



Newsletter

NIHSAD

ISO 9001:2008 CERTIFIED

From the Director's Desk

A large number of new infections with severe animal and human health and economic consequences have emerged over the past three decades. Of the 30 new infections detected worldwide, 60 per cent are of zoonotic origin. The emerging diseases are either new or previously undefined diseases as well as old diseases with new features. These new features may include the introduction of a disease to a new location, a new population, new clinical features, including resistance to available treatments and rapid increase in the incidence and spread of the disease. In most of the emerging diseases, animals play an essential role in maintaining the infection in nature and contribute in varying degrees to the distribution and the actual transmission of infection in human and animal populations. Hence, the burden of animal diseases has shifted towards emerging infections, which warrants re-shaping of our approach with tools that not only provide solutions for better productivity and survivability of our livestock but also reduce the public health threat and protect the environment.



With this changing scenario of growing importance of the emerging diseases in livestock, ICAR-NIHSAD is poised to maintain preparedness for rapid diagnosis of these diseases and to update the diagnostic tools with changing characters of pathogens. In this direction, we are continually studying the evolution of the H5N1 avian influenza viruses (AIVs) so as to keep vigil over its changing character and disease pattern. Two new H5N1 clades having substantial antigenic divergence have been identified. In addition, characterization of H6N2 viruses which have donated genes to the human influenza viruses highlights the critical role of low pathogenic AIVs in the emergence of new variants. Although the country is free of H7N9AIV which has been infecting the human population in China and other south eastern countries, we have initiated steps toward its diagnostic preparedness also. Similarly, newly emerging strains of bovine viral diarrhoea virus (BVDV) in cattle have been detected earlier with the recent identification of the BVDV-2a subtype from bull semen samples of Tamil Nadu. Detection of unknown infections in animals is an important area and has been a tough challenge for diagnosticians and researchers. In the recent past, the work initiated in this direction has shown promising results and detection of multiple pathogens in a single test may soon be a reality for certain important pathogens.

For the last 15 years, providing diagnosis and development and improvement of diagnostics for exotic and emerging diseases has been the major focus of the institute. However, keeping in view of the current and future requirement of the country's livestock sector, developing vaccines for two economically important emerging viral diseases, namely BVD and porcine reproductive and respiratory syndrome (PRRS), will be an important priority in the research program of the institute. While we have already prepared a reverse genetics based vaccine for highly pathogenic avian influenza H5N1, the identification of new antigenic variants of H5N1 will help us update the vaccine strains for the poultry.

It is a matter of great pleasure for me to inform that ICAR-NIHSAD has been conferred with ISO 9001: 2008 Certification on 26th April, 2016. On this significant achievement, I congratulate my team for achieving this new milestone.

I feel happy to present this issue of the Newsletter to its readers.


(V.P. Singh)

What's Inside

- Research Highlights 2-4
- Initiatives Accomplished 4
- Celebrations 4-6
- Extension Activities 6
- Trainings/ Workshops Organized 6-7
- Meetings 7
- Capacity Building 7-8
- Honours/ recognitions 8
- Visit Abroad 8
- Personalia 8

Editorial Board

Editors

- Dr. Richa Sood, Sr. Scientist
- Dr. Naveen Kumar, Scientist

Photo Courtesy

- Mr. Somkuwar, STA

Antigenic analysis of highly pathogenic avian influenza H5N1 viruses isolated from poultry in India

C. Tosh, S. Nagarajan, M. Kumar, H.V. Murugkar, G. Venkatesh

Highly pathogenic avian influenza (HPAI) is a major animal and human health concern worldwide. Antigenic analysis of HPAI H5N1 viruses isolated from poultry during 2006 to 2015 in India comprising of 25 isolates from four phylogenetic clades, 2.2, 2.2.2.1, 2.3.2.1a and 2.3.2.1c was carried out. Seven H5N1 isolates from all four clades were selected for production of chicken antiserum, and antigenic analysis was carried out by hemagglutination inhibition (HI) assay. HI data indicated antigenic divergence (6-21 fold reduction in cross-reactivity)

