



NIHSAD

NEWSLETTER



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ICAR - NATIONAL INSTITUTE OF HIGH SECURITY ANIMAL DISEASES, BHOPAL

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Greetings from NIHSAD!

NIHSAD has come a long way to establish itself as the torch-bearer of biosafety and biosecurity, and now, we are happy to be among the numero uno veterinarians from India in exploration of the fauna of Antarctica! The institute has evolved over the years with changing trend in emergence of diseases and rapid advancement in technology driven disease diagnosis and pathogen discovery. Beginning with the vigilance of the exotic and emerging pathogens over the country's mainland in early years of 21st century, NIHSAD has driven itself to look for unknown pathogens in the frozen environs of Antarctica. A team of scientists of the institute have taken up this new challenge in view of the recent increase of footsteps in Antarctica as well as consequent to global warming which have increased the possibilities of spreading of biological material from there to the other continents. I wish the team good luck for this new venture.

Another factor that is especially important in the re-emergence of diseases is drug resistance. Bacteria, viruses, and other microorganisms can change over time and develop a resistance and therefore, drugs that were effective in the past may no longer be useful in controlling disease. A continuous surveillance is carried out in avian Influenza for national preparedness at NIHSAD.

We are all aware that Nipah virus was first recognized in 1999 during an outbreak among pig farmers in Malaysia. Since then, there have been number of outbreaks, all in South Asia. It is known now that geographical distribution of Henipavirus (Nipah and Hendra) overlaps with that of Pteropus. Over the years, the epidemiology of Nipah appears to have changed. Evidence of person to person transmission and a high case fatality rate (60-70%) were some of the alarming developments seen in Nipah outbreaks in India, Siliguri (2001) and recently in Kerela (2018).

The bio-engineering components of NIHSAD's unique bio-containment facilities have been continuously running for 20 years. The engineering staff of the institute has been doing excellent job in maintenance of the infrastructure as well as upgrading with new components. Every year, we are timely restructuring and replacing the parts to ensure continuity in operation of bio-safety infrastructure of the laboratory.

At NIHSAD we are constantly working in liaison with relevant sectors, both governmental and non-governmental to share best practices and expand collective knowledge base. Concerted efforts are also made to develop advanced counter measures such as surveillance tools, diagnostic tests and vaccines through basic, translational and applied research.

It gives me pleasure to present the newsletter to its readers!!

(V.P. Singh)

FROM CENTRAL MAIN-LAND TO THE SNOWY TERRENE OF ANTARCTICA

NIHSAD added a feather in its cap when one of its principal scientist Dr Ashwin Ashok Raut visited Antarctica in a proposal "Investigation of Antarctic Animal Metavirome: An initiative for Pathogen Discovery with special reference to globally emerging avian influenza and high risks viruses". The visit was made under 37th Indian Scientific Expedition to Antarctica (ISEA), Voyage team by Expedition Vessel IVAN PAPANIN during 15th Dec 2017 to 27th Feb 2018. Samples of feces, cloacal and oropharyngeal swabs, feather and hair

follicles, orinithogenic and lake sediment soils, as well as lake and sea water were collected from Adele Penguins, Emperor Penguins, South Polar Scuas, Snow Petrels, and Weddell Seals. All the samples were transported in cold chain from Antarctica to India and stored in NIHSAD repository for further processing. The viral diversity of the Antarctic ecosystem will be identified by high throughput sequencing of the isolated metaviromes.



RESEARCH HIGHLIGHTS

Drastic reduction of pro-inflammatory cytokine response of H5N1 AIV challenge by M2e-HA2 fusion protein immunization in chicken

