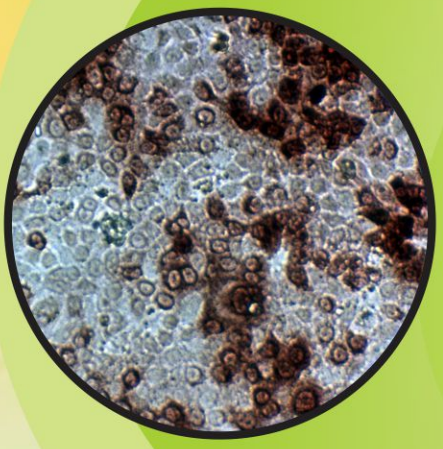
Annual Report
2015-16



ICAR-National Institute of High Security Animal Diseases
Anand Nagar, Bhopal - 462 022

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Anand Nagar, Bhopal

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Contents

Director's Foreword

I feel immense pleasure in presenting the Annual Report (2015-16) of National Institute of High Security Animal Diseases (NIHSAD). Within a short period of one and a half years since its establishment as National Institute on 8th Aug., 2014, NIHSAD has crossed important milestones and also prepared itself for delivery in diverse areas of country's animal health system. Establishment and strengthening of Prioritization, Monitoring and Evaluation (PME) cell, Institute Technology Management Unit (ITMU), Agricultural Knowledge Management Unit (AKMU) and various other functional units that are required at the institute level, have been at the focus of attention. However, the diagnostic services for the samples received from field as well as from quarantine were carried out earnestly with vigor.

The current research agenda of the institute is robust and covers wide areas of research on exotic and emerging animal diseases viz. ruminant diseases, swine diseases, avian diseases and multiple species diseases (mostly zoonotic). The institute has continued its research on various aspects such as host-pathogen interactions, molecular epidemiology, pathogenesis and development of diagnostics and vaccines. During the last year, the institute has completed an important task of preparedness on vaccine front for highly pathogenic avian influenza H5N1 virus. In a limited survey in NE states, first evidence of BVDV in free ranging and semi-domesticated mithuns (Bos frontalis) was recorded.

Apart from research, the institute is involved in human resource development in the field of biosafety and laboratory diagnosis of exotic and emerging animal diseases. Further, as an institution of international repute, the institute has strong linkages with OIE, OFFLU and WHO and provided diagnostic services to Bhutan and participated in the process of global vaccine strain selection for avian influenza. Further, the institute also conducted regular activities under “Swatch Bharat Abhiyan” to emphasize on cleanliness within and outside the premises and various social programs with the active and enthusiastic participation from the NIHSAD family. I hope the information presented in this annual report is useful to all readers especially to the stakeholders such as farmers, diagnosticians, field veterinarians, policy makers at the state and union government, NGOs and scientists in the NARS. I request all the readers to send their suggestions, if they have any, to improve the presentation of information in the annual report.

June, 2016

(V. P. Singh)

Mandate

To conduct research on basic & applied areas related to exotic, emerging & re-emerging animal diseases of national importance including-

- Basic mechanisms for disease occurrence, pathogen characterization, their transmissibility under various ecological factors, host-pathogen interactions, development of diagnostics and vaccines, fundamental aspects arising out of work on exotic/emerging animal pathogens.
- Updating on biorisk management and to train the manpower in the areas of biosafety, biosecurity and biocontainment for handling high risk pathogens.

Vision Statement

"Mitigating risks of known and unknown emerging infectious diseases in animals including zoonotic infections at human-animal interface through forecast, early detection of pathogens, emergency preparedness with diagnostics and vaccines while keeping vigil on changing host pathogen and environment interactions and creating understanding of potential bio-risks and disease threats among stakeholders."

Mission

Reducing threats of emerging and new pathogens for sustainable animal husbandry sector and safeguarding public health.

Institute Profile

National Institute of High Security Animal Diseases (NIHSAD) of Indian Council of Agricultural Research (ICAR) is a premier institute of India for research on exotic and emerging pathogens of animals. NIHSAD came into existence on 8th Aug., 2014 as an independent institute under ICAR from its original status as High Security Animal Disease Laboratory (HSADL), a regional station of Indian Veterinary Research Institute (IVRI), Izatnagar. The institute has contributed significantly by detecting many animal diseases of exotic origin and preventing them from entering our country. The fully functional bio-containment laboratory at NIHSAD was dedicated to the Nation on June 23, 2000. Since then the lab has been functional round the clock with unmatched safety record. The committed lot of scientists and staff of NIHSAD, with their single minded devotion to protect the country's livestock and poultry health, have played an important role in checking the entry of many exotic animal diseases into India. Considering the challenging need of stringent biological safety measures and growing threat of novel pathogens in the era of globalized animal trade and rapid climate change, the institute has important role to play in providing services to the nation in animal health through its mandate and objectives.

The institute has been a forerunner in providing the diagnostic services for emerging diseases to all the animal quarantine centres as well as disease outbreak areas in various parts of the country. The research programs and activities of the institute are multifarious and include rapid disease diagnosis of established pathogens, development of diagnostics and vaccines against emerging pathogens, host-pathogen interactions studies, risk analysis, molecular epidemiology and environmental studies for pathogen survival etc. The institute stands as the epitome of prompt diagnosis and is like a lighthouse for tackling the emerging and exotic diseases including the high risk pathogens entering the country.

The institute was recognised as OIE Reference Lab for Avian Influenza in May 2009 by OIE in view of its achievement towards diagnosis and control of bird flu using its own expertise, facilities and resources. This recognition could be achieved only after meticulous and unrelenting contributions of this institute's scientists and the timely and encouraging support of ICAR and the Department of Animal Husbandry, Dairying and Fishery (DADF), Ministry of Agriculture GOI. With this status NIHSAD is having upward linkages with other OIE recognized labs in frontier areas of bird flu research and participating with OFFLU (OIE-FAO common platform to handle avian influenza on global basis). Simultaneously, this institute also has downward linkages with neighbouring countries like Bangladesh, Myanmar, Afghanistan, Pakistan and Nepal and extends support for bird flu diagnosis.

Expenditure Statement 2015-16

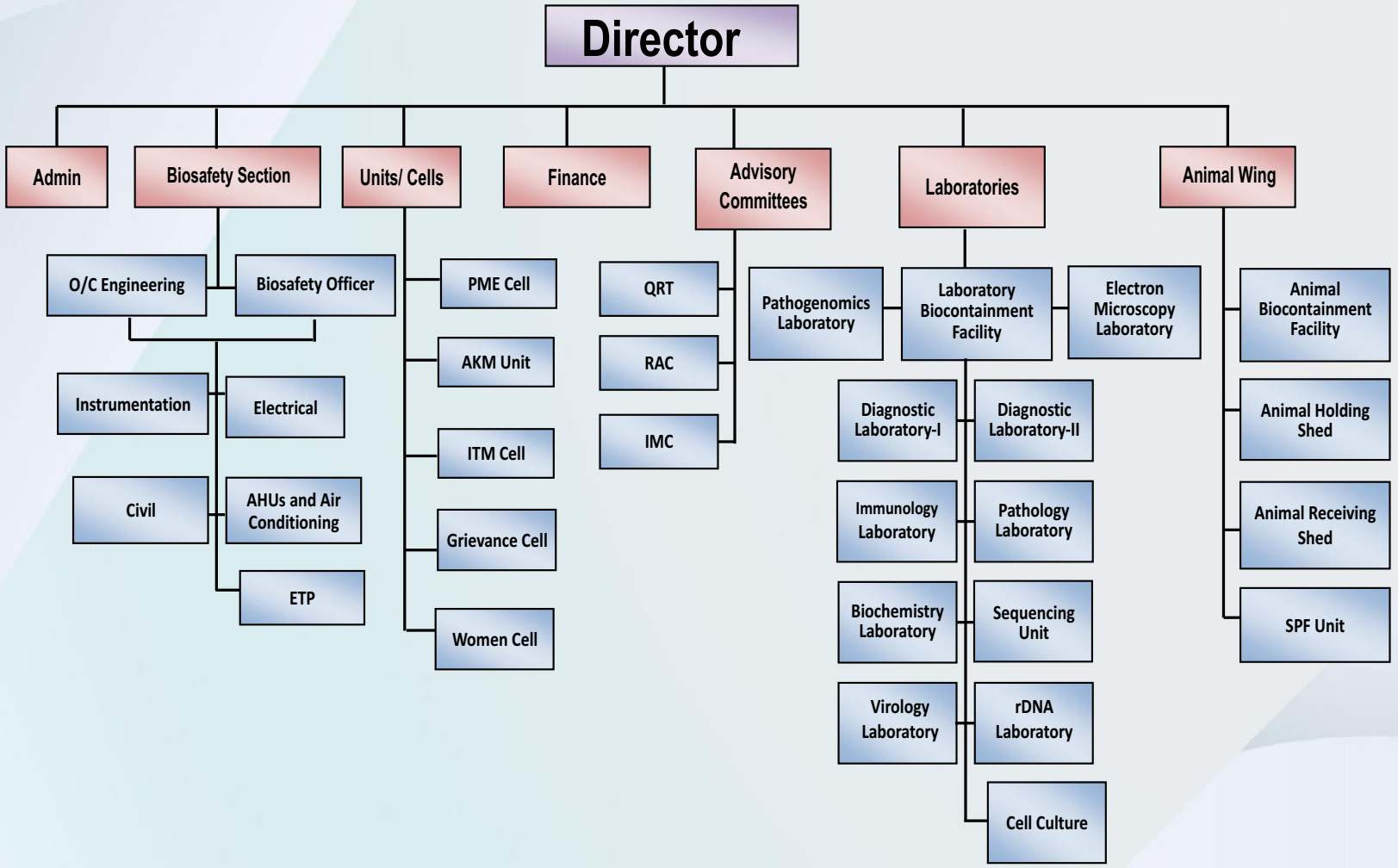
Financial statement showing receipts and expenditure of Institute (Rs. in lakhs)

Head	Plan		Non - Plan	
	2015 - 16	Expenditure	2015 - 16	Expenditure
Grants for creation of Capita Assetsl				
S.No. Works				
1. Work (office building)	25.50	25.50	0.00	0.00
2. Equipments	44.50	39.34	1.75	1.65
3. Information Technology	0.00	0.00	0.00	0.00
4. Library Books and Journals	0.00	0.00	0.00	0.00
5. Vechicle & Vessels	0.00	0.00	8.00	7.67
6. Livestock	0.00	0.00	0.00	0.00
7. Furniture & Fixtures	1.00	0.93	0.25	0.15
8. Others	0.00	0.00	0.00	0.00
Total Capital (Grants for creation of capital Assets)	**71.00**	**65.77**	**10.00**	**9.47**
Grants in Aid-Salaries(Revenue)				
1. Establishment Expenses	0.00	0.00	0.00	0.00
A. Salaries	0.00	0.00	0.00	0.00
i. Establishment Charges	0.00	0.00	492.25	492.24
ii. Wages	0.00	0.00	0.00	0.00
iii. Overtime Allowance	0.00	0.00	0.00	0.00
Total Esttablishment Expenses (Grants in Aid-Salaries)	**0.00**	**0.00**	**492.25**	**492.24**
Grants in Aid-General (Revenue)				
1. Pension & Other Retirement Benefits	0.00	0.00	**64.50**	**64.32**
2. Travelling Allowances				
A. Domestic TA/Tranfer TA	6.00	5.98	3.50	3.49
B. Foreign TA	0.00	0.00	0.00	0.00
Total Traveling Allowances	**6.00**	**5.98**	**3.50**	**3.49**
Research and Operational Expenses				
A. Research Expenses	80.50	80.22	50.00	49.73
B. Operational Expenses	0.00	0.00	13.00	13.00
Total Research and Operational Expenses	**80.50**	**80.22**	**63.00**	**62.73**
Administrative Expenses				
A. Infrastructure	210.00	209.87	107.00	106.99
B. Communication	0.00	0.00	3.00	2.94
C. Repairs/Maintenance				
i. Equipments, Vehicles & Vessels	49.25	49.02	48.00	47.81
ii. Office building	0.00	0.00	36.34	36.25
iii. Residential building	0.00	0.00	2.75	2.70
iv. Minor Works	0.00	0.00	3.00	2.75
D. Others (excluding TA)	103.25	103.16	20.00	20.00
Total Administrative Expenses	**362.50**	**362.05**	**220.09**	**219.44**
Miscellaneous Expenses				
A. HRD	1.00	0.95	0.00	0.00
D. Guest House – Maintenance	0.00	0.00	5.25	5.24
E. Other Miscellaneous	0.00	0.00	0.50	0.50
Total Miscellaneous Expenses	**1.00**	**0.95**	**5.75**	**5.74**
Total Grants in Aid-General	**450.00**	**449.20**	**849.09**	**847.96**
Grand Total : Capital + Revenue	**521.00**	**514.97**	**859.09**	**857.43**
NEH Expenditure	5.00	4.63	0.00	0.00
Grand Total : Plan + NEH	**526.00**	**519.60**	**0.00**	**0.00**
B. Loans & Advances				
Grand Total : Non-Plan + Loans & Advances			**864.09**	**858.22**

Revenue Generation (2015-16)

S.No.	HEAD OF ACCOUNT	AMOUNT (Rs. In Lakhs)
1.	Sale of farm produce	0.07
2.	Licence fee	1.69
3.	Interest earned on loans & advances	1.05
4.	Analytical and testing fee	108.18
5.	Interest earned on short term deposits	81.96
6.	Recoveries of loans and advances	5.40
7.	Miscellaneous receipts	18.88
	Total	**217.23**

Organizational Setup of NIHSAD



EXECUTIVE SUMMARY

Research Accomplishments

Characterization of Pathogens

- Thirteen Avian Influenza H5N1 viruses were isolated from the different outbreaks in India during 2015-16 viz. Telangana (1-chicken), Manipur (1-chicken), Tripura (1-duck and 1-chicken), Kerala (4-duck and 1-Turkey), Chandigarh (1-duck) and Uttar Pradesh (1-duck and 2-crow) were sequenced and characterized on molecular basis. All the H5N1 viruses were found to be highly pathogenic and sensitive to neuraminidase inhibitors as evidenced from the absence of known molecular markers of resistance.
- The phylogenetic analysis showed that H5N1 viruses isolated till early 2014 and that isolated in Tripura (2016) grouped with clade 2.3.2.1a, whereas the H5N1 viruses isolated from Kerala, Chandigarh and Uttar Pradesh grouped with a new clade 2.3.2.1c.
- A total of 15 H9N2 viruses (2 each from Uttrakhand, Gujarat, Kerala, Odisha and Rajasthan, One each from Uttar Pradesh and Madhya Pradesh and 3 from Maharashtra) have been sequenced and characterized on molecular basis. The phylogenetic analysis indicated that all the H9N2 viruses are reassortants like the previously reported Indian viruses and belonged to G1 lineage.
- One H4N6 virus from Jharkhand, four H3N8 and one H6N2 viruses from Kerala, were sequenced and characterized on molecular basis. The H6N2 virus from water fowl was found to be reassortant by phylogenetic analysis.
- The intravenous pathogenicity index of three H5N1 viruses (one each from Chattisgarh, Telangana and Kerala) ranged from 2.925 to 2.97 indicating that they are highly pathogenic to poultry. The intravenous pathogenicity index of one H9N2 virus from Uttarakhand was 0.18 and that of H3N8 virus from Kerala and H4N6 virus from Jharkhand was 0.00 indicating that they are low pathogenic to poultry.
- Border disease virus (BDV), the principal causative agent of border disease in sheep and goats was identified and isolated from Indian sheep for the first time. Genetic and antigenic analysis of eight Indian BDV isolates revealed that they belong to BDV-3 genotype and indicated probable introduction through sheep trade from other countries. Real time RT-PCR was found to be more useful than commercial BVDV Ag-ELISA for laboratory diagnosis of BDV.
- Bovine viral Diarrhoea virus -3 (Hobi-like virus), an emerging bovine pestivirus was identified and isolated for the first time in naturally infected Indian cattle in organized dairies thereby establishing the fact that BVD in India is caused by BVDV-1, BVDV-2 and BVDV-3. Molecular characterization identified the circulation of two lineages of BVDV-3 viruses in India that are novel and divergent to those circulating in other countries.
- Genetic analysis of the complete genome of the two Porcine Reproductive and

Respiratory Syndrome virus (PRRSV) isolates from Mizoram (Ind-297221 and Ind-299830) showed that the two are closely related and shared more than 99% sequence homology and grouped phylogenetically with highly pathogenic PRRSV genotype 2 (2010) isolates from China.

Diagnostic and Vaccine development

- Recombinant nucleoprotein was expressed and purified in soluble form for specific detection of avian influenza by indirect ELISA. Monoclonal antibodies against HA H5 protein were characterized and purified for developing H5 specific rapid antigen detection test. Synthetic peptides from H5 specific amino acid sequences were designed and synthesized for use in peptide based H5 specific antibody detection test. A quantitative multiplex real time RT-PCR for the simultaneous typing and sub typing of Influenza type A viruses was developed.
- An rgH5N2 vaccine for clade 2.2 was successfully tested in commercial chickens. The vaccine induced protective HI titres in the vaccinated chickens within 14-21 days and protected chickens from highly lethal dose (10^9 EID_{50}) of HPAI H5N1 field virus isolate. The clade 2.2 vaccine was tested for its stability for 6 months. An rgH5N2 vaccine for clade 2.3.2.1 was tested in SPF chickens for its potency.
- Using reverse genetics, a reassortant H3N8 virus was generated as potential vaccine candidate using HA gene and NA gene isolated from field isolate of equine influenza virus EIV [A/equine/Jammu-Katra/2008/H3N8].
- Immunoperoxidase monolayer assay (IPMA) was optimized on MARC-145 cells for PRRSV antibody detection in porcine serum samples. The assay was found to be more sensitive and specific for detection of PRRSV genotype 2 antibodies. Indian PRRSV grown in porcine pulmonary alveolar macrophages (PAM) was inactivated using BEI and tested for absence of residual infectivity for use as an inactivated vaccine.
- Recombinant nucleoprotein (rNp) of Crimean congo haemorrhagic fever (CCHFV), Schmallenberg virus (SBV), Nairobi Sheep Disease virus (NSDV) and Hantaan virus were expressed in prokaryotic system for use in immunodiagonstic development.

Disease monitoring and Epidemiology

- Testing of 277 domestic duck serum samples from 3 districts (Alappuzha, Kottayam, Pathanamthitta) in Kerala by ELISA and virus neutralization test demonstrated serological evidence of West Nile virus infection in 46 ducks (16.6%) indicating circulation of WNV in the sampled region and its zoonotic implication. All the 1489 fecal/oral samples from wild birds in Kerala, Gujarat, Madhya Pradesh and Manipur were found negative for WNV by RT-PCR.
- Serological evidence of BVDV infection in free ranging and semi-domesticated mithuns (Bos frontalis) was observed for the first time, with a seroprevalence rate of 16.3% in Nagaland, Arunachal Pradesh and Mizoram.
- In samples recieved from Rajasthan two serum out of 31 samples were positive for CCHFV antibodies by indirect ELISA (NIV, Pune) and all three tick pools were found negative for CCHFV genome by Real Time RT-PCR.

- Out of 271 serum samples received from Mizoram, Assam and Sikkim 103 (38%) were positive for PRRSV antibodies by indirect ELISA. Serum samples (129) and tissue samples (4) processed for virus isolation in PAM cultures were negative for PRRSV.
- Accumulation of H9N2 avian influenza virus in shrimps was studied in view of the zoonotic potential of the virus. Bamboo shrimps (*Atyopsis*) were exposed to 10 EID_{50}/ml of H9N2 avian influenza virus by introducing these shrimps in the virus spiked water in aquarium tanks. The water without shrimps retained infectivity up to 8 days after decreasing gradually. Bamboo shrimps retained infectivity up to the 11 day of the experiment. The study revealed that the presence of bamboo shrimps aids in maintaining avian influenza virus in water.

Host-pathogen Interactions

- Pathogenicity of H9N2 avian influenza virus in mice was studied, wherein significant reduction in body weight in the infected mice indicated deleterious effect of the infection till seven days post infection (dpi). Tissue tropism of virus was mainly confined to lungs and trachea and it caused significant pathognomonic lesions in lungs at 3 dpi. Pathogenicity of H9N2 viruses to mammals in spite of its failure to adapt to the mammalian system during initial passages indicates zoonotic potential of the virus.
- Crow derived H5N1 virus produced systemic disease leading to death in crows but excreted minute level of virus to initiate productive infection in chickens. Genetic analysis has confirmed high pathogenicity of the virus to chickens and indicated that the crow virus can initiate fatal infection in chickens without adaptive changes.
- An initial unbiased measure of the viral diversity in the enteric tract of duck, which is one of the principal natural reservoirs for influenza virus, was generated from the virome purified from the cloacal swabs of ducks. Metagenomic analysis of viruses in duck gut using second-generation sequencing revealed the presence of previously unidentified viral species infecting vertebrates, insects, plants or bacteria and expands the knowledge of spectrum of viruses harbored by ducks in the gut.
- Experimental infection of piglets with Indian PRRS virus showed clinical effects between 2 and 8 days post infection

Diagnostic Services

- ***Avian influenza***- During the year 2015-16, out of 38,882 morbid/swab/fecal samples tested, 16 samples from three States (10 from Telangana, 5 from Tripura and 01 from Manipur) have tested positive for H5N1. Further, 28 H9N2 (7 from Uttarakhand, and 21 from Gujarat) viruses were isolated. A total of 9,465 random serum samples had been tested, five (from chicken) and 288 (285 from chicken and 3 from ducks) serum samples were positive for antibodies to avian influenza virus subtype H5 and H9 respectively. On request from Bhutan, AIV diagnostic services were provided. Out of the five states from which post outbreak surveillance plan (POSP) samples have been received, 103 sera were found positive for H9N2 AIV antibodies (11 from Kerala, 2 from Manipur and 90 from Telangana) and five chicken sera samples from Kerala State were found positive for H5N1 AIV antibodies.

