be 7.60%, 35.14% and 34.32% respectively in the lymphocytes isolated from infected animal and 5.65%, 30.27% and 3.13% respectively from control animal. Hence, CD19+ cells showed maximum infiltration after infection.

MVP-27 Molecular Characterization of Coxsackie A-16 (CA-16) Isolated from Hand, Foot and Mouth Diseases (HFMD), West Bengal, India

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Hand, Foot and Mouth Disease (HFMD) is a common childhood viral infection. The disease is characterized by fever followed with pharyngitis, mouth ulcers and rashes on hand and feet. Outbreaks of HFMD have been mainly caused due to Coxsackie Virus A-16 (CA-16) or Enterovirus-71 (EV-71). However, other enterovirus (EV) types such
as CA-5, CA-6, CA-10 are found associated in sporadic infections of HFMD. During August–October 2009, HFMD cases were reported from Kolkata, West Bengal, India. The present study was carried out to isolate and characterize the viral etiological agents associated in HFMD by molecular approach. A total of 8 HFMD cases were investigated from the study. Age distribution of patients ranged from 10 months to 3.5 years. Clinical samples such as vesicular, throat swab, stool and serum were collected. All the clinical samples were tested for the presence of enterovirus by RT-PCR using 5′NCR gene. Among these, 5 vesicular swab PCR positive samples were further subjected to virus isolation in Vero, RD and HEP-2 cell-lines. Blind passage was carried out up to P-3 level. Cultures showing 4+ CPE were tested for the presence of EV and were subjected to molecular serotyping using RT-PCR of VP1 gene. Positive amplicons were sequenced and phylogenetic analysis carried out using MEGA-4 software. Isolates were also tested for the virus by Electron-microscopy. Of the five vesicular swabs inoculated in RD cells four of them showed 4+ CPE at 3rd passage level. Culture supernatant tested for RT-PCR showed presence of EV by 5′NCR gene. Positive amplicons sequenced and BLAST analysis carried out with standard strains indicated, isolated strains showed homology with Coxsackie virus A-16. Molecular typing of VP1 gene using type specific CA-16 primers and their sequences confirmed the presence of CA-16. Phylogenetic analysis indicated Indian strains showed 95.7–96.4% homology with Sarawak Malaysian strain isolated in 2005. Electron microscopic analysis in isolated strain showed the presence of Picornavirus particles. The present study indicates circulation of CA-16 strain associated with HFMD in the reported region in West Bengal, India.

MVP-28 Nucleic Acid (siRNA) Mediated Inhibition of Influenza A Virus Replication in Mammalian Cell Line
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Influenza A virus, since time immemorial, has posed an acute worldwide threat to human health and has been the cause of frequent epidemics and reoccurring pandemics. Various RNA interference (RNAi) studies have been carried out for the RNA-mediated RNA degradation in a sequence-specific manner. NS1 gene of influenza viruses plays a crucial role in inhibiting the interferon-mediated responses in the host. We have studied the viral replication inhibition using siRNAs targeted against the conserved regions of the NS1 gene of influenza A Virus. The NS1 gene was cloned in pSecTag 2A vector and was co-transfected with 30, 40 and 50 pmol of the designed siRNAs in MDCK cells. The same concentrations of siRNAs were also transfected with the whole virus (Influenza A/PR/8/34) to study the inhibition of replication. RT-PCR and real-time RT-PCR assays followed by western blot analysis confirmed an increase in the inhibition of the expression of NS1 gene with an increase in the concentration of siRNA. The maximum inhibition (75%) of the virus replication was observed at 50 pmol of siRNA. Our study demonstrates that siRNAs can be potently used as an effective agent for down regulating the NS1 gene of the virus.

MVP-29 Accurate Analysis of Hepatitis B Virus can be Possible Through PCR Rather than RIA in Blood Transfusions
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Most of the jaundice patients (about 40%) are positive for hepatitis B in Rayalaseema region. Hepatitis B is a disease caused by hepatitis B virus (HBV) which infects the liver of hominoidae, including man and causes an inflammation called hepatitis, originally known as “serum hepatitis”. During blood transfusions patients are at high risk of getting the disease through blood. Recent studies states that about 10% of hepatitis B cases are developed due to the unscreened blood transfusions. To avoid this technicians have to follow well established screening procedures to minimize the rate of transmission. For this the conventional method like radioimmunoassay (RIA) is being applied by looking for the presence of HBV infection (HBV antigen) and antibodies that would be produced if there was an infection. Latest knowledge of science exploited a sensitive and accurate method by using the genetic material (DNA) present in a single tissue, is said to be polymerase chain reaction (PCR). By analyzing the viral DNA in the patient’s blood sample we can give an accurate result for HBV positive. The current methods used for screening blood samples are still more practical, in terms of time and money, than the PCR method. However, more sensitive methods for screening donor blood samples should be developed, since radioimmunoassay does not identify all infected blood samples.

MVP-30 Molecular Characterization of Complete NSP4 and Structural Genes of Chikungunya Virus Causing Epidemic in Chittoor District of Andhra Pradesh, India 2009
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Chikungunya, an arboviral disease, which belongs to the genus Alphavirus and family Togaviridae, had an estimated 1.3 million people in India as its victims during the recent epidemic. Andhra Pradesh was the first state to report chikungunya epidemic during the recent outbreak in December 2005 and one of the worst affected states. A single mutation (A226V) in E1 gene of chikungunya virus was responsible for the severity of the current epidemic. This A226V shift which was absent in all 2006 Indian isolates (Pradeep et al. 2006; Arankalle et al. 2007) is found to be present in 2007 isolates from Kerala (Santosh et al. 2008; Pradeep et al. 2008). In the Present study CHIKV suspected blood samples were collected and the acute phase samples were subjected to RT-PCR for the presence of virus specific RNA by using the primer pair DVRChk-F/DVRChk-R as described by us earlier (Naresh Kumar et al. 2007). The convalescent phase samples were screened for CHIKV specific antibodies by using SD Bioline Chikungunya IgM rapid test. Six sets of primers were designed to amplify the complete NSP4 and Complete structural genes of Chikungunya virus. The products were further gel purified, cloned in pTZ57R/T vector and the recombinant clones were sequenced and submitted to the Genbank. The complete NS4gene and Structural genes were compared with other available sequences in the Genbank. Sequence analysis results will be presented. The present study discusses these aspects in detail.

MVP-31 Association of ABO Blood Groups with Chikungunya Virus
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Chikungunya, an arboviral disease transmitted by the Aedes species of mosquitoes (Ae. aegypti and Ae. albopictus) belongs to the genus Alphavirus, family Togaviridae. The recent outbreaks of CHIKV have caused severe morbidity where the symptoms ranged from mild febrile illness to severe polyarthritis. The major symptom being prolonged arthralgic syndrome affecting primarily the peripheral small joints. During the current outbreak Andhra Pradesh was the first state to report CHIKV epidemic in India and one of the worst affected states (Ravi 2006). There have been some controversies regarding the association of blood groups with the disease. Susceptibility of populations with specific blood type to diseases like plague, smallpox, malaria, cholera etc. suggests the possible role of blood group antigen and disease occurrence. In the present study we undertook a questionnaire based survey in Sri Venkateswara University campus, Tirupati. The questionnaire enquiring patient’s name, age, gender, blood group and CHIKV status from 1031 students were collected. Among them 626, were males (60.71%) and 405 were females (39.29%). 246 (23.86%) subjects reported to have acquired CHIKV disease and 785 (76.14%) subjects reported the absence of Chikungunya fever. Based on the blood group antigens the individuals were kept in four groups (A, B, AB, and O). Cross tabulations and odds ratios were reported separately for each and every blood group with respect to their disease status. It is observed that, in Blood group O, the odds ratio of positive over Negative in males is OR = 12.710 (C.I: 1.395–115.836) and in females is OR = 0.622 (C.I: 0.127–3.044), Blood Group A, the odds ratio of positive over Negative in males is OR = 1.504 (C.I: 0.275–8.217) and in females is OR = 0.750 (C.I: 0.078–7.180), Blood Group B, the odds ratio of positive over Negative in males is OR = 3.241 (C.I: 0.831–12.636) and in females is OR = 1.307 (C.I: 0.241–7.082) and in case of AB results cannot be interpreted because only one subject was included in the study in random selection. It was observed that in O and A blood groups, male subjects possessing Positive group are likely to acquire the CHIKV disease when compared to their female counterparts. The subjects (both male and female) possessing B positive blood groups are more likely to acquire CHIKV disease than the B negative blood group subjects. The present study discusses all these aspects in detail.

MVP-32 Ticks as Vectors of Arboviral Diseases in India: An Ecological and Epidemiological Perspective
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Ticks are obligate blood sucking arthropods found in almost every region of the world. They belong to the subfamily Ixodoidea of the order Acarina of class Arachnida. They are very important vectors of human and animal diseases, causing viral, protozoan, bacterial, rickettsial diseases all over the world including India. A total of more than 850 species have been recorded from all over the world out of which about 106 from India. In India there are ticks which inhabits forested and non-forested areas, parasitizing on wild and domestic animals. There are also a few species which inhabits the cave dwelling animals and bats. Of all the human viral diseases recorded so far, Kyasunur Forest Disease is the most important viral disease existing in India. Studies carried out at NIV have shown that tick fauna varies depending up on the type of the forest, like semi-evergreen or semi deciduous forests. Similarly tick fauna also varies in different ecological zones of non-forested areas. The KFD is restricted to forested areas of five to six districts of Karnataka state. The man get the infection while he get into the forested region for collected wood and food during the summer seasons and the virus is predominantly transmitted by nymphal stages of Haemaphysalis ticks, especially Haemaphysalis spinigera. Among the protozoan diseases thileriasis, affecting domestic animals, is the most important diseases transmitted by ticks in India. Studies carried out at National Institute of Virology have shown that application of repellents like DMP can prevent man and animal from tick bites.

MVP-33 Development of a Method for Isolation and Propagation of Mycobacteriophages
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Two Mycobacterium smegmatis strains (ARI Lab Nos. V842 and V946) were employed for the isolation of mycobacteriophages from soil and sewage samples. Mycobacteriophages were isolated from soil samples collected from an area surrounding the Tuberculosis (TB) ward, Naidu hospital, Pune, against *M. smegmatis* strain V842. These were numbered as V942, V943 and V944 and were isolated by using washed-cell preparation method. The Bacteriophages against the other *M. smegmatis* strain, i.e. V946, were isolated from soil samples (collected from around TB ward, Sassoon hospital, Pune). Some of these phages (viz. V953, V954) showed plaques at 42°C but not at 37°C. Thus they seem to be lysogenic. For propagating and increasing the titre of all the above isolates, various previously described methods were attempted, but none of these methods were satisfactory. But when siliconized glassware and plastic-ware were used, propagation was successful. We showed that siliconization of glassware and plastic-ware was essential for the propagation of our mycobacteriophage isolates V951, V952, V953, V954 and V955. Also, phage dilution medium (PDM) as described by Chaterjee et al. (2000) was found to be effective for picking out of the plaques made by the phages. In this way, the phage isolates were propagated up to P3. The various passages of the phage isolates V951, V952, V953, V954 and V955 (i.e. original, P1, P2 and P3) were stored at −80°C.

MVP-34 Detection of Human Papilloma Viral proteins in Cervical Carcinoma After Treatment
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Papilloma viruses are non enveloped viruses with 72 capsomeres around the viral genome. Human papillomavirus (HPV) is one of the most common causes of sexually transmitted disease (STD) in the world. In India more cases of genital HPV infections (>10 million) were prevalent when compared to any other STD. The incidence of these cervical lesions is on the rise in the India and is associated with increased risk for the development invasive cervical carcinoma, one of the most prevalent of all human cancers. HPV types such as 16, 18, 31, 33, 35 and 68 are high and intermediate risk carcinogenic types. HPV infection with high-risk types increases relative risk for high-grade squamous intraepithelial lesions (HSIL). In the present study we investigated the presence of Human papilloma viral proteins, E6 and E7 of high risk type viruses in cervical carcinoma patients’ serum after radiation treatment. In cervical
Veterinary Virology

Poster Session

VVP-1 Molecular Detection and Phylogenetic Analysis of Recent Out-Break of Classical Swine Fever Virus in Bihar

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Classical swine fever (CSF) also known as hog cholera is a highly contagious and fatal disease of swine. CSF became rapidly a major issue of pig industries. It still causes important economical losses worldwide. It is considered as a major health problem of swines in India. During the month of August to October 2009 there was an outbreak of classical swine fever in Bihar. From three districts Darbhanga, Patna and Supol, total 36 numbers of different infected tissue samples like kidney, spleen and lymphnode were collected from the dead morbid/pigs. Total RNA was isolated from 20% homogenate of infected tissues in sterile PBS by Tri-reagent (Sigma, USA) according to the manufacturer’s instructions and cDNA was prepared using commercial available kit. The cDNA was stored frozen at −20°C until used. For the molecular detection of classical swine fever virus specific nested PCR amplification of E2 and 5’NTR was done along with NS5B and E\textsuperscript{ms} amplification. Primarily these samples were found positive with these primers. Further confirmation by sequencing was done by cloning of these PCR products in pGEM-T easy vector. E2 and 5’NTR sequences were considered for phylogenetic analysis along with 20 complete available sequences of CSFV. Nucleotide sequence alignments were carried out using the ClustalW program (DNASTAR) and Phylogenetic tree analysis (DNASTAR) showed that 5’NTR have close proximity with Taiwan strain (Accession No. AY568569) and E2 shows close proximity with Chinese isolate CSFV-39 (Accession No. AF407339).