S. Kalaiyarasu, S. Bhatia, N. Mishra, K. Rajukumar, D. Senthil Kumar, M. Kumar, R. Sood

Host pro-inflammatory response is one of the major contributing factor to the pathogenesis of H5N1 HPAI virus infection in chicken and the fatal outcome is due to hyper-acute dysregulation of pro-inflammatory cytokines. Pro-inflammatory cytokine genes (IL-1 β , IL-6 and CXCLi2) were highly upregulated in H5N1 HPAI infected group of chickens whereas the same were drastically reduced in M2e-HA2 fusion protein immunized group as a protective response. Reduction of IL-6 and CXCLi2 was also noticed in M2e

synthetic peptide group with lesser percentage than M2e-HA2 group (Figure 1). Upregulation of IL-4 and type-I and type-III IFNs in M2e-HA2 group also ensures the development of cellular immune response. Most of the TLRs genes were down regulated in H5N1 HPAI infected group at 24hr of infection whereas significant upregulation was noticed in M2e-HA2 fusion protein immunized group than M2e synthetic peptide and control group. FACS analysis of CD4⁺ and CD8⁺ cell population after 24 hr of virus infection revealed that AIV induced the depletion of both the populations. Here, the combination of M2e and HA2 conserved regions of H5N1 shows enhanced humoral and CMI response than M2e synthetic peptide alone which will be helpful in future development of universal vaccines against avian influenza.

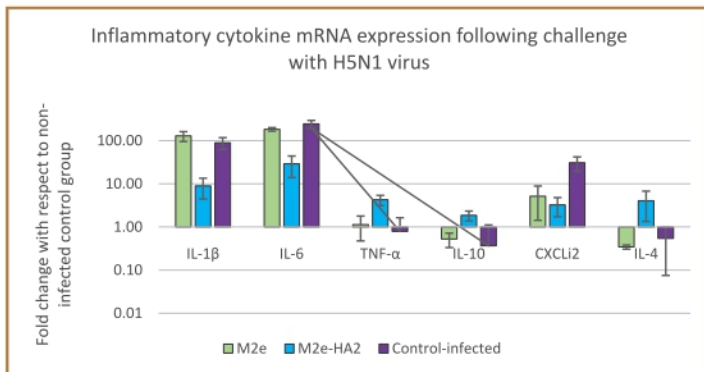


Figure 1: Inflammatory cytokine mRNA expression following challenge with H5N1 virus

Neuraminidase inhibitors susceptibility profiles of highly pathogenic influenza A (H5N1) viruses isolated from avian species in India (2006–2015)

R. Sood, N. Kumar, S. Bhatia, A. K. Pateriya, A. Mishra, V. P. Singh

A total of 65 highly pathogenic avian influenza (HPAI) A(H5N1) viruses, isolated from avian species in India between 2006 and 2015, were tested for susceptibility to the FDA approved neuraminidase (NA) inhibitors (NAIs), oseltamivir and zanamivir using a phenotypic fluorescence-based assay. The overall incidence of resistant variants among HPAI A(H5N1) viruses was 7.69% (5/65). The NA inhibition assay identified 3 viruses resistant to oseltamivir (N294S substitution, N2 numbering) and 2 cross-resistant to oseltamivir and zanamivir (E119A or I117V+E119A substitutions), all of which belonged to hemagglutinin (HA) clade 2.2 (5/17) and predominantly circulated in Indian poultry during 2006–2010 (Figure 2). In comparison to E119A substitution alone, viruses with I117V +E119A double substitutions showed greater reduction in susceptibility to both oseltamivir and zanamivir.

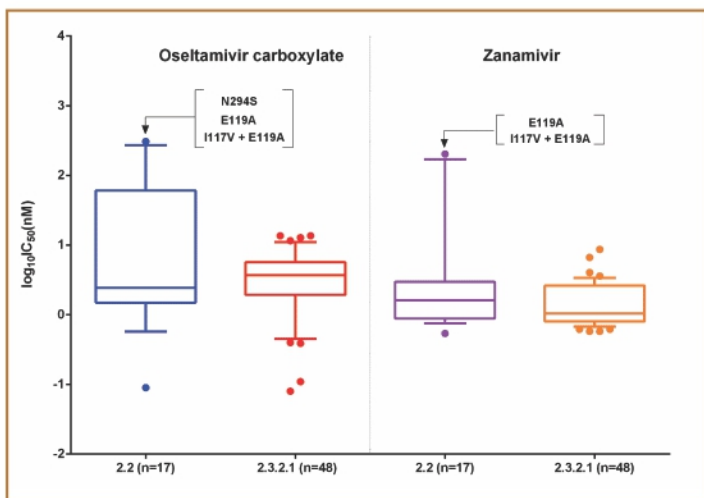


Figure 2: Box-and-whisker plot analyses of 65 highly pathogenic avian influenza A (H5N1) viruses.

