



NIHSAD

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Newsletter

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Change is constant! There is no better example of this than the avian influenza virus. In October 2016, a new subtype H5N8 highly pathogenic avian influenza virus was detected in Delhi zoo and subsequently in many states of the country. ICAR-NIHSAD rose to the occasion and promptly diagnosed the disease as and when the suspected samples were submitted, in time bound manner, to enable early initiation of control measures. This virus was detected for the first time in wild birds and poultry in eastern Asia and Europe. The virus was subsequently detected in North America in late 2014 and sporadically in Canada and the USA until mid-2015. Since June 2016, many countries of Europe and Asia have also detected this virus. Our neighboring country, China has experienced a sharp increase in human cases of avian influenza H7N9 since late 2016 and chances of it crossing the border also loom large. At NIHSAD, diagnostic preparedness for such eventuality is already in place. The Platform for the reassortant vaccine development has already been established at the institute to cater to any need of the vaccine. At NIHSAD, we also keep preparedness and a constant vigil for other emerging animal and zoonotic diseases. Sero-prevalence of BVD infection was detected in the Mithun population in various states of northeast and it is indeed a matter of relief that suspicion of MERS-CoV infection in camel in our country was proved negative in the tests.

This year the cleanliness drive taken up in the Swachhta Abhiyan by the institute marked the spreading of awareness of Gandhiji's dream in the nearby surroundings and the same will continue to be taken up in a mission mode during 2017. The digital India dream has been addressed with the complete implementation of ERP developed by ICAR and will be taken forward with the development of an app for avian influenza at grass-root farmer level.

It is my pleasure to release the biannual newsletter for the readers.

(V.P. Singh)



Director's Desk

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Editorial Board

Editors

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

Photo Courtesy

- Mr. Somkuwar, STA

Novel reassortant highly pathogenic Avian Influenza H5N8 (clade 2.3.4.4) virus in zoological parks in India

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

Highly pathogenic avian influenza H5N8 virus was detected in water fowls, ducks, crows and chicken at National Zoological Park, Delhi; Gandhi Zoological Park, Gwalior (Madhya Pradesh); Karnataka, Kerala; Gujrat; Haryana and Punjab during October to December, 2016. One representative virus isolated from domestic duck (*Anas platyrhynchos domesticus*) and painted stork (*Mycteria leucocephala*) from Delhi and Gwalior, respectively was processed for molecular and pathogenic characterization. Both the isolates

were highly pathogenic based on amino acid sequence at the hemagglutinin (HA) cleavage region (PLREKRRKR/GLF), which was corroborated by intravenous pathogenicity index. Amino acid markers in the NA and matrix protein indicated sensitivity to neuraminidase inhibitors and amantadine. Except the PA and NP genes, all other gene segments of both isolates shared high nucleotide identity ranging from 99.2% to 99.5%. The nucleotide identity of the PA and NP genes was 95.8% and 94.8%, respectively, suggesting involvement of 2 gene pools of H5N8 virus in the waterfowl outbreaks at Delhi and Gwalior. The phylogenetic analysis (Figure 1) suggested that both isolates are 7:1 reassortant of the Tyva Republic and Uvs-Nuur Lake H5N8 viruses with different gene constellations. A median-joining network analysis indicated a closely related precursor gene pools of the above viruses as a source of the H5N8 viruses that caused outbreaks in waterfowls at the two zoological parks in India.

The reverse genetics-based reassortant H5N2 vaccine virus protects chickens against lethal challenge with HPAI H5N1 viruses

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

In a further step towards vaccine development for HPAI avian influenza, two reassortant viruses using reverse genetics were generated. A 5+3 reassortant H5N2 strain containing the modified HA gene (H5) of A/chicken/India/CA0302/H5N1/2011 (clade 2.3.2.1), the neuraminidase (N2) of A/Chicken/Uttar Pradesh/2543/H9N2/2004, the matrix (M) of A/chicken/West Bengal/239022/H5N1/2010 and the 5 other internal genes of A/WSN/33/H1N1, and a 6+2 reassortant virus containing modified HA from A/chicken/India/CA0302/H5N1/2011 (clade 2.3.2.1), the N2 from A/Chicken/Uttar Pradesh/2543/H9N2/2004 and all other internal genes of A/WSN/33/H1N1. The 5+3 reassortant H5N2 virus had the ability to grow to high titer in both MDCK cells and chicken