VVP-2 Sheep Vaccinated with Dual Vaccine Containing the Thermo Stable PPR and Attenuated Sheeppox Viruses Elicited Protective Immune Response

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Peste des petits ruminants (PPR) and sheeppox are OIE notifiable diseases of small ruminants especially sheep and goat. Both the diseases are economically important, in enzootic countries like India and are major constraints in the productivity of animals. Considering the geographical distribution of both PPR and sheeppox infections and prevalence of mixed infection, in the present study, safety and potency of the experimental dual vaccine comprising attenuated strains of thermostable-PPR virus (PPRV-Revati, P-50) grown at 40°C and attenuated sheep pox virus (SPPV-Srinagar, P40) was evaluated in local non-descript sheep. Experimental animals were grouped into four groups and each group was comprising six animals, received 100 doses (10\textsuperscript{5} TCID\textsubscript{50}), 1 dose (10\textsuperscript{5} TCID\textsubscript{50}) and 1/10th dose of vaccines and normal saline as control in 1 ml volume subcutaneously, respectively. Serum samples were collected on 0, 7, 14, 21 and 28th day post vaccination. Sheep simultaneously immunized with 1 ml of vaccine consisting of either 100 or 1 doses of each of PPRV and SPPV were monitored for clinical and serological responses for a period of 3–4 weeks post-immunization (pi) and post

MVP-35 Seroprevalence of Human Immuno Deficiency Virus Among the People Living in Northern Hills of India

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Human immunodeficiency virus (HIV) is a lentivirus (a member of the retroivirus family) that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The four major routes of transmission are unsafe sex, contaminated needles, transmission from an infected mother to her baby at birth (vertical transmission) and breast milk. Screening of blood products for HIV has largely eliminated transmission through blood transfusions or infected blood products in the developed world. In 2008, globally, about 2 million people died of AIDS, 33.4 million were living with HIV and 2.7 million people were newly infected with the virus. HIV infections and AIDS deaths are unevenly distributed geographically and the nature of the epidemics vary by region. More than 90% of people with HIV are living in the developing world. There is growing recognition that the virus does not discriminate by age, race, gender, ethnicity, socioeconomic status—everyone is susceptible. However, certain groups are at particular risk of HIV, including men who have sex with men (MSM), injecting drug users (IDUs), and commercial sex workers (CSWs). The present study indicates the prevalence of HIV infection among the people residing in the northern region of India predominantly among the foothills of the Himalayas. The study was carried out on the patients visiting Herbertpur Christian Hospital (A unit of Emmanuel Hospital association) under the integrated counselling and testing centre scheme at the respective hospital during the 2009–2010. The study indicates the screening of people groups residing in the respective area through community health schemes. The diagnosis of the HIV infection is done by three types of assays namely the tridot method which is the rapid method of diagnosis followed by the HIV Coombs test which involves the DOT immunoassay principle. The third assay is the enzyme linked immunosorbent assay (ELISA). The number of patients screened during the period of September 2009 to March 2010 is 635 which include patients coming from four different states namely Haryana Uttarakhund Uttarakhand and Himachal Pradesh. The number of people who were tested positive are 8 and the number of people who were tested negative are 627. The people tested positive are sent to the higher centre for other confirmatory tests such as PCR and western blot analysis. These patients are sent for treatment and prophylaxis at a respective recognised centre in Dehradun. The present study determines a consistent community HIV screening and treatment approach through diagnostics counselling and awareness programmes.
challenge (pc). Specific immune responses i.e., antibodies directed to both PPRV and SPPV could be demonstrated by PPR competitive ELISA kit and capripox indirect ELISA, respectively following immunization. All the immunized animals resisted infection when challenged with virulent strain of SPPV (Srinagar isolate at P-6) on day 28 dpi, while in contact control animals developed characteristic signs of sheeppox. The challenge of the sheep against PPR was not carried out, however, the antibody titre after immunization determined by SNT and ELISA, indicated that protective titre, as per earlier report on the goats. Dual vaccine was found safe at higher dose and induced protective immune response even at lower dose ($10^2$ TCID$_{50}$) in sheep, which was evident from sero-conversion as well as challenge study with SPPV. The study indicated that both the viruses are compatible and did not interfere with each other in eliciting immune response, paving the feasibility of use of this dual vaccine in combating both infections simultaneously.

**VVP-3 Long Term Potency Trials of a Live Attenuated Goat Pox Vaccine**

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Goatpox is one of the highly contagious, OIE notifiable and economically important viral diseases of goats. The disease is caused by goatpox virus (GTPV) is classified of the genus Capripoxvirus in the family Poxviridae. The disease incurs severe economic losses in terms of high morbidity in adults and heavy mortality in young kids and is a major constraint in goat farming in India. Considering the enzootic nature and economic impact of the disease, it is all important to control the infection by developing an effective vaccine. Recently, Vero cell based a live attenuated goat pox vaccine; using GTPV Uttarkashi isolate (P60) has been developed in authors’ laboratory and evaluated in goats. The vaccine was found safe, potent and immunogenic experimentally and even at field trials. The vaccine has been evaluated at large-scale at different regions of the country and found suitable for mass vaccination. However, the longevity of potency was not evaluated. Therefore, a long term potency trials were studied for a period of 4½ years with annual challenge by using virulent goatpox virus and sero-monitoring. A sufficient number of hill goats has been vaccinated with 1 dose of vaccine ($10^{3.0}$ TCID$_{50}$/ml) and monitored for clinical and serological response. Every year, significant number of vaccinated ($n=5$) and control animals ($n=2$) were used for challenge with virulent strain ($2 \times 10^{10}$ SRID$_{50}$/ml, GTPV Uttarkashi). Sera of pre- and post-challenged (14 dpc) animals including controls have been collected and monitored for serological response in the form of specific antibody production by SNT and indirect ELISA. All the vaccinated animals were protected on challenge, whereas, all unvaccinated controls developed infections. The same has been reflected in sero monitoring of collected sera. So the developed live attenuated goat pox vaccine was found safe, immunogenic and potent for a period of 4½ years of immunization and suitable for mass scale vaccination in control and eradication of goat pox along with a are suitable diagnostic tool/s in goatpox enzootic country like India.

**VVP-4 Prevalence of Rotavirus Antibodies in Field Chicken Sera**

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Rotavirus infection in avian species varies from subclinical infections to outbreaks of diarrhea. The economic significance of rotaviral enteritis to the poultry industry has not yet been defined, but by analogy to the situation in mammals, it is likely to be significant. Unlike the extensive studies performed on rotavirus infection in humans and animals, limited studies have been carried out to determine the extent of exposure of poultry birds to rotaviruses. To determine the prevalence of avian rotavirus antibodies in commercial broiler chickens. A total of 120 chicken serum samples were collected from the lairage of a poultry slaughter house where birds from four different broiler farms in and around Pune city were supplied to. The serum samples were tested by an IgG antibody capture ELISA wherein purified chicken rotavirus Ch2 was used as coating antigen. Sera from specific pathogen free (SPF) chick ($n=20$) served as negative control in the test. Cut off was calculated as Mean negative control + 3SD (standard deviation). S/CO (mean sample OD 450/cut off) values above 1 (1.113–4.445) in 60% (72/120) serum samples were indicating positivity to rotavirus antibodies. The result of the study indicates exposure of the birds to avian rotavirus or similar agent that is circulating in Pune city.

**VVP-5 Standardization of RT-PCR for Typing of BTV 2, 9 and 15**

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Bleutongue has become established in south India causing regular outbreaks in sheep. BTV serotypes 2, 9, 15 and 21 were isolated from native sheep of Andhra Pradesh. The other serotypes circulating in the state need to be identified. However the major constraint is the serotype identification. To overcome the difficulties of traditional serotyping methods (neutralization tests), nucleic acid based tests are being tried. RT-PCR for serotyping was standardized using primers specific to VP2 gene of BTV-2, 9 and 15 serotypes. RT-PCR resulted in 653 bp product of BTV-2, 1241 bp product of BTV-9 which was defined by specific primers. However non specific amplification at two different sites i.e. 700 bp and 1500 bp was noticed for BTV-15. Specificity of RT-PCR was evaluated. BTV-2 and BTV-9 specific primers could amplify only BTV-2 and BTV-9 respectively wherein BTV-15 type specific primers amplified not only BTV-15 but also BTV-2 and BTV-9. Nucleic acid sequence data obtained from BTV-2 PCR product and BTV-9 cloned products were specific to VP2 gene of BTV-2 and BTV-9 respectively. However, 700 and 1500 bp products of BTV-15 were identical to VP4 gene of BTV-2, 8, 10, 11, 13 and 18 and VP1 gene of BTV-2, 8 and 10 respectively, indicating the non specific amplification of BTV-15.

**VVP-6 “Production of Chimeric Foot and Mouth Disease Virus Serotype ‘O’ Using Serotype Asia 1 Genome as a Backbone Through Reverse Genetics Approach”**

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Foot and mouth disease is the most contagious and highly economically impotent disease of cloven footed animals. The disease is controlled by regular vaccination using the vaccine produced from the virus grown in the cell culture. The vaccine strain used for vaccine production is selected from the field isolates based on the adaptability and growth kinetics in BHK21 cells and antigen coverage. However the field viruses need to be passaged several times to adapt in tissue
VVP-7 Expression of Polyvalent Immunoactive Protein Genes Constructed by Linking 3 Foot and Mouth Disease Virus Serotype-1D (VP1) Protein Genes: A Strategy for Development of Polyvalent Subunit Vaccine

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Foot and mouth disease (FMD) is the most contagious viral disease and a potential threat to cloven hoofed wild and domesticated animals. The virion (FMDV), a non-enveloped icosahedral capsid consists of infectious single stranded positive sense RNA of 8.5 kb. The FMDV occurs as 7 serotypes (A, O, Asia 1, C and Sat 1-3) of which Sat 1-3 are confined to only South Africa. The remaining 4 (A, C, Asia 1, C) which are called as European strains are distributed world over. In India serotypes A, O and Asia 1 are in circulation. The genome of FMDV has a single open reading fame coding for 3 polyprotein, P1, P2 and P3. The P1 is composed of 1A, 1B, 1C and 1D (VP4, VP2, VP3, and VP1) respectively of which the VP1 is the most immunogenic and subunit vaccine produced with VP1 alone was able to induce high level of neutralising antibodies. Thus to control the disease in India polyvalent vaccine consisting of the inactivated virus of all the three serotypes are in use. However the conventional vaccines have several drawbacks which include safety and temperature sensitivity. Hence alternatively sub-unit vaccines consisting of VP1 protein has been tried. However this showed limited success due to the antigenic variations occurring in the field viruses thus escaping the neutralization from the antibodies generated from single cloned protein. Hence the present study was undertaken with an objective to include all the neutralizing epitopes present in the three serotypes by linking VP1 (1D) genes and produce a poly valent protein for using as poly subunit vaccine. In this study we have constructed a cassette by linking the genes of three serotypes ‘O’ (622 bp), ‘A’ (640 bp) and ‘Asia 1’ (622 bp). These genes were cloned individually in commercially pBSk vector and confirmed by sequence analysis before linking in pC DNA vector. The linked gene construct was sub-cloned in pET32 expression vector. The expression of the protein gene from the pET vector was induced with IPTG and analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). A fusion protein of size 72 kDa was observed in PAGE gels. Since the protein contains 6 His residues from the vector at the N-terminal end, affinity purification was carried out using nickel nitrilo-tri-acetic-acid (Ni–NTA) agarose matrix. The immunoreactivity of the purified protein was assayed by western blot with the anti FMDV type ‘O’ and ‘Asia 1’ specific sera. The may be used as a subunit vaccine.

VVP-8 Recent Trends in Disinfection for Viral Disease Management in Sericulture

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Silkworm diseases caused by viruses, bacteria, fungi and protozoans form major constraints for the silk cocoon production in all the sericultural countries and among these silkworm viral diseases viz., nuclear polyhedrosis and infectious flacherie caused by BmNPV and BmIVF cause severe crop loss. The traditional disease management strategies include prophylactic measures and use of disease free silkworm eggs. The prophylactic measures such as disinfection of silkworm rearing house and appliances, egg surface, silkworm bed disinfection and rearing surroundings. The disinfectants used presently in sericulture are either formaldehyde or chlorine based products, but these chemicals are neither eco-nor user-friendly. The awareness about health hazards caused by formaldehyde and environmental pollution caused by Cl2 necessitated the development of eco- and user-friendly disinfectant products for use in sericulture. These include alternative disinfectant products developed using biodegradable chemicals and plant based ingredients by APSSRDI, Hindupur and Central Silk Board for the management of silkworm diseases in India. The ideal disinfectant for sericulture would be the one which can inactivate silkworm pathogens of diverse origin and economical for sericulture. The paper discusses on the disadvantages of HCHO and Cl2 based disinfectants and advantages of eco- and user-friendly disinfectant for the management of silkworm diseases especially the ones caused by viruses.

VVP-9 Baculovirus Expression System-Silkworm as Bioreactor

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The Baculovirus Expression Vector System (BEVS) is widely used for the production of high levels of properly post-translationally modified, biologically active and functional recombinant proteins and has facilitated basic biomedical research on protein structure, function, drug discovery and the roles of various proteins in disease. BEVS is based on the introduction of a foreign gene into nonessential for viral replication genome region via of homologous recombination with a transfer vector containing target gene. The resulting recombinant baculovirus lacks one of nonessential gene (polh, v-cath, chiA etc.) replaced with foreign gene encoding heterologous protein which can be expressed in cultured insect cells and insect larvae. Insect cell-BEV system is widely used to produce recombinant proteins. BEVS also eliminates concerns regarding pathogens that could potentially be transmitted to humans as it is non-infectious to vertebral animals.
These features make silkworm system an ideal expression and delivery package for producing proteins of medicinal importance. The efficiency, low cost and large-scale production of proteins using BEVS represents breakthrough technology that is facilitating high-throughput proteomic studies. The BEVS has become a core technology for cloning and expression of genes for study of protein structure, processing and function; production of biochemical reagents; study of regulation of gene expression; commercial exploration, development and production of vaccines, therapeutics and diagnostics; drug discovery research; exploration and development of safer, more selective and environmentally compatible biopesticides. Utilization of silkworm larvae and pupae as bioreactor with recombinant BmNPV producing foreign proteins extends the usages of silkworms. Due to its large-size and high protein synthesis ability as well as the expediency in mass culture, silkworm is considered as good candidate for producing recombinant proteins.

**VVP-10 A Study on Some Ecological Factors in the PCR Screened White Spot Syndrome Virus in *Penaeus monodon***

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Shrimp farming has made rapid progress during the decade. However the sector suffered a serious setback mainly due to disease problems in recent times. Cultured shrimp suffer from various infections and non-infections. Diseases that have caused havoc in shrimp farming during the past 20 years (in coastal region of AP). The present study is intended to examine the various physic-chemical parameters of water in various *Penaeus monodon* culture ponds of different regions of Guntur and Prakasam districts, Andhra Pradesh, India. White spot syndrome virus (WSSV) is very important and responsible for huge economic loss in Indian shrimp culture industry. White spot syndrome associated Bacculo virus (WSSBV) is the other name of WSSV. WSSBV is the causative agent of a disease, which has recently caused high shrimp mortalities and severe damage to shrimp culture. WSSBV has been found across different penaeid shrimp species. In order to develop a effective diagnostic tool, a WSSBV genomic library was constructed by cloning WSSBV genomic DNA extracted from purified virions. In the present study WSSV disease free (confirmed by PCR analysis) were collected from hatcheries from different areas of Guntur and Prakasam districts and analysed to study the effect of various physical parameters like temperature, \(pH\), salinity and turbidity on the prevalence of above disease. The studies on the surface water temperature revealed fluctuations in the ponds ranging between 19 to 30.2°C in diseased ponds and 25.2 to 34.5°C in healthy ponds. These results show definite influence of temperature on the prevalence of WSSV.