The NAI resistance-associated NA markers, identified in this study, were as a result of naturally occurring mutations. Of note, 48 viruses of HA clade 2.3.2.1 that circulated in Indian poultry during 2011–2015 were susceptible to both oseltamivir and zanamivir. It is essential to monitor NAI susceptibility among human and avian HPAI A(H5N1) viruses that would provide baseline data to develop strategies for pandemic preparedness and therapeutic interventions.

Genome-wide analyses of henipaviruses reveal natural selection mediated evolution of codon usage

N. Kumar, D.D. Kulkarni, S. Bhatia, R. Sood, A.K. Pateriya, and V.P. Singh

A group of emerging bat-borne paramyxoviruses, which includes Hendra virus (HeV) and Nipah virus (NiV), have been responsible for serious disease outbreaks in humans, pigs and horses with huge death rate. The dependence of viruses on host's cellular machinery governs the codon usage patterns in viruses for their evolution, adaptation and survival. Bringing this into consideration, we performed the first ever genome-wide codon usage bias analyses of henipaviruses isolated from different host species. An entirely discrete evolutionary pattern for NiV and HeV were observed in phylogenetic and multivariate statistical analyses. Relative synonymous codon usage (RSCU) analysis showed that henipaviruses preferred A/U-ended synonymous codons, while CGN/NCG codons were severely suppressed indicating CpG deficiency in their genomes. A slightly lower codon usage bias was found in the coding gene sequences of henipaviruses. Moreover, the evolution of codon usage patterns in henipaviruses is predominantly dictated via natural selection, however, the codon usage patterns in P-gene products (W and V in HeV; C in NiV) are influenced predominantly by the mutational pressure (Figure 3). Several codon usage methodologies showed that henipaviruses might also have co-evolved with microbat of the genus *Myotis* besides the established reservoir, megabat of genus *Pteropus*. Furthermore, high tRNA adaptation index of henipaviruses for human cells was mapped to F, G and L proteins in comparison to recently identified henipalike viruses. Consequently, henipaviruses might create a substantial emergence potential in humans, particularly when paired with frequent exposure to direct/indirect bat excretions.

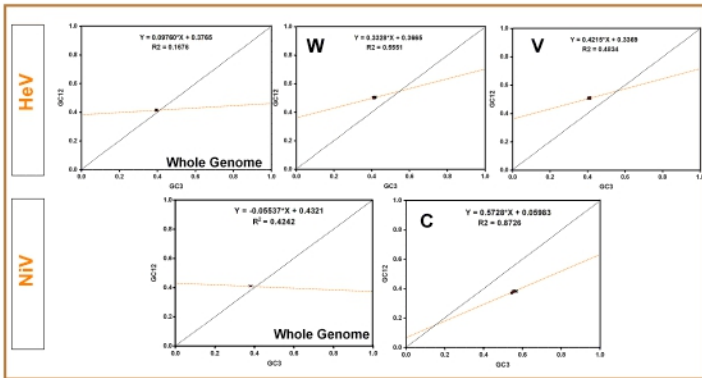


Figure 3: Neutrality plots analyses of coding sequences of HeV and NiV.