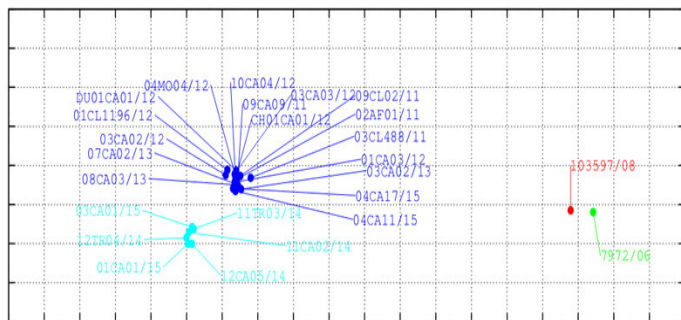


Figure 1. Antigenic cartography of H5N1 viruses based on Mean HI titre with polyclonal sera. The spacing between grid lines is 1 unit of antigenic distance corresponding to a 2-fold dilution of antiserum in the HI.

between the two recently emerged clades 2.3.2.1a and 2.3.2.1c. These two clades are highly divergent (21-128 fold reduction in HI titre) from the earlier clades 2.2/2.2.2.1. However, a maximum of 2-fold and 4-fold reduction in cross-reactivity was observed within the isolates of homologous clades 2.3.2.1c and 2.3.2.1a, respectively (Fig. 1). The molecular basis of inter-clade antigenic diversity was identified on the HA antigenic sites of the virus. Between clades 2.3.2.1a and 2.3.2.1c, amino acid substitutions at 6 HA antigenic positions was observed, whereas between clades 2.3.2.1a/2.3.2.1c and 2.2/2.2.2.1, amino acid substitutions were detected at ≥ 9 HA positions.

Therefore, a systematic analysis of antigenic drift of the contemporary strains will help to determine the strain(s) suitable for vaccine candidate.

Genetic characterization of H6N2 avian influenza virus isolated from ducks in live market of Assam

M. Kumar, S. Nagarajan, G. Venkatesh, H.V. Murugkar, C. Tosh

A H6N2 avian influenza virus isolated from healthy ducks in live bird markets of Assam in 2015 was characterized by complete genome sequencing. Haemagglutinin (HA) gene analysis revealed it to be a low pathogenic avian influenza virus. Further HA gene phylogeny revealed that the Assam virus grouped closely with H6N2 virus isolated from a wild bird in China, whereas the Kerala H6N2 virus isolated in the previous year, 2014 displayed close relationship with H6N6 virus isolated from duck in Japan in 2014 and formed a separate lineage within Eurasian region (Fig. 2). As H6 virus has contributed genome segments for the emergence of avian influenza viruses with public health significance, surveillance and complete genome sequencing of emerging strains will help in the control of avian influenza.

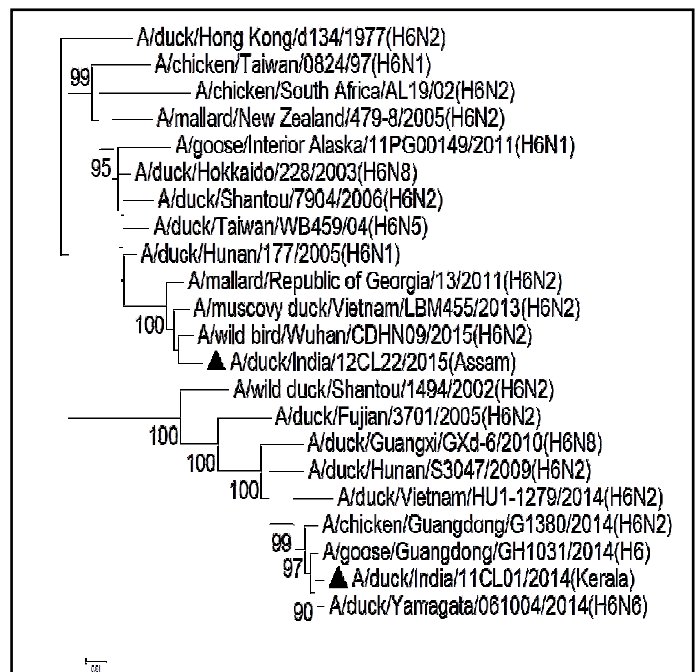


Figure 2. Haemagglutinin phylogeny of H6N2 avian influenza viruses. Indian isolates are highlighted with solid triangle.

