

Director's Desk



Greetings from NIHSAD!

Emergence of new and unknown pathogens is one of the greatest challenges the country's agriculture is facing today. In livestock sector, this problem is threatening its prosperous growth and creating trade barriers for export of livestock /livestock products and is becoming a major factor of economic losses to the sector. The institute constantly keeps vigil on these emerging infections and takes necessary steps for providing the laboratory diagnostic services. The enzootic bovine leukosis (EBL) in cattle and porcine epidemic diarrhoea (PED) in pigs are recent diseases for which

diagnosis is now available at the institute.

In recent years, the government has sought greater emphasis on farmer or field oriented research and generation of farmer-friendly technologies to enable doubling of farmer's income by 2022. In line with the current focus of the government, the institute has given top priority to development of diagnostic technologies that can be applied in the veterinary laboratories with minimal infrastructure and training. At this juncture, we are reaching a milestone for releasing the first technology from this institute, Avian Influenza Antibody Detection Kit which can replace the costly imported kits that are currently being used for surveillance purposes in the regional disease diagnostic laboratories (RDDLs). Another diagnostic kit for porcine reproductive and respiratory syndrome (PRRS) antibody detection in pigs will be soon ready for commercialization after inter-laboratory validation.

The institute has also embarked on an uncharted territory of Antartica where Dr. A.A. Raut, Principal Scientist has gone with an aim of investigation of Antarctic Animal Metavirome as part of 37th Scientific Expedition to Antartica organized by Ministry of Earth Sciences, GoI. The metavirome of the Antartic region will give an insight to the global spread of diseases. This is the first venture of Animal Sciences from India to research on Antarctic fauna. I wish him good luck on this mission.

Besides this, the staff of institute takes pride in celebrating the foundation day of the institute and other festivities like Independence Day and takes active participation in all other activities like Swachhta Abhiyaan, International Yoga Day, Antimicrobial Resistance Awareness Week and Hindi Pakhwara etc. The institute being a forerunner in the bio-containment laboratories has also taken up an important onus of training various researchers and life scientists in the latest nuances of biosafety and biosecurity.

It gives me pleasure to release this biannual newsletter to the readers and wish one and all a fruitful year ahead!

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- Dr. Naveen Kumar, Scientist

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RESEARCH HIGHLIGHTS

Inter-laboratory validation of Indirect ELISA Kit for Avian Influenza antibody detection

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

The Avian Influenza Antibody ELISA kit is an indirect Enzyme Linked Immuno-sorbent Assay for the qualitative detection of antibody against H1-H16 AIV subtypes in chicken serum samples. The indirect ELISA kit has been validated as per the OIE Manual for Antibody Detection Assays. The kit showed high diagnostic performance (diagnostic specificity- 99.0% and diagnostic sensitivity- 99.1%) based on results of 715 chicken serum samples and exhibited high accuracy (Area under the ROC- 0.998/ 1.000). The inter-laboratory validation within the institute (ICAR-NIHSAD, Bhopal) was carried out successfully with a panel of 25 sera (coded R1-R25) at three different labs by different operators. The validation of the kit was also carried out successfully in three institutes' viz. National Institute of Virology, Pune at





Figure 1. Validation of Indirect ELISA Kit at serodiagnosis Lab for AIV (NABL accredited for ISO17025) at National Institute of Virology, Pune.

NABL ISO17025:2005 accredited sero-diagnosis laboratory for Avian Influenza (Figure 1), ICAR-IVRI, Izatnagar at CADRAD and ICAR-NRCE, Hisar at Equine Influenza lab. The ELISA kit is being tested and demonstrated at Regional Disease Diagnostic Labs (RDDLs) of the country. The trainees from NE states were given hands-on-training for using the kit. The kit is now ready to be released and distributed to the RDDLs and other veterinary labs of the country where serosurveillance for AIV is being done using costly imported kits.