**VVP-11 A Novel Strategy to Develop Positive Marker Vaccine Against Foot and Mouth Disease**

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Present day strategy in vaccine development is to include marker facility that helps in distinguishing antibody response due to vaccination vis-à-vis infection in vaccinated animals. Such information becomes relevant for effective disease control programmes especially when using inactivated virus vaccines like foot and mouth disease (FMD). The antibodies generated in the animals, only through vaccination, is the measure of vaccine efficacy and safety. Presently inactivated FMD virus (FMVDV) vaccines are used to control the disease in the endemic countries like India. The quality assurance of the vaccine depends on the efficacy of the vaccine in generating protective antibody without causing subclinical disease due to improper inactivation. Since protective antibody response in vaccinated animals can not be distinguished from that of infected animals one needs to assay the antibody response against non structural proteins (NSPs) and the vaccine must be free of contaminated NSPs. Production of vaccine free of NSPs requires the cumbersome method of virus purification which adds to the cost of the vaccine. Alternatively one may develop a positive marker vaccine by including a foreign protein or epitope which is not expected to be present in the vaccine and the antibodies generated against which helps in detecting the vaccine related response. Here we report a molecular approach by which we introduced an immuno-dominant epitope of green fluorescent protein (GFP) into the structural protein gene of foot and mouth disease virus strain Asia 1 (63/72). Our laboratory has produced a mini-genome of FMDV Asia 1 that lacks structural protein gene (P1-2A) coding for all the structural proteins (VP-1-4) of FMDV Asia 1 as a vector (pCFL /AAsia 1). The P1-2A of the Asia 1 vaccine strain was cloned separately into a plasmid vector and by successive PCR mutagenesis and cloning we have introduced nucleotide sequence corresponding to 9 amino acid epitope of GFP into P1-2A gene. GFP epitope was inserted at N-terminal region of VP-2 which is not immunogenic. The modified P1-2A was expressed in *E. coli* and studied. The modified P1-2A gene with GFP epitope was inserted into the pCFL AAsia 1 to get full length replication competent cDNA cloned under CMV promoter in pCDNA (pCFLAsiaGFP). This can be used to produce synthetic virus with GFP epitope that can generate antibodies not only against neutralizing epitopes but also against GFP epitope. Presence of antibody against GFP epitope in the vaccinated animal will reveal vaccine efficacy. ELISA against GFP can be used as a companion test not only for safety evaluation but also for quick evaluation of efficacy. Further absence of NSP antibodies in the serum may reveal the quality of the vaccine in respect of safety.

**VVP-12 Immunological Evaluation of Self Replicating DNA Vaccine (Constructed for Humoral Response) carrying 1D Gene of Three Foot and Mouth Disease Virus Serotypes in Guinea Pigs**

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Self replicating DNA vaccines are developed to achieve robust immune response through enhanced antigen production and gamma interferon expression in vaccinated animals. Since self replicating DNA vaccines induce gamma interferon expression which helps in viral clearance such vaccines are expected to be useful to cure even the carrier and persistently infected animals. Understanding the events that help in the elicitation of both the arms of immune response in vaccinated animals is necessary to understand the effectiveness of the vaccine. The work presented here deals with the immunological evaluation of a Sindbis virus replicase based DNA vaccine carrying linked FMDV VP1 genes in vaccinated guinea pigs. We have constructed self replicating DNA vaccine vector and to the down stream
of a sub genomic promoter we have inserted secretory signal followed by linked-VP1 genes of 3FMDV serotypes (O-A-Asia 1) with glycine and proline bridge in between. Guinea pigs were vaccinated with the construct and the sera at 28 days post vaccination were evaluated both for cellular response by studying the CD8 levels and by MTT and cytokine profiles by real time assays. The humoral response was evaluated by studying CD4 levels in the whole blood by Facs analysis and serum antibody levels by SNT and ELISA. The animals were challenged with 100 GP infective dose of FMDV type ‘O’ virus lesions were scored. Further the replicative efficiency of the challenge virus was studied by 3AB ELISA. The results showed that all the assays except antibodies against 3AB protein have positive correlation with the protection. As expected the titre of the antibodies against 3AB protein was lower indicating that the challenge virus replication was inhibited in the vaccinated animals. The limited studies conducted by us showed that self replicating vaccine has a potentiality to emerge as potent vaccine for FMD.

VVP-13 Phylogenetic Comparison of Ganjam Virus Isolated from India with Other Nairoviruses
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Ganjam virus (GANJV) belongs to the genus Nairoviruses (family Bunyaviridae). These viruses cause diseases in livestock. It has been isolated from different animal hosts and tick vectors from India. Genus Nairoviruses includes a total of 34 tick-borne viruses, classified into 7 serogroups. The important serogroups are Crimean Congo hemorrhagic fever (CCHF) and the Nairobi sheep disease (NSD). The main members of the NSD group are NSD and Dubge viruses. Their genome consists of three segments of single stranded RNA, viz. S, M and L that encodes viral nucleocapsid protein, Viral glycoprotein G1 and G2 and the Viral polymerase respectively. GANJV is very closely related to (NSDV). NSDV is found in East and central Africa, causes very high morbidity and mortality in livestock. The present study involves phylogenetic comparison of GANJV isolates from India with other nairoviruses based on complete N gene. It will help to understand the kind of nucleotide (nt) and amino acid (aa) changes that have occurred in GANJV strains from different geographical areas. Eight strains of GANJV isolated at NIV during 1954–2002 from different parts of India were used in this study. Virus stocks were prepared in Vero E6 cell line these were used as the source of viral RNA. The N gene was amplified either as a complete gene in one reaction or in fragments whenever necessary. Thus obtained sequences were analyzed; annotated to get a consensus sequence, aligned against the sequence of prototype strain of GANJV and other representative nairoviruses. The nt sequences were converted to aa sequences and analysis was done at both nucleotide and amino acid levels. Based on what nt or aa phylogenetic tree was constructed and compared with other nairoviruses (CCHF, DUGV, HAZV, KUPV and NSDV) where complete S segment sequences were available GenBank database (NCBI). The phylogenetic data at both the nt and aa levels showed that all the strains of GANJV form monophyletic lineage with the NSDV. CCHFV and HAZV together formed another clade, whereas DUGV and KUPV made a separate branch in the tree. The different GANJV strains showed 9–10% difference with NSDV at the nucleotide level and 3–4% difference at the amino acid level. HAZV showed 37–38% difference at the nt level and 37% difference at the aa level with GANJV as well as NSDV. The present data obtained suggests that GANJV and NSDV are minor variants of the same virus.

VVP-14 Electropherotyping and RT-PCR Detection of Bovine Rotavirus Associated with Diarrhea in Dairy Calves in Tarai Region of Uttarakhand
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Diarrhoeal syndrome is one of the major concerns of the livestock industry. Most of the diarrhoeic cases in animals go unnoticed and limited attention is paid on viral etiology. Presence of large amount of fecal matter in animal shed acts as a source of infection for calves via drinking water, feed, or contaminated soil. Keeping this in view, investigation was planned to detect the association of rotaviruses with diarrhea in dairy calves and to observe the genomic diversity among the circulating viruses in Tarai area of Uttarakhand. A total of 63 diarrhoeal fecal samples collected from Instructional Dairy Farm, Nagla, Pantnagar, Uttarakhand were screened during the study. Samples were collected from both cow calves and buffalo calves in 0–3 months of age. For the diagnosis of rotavirus, all the fecal samples were subjected to RNA-electrophoresis after nucleic acid extraction. Viral genome segments were visualized by silver staining. Out of the total 63 samples tested, seven were found positive in RNA-PAGE showing typical 11 genome segments migration pattern of bovine rotavirus. In the given samples prevalence of bovine rotavirus was 11.32% and 10% in cow and buffalo calves, respectively. On the basis of migration patterns of rotavirus in RNA-PAGE, group A were identified with typical 4:2:3:2 pattern. Variation within movement of various genome segments among isolates of bovine rotaviruses was observed during the study that may be indicative of emergence of mutants in the circulating isolates. The VP6 gene based group A specific RT-PCR was standardized and all the isolates in this area were confirmed to be of group A type. Work is in progress to genotype the bovine rotaviruses of this region based on VP7 and VP4 genes. This study emphasizes the need to explore the prevalence of bovine group A rotaviruses in different places of Uttarakhand and their genetic characterization which could help in selection of control strategies for rotavirus infections.

VVP-15 Significance of Molecular Diagnosis of Foot and Mouth Disease
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Foot-and-mouth disease (FMD) is endemic in India causing enormous economic loss to the animal keepers and trade embargo with FMD free countries in livestock and animal products. Rapid diagnosis of FMD is of immense importance in prevention and control of the disease. FMD is initially diagnosed clinically and confirmed by laboratory tests. Virus isolation in cell culture and sandwich ELISA for antigen detection are commonly practiced in laboratories. The virus isolation though is very sensitive but it can be slow and analytical sensitivity of the ELISA is lower and can not be used with certain sample types. The use of molecular techniques in the diagnostic laboratory has greatly increased the speed, specificity and sensitivity of FMD diagnostic tests. Molecular techniques like RT-PCR, PCR-ELISA and dot hybridization can be used with more success for detecting carrier animals and animals harboring sub-clinical infection and can be applied in a wide range of clinical sample types. These
techniques can be used as genus and serotype specific test including detection of particular lineage/genotypes with in the serotype. Multiplex PCR has been used to differentiate serotypes of FMDV and the technique is sensitive, experimentally simpler, cost effective and less time consuming. The assay can be used for serotyping on ELISA negative samples. The molecular techniques not only help in diagnosis but also useful for epidemiological studies. Lineage differentiating RT-PCR has been useful in identifying different lineages of serotype Asia 1 (Lineage B, C and D) before proceeding with sequencing of 1D region. Similarly genotype differentiating RT-PCR has been developed and used in differentiating two different genotypes of serotype A (Genotype VI and VII). These assays have the potential to be applied on clinical samples directly, thereby saving much time needed for sample processing and nucleotide sequencing. Recent development of real time RT-PCR methodology has allowed the diagnostic potential of molecular assays to be realised. Advancement in real time PCR technology made it possible to combine several assays within a single tube which is in the progress in our laboratory. Integration of these assays onto automated high throughput platforms provides diagnostic laboratories with the capability to test large numbers of samples. Microarray technology was provided greater screening capabilities for pathogen detection. The microarray allows the addition of large number of oligonucleotide probes for identification of mutant pathogen and also for subtype determination. The combined properties of high sensitivity and specificity, low contamination risk, and speed has made real time PCR and microarray technology a highly attractive alternative to conventional methods in increasing percentage of outbreaks confirmed and analyzed and for tracing the origin of FMD virus responsible for outbreaks.

VVP-16 Sindbis Virus Replicase Based DNA Vaccine Construct Carrying Polyvalent Immunoreactive Protein Gene of Foot and Mouth Disease Virus, Developed for Humoral Response Shows Protective Response in Guinea Pigs Against Challenge Virus

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DNA vaccines are expected to elicit both humoral and cellular responses, cellular response being long lasting. However the approach has several limitations like poor stability of DNA, poor expression and risk of integration. Poor expression becomes the major limitation in the case of FMD as FMDV proteins are poor immunogens. Also DNA vaccine vectors carrying only eukaryotic promoters elicit strong CMI response and weak humoral response. The methodology to achieve humoral response involves the expression and secretion of the expressed protein so that the antigen presenting cells will be able to process the antigen and produce humoral response. In case of FMD humoral response is as important as cellular response. The present project aims at addressing these issues; achieving higher expression and getting the protein secreted out by constructing self replicating gene vaccines for FMD and studying their efficacy. The vector for humoral immune response contains eEF1 promoter, Sindbis virus polymerase gene and secretary and anchoring signals. The integrity of the vectors was confirmed by sequence analysis. The linked polyvalent protein genes of FMDV serotype A, O and Asia 1 were cloned into the vectors and the presence of the insert was confirmed by restriction enzyme digestion. The functionality of the constructed DNA vaccine vector (pVac Self Rep 990) was assayed by transfecting the DNA into BHK 21 cell monolayer and studying the 35S labeled proteins in immuno-precipitation assays. The studies showed high level of expression in case of constructed vector as compared to infected virus for the specific protein. The secretion of the expressed protein was assayed by Immuno-fluorescence assay and found to be positive. Encouraged with these studies the preliminary studies were conducted on vaccine efficacy studies in guinea pig model. The immunized guinea pigs showed high antibody titre by SNT and ELISA, as compared to conventional DNA Vaccines (pUP3CD) even at 1/10th of the dose. This approach of constructing self replicating DNA vaccine for humoral response is the first report.

VVP-17 Chloroplast Mediated Foot and Mouth Disease Virus Gene Transfer into Sunnhemp: A Strategy for Development of Edible Vaccines

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Genetically engineered microorganisms are important sources of industrial and medicinal proteins. Over the past decade, plant host system has been investigated as potential host system for expressing proteins of therapeutic and diagnostic use. However concerns regarding the stability and environmental safety need to be addressed. Chloroplast engineering is expected to resolve some of these issues since, plastids/chloroplasts are inherited maternally and are not disseminated through pollen. This makes plastid transformation a valuable tool for transgenic creation besides offering biological containment. Since foot and mouth disease (FMD) of cloven footed animals is a major concern in the world over. Presently virus based inactivated vaccines are used to control the disease. However there are several draw backs with the conventional vaccines, like risk of handling live virus, safety and temperature sensitivity of the vaccine and the vaccine can not be used at the phase of outbreak. To overcome these attempts have been made towards development of alternative vaccines for the conventional virus based immunoprophylactics. Several alternative vaccines have been developed and studied. However none of them can be used as therapeutic vaccine at the phase of outbreak. The present investigation deals with the strategy to develop an edible vaccine by transferring the immunogenic protein genes into forage crop through chloroplasts. To carryout this transfer vector was constructed with the commercially available pBSk plasmid as backbone. Promoter region of Ribulose dicarboxylase (large) gene (rbcL) was amplified and cloned in pBSk and sequenced. Multiple cloning sites and left and right border sequences were added. FMDV serotype ‘O’ P1-2A gene was inserted. Since promoter sequence showed homology with prokaryotic promoter, expression of the P102A immunogen under the rbcL promoter was checked in the E. coli host DH5α. This transfer vector was then coated with tungsten particles and bombarded on to the sunn hemp callus raised from the leaf explant. The calluses were then screened for the presence of the transgene by PCR. Out of 7 calli 2 showed the presence of the transgene. The positive calli are to be further subjected to organogenesis before evaluation as edible vaccine for FMD.