Pathology of reassortant highly pathogenic avian influenza H5N1 virus in BALB/c mice

M. Kumar, H. V. Murugkar, S. Nagarajan, C. Tosh

The pathogenicity of H9N2-PB2 reassortant H5N1 virus was studied in BALB/c mice and compared with non-reassortant H5N1 virus at 3rd, 7th and 8th dpi. In lungs of mice, both viruses caused severe congestion, oedema, haemorrhage and consolidation as time elapsed, the lesions being more pronounced in non-reassortant group. Microscopically, lungs showed severe congestion, haemorrhage, thrombus, fibrinous exudate in perivascular area, interstitial septal thickening, bronchiolitis and alveolitis leading to severe pneumonic changes lesions being more severe in non-reassortant virus infected group (Fig. 3a and b). Viral antigen and RNA were detected by immunohistochemistry and RT-qPCR, respectively in the nasal mucosa, lungs, trachea and brain at all the three intervals in both the groups. In reassortant virus infected mice, proinflammatory cytokines IL-1, IL-6 and KC were upregulated in the brain and lungs and innate sensors *viz.*, TLR-3 and TLR-7 mRNA was also expressed more during infection. In non-reassortant H5N1 virus infected mice, cytokine responses were less pronounced than reassortant H5N1 virus infected mice. In conclusion, the H9N2-PB2 reassortant H5N1 virus caused milder lesions in mice and the innate immunity might have played a major role in its low pathogenicity.

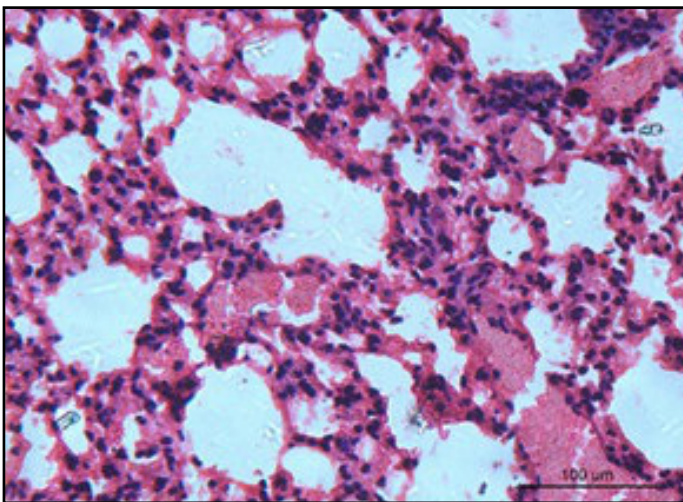


Figure 3a. H9N2-PB2 reassortant H5N1 virus infected mice (8 dpi)- Lung- Vascular congestion, haemorrhages, fibrin in alveoli and infiltration of polymorphs in interstitial septa (Bar-100 µm).

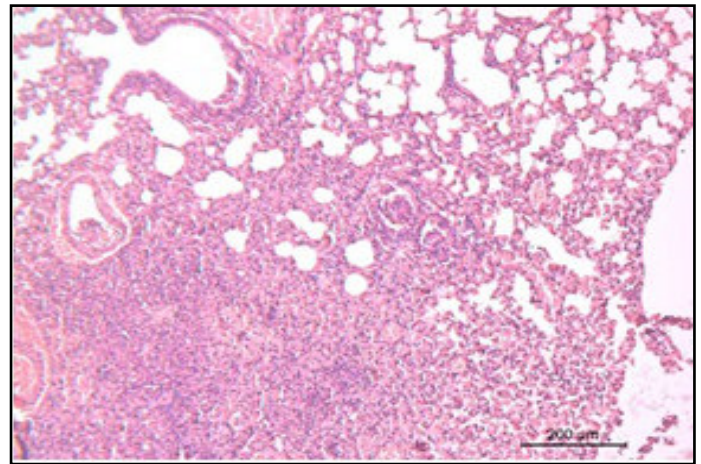


Figure 3b. Non-reassortant H5N1 virus infected mice (8 dpi)- Lung- Severe interstitial thickening due to polymorphs and mononuclear cells, alveolitis and alveolar lumen completely filled with inflammatory cells (Bar-200 µm).