Genetic diversity in Avian Influenza viruses (subtypes H5N1 & H9N2) isolated in India during 2016 to 2017

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

Complete genome sequence of nine avian influenza viruses (H5N1: 07 & H9N2: 02) from the States of Gujarat, Madhya Pradesh, Odisha and the Union Territory of Daman & Diu were determined. Phylogenetic analysis of the hemagglutinin (HA) gene revealed that the H5N1 viruses formed two distinct groups (>4.5% divergence between the groups) within the HA clade 2.3.2.1a of H5N1 viruses (Figure 2a). One of the groups comprised of five H5N1 virus isolates from Gujarat, Odisha and Daman & Diu clustered together and are closely related (98.3% homology) to a crow virus (A/crow/India/01CA02/2014) of 2014 indicating epidemiological link between the outbreaks. In the second group, the viruses isolated from two outbreaks in Odisha (Dec. 2016 and Feb. 2017) are closely related (99.9% homology) which are in turn related to a duck virus from Bangladesh (A/duck/Bangladesh/28250/2016) indicating link between the outbreaks. These findings indicate cross border movement of the H5N1 viruses circulating in South Asia, thus highlighting the need for continuous monitoring. Both the H9N2 viruses analyzed are highly divergent (4.8%) from each other and related to a virus isolated in Gujarat (A/chicken/India/11TI01/2015) in 2015. In the HA phylogeny, these three isolates formed a distinct group (Figure 2b) within G1-like lineage of H9N2 viruses isolated in Eurasian region. Other H9N2 viruses isolated during 2003-2015 in South Asia including Bangladesh, India and Pakistan are highly divergent (7.2-11.9%) from this new group. These findings indicate that the H9N2 viruses are evolving in India and hence continuous surveillance is needed.



Figure 2. HA gene phylogeny of (a) H5N1 subtype and, (b) H9N2 subtype avian influenza viruses. Genetic clades/lineages are shown to the right

Evidence of Ovine Herpesvirus -2 infection in multiple species housed in a farm of Tirunelveli, Tamil Nadu

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

Malignant catarrhal fever (MCF) is a clinically noticeable and lethal infection of domestic cattle, buffalo, wild ruminants and occasionally pigs. Ovine Herpesvirus -2, the causative agent of MCF produces subclinical infection in sheep and goat, with lack of characteristics clinical signs of the infection. In the month of October 2017, blood samples of a morbid 50-day old calf (showing typical MCF clinical signs) along with in-contact susceptible animals; cattle (n=1), sheep (n=4) and goat (n=1) housed in a farm of Thirunelvelli, Tamil Nadu were received. All these samples were tested by OIE approved nested PCR test, which detects highly conserved tegument gene of OvHV-2. The samples from three sheep and one each from goat and calf were detected as positive for OvHV-2. To elucidate the species-wise differences and phylogenetic relationship in the glycoprotein B (gB), full length gB (ORF-8, 2.8kb) from the sheep, goat and calf was amplified and sequenced (Figure 3). Of note, gB of Indian isolates clustered separately from reported isolates from other countries, and Indian isolates specific amino acids substitutions were identified. Four amino acid substitutions, not present in any of Indian or other reported isolates were detected in gB of dead calf. Whether these four amino acid substitutions had any role in pathogenicity (by virtue of enhanced spreading among the cells), need further experimental studies.



Figure 3. Agarose gel electrophoresis showing amplification of full length gB (ORF-8, 2.8kb) of Ovine Herpesvirus -2 in multiple species.

Pathogenic characterisation of Indian Porcine Reproductive and Respiratory Syndrome virus in young piglets reveals its highly pathogenic nature

Senthilkumar D, Rajukumar K, M. Kumar, Kalaiyarasu S, S.Gautam, D.D. Kulkarni, and V.P. Singh

The study was aimed to assess the pathogenic potential of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) belonging to genotype 2 that emerged in India in 2013. Nine six-week-old piglets were inoculated intranasally with PRRSV (Ind-297221/2013). Blood and nasal swabs were collected daily up to 7 days post infection (dpi) and on alternate days subsequently. Piglets were necropsied for tissue sample collection. The piglets showed the typical signs of PRRS; high fever, blue ear, weight loss, respiratory distress, diarrhoea and leucopenia between 2 and 8 dpi. Two infected piglets died during the course of study. Shedding of virus in serum and nasal



Figure 4. Quantification of PRRSV RNA copies in serum and nasal secretions of infected piglets

secretion was observed up to 19 and 17 dpi respectively with maximum load between 4 and 7 dpi (Figure 4). Seroconversion started by 6 dpi and the mean PRRSV antibody titre reached up to 640 by 21 dpi. Major lesions in PRRSV infected piglets included moderate to severe interstitial pneumonia lymphoid depletion in tonsils and lymph nodes (cystic), thymic atrophy, reactive hyperplasia followed by lymphoid depletion in spleen. PRRSV's antigen was consistently demonstrated by immunoperoxidase test in lungs, spleen (Figure 5), tonsils and lymph nodes. The findings establish that the Indian PRRSV is highly pathogenic to piglets.