VVP-18 Development of Herbicide Resistant Transgenic Peanut (Arachis hypogea) Plant Carrying Foot and Mouth Disease Virus Serotype ‘O’ Structural Protein (P1-2A) Genes, for Edible Vaccine Development

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Foot and mouth disease (FMD) is the most feared, viral disease of the cloven hoofed animals causing heavy losses to the live stock industry. The disease is enzootic in many parts of the world including Asia. The conventional vaccines for FMD have several limitations which include safety, temperature sensitivity and duration of immunity. Attempts have been made to overcome these limitations using recombinant DNA technology. amongst the newer vaccines, edible vaccines are cost effective and easy to administer. Since the stability of the gene of interest is the major concern in the case of plant transgenics, marker genes are used for regular selection. The detection methods based on the available marker proteins like β Glucoronidase (GUS) protein/antibiotic selection are cumbersome and cost intensive. However selection based on herbicide resistance is much simpler and easy. Hence in the present study, the 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) gene was used as a marker along with the immunogen gene of FMDV. EPSP is the key enzyme in the shikimate biosynthesis pathway necessary for the aromatic amino acids production. The herbicide Glyphosate is a competitive inhibitor of EPSP. Thus the gene product titrates the herbicide. The GUS gene was replaced with EPSP gene in the binary vector pCambia 2301 to get pCambiaEPSP. P1-2A of FMDV serotype ‘O’ was cloned under CAMV promoter separately in pBSk and the cassette consisting of CAMV-P1-2A was mobilized into pCambiaEPSP to get pCambiaEPSP-P1-2A ‘O’. The clones were confirmed by sequence analysis. The construct was then transferred into Agrobacterium (LBA4404). The bacteria was then co-cultivated with the injured groundnut cotyledons (containing the embryos) and the plants were raised. The fully grown matured leaves of these groundnut plants were swabbed with Glyphosate at the concentration of 3000 ppm. The plants which showed tolerance to Glyphosate were further screened for the presence of the FMDV gene by PCR. Out of 45 transformants, 8 were found to be positive both for marker gene and the FMD immunogen at T1 level. The seeds from positive plants are now under further propagation for immunological evaluation as edible vaccines.

**VVP-19 Cationic Micro Particle (PLG) Coated DNA Vaccination Induces a Long Term Immune Response and Protective Immunity Against Foot-and-Mouth Disease Virus**

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In order to investigate the mechanism of long term immunity and the effect of protective immunity induced by cationic PLG micro particle coated DNA vaccination. We constructed the expression plasmid containing a Foot-and-mouth disease virus (FMDV) ID gene sero type A. Intramuscular vaccination of Guinea pigs with the micro particles coated plasmid DNA induced a strong antibody response and neutralization antibodies, cellular mediated immune response which lasted 1 year. We further analyzed the persistence and expression of ID gene by polymerase chain reaction and reverse transcriptase polymerase chain reaction and quantitative PCR. The results showed that ID gene was present and expressed in the muscle cells up to 1 year after days post vaccination. Furthermore, Guinea pigs vaccinated with micro particles coated plasmid DNA were protected against a Challenge with FMDV virus. Therefore the micro particles coated plasmid DNA vaccination dose induce a protective immunity and long term humoral, cellular immune responses against FMDV, which could be maintained by persistent expression of ID gene in muscle cells.

**VVP-20 Bovine Interleukin-18 Inhibits Foot-and-Mouth Disease Virus Replication in BHK-21 Cells**

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Foot and mouth disease virus (FMDV) causes a highly contagious viral disease of cloven hoofed animals, which has a considerable socio-economic impact on the countries affected. Interleukin-18 (IL-18) enhances the IL-12 driven Th1 immune response that is important in immunity against intracellular pathogens. The multiple roles of IL-18 in many physiological and pathological processes have generated a great deal of interest in recent years. Antiviral effects of IL-18 have been reported. We evaluated the effects interleukin-18 (IL-18) on the replication of FMDV in vitro in BHK-21 cells. Bovine IL-18 mature protein coding sequence was amplified from the bovine PBMC cells and cloned into prokaryotic expression vector pET32α. Protein expressed was purified and specificity was confirmed by immunoblotting. BHK-21 cells were treated with purified expressed IL-18 protein with (2 μg/ml) 4 h prior to FMD infection. Cell culture supernatants were collected at 24 h post infection were subjected for ELISA and virus titration assay. RNA extracted from the cells was subjected to Real Time PCR for viral RNA quantification. 2 log titer reduction was observed in the FMD virus titer in IL-18 treated cells compared to the untreated cells where as virus antigen quantified by ELISA has shown a reduction of 60-folds. 69-fold reduction in the FMD viral RNA copy number was observed in the IL-18 treated cell compared to the untreated measured by qPCR. Current study demonstrated the potent anti viral activity of IL-18 on FMDV by inhibiting the viral replication. These results further suggests that IL-18 has the potential role of IL-18 as molecular adjuvant in FMD vaccine development and development of therapeutic for FMD.

**VVP-21 Bio-Distribution of Nanoparticle Based DNA Vaccines Against FMD**

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Foot and mouth disease is the most contagious viral disease of farm animals. Control of the disease in animals is by vaccination and slaughtering of infected animals. Conventional oil adjuvant vaccine has its own limitation. Alternate to this genetic vaccines where the DNA encoding viral antigen may be a promising approach. Naked DNA vaccine has limitations like poor uptake of DNA by cells and more importantly by nucleus. As a result delivery of naked DNA through calcium phosphate nanoparticle was attempted. Calcium phosphate nanoparticle is a potential delivery agent which proved to enhance the immune response. FMDV P1-3CD “O” vaccine gene constructs in pCDNA3.1+ entrapped by the nanoparticles was prepared by using different molarity of calcium chloride and disodium hydrogen orthophosphate. The nanoparticles entrapping FMDV P1-3CD “O” and naked DNA were presented to the guinea pigs through intramuscular injection to study the mRNA expression of antigen by RT-PCR. Animals were sacrificed at defined time to collect different organs and total RNA was extracted. Each time blood was collected to analyse the FMDV specific serum antibodies. DNA vaccines presented through calcium phosphate produced transcripts in the injected muscle up to 240 days whereas naked DNA up to 120 days.
Serum antibody levels of naked DNA vaccine showed antibody titre till 60 days. Whereas nanoparticle injected animals showed serum antibody till 120 days. Serum neutralization titres of 1.5 were observed in calcium phosphate DNA Vaccines at about 28–150 days, where as naked DNA SN titers were observed for short period of 30–90 days. The study clearly showed calcium phosphate nanoparticle entrapping FMDV vaccine DNA may be a better delivery system for DNA vaccines as it confirms availability of the antigen and persistence of antibody for longer duration than naked DNA.

VVP-22 Isolation of Bluetongue Virus (BTV) Serotype 21 from India
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Bluetongue was most rampant during 2005 in Andhra Pradesh, India with 880 outbreaks affecting 2,72,415 sheep and 62,938 deaths. A total of 93 heparinized blood samples were collected during the outbreaks in the state. Twelve of these samples caused death in embryonating chicken eggs (ECE) with subcutaneous haemorrhages. However, only one of these samples (KMN 07/05) collected from Karimnagar district caused cytopathic effect (CPE), on further passage, in Baby Hamster kidney 21 (BHK21) cell line. The CPE consisted of rounding of cells, aggregation of cells and peeling of the monolayer at about 72 h post inoculation. Preliminary confirmation of bluetongue virus in the sample was based on segmented RNA transcription polymerase chain reaction (RT-PCR). The virus was typed as BTV21 based on RT-PCR using BTV21 specific nested primers by Typing centre of All India Network Programme on Bluetongue at Haryana Agricultural University, Hisar. This is the first report of isolation of BTV21 from Andhra Pradesh. There is only one report of isolation of BTV21 from India, in December 2009, from migratory sheep flocks in Kolkata, West Bengal.

VVP-23 Comparative Sequence Analysis of Indian Capripox Isolates Based on Attachment Gene
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Capripox is highly infectious, contagious, and OIE notifiable disease of small ruminants, caused by sheeppox and goatpox viruses which are members of capripoxvirus genus of the family Poxviridae. In the present study, we analyzed the partial gene sequences of P32 protein, an immunogenic envelope protein of Capripox viruses (CaPV) to assess the genetic relationship among different sheep pox and goat pox virus isolates from different geographical areas of the country. Product of this gene has been shown to be important in attachment of CaPV to host cell surface receptors during viral entry and host immune response. The following virus isolates have been used in the analysis: GTPV-Uttarkashi, P60, vaccine virus; GTPV Mukteswar, P10, Challenge virus; GTPV (Akola), GTPV Bareilly/00, GTPV Ladakh/01 and GTPV Sambalpur/82, field isolates and SPPV Srinagar, P40; SPPV Ranipet, P50; SPPV-RF, P50, vaccine viruses and SPPV Makidhoom/07, SPPV CIRG/08, SPPV Pune/08, SPPV Bareilly, SPPV 183/03 and SPPV 125/02, field isolates. In this study, all virus isolates were confirmed by PCR amplification and analysed in PCR-Restriction fragment length polymorphism (PCR-RFLP) using EcoRI enzyme to confirm their specificity. Further, the amplicons were cloned and sequenced commercially. Nucleotide and the deduced amino acid (aa) sequences were compared with published sequences of the members of the genus Capripox virus. Sequence analysis of partial 172 bp sequence has shown high sequence identity among all Indian SPPV and GTPV isolates at both nt and aa levels. It revealed a 99.4–100% and 98.2 for GTPV field isolates where as, 100% for SPPV field isolates at both the nt and aa levels. In general, CaPV isolates in this study showed 98.3–98.8 and 96.5% homology between GTPV and SPPV at nt and aa levels as reported earlier. Further, it revealed a unique change of G120A in all GTPV isolates resulting in formation of DraI site in place of EcoRI and possible development of restriction enzyme specific PCR-RFLP for differentiation of SPPV and GTPV from field isolates.

VVP-24 Sequence and Phylogenetic Analysis of B2L Gene of Orf Virus from Camel (Dromedarius camelus) in a Sporadic Infection at Bikaner, India
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Orf or contagious ecthyma is considered as non-contagious, proliferative disease and is caused by Orf virus of the genus poxvirus of the family poxviridae. It is reported most commonly in sheep and goats and also a zoonotic agent. Camels are also infected by Orf virus and reported in camel rearing countries as a mixed infection with camel pox, the later is caused by an orthopox virus. In India, there are few reports of the Orf virus infection in camels and identified by clinical signs and PCR. In this study, we identified the presence of Orf virus from clinical samples of suspected case of sporadic infection in camels by serological and molecular techniques. Viral DNA isolated from processed scabs used initially in nested polymerase chain reaction as diagnostic PCR which successfully amplified 235 bp fragments and also sequenced to check the fidelity of the product. After confirming the infection by PCR, some of the structural and non-structural genes were amplified for sequence analysis. Out of the five genes characterized, the major important one selected for sequence and phylogenetic analysis is B2L gene which is homologous to a major envelope protein P37k of vaccinia virus. Full open reading frame of 1137 bp from Orf B2L was amplified by PCR, cloned and sequenced commercially. Nucleotide and deduced amino acid sequences of B2L were compared with other published sequences of the members of the genus papapox virus. Sequence analysis shows a maximum percent identity of 94.8 and 95 (Indian Orf virus isolates); 94.7 and 94.5 (other Orf isolates); 98.8 and 98.7 (Orf-camel/Jodhpur/08); 85 and 82.8 (bovine popular stomatitis virus) and finally 97.4 and 97.6 (pseudo cowpox virus) respectively at nt and aa levels. Phylogenetic analysis of the isolate was also performed using the neighbour joining method in MEGA 4 program to know the phylogeny relatedness of the virus, which revealed that the isolate is well grouped with the Jodhpur isolate and closely related to pseudo cowpox virus. It warrants further analysis of other potential genes to confirm the causative agent of the contagious ecthyma in camels as pseudo cowpox virus.

VVP-25 Screening of Domestic Animals for Chikungunya Virus in Andhra Pradesh, India
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Chikungunya an arboviral disease is transmitted through the bite of an infected Aedes mosquito. It causes a self limited febrile illness along with arthralgia and myalgia. In some cases neurological and severe hemorrhagic manifestations has been observed. CHIKV epidemic has been reported in Africa, India, South East Asian countries and during the current out break imported cases of CHIKV has been encountered in most of the European countries. The causative agent belongs to the genus Alphavirus family Togaviridae. Human beings serve as the chikungunya virus reservoir host during epidemic periods. Outside these periods the main reservoirs are monkeys, rodents, birds, and other unidentified vertebrates. Antibodies to CHIKV have been detected in domestic animals. In the present study we surveyed Madanapalli, Palamaner, B. Kotta Kota and Tirupati and collected a total of 67 rodent samples, 75 bovine samples; 20 sheep samples and 15 canine samples. Total RNA was isolated from all these samples and subjected to RT-PCR using a primer pair DVRChk-F/DVRChk-R which could amplify a 330 bp E1 gene product specific to Chikungunya virus (Naresh Kumar et al. 2007). All the Serum samples were further screened for CHIKV specific IgM antibodies using commercially available CTK biotech strips. None of the samples were found positive either for CHIKV specific RNA or CHIKV specific IgM antibodies. More number of samples from domestic animals as well as rodents are being screened to study their possible role if any in the maintenance of CHIKV in nature and during the inter epidemic periods. The present study discusses these aspects in detail.

**VVP-26 Characterisation of Proteins in the Haemolymph of Silkworm, Bombbyxmori During NPV and CPV Infection**

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Effective dose of free viral inoculums of cytoplasmic polyhedrosis virus (CPV) and Nuclear polyhedrosis virus (NPV) on bombyxmori as been evaluated. Proteins are in control and infected worms through 2D polyacrylamide gel electrophoresis (2D page). The amino acids like glycine and histidine seems to be increased as in pathological state. They may act as detoxifying agents in pathological conditions of CPV and NPV infection.

**Plant Virology**

**Poster Session**

**PVP-1 Petunia hybrida: A New Natural Host for Tomato Leaf Curl Virus in India**

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*Petunia hybrida* is widely used as experimental host plant for begomovirus identification and its characterization. Hitherto, natural infection of begomovirus on Petunia has not been reported in India. Recently, *Petunia hybrida* grown in and around Ludhiana were found to be depicting typical symptoms caused by begomovirus. The symptoms include severe reduction in leaf size, downward curling and distorted leaves. Severely infected plant became bushy, stunted and produces no flower. Total genomic DNA was extracted from the plants showing symptoms of begomovirus, by CTAB method. The presence of virus was confirmed by using degenerated primers, designed to identify all the begomovirus prevailing in the world. To identify the strain associated with the disease, the positive samples along with healthy control were tested against different strain specific primers of tomato leaf curl virus, so far reported in India i.e. tomato leaf curl New Delhi virus, tomato leaf curl Palampur virus, tomato leaf curl Banglore virus, tomato leaf curl Karnataka virus and tomato leaf curl Gujarat virus. Among these, only tomato leaf curl New Delhi virus specific primer was able to give the desired amolicion of ~ 1180 bp. Hence, it is confirmed that the leaf curl disease of *Petunia hybrida* is associated with tomato leaf curl New Delhi virus. This disease of petunia can become a sever production constraint in coming years.