Complete genome sequences of cattle BVDV-2 isolate from India

N. Mishra, S. Kalaiyarasu

Complete genome sequence analysis can provide better insights into molecular epidemiology of BVD, complete genome sequences of an Indian BVDV-2 isolate from cattle was determined. The full-genome of isolate Ind 141353 contained 12,285 nucleotides (nt) with a single large open reading frame which codes for 3898 amino acids. The genomic organization of Ind 141353 was found consistent with that of other BVD viruses and based on the putative polyprotein processing sites has the following order from 5'-end to 3'-end: 5'-UTR, 1-387; Npro, 388-891; C, 892-1197; Erns, 1198-1878; E1, 1879-2463; E2, 2464-3579; p7, 3580-3789; NS2, 3790-5148; NS3, 5149-7197; NS4A, 7198-7389; NS4B, 7390-8430; NS5A, 8431-9921; NS5B, 9922-12081; 3'-UTR, 12082-12285. Phylogenetic analysis indicated that this strain belongs to the BVDV-2a subtype and has highest (93%) level of genetic identity with the Chinese cattle strain JZ05-1. It was demonstrated that two genetically closely related BVDV-2a viruses, one of ncp biotype and the other cp biotype, having identical length of complete genome are circulating in cattle in India and China. The results extend the spectrum of pestivirus molecular data and provide important insights into BVDV molecular epidemiology.

CELEBRATIONS

Republic Day Celebration

The celebration of Republic Day started with flag hoisting by Dr. V.P. Singh, Director of the institute with enthusiasm and patriotic fervor on January 26, 2018. In



his motivational address, he remembered and saluted the sacrifices of freedom fighters, soldiers and great leaders of the country. Also, he recapitulated the achievements of the institute during past year and laid emphasis on the future scientific challenges. On this occasion, different sports activities were also organized.

International Yoga Day

The International Yoga Day was observed at the NIHSAD on June 21, 2018. To mark this occasion, preparatory yoga sessions were organized from June 19-20, while on June 21, Shri Anshul Jain and Shri Paras Dhawan from 'Art of Living', Bhopal demonstrated the Yoga Asana as per Common Yoga Protocol (CYP) and staff and their family members participated in all the sessions. A lecture-cum-practical session on "Role of Physiotherapy in Body Pain Management" was delivered by Prof. P.R. Suresh,

EVENTS

Krishi Unnati Mela

The Krishi Unnati Mela-2018 with the theme of “Doubling Farmers Income by 2022” was held at ICAR-IARI, New Delhi during March 16-18, 2018. On the occasion, Hon’ble Prime Minister and Hon’ble Union Minister of Agriculture and Farmers’ Welfare addressed the farmers, KVKs, Agriculture scientists, Vice-Chancellors, Directors of ICAR institutes and students.



Principal, People's college of Paramedical Sciences, Bhopal and practical session was performed by group of students of his college. He explained the basics of identification of pain and its evaluation and management. Both the programs would definitely benefit in bringing harmony between the body, mind, and spirit, making one physically, mentally and spiritually strong. Dr. V.P. Singh appreciated the faculties and all the staff members for their participation and exhorted them to continue the organization of yoga sessions on regular basis in the institute campus.

Research Advisory Committee (RAC) Meeting

The fourth RAC meeting of ICAR-NIHSAD was conducted on March 20, 2018 at the institute under the chairmanship of Prof. M.P. Yadav, Former Vice Chancellor, SVBPUA&T, Meerut and Ex. Director, IVRI, Izatnagar. On this occasion, RAC members, Dr. Ashok Kumar, ADG (AH), ICAR; Dr. S. K. Rana, Head Animal Health, NDDDB, Anand and Dr. Mala Chhabra, Consultant, Dr. Ram Manohar Lohia hospital, New Delhi were present along with all scientists of the institute. Major achievements of the institute were presented by Dr. V. P. Singh, Director, NIHSAD. Action taken report on previous RAC recommendations was presented by Dr. S. Bhatia, Incharge PME & Member Secretary, RAC.



Institute Research Committee (IRC)

Annual IRC meeting of the institute was held on April 19, 2018. All the projects were presented by respective principal investigators of each project. Research priorities of the institute were discussed at length with main emphasis given on development of diagnostics and vaccines for the country.

EXTENSION ACTIVITIES/ VISITS

Scientists from NAARM, Hyderabad

Six scientists on probation of FOCARS-107 batch from ICAR-National Academy of Agricultural Research Management (NAARM), Hyderabad visited the institute on March 3, 2018 as part of Field Experience Training at ICAR-Central Institute of Agricultural Engineering, Bhopal. The scientists were made aware of different aspects of biosafety and biosecurity, exotic & emerging diseases and containment facility.