Figure 5. Immunohistochemistry for PRRSV antigen distribution in infected piglets in lungs

Generation of reassortant Equine Influenza Virus through reverse genetics approach

S. Bhatia, R. Sood, N. Kumar

With the increasing significance of plasmid based generation of reassortants for quick vaccine production especially in pandemic/ epidemic situations caused by

novel strains, a reassortant Equine Influenza Virus (EIV) of subtype H3N8 was generated through reverse genetics approach in a collaborative project with ICAR- NRCE, Hisar. The HA and NA genes of MDCK cell-culture adapted EIV strain, A/equine/Jammu-Katra/2008/H3N8 (Clade 2 of Florida sublineage) were amplified and cloned in pHW2000 vector. The recombinant vectors were transformed in *E.coli* Dh5-alpha competent cells and colony PCR positive clones were selected for plasmid isolation. The reassortant viruses were rescued by 6:2



Figure 6. Rescue of reassortant viruses in MDCK-293T co-culture.

reassortant approach carrying HA-pHW2000 and NApHW2000 plasmids on WSN/33 H1N1 backbone. Briefly, MDCK and 293T cells, in the ratio of 1:1 were cocultured in MEM. Plasmid transfection mixture containing 1µg each of pHW-2000 cloned HA and NA of equine/H3N8; and 1µg each of rest of the six pHW-2000 cloned genes of WSN/33 (PB1, PB2, PA, NP, M and NS) was prepared in OptiMEM media. Plasmid transfection mixture was used for transfection of MDCK/293T cells at the density of 80-90% confluence in the presence of Lipofectamine. After 6h incubation post-transfection, transfection media was changed and replaced by DMEM containing 0.3% BSA and antibiotics. After 72 hrs, cells were lysed by freeze-thawing and supernatant after centrifugation was inoculated in 10 days old chicken embryonated eggs through allantoic cavity route. The characteristic CPE was observed for the rescued virus carrying all the genes from WSN/33, while no CPE were observed even after 72 hr post-transfection in the case of rescued EIV (Figure 6). HA titer of 2⁶ for rescued RG-EIV was obtained in the harvested allantoic fluid. It was further confirmed by HI test for the presence of RG-EIV using H3 specific sera.

Influencing factors in overall codon usage bias of Equine Influenza Viruses

N. Kumar, S. Bhatia, R. Sood

Equine influenza viruses (EIVs) of H3N8 subtype are culprits of severe acute respiratory infections in horses, and are still responsible for significant outbreaks worldwide. Adaptability of influenza viruses to a particular host is significantly influenced by their codon usage preference, due to an absolute dependence on the host cellular machinery for their replication. In the present study, we analyzed genome-wide codon usage patterns in 92 EIV strains, including both H3N8 and H7N7 subtypes by computing several codon usage indices and applying multivariate statistical methods. Relative synonymous codon usage (RSCU) analysis disclosed bias of preferred synonymous codons towards A/U-ended codons. The overall codon usage bias in EIVs was slightly lower, and mainly affected by the nucleotide compositional constraints as inferred from the RSCU and effective number of codon (ENc) analysis. The primary selective pressure on the CpG dinucleotides can drive codon usage patterns through mutational biases over time (Figure 7). Interestingly, during the course of evolution, H3N8 subtype gradually reduced GC content over the time



Figure 7. The selective force on the CpG dinucleotide as a function of time for H3N8 viruses.

period of study, which is also reflected in the patterns followed by the positions of individual strain on first two major axes (Figure 8). Our data suggested that codon usage pattern in EIVs is governed by the interplay of mutation pressure, natural selection from its hosts and undefined factors. The H7N7 subtype was found less fit to its host (horse) in comparison to H3N8, by possessing higher codon bias, lower mutation pressure and much less adaptation to tRNA pool of equine cells. To the best of our knowledge, this is the first report describing the codon usage analysis of the complete genomes of EIVs. The outcome of our study is likely to enhance our understanding of factors involved in viral adaptation, evolution, and fitness towards their hosts.