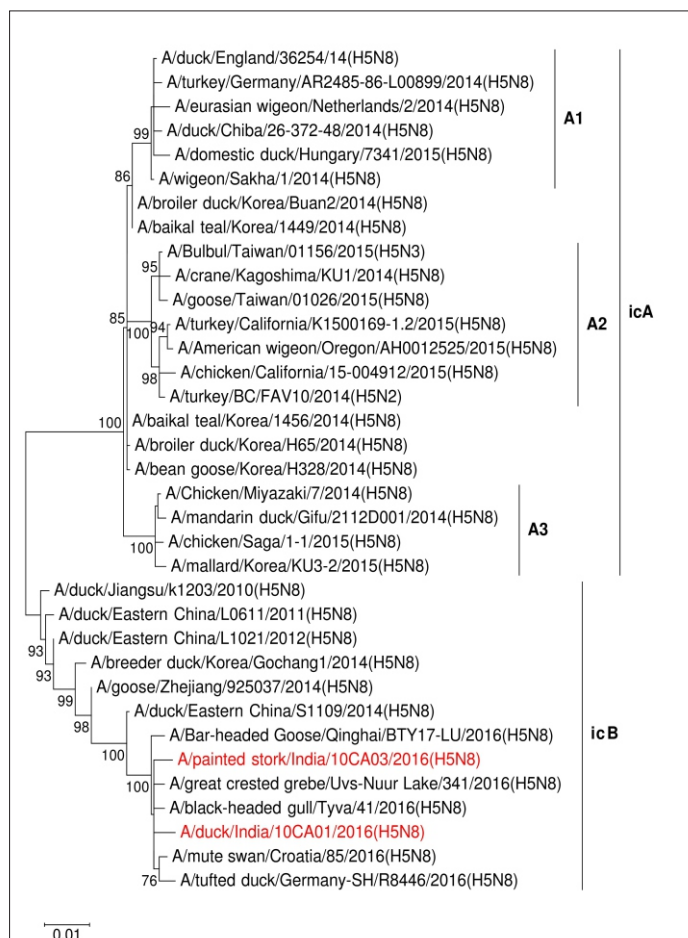


Figure 1. Molecular phylogeny of avian influenza H5N8 (clade 2.3.4.4) virus. Intercontinental groups (ic) and subgroups are shown to the right.

embryonated eggs compared to 6+2 reassortant H5N2 virus. An inactivated vaccine was developed with 5+3 reassortant H5N2 virus (2.3.2.1 clade) was used in a two-dose immunization regime. It protected SPF chickens challenged with homologous 2.3.2.1 clade and heterologous 2.2 clade HPAI H5N1 viruses. These challenged survived chickens remained protected after a second cross challenge with ten-fold higher dose of HPAI H5N1 viruses. There was no virus shedding through nasal or cloacal route in the surviving chickens up to 7 days post cross challenge. These data show that the virus generated in this study is effective in providing protection even in the case of an outbreak caused by antigenically mismatch clade 2.2 HPAI viruses and opens the way to explore its applicability as potential vaccine candidate especially in the Asian continent or countries reporting these clades frequently.

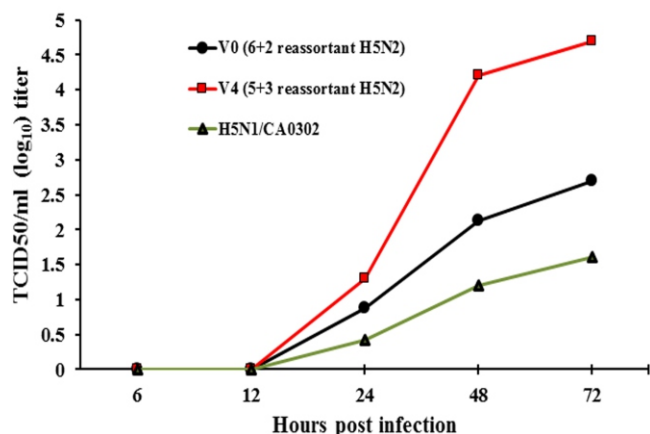


Figure 2. Growth kinetics of rescued 5+3 reassortant (V4), 6+2 reassortant (V0) and wild type H5N1/CA0302 virus in MDCK cells. Titre was expressed as Tissue culture infective dose (TCID 50) per ml.