- ***Swine influenza***- Porcine serum samples from Gorakhpur (68) and Bhopal (18) were tested negative for swine influenza (H1N1) antibodies. Porcine swab samples from Gorakhpur (68) and Bhopal (02) were tested negative for swine influenza (H1N1) virus.
- ***Bovine viral diarrhea***- A total of 337 diagnostic samples from cattle, buffaloes, deer, elephant and imported biologicals (bull semen, embryo, FBS, ABS etc.) during 2015-16 were tested for BVDV following the OIE prescribed diagnostic tests. Testing of 123 diagnostic specimens within India revealed that semen from 3 cattle bulls in Tamil Nadu were positive for BVDV-2 by virus isolation and Real-time RT-PCR, while BVDV neutralizing antibodies were detected in 25 cattle of Gujarat and Tamil Nadu. All the 50 samples from imported biologicals tested negative for BVDV. Laboratory testing for BVD was carried out for blood and serum samples from 82 imported Danish Holstein and Jersey purebred bulls by NDDB and stationed at AQCS, Chennai and Kolkata in Dec. 2015 by real-time RT-PCR, BVDV antigen ELISA and antibody ELISA. All the imported animals were found free of BVDV infection.
- ***Malignant catarrhal fever***- A total of 199 samples including imported cattle, buffalo, elephant, black buck, deer and sheep from AQCS, Bengaluru, Gujarat and Andhra Pradesh were screened for MCF. Fifty samples including that of cattle from Gujarat, and Bengaluru, buffalo from Andhra were positive by OIE approved test for genomic detection of Ovine Herpesvirus 2, PCR and nested PCR.
- ***Porcine reproductive and respiratory syndrome***- A total of 102 imported porcine meat samples received from AQCS screened for PRRS were found to be negative for PRRSV by RT-PCR and/or virus isolation.
- ***Cremian congo haemorrhagic fever***- A total of 35 serum samples were positive for CCHFV antibodies by iELISA (NIV, Pune) and 05 tick pools were positive for CCHFV genome by Real Time RT-PCR out of 347 post CCHF outbreak samples (serum: 171/blood: 131/ticks pools: 45) of suspected animals received from Gujarat for the detection of CCHFV.

A number of avian diseases have emerged in last few decades and affected poultry and wild avian species, threatening the fast growth of poultry industry and in few cases becoming public health threat in case of infections with zoonotic potential such as avian influenza. However, the overwhelming threat to the poultry industry from avian influenza viruses in comparison to other emerging diseases and endemic diseases of avian hosts underscores the importance of this disease. Emergences of avian influenza (highly pathogenic as well as low pathogenic types) have changed the priorities of the country for control of avian diseases.

Avian influenza (AI), caused by type A influenza viruses (family *Orthomyxoviridae*), imposes a large burden on both animal and human health globally. Influenza A viruses are categorized into different subtypes based on genetic and antigenic differences on their surface glycoproteins, the haemagglutinin (HA) and neuraminidase (NA). AI viruses are unique in many ways including the diversity of host species they infect, capacity to evolve and adaptation following interspecies transmission. Highly virulent pathotypes (H5 & H7) of AIV have devastating effects with threat to human life due to their zoonotic nature. AIV, particularly the Asian-lineage of highly pathogenic AI (HPAI) H5N1 viruses since late 2003 is continuing its spread by infecting domestic poultry, other birds and occasional transmission to humans thereby revealing its pandemic potential. The virus has spread from Asia to Europe and Africa, and recently to the North America leading to death or culling of millions of birds to contain this dreaded transboundary disease.

Avian influenza

Live bird market surveillance

A total of 1057 samples (86 tissues and 971 swabs) were collected from live bird markets of Assam, Manipur, Meghalaya, Mizoram, Kerala, Madhya Pradesh, Chattisgarh and Maharashtra. The samples were tested by RT-qPCR and virus isolation in embryonated specific pathogen free chicken eggs for detection of avian influenza. A total of 31 samples were found positive for avian influenza by RT-qPCR. Avian influenza viruses could be isolated from all the 31 samples and three subtypes of avian influenza viruses viz. H9N2 (26), H3N8 (4) and H6N2 (1) have been identified. In addition, five Newcastle disease viruses were also isolated. The details of the samples are given in Table 1.

Pathogenic Characterization of avian influenza viruses

The intravenous pathogenicity index of three H5N1 viruses (one each from Narayanpur, Chattisgarh, Hyderabad, Telangana and Alapuzha, Kerala) ranged from 2.925 to 2.97 indicating that they are highly pathogenic to poultry. The intravenous pathogenicity index of one H9N2 virus from Uttarakhand was 0.18 and that of H3N8 virus from Kerala and H4N6 virus from Jharkhand was 0.00 indicating that they are low pathogenic to poultry.

Table 1: Samples (tissues & swabs) screened for avian influenza virus infection from live bird markets.

State	Chicken	Duck	Total	Subtypes isolated
Assam	50	60	110	H3N8 (04-D) H6N2 (01-D) H9N2 (02-D, 01-C)
Manipur	22	104	126	H9N2 (01-C)
Mizoram	16	0	16	Nil
Kerala	81	0	81	H9N2 (04-C) NDV (05-C)
Meghalaya	84	0	84	H9N2 (02-C)
Madhya Pradesh	82	0	82	Nil
Chhattisgarh	28	0	28	Nil
Maharashtra	530	0	530	H9N2 (16-C)
Total	**893**	**164**	**1057**	**36**

- **Figures in parenthesis represent positive samples and species**
- **C - Chicken D - Duck**

Pathogenicity of H9N2 avian influenza virus in mice

To study the pathogenicity and adaptation of H9N2 isolate (A/chicken/india/100260/2013) in mice and to study the presence of molecular signatures indicative of potential adaptation of viruses to mammalian host species, groups of 6-8 week old BALB/c mice were inoculated with H9N2 virus with an EID_{50} of $10^{6.2}$ and three serial lung to lung passages were performed to study the adaptability of the virus. Pathogenic effect of the virus in mice was determined through reisolation of the virus in different tissues and reduction in body weight which is significant indicator of the infection. Body weights of the mice in first adaptation passage showed significant decline indicating the deleterious effect of the infection till seven dpi and thereafter started gaining weight upto 14 days d.p.i., indicating that the virus had started clearing from the system. The mean body weight loss in the mice sacrificed on three days post infection was shown to be upto 11.50%. In the mice sacrificed on seven days post infection, the mean body weight loss between zero day and 3dpi and between 3dpi and 7dpi was11.28% and 19.09%, respectively.

Significant virus titre was noticed in first passage by egg inoculation in lungs at 3dpi (n=4/6), 7dpi (n=4/6) and 14dpi (n=4/6) and in trachea at 3dpi (n=1/6), 7dpi (n=3/6) and 14dpi (n=0/6) with percent egg infectivity ranging between 60-100%. Tissue tropism of virus was mainly confined to lungs and trachea.

Serology was confirmed by HI titre of 1:128 and 1:256 at 7 and 14dpi of first passage respectively, reiterating the facts reminiscent to seroprevalence studies on humans regarding H9N2 both in our country and abroad. The virus caused significant microscopic lesions in lungs at 3 dpi without prior adaptation detected by histopathology and immunoperoxidase test. Microscopic lesions observed were capillary congestion, hemorrhages, thickened alveolar wall with mild alveolar collapse and infiltration of inflammatory cells (Fig. 1 and 2). The same were processed for detection of presence of Influenza A viral antigen by Immunoperoxidase test which were positive affirmatively. This acute lung injury at three dpi is a significant outcome of this study highlighting the capability of H9N2 avian influenza virus to cause mild to severe lung injury without prior adaptation in murine model implicating its zoonotic perspective. (Fig. 3 and 4).

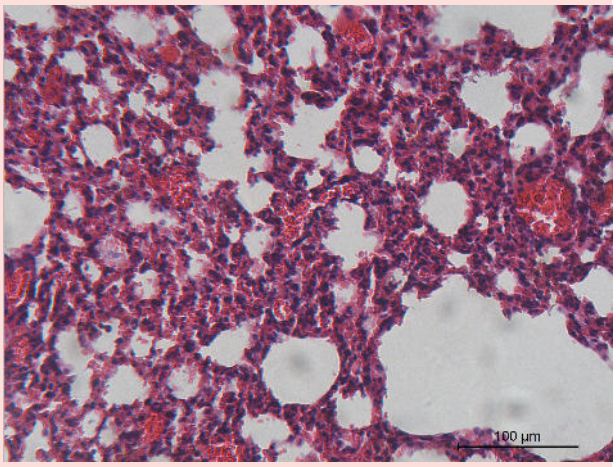


Fig.1: Lung 3 DPI (H9N2): Capillary congestion hemorrhages and thickened alveolar wall with inflammatory cells.

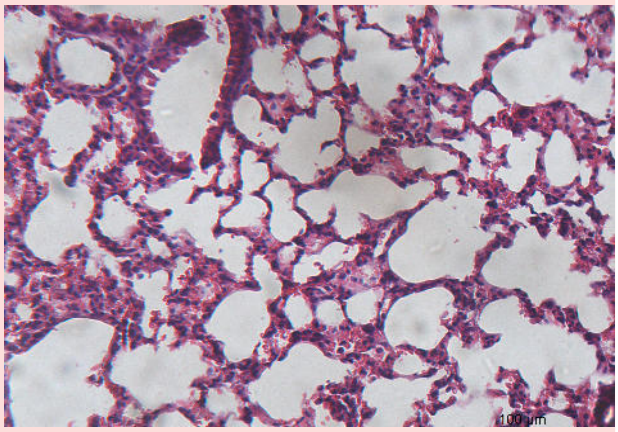


Fig. 2: Control Lung: Normal alveoli and alveolar wall.

In contrast, there was a constant increase in the body weight of the mice which were inoculated with the infected lungs of the first passage mice and for the second passage and also in the third passage. In addition, no positive virus detection either through RT-qPCR and egg inoculation was observed in the tissues obtained from the sacrificed mice subjected to the second and third passages. Genetic analysis of the virus was carried out to find molecular signatures that confer mammalian adaptation i.e. glutamine to leucine (Q226L) substitution in HA gene; glutamic acid to lysine (E627K) substitution in PB2 gene. The sequencing of HA and PB2 genes revealed no molecular changes related to mammalian adaptation.

The results in this study provide helpful insights into the pathogenic potential of H9N2 viruses to mammals that deserves further attentions. This study reemphasises the need of carrying out transmission studies and seroepidemiological studies amidst human-animal-avian interface in India in order to envisage the public health risk of avian influenza H9N2 viruses.

Accumulation of avian influenza virus in shrimps

Fishes and other aquatic fauna in water bodies can easily be exposed to the avian influenza virus and aquatic animals play an important epidemiological role in spread and maintenance of avian influenza virus in the aquatic habitat. Avian influenza viruses have been reported to survive in the water, soil, lake sediments and fomites for variable durations ranging from a few hours to years. Water bodies inhabited by ducks and other wild waterfowl

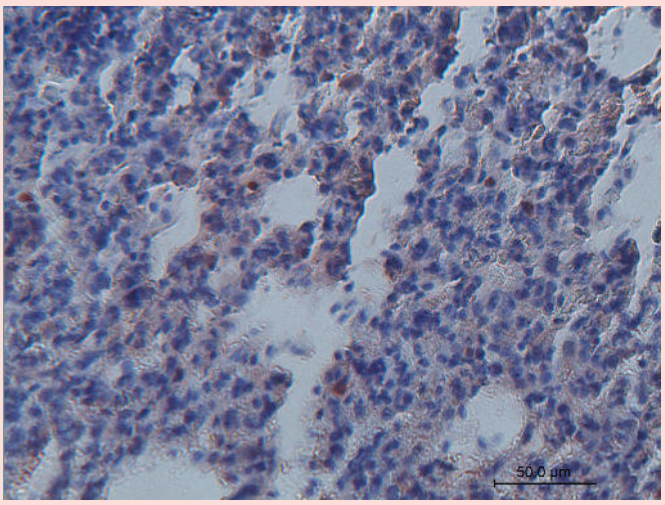


Fig. 3: Immunohistochemistry: Lung 3 DPI (H9N2): Viral antigen in alveolar epithelial cells.

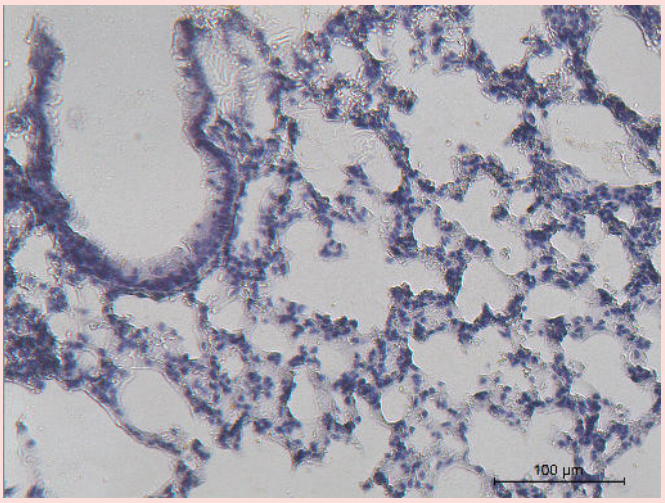


Fig. 4: Immunohistochemistry: Control Lung: Negative for viral antigen.

and the water bodies that are stopovers and wintering stations of migratory birds can harbor avian influenza virus. Fishes and other aquatic fauna of these water bodies can easily be exposed to the avian influenza virus and aquatic animals play an important epidemiological role in spread and maintenance of avian influenza virus in the environment. India is considered as one of the world leaders in shrimp production and exports. There are more than 50 varieties of shrimps in the country and shrimps are nowadays extensively being used with pigs and ducks in integrated farming. The objective of present work was to study the accumulation of H9N2 avian influenza virus in shrimps in view of the zoonotic potential of the virus. Bamboo shrimps (*Atyopsis)* were exposed to 10^6 EID_{50}/ml of Low Pathogenic Avian Influenza virus - A/chicken/India/50438/2007 by introducing these shrimps in the virus spiked water in aquarium tanks. The shrimps and the water samples were collected at 24 hours intervals. Percent infectivity of the virus in these samples was estimated by inoculating these samples in 9-11 day old embryonated eggs and determining the infectivity of the samples in infecting the egg embryos as determined by haemagglutination test carried out with the amnio-allantoic fluid of these eggs.

The percent infectivity was calculated 24 hours after spiking the water with avian influenza virus to be 100% in the shrimp samples and water samples both with shrimps and without shrimps. The control water without shrimps retained the infectivity up to 8 days and decreasing gradually. The Bamboo shrimp samples retained the infectivity up to the 11^{th} day of the experiment. From the day 5 to day 11 the shrimp samples possessed maximum percent infectivity when compared to the water samples. The average percent infectivity of the water samples with shrimp was higher at all times than the water without shrimps and it retained the infectivity up to 11 days.

For the evidence of the presence of the virus in the samples, RT-qPCR was used to detect the viral RNA. The water samples with and without shrimps and also the shrimp samples were positive from the first day till the 12^{th} day of the experiment. In order to compare the concentration of the virus in the spiked water with that of the shrimps containing spiked water, RNA copy numbers in these two water samples were calculated by extrapolation of the values taking IVT RNA readings as standard. The concentration of the virus reduced gradually in all the samples even though they remained positive upto 12 days, which was the duration of the experiment, however, the values remained higher in the water in which shrimps were reared as compared to the water without shrimps. The initial viral load in terms of log 10 values was 8.472 in the water with shrimps and 8.228 in the water without shrimps on the day 12 the viral load in the water with shrimps was 5.103 which was still higher than the control water load i.e. 3.833.

This study has explored further the role of aquatic biotic community in transmission and persistence of LPAI. Shrimps are important members of aquatic fauna as they are consumed by large fishes, birds and human beings. Their role in persistence of avian influenza virus in the environment cannot be neglected. This study shows that shrimps under experimental conditions are able to accumulate the virus. The virus remains infective in the body of the shrimps for a few days. The presence of bamboo shrimps in the water aids in maintaining higher virus counts as compared to water without bamboo shrimps.

Screening of avian influenza viruses for drug resistance

Identification of H5N1 possessing mutations for resistance to oseltamivir and zanamivir is rapidly detected by an enzymatic assay which can identify the increased inhibitory concentration (IC_{50}) of the drug. This work is being carried out as a regular monitoring for emergence of drug resistance in the currently isolated viruses from the outbreaks in the country. A total of 17 isolates were screened for susceptibility to currently approved FDA drugs oseltamivir and zanamivir by enzyme based Neuraminidase inhibition assay (Table 2). All isolates of Highly Pathogenic Avian Influenza H5N1 isolated so far in 2015 were found to be susceptible to the two drugs except one which revealed higher IC_{50} values than the cut off, however, sequencing analysis revealed that all the known markers of resistance were absent from it resistance. Low pathogenic avian Influenza isolates, of other subtypes, H9N2, (7) H3N8 (3), H4N6 (1) H6N8 (1) and H11N9 (1) were also tested and found to be susceptible to oseltamivir and zanamivir (Table 2).

Table 2: Inhibitory Concentration 50 (IC 50) values of Oseltamivir and Zanamivir against Avian Influenza viruses.

Accession number	Subtype	IC_{50} (nM) Oseltamivir	IC_{50} (nM) Zanamivir
A/ck/India/04CA07/2015	H5N1	6.24±0.50	4.05±0.07
A/ck/India/04CA08/2015	H5N1	5.87±0.12	2.75±0.04
A/ck/India/04CA09/2015	H5N1	4.97±0.05	2.39±0.24
A/ck/India/04CA10/2015	**H5N1**	**33.85±0.04**	**2.61±0.09**
A/ck/India/04CA11/2015	H5N1	5.52±0.06	3.32±0.34
A/ck/India/04CA12/2015	H5N1	5.29±0.29	2.80±0.13
A/ck/India/04CA13/2015	H5N1	5.14±0.35	2.60±0.26
A/ck/India/04CA14/2015	H5N1	3.71±0.10	2.95±0.07
A/ck/India/04CA15/2015	H5N1	6.17±0.09	2.43±0.50
A/ck/India/04CA16/2015	H5N1	4.40±0.08	2.65±0.34
A/ck/India/04CA17/2015	H5N1	3.23±0.15	1.85±0.26
A/tu/India/01CA01/2015	H5N1	5.73±0.07	3.60±0.25
A/ck/India/01CA01/2016	H5N1	3.6164	0.95
A/du/India/01CA01/2016	H5N1	3.138	0.68
A/ck/India/01CA02/2016	H5N1	4.0756	1.54
A/ck/India/01CA03/2016	H5N1	4.9437	1.84
A/ck/India/01CA04/2016	H5N1	4.1616	1.60
A/ck/India/01Tr101/2014	H9N2	1.14±0.05	5.8±0.10
A/ck/India/01Tr04/2014	H9N2	0.71±0.10	4.43±0.34
A/ck/India/11TI01/2015	H9N2	0.38±0.05	3.81±0.44
A/ck/India/11CA01/2015	H9N2	0.82±0.10	1.70±0.03
A/ck/India/11TI02/2015	H9N2	0.36±0.01	2.30±0.27
A/ck/India/11TI03/2015	H9N2	0.39±0.08	3.05±0.09
A/du/India/11CL10/2014	H3N2	5.40±0.22	4.97±0.06
A/du/India/11TR09/2014	H3N2	9.44±0.01	6.59±0.27
A/du/India/11TR31/2014	H3N2	10.18±0.08	5.54±0.49
A/du/India/12CL01/2014	H6N8	0.73±0.07	4.84±0.07
A/du/India/11CL1044/2011	H11N9	0.08±0.01	3.45±0.32

Molecular characterization of Avian influenza virus

H5N1 subtypes

In India, since its first report in february 2006, H5N1 HPAI virus has been isolated almost every year from poultry and/or peri-domestic birds including crows. During the period under report, three AI outbreaks of H5N1 virus infection in domestic poultries were reported in Telangana, Manipur and Tripura. For molecular characterization genome sequencing of 4 H5N1 viruses isolated during 2015-16 from poultry in Telangana, Manipur and Tripura and 4 H5N1 viruses isolated during 2014-15 from poultry and crows in Kerala and Uttar Pradesh, respectively were carried out. Analysis of HA revealed that the viral isolates possessed molecular signatures (polybasic amino acid motif RRRKR*GLF at the HA cleavage region) that defines HPAI, which was corroborated with intravenous pathogenicity (IVP) index of 2.93 and 2.96 (out of 3.00) in 2 representative isolates. Sequence analysis of NA revealed sensitivity of the virus to neuraminidase inhibitors. However, emergence of amantadine resistant due to amino acid substitutions S31N and V27A in M2 was observed in the isolates from Manipur and Telangana, respectively. Presence of amino acids Q222 and G224 in the receptor binding sites indicated avian receptor specificity of the H5N1 isolates.

Other subtypes

Besides H5N1, other type A influenza virus subtypes such as H9N2, H6N2 and H3N8 have been isolated from poultry. For molecular characterization genome sequencing of 15 H9N2 (Uttarakhand-02, Gujarat- 02, Kerala-01, Odisha-02, Rajasthan-02, Uttar Pradesh-01, Madhya Pradesh-01 and Maharashtra-04), 4 H3N8 (Kerala) and one H6N2 (Kerala) isolates have been carried out. All the isolates possessed single basic amino acid (H9N2: RSSR*GLF, H6N2: IETR*GLF, and H3N8: KQTR*GLF) at the HA cleavage region indicating low pathogenic avian influenza (LPAI), which was corroborated by IVP index of 0-0.19 (H9N2) and 0 (H3N8 and H6N2). Presence of amino acids Q and G in the receptor binding sites indicated avian receptor specificity of the H3N8 and H6N2 isolates; however, presence amino acids L and G at the same sites in H9N2 isolates indicate human receptor specificity of H9N2 virus.

Molecular epidemiology of Avian influenza virus

H5N1 subtype

In the HA gene phylogeny, the H5N1 viruses isolated from poultry in Telangana, Manipur and Tripura during 2015-16 grouped with clade 2.3.2.1A viruses (Fig. 5). Within clade 2.3.2.1A, all these isolates grouped separately indicating multiple sources. For example, the Telangana virus grouped with H5N1 virus isolated from chicken in Chhattisgarh in 2013 and the Manipur virus shared grouping with H5N1 virus isolated from chicken in Bhutan in 2015. Similarly, the H5N1 viruses isolated from ducks and chickens from Tripura in 2016 grouped with H5N1 virus isolated from duck in Bangladesh in 2014. The results indicated cross-border movement of virus in South Asia.

Besides poultry, H5N1 virus has been isolated from crows during H5N1 outbreaks in ducks in Uttar Pradesh during 2015. In the phylogenetic tree (Fig. 5), the crow virus grouped with clade 2.3.2.1C viruses. Within clade 2.3.2.1C, the virus grouped closely with H5N1 viruses isolated from ducks in Chandigarh and Uttar Pradesh, and ducks and turkeys in Kerala

isolated during 2014-15 along with H5N1 viruses isolated from wild birds in Dubai in 2014 indicating epidemiological link between the outbreaks. These closely related viruses from India and Dubai shared ancestry with H5N1 viruses isolated during 2012-13 in China and Vietnam. A Median Joining Network analyses indicated multiple introductions of H5N1 virus of clade 2.3.2.1C which was detected for the first time in India during November 2014 to March 2015 (Fig. 6). One of the outbreaks in ducks in Kerala was linked to the H5N1 virus isolated from wild birds in Dubai suggesting movement of virus probably through migration of wild birds, although the role of poultry cannot be ruled out. Internal spread of virus in Kerala was also observed. Outbreaks in ducks in Chandigarh and Uttar Pradesh were probably from Asia.