Figure 8. Correspondence analysis of the synonymous codon usage in two subtypes of EIVs.

CELEBRATIONS

Foundation Day Celebration

ICAR-National Institute of High Security Animal Diseases celebrated its 3rd Foundation Day on 8th Aug., 2017. The foundation day function was graced by chief guest Dr. H. Rehman, Regional Representative for South Asia, International Livestock Research Institute (ILRI)







and former DDG (AS), ICAR. Dr. Rehman was accompanied by Dr. Ashok Kumar, ADG (AH), ICAR; Dr. K.K. Singh, Director, Central Institute of Agricultural Engineering (CIAE); Dr. A.K. Patra, Director, Indian Institute of Soil Science (IISS); Dr. R.K. Rokde, Director, Animal Husbandry, Govt. of Madhya Pradesh and Dr. S.C. Dubey, Former Joint Director of ICAR-NIHSAD. Dr. V.P. Singh, Director, ICAR-NIHSAD briefed about the genesis of the institute and the achievements made by the Institute in the area of diagnostics and vaccine development and basic research on exotic and emerging animal diseases. Dr. H. Rehman applauded the efforts of the scientists in doing quality research and publications. Dr. Rehman shared his ideas on prospective vision of the institute in view of the changing scenario of livestock production in Asian countries and emerging threats to the animal husbandry sector. Other dignitaries also shared their views on the critical role of the institute in animal health in light of emerging animal disease threats. A documentary film on NIHSAD and a technical leaflet were released by the dignitaries on this occasion. The day's program of deliberations was followed by a cultural program in the evening with participants from the staff, their family members, students and trainees.

Independence Day Celebration

The 71st Independence Day was celebrated on 15th Aug., 2017 with enthusiasm by the staff of NIHSAD along with their family members. On this occasion, Dr. V.P. Singh hoisted the national flag and congratulated the staff for



making excellent work in the area of research and other institute activities.

Vishwakarma Diwas

Vishwakarma Diwas, a unique Hindu festival is



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celebrated every year at NIHSAD on 17th September with prayers offered to the lord Vishwakarma who is considered the lord of architecture and engineering. The complete engineering unit of NIHSAD organized the event which was also attended by the families of NIHSAD staff.

EVENTS

Swachhta Hi Seva

'Swachhta Hi Seva' programmewas accomplished under the campaign Swachh Bharat Mission from 15th Sept.- 2nd Oct. 2017. Various activities like celebration of Sewa







Diwas (17th Sept.), celebration of Samagra Swachhta Diwas (24th Sept.), Sarwatra Swachhta (25th Sept.), Swachhta of nearby tourist spot, Hathai Kheda Lake (1st Oct.) and Award Ceremony (2nd Oct.) were held with enthusiastic participation by the staff. The plantation of fruit trees such as mango, orange, coconut etc. was also carried out within the campus. Dr. H.V. Murugkar, Principal Scientist of the institute delivered a lecture on "10 steps for Swachh Bharat". Prizes were distributed to five outstanding performers during the Swachhta Hi Seva programme.

Vigilance Awareness Week

The Vigilance Awareness Week was observed at the institute from 30th Oct. to 4th Nov., 2017. This week began with administering of the Integrity Pledge to the staff and research scholars of the institute in the presence of Dr. V.P. Singh. In his statements, he emphasized to indoctrinate honesty, transparency and regularity among all sections of the institute. Banners depicting vigilance awareness in public life were displayed prominently at different locations of the Institute. A debate competition was also organized on the theme 'My Vision- Corruption Free India' in which scientific, technical, administrative and



contractual staff actively participated. The prizes were distributed to the best debaters.

