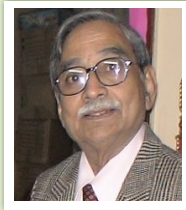
VirusResearch News

ISSN : 2394-4536 (Print)

Vol. 4 No. 1 July 2015

A Half Yearly News Letter of Indian Virological Society

From IVS President Desk



I will fail in my responsibility if we do not recognize the Team Spirt of the Editor, Dr. Bikas Mandal and his associates in establishing the IVS: VirusReseasrch News in its well defined objectives. Today the need is high to reach the researchers in the field of Medical, Animal Sciences including the Marine Biology and the important Plant Viral diseases. An important part of the country economy rests in the ever non-ending solutions for the disease control. Our small effort to bring this through the newsletter is a step ahead.

Emerging trends in host microbe interaction today is fast drawing attention of not only Clinicians but all concerned including the patients. In the ever demanding for the betterment of disease control, reduction in load on the health national budget, planners, advisers all have to join hands for a right solution in the existing situation. The easiest is achieved by the vaccines available. This not only prevents but protects from the infectious diseases, and the easiest cheapest.

The days of antibiotics today has been limited due to resistant strains cropping up due to miss and over use of this wonderful medication found in forties. If we look back there is hardly any new antibiotic worth naming discovered in the past 30 years. The need today is for prevention, protection and creating immunity for most of the available common infectious diseases. I strongly wish advocacy for using preventive methods if available.

A.K. Prasad
President, Indian Virological Society

In this Issue

RESEARCH NEWS

Black gram germplasm resistant to mungbean yellow mosaic virus

Anirban Roy[1*], Manas Kumar Bag[2], N.K. Gautam[2]

[1]Advance Centre for Plant Virology, Indian Agricultural Research Institute; [2]National Bureau of Plant Genetic Resources, New Delhi-110012, India

*E-mail: anirbanroy75@yahoo.com

Black gram (*Vigna mungo* (L.) Hepper) is the third important pulse crop of India and is contributing 70% of world's total black gram production. Biotic stresses particularly yellow mosaic disease (YMD) accounted mostly for the low harvest index of the present day black gram cultivars.

Use of resistant varieties, is the only feasible, effective, economical, environment friendly and sustainable approach to alleviate occurrence of YMD in areas where it is a major constraint to grain legume cultivation.

We have screened 344 germplasm of black gram to identify the resistant source. 32 accessions which showed resistant or highly resistant response were selected and evaluated further under field condition for consecutive two more years. Eight accessions showed consistent resistant response for consecutive three years and also showed better agronomic performance over the best agronomic check T-9. These eight accessions were selected for evaluating their performance under challenged inoculation condition with viruliferous whiteflies. Of the eight field resistant accessions, two accessions (IC144901 and IC001572) were grouped into HR category and two other accessions viz. IC011613 and IC485638 were grouped into R category. The resistance (HR or R) of four accessions, viz. IC144901, IC001572, IC011613 and IC485638, was further evaluated by agroinoculation with the infectious cloned DNA-A and DNA-B components of the New Delhi isolate of MYMV and the result of the whitefly transmission experiment was confirmed. Ic144901 has already been registered with NBPGR.

These four accessions could be used in YMD resistant breeding programme or could also be released directly for cultivation after verifying their adaptation to various regions and other acceptable quality traits.

RT-PCR ELISA, a rapid and cost effective molecular test for detection of pestiviruses (BVDV-1, BVDV-2 and BDV) in ruminants

Dr. Niranjan Mishra

National Institute of High Security Animal Diseases (formerly HSADL), Indian Council of Agricultural Research, Bhopal, India
Correspondence: mishranir@rediffmail.com

Pestiviruses are important pathogens of livestock and cause substantial economic losses in farming. The genus *Pestivirus* belonging to the family *Flaviviridae* consists of four recognised virus species: Bovine viral diarrhea virus 1 (BVDV-1), Bovine viral diarrhea virus 2 (BVDV -2), Classical swine fever virus (CSFV) and Border disease virus (BDV). Cattle, sheep and goats can be infected with BVDV-1, BVDV-2 or BDV. We recently developed and evaluated a RT-PCR ELISA for the simultaneous detection of BVDV-1, BVDV-2 and BDV in ruminants.