**PVP-2 Tomato Leaf Curl New Delhi Virus Naturally Infecting Brinjal in India: A First Report**

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From last 2 years (2008 and 2009) it was observed that some varieties of brinjal grown in rainy season, showed typical leaf curl type of symptoms. The symptoms include upward curling of the leaves, cupping, vein thickening, reduction in leaf size and distortion of leaves. The severely infected plant remains stunted and bushy, became unproductive or produces only few fruits. The disease was experimentally transmitted from naturally infected brinjal to healthy seedlings by whiteflies (*Bemisia tabaci*) and grafting, but not by mechanical or aphid transmission. To detect the begomovirus associated, total genomic DNA was extracted from the plants showing disease symptoms. The presence of virus was confirmed by using PCR based begomovirus geneus-specific primers designed by Deng et al., Wyatt and Brown and Rojas et al. These degenerated primers gave the expected product size of ~530, ~575 and ~1280 bp, respectively. Core coat protein (CP) gene and DNA-β was also amplified in the samples using specific primers. To identify the strain associated with leaf curl virus, DNA was subjected against primers of different Indian tomato leaf curl virus strain i.e. Tomato leaf curl New Delhi virus, Tomato leaf curl Palampur virus, Tomato leaf curl Banglore virus, Tomato leaf curl Palampur virus, Tomato leaf curl Karnataka virus and Tomato leaf curl Gujarat virus, using PCR. Among these, only Tomato leaf curl New Delhi virus primer was able to show the desired product size of ~ 1180 bp. Therefore, it was confirmed that leaf curl disease of brinjal is caused by Tomato leaf curl New Delhi virus in association with satellite β-DNA.

**PVP-3 Tomato Leaf Curl Palampur Virus Infecting Calendula and Marigold in Punjab**

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*Calendula officinalis* (family Asteraceae) and Marigold is grown as annual ornamental plants in India for their beautiful flowers. Leaf curl like disease of Calendula and Marigold was observed on several plants growing in campus of Punjab Agricultural University, Ludhiana. Symptoms of the disease consist of stunting of plants, thickening of veins, curling and distortion of leaves in case of Calendula, while, the infected plants of Marigold showed upward curling of the leaves and leaf distortion. The total genomic DNA was extracted from the plants showing typical symptoms of leaf curl disease. A whitely transmitted begomovirus was detected by PCR using begomovirus-specific primers from naturally infected Calendula and Marigold plants. To identify the strain associated with the disease, all samples were further subjected to the specific primers, designed to amplify all the tomato
leaf curl virus strains, so far reported from India i.e. tomato leaf curl
New Delhi virus, tomato leaf curl Palampur virus, tomato leaf curl
Banglore virus, tomato leaf curl Karnataka virus and tomato leaf curl
Gujarat virus, using PCR. Among these, only tomato leaf curl Palampur virus specific primer was able to give the expected product size of ~900 bp. This shows the association of tomato leaf curl Palampur virus with leaf curl disease of Calendula and Marigold. Thus, Calen-
dula and Marigold can act as a reservoir for the tomato leaf curl Palampur virus and may cause severe constrain in the production of these important ornamental plants.

PVP-4 Mapping the Domains Involved in Oligomerization, RNA Binding and Phosphorylation of Nucleocapsid Protein of Groundnut bud necrosis virus

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Groundnut bud necrosis virus (GBNV) belongs to serogroup IV of the genus Tospovirus in Bynaviridae family and infects several econ-
ómically important crops all over India. The nucleocapsid protein (NP) encoded by the small RNA of GBNV encapsidates the viral RNA. Apart from this structural role, the NP has also been implicated in the replication, transcription, maturation and cell to cell movement. With a view to study the structure and function, the NP of GBNV-tomato isolate from Karnataka was over expressed in E. coli and purified by Ni–NTA chromatography. The purified NP was present as ribonucleoprotein complex and as heterogeneous mixture containing monomers, tetramers and higher order multimers. In order to determine the regions involved in oligomerization and nucleic acid binding, mutational approach was taken. N- and C-terminal deletion clones were generated (N20NP, N40NP, C15NP and C37NP), over expressed in E. coli, and were purified by a procedure identical to that used for the wild type protein. Initial studies on oligomeric status suggested that in addition to N- and C-terminal regions there may be additional regions or residues which contribute to multimerization of NP. The amount of RNA bound to the truncated proteins was reduced in case of N20NP, N40NP and C15NP. Interestingly removal of 37 amino acid residues (natively unfolded region) from the C terminus resulted in complete loss of nucleic acid binding suggesting that the RNA binding domain was located in C-terminal region of NP. Further NP was observed to get phosphorylated in in vitro kinase assays by a kinase present in the soluble fraction of tobacco plant sap. Both ATP and GTP were utilized as phosphoryl donors and Mn2+ was the preferred metal ion which suggests that NP might be phosphorylated by a CK2-like protein kinase. Phosphorylation studies with N- and C-terminal truncated proteins revealed that the site of phosphorylation lies within the amino acid residues 40–239. By Mass spectrometric analysis of the protein Threonine-84 and Serine-202 were identified as possible phosphorylation sites.

PVP-5 Genomics of Citrus yellow mosaic virus (CMBV) Isolates and Tests for Detection of the Virus: A Stop of Control of Virus Spread in India

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Citrus yellow mosaic virus (CMBV) is a member of Badnavirus genus; Family Caulimoviridae infects various citrus species causing mosaic disease in India. Various citrus species like Citrus sinensis (Sweet Orange), Citrus aurantifolia (Acid lime), Citrus grandis (Pumello), Citrus reticulata (Mandarins) and other citrus cultivars show variant symptoms of virus infection. Losses up to 77% very observed on survey, physiological and biochemical studies. Causative of the virus spread was found to be chiefly bud wood selected for grafting in case of Sweet Orange where as the vector transmission (Citrus mealy bug Planococcus citri) was experimentally observed but plays a minor role in field level spread of the virus. Survey in the citrus growing regions of India revealed the occurrence of virus is confined chiefly to Andhra Pradesh state. The virus is becoming a threat to the citrus industry in India. As traditional Immunological tests fail to detect virus efficiently, Molecular tests like PCR and Radioactive and Non radioactive NASH (Nucleic acid Specific Hybridisation) were developed for the virus detection. In order to understand the virus biodiversity among different isolates that affect different hosts, full genomes of various CMBV isolates were sequenced and analyzed. Phylogenetic analysis of the virus reveals its close relationship to Cacao swollen shoot virus, a member of Badnavirus genus. Diagnosis of the budwood would control the possible spread of virus to other citrus growing regions of India in the mere future.

PVP-6 First Report on Natural Occurrence of Isolate of Zucchini yellow mosaic virus Infecting Cucumis anguina (Gherkin) from India

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A naturally occurring isolate of virus infecting Gherkin (Cucumis anguina) showing mosaic symptoms of mosaic, leaf distortion and dark green islands in the lamina was identified in the export cultivars of gherkin grown in commercial fields of Kuppam rural, Chittoor District, Andhra Pradesh. The virus infection was deadly prevalent among the field that caused a lot of economic damage to the crop that resulted in yield losses and reduced quality of fruits meant for export. Symptoms of the infected fruit included blistering and malformation of the fruit. The virus infected leaf samples were collected and initial host range tests were conducted with different cucurbit species, but plays a minor role in field level spread of the virus. Survey in the citrus growing regions of India revealed the occurrence of virus to other citrus growing regions of India in the mere future.

Electron microscopy of the leaf dip preparations stained with 2% Uranyl acetate from the pumpkin leaves showing symptoms revealed the presence of a long flexuous filamentous particle measuring 750 × 12 nm. The virus positively reacted to the polyclonal antisera of Papaya ringspot virus-W, Potato virus Y, Tobacco etch virus and also strongly reacted with the polyclonal antiserum of Zucchini yellow mosaic virus in Direct Antigen Coated-Enzyme Linked Immunosor-

bent Assay (DAC-ELISA). Because of very strong reaction to polyclonal antiserum of Zucchini yellow mosaic virus, we tried to amplify the partial Nib and CP genes of the virus along with the 3’UTR by using two primers ZY2 5’GCTCCATACATACGTAGGACACG3’ and ZY3 5’TAGGCTTTTTTGCAACGAAGTCTA AT C3’. Total RNA from Gherkin infected leaves was isolated using Trizol LS reagent (Sigma). RT-PCR was performed to obtain an amplicon of ~1.2kbp, cloned into Fermentas pTZ57R/T vector and sequenced at MWG biotech, Bangalore. Sequence analysis revealed that the virus was isolate of Zucchini yellow mosaic virus and was
showing 98% of homology to that of the Zucchini yellow mosaic virus strain B genome AY188994 and Zucchini yellow mosaic virus NAT genome EF062582 which were strains reported from Israel. The sequence of the present study was submitted to the Genbank Q4482976. The results state a suspicion that the virus could have been mobilized by some infected source brought by the Commercial Israeli based companies into India due to poor quarantine regulations as the Gherkin cultivation in these regions is chiefly supported, purchased, exported and marketed by these private companies that are based from Israel. This is the first report on Molecular characterisation of Zucchini yellow mosaic virus infecting Cucumis anguria (Gherkin) from India.

PVP-7 Occurrence of Carlavirus in Brinjal (Solanum melongena L.) in India
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Reconnaissance studies were carried out during 2007–2010 for the occurrence and diversity of tospoviruses in vegetable crops in 14 major states in India. Surprisingly many viral symptomatic samples were negative to tospovirus broad spectrum Fab and Mab to Watermelon silver mosaic virus group. ELISA and multiplex RT-PCR for different virus species of Potyvirus, Cucumovirus, Ilarivirus, Tobamovirus, Tombusvirus and Carlavirus were done to identify the pathogen. Most of the Peanut bud necrosis virus (PBNNV) negative samples were positive for Cucumber Mosaic virus (CMV), carlavirus and potyvirus but the carlavirus was most prevalent in all the regions (TN, AP, MH). They also exhibited synergism with other virus which was region specific. Fifty percent of the total symptomatic plant population was found to be positive only for carla while remaining showed mixed infection of Carla with Tospo in some regions while in others carla virus was found to be associated with CMV. Presence of only carlavirus was up to 10–20% incidence, without association of Tospo, CMV, Poty or Tobami viruses was also observed in some fields.

PVP-8 The Role of Recombination Events in Genetic Diversity and Evolution of Citrus tristeza virus in India
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Citrus tristeza virus (CTV), a brown citrus aphid (Toxoptera citricidus) transmitted Closterovirus under family Closteroviridae, is one of the major limiting factors in cultivation of citrus worldwide. CTV is a longest known plant virus having flexuous particle of 2000 x 11 nm in size. CTV genome is a positive sense ssRNA of about 20 kb nucleotide containing 13 open reading frames (ORFs) encoding 17 proteins. Several biological as well as genetic variants of CTV are reported in all the citrus growing countries in the world. CTV causes decline and death of millions of citrus trees in the world. In India, CTV is a century old problem, and has killed an estimated one million citrus trees till today. In molecular and genetic level, CTV isolates from India were not fully characterized. Genetic diversity and sequence divergence in CTV isolates of India are not fully established. Further, evidence of recombination and causes of evolution of CTV variants in India have not been studied till date. Therefore, in the present study, effort has been made to characterize several Indian CTV isolates in genetic level, examine their genetic diversity, identify recombination events and analyze evolution of divergent CTV. A total number of 73 CTV isolates from different regions of India (35 from Darjeeling hills, five from Bangalore, 15 from Delhi and 18 from Vidarbha) were taken for genetic study. Two genomic regions of CTV, i.e., entire CP gene (CPG) (672 nt) and a gene fragment of 5‘ORF1a (ORF1a) (404 nt) were amplified, cloned, sequenced and nucleotides were analyzed. Based on CPG, Indian isolates shared 88–99% nucleotide identity, and based on ORF1a they shared 82–99% identity, among them. Incongruence of phylogenetic relationship was observed as on sequence analysis five phylogenetic clades based on CPG, and eight clades based on ORF1a, were generated suggesting the recombination events have been occurred between the sequences of Indian CTV isolates. Thus, to identify the potential recombination events, and determine the parental sequences in CTV isolates, six recombination detecting algorithms, namely, RDP, Gencov, Bootscan, Maxchi, Chimera and Siscan were used. Out of 73 Indian CTV, CPG of 18 and ORF1a of 47 isolates of CTV showed recombination events suggesting ORF1a was more prone and fragile to RNA recombination as compared to CPG. This findings indicated that high degrees of genetic diversity and incongruent relationships of Indian CTV isolates are due to genetic recombination occurred, which may be the important factors in driving evolution CTV variants in India, that was also supported by a splitTree decomposition analysis.

PVP-9 Molecular Characterization of Tobacco streak virus infecting Sunflower in Andhra Pradesh
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Sunflower (Helianthus annuus L.) is one of the most important oilseed crops in the world which ranks third in area after Soyabean and Groundnut. The Sunflower Necrosis Disease (SND) is characterized by necrosis of leaves, necrosis streaks on petioles, stem, floral parts and stunted growth. The causal agent of the disease has been identified as Tobacco streak virus (TSV) which belongs to genus Ilarvirus of the family Bromoviridae. The suspected TSV infected sunflower samples collected from chittoor district in Andhra Pradesh were found positive for TSV-DAC ELISA. Total RNA was extracted from sunflower using RNAeasy isolation kit (QIAGEN). The TSV coat protein (CP) gene, movement protein (MP) gene and replicase (Rep) gene were amplified by RT-PCR with specific primers, cloned in pTZ57R/T vector, sequenced and deposited in GenBank (GU355899, GU355900 and GU371445). The size of cloned CP gene was 717 bp and codes for 239 amino acids. The CP gene sequence analysis revealed that the TSV-TPT infecting sunflower has 98–100% homology at nucleotide level with soybean, tagieties-TPT and okra-TN isolates and 93–99% homology at amino acid level. The movement protein gene was 615 bp and codes for 205 amino acids. The MP gene sequence analysis showed that it has 94–97% homology at nucleotide level and 92–95% at aminoacid level.