Rashtriya Ekta Diwas

Rashtriya Ekta Diwas was observed on 31st Oct., 2017 in the institute. On the birth anniversary of SardarVallabh bhai Patel, this day was celebrated to foster and reinforce our dedication to preserve and strengthen unity, integrity and security of our nation. The Rashtriya Ekta Diwas pledge to all the employees was administered by the Director. On this occasion, he spoke about the duties and

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responsibilities of institute staff to foster and preserve the integrity and security of our country and reminded the



inherent strength and resilience of our nation to withstand the actual and potential threats to the unity.

Antimicrobial Resistance Awareness Week

A public awareness week dedicated to antimicrobial resistance (AMR) was celebrated at the institute from 13th-19th Nov., 2017. Twenty five AMR awareness tool kits were distributed among the staffs of the Institute as per the request by FAO. A program on 'AMR: Role of Food and Agriculture' was organized on 17th Nov. in the auditorium of the Institute. Dr. V.P. Singh, chaired the session and addressed the challenging issues on AMR. He suggested the audience to judicious use of antibiotics. Dr. D.D. Kulkarni, Principal Scientist, elaborated the different ways of indiscriminate use of antibiotics with the



prescription of experts. A lecture on 'Antimicrobial resistance: control strategies' was delivered by Dr. Fateh Singh, Scientist of the institute.

Constitution Day

The Constitution Day was observed at the institute on 27th Nov., 2017. Dr. V.P. Singh, Director of the Institute read and explained the meaning and philosophy of the Preamble to the Constitution in Hindi and English to the staff of the Institute.

Hindi Pakhwara

Hindi Pakhwara was celebrated at the institute from 14th-28th Sept., 2017. Dr. Fateh Singh, Scientist organized



various competitions during this two week long celebration. The prizes were distributed to winners by Dr. S.C. Dubey, the former Joint director of NIHSAD, who presided over the closing ceremony as chief guest.

Agriculture Education Day



Agriculture education day was celebrated on 5th Dec., 2017. On this occasion, Dr. A.K. Pateriya, Scientist delivered an interactive lecture on the topic 'Importance of Agriculture Education in India'.

ICAR Sports Tournament

A sports team comprising 18 contestants from the institute

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participated in the ICAR Zonal Tournament- Central Zone from 10th-13th Nov. 2017 at ICAR- Central Institute of Agricultural Engineering, Bhopal.

MEETINGS

Institute Technology Management Committee Meeting

The Institute Technology Management Committee (ITMC) meeting was held under the Chairmanship of Director, ICAR-NIHSAD on 13th Oct., 2017 to review the



status of technology, Indirect ELISA kit for detection of avian influenza virus antibodies in chickens. The ITMC recommended to explore the possibility of the IP protection of the technology and its early commercialization.

Half Yearly Institutional Research Committee Meeting

The Half yearly IRC meeting was held on 21st Dec.,2017. This meeting began with welcome address by Dr. Sandeep Bhatia, Incharge, PME cell. The meeting was chaired by Dr. V.P. Singh, Chairman IRC & Director, NIHSAD. In his opening remarks, he stated that the scientists should focus on impact based research and



stressed upon development of diagnostics & vaccines for emerging and exotic diseases and conversion of those in pipeline into deliverable. He advised to concentrate on the time line given in the previous IRC proceedings to give deliverables and publications.

TRAININGS/WORKSHOPS ORGANIZED

Workshop on Laboratory Biosafety and Biosecurity A two-day workshop on Laboratory Biosafety and



Biosecurity under 'Outreach Programme on Zoonotic Diseases' was organized at the institute from 12-13thDec., 2017. Seventeen participants from sixteen different collaborating centers of the project from the research institutes and universities participated in the programme. The workshop was inaugurated by Dr. Ashok Kumar, ADG (Animal Health), ICAR. The workshop covered various topics on laboratory biosafety and biosecurity including biosafety laboratory design, biosafety work practices, pathogen inventory management and biomedical waste management in a microbiology laboratory. The participants also visited inside the biocontainment laboratory where practical demonstrations on validation of biosafety cabinets and use of personal protective equipment were carried out.