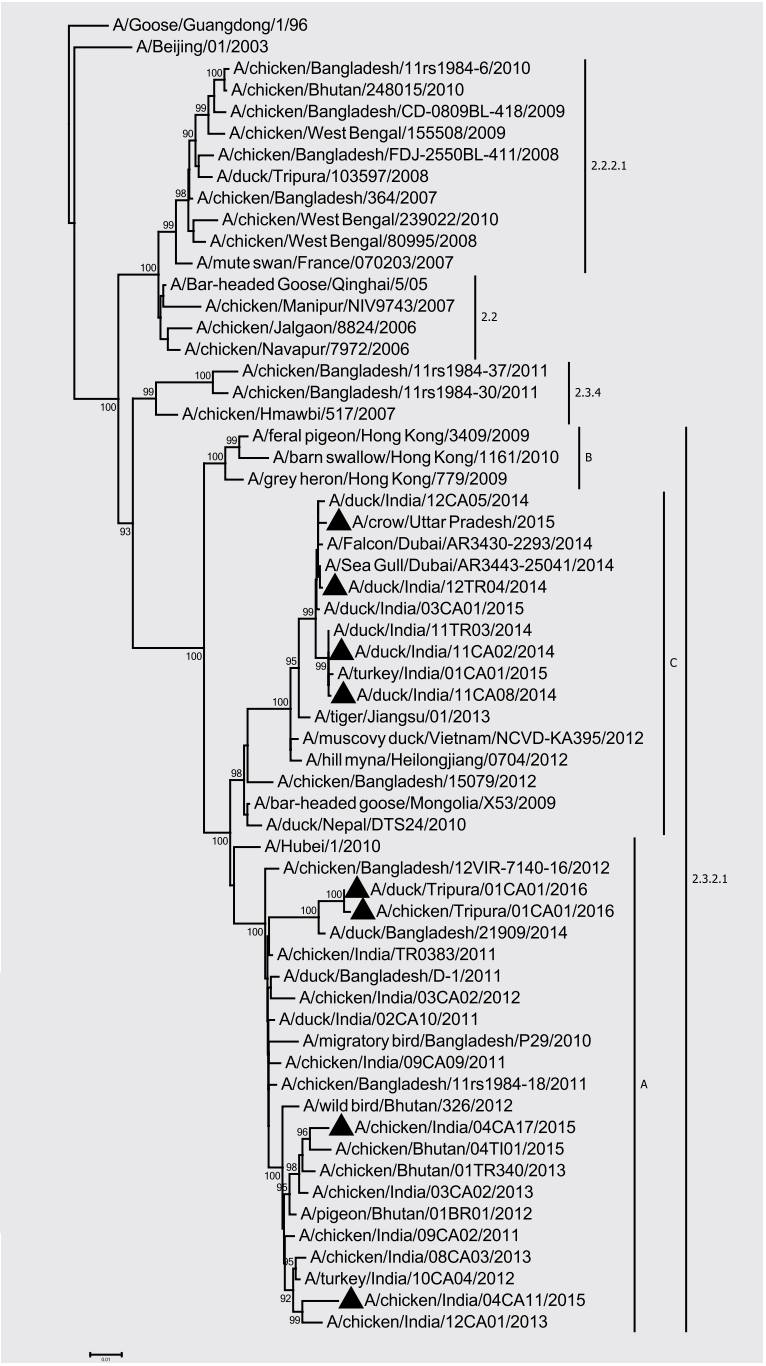


Fig. 5: Phylogenetic analysis of H5N1 AIVs (based on HA gene). HA clade classifications are shown to the right. We wish to acknowledge the authors, and the originating and submitting laboratories of the sequences (A/Falcon/Dubai/AR3430-2293/2014 and A/Sea Gull/Dubai/AR3443-25041/2014) from EpiFlu™ Database of GISAID.

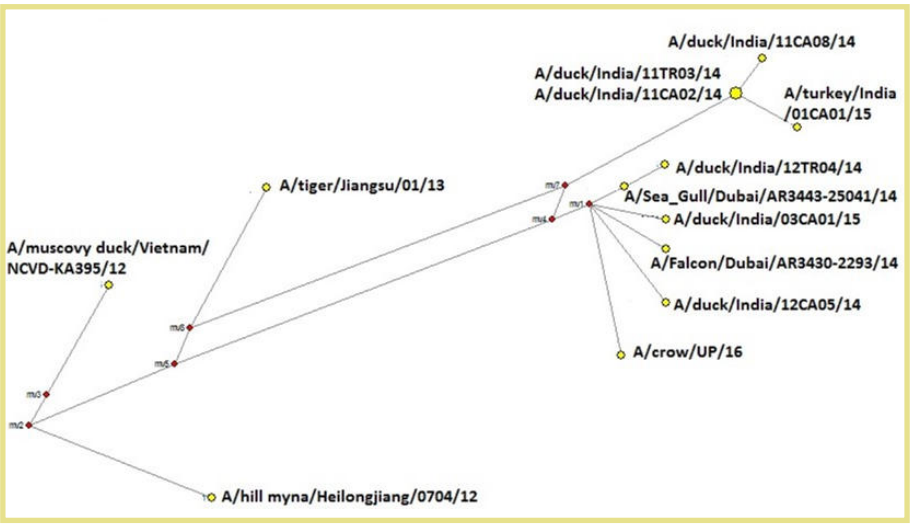


Fig. 6: Median-Joining network based on HA gene of H5N1 AIVs focusing Indian isolates. Nodes in red color represent median vectors, which often represent ancestral sequences and may or may not have existed but are not represented in the sequence database. Numbers along the branch represent the number of nt. substitutions distinguishing different nodes. Branches without number indicate 1-2 mutations.

H6N2 subtype

The H6N2 virus isolated from duck in Kerala during 2014 was sequenced, and in the phylogenetically, H6N2 virus grouped with viruses circulating mostly in water birds in the Asian region. In the tree, the virus grouped closely with contemporary H6 viruses isolated from ducks, chicken and goose in China, Japan and Vietnam (Fig. 7). The HA and NA genes grouped closely with duck strains of H6N6 from Japan (A/duck/Yamagata/061004/2014) and H3N2 from Norway (A/Teal/Norway/ 10_1037/2010, data not shown), respectively. The internal genes (PB2, PB1, PA, M and NS) were contributed by H2N3, H4N9, H5N3/H2N9, H10N8 and H6N6 AIVs, respectively found in different duck species (mallard and tufted) in Eurasian region, except the NP gene which was contributed by H5N2 virus from a gull (data not shown). The phylogenetic analysis indicated that the virus is a reassortant and is of waterfowl origin.

H9N2 subtype

Phylogenetic analysis of the HA genes of H9N2 viruses isolated from chickens during 2012-1015 in Uttarakhand, Gujarat, Kerala, Odisha, Rajasthan, Uttar Pradesh, Madhya Pradesh and Maharashtra along with previously isolated H9N2 viruses in India during

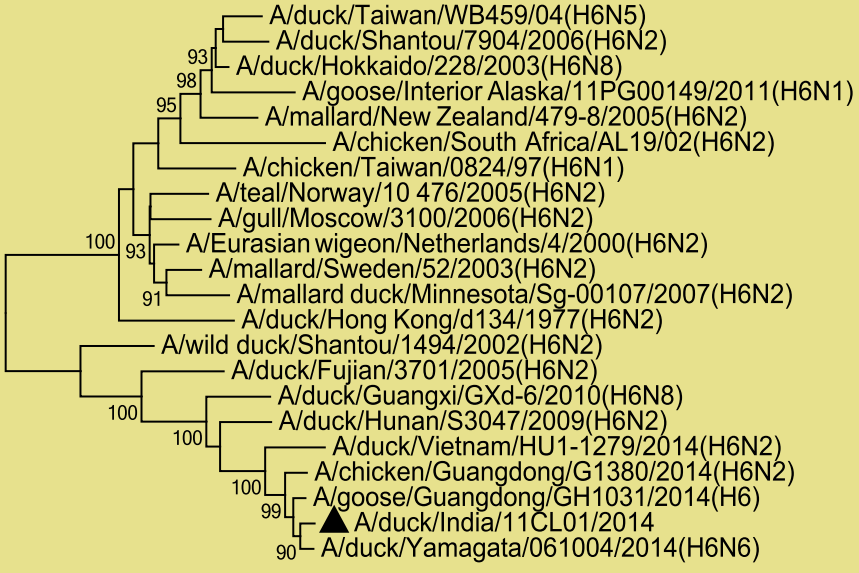


Fig. 7: Phylogenetic analysis of H6N2 AIVs (based on HA gene).

Fig.8: Phylogenetic analysis of H9N2 AIVs based on HA gene). Sub-lineages are shown to the right.

2003-2011 grouped with G1-like sub-lineage (Fig. 8). Within the sub-lineage, Indian isolates grouped with isolates from Bangladesh, Nepal and Pakistan indicating movement of virus across the borders. As in the HA, the molecular phylogeny of NA, NP and Matrix genes, showed close relationship with H9N2 viruses, whereas the polymerases (PB2, PB1 and PA) and NS genes are of unknown origin (data not shown).

H3N8 subtype

Four H3N8 viruses isolated from chickens and ducks in Kerala during 2014 formed two phylogenetic groups (Fig. 9), indicting multiple introduction of the virus. One of the duck isolate (A/duck/Kerala/Renny2CL/2014) grouped closely with H3N8 viruses isolated in Vietnam and Mongolia during 2013 and 2015, whereas the other three isolates formed separate group closely associated with duck viruses isolated during 2010 in China and Mongolia.

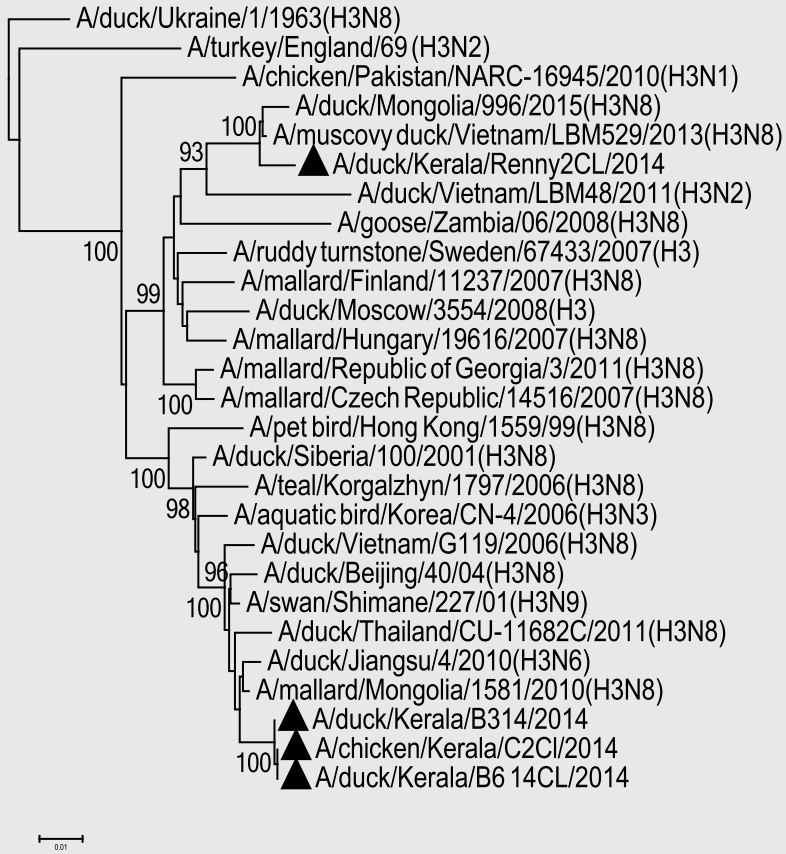


Fig. 9: Phylogenetic analysis of H3N8 AIVs (based on HA gene).

Diagnostic development for avian influenza

Characterization of H5 specific MAbs

The characterization of monoclonal andtibodies (MAbs) and mapping of their binding sites aid in the application of MAbs for any diagnostic test development. The binding sites of four MAbs (Mab 1, 2, 3 and 4) specific to AIV H5 HA which possessed neutralizing activity has been elucidated by selection of monoclonal antibody resistant mutants (MARM), sequencing and analysis. The sequence analysis of HA gene of the MAR mutants revealed that the position of amino acid changes due to each Mab were different. The MARM obtained with Mab1 showed amino acid change in close proximity to epitope site D in HA protein. The Mab 2-MARM showed amino acid change at two different sites. One of the deduced site was part of epitope site A and another was in close proximity to epitope site D. The Mab 3-MARM showed amino acid change within one of the antigenic peptide reported earlier. The Mab 4-MARM also showed amino acid change at two different sites. One of the deduced site was part of epitope site A and another was part of antigenic peptide reported earlier and different from Mab 3-MARM. All the MAb binding sites elucidated in this study were mapped in HA crystal structure to define exact location of these sites and was shown that all the sites were located in the globular head of the HA protein (Fig. 10).

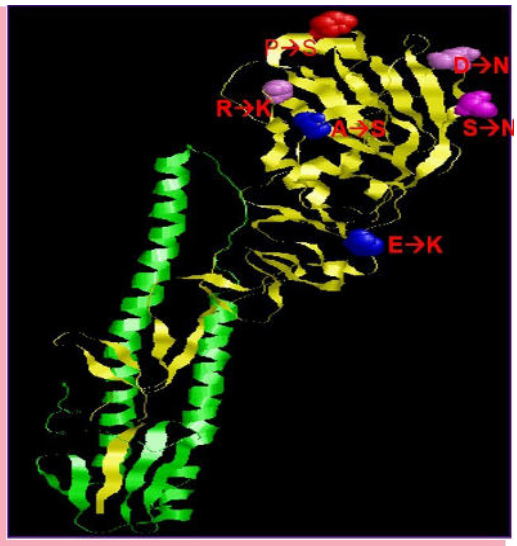


Fig. 10: Location of mutated anino residues of MARM in HA crystal structure built up by using PyMOL V.1.5x

The reactivity of each MARM against its corresponding MAb and other MAbs in HI, VNT, western blotting and IPMA were studied. The reactivity in all the tests were similar. Based on these reactivity and deduced Mab binding sites it can be inferred that the Mab 1, 3, and 4 recognize or bind to different epitope sites on HA. The Mab 2, though recognizes different site, bind to the site adjacent to Mab1 and showed stearic hindrance with binding of Mab 1.

Further, the MAbs were tested in HI with three different clades of H5N1 viruses and other influenza viruses available in the lab. It was found that Mab 1 and 4 reacted with three clades of H5N1 virus available in the lab but did not react with other influenza viruses available in the lab. The Mab 2 reacted only with clade 2.2 H5N1 virus. The Mab 3 showed mild cross- reactivity with H9N2 viruses also.

Recombinant expression of AIV H5HA protein

The HA protein of clade 2.3.2.1 H5N1 virus (A/CK/india/03CL488/2011) was cloned and expressed in yeast expression system. The recombinant protein was intended to be used along with MAbs for development of a diagnostic test. The wild type HA gene and codon optimised HA gene were cloned in two yeast expression vectors. The recombinant plasmids were transformed into *Saccharomyces cerevisiae* and *Pichia pastoris*. Attempts were made for expression of the recombinant HA (rHA) protein by induction with galactose and methanol respectively. The full length rHA

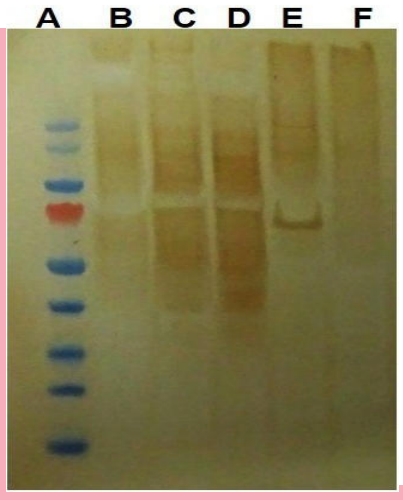


Fig. 11: Western blot of Recombinant H5HA in S.cerevisiae

Lane B - *S.cerevisiae* transformed with vector alone
Lane C - *S.cerevisiae* transformed with recombinant plasmid

protein were expressed in *Saccharomyces cerevisiae* as evidenced by western blot with polyclonal serum against AIV H5N1 clade 2.3.2.1 virus (Fig. 11).

Production and optimization of recombinant NP antigen

Production and purification of rNP from pET28a-NP clone- The expression clone of nucleoprotein gene in pET-28a vector was revived from the glycerol stock. The expression clone was induced at an OD of 0.6 using 1, 2 and 3 mM of IPTG and high level expression was optimized in Rosetta pLysS cell at 1mM IPTG concentration in terrific broth medium after 6 hr post induction. The pellet was lysed using the bugbuster reagent for 30 min at 37 C and after spin at full speed soluble (supernatant) and inclusion bodies fraction (pellet) was collected. The pellet was solubilized in 1x solubilization/binding buffer containing 6M urea. The expression of rNP protein was confirmed by SDS-PAGE analysis followed by western blotting in both the fraction yield in soluble fraction was better. The soluble fraction was produced in bulk and purified using immobilized metal affinity chromatography using Ni-NTA resin. The elution of protein could be optimized in 200 mM Imidazole. The rNP protein was purified further by. The purified rNP protein was quantified and stored at -20^{0}C.

Construct of recombinant bacmid containing NP gene (Baculovirus expression system)- Complete coding region of NP of AIV was codon optimized for insect cell line to achieve high level of protein expression using JCAT (Java Codon Adaptation Tool) online server. The codon optimized NP gene was synthesized from GeneScript and then cloned into pFastBacTMHT-A vector in frame. This recombinant vector was transformed in DH10Bac™ E. coli competent cells and positive clones (recombinant bacmid) were selected based on blue-white screening on LB agar plates. Further confirmation of NP gene insertion through homologous recombination into bacmid was done with three sets of primers targeting different regions of codon optimized NP gene.

Optimization of an indirect ELISA

The rNP purified protein was coated in variable concentration (10ng to 2µg per well) and checkerboard titration was done using varying dilution of positive and negative sera (1:10 to 1:500) to derive the positive/negative ratio. optimization of blocking reagents was carried out using BSA, skimmed milk powder, casein hydrolysate etc. at different concentrations . Many AIV positive and negative sera have been tested accurately but due to higher background in negative sera (OD 0.4) further refinement is underway before deriving the cut-off. To reduce such background that may be due to bacterial protein contamination, expression of NP has been initiated in baculovirus expression system.

Generation of RAb (single chain fragment variable) enzyme conjugate

AIV NP specific ScFv (single chain fragment variable) gene was expressed in prokaryotic system with alkaline phosphatase fusion tag (rNPScFv-AP) in inclusion bodies using pEP-AP vector (procured under MTA). Expression of rNPScFv-AP was confirmed by SDS-PAGE analysis and western blotting. Alkaline phosphatase activity was confirmed with BCIP/NBT and pNPP (soluble) substrates. The color development time was specific but observed after 6 h to overnight may be due to folding issue in inclusion bodies fraction. Optimization is underway to express the same in native form (soluble fraction) to ascertain the enzymatic activity of alkaline phosphatase.

Assessment of multiplex compatible functional primers and probes for simultaneous typing and subtyping H9N2 Influenza A virus

Development of a multiplex assay was initiated for simultaneous typing and subtyping of H9N2 influenza A viruses. Consensus representative sequences was determined for the matrix (M), nucleoprotein (NP), H9 and N2 genes after assessing thousands of nucleotide sequences from NCBI database as well as unpublished sequences of Indian isolates . Novel target regions (unreported) within these genes were identified after comparative analysis of existing literature and within these regions multiplex compatible primer and probe sets were designed. All the designed oligos were further verified with multiple sequence alignment dataset and degenerate bases were substituted wherever required for extending the scope of diagnosis. Using online tools, the modified oligos were further analyzed statistically and thermodynamically for G+C content, primer dimer formation, hairpin loop etc to determine the functional stability. Primarily, all the probes were integrated and synthesized with 5'-FAM and 3'-BHQ1 reporter and quencher dyes to evaluate their functionality in wet experiment. On assessment, all the probe and primer sets produced clear sigmoid amplification curve with high fluorescence in Taqman RT-qPCR assay with RNA from AI H9N2 reference viruses (Fig. 12). The probe and primer sets designed for matrix and nucleoprotein genes were also tested with viruses of other subtypes (AI H5N1 and others). All the probe and primer sets were optimized with 20 and 10 pmol of each primers and probe respectively in 20 µl reaction volume. After determination of sensitivity and specificity of all the individual assays, a multiplex assay will be approached by integrating different reporter and quencher dyes for simultaneous typing and subtyping of H9N2 avian influenza A virus.