The institute also participated in a big farmers-interaction meet held at ICAR-CIAE, Bhopal on March 17, 2018 and created awareness among the livestock farmers about the emerging diseases in animals. The live web-telecast of these addresses were arranged at the institute auditorium for the farmers, scientists, faculty, students and staff.

Web telecast of the Hon'ble Prime Ministers' address

Hon'ble Prime Minister Shri Narendra Modi Ji interacted directly with farmers across the country on June 20, 2018. The institute had arranged for live telecast of this event in the auditorium for staff and local farmers. Before the beginning of PM's interaction, Dr. V.P. Singh, Director of the institute briefed the gathering about the programme and policies of the Government.



Health Camp on World Health Day

A health camp was organized on April 7, 2018 on the occasion of World Health Day at the institute. Dr. Atul Gupta, AMA of NIHSAD, with his team measured blood pressure, sugar and also checked for any indications of neuropathy for all the staff members of NIHSAD.





SSB trainee's officers, Bhopal

The Sashastra Seema Bal (SSB) trainees team of six veterinarians ranked as assistant commandants visited the institute on March 6, 2018.



Researchers from African countries

International trainees from Indian Institute of Soil Science, Bhopal under the Feed and Future India Triangular Training visited NIHSAD Campus on Feb., 9, 2018. They were given an opportunity to acquaint themselves with the State of Art Laboratory and the facility at NIHSAD.



CAPACITY BUILDING

Training/Workshops attended

- Dr. C. Tosh attended the workshops; "Transition of ISO/IEC 17025: 2005 to ISO/IEC 17025: 2017, for NABL assessors organized by NABL, Gurgaon, on April 21-22, 2018, and "Right to Information for Public Information Officers", organized by Institute of Secretariat Training and Management, New Delhi, June 11-13, 2018.
- Dr. Manoj Kumar attended a training programme on "Laboratory Quality Management and Internal Audit as per IS/ISO/IEC 17025: 2017" during June 25-28, 2018 at National Institute of Training for Standardization, Bureau of Indian Standards, Noida.

Meetings attended

- Dr. R. Sood attended Institute Animal Ethics Committee meeting as CPCSEA Nominee at Chirayu Medical College on January 9, 2018.

DISTINGUISHED VISITORS

Dr. Samia Metwally, a Senior Animal Health Officer visited the institute on Feb. 8, 2018. She is a virologist and one of the experts at FAO/OIE involved in the maintenance of global freedom from Rinderpest in the post-eradication era.



Dr. Panjab Singh, President, National Academy of Agricultural Sciences, former Secretary DARE and DG, ICAR, visited NIHSAD on March 24, 2018. During his visit, he was impressed with the elaborate biosafety infrastructure of the institute. He interacted with the scientists on the research work done on exotic and emerging animal diseases. In his address, he appreciated the quality research work carried out in the institute and highlighted the need of such institutes in handling emerging animal diseases. He also stressed the need for the establishment of the BSL-4 laboratory at NIHSAD in view of the rising threat from zoonotic infections. Dr. V. P. Singh, Director, NIHSAD thanked Dr. Panjab Singh for his keen interest in the activities of the institute and continued support for the institute's needs.



John Weaver and John Staton visited NIHSAD on April 27, 2018 as part of the OIE Team visiting the animal husbandry and practices networking workflow of the state. They were impressed to see the work carried out at NIHSAD and bio-containment facility available in the animal sector in India.

PERSONALIA

Promotions:



S. Nagarajan, Senior Scientist (Animal Biotechnology) at ICAR- National Institute of High Security Animal Diseases promoted to Principal Scientist w.e.f. Feb. 10, 2017.



G. Venkatesh, Senior Scientist (Animal Biotechnology) at ICAR- National Institute of High Security Animal Diseases promoted to Principal Scientist w.e.f. Feb. 10, 2017.

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