DBT-NER-ADMaC Training

A training program titled 'Hands-on Training on Diagnostic Techniques for Emerging Animal Diseases' was organized at the institute under the DBT-NER-Advance Animal Diseases Diagnosis and Management Consortium (ADMaC) programme from 3rd-8thAug.,2017, as a part of the capacity building initiative for North Eastern (NE) partners. The training was attended by 10 participants from five NE States of India, namely, Assam, Manipur, Mizoram Sikkim and Tripura. During the training, the participants were exposed to the general biosafety concepts and practices required to work inside the containment facility and subsequently were given hands-on training for various molecular and serological techniques used in the diagnosis of emerging animal pathogens. Many of the tests developed in-house



at NIHSAD were handled successfully by the participants. The trainees participated enthusiastically in the programme, and enjoyed enhancing their knowledge and experience on conventional and new generation diagnostic techniques for emerging animal pathogens.

Demonstration of Transmission Electron Microscopy

Twenty participants of a ICAR sponsored short course entitled 'Advances in nutrient dynamics for improving nutrient and water use efficiency of crops' organized by



ICAR-Indian Institute of Soil Science, Bhopal from 5th-14th Sept., 2017 visited EM unit of ICAR-NIHSAD. Dr. K. Rajukumar and Dr. Manoj Kumar demonstrated applications of transmission electron microscopy for ultra-structural studies to all the participants on 11th Sept. 2017.

CAPACITY BUILDING

Meetings/Trainings Attended

Dr. V.P. Singh, Director attended WHO Regional Consultation workshop on Networking and Coordination of Health Partners for Emergency Response at Bangkok from 28th-29th Nov., 2017 and Dr. Singh also participated in FAO workshop on Establishment of a South Asia One Health Disease Surveillance Network at Bangkok from 11th-13th Dec, 2017.

Dr. C. Tosh, Principal Scientist attended a workshop on Avian Influenza: Monitoring, Surveillance and Biosecurity; as Subject Matter Expert organized jointly by USDA/APHIS and DADF/ Ministry of Agriculture and Farmers Welfare, Government of India at Chennai, from 4th-8th Sep., 2017.

Dr. Manoj Kumar, Scientist attended a training programme on "Training on biosafety and risk assessment" organized by Integrated Quality Laboratory

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Services in Collaboration with CDC Global Disease Detection Centre at Delhi from 9^{th} -13th Oct., 2017.

Dr. S.B. Sudhakar, Scientist attended a training programme on "Application of Bioinformatics in Agricultural Research and Education" at NAARM, Hyderabad from 14th-23th Sept, 2017.

Dr. Naveen Kumar, Scientist attended a training programme in the area of Bioinformatics, organized at Indian Institute of Technology, Delhi from 10th-20th July, 2017.

DISTINGUISHED VISITORS

Shri Chhabilendra Roul, Secretary, ICAR & Additional Secretary, DARE visited the ICAR-NIHSAD, Bhopal on 18thAug., 2017. During his visit, he saw the elaborate biosafety infrastructure inside the bio-containment 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 scientists, he appreciated the work being carried out in the

institute and highlighted the need for high end research and technology to make the institute a world leader in the area of its work. Dr. V.P. Singh, Director, ICAR-NIHSAD thanked Secretary, ICAR for his keen interest in the activities of the institute and urged for his support for the



institute's need of a BSL-4 laboratory in view of the rising threat from zoonotic infections.

Shri S.K. Singh, Additional Secretary & Financial Advisor (DARE/ICAR) visited ICAR-NIHSAD on 30th July, 2017.



PERSONALIA

Promotions



Dr. Rajukumar, K., Senior Scientist (Veterinary Pathology) at ICAR-National Institute of High Security Animal Diseases promoted to Principal Scientist w.e.f. 26thNov., 2016.



Mr. K.S. Tantuway, Upper Division Clerk at ICAR-National Institute of High Security Animal Diseases promoted to Assistant w.e.f. 17thJuly, 2017.



Dr. Richa Sood, Senior Scientist (Veterinary Medicine) at ICAR-National Institute of High Security Animal Diseases promoted to Principal Scientist w.e.f. 1st Dec., 2016.

Retirement

Mr. R.K. Kaushik, Chief Technical Officer (Instrument) retired from his service at ICAR-NIHSAD w.e.f. 31st July, 2017.



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