Enzyme immunoassay for sero-surveillance of Avian Influenza H7N9 virus using truncated recombinant HA protein

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

The threat of H7N9 remains large with ever increasing incidents of H7N9 in China, making it essential for us

in India to be prepared with diagnostics and a vaccine candidate. A consensus coding sequence of HA gene of H7N9 viruses that caused outbreaks in humans in 2013 in China was derived by aligning 55 nucleotide sequences retrieved from GISAID databank and was chemically synthesized. It was further expressed in-house, purified and confirmed with western blot analysis using anti-rgH7N9 polyclonal serum. Indirect ELISA using recombinant HA1 protein has been developed and optimized. A total of 152 sera samples (40 sera from chicken experimentally vaccinated with inactivated rgH7N9 & rgH7N2 and remaining 112 AGID negative field sera) were screened using this assay, and this assay showed 100% diagnostic sensitivity and specificity. The analytical sensitivity of the assay was $2^{0.5}$ HIU which is comparative high to gold standard HI assay. This assay showed cross-reactivity with OIE reference serum of H1, H2, H8, H10, H12 and H15 subtypes. However, no cross-reactivity with H5 and H9 subtypes was detected. Following stringent validation of this test, it could serve as suitable assay for sero-surveillance of common AIV subtypes, H5 and H9 in chickens.

Serological evidence of BVDV infection in mithuns (*Bos frontalis*) in the NER, India

N. Mishra, S. Kalaiyarasu, K. Rajukumar

Serological evidence of bovine viral diarrhoea virus infection has been identified in mithun (*Bos frontalis*), a unique bovine species and important to the farmers in the North Eastern region of India indicating a broadening of BVDV host range in ruminants. Blood and serum samples were collected between 2013 and 2016 from mithuns (n = 466) belonging to the states of Nagaland, Mizoram and Arunachal Pradesh. Serum samples were tested for BVDV antibodies by a commercial ELISA and leukocytes were tested for BVDV by real-time RT-PCR. The overall true seroprevalence rate was found to be 13.1% with higher prevalence in mithuns reared under semi-intensive system (27.5%) than in free ranging mithuns (7.6%). Among the three states, seroprevalence (16.2%) was highest in Nagaland, while prevalence rates varied

markedly among geographical locations. Age wise data showed highest seroprevalence rate in > 6 years old animals (20.6%) than 2-6 yrs. old (16.9%), 6 months-2 yrs. old (8.5%) and < 6 months old animals (11.3%). The seroprevalence was higher in males (20.9%) than in females (12.1%). Among the four mithun strains, higher prevalence was evident in Manipur (30.3%) than Arunachal (21.3%), Nagaland (11.7%) and Mizoram strain (10.2%). The baseline data generated in this study will help further investigations on epidemiology of BVD in mithun and impact of BVDV on mithun production.

HPAI H5N1 virus induces cytokine dysregulation and suppression in maturation of chicken monocyte-derived dendritic cells

S Kalaiyarasu, M. Kumar, D Senthil Kumar, S. Bhatia, S. Nagarajan

One of the major causes of death in highly pathogenic avian influenza virus (HPAIV) infection in chickens is acute induction of pro-inflammatory cytokines, which leads to severe pathology and acute mortality. Infection of chicken monocyte-derived dendritic cells (chMoDCs) with H5N1 HPAIV produced high titers of progeny virus with more rounding and cytotoxicity than with H9N2 LPAIV. Expression of maturation markers (CD40, CD80 and CD83) was weaker in both H5N1 and H9N2 groups than in a LPS control group. INF- α , - β and - γ were significantly upregulated in the H5N1 group. Pro-inflammatory cytokines (IL-1b, TNF- α and IL-18) were highly upregulated in early, mid (IL-1), and late (IL-6) phases of H5N1 virus infection. IL-8 (CXCLi2) mRNA expression was significantly stronger in the H5N1 group from 6 hr of infection. TLR3, 7, 15 and 21 were upregulated 24 hr after infection by H5N1 virus compared with H9N2 virus, with maximum expression of TLR 3 mRNA. Similarly, greater H5N1 virus-induced apoptotic cell death and cytotoxicity were found. Thus, both H5N1 and H9N2 viruses evade the host immune system by inducing impairment of chMoDCs maturation and enhancing cytokine dysregulation in H5N1 HPAIV-infected cells.

Investigation of Middle East Respiratory Syndrome Corona Virus (MERS-CoV) in Camels and Crimean-Congo Haemorrhagic Fever Virus (CCHFV) in livestock

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

A mortality of more than 80 camels in the herd of 125 camels within a week was reported in camel caravan at Rinjpar village of Jamnagar district of Gujarat. Sixteen morbid samples were tested by WHO recommended one step RT-qPCR and found negative for MERS-CoV. Post human incidence of CCHF in Gujrat; blood, sera and tick pools from livestock population inhabiting the area surrounding index cases were tested for by one-step RT-qPCR for presence of CCHFV genome and anti CCHFV antibodies by iELISA and found negative.