The test optimization was carried out by serial dilution of home-made digoxygenin-labelled RT-PCR product standards obtained from pestivirus isolates and pestivirus infected animals. The results showed that 2 µl of DIG-labelled RT-PCR product, 2.5 pM of biotinylated probe, conjugate anti-DIG peroxidase and substrate ABTS with a substrate reaction time of 10 min were optimum for all types of the samples analyzed. The RT-PCR ELISA showed no positivity in any of the known negative samples and no cross reactivity was found with the unrelated viruses tested suggesting that the assay was specific.

The detection limit of the assay was found to be 10 $TCID_{50}$/ml for BVDV-1 and BVDV-2, similar to virus isolation and real-time RT-PCR but 10-fold higher than RT-PCR. The limit of detection of the RT-PCR ELISA for BDV was 200 copies of RNA. The assay had a good reproducibility. The validation of the RT-PCR ELISA was carried out on clinical samples obtained from field or BVDV-1 / BVDV-2 infected sheep and goats. A total number of 121 samples were tested by RT PCR ELISA, RT-PCR and virus isolation. The results showed that RT-PCR ELISA showed a high diagnostic sensitivity (95.9%) and specificity (98.6%) when compared with virus isolation and there was strong agreement (97.5% concordance) between the two tests. In contrast, RT-PCR showed a sensitivity of 78.2% and specificity of 96% when compared with virus isolation. Hence, RT-PCR ELISA displayed an increased diagnostic specificity and sensitivity over RT-PCR. Additionally, when a few samples (n=26) were tested by RT-PCR ELISA and real-time RT-PCR, 100% concordance was obtained between them.

Similar analytical sensitivity as found for virus isolation and real-time RT-PCR methods and a slightly lower diagnostic sensitivity and specificity than virus isolation showed that RT-PCR ELISA developed recently in our laboratory can be an effective alternative test having several other advantages. First, the test is rapid (one day), while virus isolation from clinical samples generally requires 10-14 days. Secondly, virus isolation requires maintenance and culture of cells and other infrastructural facilities that are not available in most of the diagnostic laboratories in developing countries. Thirdly, although both RT-PCR ELISA and real-time RT-PCR have two levels of specificity.

Peste des Petits Ruminants (PPR), the next animal disease to be eradicated?

Dr. V. Balamurugan

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bangalore-560064, India
(balavirol@gmail.com; b.vinayagamurthy@icar.gov.in)

Peste des petits ruminant (PPR) also known as "*Goat Plaque*" is a highly contagious and economically important transboundary viral disease of domestic sheep and goats and wild small ruminants and is currently emerging to cause infections in camels. It is considered as one of the main constraints in augmenting the productivity of small ruminants in endemic countries such as Africa, the Middle East and Parts of Asia including India and its control and eradication is a priority. Rinderpest virus (RPV) caused catastrophic losses in large animals, which played a killing instinct for period of a century causing a major impact on rural economy and health status of poor farmers to livestock.

Given the similarities between RPV and PPRV, it is believed that any future plans for PPRV elimination will require the implementation of the same principles which led to RP eradication. The shorter life-span of small ruminants compared to cattle means that their turnover rate is higher. This in turn results in the need for more trained veterinary services to carry out vaccine administration. Another major gap for the success of PPR control is the lack of economical assessment of control strategies. Such information would be useful to help veterinary services in convincing governments and international organizations to support and fund PPR control. All of these factors need addressing before formulating a viable eradication programme.

A better knowledge of sheep and goat population dynamics, herd management practices and animal movements will be a critical condition for success of control programme. Another issue is the local management of the PPR control program by farmers, community based animal health workers, veterinary professionals and services, and research organizations.Improvements to achieve in the governance of the surveillance systems and

veterinary services in order to better coordinate the private and public sectors of animal health management is highly significant.Though a good PPR vaccine is available, the definition and implementation of a relevant vaccination strategy and vaccination monitoring might be tricky. Global Framework for the progressive control of transboundary animal diseases (GF-TADs-joint FAO/OIE initiative) is an ideal international forum where PPR control strategies can be elaborated and decided in collaboration with national veterinary services for involving in the coordination and implementation of control programme.