PVP-10 Molecular Characterization of Viruses Infecting Capsicum annuum in Himachal Pradesh
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Chilli (Capsicum annuum), the important commercial vegetable/spice of Himachal Pradesh, is affected by several viral diseases; of them Cucumovirus, Tospo, Poty and Gemini viruses are the most common genera. However, these viruses are not identified clearly and
characterized fully, which are foremost needed to formulate the management strategy. Therefore, in the present study, effort has been made to identify and characterize the important viruses causing diseases in chilli. In this study, several farms in major chilli growing areas of Bilaspur and Kangra districts in Himachal Pradesh were surveyed and infected plant samples were collected randomly. Virus infections in these samples were detected by DAS-ELISA using antisera to *Cucumber mosaic virus* (CMV) and potyvirus (group specific) and through slot-blot hybridization (SBH) using CMV, *Iris severe mosaic poty virus* (ISMV), *Tomato spotted wilt tospovirus* (TSWV) and *Chilli leaf curl gemini virus* (CLCuV). Based on DAS-ELISA and SBH, the incidence of disease was estimated and ranged from 18.2 to 21.8% by CMV and 3.5 to 5.4% by potyvirus. To detect *Tospo* and *Geminivirus* in the infected chilli, SBH test was carried out. Infected samples showed maximum virus titer in both DAS-ELISA and SBH test were further confirmed by PCR using specific primers. Desired sizes of amplicons; ~540 bp, ~800 bp, ~570 bp and ~460 bp of CMV, *Poty*, *Gemini* and *Tospo* viruses, respectively, were obtained. As the present study clearly indicated that CMV appeared as a major one among the viruses infecting chilli in the hilly region of Himachal Pradesh, two isolates of CMV were characterized in genetic level. Thus the amplified products (~540 bp) of CMV, Palampur 1 and Palampur 2 were cloned in pGEMT cloning vector, sequenced and the sequences were submitted to NCBI database (Palampur 1: Acc-FM209497 and Palampur 2: Acc-FM209498). The sequences were then analyzed and compared with other sequences available in the data base. Based on sequence analysis, it was found that present CMV isolates shared 99% nucleotide identity between them, are closely related with Australian CMV isolate CMV-Ly (Acc-AF198103) by 98% nucleotide identity. In phylogenetic tree analysis, it was observed that Indian CMV isolates formed same cluster along with CMV-Ly. As it is known that CMV subgroup II comprises CMV-Ly, it is concluded that the CMVs of this hilly region of Himachal Pradesh belong to subgroup II.

PVP-11 Multiple Resistance to CMV and TYLCV by the Screening of Different Cultivars of *Capsicum annuum* L. in Agroclimatic Conditions of Aligarh

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Chilli is essentially a crop of the tropics and grows better in hotter regions. Chili (*Capsicum annuum*), a member of family solanaceae is an important vegetable and spice crop of immense commercial importance. The pungency in pepper is due to an alkaloid known as capsaicine and peppers are characterized as sweet, hot or mild depending on capsaicine content. The present investigation were conducted to find out the highly resistant cultivars of *Capsicum annuum* against CMV and TYLCV among ten cultivars of chilli in agroclimatic condition of Aligarh. The highest (70 and 80) percentage of infection was observed in HC-201 and Kalyanpur type-1 by showing the positive reaction to CMV by ELISA test. No symptoms was recorded in case of BC-16, LCA-235 and JCA-154 and showed negative reaction to CMV by ELISA. BC-16 and LCA-235 also showed negative reaction to TYLCV by ELISA and these were symptomless. Maximum infection (70 and 80) was registered in HC-201 and Cg, cultivar. So, the BC-16, LCA-235 and JCA-154 has proved highly resistant varieties against CMV and TYLCV and these may be used in breeding programmes against viruses.

PVP-12 Molecular Characterization of AC4 Protein of Cotton Leaf Curl Kokhran Virus-Dabawali Isolate

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*Cotton leaf curl virus* belongs to the family Geminiviridae, genus Begomovirus. The members of this family contain circular single stranded DNA molecules as their genomes. There are two kinds of begomoviruses—bipartite viruses with genomes consisting of two DNA molecules designated DNA-A and DNA-B and the monopartite viruses which contain only DNA-A but not DNA-B. In monopartite viruses, the DNA-A is accompanied by a small circular DNA molecule called DNA-β which is essential for the development of typical disease symptoms. *Cotton leaf curl virus* is a monopartite virus and causes the cotton leaf curl disease which has emerged as a major disease of cotton in the Indian subcontinent. The non-structural protein AC4 of Cotton leaf curl Kokhran virus-Dabawali isolate (CLCuKV-Dab) was cloned into PGEX5X2 vector and overexpressed in BL21(DE3)PhysS *E. coli* cells. The overexpressed GST-AC4 protein was purified by glutathione Sepharose chromatography. The purified GST-AC4 protein was found to possess ATPase activity. The optimum temperature and pH for the activity were 37°C and 7.4 respectively. The ATPase activity was inhibited in presence of EDTA, showing that it is dependent on divalent metal ions. The activity was supported by magnesium, manganese and zinc ions but inhibited in presence of calcium ions. It was also inhibited by the non-hydrolyzable ATP analogue adenosine-β, γ-imido triphosphate and in the presence of other nucleotides like CTP and GTP. The Km and the Vmax of the reaction for ATP as the substrate are 1.54 mM and 95.2 nmol/min/mg of the protein respectively. The enzyme could also utilize GTP as the substrate. The fact that AC4 is specifically an NTPase and not a general phosphatase is revealed by the finding that it does not hydrolyze p-Nitrophenyl phosphate to yield yellow colour while a similar reaction carried out in parallel with alkaline phosphatase readily yields the colour. It has been suggested earlier that AC4 may be involved in cell to cell movement of the virus (Rojas et al. 2001). It is possible that by its ability to hydrolyze ATP, AC4 serves to power viral movement in the plant.

PVP-13 Existence of Two Strains of Sugarcane yellow leaf virus in India and Its Secondary Spread in Nature Through Aphids

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Thirteen *Sugarcane yellow leaf virus* isolates causing yellow midrib and irregular yellow spot pattern from six states of India were characterized by RT-PCR assays. SCYLV-615F and SCYLV-615R primers were used as forward and reverse primer pairs and the amplified products were cloned and sequenced. Comparative coat protein sequence analysis confirmed that all the SCYLV-Indian isolates were clustered into two major groups confirming the existence of two strains of SCYLV affecting sugarcane crops of India. In a separate experiment, the member of both of the phylogenetic groups were found to be transmitted by the sugarcane aphid, *Melanaphis sacchari* from infected to healthy sugarcane suggesting its secondary spread in nature.
PVP-14 Occurrence of Tobacco streak virus (Ilar Virus) on Cotton in Maharashtra

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The symptoms produced by the virus causing cotton mosaic disease were little bit different in both sap inoculation and under natural field condition. In natural field condition it has shown clear chlorosis type of symptoms on major leaves of plants but in sap inoculated plants veinal chlorosis and mosaic type of symptoms are found to be common. In field conditions infected plants grows erect and have less boll formation. There is no effect found on seed shape or seed size. The initial symptoms produced on cotton leaves after inoculation were wonderful. Local lesions observed in second week from inoculation and then they changes to chlorotic type of symptoms and some are necrotic symptoms also. The plants at early stage are found to be affected, has less lateral branch development and hence reduction in yield production. The naturally field infected plants showing good symptoms are also difficult to identify in lateral stage of plant. Because they disappear with time. The virus is very easily sap transmissible. The virus is found to be transmitted by Thrips palmi and Thrips tobacci in persistent manner. No seed transmission is observed. Virus showed same physical properties as it shows in stem necrosis of peanut or sunflower necrosis disease. The physical properties are found to be thermal inactivation point (TIP) 55–60°C, dilution end point (DEP) 10^-2 to 10^-3 and longevity in vitro (LIV) 5 h, virus infecting nineteen different host plants are identified belonging to five different types of families viz. malvaceae, cheno-podiaceae, compositae, leguminaceae and solanaceae. However they found to produce same types of symptoms as in most of the host that have been tested before. In ELISA test report it is found that the virus showing positive test only with anti serum of TSV of a cowpea and cotton but negative reaction with PBNV of cowpea and cotton which clearly denied possibility of presence of PBNV in cotton producing these kinds of symptoms ELISA report clearly shows that TSV antiserum of cowpea is showing positive results with clear chlorotic types of symptoms.

PVP-15 Development of Virus Induced Gene Silencing Vectors based on Bhendi yellow vein mosaic virus

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A powerful approach to functional genomics, and an alternative to the massive generation of transgenic plants, is the use of the recently described Virus Induced Gene Silencing (VIGS) process, which allows viral vectors to knock out the function of a gene-of-interest. VIGS is based on a silencing mechanism that regulates gene expression by the specific degradation of RNA. As a tool for reverse genetics, VIGS has many advantages over other common ways to study gene function because of the ability of viruses to replicate and move systemically within a plant. VIGS can generate a phenocopy of a mutant without all the troubles of traditional methods of mutagenesis. Geminiviruses with their small DNA genomes and ease of inoculation through agrobacterium, are excellent candidates for VIGS vector development. As a first step, the geminivirus Bhendi yellow vein mosaic virus, characterized in our lab (Jose and Usha, Virology 305:310–317, 2003) has been chosen. The satellite C1 ORF, the plant Phytoene desaturase gene has been cloned and the resulting construct was used for agroinfiltration along with the partial tandem repeat clone of the begomovirus (DNA A component). The results show that the Del C1/BPTR carrying the PDS gene acts as VIGS vector and silences the endogenous PDS, showing the bleached phenotypes. The VIGS vector was further tested by cloning the gfp gene and gfp-expressing Nicotiana benthamiana plants (16c) were agroinfiltrated. Confocal microscopy of the agroinfiltrated plants show that the gfp transgene was silenced by the gfp present in the VIGS vector. Thus an efficient VIGS vector based on the β DNA of Bhendi yellow vein mosaic virus has been developed.

PVP-16 Molecular Characterization of Begomovirus from Two Isolates of Chilli (Capsicum annuum L.) in India

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Chilli (Capsicum annuum L.) plants exhibiting prominent symptoms of begomovirus like: leaf curl, vein swelling, shortening of petioles, crowding of leaves and stunting of plants were collected from Korkee, Uttarakhand and Dhaulpur, Rajasthan, India. Total genomic DNA was isolated from naturally infected chilli samples and PCR was carried out with Coat Protein (located in DNA-A) gene specific primers. As expected to the primers, ~800 bp DNA fragments were amplified from the infected chilli samples. To know the bipartite nature of the virus isolates, Nuclear Shuttle Protein (located in DNA-B) gene specific primers were employed which also resulted in positive amplification of ~850 bp DNA bands with all the Coat Protein tested positive samples. To ascertain the association of DNA-β component with the virus isolates, a set of DNA-β specific primers were used which resulted in positive amplification of full length (~1.3 kb) DNA bands in the chilli samples collected from Korkee, Uttarakhand, however, multiple sizes bands were resulted with the samples collected from Dhaulpur, Rajasthan. These findings confirmed that both the virus isolates under study are bipartite begomoviruses associated with DNA-β satellite. The sequencing of the PCR products is under progress which analysis will be discussed.

PVP-17 Nonstructural Protein, NSs Encoded by Ground nut bud necrosis virus (Tomato) is an ATP Dependent Helicase

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Groundnut bud necrosis virus (GBNV) belonging to the genus Tospovirus, which is a unique member of the family Bunyaviridae, infects several economically important crops. The virus has three genomic ssRNA segments namely S (ambisense), M (ambisense) and L (negative sense). The S RNA codes for Nucleoprotein (NP) and Non-Structural protein (NSs) from viral complimentary and viral strands respectively. Many viral nonstructural proteins namely S (ambisense), M (ambisense) and L (negative sense). The S RNA codes for Nucleoprotein (NP) and Non-Structural protein (NSs) from viral complimentary and viral strands respectively. Many viral nonstructural proteins such as NS3 of Hepatitis C virus, Yellow fever virus, Dengue virus, SV40 large T antigen and cytoplasmic inclusion protein of Tamarillo mosaic potyvirus are known to exhibit RNA/DNA stimulated NTPase, dNTPase and helicase activity. NSs of GBNV does not have any sequence similarity with any of the above mentioned viral RNA/DNA helicases but...
traditional medicinal uses in several countries of the world and also reputed to possess varied medicinal properties including the treatment of wounds and burns. In Cameroon and Congo, it is used traditionally to treat fever, rheumatism, headache, and colic. During survey in and around Gorakhpur in 2009, Ageratum plants were found affected with the symptoms of leaf curling, mosaic mottling and leaf yellows. The infected leaf samples were processed for virus identification and association with PCR assays. Total DNA was extracted and PCR were performed with Begomovirus specific primers (TLCV-CP). A ~ 800 bp band was consistently amplified on 1% agarose. The PCR products were directly sequenced and sequence was submitted in GenBank with the accession no. GQ412352. The blast search analysis showed highest similarity of 98% with the Ageratum enation virus. Our result suggests the association of begomovirus with Ageratum plants showing yellows symptoms.

PVP-20 Molecular Characterization of a Distinct Begomovirus Species and Its Associated Satellite β-DNA from Vernonina cinerea

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Vernonia cinerea leaves with yellow vein symptoms were collected around crop fields in Madurai. A 550 bp product amplified from total DNA extracted from symptomatic leaves with degenerate primers designed to amplify a part of the AV1 gene from begomoviral DNA A component was cloned and sequenced. Based on the above sequences, specific primers were designed and the full length DNA A of 2745 nucleotides with typical genome organization of begomoviral DNA A was obtained and was submitted to EMBL data base (Acc No: AM182232). The sequence comparison with other begomoviruses revealed the closest identity (83%) with Emilia yellow vein virus from China and less than 80% with all known begomoviruses. The International Committee on Taxonomy of Viruses (ICTV) has therefore recognized Vernonia yellow vein virus (VYVV) as a distinct begomovirus species. Conventional PCR could not amplify the DNA B or DNA β from the infected tissue. However, the β DNA (1364 bp) associated with the disease was obtained (Acc No: FN435836) by the Rolling circle amplification–Restriction fragment length polymorphism method (RCA–RFLP) using Phi29 DNA polymerase. Sequence analysis shows that DNA β of VYVV has the highest identity (81%) with DNA β of Ageratum leaf curl disease and 58–77% with the β DNA associated with other begomoviruses. Infectious clones of VYVV DNA A and DNA β as dimers were made using the products of RCA–RFLP. These infectious clones will be used for agroinfection of Vernonia and the results will be discussed. This is the first report of the molecular characterization of Vernonia yellow vein virus (VYVV) from Vernonina cinerea in India.