CELEBRATIONS

2nd Foundation Day Celebrations

The second anniversary of foundation day of ICAR-NIHSAD was celebrated on 8th August, 2016. Prof. P. D. Juyal, Hon'ble Vice Chancellor, Nanaji Deshmukh Pashu-Chikitsa Vigya Vishwavidyalaya (NDVSU), Jabalpur was the Chief Guest and Dr. A. K. Patra, Director, ICAR-Indian Institute of Soil Sciences (IISS), Bhopal; Dr. S. K. Jain, Registrar, NDVSU and Dr. S. K. Goel, Prof. & Head, Department of Biochemistry, AIIMS, Bhopal were the Guests of Honor. The scientists from ICAR-IISS and ICAR-Central Institute of Agricultural Engineering, Officials of State Disease Diagnostic Laboratory and Professors from Barkatullah University, Bhopal also graced the function along with the staffs and students of ICAR-NIHSAD. Dr. V. P. Singh, Director, ICAR-NIHSAD welcomed the guests and focused the achievements and initiatives taken during the year. The Chief Guest, Dr. P. D. Juyal congratulated all staff of ICAR-NIHSAD for bringing up the laboratory to the institute level by their hard work, devotion and collaboration with national and international

agencies. The key note address on 'One Health' was delivered by Dr. S. K. Goel on this occasion. The Hindi leaflets on “बोवाइन वायरल डायरिया-लक्षण एवं बचाव ” and “बर्डफ्लू और जनस्वास्थ्य” were also released as the first hand information for the farmers. In the evening, a cultural program was organized by the staff and students of ICAR-NIHSAD and their family members.



Glimpses of second foundation day of ICAR-NIHSAD

Independence Day Celebrations

The 70th Independence Day was celebrated with the festivities and fervor. Dr. V.P. Singh, Director, ICAR-NIHSAD hoisted the national flag and addressed and



Independence day celebrations at ICAR-NIHSAD

congratulated the staff on the progress made by an efficient group. The way forward for goals set for next year was elucidated.

EVENTS

Swachhta Abhiyan

Taking one more step and oath towards fulfilling the Mahatma Gandhi's dream of a clean and hygienic India his 150th birth anniversary in 2019, ICAR-National Institute of High Security Animal Diseases celebrated “Swachhta Pakhwada” programme from October 16-31, 2016. The staff of NIHSAD worked in different groups to clean the campus premises as well as outside adjoining areas. Various events were organized. A lecture on “Stress Management” by B.K. Kiran, Regional coordinator of Madhya Pradesh for Value Education & Spirituality cell of Brahmakumaris' Rajyoga Education & Research Foundation was arranged in the institute auditorium on October 21, 2016 keeping in view the 'burgeoning stress' on our day to day life. Each and every member of the institute





Various activities carried out during Swachhta Pakhwada

participated enthusiastically in the programme with an assurance to maintain cleanliness throughout the year.

Vigilance Awareness Week

The Vigilance Awareness Week was observed at the institute from 31st October to 5th November, 2016. The week began with administering of the pledge to the staff and students of the Institute by Dr. V.P. Singh, Director, ICAR-NIHSAD on 31st October in the Institute auditorium. In his remarks, he stressed upon the importance of transparency, punctuality and

integrity at all working levels of staff for effective implementation of preventive vigilance. He also emphasized on inculcating moral values among all sections of the society so that corruption at all levels can be eliminated and our country can progress at a rapid pace. Dr. Niranjan Mishra, Principal Scientist and Vigilance Officer, deliberated upon the importance of “Public Participation in Promoting Integrity and Eradication of Corruption” and advocated that the fight against corruption should involve active involvement of all the citizens of the country and active vigilance by all concerned. Banners depicting vigilance awareness and corruption in public life were displayed prominently at several locations of the Institute. All the staff of the Institute actively participated in the vigilance awareness programme.

Hindi Pakhwara

Various competitions marked the beginning of Hindi Pakhwara, starting from 14th September, 2016. Incharge Raj Bhasha and Senior Administrative Office, Mr. S.K. Gupta delineated the road map for two week long celebration in the inaugural function. Nine different competitions were held and prizes were distributed on 7th October by Dr. S.C. Dubey, the former Joint director of ICAR-NIHSAD, who presided over the closing ceremony as chief guest.