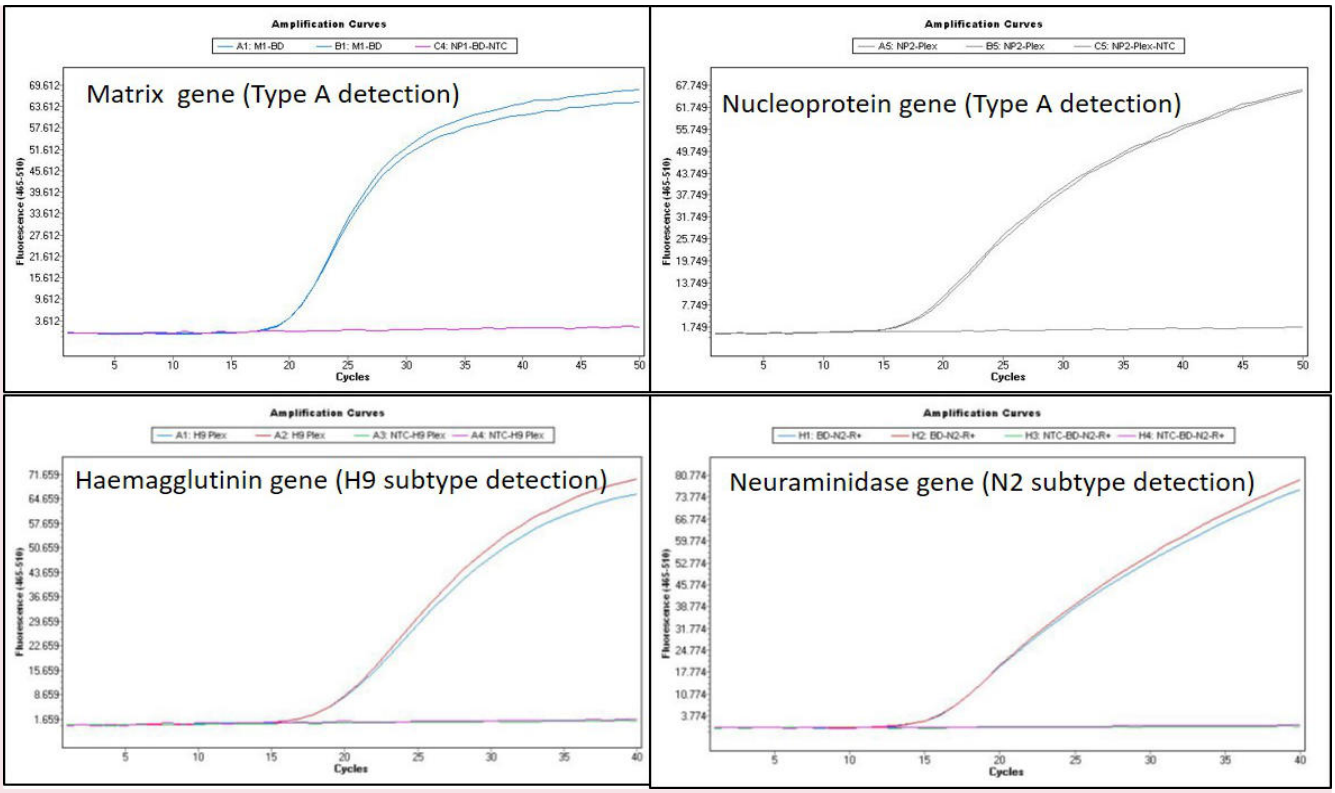


Fig 12 Amplification curve for matrix and nucleoprotein genes for influenza A typing and subtyping of AI H9N2 viruses

Standardization of multiplex PCR assays for simultaneous detection of H5, H7, H9 and N9 sub-types of Avian Influenza viruses

Among the different hemagglutinin (HA) subtypes of avian influenza viruses, H5, H7, and H9 are of major interest because of the serious consequences for the poultry industry and the increasing frequency of direct transmission of these viruses to humans. Availability of new tools to rapidly detect and subtype the influenza viruses can enable the immediate application of measures to prevent widespread transmission of the infection.

In this study, singlex, duplex and triplex PCR assays were standardized to detect H5, H7, H9, M, and N9 individually in singlex format; H5 and M, H7 and M, H9 and M, N9 and M, H7 and N9 together in duplex format along with H7, N9, and M gene of AI viruses simultaneously in triplex format. This is a novel PCR assay for simultaneous detection of AI viruses belonging to subtypes H5, H7 and H9, and so far the results obtained indicated its suitablity as a routine laboratory test for the rapid detection and differentiation of the three most-important AI virus subtypes in samples of avian origin. However, the assays are under further optimization process and subsequently be transformed into PCR based nucleic acid lateral flow (NALF) assay which is becoming a popular podium for fast, robust, nucleic acid testing in point of need environments with its wide applicability in diagnosis of infectious disease.

Vaccine development and validation for avian influenza

Validation of the rgH5N2 (clade 2.2) vaccine in commercial chickens

The rgH5N2 vaccine developed earlier against clade 2.2 H5N1 avian influenza virus was validated in commercial chickens procured from Central Poultry Development Organization (CPDO), Mumbai. Four week old chickens (n= 100) were vaccinated with inactivated rgH5N2 (clade 2.2) vaccine and were kept in animal house under normal conditions. Unvaccinated control chickens (n = 10) were kept in a separate room. H5 specific immune response in the vaccinated chickens was observed in the vaccinated chickens in 7-14 days post vaccination with a mean HI tire of $10^{5.8}$ on 14^{th} day. On 28 days post vaccination, 20 vaccinated chickens and 10 unvaccinated controls were challenged with 10^{9} EID_{50} of H5N1 virus. All the control chickens developed the disease and died within 48 to 72 hrs of challenge. All the vaccinated chickens, except one with nil HI titre, were fully protected from the disease. The chickens with HI titre as low as 10^{4} were also protected. Safety of the vaccine was validated by conducting OIE prescribed safety test. Briefly, 10 chickens were vaccinated with double dose (1 ml) of the vaccine and were observed for 15 days for development of any illness and/ or lesions at the site of vaccination. The vaccine cleared the safety test. The vaccine used in all the experiments was prepared six months before the actual use and was kept in ordinary refrigerator (4-8° C). Therefore, the stability of the vaccine was tested for six months.

Evaluation of rgH5N2 virus vaccine (clade 2.3.2.1) in SPF chickens

An oil adjuvanted vaccine with inactivated low pathogenic reassortant H5N2 virus, generated by reverse genetics using mutated HA gene of clade 2.3.2.1 H5N1, M gene of clade 2.2 H5N1, NA gene of H9N2 virus and internal genes of H1N1 (WSN/33), was

evaluated in SPF chickens. The birds (4 weeks old) were vaccinated with 1075 HAU of inactivated antigen followed by a booster (144 HAU) on 28th day. The mean antibody response to the heterologous clade 2.2 virus in vaccinated birds (n=20) was significantly lower (HI titer: $2^{3.67}$-$2^{8.0}$) than to the homologous clade 2.3.2.1 (HI titer: $2^{4.67}$-$2^{10.31}$) between 14th day post primary immunization and 19th day post booster (Fig. 13). Two groups of vaccinated birds (n=7) were challenged with 106.5 EID_{50} of either clade 2.3.2.1 (Group A) or clade 2.2 (Group B) H5N1 viruses and were fully protected. Group B challenged with heterologous clade 2.2 H5N1 virus showed prolonged virus shedding in more birds compared to Group A challenged with homologous clade 2.3.2.1 virus. The mean HI titer at 7 days post challenge remained higher (Group A=$2^{7.85}$; Group B=$2^{7.43}$) for clade 2.3.2.1 than for clade 2.2 (Group A=$2^{6.28}$; Group B=$2^{6.43}$). On subsequent challenge of Group A with $10^{7.5}$ EID_{50} of clade 2.2 H5N1 virus and Group B with $10^{7.5}$ EID50 of clade 2.3.2.1, the birds were fully protected without any virus shedding in both groups. However, the mean HI titer at 5 days post challenge was significantly higher in Group B with lesser difference between the two clades (clade 2.3.2.1= $2^{9.86}$; clade 2.2= $2^{9.0}$) than in the Group A (clade 2.3.2.1= $2^{8.86}$; clade 2.2= $2^{6.86}$). The study demonstrates that two dose vaccination with clade 2.3.2.1 HA may protect against heterologous clade 2.2 H5N1 viruses and may be useful to limit virus spread in regions where both the clades are circulating.

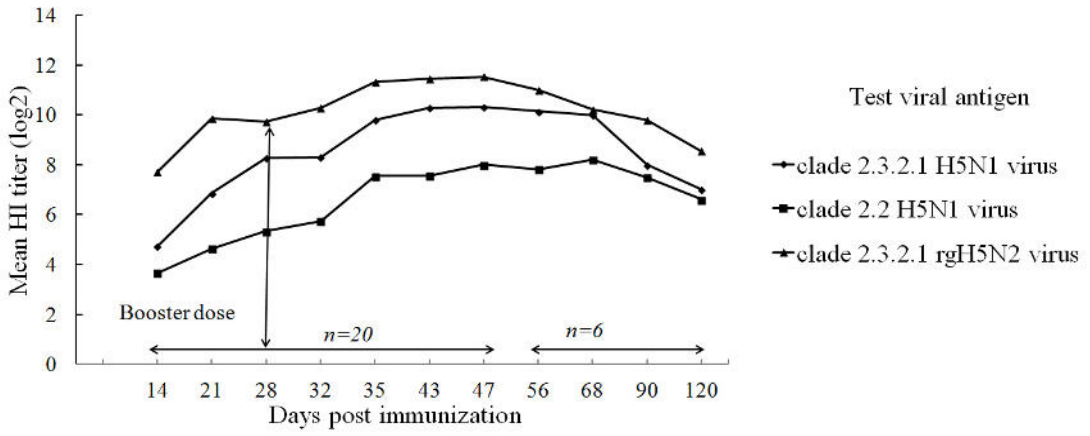


Fig. 13: Differential clade-specific anti-HA antibody response in chickens immunized with rgH5N2 (2.3.2.1 clade) vaccine

India ranks first in dairy milk production in the world contributing 146.3 million tonnes during 2014-15 and has the largest bovine population in the world. Diagnosis and control of prevalent and emerging diseases in ruminants is of utmost importance for increase in production of milk and other livestock products which provide income to millions of rural farmers in India. Among the infectious agents infecting ruminants, emerging diseases in ruminants are being reported from many countries posing new challenges to animal health with respect to their diagnosis and control. BVDV-3 (HoBi-like) viruses are emerging pestiviruses and are associated with respiratory, enteric and reproductive diseases in cattle and water buffaloes and mucosal disease in cattle. Recently, two lineages of highly divergent BVDV-3 strains circulating in Indian cattle have been identified at NIHSAD. Border disease in sheep caused by BDV was considered exotic to India, however identification and isolation of BDV at NIHSAD has confirmed its presence in India. Schmallenberg virus, a vector borne orthobunyavirus emerged in 2011 in cattle in Germany and Netherlands and has since then spread to vast parts of Europe. Diagnostic preparedness for this disease has been initiated at NIHSAD. Of the several OIE listed diseases and diseases important for international trade in cattle, buffaloes, sheep and goats at present, research work is being carried out on bovine viral diarrhea virus (BVDV) in cattle and other ruminants including mithun. Laboratory diagnostic services are also being provided for BVD, border disease (BD), malignant catarrhal fever (MCF) and Schmallenberg virus (SBV) infections. Diagnostic preparedness for enzootic bovine leucosis (EBL) in cattle has been initiated as this is an OIE listed disease and important for international trade.

Border Disease

Identification of border disease virus (BDV) in sheep in India for the first time and its genetic and antigenic analysis

Border disease (BD) is primarily a reproductive disease of sheep, and rarely of goats, which causes significant economic losses and occurs world-wide. The clinical manifestations of the disease include barren animals, abortion, birth of stillborn or mummified fetuses and birth of small weak lambs with tremor and abnormal fleece. BDV belongs to the genus *Pestivirus* in the family *Flaviviridae* and is widely distributed in most of the sheep rearing countries. At least 7 genotypes of BDV have been reported from all over the world. BDV-1 genotype has been identified in sheep from Australia, USA, U.K. and New Zealand, BDV-2 from Germany, BDV-3 from Germany Switzerland, Austria, France, Slovakia, Italy and China, BDV-4 from Spain, BDV-5 and BDV-6 from France and BDV-7 from Italy. In addition, ovine isolates of other possible BDV genotypes have been reported from Turkey and Tunisia. The pestiviruses identified and isolated from sheep and goats thus far in India have been BVDV-1 or BVDV-2, while BDV was considered exotic. The first identification and molecular characterization of BDV isolated from sheep in India is reported herewith.

During routine genetic typing of pestiviruses in the years 2009-10, BDV was detected by real time RT-PCR in eight Indian sheep of a flock in Jammu and Kashmir showing clinical signs of BD. Clinically, many lambs were born with hairy fleece (Fig.14) and a few had fleece with abnormal brown or black pigmentation . The flock had a history of barren

ewes, abortions during 2 to 2.5 months following gestation, stillbirths (Fig. 15) and birth of small weak lambs. When propagated on SFT-R cells, eight pestiviruses were isolated from the eight sheep that had been found BDV positive by Taqman RT-PCR and all were of non-cytopathic biotype. All the BDV positive sheep were found to be negative for pestivirus neutralizing antibodies, while 52.9% (144 of 272) of the animals sampled in the flock had neutralizing antibodies against BDV (> 4-8 fold titre difference as compared to BVDV-1 and BVDV-2).

Fig. 14: BVDV infected lamb with hairy fleece (right) seen near a healthy lamb (left)

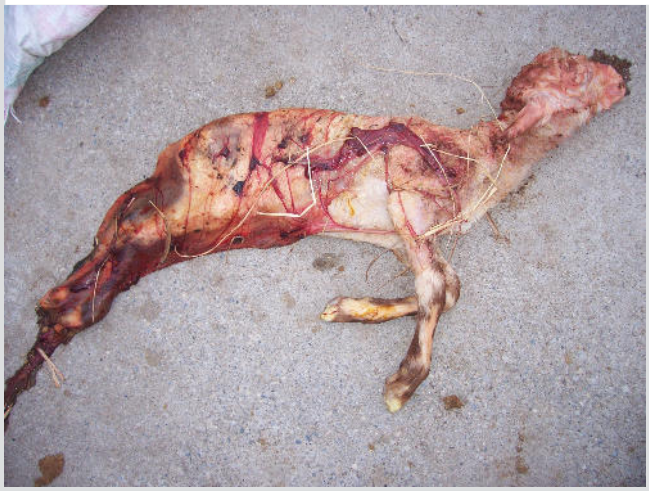


Fig. 15: Stillbirth in a BDV infected flock

Genetic analysis of partial 5'-UTR showed that the sequences obtained from all the eight original blood samples were almost identical providing evidence that the same strain was circulating in the flock. Hence, a representative BDV isolate (Ind 830-09) was studied. Phylogenetic analysis of 243 bp 5'-UTR sequences typed the Indian ovine isolate as BDV and clustered within the BDV-3 isolates together with strains Gifhorn from Germany, B300/06 from Austria and 85-F-588 from France. Phylogenetic analysis based on full length N^{pro} coding sequences showed that indeed the Indian BDV isolate (Ind 830-09) fall into BDV-3 genotype within the BDV but in a branch clearly separated from BDV-3 strains, Gifhorn and CHBD1 (Fig. 16).

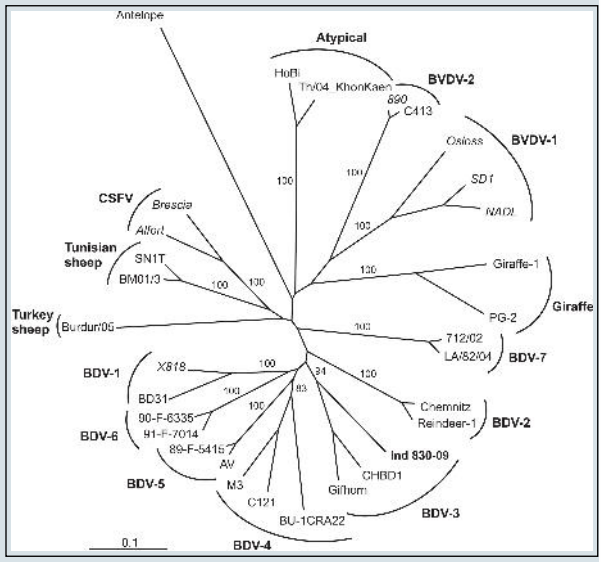


Fig. 16: Phylogenetic analysis of BDV (based on full length N^{pro} coding sequences)

Analysis of nucleotide and deduced amino acid sequences of all the structural proteins clearly demonstrated that the genomic organization of Indian ovine pestivirus isolate, Ind 830-09 is similar to BDV. The C (100 aa), E^{rns} (227 aa), E1 (195aa) and E2 (373 aa) proteins were of size characteristic for BDV reference strain X818. The relationship of Indian BDV isolate (Ind 830-09) with other pestiviruses is shown in Table 3.

Table 3: Percentage of nucleotide and amino acid identity between Indian sheep BDV isolate Ind 830-09 (KT934377) and selected representative pestivirus strains.

Genomic region Pestivirus strain	5'-UTR*	N^{pro}	C	E^{rns}	E1	E2
SD1 (M96751) BVDV-1	71.2	66.7 *72.6*	55.0 *71.0*	71.0 *78.0*	72.1 *76.4*	63.7 *61.9*
890 (U18059) BVDV-2	72.8	68.3 *69.6*	56.0 *71.0*	73.6 *78.9*	71.5 *78.5*	62.5 *57.0*
Alfort (J04358) CSFV	83.9	69.8 *75.0*	64.3 *75.8*	76.9 *83.7*	75.6 *87.7*	68.4 *67.8*
X818 (AF037405) BDV-1	85.6	75.4 *81.0*	77.7 *87.0*	78.0 *93.0*	77.1 *89.2*	73.8 *77.7*
Reindeer-1 (AF144618) BDV-2	85.1	74.0 *76.8*	76.7 *87.0*	81.4 *93.8*	78.5 *90.8*	73.1 *76.9*
Gifhorn (GQ902940) BDV-3	88.9	79.0 *86.9*	74.7 *91.0*	81.9 *96.5*	79.5 *92.8*	78.3 *83.1*
C121 (DQ275625; DQ273159) BDV-4	81.9	75.2 *79.2*	NA	NA	NA	NA
AV (EF693984; EF693962) BDV-5	81.9	75.8 *78.0*	79.1 *82.3*	83.2 *90.5*	79.2 *88.6*	75.0 *77.1*
90-F-6335 (EF693990; EF693968) BDV-6	86.8	73.8 *78.6*	NA	NA	NA	NA
712/02 (AJ829444) BDV-7	83.1	71.4 *77.4*	NA	NA	NA	64.0 *60.0*
Burdur/05 (AM418428; EU930015) Turkey sheep	79.2	68.7 *72.0*	NA	NA	NA	NA
SN1T (AY452484) Tunisian sheep	81.1	71.6 *73.8*	61.3 *72.7*	78.6 *91.2*	76.1 *87.7*	NA
Giraffe-1 (AF144617)	72.4	69.8 *69.0*	59.5 *68.4*	75.2 *81.5*	71.5 *80.5*	62.4 *56.6*

The antigenic characterization revealed that although the mAb reactivity pattern of Indian BDV isolate Ind 830-09 has an antigenic profile quite similar to BDV reference strains, the difference in reactivity from earlier reported BDV-1, BDV-5 and BDV-7 isolates showed that Indian BDV-3 isolate is antigenically different from those genetic groups of BDV. Results of cross neutralization tests employing antisera raised to currently circulating Indian BVDV strains showed significant antigenic differences between BDV, BVDV-1 and BVDV-2 isolates (Table 4). This should be taken into account while developing prevention and control strategies against BD in India, since all the three recognized species of ruminant pestiviruses, BVDV-1, BVDV-2 and BDV have now been identified.

Table 4: Coefficients of antigenic similarity (R) of BDV Ind 830-09 with other circulating BVDV strains.

Pestivirus strain	Ind 141353	Ind 51966	Ind 5197	Yak	IndS1449
Ind 830-09 (sheep, BDV-3)	0.4	0.6	0.8	0.6	0.6
Ind 141353 (cattle, BVDV-2a)		35.3	50.0	0.7	2.2
Ind 51966 (sheep, BVDV-2b)			50.0	0.5	1.5
Ind 5197 (goat, BVDV-2a)				1.5	1.1
Yak (BVDV-1c)					35.3

Until now, BDV-3 has been found in sheep in Germany, Switzerland, Austria, France, Slovakia, Italy and China and in cattle in Austria. Identification of BDV-3 genotype in sheep in India in this study provides evidence that BDV-3 is not only found in several countries in Europe but also in Asia and this genotype may be more widespread than previously thought. The origin of BDV in sheep in India is unknown. However, spread through trade is possible since over the years, many exotic breeds of sheep of fine wool, mutton, dual-purpose and pelt have been introduced in India from Europe, North America and Australasia for improving indigenous sheep. Although it is not possible to find any epidemiological link between Indian BDV-3 isolate with other BDV-3 isolates, the present finding reflects probable trade in sheep between India and other countries and emphasizes the need of strict sanitary measures during import.

Bovine Viral Diarrhea

Prevalence of BVDV infection in mithuns (*Bos frontalis*)

Mithuns (gayals) contribute significantly in socio-economic life of the people in the North Eastern states of India. Testing of 433 mithuns from the states of Nagaland, Arunachal Pradesh and Mizoram by BVDV antibody ELISA revealed that 71 mithuns were positive for BVDV antibodies. Serological evidence of BVDV infection in free ranging and semi-domesticated mithuns (*Bos frontalis*) was detected for the first time in this bovine species. Prevalence of BVDV neutralizing antibodies were also found in 12 out of 373 cattle and 3 out of 65 goats from the NER indicating a low prevalence of BVDV infection.

Host Pathogen Interaction

Bovine viral diarrhea virus (BVDV) does not use macropinocytosis or claveolae-mediated endocytic pathway for entry into bovine or ovine cells while brefeldin A inhibits its release in ovine cells

Animal viruses use several routes of entry for entering susceptible host cells. Some viruses use fusion with cell membrane, whereas others use receptor-mediated endocytosis. The endocytic pathways exploited by animal viruses include macropinocytosis, clathrin-dependent endocytosis, and caveolae-dependent endocytosis, as well as poorly characterized routes such as clathrin- and caveolae-independent endocytosis. BVDV entry into bovine and ovine cells occurs by clathrin-dependent endocytosis and endosomal fusion. Alternate routes of entry such as such as caveolae-mediated endocytosis or micropinocytosis have not been explored for BVDV entry into ovine or bovine cells. Similarly, no information is available on the assembly and release of BVDV from sheep cells.

Effects of drug cytochalasin D and nystatin on BVDV entry in bovine and ovine cells and the effect of Brefeldin-A (BFA), on BVDV release in ovine cells was studied. The bovine (MDBK) and ovine (SFT-R) cells were treated with various concentrations of cytochalasin D or nystatin before infection with BVDV and at 16 hpi, the number of infected cells was determined by immunochemistry. The ovine cells were infected with BVDV before addition of different concentration of BFA at 8 hpi and the supernatants at 24 hpi were subjected to RT-PCR or immunochemistry.

Cytochalasin D, is an actin-disrupting drug, specifically affects the actin cytoskeleton by preventing its proper polymerization into microfilaments and promoting microfilament disassembly. Only a minor inhibitory effect (0-2.5%) was observed on the entry and infectivity of BVDV in both ovine and bovine cells even at highest concentration (0.4µg/ml) of cytochalasin D (Fig. 17). Almost identical results were obtained when ovine (SFT-R) or bovine (MDBK) cells were treated with 0.4 µg/ml of cytochalasin D at 1 hpi. These results revealed that BVDV entry remained unaffected when macropinocytosis is inhibited, suggesting that BVDV does not use macropinocytosis for entry into ovine or bovine cells.

Nystatin acts on caveolae, characterized by high levels of cholesterol and glycosphingolipids and also by the presence of caveolin, an integral membrane protein.