PVP-19 First Report of Begomovirus Infection on Ageratum conyzoides in North-Eastern Uttar Pradesh, India

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Ageratum conyzoides is commonly known as Billygoat-weed, Chick weed, Goatweed and Whiteweed. In India it is popularly known as bill goat weed. It is an annual herbaceous plant with a long history of

PVP-21 Losses caused by Onion yellow dwarf virus and Iris yellow spot virus in Onion Crop in Northern India

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Production of bulb and seed crop of onion (Allium cepa L.) is hampered by onion yellow dwarf virus (OYDV) and iris yellow spot virus (IYSV) with an incidence of 83.22% and 89.97% in bulb crop and 90.65% and 89.58% in seed crop, respectively in the popularly grown cv. Hisar-2. Four symptom-based variants of OYDV designated as Grade A, B, C and D produced varied types of symptoms in onion crop
incurring heavy losses in bulb and seed production. IYSV caused tiny hay coloured spots of different shapes and sizes on leaves and scapes which later coalesced and led to drying and lodging of scapes. The plant height, bulb weight and bulb size were 37.7 cm, 75.5 g and 24.2 cm² in plants infected with OYDV, 39.6 cm, 79.7 g and 25.5 cm² in IYSV infection, 35.1 cm, 68.4 g and 22.1 cm² due to their combined infection, as compared to 40.6 cm, 88.4 g and 27.6 cm² respectively, in healthy plants of bulb crop. In plants infected with OYDV Grade A the plant height was minimum (90.33 cm) whereas the number of umbels was maximum (9.20 umbels/pl.) but other yield parameters viz., weight/umbel (2.32 g), number of seeds/umbel (209), seed weight/umbel (0.64 g) and seed yield/plant (5.88 g) were recorded to be the lowest. The minimum reduction in plant height (100.26 cm), weight/umbel (6.72 g), number of seeds/umbel (633), seed weight/umbel (2.36 g) and seed yield/plant (11.90 g) were recorded in OYDV Grade D. The plant height was 98.84 cm with 5.10 umbels per plant, 4.24 g weight/umbel, 428 seeds/umbel, 1.25 g seed weight/umbel and 6.37 g seed yield/plant in IYSV infected plants. The plant height (96.26 cm), umbels/plant (5.97), weight/umbel (4.60 g), number of seeds/umbel (432), seed weight/umbel (1.42 g) and seed yield/plant (7.82 g) were found to be the lowest in combined infection of OYDV and IYSV diseases in comparison to higher values in healthy controls (104.50 cm, 4.90, 7.84 g, 677, 2.60 g, 12.74 g, respectively). A minimum reduction in the test weight, germination and seed vigour index were found (3.06 g, 75.68% and 926) due to OYDV grade A infection, whereas these were 2.92 g, 70.42% and 788 in IYSV disease infected plants and 2.62 g, 70.4% and 776 in combined infection of OYDV and IYSV diseases in comparison to 3.84 g, 88.67% and 1276 in healthy plants. The maximum hampering of seed vigour parameters was recorded due to IYSV infection. Lodging of scapes caused by this disease was responsible for heavy losses in seed production and seed quality.

PVP-22 Molecular Diversity of Cotton leaf curl virus (CLCuV) in Northern India
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Cotton leaf curl disease is one of the major threats to cotton cultivation from Northern India. Survey conducted during 2009, observed the disease incidence ranged from 70 to 90% from Bhatinda, Abohar, Fazilka, Sri ganganagar, Hanumanghar. In order to study genetic variability in the virus, twelve CLCuV isolates were partially characterized (700 bp common region, full length AV2 gene and partial sequences of AC1 and AV1 gene). Full length characterization of representative isolates from Bhatinda, Abohar, Fazilka, Sri ganganagar, Hanumanghar is under progress. Partial sequence analysis of CLCuV isolates revealed that, the virus isolates collected during 2009 cropping season are closely related to Cotton leaf curl Burewala virus from Pakistan and results were discussed.

PVP-23 Biochemical Characterization of GBNV Encoded NSm
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Tospoviruses, belonging to the family Bunyaviridae, infect economically important plants such as groundnut, tomato, watermelon etc. They have a tripartite genome, with L, M and S segments of RNA, in pseudo circular (panhandle) form. The viral genomes encode four structural proteins (L, N, G1 and G2) in the antissepsis orientation, and two non structural proteins NSs and NSm in the sense orientation. The NSm is the only protein unique to Tospoviruses that infect plants in the bunyaviridae family and hence is proposed to be important for cell to cell movement. Ground nut bud necrosis virus (GBNV), a member of the tospovirus genus, is the most prevalent virus infecting several species of Leguminosae and Solanaceae plants in India. Total RNA was isolated from GBNV infected tomato leaves and RT-PCR was performed using appropriate primers to amplify the NSm gene. The PCR product was cloned in pGEX5X2 vector. The recombinant NSm clone was transformed into BI21 (DE3) E. coli cells and over-expressed by induction with 0.3 mM IPTG. SDS-PAGE analysis of induced and uninduced fraction revealed the presence of overexpressed protein of expected size. The soluble GST-NSm was purified by GSH Sepharose affinity chromatography. Purified GST-NSm was shown to interact with in vitro transcribed RNA transcript by electrophoretic mobility shift assay. Further NSm was shown to interact with viral encoded proteins NP and NSs using ELISA and yeast two hybrid system. NSm was also shown to be phosphorylated in vitro by pellet fraction of plant sap. Thus the recombinant GBNV Nsm possesses the characteristic features of a movement protein such as nucleic acid binding, interaction with nucleocapsid protein, and ability to undergo posttranslational modification.

PVP-24 Studies on Cucumber mosaic cucum virus (CMV) on Brinjal-Collection, Screening and Biochemical Analysis in and Around Tirupati Region Chittoor (AP)
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Solanum melongena, commonly called as Egg plant is one of the most important vegetable crop in the world. It is cultivated widely in the tropical and sub tropical regions. Several viruses such as Cucumber mosaic cucum virus (CMV), Potato virus-Y (PVY), Potato virus-X (PVX) and Tobacco ring spot virus (TRSV) infect egg plant under natural conditions. In India major crop losses due to CMV infection in brinjal is 57% (FAO STAT-2008). In the present study the infected leaf samples were collected from local fields of Ramapuram, Chandamama palli, Chandragiri, Madanapalli, Yadhamari, Durga samudram villages in and around Tirupati, were tested for CMV infection by DAC-ELISA with CMV antisera. The resulting positive samples were further inoculated to the raised brinjal seedlings of selected varieties through mechanical sap inoculation. Different varieties of brinjal like Mullabadhine, Ankur, Rayva, Mattigulla, Casper and Easter egg were used for monitoring the susceptibility to CMV infection. The mosaic symptoms were observed after 2 weeks of inoculation in all varities of brinjal except Mullabadhina. Among all these susceptible varieties Ankur variety is selected to study induced biochemical changes such as Chlorophylls, Carbohydrates, Proteins, Nucleic acids and Polyphenol oxidases in CMV infected brinjal leaves. In the infected leaves considerable reduction in chlorophyll and starch and increase in total Proteins, Sugars, RNA and Polyphenol oxidases was observed when compared to healthy leaves. The amount of total Starch, protein and DNA decreased to about 25, 136 and 645 μg/g respectively in infected leaves, where as sugars (75 μg/g), RNA content (754 μg/g) and Polyphenol oxidase activity was increased as compared to healthy leaves. The above results suggests that there is an altered concentrations of chlorophyll, proteins, nucleic acids, carbohydrates and Polyphenol oxidase activity in the brinjal leaves due to the effect of Cucumber mosaic cucum virus
infection. Leaf analysis was found to be used as widely accepted diagnostic tool to assess the nutritional status of the vegetables. The present study deals with these aspects in detail.

PVP-25 Mixed Infection of Virus and Phytoplasma Associated with Sugarcane Yellow Mid Rib and Sugarcane Leaf on Sugarcane Crops of Uttar Pradesh and Uttarakhand, India

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Sugarcane yellow leaf virus and Sugarcane yellow leaf phytoplasma were detected on sugarcane leaf samples exhibiting midrib yellowing symptoms from Uttar Pradesh and Uttarakhand states of India on five sugarcane var. CoS 8436, CoS 8432, CoLk 8102, CoS 767 and CoS 95255 during 2008–2009. The total RNA and DNA was isolated from infected leaf samples. RT-PCR assays were performed using Sugarcane yellow leaf virus (SCYLV) specific primers (SCYLV-615F and SCYLV-615R). The infection of SCYLV was detected in all the collected samples, which showed the expected size (~610 bp) amplicon during RT-PCR. In another experiment with nested PCR analysis, a phytoplasma characteristic 1.2 kb rDNA PCR product were amplified from DNAs of all infected samples but not in healthy sugarcane plants tested using phytoplasma universal primer pairs P1/P7 and IU3/eU5. DNA extracts from plants with yellow mid rib and leaf yellows produced products of 1250 bp, which gave typical phytoplasma profiles when digested with Hae III and Hha I. No PCR amplifications were produced using DNA from symptomless plants. Our results suggest that the yellow mid rib and leaf yellows symptoms on sugarcane varieties in Uttar Pradesh and Uttarakhand states of India exhibiting midrib yellowing and leaf yellows symptoms is mainly caused by mixed infection of SCYLV and SCYLP. The affected clumps showed reduction in stalk height as compared to healthy fields.

PVP-26 Genetic Diversity in the Coat Protein Coding Region of Thirty-One Sugarcane mosaic virus Isolates from China and India

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Thirty-one sugarcane mosaic isolates belonged to Sugarcane mosaic virus (SCMV) and Sugarcane streak mosaic virus (SCSMV were collected from China and India), confirmed in indirect ELISA and RT-PCR amplification with SCMV and SCSMV-specific primers. The amplicons (0.8 kb) from the coding region of coat protein (CP) were cloned, sequenced and compared to each other as well as to the sequences of 13 SCMV isolates from sugarcane (Australia, USA, China, Brazil, Mexico and South Africa), maize (Australia, China, Iranian) and one SCSMV isolate from sugarcane (India) in Genbank. Maximum likelihood and maximum parsimony analyses robustly supported two major monophyletic groups that were correlated with the host of origin: the SCMV subgroup that included 18 isolates from China and only 13 isolates from India, and the SCSMV subgroup that contained all isolates from India. Maize dwarf mosaic virus (MDMV) and Johnsongrass mosaic virus (JGMV) were not detected in any of the samples tested. A strong correlation was observed between the sugarcane groups and the geographical origin of the SCMV isolates. The 11 millable sugarcane samples from China contained a virus tentatively described as Sorghum mosaic virus (SrMV). Three isolates from nine chewing canes in Fujian, Yunnan and Guizhou provinces of China also contained SrMV, and the other 12 samples including five isolates from India was found infected with SCMV. No SrMV infection has been detected in sugarcane mosaic samples from India. Sequence comparisons and phylogenetic analysis indicated that SrMV can be considered as the most common and prevalent potyvirus infecting sugarcane in China, however in India *Sugarcane streak mosaic virus* is dominant in causing mosaic symptoms on sugarcane.

PVP-27 Digoxigenin-Labelled DNA Probe for Broad Spectrum, Sensitive and Rapid Detection of the Tobacco streak ilarvirus Isolates in India by Dot-Blot and Tissue Print Hybridization

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DIG-labeled DNA probe complementary to coat protein (CP) region of Tobacco streak virus (TSV) Sunflower isolate was designed for the sensitive and broad-spectrum detection of TSV isolates, the most devastating virus in India. Dot-blot and tissue print hybridizations with the digoxigenin labeled probe were performed for the TSV detection at field levels. Here, dot-blot hybridization was used to check a wide number of TSV isolates with a single probe and sensitivity with different sample extraction methods. The probe with CP conserved region prepared from sunflower PCR amplicon was hybridized with the TSV field isolates of Gherkin, Pumpkin, Sunflower, Marigold and Globe amaranth samples because of highly conserved with little variability in CP region. The sensitivity limits were decreased from total nucleic acid to partially purified and crude extract preparations. In particular, tissue blot hybridization offers a simple, reliable procedure as dot-blot, but requires no sample processing. Because there is minimal sample preparation, tissue-print hybridization could be an important component of TSV management programs. Thus, the above non-radioactive labeled probe techniques can facilitate in screening the samples during TSV outbreaks and in quarantine services.

PVP-28 Development of a Method for Isolation and Propagation of Mycobacteriophages

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Two Mycobacterium smegmatis strains (ARI Lab Nos. V842 and V946) were employed for the isolation of mycobacteriophages from soil and sewage samples. Mycobacteriophages were isolated from soil samples collected from an area surrounding the Tuberculosis (TB) ward, Naidu hospital, Pune, against *M. smegmatis* strain V842. These were numbered as V942, V943 and V944 and were isolated by using washed-cell preparation method. The Bacteriophages against the other *M. smegmatis* strain, i.e. V946, were isolated from soil samples (collected from around TB ward, Sassoon hospital, Pune). Some of these phages (viz.V953, V954) showed plaques at 42°C but not at 37°C. Thus they seem to be lysogenic. For propagating and increasing the titre of all the above isolates, various previously described methods were attempted, but none of these methods were satisfactory. But when siliconized glassware and plastic-ware were used,
propagation was successful. We showed that siliconization of glassware and plastic-ware was essential for the propagation of our mycobacteriophage isolates V951, V952, V953, V954 and V955. Also, phage dilution medium (PDM) as described by Chaterjee et al. (2000) was found to be effective for picking out of the plaques made by the phages. In this way, the phage isolates were propagated up to P3. The various passages of the phage isolates V951, V952, V953, V954 and V955 (i.e. original, P1, P2 and P3) were stored at −80°C.

**PVP-29 Effect on Pigments Due to Geminivirus Infection on Cowpea (Vigna unguiculata)**

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Geminiviruses are one of the most important group of viruses causing economic losses in tropics. The symptom produced are yellowing of leaves which directly affect the pigments of diseased plants it in turn affects productivity and yield of diseased plant. Cowpea Vigna unguiculata is one of the important crop cultivated throughout India for its green pods which are used as vegetables and seeds are used as pulse. Cowpea is affected by many viruses amongst them geminiviruses are one of the important virus on the cowpea plant. In the present study total chlorophyll content was studied in leaf of cowpea of diseased and healthy plants using Arnon’s method. Carotenoids were also studied using Ikan’s method. It was found that chlorophyll content in diseased plants were lower compared to healthy plant similar results were found with carotenoids so the geminiviruses infection lowers the chlorophyll and carotenoid content in diseased plants which reduces yield of diseased cowpea plant.

**PVP-30 Cloning and Sequence Analysis of Two Complete Genomic Sequences of Rice tungro bacilliform virus from India**

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Rice tungro disease is an important disease of rice, caused by a joint infection by two viruses: Rice tungro spherical virus (RTSV) and Rice tungro bacilliform virus (RTBV) in South and Southeast Asia. The complex of RTBV and RTSV is transmitted by an insect vector Green Leaf Hopper (GLH). Previously we reported complete genomic sequences of two geographically distinct isolates of RTBV; RTBV-80 from India and the recently reported one, there is very little sequence variability from those reported from The Philippines. Frequent outbreaks of tungro were reported near Kanyakumari in the last 2–3 years. The present work was undertaken to clone and sequence the full-length RTBV and RTSV genomes from the infected rice plants collected from above region and to analyze the similarity of its genetic material with the existing Indian isolates of RTBV and RTSV. A 1.1 kb DNA fragment encoding the Reverse transcriptase gene of RTBV genome was amplified and cloned in T/A vector and was sequenced commercially. Homology search at the nucleotide level using BLASTn program with the previously existing RTBV isolates revealed a very high percentage identity of 99% with the RTBV West Bengal isolate and 95% with the RTBV Andhra Pradesh isolate. This further strengthens the earlier reports that there is not much genetic variability in the RTBV genomes in Indian subcontinent.