Director addressing the staff on the occasion of Hindi Pakhwara

ICAR Sports Tournament

A sports team comprising of thirteen participants were selected for participation in the ICAR Zonal Tournament-Central Zone from 8-11th November,

2016 at IARI, New Delhi. Dr. Siddharth Gautam, Scientist won the gold medal for the institute in the event of Javelin throw.



Dr. Siddharth Gautam, scientist received gold medal in the Javelin throw event

Constitution day

The Constitution Day was observed at the institute on 26th November, 2016. Dr. V.P. Singh, Director, ICAR-NIHSAD read the Preamble to the Constitution in Hindi and English to the staff of the Institute. After reading the Preamble, he explained the meaning and philosophy of the Preamble to the Constitution of India.



Reading the preamble to the constitution

Agriculture Education Day

The Agriculture Education Day was celebrated on 3rd December, 2016. An essay writing competition was conducted on the topic “Impact of agriculture education on farming practices in India” at the auditorium hall of the institute. Eighteen participants including, administrative staff, students, research

associates, project assistants, young professionals and other staff enthusiastically participated in the competition.

MEETINGS

Institute Management Committee Meeting

The 3rd IMC meeting of the institute was conducted on 3rd December, 2016 under the chairmanship of the Dr. V. P. Singh. On this occasion, Dr. Ashok Kumar, ADG (AH), ICAR, Dr. R. K. Rokde, Director, Animal Husbandry Department, MP; Dr. R. P. Singh, Head, Division of Biological Products, IVRI; Dr. (Mrs.) Madhu Swamy, Professor & Head, Vet. Pathology, NDVSU, Jabalpur were present. Dr. R. K. Rokde specially mentioned the timely help by the institute in detecting the avian influenza in captive birds of Gwalior zoo in recent outbreaks. The issues and problems of the infrastructure of the institute and its maintenance, research priorities in the changing scenario of rapidly advancing emerging zoonotic infections, the new facilities required for meeting the challenges were discussed at length. The provisions made in the SFC (2017-20) for meeting the targets of the institute were also discussed and approved by the IMC. A lecture on IPR issues was also delivered by Dr. R.P. Singh, Principal Scientist and In charge, ZTMU, ICAR-IVRI, Izatnagar (U.P.).



IMC meeting

Half Yearly Institutional Research Committee Meeting

The half yearly institutional research committee meeting was held on 5th December, 2016. All the PIs of

the ongoing institute and externally funded research projects presented their research findings. The progress of the ongoing institute and externally funded research projects was discussed and prospects of new areas were evaluated in the meeting.

Institute Biosafety Committee Meeting

The biannual Institute Biosafety Committee Meeting was held on 22nd December, 2016. DBT Nominee, Dr. Himanshu Kumar Associate Professor, Dept. of Biological Sciences from Indian Institute of Science Education and Research Bhopal discussed all the projects in detail.



IBSC meeting

TRAININGS ORGANIZED

Capacity building program for the researchers of the NER region in their skills and potentials to handle emerging zoonotic pathogens and orient their awareness towards the technological advances in the area of pathogen discovery, three training programmes on '*Biosafety and Biosecurity for handling and identification of emerging zoonotic pathogens*' were organized in three batches, from 18 -22th July, 3- 8th August & 3- 7th October, 2016 for selected researchers of the north-eastern states for Pathogen Discovery program of DBT under DBT-ADMaC, project at ICAR- NIHSAD, Bhopal. A total number of 21 participants from DBT Biotech Hubs under different NER organizations from the states of Assam, Tripura, Nagaland, Arunachal Pradesh, Meghalaya, Mizoram

and Manipur participated. Different aspects of Biological Safety and Security under field and laboratory conditions, strategies for identification of emerging zoonotic pathogens including Real-time PCR test to PCR Array for simultaneous identification of multiple emerging viruses were demonstrated.



Glimpses of training programmes

CAPACITY BUILDING

Trainings attended

Dr. Manoj Kumar, Scientist attended “Basic Epidemiology for Veterinarians” at National Institute of Epidemiology (ICMR), Chennai from 22-26th August, 2016.

Dr. K. Rajukumar, Senior Scientist as Nodal Officer of the Public Authorities/Institutes under DARE/ICAR attended training on RTI–MIS on October 21, 2016 at New Delhi.