Investigation of Crimean-Congo Haemorrhagic Fever Virus (CCHFV)

P.N. Gandhale, A.A. Raut, S.B. Sudhakar, A. Mishra, D.D. Kulkarni

Post human CCHF outbreak, livestock and ticks sample lots from Gujarat from the surrounding index case areas were received from Gujarat state animal husbandry departments for investigation. Five sample lots were comprised of 38 blood, 38 sera and 20 tick pools. The blood and tick pools were tested by one-step Real Time RT-PCR for presence of CCHFV genome and serum samples were tested by iELISA for the presence of anti-CCHFV antibodies. All the samples tested were found negative by respective tests.

Identification of BVDV-2 in cattle bull semen from Tamil Nadu

N. Mishra, S. Kalaiyarasu, K. Rajukumar

During routine screening for pestiviruses, BVDV antigen and genomic RNA were detected in three cattle bull semen passaged cell supernatants originating from Tamil Nadu state. All the three strains were typed as BVDV-2 by TaqMan real-time RT-PCR. Genetic analysis in 5'-UTR and N^{pro} gene revealed that they belong to the BVDV-2a subtype, but clustered in a clade separate from previously reported BVDV-2a strains in northern India. This

study highlights mandatory testing of the bulls and bull semen for BVDV before the cryopreserved semen is distributed for artificial insemination so as to prevent BVDV transmission in India.

Serological evidence of widespread West Nile virus and JEV infection in native domestic ducks in Kuttanad region, Kerala

S. Kalaiyarasu, N. Mishra, V.P. Singh

While investigating the extent of WNV infection in domestic ducks in Kuttanad region, Kerala, 95 of 209 ducks were found positive in WNV Ab ELISA. End point neutralizing antibody titre against WNV and JEV revealed WNV specific antibodies in 24 (11.5%) ducks in 3 districts, JEV specific antibodies in 21 (10%) ducks in 2 districts and flavivirus specific antibodies in 19 (9%) ducks. This is the first report of high seroprevalence of WNV infection in two most popular local breeds of Chara and Chemballi domestic ducks (*Anas platyrhynchos* var *domesticus*) in Kuttanad region, having a history of recent human outbreaks. Additionally, detection of both WNV specific and JEV specific neutralizing antibodies in domestic ducks in wide geographical areas indicate co-circulation of these viruses in the region. Persistence of WNV and JEV activity in wider areas in Kerala than previously reported may help the public health authorities in formulating surveillance programs in the region and domestic ducks may be useful captive sentinels in monitoring WNV/JEV activity.

Sero-diagnostic preparedness against avian influenza H7N9 virus

S. Bhatia, N. Kumar, A.K. Pateriya, R. Sood

The first incidence of H7N9 avian influenza virus to cause mortality in human population of China in 2013 raised the concern and emphasized the high probability of further outbreaks in birds, especially where close interactions between poultry and humans are present. Therefore, with an aim to initiate sero-diagnostic preparedness in our country, the consensus coding sequences of HA gene was expressed from AIV

H7N9 viruses detected from fatal human cases in China in 2013 in prokaryotic systems. Western blot analysis using anti-rgH7N9 polyclonal serum confirmed the expression of rHA₀. The recombinant protein will be used in developing sero-diagnostic which would reveal the status of anti-H7 antibodies in the birds, critical in risk assessment of emergence of novel viruses.

INITIATIVES ACCOMPLISHED

ISO 9001: 2008 Certification

ICAR-NIHSAD was conferred “ISO 9001: 2008 Certification” on 29th April, 2016 for the quality management system applicable to the scope of “research on exotic & emerging animal diseases of national importance, providing animal disease diagnostic services as well as human resource development in these areas and in biorisk management.” A two day ISO general awareness cum training was organized for all the employees of ICAR-NIHSAD.

Commencement of newsletter publication

In an initiative to publish biannual newsletter of the institute, the first newsletter for the year 2015 was released.