Rice tungro disease has become the major cause of production losses in rice during last three decades in several rice growing states of India. Here, we report, for the first time the complete sequence analysis of two geographically distinct Indian isolates of RTSV. We analyze the deduced protein sequences and their phylogenetic relationship with the two complete RTSV sequences from Philippines as well as with other members of Sequiviridae family. We provide molecular evidence that the Indian isolates of RTSV are closely related to those from the Philippines. We had earlier reported that RTBV isolates between India and Philippines differ significantly from each other [18]. This study was undertaken in order to see whether RTSV isolates from India also show similar difference from those reported from The Philippines. Frequent outbreaks of tungro were reported near Kanyakumari in the last 2–3 years. The present work was undertaken to clone and sequence the full-length RTBV and RTSV genomes from the infected rice plants collected from above region and to analyze the similarity of its genetic material with the existing Indian isolates of RTBV and RTSV. A 1.1 kb DNA fragment encoding the Reverse transcriptase gene of RTBV genome was amplified and cloned in T/A vector and was sequenced commercially. Homology search at the nucleotide level using BLASTn program with the previously existing RTBV isolates revealed a very high percentage identity of 99% with the RTBV West Bengal isolate and 95% with the RTBV Andhra Pradesh isolate. This further strengthens the earlier reports that there is not much genetic variability in the RTBV genomes in Indian subcontinent. Similarly, the CP3 region of RTSV was amplified by RT-PCR and was cloned in T/A vector. Recently, rice tungro disease has been reported from Kanyakumari district of Tamil Nadu. It is important to determine the genetic nature of this isolate in order to develop resistance strategies. It is thus necessary to clone and characterize the viruses from...
infected the begomovirus infection, the total DNA from leaf samples of experimental plots of NBRI and *J. curcas* incidence was observed in 2006–2009 on disease. Recently, a severe mosaic disease with significant disease incidence was observed during the survey of different geographical conditions like those present in India select for genotypically variable strain and to design for transgenic resistance strategy, information on RTSV from India is absolutely essential. The objective of this study was to clone RTSV isolates from India and compare the genetic diversity of Indian isolates from other Southeast Asian isolates and amongst each other. Also develop strategy to impair the attack of virus-complex on rice. The achieve this, complete genomes of two isolates from India were cloned by amplifying different genes by RT-PCR and subsequently cloned in TA vectors, followed by sequencing. Subsequently constructs containing CP1-3, antisense Replicase, sense Replicase and double stranded Replicase were cloned in plant transformation vector. These constructs were used to transform aromatic rice variety from Indian-Pusa Basmati (PB1). PCR analysis of the above plants was done to check the stable insertion of insert in the transgenics.

**PVP-31 Genetic Diversity of Four Begomovirus Isolates Associated with Mosaic Disease of *Jatropha* Species in India**

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Jatropha (*Jatropha curcas*) of the family *Euphorbiaceae* is being grown in India as a major commercial fuel (bio-diesel) crop. Jatropha is cultivated in 200 districts of 19 potential states of India. Unfortunately, the cultivation of Jatropha is limited by the severe mosaic disease. Recently, a severe mosaic disease with significant disease incidence was observed in 2006–2009 on *J. curcas* grown in experimental plots of NBRI and *J. gossypifolia*, a weed growing road side around Lucknow and Kathaupahadi, Madhya Pradesh. The disease consisted of the symptoms of severe mosaic, blistering, leaf distortion and stunting of whole plant and no fruit/seed production in severely affected plants. Symptomatology and whitely population observed on them suggested the occurrence of *Begomovirus* infection. To detect the begomovirus infection, the total DNA from leaf samples of infected *Jatropha* plants was extracted and polymerase chain reaction (PCR) were performed using three sets of begomovirus genus specific (CPIT-1/CPIT-T, PAlIV 1978/PARc 496 and PAlIV 722/PARc 1960) primers and the expected size ~800 bp, 1.2 kb and 1.2 kb amplicons were obtained which confirmed the begomovirus infection. Further to identify the begomovirus/es and investigate the genetic diversity among them exists if any, the ~1.2 kb amplicons were cloned and sequenced. The sequence data were deposited in the GenBank database under accession nos.: GQ847545 and FJ146232 (from *J. curcas*) and EU727086 and FJ177030 (from *J. gossypifolia*) was performed along with some selected isolates of begomovirus which showed >90% sequence identities during BLAST analysis. The isolate EU727086 showed closest relationship with *Croton yellow vein mosaic virus* while FJ177030 showed separate clustering of all the four begomovirus from Jatropha species. During phylogenetic analysis these isolates formed three separate clusters, therefore, they were considered as three distinct begomoviruses. The above data clearly show that some genetic diversity exists among the begomoviruses infecting *Jatropha* species in India.

**PVP-32 Elimination of Sugarcane mosaic virus and Sugarcane streak mosaic virus by Tissue Culture**

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Elimination of Sugarcane streak mosaic virus and Sugarcane mosaic virus from two commercial varieties of sugarcane viz. CoLk 8102 and Col 83 was attempted using in vitro culture technique. Plantlets were produced using meristem explants collected from virus infected field grown plants. RT-PCR assay of regenerated plants was carried out at monthly interval for six months using primer pairs for *Sugarcane mosaic virus* (SCMV-F3, SCMV-R3) and *Sugarcane streak mosaic virus* (SCSMV-S2, SCSMV-P1). No virus could be detected in regenerated plants of both the varieties plantlets transferred to the greenhouse during the studies indicating the elimination of SCMV and SCSMV.

**PVP-33 Variability of Viruses on *M. charantia* in Eastern UP, India**

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Bitter gourd (*Momordica charantia*) of the family Cucurbitaceae, also known as bitter melon is extensively cultivated in north eastern region of Uttar Pradesh, India. It is regarded as one of the world’s major vegetable crops and has great economic importance. A severe yellow mosaic disease on bitter gourd (*Momordica charantia*) with a significant disease incidence was observed during the survey of different locations of Eastern UP, India in the year 2007. The whitely (*Bemisia tabaci*) population was also observed in the vicinity. The characteristic disease symptoms and whitely population indicated the possibility of begomovirus infection. Total DNA were isolated from infected as well as healthy leaf samples. Two primer pair (TLCV-CP and Roja’s Primer) were used to study, which resulted ~ 800 bp with TLCV-CP in 3/3 samples and ~ 1.3 kb amplicons with Roja’s primer in 3/4 samples. For further identification of the begomovirus, the PCR amplicons were cloned and sequenced (GenBank accession no. EU439260 and EU888908, respectively). The BLASTn search
Infecting groundnut PVP-34 biological and molecular characterization of tobacco gourd in eastern UP, India. These results confirmed the association of PepLCBV with selected begomovirus isolates revealed a closest relationship (PepLCBV) isolates. The phylogenetic analysis of the virus isolate virus gourd was considered as an isolate of closed relationships with ToLCNDV the virus isolated from bitter with ToLCNDV isolates. Based on highest sequence identity and analysis also showed closest relationships of the isolate (EU439260) highest 99–97% identities with Pepper leaf curl Bangladesh virus (PRSV-P) is the most important virus. The detection of virus infection in plants has traditionally involved either bioassay on indexing plants and or immunological methods (Hill 1981, Torrence and Jones 1981). Use of nucleic acid probes has improved the detection and sensitivity of viruses. The most common non-radioactive probes are Biotinylated probes, which are very specific and sensitive. Papaya ring spot virus (PRSV-P) is a positive sense ssRNA virus belonging to the genus potyvirus Family potyviridae and transmitted by aphids. PRSV-P coat protein gene region was used as template cDNA for probe preparation. Dot-blot hybridization with the biotin labeled probe were performed for PRSV-P detection. The clarified sap of healthy and infected plants were serially diluted and spotted onto the nitrocellulose membrane, hybridized to biotin labeled probe. Biotin labeled RNA’s are employed as probes, with a subsequent detection based on streptavidin–alkaline phosphatase conjugates. The sensitivity for viral detection of the biotin labeled probe was found to be sensitive than Enzyme Linked Immunosorbsent Assay (ELISA).

PVP-35 biological and molecular characterization of Tobacco streak virus infecting groundnut

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Tobacco streak virus groundnut isolate was characterized biologically by taking six cultivars (JL24, TMV2, K6, K7, K9) and one pre-release culture (K1271) using seedlings of 7–84 days old under glasshouse conditions. There were clear differences were observed among cultivars tested regarding incubation period, percent seedling wilt and time taken to death of seedlings. K-7 was least susceptible among all the cultivars tested and it supported least virus titer (A405 nm: 0.11–1.23). Both localized (necrotic lesions on leaf, vein necrosis, leaf yellowing, wilting) and systemic (petiole necrosis, necrotic lesions on young leaves, death of top growing buds not only on main stem but also on all primaries (side shoots), followed by stem necrosis, stunted growth, axillary shoot proliferation with small leaves having general chlorosis, peg necrosis, pod necrosis, pod size reduction, wilt of plants) symptom were observed in all cultivars tested. Biological differentiation of TSV and GBNV was made by sap inoculation of both viruses separately using susceptible groundnut cultivar JL24 under glasshouse conditions. There were certain similarities and differences were observed between these viruses infecting groundnut. Seed infection of TSV ranged from 18.9 to 28.9% in seeds collected from naturally infected and sap inoculated groundnut cultivars/pre-releases (JL24, TMV2, K-6, K-7, K-9 and K-1271) belonging to Spanish and Virginia types. TSV was detected both in pod shell and seed testa from pod samples produced by sap inoculation under glasshouse conditions. However, seed transmission of TSV was not observed in groundnut. Coat protein (CP) gene of three groundnut TSV isolates (GA-AP1-100; GA-AP2-04; GA-AP3-07) were sequenced and all the three isolates contained a single open reading frame (ORF) of 717 bp nucleotide and could potentially code for 238 amino acids (aa). CP gene of TSV isolates originating from different hosts shared high degree of sequence identity both at nucleotide (97.6–100%) and amino acid (95.7–100%) levels respectively.

PVP-36 natural occurrence of Peanut bud necrosis virus (PBNV: Tospovirus) in Brinjal

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In recent years tospovirus is causing devastating damage to the yield of vegetables in India. It infects economically important crops viz., tomato, chilli, peppers, groundnut, watermelon and various legumes. Now it is emerging as severe disease in brinjal also. In order to monitor the natural occurrence and distribution of Tospovirus in vegetable, surveys were conducted in the predominant brinjal growing areas of Gujarat, Karnataka, Maharashtra and Andhra Pradesh during 2008–2010 incidence ranging from 5 to 10%, 0 to 80%, 1 to 40%, and 0 to 55.78% respectively. Samples collected from different places of India were found positive to PBNV in direct antigen coating-enzyme linked immunosorbsent assay (DAC-ELISA). PBNV infected brinjal plants showed mosaic motting of leaves with leaf distortion, longitudinal streaks on the stem and necrotic rings on leaves and fruits. Early infection led to severe stunting and abnormal fruiting. Biological and molecular characterization of PBNV-brinjal isolates were compared with other isolates and results are discussed.

PVP-37 studies on host range and physical properties of virus causing mosaic in soybean

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For identification of virus causing mosaic symptoms on soybean various host plants were tested. Plants species belonging to the different families viz. caricaeae, graminae, leguminoseae, malvaceae and solanaceae were tested. The virus produced symptoms on diagnostic plant species like Chenopodium album, C. quinoa, Helianthus anus, Phaseolus vulgaris and Vigna unigulculata. Among tested families the leguminoseae that were the host of virus included Arachis hypogaea,

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Glycine max, Phaseolus vulgaris, Pisum sativum, Vigna mungo, Vigna radiata and Vigna angliculata. The virus causing mosaic symptoms in soybean is inactivated between 50 and 55°C and between dilution of $10^{-4}$ to $10^{-5}$. All the inoculated plants of assay host showed the symptoms at 50°C but not at 55°C. Similarly local lesions produced at $10^{-4}$ but not at $10^{-5}$. The virus in crude sap was infectious up to 72 h but not at 96 h at room temperature. However, the percentage infectivity decreased progressively as the aging of the sap was increased at room temperature. On the basis of reactions on diagnostic hosts, host range and physical properties the virus causing mosaic in soybean appears to be strain of soybean mosaic virus (SMV).

PVP-38 Identification and Characterization of Potyvirus Infected Chilli (Capsicum annum L.) in North-Eastern Uttar Pradesh

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Chilli (Capsicum annum L.) is an important medicinal plant, used as stomachic, analgesic, carminative and in dyspepsia etc. In a survey of Chilli (Capsicum annum L.) in North-Eastern Uttar Pradesh PVP-38 Identification and Characterization of Potyvirus Infected Chilli (Capsicum annum L.) in North-Eastern Uttar Pradesh

PVP-39 Elimination of Sugarcane yellow leaf virus and Sugarcane grassy shoot disease Through Apical Meristem Culture

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Phytoplasma causing Grassy shoot disease and Sugarcane yellow leaf viruses are important pathogens of sugarcane. These pathogens are causing severe losses in sugarcane productivity. With a view to producing virus and phytoplasma free planting material of sugarcane, experiments were undertaken using infected varieties of sugarcane growing at the farms of Sugarcane Research Institute. Apical meristem tissue was collected from the farms of Sugarcane Research Institute. Apical meristem tissue was collected and maintained on agar gelled Murashige and Skoog’s (MS) medium containing growth regulators for shoot induction. The established shoot cultures were multiplied through repeated subcultures on fresh media at 10–12 days interval. Elimination of GSD and SCYLV was confirmed through molecular analysis of regenerated plants using specific primers of SCYLV and GSD. Results revealed that apical meristem culture technique is effective in eliminating the pathogens like SCYLV and Phytoplasma (GSD) from the infected clones. This is probably the first report on elimination of Grassy shoot disease in sugarcane through meristem culture.

PVP-40 Development of Recombinant Coat Protein Antibody Based IC-RT-PCR and Comparison of Its Sensitivity with Other Immunoassays for the Detection of Papaya ringspot virus Isolates from India

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Papaya ringspot virus (PRSV), which causes the most widespread and devastating disease in papaya, isolates originating from different geographical regions in south India were collected and maintained on natural host papaya. The entire coat protein (CP) gene of Papaya ringspot virus-P biotype (PRSV-P) was amplified by reverse transcription-polymerase chain reaction (RT-PCR). The amplicon was inserted into pGEM-T vector by T-A cloning method, sequenced and sub cloned into a bacterial expression vector pRSET-A using directional cloning strategy. The PRSV coat protein was over expressed as fusion protein in E. coli. SDS-PAGE gel revealed that CP expressed as a ~40 kDa protein. The recombinant coat protein (rCP) fused with 6× His-tag was purified from E. coli using Ni–NTA resin. The antigenicity of the fusion protein was determined by western blot analysis using antibodies raised against purified PRSV. The purified rCP was used as an antigen to produce high titer PRSV specific polyclonal antiserum. The resulting antiserum was used to develop an immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) assay and compared its sensitivity levels with ELISA based assays for detection of PRSV isolates. IC-RT-PCR was shown to be the most sensitive test followed by dot-blot immunobinding assay (DBIA) and plate trapped ELISA.