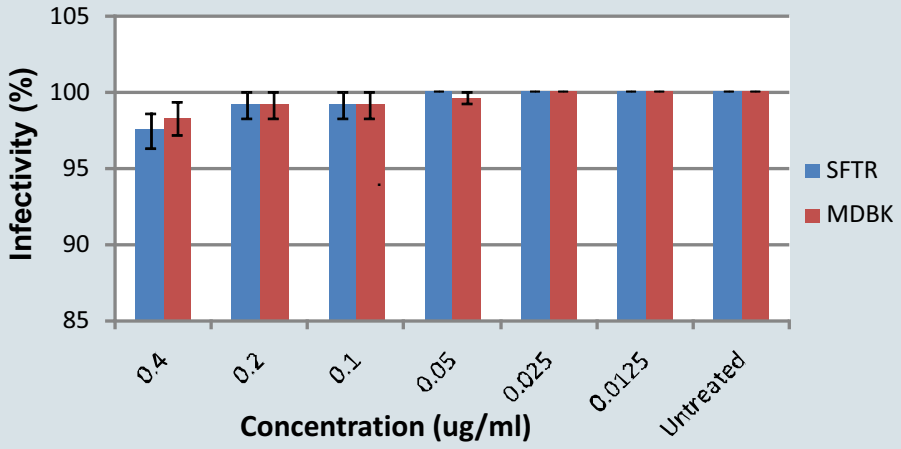


Fig. 17: Effect of cytochalasin D (various concentrations) treatment before infection on infectivity.

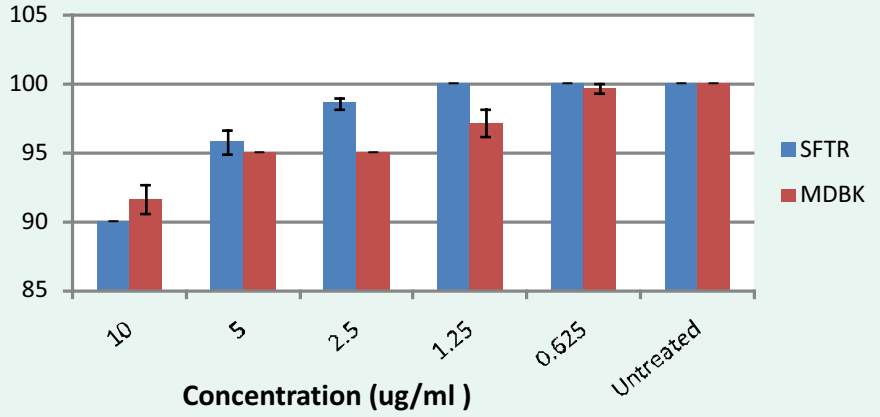


Fig. 18: Effect of nystatin (various concentrations) treatment before infection on infectivity.

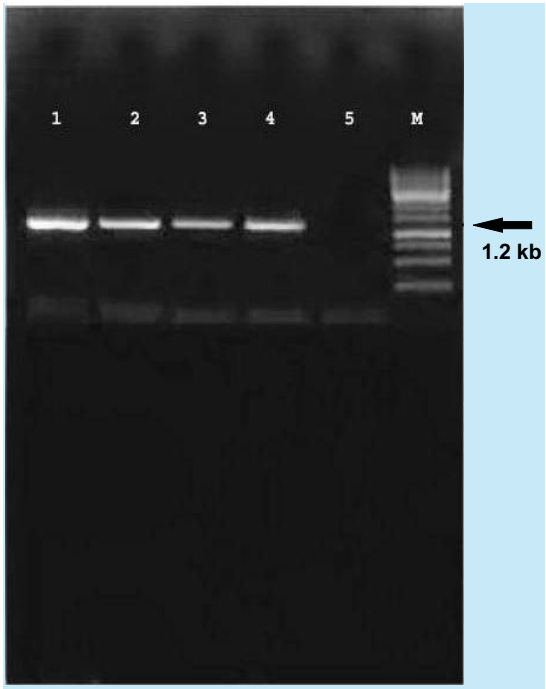


Fig. 19: BFA treatment inhibited the BVDV secretion from ovine cells in a dose-dependent manner

The results of BVDV infectivity assay using immunochemistry showed that only a slight inhibitory effect (0-9.4%) was observed on the entry and infectivity of BVDV in both ovine and bovine cells when treated even with the highest concentration (10 µg/ml) of caveolae disrupting drug nystatin (Fig. 18). These results showed that BVDV does not use caveolae-mediated endocytosis for ovine or bovine cell entry during infection.

To characterize the intracellular route taken by mature BVD virions in ovine cells for secretion, brefeldin A (BFA) was used, which blocks the transport of newly synthesized proteins from the ER to the golgi complex. Since a 20 h incubation with >2 µg/ml BFA resulted in visible cytotoxic effects in ovine cells, a lower (2 or <2 µg/ml) concentrations of BFA was used with reduced ~~the~~ time of drug exposure (16 h). As revealed by RT-PCR assay, BFA treatment inhibited the BVDV secretion from ovine cells in a dose-dependent manner (Fig. 19). The highest BFA concentration used (2µg/ml) completely inhibited ncp BVDV secretion without affecting the SFT-R cell viability, since no infectious BVD virus was detected in extracellular fluid obtained from infected cells treated with 2µg/ml of BFA by virus titration (Fig. 20). The results suggested that BFA inhibits release of BVDV in ovine cells without affecting BVDV assembly and infectivity.

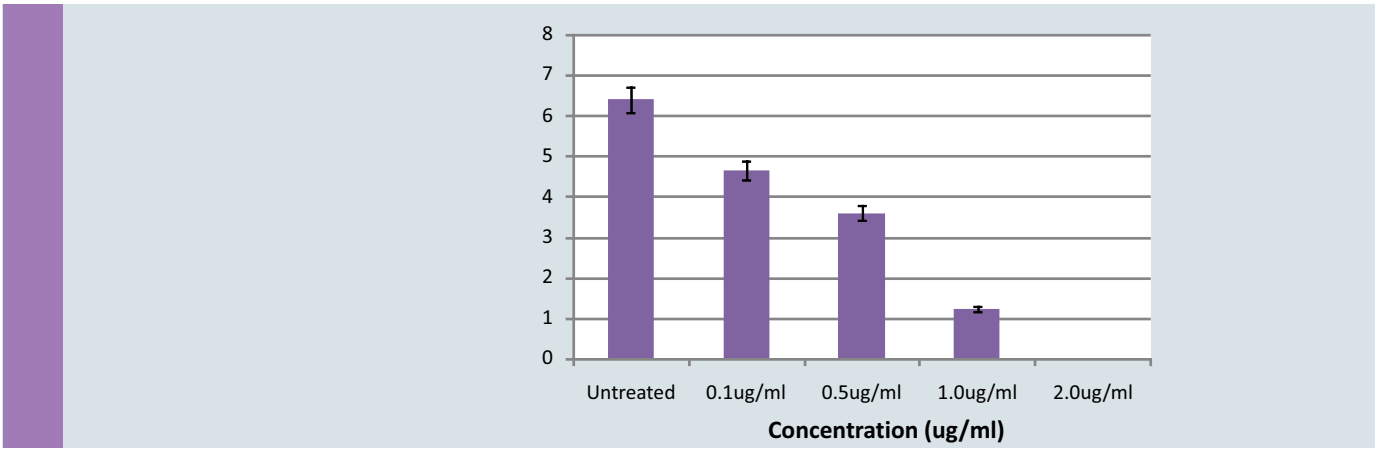


Fig. 20: BVDV-1 infectivity titre in tissue culture fluids collected at 24 h p.i. from BFA treated and untreated SFTR cells. (Columns represent mean values of three independent experiments assayed in duplicate with error bars

Evaluation of diagnostics tests

BVDV E^{rns} based antigen ELISA is able to detect the highly divergent Indian BVDV-3 strains

Evaluation of BVDV antigen ELISA using clinical samples and isolates demonstrated that commercially available BVDV E^{rns} based antigen ELISA is able to detect the highly divergent Indian BVDV-3 strains, but NS3 based antigen ELISA fails to detect them. Hence, it is recommended that for detection of BVDV persistently infected animals E^{rns} based antigen ELISA which has the ability to detect BVDV-1, BVDV-2 and BVDV-3 viruses.

Real time RT-PCR is more useful than BVDV Ag-ELISA for identification of BDV in sheep

BDV was isolated from eight sheep of the same flock in Jammu and Kashmir state, that were initially tested positive for BDV by real time RT-PCR but negative by BVDV Ag-ELISA. Failure of detection of BDV antigen in blood of sheep and even the isolates by one of the commercially available BVDV Ag-ELISA kits has significant implications with regard to pestivirus diagnosis and control in ruminants, as a few cases of natural BDV infection have also been reported in cattle. The low sensitivity of BVDV Ag-ELISA in detecting BDV compared to BD virus isolation has also been reported. Although it is not surprising that BVDV Ag-ELISA specific for BVDV does not cross react with BDV, it is pertinent to suggest that until a suitable validated BDV Ag-ELISA becomes available, either virus isolation or real time RT-PCR should be used for detection of BDV in field samples.

BVDV NS3 recombinant antigen based indirect ELISA detects pestivirus antibodies in sheep and goats

Detection of pestivirus antibodies is still the most rapid and cost effective method for determining exposure to pestiviruses in unvaccinated herds. Virus neutralization test (VNT) is considered as the gold standard test for serological diagnosis of pestivirus infections. However, it can be performed only in specialized laboratories besides being labor and time intensive. Since the NS3 protein is immunodominant and highly conserved among pestiviruses, it is a preferred target to develop immunoassays for detection of pestivirus antibodies. The 1152 bp DNA fragment of NS3 gene of Ind S-1449 was cloned in pTriEx-2-Neo expression vector (Novagen) and expressed in ***E.*** *coli* strain of Origami (DE3) pLac1. Expressed NS3 protein was purified by His-bind affinity chromatography under denaturing conditions (Novagen) and eluted in elution buffer (1M imidazole in 0.5 M Nacl, 20mM Tris-Hcl, pH 7.9). The concentration of purified Rc-NS3 protein was estimated using Qubit® fluorometer. The purified 50 kDa recombinant NS3 protein was used as the coating antigen in the ELISA. The optimal concentration of antigen was 320 ng/ well at a serum dilution of 1:20 and the optimal positive cut-off optical density (OD) value was 0.40 based on test results of 418 VNT negative sheep and goat serum samples. A total number of 619 serum samples from sheep and goats were subjected to Rc-NS3 ELISA and the cut-off value was calculated based on the results of BVDV/BDV VNT positive (n= 201) and negative (n= 418) serum samples, a mean O.D. value of 0.220 with a standard deviation of 0.089 was obtained. Hence, the cut-off value was set at O.D. value of 0.40.

A total of 569 field sheep and goat sera were tested for presence of virus neutralizing antibodies against BVDV-1 or BVDV-2 by VNT using 200 $TCID_{50}$ of BVDV-1 cattle isolate Ind S-1449, BVDV-2 sheep isolate Ind 51966 in SFT-R cells. 168 (36.28%) sheep and 19 (17.92%) goats were found positive for BVDV antibodies by VNT. The same sera were tested with rc-NS3 ELISA which showed a sensitivity of 91.71% and specificity of 94.59% as compared to VNT (Table 5).

Table 5: Sensitivity and specificity of Rc-NS3 indirect ELISA in comparison with VNT.

		Sheep VNT			Goat VNT		
		Positive	Negative	Total	Positive	Negative	Total
Rc- NS3 ELISA	Positive	152[a]	16[b]	168	14[a]	5[b]	19
	Negative	15[c]	280[d]	295	0[c]	87[d]	87
	Total	167	296	463	14	92	106
	Sensitivity	91.02% (95% CI-85.71, 94.48)			100 (95% CI-78.47,100)		
	Specificity	94.59% (95% CI-91.4, 96.65)			94.57 (95% CI-87.9, 97.66)		
	Agreement	93.3% (95% CI-90.65, 95.24)			95.28% (95% CI- 89.43, 97.97)		
	Kappa value	0.856 (95% CI-0.81, 0.91)			0.82 (95% CI-0.67, 0.97)		
prevalence	VNT	36.07 %			13.21 %		
	Rc-NS3 ELISA	36.29 %			17.92 %		

The sensitivity and specificity of Rc-NS3 indirect ELISA developed and evaluated correlated well with the reference virus neutralization test for detection of BVDV antibodies in sheep and goats. Being a simple and rapid test, the developed NS3 ELISA may be useful during sero-monitoring of pestivirus infections in sheep and goats.

Swine diseases

Pig rearing is one of the important occupations for many sectors of society in India, providing a source of income and a choice of meat for consumption. As per the 19th livestock census, the total pig population in India is about 10.29 million, out of which almost 40% is concentrated in the North-Eastern Region. In the past few decades, a number of viral diseases have emerged in the global swine population. These constantly pose a major concern for swine health and have significant economic impact on the swine industry. While some emerging viruses of pigs such as porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) cause economically important diseases only in pigs, others like Nipah virus, Bungowannah virus and Menangle virus pose important zoonotic threat to humans also. In line with the mandate of the institute, the current focus of research on swine diseases include PRRS and swine influenza. PRRS is an economically important transboundary viral disease of pigs characterized by reproductive problems in the adults and respiratory problems in piglets. It is caused by PRRS virus belonging genus *Arterivirus* of family *Arteriviridae.* The first outbreak of PRRS in India was recorded in 2013 in Mizoram. Apart from the routine diagnostic services offered by NIHSAD for detection of PRRS, the current areas of research work include genetic characterization and molecular epidemiology of PRRSV isolated from North Eastern states, development of diagnostic assays, evaluation of pathogenic potential of Indian PRRSV, understanding its pathogenesis and evaluation of prospects of an inactivated vaccine for PRRS.

Swine influenza is an acute respiratory infection of pigs caused by one of the several subtypes of Type A and C influenza viruses under the family *Orthomyxoviridae*. Pigs play an important role in epidemiology and transmission of influenza viruses due the presence of siali cacid receptors for both avian and mammalian influenza viruses, and act as “mixing vessels”, mediating the emergence of novel influenza viruses to human population with pandemic potential. The current area of work is on diagnosis of swine influenza in suspected samples and genetic characterization of the virus isolates.

Recently, with the emergence of Porcine Epidemic Diarrhea Virus (PEDV) and African Swine Fever virus (ASFV) as the major threat to the global swine industry, the future efforts of NIHSAD will be on the diagnostic preparedness for these two diseases to facilitate their quick detection, confirmation and control in case of their occurrence in India.

Porcine Reproductive and Respiratory Syndrome (PRRS)

Genetic characterization of Indian PRRS viruses isolated from Mizoram and Meghalaya states indicate the possibility of independent introductions

Two PRRSV isolated in 2014, Ind-299830 from Mizoram and Ind-299983 from Meghalaya, were genetically characterized. Whole genome sequence of the Mizoram isolate was derived through nucleotide sequence assembly of various RT-PCR amplicons covering the entire length of PRRSV genome. About 4.2 kb of Meghalaya isolate genome in ORF1, ORF6 and ORF7 regions was also sequenced. Analysis of

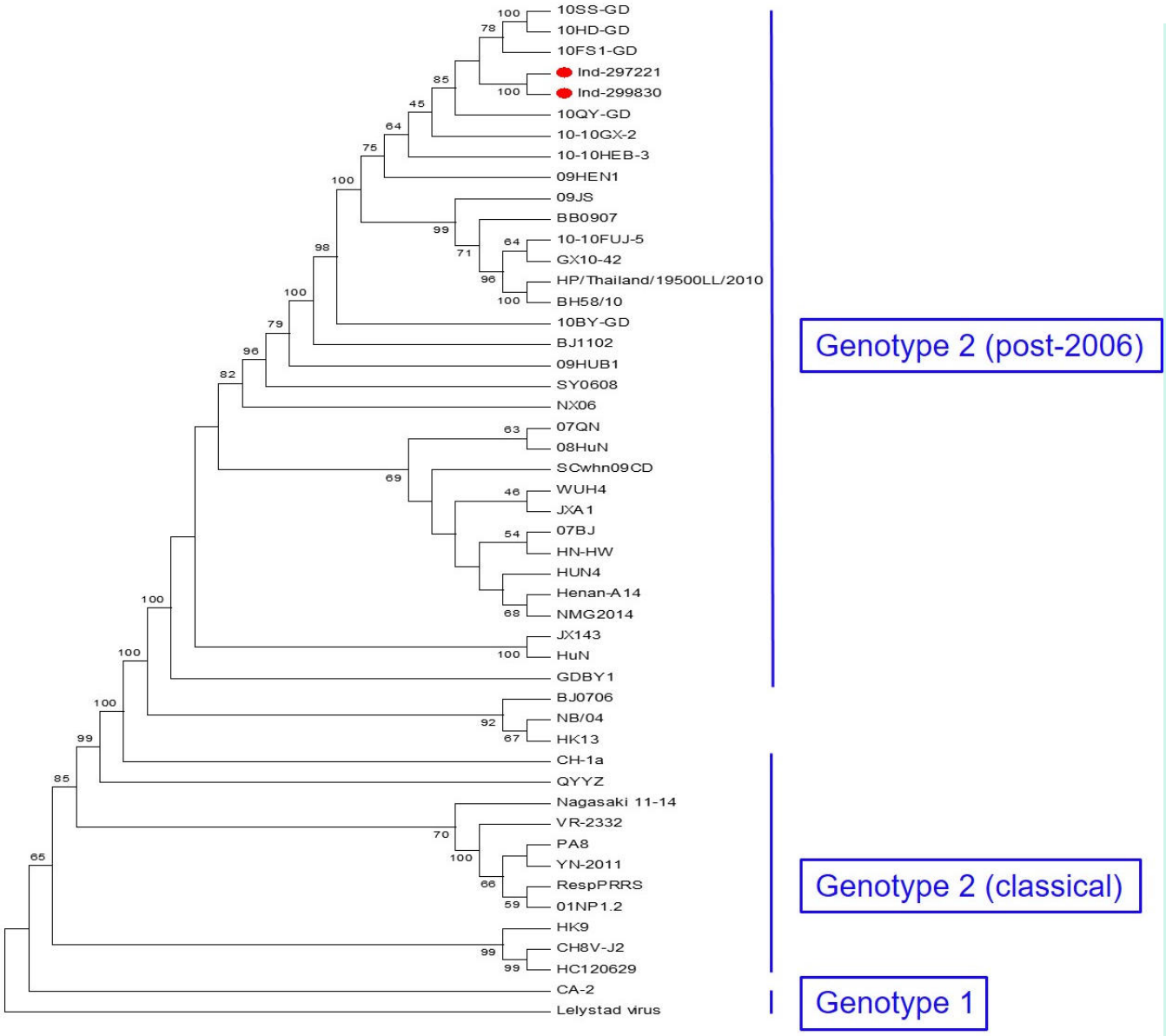


Fig. 21: Phylogenetic analysis of Indian PRRSV (based on complete genome)

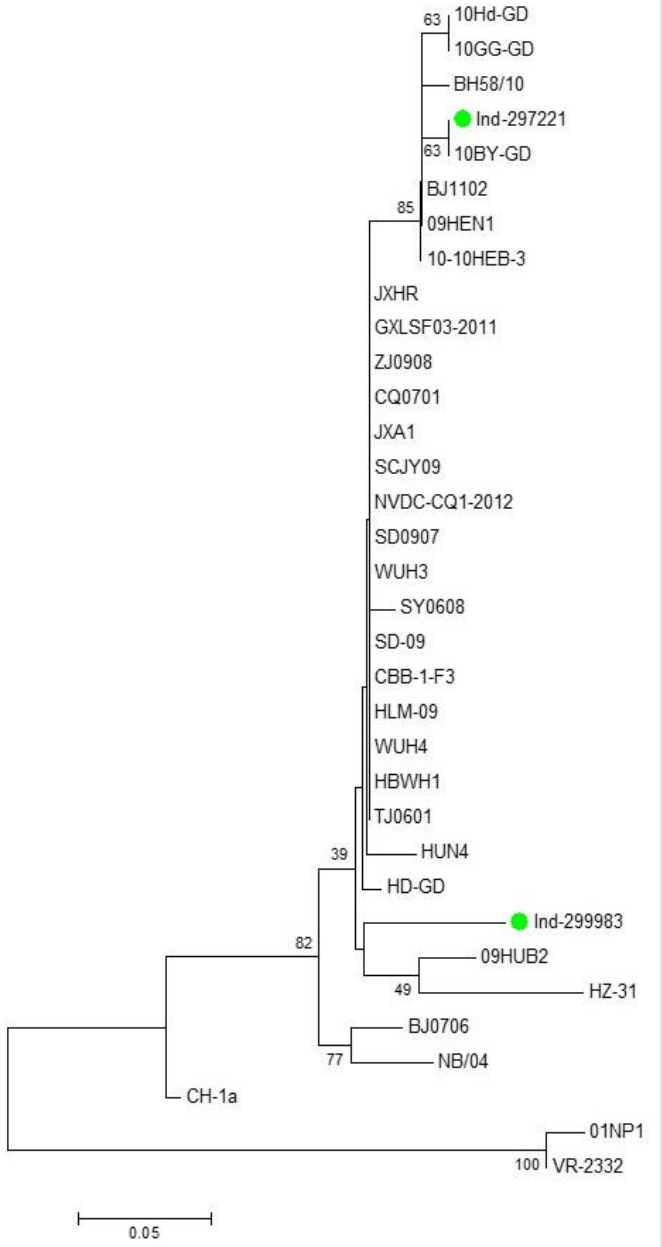


Fig. 22: Phylogenetic analysis of Indian PRRSV (based on ORF 7)

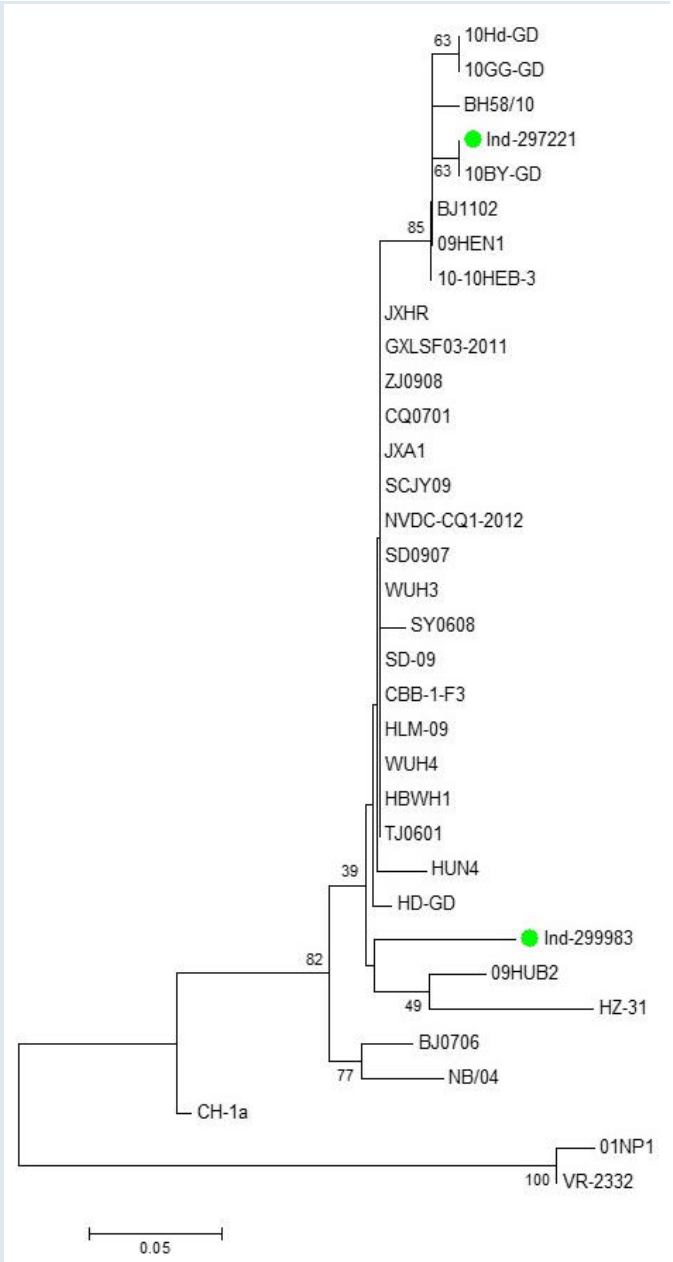


Fig. 23: Phylogenetic analysis of Indian PRRSV (based on partial nsp-2)

the nsp-2 of both the isolates showed the presence of the 30-amino acid-deletion typical for highly pathogenic PRRSV (HPPRRSV) as seen in the first Indian PRRSV isolated in 2013. Genetic analysis of the complete genome of the two PRRSV isolates from Mizoram (Ind-297221 and Ind-299830) showed very little divergence between the two, with a sequence identity of 99.7%. In the phylogenetic tree for complete genome, both the Mizoram isolates were grouped together with highly pathogenic PRRSV genotype 2 isolated from China around the year 2010 (Fig. 21). Analysis of percent identity matrix of selected PRRSV isolates showed that the Indian PRRSV from Mizoram shared sequence identity between 88 and 93% with the classical genotype 2 isolates (VR-2332, PA-8 and CH1a), 97 to 98% with post-2006 HPPRRSV isolates and 59% with genotype 1 prototype Lelystad virus. Genetic analysis of the Meghalaya isolate in ORF 7 and partial nsp-2 coding regions showed the presence of nucleotide sequence homology of only about 96%

between the Meghalaya and Mizoram isolates. They were found to group separately in the phylogenetic trees for ORF 7 and partial nsp-2 (Fig. 22 and 23). Interestingly, in the nsp-2 tree, Ind-299983 grouped closer to PRRSV strain HZ-31 which is known to be a novel variant. This may suggest that the Indian PRRSV isolated from Mizoram and Meghalaya states evolved independent of each other and that they may have separate introductions. Further genetic analysis is underway in this direction.