In India, Department of Animal Husbandry, Dairying and Fisheries (DADF, GOI), Government of India launched a national control programme on PPR (NCP-PPR) during the year 2010-11 with a sum of INR 432.5 million in first phase with a mass immunization vaccination programme using live attenuated PPR vaccine with an aim to control and eradicate this disease from India in a time bound manner on the lines of RP eradication. At present, the disease occurrence, severity of the clinical disease and number of outbreaks have progressively and substantially declined in areas under regular vaccination mostly under NCP-PPR and partly under ASCAD (Assistance to States for Control of Animal Diseases) of the Government of India.Finally, it is hoped that PPR in the direction of RP will be eradicated in India within a decade.

Ranaviruses in ornamental and cultivable fishes: a looming threat to aquaculture sector in India

K. Riji John*, M. Rosalind George, M. Mohamed Mansoor, P. Magesh Kumar and P. Sivasankar
Department of Fish Pathology and Health Management, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu 628008
Email: rijijohn@gmail.com

Similar to culture of food fishes, ornamental fish culture is also undertaken by marginal fish farmers and well established industrialists. Currently, the entire industry is estimated to be worth around US$ 15 billion. Ornamental fish exports from India have increased from Rs. 3.2 crore in 2001-02 to Rs.10 crores in 2010. Compared to aquaculture of cultivable fishes, ornamental fishes require modest infrastructure and hence form lively hood option of many fish farmers. There are about of 250 species of freshwater and 150 species marine exploitable ornamental fishes available in India.

Marine cultivable and ornamental fishes are prone to serious viral infections especially during the larval and early juvenile stages of their life cycle. Viral pathogens are by far the most serious infectious agents in aquaculture with very little therapeutic interventions possible. Without immediate therapeutic remedies, the control measures solely depend up on prophylactics and extermination. While prophylactics do considerable improvement in the culture of fishes, diagnosis of a disease is the first step towards the control of viral infection in aquaculture.

Epizootic ulcerative syndrome (EVS) is one of the most serious infections that caused large scale mortalities in the finfish during the last three decades. While the exact aetiology of EUS is still

Infected Koi (*Cyprinus carpio carpio* Linnaeus 1758)

under contention, several viral agents including rhabdoviruses, birnaviruses, a reovirus and a ranavirus were isolated from the EUS infected fishes from various countries.

Our separate investigations of ornamental and cultivable fishes suffering from mortality led to the isolation of ranaviral agents in cell culture and also detection of the capsid gene of the virus in the tissue extracts of the infected fishes. Biophysical, biochemical and molecular characterisation of the viral agents from koi (KIRV) and damselfish (SRDV) showed that they belong to *Ranavirus* genus of the family *Iridoviridae*. While the isolated ranaviruses are from ornamental fishes of South India, we found the presence of these viral agents by PCR in the cultivable fishes of Northeast region. Pathogenicity studies using koi ranavirus (KIRV) and similar damsel virus (SRDV) showed that while KIRV was unable to induce mortality, SRV inflicted over 90 % mortality in experimentally infected fishes. Since the ranaviruses are genetically divergent and were found both in freshwater and marine fishes, immediate attention is required for early diagnosis of infections, development of robust and specific diagnostic methods and prophylactic measures to deal with emergencies arising out of spread of such infections.

Sub-viral particles based vaccine affords complete protection to chickens against Infectious bursal disease virus

Sohini Dey and C. Madhan Mohan
Division of Veterinary Biotechnology,
Indian Veterinary Research Institute, Bareilly 243122, UP
Correspondence: sohinimadhan@yahoo.com

Infectious bursal disease virus (IBDV) causes major economic losses to the poultry industry worldwide. The actively dividing and differentiating lymphocytes of the B-cells lineage of the bursa of Fabricius of particularly young chickens get affected, resulting in morbidity, mortality and immunosuppression along with ineffective response to vaccines. The major capsid protein, VP2 is the host protective antigen against IBDV and induces virus neutralizing antibodies that protect the susceptible chickens from IBDV infection. Moreover, complete protection against the IBDV

requires the production of neutralizing antibodies against several VP2 conformational epitopes.

The commercially available live vaccines based on classical virulent strains induce both cellular and humoral immunity but may also show necrosis and lymphoid depletion. They may show reversion to virulence and vaccine associated reactions resulting in clinical disease and production losses. Non-replicating antigens such as inactivated whole viruses, viral subunits or recombinant viral antigens are not efficacious unless combined with adjuvants and administered in repeated injections. When chickens were vaccinated with a recombinant live fowl pox virus expressing the VP2 antigen, it actively protected the birds against mortality but not against bursal atrophy.