CELEBRATIONS

World Veterinary Day

World Veterinary Day was celebrated at NIHSAD on 30th April, 2016 with a theme “Continuing Education with One Health Focus”. Dr. B. N. Singh, Registrar, Ram Krishna Dharmarth Foundation (RKDF)

University and former Managing Director, MP State Livestock and Poultry Development Corporation as Chief Guest and Dr. R. K. Rokade, Director, MP State Animal Husbandry and Veterinary Services was invited as the Guest of Honor on this occasion. More than 125 undergraduate students from AIIMS, Bhopal and postgraduate students from Rajiv Gandhi Proyodiki Vishwavidyalaya (School of Biotechnology) with the faculty of these institutions along with Veterinary Officers posted in various districts of Madhya Pradesh and the NIHSAD staff participated in the event. 'Biosafety and Biosecurity awareness program' was also conducted as part of this celebration.

the employees. The cleaning drive was organized for working areas in the offices, laboratory, engineering section including substation, boiler, EM unit etc. including outside premises of the institute.



Cleaning of the ICAR-NIHSAD campus



Director, NIHSAD addressing the staff on the occasion of World Veterinary Day

International Yoga Day

ICAR-NIHSAD celebrated “International Yoga Day” on 21st June, 2016 with preparatory yoga sessions on 19th and 20th June as per the directives of ICAR and Ministry of AYUSH, Government of India. On 21st June, 2016, morning yoga session was conducted in the presence of an expert yoga teacher, Mr. Manoj Patil, Art of Living, Bhopal. He elucidated various asanas and highlighted the importance of yoga in human life for holistic development. More than 50 participants performed various yoga asanas at the playground of the institute on this occasion. A lecture on “Yoga and Dietary Interventions in Metabolic

Swachhta Pakhwada

Moving another step towards fulfilling Mahatma Gandhi ji dream of a clean and hygienic India by 150th birth anniversary in 2019, ICAR-National Institute of High Security Animal Diseases celebrated “Swachhta Pakhwada” programme from 16 - 30th May, 2016. The programme started with a pledge for cleanliness administered by the Director, ICAR-NIHSAD to all



Director, ICAR-NIHSAD administering the pledge on inauguration of special “Swachhta Pakhwada” programme



Invited yoga expert from Art of Living, Bhopal explaining the various yoga asanas

Diseases” by Dr. Charu Bansal, Professor and Head, Dept. of Swasthavritta, Pandit Khusilal Sharma Govt. College of Ayurvedic Medicine, Bhopal was arranged in the institute auditorium, which was attended by the staff and their family members. The emphasis was on the urgent need to adopt good food habits and yoga in our day to day life for healthy mind and body.



Dr. Charu Bansal delivering a lecture on the importance of Yoga and Diet for a healthy body and mind



Participants performing asanas

EXTENSION ACTIVITIES

- Students of College of Veterinary Science and Animal Husbandry, Jabalpur visited ICAR-NIHSAD for an educational tour of the institute under the DBT, New Delhi sponsored programme “Star College Scheme” on 18th June, 2016. The students were given a glimpse of biocontainment laboratory and its workings.
- Field Veterinary Doctors of Animal Husbandry Department, Govt. of Madhya Pradesh, undergoing training at Disaster Management

Institute visited the institute on 15th Jan., 2016 as part of a three day training program on Disaster Management.



Participants of the Star College scheme from Jabalpur

- Students from two colleges, SAM College and RKDF College, Bhopal visited the institute on 16th April and 27th May, 2016, respectively. An awareness tour of the supporting infrastructural facility of the containment laboratory and the animal wing was conducted for them. They were given a demonstration of the working of Air Handling Units and their application in the maintenance of the negative air pressure in the containment facility.



SAM and RKDF college students during an awareness programme of biocontainment Lab

TRAININGS/ WORKSHOPS ORGANIZED

Under the umbrella of DBT-NER-Advanced Animal Disease Diagnosis and Management Consortium, a five day training program titled “Biosafety and Biosecurity for handling and identification of zoonotic

pathogens” was organized at ICAR-NIHSAD for North Eastern Region partners from 16 to 20th Feb., 2016. The training was attended by participants from Assam, Arunachal Pradesh, Sikkim, Nagaland, Meghalaya and Mizoram states.



Inaugural function of the training



Participants from Northeastern states with NIHSAD scientists

MEETINGS

Institutional Research Committee meeting

The institutional research committee meeting of NIHSAD was held on 5th May, 2016 under the chairmanship of Dr. V.P. Singh, Director, NIHSAD.