RADIO/TELEVISION TALK

Dr. Ashwin Raut, Principal Scientist, participated as panel expert on “Zoonotic Diseases” for Vichar Vimarsh of DD Kisan at Doordarshan, New Delhi.

HONOURS & AWARDS

Dr. H.V. Murugkar, Principal Scientist, received prestigious “*Biosafety Hero's Award*” from International Federation of Biosafety Association for making significant contributions in the field of biosafety and biosecurity.

Dr. K. Rajukumar, Senior Scientist and Dr. Shailesh Kumar Patel (student) received '*Dr. Ram Raksha Kiran Shukla Best M.V.Sc. Thesis Award*' from the Indian Association of Veterinary Pathologists for 2016.

Dr. Naveen Kumar, Scientist, received “*IVS Young Scientist Award*” for the year 2016 at the 5th International Conference of the Indian Virological Society-*VIROCON* 2016, Bengaluru.

VISITS

A team of newly recruited ARS scientists from 104th FOCARS batch, National Academy of Agricultural Research Management, Hyderabad visited the institute on September 6, 2016 as part of their 21 days Field Experience Training at ICAR-Central Institute of Agricultural Engineering, Bhopal. The scientists were given glimpse of biosafety, biosecurity and

research activities of the institute.



Newly recruited ARS scientists from NAARM, Hyderabad

The officials of Animal Husbandry & Fisheries Department, Govt. of Madhya Pradesh visited the institute as trainees of Disaster Management Institute, Govt. of Madhya Pradesh on September 7, 2016 as part of four day training program on Disaster Management.



Disaster management trainees

DISTINGUISHED VISITORS

Dr. Trilochan Mohapatra, Secretary (DARE) and DG, ICAR visited the Biosafely Level -3+ laboratory at ICAR-NIHSAD on October 6, 2016. During his visit, he witnessed the elaborate biosafety infrastructure inside the laboratory and interacted with the scientists about the diagnosis and research work being conducted on exotic and emerging animal diseases. In his address to the staff of NIHSAD, DG, ICAR appre-

ciated the quality research publications of the institute and highlighted the need to find out ways to make interventions in the replication of viral pathogens at the molecular level. Two Hindi leaflets, “बर्ड फ्लू” and “शूकरों का श्वसन एवं प्रजनन सिंड्रोम” were also released by the DG, ICAR along with other dignitaries from ICAR; Dr. S.K. Chaudhari, ADG (Soil Management); Dr. P.K. Agrawal, ADG (National Agricultural Science Fund) and Directors, Dr. K.K. Singh, Director, CIAE; Dr. A.K. Patra, Director, IISS and Dr. N. P. Singh, Director, IIPR. Dr. V.P. Singh, Director, NIHSAD thanked Secretary (DARE) and DG, ICAR for his keen interest in the activities of the institute and urged for his support for the institute in view of the rising threat from zoonotic infections.



Tree plantation at NIHSAD by DG, ICAR



Release of Hindi leaflets by DG, ICAR



Interaction with NIHSAD scientists inside the BSL-3+ facility

Dr. Paul Huntly, Managing Director, Riskren, Indonesia visited the institute to discuss the Biosafety level-2 checklist for the Indian laboratory setup working on infectious diseases.



Discussion on BSL-2 checklists for Indian laboratory setup

Dr. Moirangthem Premjit Singh, Hon'ble Vice-Chancellor, Central Agricultural University, Imphal visited the Institute on 17th August, 2016. He was very impressed with the facility and biosafety infrastructure and appreciated the efforts made by staff towards training programs conducted especially for the North-East people, in the area of biosafety and biosecurity for handling and identification of emerging zoonotic pathogens.



Hon'ble Vice Chancellor being taken around the institute



Discussion on biocontainment infrastructure

Dr. Suresh S. Honnappagol, Animal Husbandry Commissioner visited the institute and looked into the details of working of the biocontainment facility.



Animal husbandry commissioner being taken around biocontainment facility

PERSONALIA

Promotions

Four Senior Technical Assistants (Mr. R.K. Shukla, Mr. Somkumar B., Mr. Asanna Badge and Mr. Mahesh Kumar) promoted to Technical Officer.



Mr. R.K. Shukla



Mr. Somkumar



Mr. Asanna Badge



Mr. Mahesh Kumar

Two Technical Assistants (Mr. R.R. Chouksey and Mr. Malkhan Singh) promoted to Senior Technician.



Mr. R.R. Chouksey



Mr. Malkhan Singh





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