Diagnostics and vaccines

Optimization of a single dilution immunoperoxidase monolayer assay for detection of PRRSV genotype-2 antibodies in porcine serum samples.

Immunoperoxidase monolayer assay (IPMA) was optimized on MARC-145 cells for detection of PRRSV genotype 2 antibodies. Indian PRRSV isolate Ind-297221 was adapted to grow on MARC-145 cells through twenty serial passages and the $TCID_{50}$ was estimated to be $10^{5.8}$. For the IPMA, sub-confluent monolayers of MARC-145 cells were infected with the adapted virus for 48 to 72 h in 96-well cell culture plates. At the end of incubation, cells were fixed by standard protocol (OIE manual, 2008) with minor modifications. Preliminary optimizations of various parameters such as virus inoculum, serum dilution, conjugate dilution, incubation time, etc was done using a set of 8 reference serum samples of known status comprising of two each of genotype 1 positive, genotype 2 positive, false positive and negative sera. Immunodetection was carried out using anti pig IgG- HRPO conjugate and H_2O_2/AEC substrate-chromogen combination. Serum samples containing PRRSV antibodies were identified by the presence of red stained foci of cells in PRRSV infected monolayer, while the negative samples showed no such stained foci (Fig. 24 and 25). A total of 383 field serum samples were tested in four dilutions (1/10, 1/20, 1/40 and 1/80) and the results were compared with that of a commercial ELISA kit (Idexx laboratories) with a recommended cut-off S/P value of 0.4 for a positive sample. The 1/20 dilution was found to be optimum. Thirty three out of 383 serum samples which were detected as negative by ELISA were found to be positive for PRRSV antibodies by IPMA. The mean S/P value of the IPMA positive-ELISA negative serum samples was 0.27 ± 0.09. The false positive samples were found to be negative by IPMA. The kappa value for agreement between

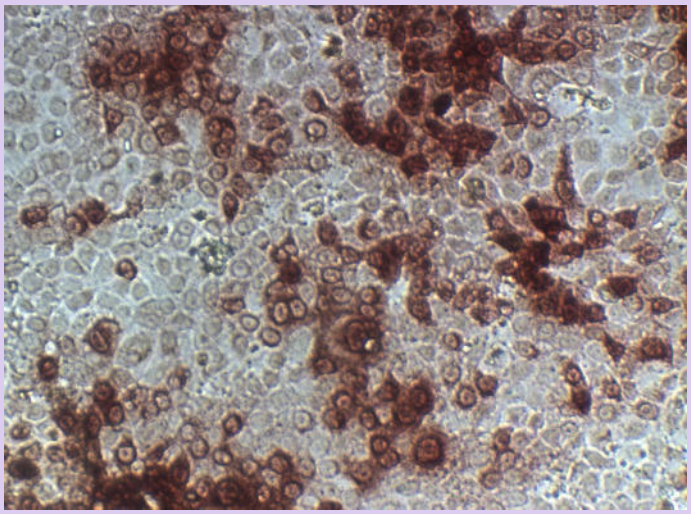


Fig. 24:PRRSV antibodies identified by the presence of red stained foci of cells in PRRSV infected MARC-145 cells.

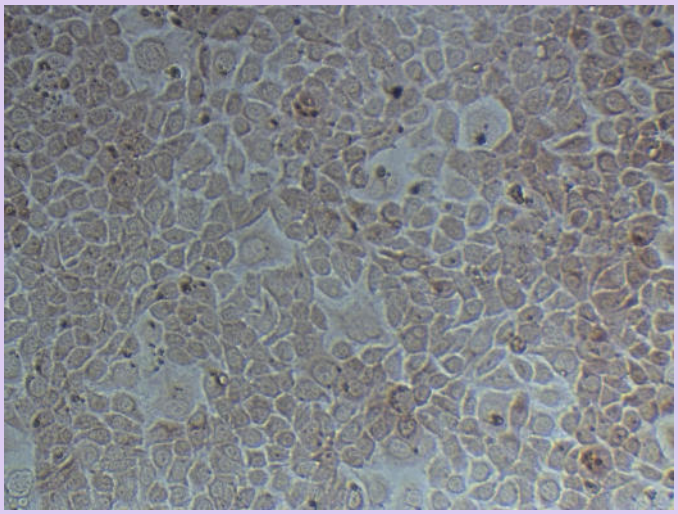


Fig. 25: Unstained foci in antibody negative samples.

two tests was found to be 0.746 ± 0.04. Although it requires more time to perform, the optimized IPMA was found to be more sensitive and specific for detection of PRRSV genotype 2 antibodies. Since the assay has been optimized for single serum dilution testing, it is suitable for large scale screening.

Host pathogen interaction

Piglets experimentally infected with Indian PRRS virus show clinical effects between 2 and 8 days post infection

The pathogenic potential of Indian PRRSV isolate was assessed in experimentally infected piglets. Six randomly selected PRRS free 6-week-old piglets were intra-nasally infected with 2 ml of clarified cell culture supernatant containing $10^{5.8}$ $TCID_{50}$ per ml of Indian PRRS virus, Ind-297221. Two piglets served as mock-infected controls. All the piglets were observed daily for clinical signs until euthanasia on 7 or 14 days post infection (dpi). Rectal temperature was recorded daily. Blood and nasal swabs were collected daily up to 7 dpi and on alternate days between 8 and 14 dpi. In the infected group, clinical signs (Fig. 26) were observed between 2 and 8 dpi. They included dullness, inappetance, high fever up to 105.2 °F, nasal discharge, cyanosis or hyperaemia of skin over inner and outer side of the ears and inner thighs, papules in the ventral abdomen, inner thighs and lower hind limbs, shivering, periorbital oedema and congestion of conjunctival mucosa. The PRRSV infected piglets had a biphasic increase in rectal temperature with an initial peak at 2 dpi and a second peak between 5 and 8 dpi. The mean rectal temperature of PRRSV infected and control piglets are shown in Fig. 27. Total leukocyte count was reduced significantly between 2 and 5 dpi in infected animals as compared to pre-infection values and uninfected controls (Fig. 28). PRRSV specific antibodies in serum could be detected from 8 dpi onwards by immunoperoxidase monolayer assay. Real time RT-PCR showed that the highest viral load in serum was on 5 dpi. Three infected and one control piglets (per interval) were euthanized for necropsy on 7 and 14 dpi. Gross lesions (Fig.29) included enlargement of spleen, petechial haemorrhage in spleen, focal consolidation in lungs, enlarged and polycystic mesenteric lymph nodes, atrophic mesenteric lymph nodes, increased peritoneal fluid, vascular congestion in pancreas, pale necrotic foci in kidneys. Further work is being carried out to elucidate the effect of PRRSV infection on host tissues and immune responses.

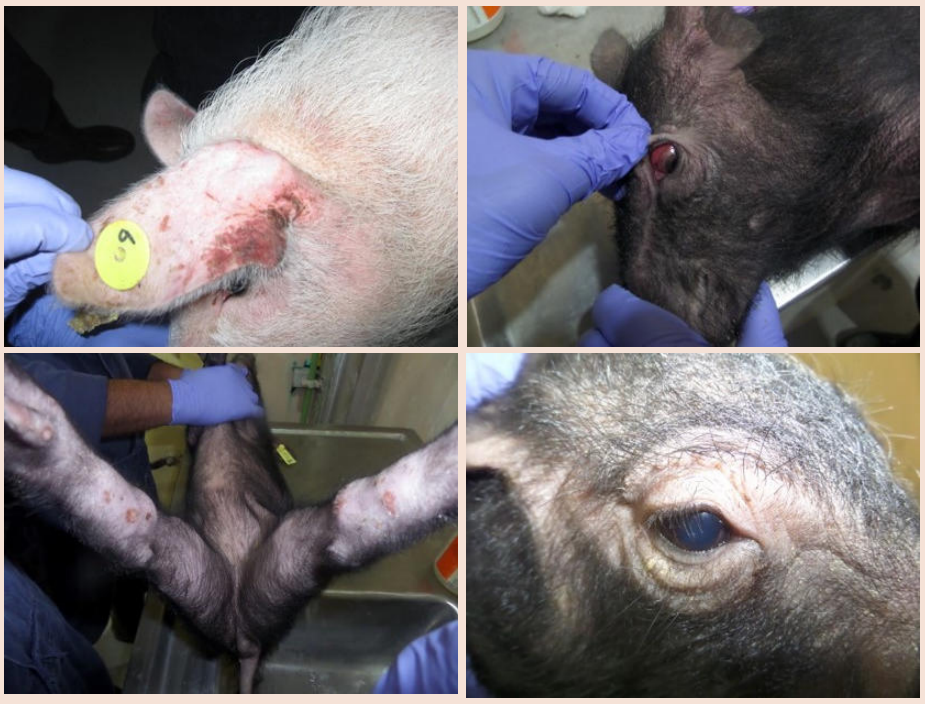


Fig. 26: Clinical signs observed in experimental PRRSV infection.

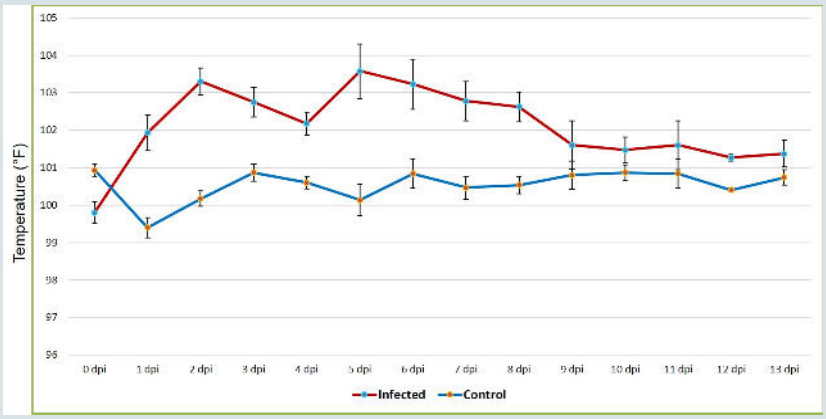


Fig. 27: Mean rectal temperature of PRRSV control and infected piglets.

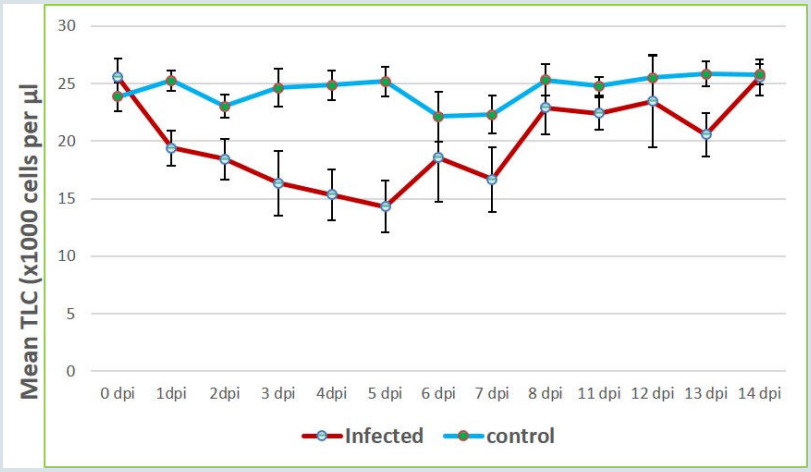


Fig. 28 Mean total Leucocyte count of infected and control animals.

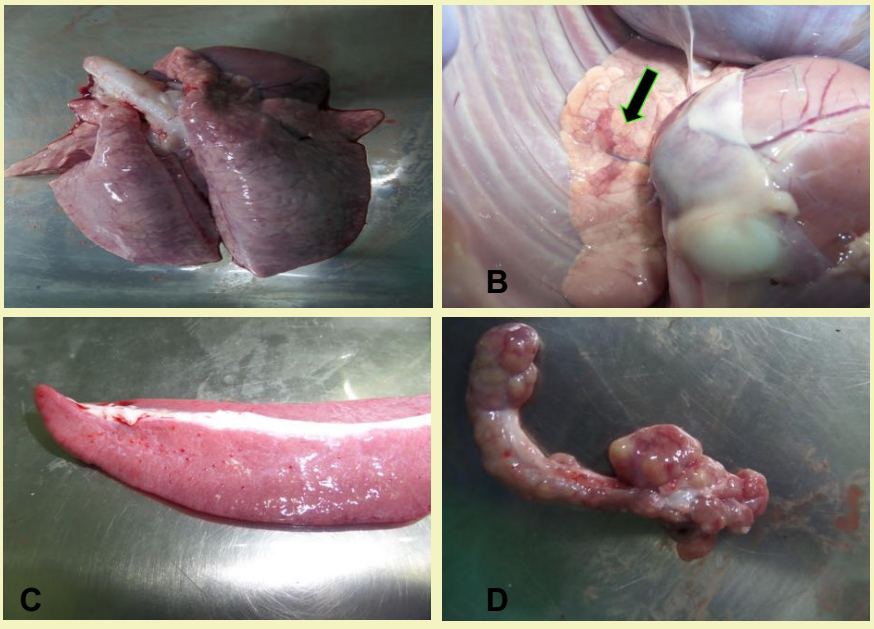


Fig. 29: Gross lesions observed in piglets experimentally infected with Indian PRRS virus

a. Petechial haemorrhage in spleen
b. Focal consolidation in lungs
c. Consolidation of lungs with emphysema
d. Enlarged and polycystic mesenteric lymph nodes

Pathogens that infect multiple species usually cause emerging and reemerging diseases since there is prolonged persistence of infectious agents in the environment and enhanced chances of spillover outbreaks in newer host species including humans. Transmission across the species barriers provides the pathogens opportunities to genetically evolve into more virulent or drug resistant forms and also to spread over larger geographic areas as well as into newer hosts. Zoonotic pathogens are the most significant ones amongst the multispecies pathogens and draw enormous attention under the one health concept. While some of the zoonotic pathogens affect both humans as well as their animal host species, others cause fatal human diseases but only subclinical infections in their animal hosts, which facilitate their evolution, amplification, environmental persistence and transmission. Diagnosis, molecular epidemiology, evolutionary characterization of such zoonotic viruses in animal populations, play an important role in the development of control and prevention strategy in human as well as animals.

For tick borne infections like Crimean Congo Haemorrhagic Fever (CCHF) and Kyasanur Forest disease (KFD) screening of tick populations are mandatory to find the footprints of the infection as they spread into newer geographic areas of the country. With a socio-cultural set up where usually livestock and human share living space, MERS-CoV in camels and CCHFV in ruminants cannot be ignored inspite of the fact that they do not affect the animals but cause fatal disease in Humans. Similarly, bats being peri-urban species with migratory habits, the information on the prevalence of Nipah and Reston Ebola viruses in bat populations need constant monitoring and surveillance for development of precision control measures. This becomes all the more urgent in view of the reports, of regular Nipah outbreaks and bats showing seropositivity for Reston Ebola virus from Bangladesh. Such threats reiterate the need for development of rapid diagnostic and sero-monitoring tools adaptable to Indian conditions along with a constant vigil over the status of prevalence in the different species that are affected. In addition to specific virus based epidemiological study, a metagenomics approach to catalogue the comprehensive virus ecology in various host species is required to keep pace with the trends of virus evolution.

Epidemiology

Seroprevalence of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) among domestic ruminants in Rajasthan

Crimean-Congo hemorrhagic fever (CCHF) is a tickborne disease caused by CCHF virus (CCHFV) of the genus *Nairovirus* of the family *Bunyaviridae* causes a fatal hemorrhagic illness in humans. CCHF was first confirmed in a nosocomial outbreak in Gujarat in 2011. During 2012–2015, several outbreaks and cases of CCHF transmitted by ticks via livestock and several nosocomial infections were reported in the states of Gujarat and Rajasthan. This disease is asymptomatic in animals. However, anti-CCHFV antibodies were detected in domestic animals from the adjoining villages of the affected area, indicating considerable seropositivity in domestic animals.

The present sero-survey was carried out to determine the seroprevalence of CCHF in cattle, buffalo, sheep and goat populations of 3 districts (Bikaner, Churu, Hanumangarh) in northern division of Rajasthan, India. A total of 410 random serum samples from these animals were screened for CCHFV antibodies using a enzyme-linked immunosorbent assay (ELISA) kit developed by NIV Pune India. Overall, 13 (9.42%) of 138 cattle samples,

28 (30.43%) of 92 buffalo samples, 50 (51.02%) of 98 sheep samples and 18 (21.95%) of 82 goat samples tested positive for CCHFV IgG antibodies.

Seropositivity levels found in these animals suggested circulation of CCHFV among livestock in these districts of Rajasthan. It would be appropriate to extend the CCHF surveillance to include other regions of the state and to study the distribution of the virus in ticks of the region to better predict and respond to CCHF outbreak in future.

Serological screening suggests presence of antibodies against simbu serogroup viruses in cattle, buffalo, sheep, goat, horse and camel in Rajasthan, Gujarat and Madhya Pradesh states of India

The Simbu serogroup of the genus *Bunyavirus*, family *Orthobunyaviridae* contains more than 25 viruses. Some viruses from the Simbu serogroup have been isolated; Sathuperi virus in 1957, Kaiklur virus in 1971 and Oyavirus from Karnataka (unpublished) and reported from India (Saeed et al., 2001; Rodrigues et al., 1977). Cross reactivity between various simbu serogroup viruses have also been reported. For the serodiagnosis of these viruses schmallenberg kit is available commercially which was employed for screening livestock population in India. Schmallenberg virus (SBV), a recently emerged *Orthobunyavirus*, is associated with abortions, stillbirths and congenital malformations in ruminants. Considering that *Culicoides* species which transmit this disease have previously been identified in India as vectors of bluetongue, another livestock disease that causes abortions, it is speculated that SBV also might be circulating in the Indian ruminant population.

In this study, we conducted a serological study to investigate the occurrence of anti-SBV antibodies in cattle (N=216), buffalo (N=160) sheep (N=118), goat (N=49), horse (N=11) and camel (N=22) in the Rajasthan, Gujarat and Madhya Pradesh states using a commercial Multispecies Competitive ELISA kit (IDVet) that detects antibodies against recombinant SBV nucleoprotein in animal sera. The results show a high percentage of antibody-positive animals. This initial serological screening suggests that SBV is possibly present in the Indian subcontinent. However, there is also the probability of cross-reactivity with other Simbu serogroup viruses, especially considering that some of these viruses have previously been reported in India. Further studies to confirm these preliminary findings using serum neutralisation assay, viral isolation or detection of SBV RNA from ruminants or *Culicoides* are underway.

Diagnostic Development

Expression of recombinant nucleoproteins of Hantaan, Nairobi Sheep Disease and Crimean-Congo Hemorrhagic Fever Viruses

The nucleotide sequence of complete CDS of nucleoprotein genes of Hantaan and Nairobi Sheep Disease Viruses retrieved from NCBI genbank database were codon optimized for expression in prokaryotic expression host system. The codon optimized genes were chemically synthesized from a commercial source and placed in frame in pET-32a vector. The CCHF virus nucleoprotein gene was PCR amplified from tick samples received from Gujarat for post human outbreak surveillance. The amplicon was cloned in pET-28a and pET-32a expression plasmids.

The expression plasmid constructs were transformed into the expression host, BL21 (*DE3*) pLysS, under host and vector-selective antibiotic pressure using TransformAid Kit (Fermentas). Transformed colonies were observed after incubation at an optimum

temperature-time combination and sub-cultured individually in LB broth under selective antibiotic pressure. The overnight broth cultures were again sub-cultured (1:100) in fresh LB broth containing the selective antibiotics and incubated till OD_{600} of 0.6 was achieved. The cells were induced by 1mM IPTG for 6 hrs at 30˚C.

The recombinant nucleoprotein expression (rNP) was confirmed by SDS-PAGE and western blot analyses that reveled proteins of expected size (Fig 30 and 31). The rNP of CCHFV was characterized for its reactivity with sera of different livestock species which revealed its specific reactivity with CCHFV antibody positive cattle, buffalo, sheep and goat sera (Fig 32).