IRC meeting

Prof. M.P. Yadav, Ex. Director cum Vice Chancellor, IVRI, Izatnagar and Ex. Vice Chancellor, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut (U.P.) was expert for reviewing the progress of the projects and evaluation of new project proposals. All the scientists of NIHSAD attended the meeting.

Research Advisory Committee Meeting

The second Research Advisory Committee (RAC) meeting of NIHSAD was held on 19th March, 2016. Dr. M.S. Oberoi, Ex-Dean, College of Veterinary Sciences, GADVASU, Ludhiana and former sub-regional manager, FAO and ECTAD was the chairman. Dr. G.K. Sharma, Head, Animal Health, NDDDB, Anand; Dr. Rajesh Chandra, Retd. Professor and Head, Microbiology, College of Veterinary Sciences, CAU, Aizwal and Dr. V.P. Singh, Director, NIHSAD were members and Dr. S. Bhatia as member secretary of the committee. The RAC appreciated the research contributions made by NIHSAD and also recommended to work on a priority basis with clear milestones and time frame. RAC also highlighted the need for the development of vaccines for PRRS and BVDV in future.



RAC meeting

CAPACITY BUILDING OF FACULTY

- **Dr. Manoj Kumar**, Scientist, participated as speaker in session “Livestock Healthcare and Management” of the 8th National Livestock Championship & Expo-2016 organized by Dept. of Animal Husbandry, Dairy Development & Fisheries, Punjab in collaboration with FICCI from 8 to 12th Jan., 2016.

- **Dr. Pradeep N. Gandhale**, Scientist, participated in a one day seminar cum interactive meet convened by ICAR-National Research Centre on Camel themed “Camel and Human medicine” on 16th May, 2016 at ICAR-NRCC, Bikaner.

HONOURS/RECOGNITIONS

- **Dr. H.V. Murugkar**, Principal Scientist, has been nominated as CPCSEA Nominee of Institute Animal Ethics Committee of the Sri Aurobindo Medical College and Post Graduate studies, Indore and Sagar Institute of Research and Technology, Bhopal by the Ministry of Environment, Forest and Climate Change.
- **Dr. N. Mishra**, Principal Scientist, was nominated as DBT Nominee, Institute Biosafety Committee, Indian Institute of Science Education and Research (IISER), Bhopal and External Member of ITMC, ICAR-Indian Institute of Soil Science, Bhopal.
- **Dr. Richa Sood**, Senior Scientist, has been nominated as CPCSEA Nominee of Institute Animal Ethics Committee of All India Institute of Medical Sciences, People's Medical College, and Chirayu Medical College Bhopal, by the Ministry of Environment, Forest and Climate Change.

VISIT ABROAD

- **Dr. C. Tosh**, Principal Scientist, attended the FAO's meeting on “Regional consultation to strengthen the Bioinformatics capacity in alignment with the Regional Strategic Framework for Laboratory Capacity Building” held in Bangkok, Thailand from 10 to 12th May, 2016.

- **Dr. S. Nagarajan**, Senior Scientist, attended the Fourth Laboratory Directors' Meeting and Workshop for SAARC Member Countries held at Dhaka, Bangladesh from 1 to 2nd June, 2016.

PERSONALIA

Promotions



Dr. Ashwin A. Raut, Senior Scientist (Animal Biotechnology) at ICAR-National Institute of High Security Animal Diseases promoted to Principal Scientist w.e.f. 1st Nov., 2014.

Joinings



Dr. Fateh Singh, Scientist (Veterinary Microbiology) at ICAR-Central Sheep and Wool Research Institute, Avikanagar got transferred and joined as Scientist, ICAR-National Institute of High Security Animal Diseases, Bhopal w.e.f. 30th May, 2016.



Mrs. Samlesh Kumari, Scientist (Dairy Microbiology) joined at ICAR-National Institute of High Security Animal Diseases, Bhopal w.e.f. 11th April, 2016.



Published by:

Director, ICAR - National Institute of High Security Animal Diseases, Bhopal - 462022, (M.P.)

Tel.: 0755-2759204 | Fax: 0755-2758842 | E-mail : director1nihsad@gmail.com, director.nihsad@icar.gov.in