Virus-like particles are one of the highly effective types of subunit vaccines that mimic the authentic structure of virus particles without containing the genetic material. In our laboratory, the IBDV major capsid protein VP2 was expressed in *Saccharomyces cerevisiae* leading to the formation of sub-viral particles (SVPs) that is structurally similar to IBDV. The SVPs, in addition to their ability to stimulate the B-cell mediated immune responses, were highly effective in stimulating CD4 proliferative response and cytotoxic T lymphocyte responses. Recently, several adjuvants have been used to improve the protective immunity of IBDV vaccines. These vaccines were able to induce antibodies and protect the specific pathogen free (SPF) chickens against very virulent IBDV challenge. In the present study, we found 100% survivability of the chickens in the groups receiving VP2 vaccine along with an adjuvant, 200 µg of recombinant VP2; VP2 alone with 100 µg and live vaccine whereas 10% mortality was observed in those birds receiving killed vaccine. Further, the chickens vaccinated with live vaccine showed highest up-regulation of IFNγ, and a significantly higher response to IFNγ in the chickens vaccinated with VP2 alone or in combination with adjuvant four days post challenge. Co-incidentally, the IL 4 was also up-regulated in the birds vaccinated with VP2 alone or in combination with adjuvant. These findings corroborate that the sub-viral particles made of VP2 were able to stimulate both the arms of immune response. The results were further supported with the findings of lymphocyte proliferative assay wherein the VP2 SVPs alone or in combination were able to generate IBDV-specific proliferation of lymphocytes in PBMCs as was observed in chickens two weeks post vaccination. The group injected with VP2 alone or with adjuvant showed a high percentage of CD4 T cells, whereas the CD8 T cells were significantly ($P<0.01$) higher in those birds that received VP2 along with the adjuvant. The antibody titres against IBDV gradually increased prior to challenge in chickens vaccinated with VP2 alone or in combination with adjuvant and there was a high rise in the antibody titres in those birds ten days post challenge.

The present study investigated the efficacy of recombinant VP2 protein along with an adjuvant and was proven to be more effective than the other treatment groups as measured by antibody titres, bursal–body weight ratio, and clearance of IBDV from the bursa, percentage of mortality, cytokine response, LTT and estimation of CD4/CD8 T cell subsets.

Upcoming Conference of IVS

National workshop on "Influenza: Risk factors, Massive Impact and Uncertain future" at IVRI, Izatnagar, on October 19, 2015 (contact: Dr. Y.P.S. Malik; malikyps@gmail.com).

A one day symposium on "Challenges in Plant Virology and our preparedness" at IARI, New Delhi on 5th December 2015 (Contact: Dr VK Baranwal; vbaranwal2001@yahoo.com).

The **8th International Geminivirus Symposium** and 6th International ssDNA Comparative Virology Workshop, 7-10 November 2016 at New Delhi (Contact: Dr. Indranil Dasgupta; indranil58@yahoo.co.in).

Editorial Board

IVS Awards-2015

Prof. K.S. Bhargava Oration Award-2015
Prof. (Dr.) S.N. Gaur
V Patel Chest Institute, University of Delhi , Delhi

IVS FELLOWS -2015

PLANT VIROLOGY
Dr. Manoj Prasad
National Institute of Plant Genome Research, New Delhi

Dr. R. Selvarajan
ICAR- National Research Centre for Banana, Tiruchirapalli

VETERINARY VIROLOGY
Dr. V. Balamurugan
ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI),
Bengaluru

MEDICAL VIROLOGY
Dr. Paban Kumar Dash
Defence Research & Development Establishment, Gwalior

Guidelines

Submit news article, which has some application prospect to any one of the editors. The article to be written in a popular format not exceeding 1000 words with a few simple table and or high quality figures. Article structure: Title, author(s), full address, email, telephone, self photo of corresponding author, running text.

Published by
INDIAN VIROLOGICAL SOCIETY
F-3, A-Block, National Societies Block,
NASC Complex, D.P.S. Marg, Pusa, New Delhi -110 012, India,
Tel: +91-9711763384
E-mail: secretaryivs@gmail.com, website: www.ivsnet.in