Prokaryotic Expression of the Nucleocapsid Protein of Schmallenberg Virus

Schmallenberg virus (SBV) is a novel orthobunyavirus that primarily infects ruminants such as cattle, sheep and goats. Infections with SBV are associated with congenital malformations in ruminants, abortions in the third trimester, central nervous system damages and musculoskeletal abnormalities. SBV is an enveloped virus that contain a tri-segmented negative-sense RNA genome, which encodes a large RNA-dependent RNA

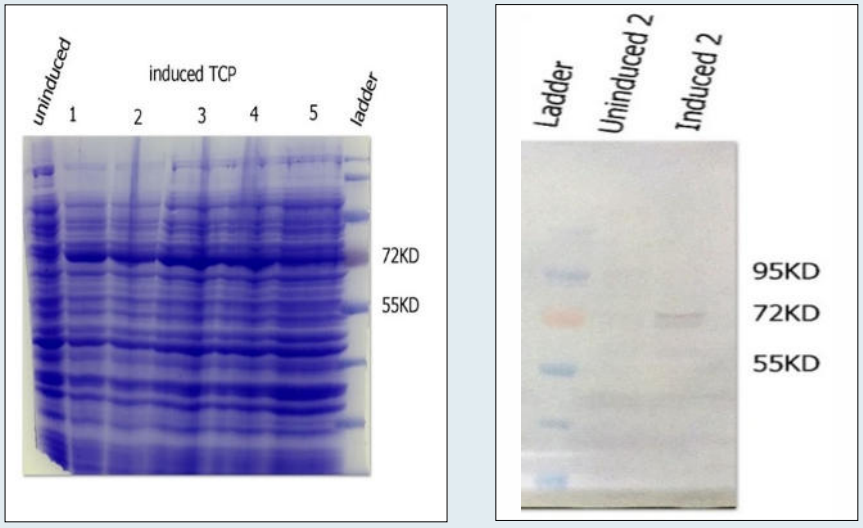


Fig. 30 SDS-PAGE and Western Blot analysis (using anti-His-antibody) of recombinant nucleoprotein of Hantaan Viru0 (~70KD) expressed from pET-32 series of Vector in Rosetta-gami (DE3) pLysS *E coli* expression hosts.

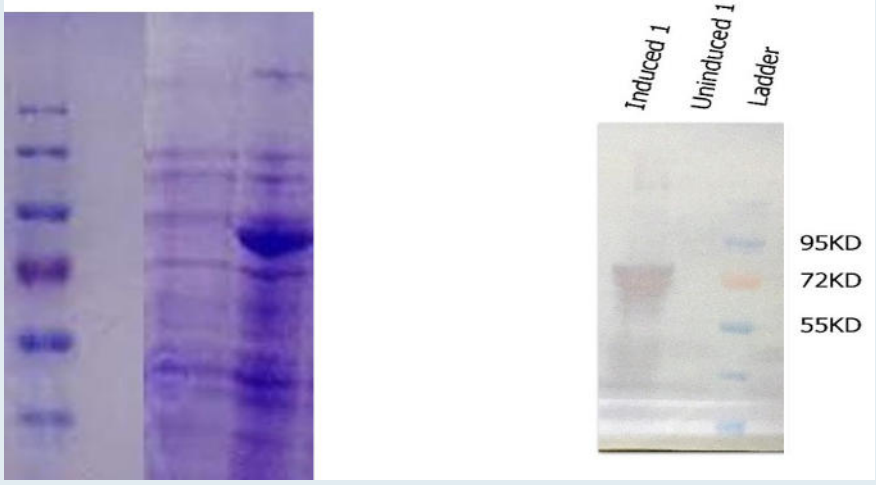


Fig. 31 SDS-PAGE and Western Blot analysis (using anti-His-antibody) of recombinant nucleoprotein of NSD Virus (~80KD) expressed from pET-32 series of Vector in Rosetta-gami (DE3) pLysS *E coli* expression hosts

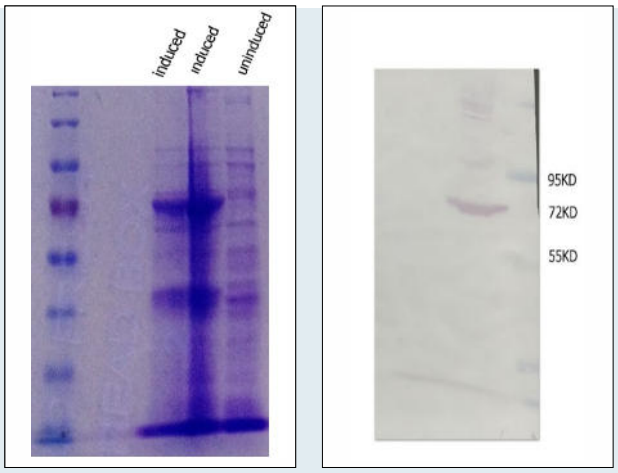


Fig. 32 SDS-PAGE and Western Blot analysis (using anti-His-antibody) of recombinant nucleoprotein of CCHF Virus.

polymerase (L protein or RdRP) on the large (L) segment, a polyprotein precursor that comprises two envelope glycoproteins (Gc and Gn) and a non-structural protein (NSm) on the medium (M) segment, and also a nucleocapsid protein (N protein) and a nonstructural protein (NSs) on the small (S) segment. The nucleocapsid (N) protein of SBV has been shown to be an ideal target antigen for serological detection. The present study was conducted to prokaryotically express the nucleocapsid (N) protein of SBV. The full-length synthetic coding sequence of the SBV N gene was PCR-subcloned from pUC 57 into the prokaryotic expression vector pET32a to construct the recombinant plasmid pET-32a-SBV-N. After verification using the double-enzyme cleavage method and sequence analysis, the recombinant plasmid pET-32a-SBV-N was transformed into the competent *E. coli* Rosetta-gami(DE3)pLysS cells which were then induced by IPTG to express as histidine (His)-tagged (His-SBV-N) fusion protein. The rNP expression was checked by coomassie brilliant blue staining of SDS-PAGE and western blotting using anti-His antibodies. Our results demonstrated that the recombinant His-SBV-N fusion protein was efficiently expressed in *E. coli* Rosetta-gami (DE3) pLysS in the form of both soluble protein and as inclusion bodies, and the molecular weight of His-SBV-N protein was 43 KDa. The successful preparation of His-SBV-N fusion protein lays a material foundation for the development of serological methods for SBV detection.

Diagnostic Preparedness

Ebola Virus

Ebola and Marburg virus are genera within the family Filoviridae and can cause a severe hemorrhagic fever with a relatively high mortality rate of 20-90% in humans. Diagnosis of Ebola virus disease relies on the detection of viral RNA in blood by real-time RT- PCR. While several of these assays were developed during the unprecedented Ebola virus disease outbreak in recent years, we adopted a commercially available real-time reverse-transcription PCR procedure (RealStar® Filovirus Screen RT-PCR Kit 1.0) for the diagnosis of Ebola virus disease in our institute .

Middle East Respiratory Syndrome Corona Virus (MERS-CoV)

The RT-PCR and realtime PCR assays recommended by WHO/OIE for detection of MERS-CoV genome in clinical samples were adapted at the institute. A specific recombinant antigen based indirect ELISA for the detection of antibodies against MERS-CoV has been standardized.

DIAGNOSTIC SERVICES

Avian Influenza

48,347 samples (38,882 morbid materials and 9,465 sera) were received from various parts of the country for avian influenza surveillance during this year.. The specimen-wise sample details and their results are shown in Table 6. The species-wise samples received and their results are presented in Table 7. The state wise samples received and their results are presented in Table 8 and 9. Out of 38,882 morbid/swab/fecal samples tested, 16 samples from three states (10 from Telangana, 5 from Tripura and 01 from Manipur) tested positive for H5N1 by RT-PCR, real time RT-qPCR and virus isolation. Out of these, 15 samples were from chicken and one sample from Tripura was from duck (Table 7). Further, 28 H9N2 (7 from Uttarakhand, and 21 from Gujarat) viruses were isolated (Table 9). A total of 9,465 random serum samples were tested, five (from chicken) and 288 (285 from chicken and 3 from ducks) serum samples were positive for antibodies to avian influenza virus subtype H5 and H9, respectively (Table 8).

The state-wise details of POSP samples received and their results are given in Table 10. Out of the 3170 samples recieved from five states , 103 sera were positive for H9N2 AIV antibodies (11 from Kerala, 2 from Manipur and 90 from Telangana) and five chicken serum samples from Kerala state were found to be positive for H5N1 AIV antibodies (Table 6).

Table 6: Specimen wise results of avian influenza surveillance (2015-16).

Specimen material	Samples Received	Positive samples			
		H5N1	H9N2	All other subtypes of AIV	NDV
Cloacal swabs/Fecal (POSP)	1156	-	-	-	-
Blood/Sera samples (Random/collected and emergency)	7632	-	185	-	NT*
Blood/Sera samples (POSP)	1833	5	103	-	NT*
Oro-pharyngeal/Tracheal swabs	4967	-	8	-	1
Cloacal swabs/Fecal	31090	-	13	-	-
Carcass/tissue/morbid samples (Emergency)	48	16	07	-	1
Oro-pharyngeal/Tracheal swabs (POSP)	181	-	-	-	-
Environmental sample (water/soil)	1393	-	-	-	-
Swab – species and type not mentioned	47	-	-	-	-
Total	**48347**	**21**	**316**	**0**	**2**

*- Not tested

Table 7: Species wise samples received for avian influenza surveillance (2015-16).

Species wise	Samples received	Positive samples			
		H5N1	H9N2	All other subtypes of AIV	NDV
Chicken					
Blood/Sera samples (POSP)	1623	5	101	-	NT*
Blood/Sera samples (Random/collected and emergency)	7630	0	184	-	NT*
Oro-pharyngeal swabs/Tracheal/Nasal	4949	-	8	-	-
Cloacal swabs/Fecal	29882	-	13	-	-
Carcass/tissue/morbid samples (Emergency)	33	15	7	-	1
Oro-pharyngeal swabs/ Tracheal (POSP)	181	-	-	-	-
Cloacal swabs/Fecal (POSP)	1118	-	-	-	-
Duck					
Blood/Sera samples (Random/collected and Emergency)	2	-	1	-	NT*
Blood/sera samples(POSP)	210	-	2	-	NT*
Oro-pharyngeal swabs/Tracheal/Nasal	18	-	-	-	1
Cloacal swabs/Fecal	12	-	-	-	-
Carcass/tissue/morbid samples (Emergency)	2	1	-	-	-
Cloacal swabs/Fecal (POSP)	38	-	-	-	-
Migratory Bird					
Oro-pharyngeal swabs/Tracheal	0	-	-	-	-
Cloacal swabs/Fecal	1196	-	-	-	-
Carcass/tissue/morbid samples (Emergency)	4	-	-	-	-
Peacock					
Carcass/tissue/morbid samples (Emergency)	3	-	-	-	-
Crow					
Carcass/tissue/morbid samples (Emergency)	3	-	-	-	-
Swab – species and type not mentioned	47	-	-	-	-
Environmental Samples (water/soil)	1393	-	-	-	-
Whale					
Carcass/tissue/morbid samples (Emergency)	2	-	-	-	-
Egret					
Carcass/tissue/morbid samples (Emergency)	1	-	-	-	-
Total	**48347**	**21**	**316**	**0**	**2**

* - Not tested.

Table 8: Statewise results of blood/serum samples screened for AIV antibodies: (2015-2016).

States	Samples received	Positive by AGID	Sub typing by HI test
Chandigarh	98	6	6 (H9)
Gujarat	280	50	50 (H9)
Haryana	655	42	42 (H9)
Himachal Pradesh	225	2	2 (H9)
Jammu & Kashmir	78	0	-
Kerala	64	16	5 (H5) ; 11(H9)
Madhya Pradesh	380	5	5 (H9)
Maharashtra	3475	28	28 (H9)
Manipur	727	0	2 (H9_ duck)
Punjab	1401	18	18 (H9)
Rajasthan	831	33	1(H9_duck); 33(H9);
Telangana	348	90	90 (H9)
Tripura	412	0	-
Uttar Pradesh	282	0	-
Uttarakhand	159	0	-
West Bengal	50	0	-
Total	**9465**	**290**	**285 (H9_Chicken)); 3 (H9_duck); 5 (H5)**

Table 9: Statewise results of tissue/swab samples screened for AIV (2015-2016).

State	Samples received	Positive samples	State	Samples received	Positive samples
Chandigarh	156	-	Manipur	852	1 (H5N1)
Goa	235	-	Odisha	3	-
Gujarat	16862	21 (H9N2)	Punjab	1747	-
Haryana	894	-	Rajasthan	4196	1 (NDV)
Himachal Pradesh	737	-	Tamil Nadu	2	-
Jammu & Kashmir	156	-	Telangana	234	10 (H5N1)
Kerala	117	1 (NDV)	Tripura	203	4 (H5N1); 1 (H5N1 - duck)
Madhya Pradesh	6468	-	Uttar Pradesh	141	-
Maharashtra	5771	-	Uttarakhand	108	7 (H9N2)
Total samples received - 38882			**Positives- 16 (H5N1); 28 (H9N2); 2 (NDV)**		

Table 10: State wise results of POSP samples screened for AI: (2015-2016).

State	Serum			Swabs			Total
	Received	AGID	Subtyping by HI test	Received	Positive*	Subtype	
Uttar Pradesh	282	-	-	141	-	-	423
Manipur	727	-	2 H9 (duck)	790	-	-	1517
Telangana	348	90	90-H9	174	-	-	522
Kerala	64	16	5-H5; 11-H9	34	-	-	98
Tripura	412	-	-	198	-	-	610
Total	**1833**	**106**	**108**	**1337**	**0**	**0**	**3170**

* - Positive by real time RT-qPCR, RT-PCR and virus isolation

Diagnostic services for SAARC Countries

Six samples from Bhutan were received for testing against AIV, of which five were positive for H5N1 AIV by RT-PCR and real time RT-qPCR.

Ruminant Diseases

Bovine viral diarhhoea/ Border disease

A total of 337 diagnostic samples from cattle, buffaloes, deer, elephant and imported biologicals (bull semen, embryo, FBS, ABS etc.) submitted by various Govt. and private agencies during 2015-16 were tested for BVDV/BDV following the OIE prescribed diagnostic tests. Testing of 123 diagnostic specimens from India revealed that semen from 3 cattle bulls in Tamil Nadu were positive for BVDV-2 by virus isolation and Real-time RT-PCR, while BVDV neutralizing antibodies were detected in 25 cattle of Gujarat and Tamil Nadu (Table 11).

All the 50 samples from imported biologicals tested negative for BVDV. Laboratory testing for BVD was carried out for blood and serum samples from 82 Danish Holstein and Jersey purebred bulls imported by NDDB and stationed at AQCS, Chennai and Kolkata in Dec. 2015 by real-time RT-PCR, BVDV antigen ELISA and antibody ELISA. All the animals were found free of BVDV infection (Table 12).

Table 11: Laboratory test results of BVD for samples from cattle in India (2015-16).

Type of Sample	State	Species	No. of sample	VNT positive	RT-PCR positive	Virus Isolation
Whole Blood	Gujarat	Cattle	18	NA	0	0
	Odisha	Elephant	2	NA	0	0
		Deer	2	NA	0	0
Serum	Tamil Nadu (TANUVAS)	Cattle	75	21	0	0
	Gujarat	Cattle	18	4	0	0
	Odisha	Elephant	2	0	0	0
		Deer	2	0	0	0
Tissue culture fluid	Karnataka (SRDDL)	Cattle	4	NA	3	3
Total			123	25	3	3

Table 12: Laboratory test results of BVD for imported cattle and biologicals (2015-16).

Type of sample	Country of origin	No. of sample	VNT positive	RT-PCR positive	virus isolation
Whole blood	Denmark	82	NA	0	0
Serum	Denmark	82	0	0	0
	Israel	1	0	0	0
	Australia	2	0	0	0
Frozen Semen straws	USA	29	NA	0	0
	France	9	NA	0	0
	Netherlands	2	NA	0	0
Embryos	Canada	7	NA	0	0
	Total	214	0	0	0

Malignant Catarrhal Fever

A total of 199 samples including imported cattle, buffalo, elephant, black buck, deer and sheep from AQCS, Bengaluru, Gujrat and Andhra Pradesh were recieved for diagnosis of MCF during 2015-16. OIE approved test for genomic detection of Ovine Herpesvirus 2, PCR and nested PCR was carried out for confirmation of the infection. 50 samples including that of cattle from Gujarat, and Bengaluru, buffalo from Andhra Pradesh were identified to be positive.

Table 13: Laboratory test result for Malignant Catarrhal Fever (2015-16).

Sample Type	place	Species	Total no of sample	Total no. of positive sample
Tissues	AQCS, Mumbai	Sheep/Pork	17	-
Whole Blood	AQCS Delhi	sheep	2	-
	SRDDL Bangalore	Cattle	4	-
	Forest officer, Raipur	Black buck	5	-
	Gujrat	Buffalo	5	5
	Vet College Tirunelveli	sheep	1	-
	Gujarat	Buffalo Cattle Mare	09 07 01	05 04 -
	AQCS Chennai	Cattle	47	-
	AQCS Kolkata	Cattle	35	-
	Bangalore	Cattle	05	04
	CADRAD IVRI	Cattle Sheep	04 02	- -
	Raipur	Black buck	03	-
	Vet college. Bhubaneshwar	Deer Elephant	01 01	- -
	Vet college, Andhra Pradesh	Buffalo	42	25
	Erode	Cattle	01	-
DNA sample	SRDDL Bangalore	Cattle	07	07
Total			**199**	**50**

Schmallenberg virus

A total of 82 bulls (HF and Jersey) imported from Denmark were screened for Schmallenberg virus antibody/antigen using competitive ELISA and real time RT-PCR were found negative.

Swine diseases

PRRS

- 102 imported porcine meat samples received from AQCS screened for PRRS were found to be negative for PRRSV by RT-PCR and/or virus isolation.
- 16 serum samples were received from Nepal for PRRSV genotyping. But none of them was positive for PRRSV by RT-PCR and virus isolation.

Swine influenza

- A total of 230 samples processed for swine influenza screening by HI test and virus isolation were found to be negative (table 14).

Table 14: Laboratory test results of Swine Influenza (2015-16).

State	Serum samples	Nasal Swabs	Tissues	HI test	Virus isolation
Uttar Pradesh	103	103	-	Negative	Negative
Madhya Pradesh	20	02	2	Negative	Negative
Total	**123**	**105**	**2**	Negative	Negative

Multiple Species Diseases

West Nile fever

Testing of 277 domestic duck serum samples from 3 districts (Alappuzha, Kottayam, Pathanamthitta) in Kerala by ELISA and virus neutralization test demonstrated serological evidence of West Nile virus infection in 46 ducks (16.6%) indicating circulation of WNV in the sampled region and its zoonotic implication. All the 1489 fecal/oral samples from wild birds in Kerala, Gujarat, Madhya Pradesh and Manipur were found negative for WNV by RT-PCR.

Table 15: Laboratory test results for NSD & RVF screening (2015-16).

SR.No.	AQCS Centre	Sample type	NSD	RVF	Result (RT-PCR)
01	Mumbai	Lamb Meat	18	02	Negative
02	Delhi	Lamb Meat	04	02	Negative
		Total	**22**		**24**

Crimean - Congo Haemorrhagic Fever

Post human CCHF outbreak, livestock and ticks sample lots (fourteen from Gujarat and one from Rajasthan) from the area surrounding index case areas were received from Gujarat and Rajasthan state animal husbandry departments for investigation. The 15 sample lots were comprised of 59 blood, 167 sera, 38 tick pools (Table No.16). The blood and tick pools were tested by one step realtime RT-PCR for presence of CCHFV genome. Of the 97 blood and tick pool samples , five tick pools, four from Kutch and one from Amreli districts of Gujarat were found positive. Likewise, the sera samples were tested by indirect ELISA for presence of anti-CCHFV antibodies. Of the 167 sera samples tested, 35 were positive.

Table 16: Laboratory test results of animal screening post human outbreak investigation.

Place	Sample Type	Species	No. of Sample	No. of sample RT-qPCR Positive	No. of sample IndirectELISA Positive
Nakhatrana, Kutch, Gujarat.	Blood	Sheep/Goat	05	00	-
	Serum	Sheep/Goat	05		00
	Tick pools	NA	02	00	-
Anjar, Kutch, Gujarat.	Blood	Bovine	08	00	-
	Serum	Bovine	08		**01**
	Tick pools	NA	01	00	-
Mandvi, Kutch, Gujarat.	Blood	Bovine	07	00	-
	Serum	Bovine	09		00
	Tick pools	NA	04	**04**	-
Liliya, Amreli, Gujarat.	Blood	Bovine	05	00	-
	Serum	Bovine	05		**04**
	Ticks	NA	04	**01**	-
Bachau, Kutch, Gujarat.	Serum	Bovine	33	-	**12**
Bayad, Sabarkantha, Gujarat.	Serum	Bovine	10	-	00
Mundra, Kutch, Gujarat.	Serum	Bovine	10	-	**06**
Mandavi, Gujarat.	Serum	Bovine	10	-	**02**
Jamnagar,Gujarat.	Serum	Bovine	05	-	**01**
Jamnagar,Gujarat.	Serum	Goat/Sheep	07	-	**06**
Nakhatrana, Kutch, Gujarat.	Blood	Bovine	10	00	-
	Serum	Bovine	10	-	00
	Tick pools	NA	01	00	-
Gondal, Rajkot, Gujarat.	Blood	Bovine	04	00	-
	Serum	Bovine	04	-	00
	Tick pools	NA	04	00	-
Ahmedabad, Gujarat.	Blood	Bovine	10	00	-
	Serum	Bovine	10	-	00
	Tick pools	NA	13	00	-
Mandavi,Kutch, Gujarat.	Blood	Sheep/Goat	10	00	-
	Serum	Sheep/Goat	10		00
	Tick pools	NA	01	00	-
Chittorgarh, Rajasthan.	Serum	Bovine	21	-	00
		Goat	10	-	**02**
	Tick pools	NA	08	00	-
	Total No. of Samples		**264**		

Lagomorph disease

Rabbit Haemorrhagic disease

A total of 27 serum samples received for the testing of rabbit haemorrhagic disease (RHDV) infection from the AQCS offices were tested negative using the INGEZIM Rabbit 1.7 RHD.K1 ELISA kit.

OTHER SERVICE ACTIVITIES

OFFLU Activity

Nucleotide sequences of 6 AIVs from India (subtypes H5N1: 04 and H9N2: 01) and Bhutan (subtype H5N1: 01) were shared with OFFLU for pre-pandemic vaccine composition meeting of the World Health Organization.

Maintenance of Virus repository

A total of 235 avian influenza viruses H5N1 (151), H9N2 (76), H6N2 (1), H4N6 (1), H3N8 (5) and H1N1 (1) and Newcalste disease virus (22) from the virus repository maintained at NIHSAD were revived.

Production of diagnostic reagents

A total of 163 ml of AGID antigen, 150 ml of antisera against six H5N1 viruses (DU/CA/14-11/100002 - Kerala – 23 ml, DU/CA/14-12/100005 - Chandigarh – 26 ml, CH/CA/15-04/100017 – Manipur – 23 ml, DU/CL/15-03/100017 – UP – 23 ml, CH/CL/11-03/100488 – Tripura – 10 ml, A/India/Nandurbar/7972/2006 – 10 ml and CR/CA/12-02/100003 – Bihar – 25 ml), 12 ml of H4N6 (DU/CL/11-11/100042), 11 ml of H3N8 (DU/CL/14-11/100009), 13 ml of H6N2 (DU/CL/14-11/100001) were produced for use in diagnosis of avian influenza.

Publications

Research papers

Research papers

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- Tosh C. and Nagarajan S. (2016) Control of Avian influenza in India: A Challenge. Presentation in Global Symposium on Animal Health: Newer technologies and

their applications and XXIX Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases, Jointly Organized by Department of Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, and ICAR-National Research Centre on Pig, Rani, Guwahati, Assam, 12-14 February, 2016.

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- Rajukumar K (2015) Emergence of PRRS in India: Concerns and Challenges, invited paper presented in XXIV National Conference of Indian Virological Society (VIROCON 2015) on 'Transboundary Viral Diseases under One Health: Perspectives and Challenges' organized at NEIGRIHMS, Shillong, 8-10 October, 2015.
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Accession numbers: KM922681-KM922688: Dinesha, K. N., Nagarajan, S., Tosh, C., Murugkar, H. V., Behera, P. and Dubey, S. C. Characterization of H9N2 avian influenza virus isolated from a chicken co-infected with H5N1 virus. (Eight nucleotide sequences of one avian influenza H9N2 virus isolate A/chicken/India/82588/2008)

Accession numbers: KM519962-KM519977: Majhi, S., Nagarajan, S., Tosh, C., Murugkar, H. V., Behera, P. and Dubey, S. C. Molecular Characterization of H9N2 Avian Influenza Viruses Isolated from West Bengal in 2008. (16 nucleotide sequences of two avian influenza H9N2 virus isolates A/chicken/India/80998/2008 and A/chicken/India/82565/2008)

Accession numbers: KM267744-KM267796: Nagarajan, S., Jain, R., Tosh, C., Murugkar, H. V., Kumar, M. and Kulkarni, D. D. Emergence of reassortant H5N1 subtype avian influenza virus with PB1 gene of endemic H9N2 subtype with low mice pathogenicity in South Asia. (53 nucleotide sequences of avian influenza H5N1 virus isolates A/pigeon/Bhutan/01BR01/2012, A/crow/India/02CA07/2012, A/crow/India/02CA06/ 2012, A/crow/India/02CA02/2012, A/chicken/India/03CA03/2012, A/chicken/India/03CA02/ 2012, A/crow/India/11TI16/2011 and A/crow/India/11TI11/2011)

Accession numbers: KM273111-KM273116: Nagarajan, S., Senthil Kumar, D., Tosh, C., Murugkar, H. V., Kumar, M. and Kulkarni, D. D. Emergence of reassortant H5N1 subtype avian influenza virus with PB1 gene of endemic H9N2 subtype with low mice pathogenicity in South Asia. (Six nucleotide sequences of two avian influenza H5N1 virus isolates A/crow/India/01CA08/2012 and A/crow/India/01CA08/2012).

Participation of scientists in conferences, workshops, symposia, trainings, etc. in India and abroad

S. No	Name of the Symposium/Seminar/ Workshop/trainings	Date and venue	Name of the Scientist/s
1.	OIE global conference on biological threat reduction "Building cooperation for efficient health and security systems worldwide"	30th June - 2nd July, 2015, Paris, France.	Dr. C. Tosh
2.	Professional attachment training for newly joined scientists	14th May to 21th August, 2015 at National Research Center on Equines,Hisar.	Dr. Naveen Kumar,
3.	Professional attachment training for newly joined scientists	14th May to 13th August, 2015 at Anand Agricultural University, Gujarat	Dr. Siddharth Gautam
4.	Diagnostic Test Course: Principles and Latest Developments of Diagnostic Test and Laboratory Quality Control Methods"	7th - 9th September 2015 at Christian Medical College, Vellore	Dr. G. Venkatesh,
5.	Sensitization workshop on "Mera Gaon Mera Gaurav"	14th October 2015 at ICAR-ATARI, Jabalpur	Dr. Atul Kumar Pateriya,
6.	Avian influenza: continuing evolution and expanding host range. Presentation in workshop on "Influenza: Risk factors, massive impact and uncertain future",	19th October 2015, ICAR-IVRI, Izatnagar, Uttar Pradesh.	Dr. C. Tosh Dr. Manoj Kumar
7.	XXIV National Conference of Indian Virological Society on Transboundary Viral Diseases under One Health: Perspectives and Challenges.	8-10th October 2015 at NEIGRIHMS, Shillong	Dr. K Rajukumar
8.	Analysis of High Throughput Sequencing and Microarray Data to Unravel Host-Pathogen Interaction	17th November to 7 th December, 2015 at IVRI, Izatnagar (U.P.).	Dr. Kh. Victoria Chanu,
9.	Indian Society for Veterinary Immunology and Biotechnology, XXII Annual Convention and National Symposium on Immunomics and Proteogenomics in Livestock Health and Productivity.	17-19th December, 2015, ICAR-NRC on Equines, Hisar, Haryana.	Dr. C. Tosh
10.	8th National Livestock Championship & Expo-2016, by Department of Animal Husbandry, Dairy Development &	8th-12th January, 2016 at Shri Muktasar Sahib, Punjab	Dr. Manoj Kumar
11.	Biorisk Management Training Global Action Plan III and Bio-Risk Management for Polio virus eradication Program sponsored by WHO	7-10th February, 2016 4 days at WHO New Delhi	Dr. D. D. Kulkarni
12.	Global Symposium on Animal Health: Newer technologies and their applications and XXIX Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases.	12-14th February, 2016, at Assam Agricultural University Guwahati, Assam.	Dr. V. P. Singh Dr. C. Tosh
13.	17th International Congress on Infectious Diseases	2-5th March, 2016 at International Convention Center, Hyderabad	Dr. Ashwin A. Raut

Trainings and Workshops

Trainings

Training on “Biosafety and Biosecurity for handling and identification of zoonotic pathogens” was organized under DBT-NER- ADMaC Consortium for 10 participants from Assam, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, and Sikkim states of North East India during 16-20th Feb., 2016 at ICAR-NIHSAD, Bhopal. The program was designed for providing training on conventional and molecular techniques for diagnosis of exotic and emerging animal diseases and biosafety procedures as a part of capacity building programme for research personnel from North East India under the ADMaC project. In tune with the consortium objective, the course included Culture of Responsibility for Biological Safety Module & Biological Security Module, Personal Protective Equipments, Implications of biosafety practices, Biosafety and biosecurity protocols for handling of suspected highly pathogenic AIV carcass, Emergency response and waste decontamination, Molecular techniques for diagnosis of emerging viruses such as PCR array, RT-PCR, RT-qPCR and ELISA, etc.

Training, program, “Laboratory Diagnosis of Avian Influenza and Bovine Viral Diarrhea” as a capacity building program for the Regional Disease Diagnostic Laboratories for preliminary testing of Avian Influenza and Bovine Viral diarrhea was conducted in two batches, from 19-26th August 2015 and 2 -9th September 2015. Representative personnel from SRDDL, Bangalore, NRRDL, Jalandhar, NERDDL Guwahati, ERDDL Kolkatta, WRDDL, Pune and CADRAD IVRI, Bareilly, were imparted training in all the conventional and molecular techniques for diagnosis of avian influenza as well as bovine viral diarrhea.

Trainees from North Eastern states with faculty.

Farmer's meet

An interactive farmer's meet was organized on 6th June, 2015 at the institute for addressing the challenges faced by the poultry farmers due to bird flu. The meet was organized in association with Telangana State Poultry Farmers for addressing their concerns in management of poultry farming in wake of recent outbreaks of highly pathogenic avian influenza H5N1 in Telangana and other states. Twenty two representatives of poultry farmers/breeders/State Associations of Poultry Farmers/National Egg Coordination Committee members arrived from 7 states (Telangana, Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu, Madhya Pradesh and Chhattisgarh) and attended the meet. The meeting provided basic information on Avian Influenza H5N1 outbreaks occurring in the country, the remedial measures and the surveillance of poultry for the disease incidences. Former FAO Expert Dr. M.S. Oberoi guided the deliberations with his wide experience of Emergency Control Program of Avian Influenza and other Trans-boundary Animal Diseases in South Asia, in addition to the scientific team from NIHSAD. The remedial measures in terms of enhancing the farm biosecurity, general hygiene the emergency protocols were also discussed at length.

Farmer's meet in progress.

Meetings & Visits

Institute Research Council (IRC) meeting

The 1st IRC meeting of ICAR-NIHSAD was conducted on 11th May, 2015 under the chairmanship of Dr. D. D. Kulkarni, Acting Director and attended by all scientists. Dr. Sandeep Bhatia, Member Secretary & PME Incharge presented the action taken report (ATR) on the recommendations of the 1st RAC meeting of NIHSAD held on 28th Oct., 2014. The progress of all the institute funded and externally funded projects was reviewed with approval of four new projects. The important issues that were discussed the IRC meeting included improvement required in reviewing and monitoring of national/international project by PME, importance of time targeted deliverables especially in research work under diagnostics and vaccines, validation of the diagnostic tests and vaccines etc.

Mid-year Institute Research Council (IRC) meeting was held on 19th Dec., 2015 under the chairmanship of Dr. V.P. Singh, Director, ICAR-NIHSAD. A total of seven institute funded, five ICAR funded, and six externally funded projects were critically reviewed and monitored during the mid-year IRC meeting. The Director emphasized the need to form research groups (each group consisting of microbiologist, biotechnologist and pathologist) to improve working efficiency and encouraged scientists to seek funding of research projects from external funding agencies.

Mid year IRC meeting with scientists of NIHSAD

Institutional Animal Ethics Committee

The **IAEC** committee was reconstituted in April 2015 by the CPCSEA with Dr. B.S.Karada, as the main nominee and Dr. Ajay, Pr. Sc, IISS, Bhopal as link nominee. The IAEC meetings were conducted on a regular interval as per the norms of CPCSEA to evaluate new projects and to review ongoing and completed projects. The annual animal house inspection report were prepared and sent to CPCSEA by the main nominee. Three meetings were conducted in 2015-16 on 24.04.15, 31.10.2015, 21.03.2016. The meetings were chaired by Dr. D.D.Kulkarni, Chairman, IAEC and attended by the CPCSEA nominees and institutional members of the committee.

NIHSAD IAEC members with CPCSEA nominee

Institute Management Committee (IMC) meeting

IMC meeting in progress

The second IMC meeting was conducted on 28th Nov., 2016 under the Chairmanship of the Director, NIHSAD. Members of committee viz Dr. A.K. Garg, Joint Director Extension, IVRI, Izatnagar Dr. Madhu Swamy, Professor and Head, Pathology JNKVV, Jabalpur, Mr. A.K. Maheshwari, Accounts and Finance officer, Directorate of Soyabean Research, Indore along with internal members Dr. D.D. Kulkarni, Dr. H.V. Murugkar and Dr. Sandeep Bhatia attended the meeting.

Research Advisory Committee (RAC) Meeting

RAC meeting of the institute

The second RAC meeting of NIHSAD, Bhopal was held on 19.3.2016. The meeting was chaired by Dr. M. S. Oberoi, Ex-Dean, COVS, GADVASU, Ludhiana and Former Sub-Regional Manager, ECTAD, RSU, FAO, UN.. Dr. G.K. Sharma, Head Animal Health, NDDB, Anand and Dr. Rajesh Chandra, Retd. Prof & Head, Dept. of Microbiology, COVS, CAU, Aizwal were also present along with the Director Dr. V. P. Singh, Member Secretary Dr. S. Bhatia and all scientists of the institute.

Visits

- Twenty one students of College of Veterinary Science and Animal Husbandry, Jabalpur visited ICAR-NIHSAD for educational tour of the institute under the DBT, New Delhi sponsored programme “Star College Scheme” on 18.06.2016. The students were given a glimpse of biocontainment laboratory and its workings.
- Forty nine veterinary doctors of Animal Husbandry Department, Govt. of Madhya Pradesh, as trainees of Disaster Management Institute, Govt. of Madhya Pradesh visited the institute on 15.01.2016 as part of three day training programme on Disaster Management. Besides the documentary of the institute the outside facility of the institute was shown to them.

Students visit from College of Veterinary Science and animal husbandry, Jabalpur

- Participants of DBT sponsored 21 day refresher course organized by MP State Council of Science and Technology visited NIHSAD facility. After the visit Dr Richa Sood delivered a guest lecture on Biosafety Rules and Regulations and Concepts of Biosecurity to participants of 21 days (15th sept- 5th October 2015) refresher course program organized by M. P State Council of Science and Technology.

Events

First Foundation Day

The First Foundation Day of ICAR-NIHSAD was celebrated on 8th August, 2015 to observe its establishment as an independent National Institute under Indian Council of Agricultural Research. One day celebration was organized, the then acting Director Dr. D.D. Kulkarni welcomed the guests and remembered the visionaries who had played key role in the establishment of the biocontainment facility which had come up to become a full-fledged institute. On this occasion, "Vision 2050" which gives a way forward to the institute for the next 3 decades was released by Dr. B.N. Singh, Registrar, RKDF University, Bhopal. The Institute profile was released by Dr. S.C. Dubey, Ex. Joint Director, HSADL.

Inaugural ceremony of First Foundation Day

Republic day celebration

The 67th Republic day (26th January, 2016) was celebrated and the National Flag was hoisted by Director, Dr. V.P. Singh. Dr Singh addressed the staff and their family members. The first Telephone Directory of NIHSAD was released on this day. Later in the day, a number of events were organized for the family members and staff of the institute like, cultural program by children, musical chair, cycle race, lemon race and volleyball match.


Release of telephone directory


Staff participating in games on Republic Day

Cleanliness crusade under Swachh Bharat Abhiyan

ICAR-NIHSAD, Bhopal had organized a cleanliness drive under “Swachh Bharat Abhiyan” from 26th September to 31st October 2015. On this occasion, the Director, NIHSAD in his inaugural speech especially emphasized to make the campus and surrounding area, a 'polythene free zone'. All the members actively participated and disposed off the non-biodegradable material scattered in and around the campus. An awareness lecture on “Environment pollution, waste disposal and its control measures” by Er. H.S. Malviya, M.P. Pollution Control Board, Bhopal was organized. In addition, all the vehicles used by staff members of NIHSAD were checked for CO emission and issued Pollution Under Control (PUC) Certificates.

Cleanliness drive outside the campus

Awareness lecture on pollution control

Vigilance Awareness Week

The Vigilance Awareness Week was observed at NIHSAD, Bhopal during 26 to 31st October 2015. The week began with administering of the pledge to the staff and students of the Institute by Dr. D. D. Kulkarni, Principal Scientist. In his remarks, Dr. Kulkarni stressed upon the importance of transparency, punctuality and integrity at all levels for effective implementation of preventive vigilance. Dr. Niranjan Mishra, Principal Scientist and Vigilance Officer, deliberated upon the importance of “Preventive Vigilance as a tool of Good Governance” and described the purpose, sources and measures of preventive vigilance. Several posters 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.

Table tennis team of NIHSAD in action.

ICAR Central Zone Sports Tournament

A 12 member team of ICAR-NIHSAD including, scientists, technical, administrative and supporting staff actively participated in different games and events in ICAR Central Zonal sports meet-2015 held at Directorate of Weed Research, Jabalpur, M.P. from Dec., 7-11, 2015.

'Jai Kisan Jai Vigyan' week

'Jai Kisan Jai Vigyan' week was celebrated from Dec., 23-29, 2015. On this occasion on the last day, 29th December, 2015, a debate competition on topic “Role of Genetically Modified Crops in Food Security: Advantages and Disadvantages” was organized with an invitation to most of the colleges of Bhopal. Dr S. Bhatia National Fellow elaborated on the immense contribution of former leaders Shri A.B. Vajpayee for promoting use of science for the welfare of farmers. The joint role of scientists in the laboratory, and farmer in the field, in ensuring food security was elaborated upon at length. The program was a grand success and prizes were distributed to those securing first, second and third positions in this debate competition.

Inaugural ceremony of Jai Kisan Jai Vigyan week

Prize distribution for debate competition.

Honours, Awards & Recognitions

- Dr. V.P. Singh nominated as President of Society for Biosafety, India.
- Dr. V.P. Singh nominated as Vice President of Indian Association for Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases (IAVMI), India.
- Dr. D.D. Kulkarni elected as Vice-President of Society for Biosafety, India.
- Dr. R. Sood elected as Secretary of Society for Biosafety, India.
- Dr. G. Venkatesh elected as Treasurer of Society for Biosafety, India.
- Dr. N. Mishra and Dr. S. Nagarajan elected as Member of Executive Committee of the Society for Biosafety, India.
- Dr. A.A. Raut nominated as member for ICAR-ICMR Joint Working group on Zoonosis.
- Dr. K Rajukumar nominated as Member of Institute Animal Ethics Committee, Barkatulla University, Bhopal, India.
- Mr. B.K. Singh awarded a *Certificate of distinction under cash award Scheme* for Administrative category Employees of ICAR. Scheme for Administrative category Employees of IC

Committees

Research Advisory Committee (RAC)

S.No.	Name	Designation
1	Dr. M.S. Oberoi, Ex-Dean, C.O.V.S., GADVASU, Sub-Regional Manager, FAO, Emergency Centre for Trans-Boundary Animal Disease, Regional Support unit for SAARC countries, Food and Agricultural Organization, UN	Chairman
2	Dr. Ashok Kumar, ADG(AH), ICAR	Member
3	Dr. D.T. Mourya, Director, NIV, Pune.	Member
4	Dr. Rajesh Chandra, Prof. & Head, Veterinary Microbiology, COVS, CAU, AIZWAL.	Member
5	Dr. R. Venkataramanan, Joint Director, IVRI, Bangalore.	Member
6	Dr. G.K. Sharma, Head Animal Health, NDDB, Anand	Member
7	Dr. V. P. Singh, Director, NIHSAD, Bhopal	Member
8	Dr. H.V. Murugkar, Principal Scientist, NIHSAD, Bhopal	Member Secretary

Institute Management Committee (IMC)

S.No.	Name	Designation
1	Dr. V. P. Singh, Director, NIHSAD	Chairman
2	Dr. Ashok Kumar, ADG(AH), ICAR	Member
3	Dr. C.R. Mehta, PC, CIAE	Member
4	Dr. R.P. Singh, PS, IVRI	Member
5	Dr. S. Bhatia, PS, NIHSAD	Member
6	Dr. H.V. Murugkar, PS, NIHSAD	Member
7	Shri S.K. Gupta, SAO, NIHSAD	Member Secretary

Institute Technology Management Committee (ITMC)

S.No.	Name	Designation
1	Dr. V. P. Singh, Director, NIHSAD	Chairman
2	Dr. H.V. Murugkar, Principal Scientist	Member
3	Dr. Sandeep Bhatia, Principal Scientist	Member
4	Dr. K. Rajukumar, Sr. Scientist	Member
5	Dr. P.C. Bargale, Project Coordinator ICAR-CIAE	External Member
6	Dr. N. Mishra, Principal Scientist	Member Secretary

Foreign Deputation Committee (FDC)

S.No.	Name	Designation
1	Dr. Sandeep Bhatia, Principal Scientist	Chairman
2	Dr. C. Tosh, Principal Scientist	Member
3	Dr. Ashwin Ashok Raut, Sr. Scientist	Member
4	Shri S.K. Gupta, SAO, NIHSAD	Member
5	Shri B.K. Kanchan, AF & AO	Member

Gender Sensitization Committee (GSC)

S.No.	Name	Designation
1	Dr. Richa Sood, Senior Scientist	Chairperson
2	Dr. H.V. Murugkar, Principal Scientist	Member
3	Mr. S.K. Gupta, Senior Administrative Officer	Member
4	Ms. Smita Shendye	Member from NGO
5	Dr. (Mrs.) Anamika Mishra, Scientist	Member
6	Ms. Mehajabin Bilgrami, LDC	Member
7	Dr. (Ms) K. Victoria Chanu, Scientist	Member Secretary

Departmental Purchase Advisory Committee (DPAC)

S.No.	Name	Designation
1	Dr. H.V. Murugkar, Principal Scientist	Chairman
2	Dr. K. Rajukumar, Sr. Scientist	Member
3	Dr. G. Venkatesh, Sr. Scientist	Member
4	Shri. R.K. Kaushik, CTO(Inst.)	Member
5	Shri S.K. Gupta, Senior Administrative Officer	Member
6	Shri B.K. Kanchan, AF & AO	Member

Technical Selection Committee (TSC)

S.No.	Name	Designation
1	Dr. C. Tosh, Principal Scientist	Chairman
2	Dr. Sandeep Bhatia, Principal Scientist/NF	Member
3	Dr. Ashwin Ashok Raut, Sr. Scientist	Member
4	Shri. R.K. Kaushik, CTO (Inst.)	Member
5	Indenting Officer	Member

Purchase Advisory Committee (PAC)

S.No.	Name	Designation
1	Dr. D.D.Kulkarni, Principal Scientist	Chairman
2	Dr. N.Mishra, Principal Scientist	Member
3	Shri R.K. Kaushik, CTO (Inst.)	Member
4	Senior Administrative Officer	Member
5	Assistant Finance & Account Officer	Member

Research Publication Committee (RPC)

S.No.	Name	Designation
1	Dr. N. Mishra, Principal Scientist	Chairman
2	Dr. C. Tosh, Principal Scientist	Member
3	Dr. K. Rajukumar, Sr. Scientist	Member

Institute Animal Ethics Committee (IAEC)

S.No.	Name	Designation
1	Dr. V.P. Singh	Chairman
2	Dr. B.S. karada	CPCSEA Main Nominee
3	Shri. Sanjeev Bhartiya Dubey	Social activist
4	Shri. D. Rathore	External member
5	Dr. Richa Sood	I/C Animal House, member
6	Dr. C Tosh	Member
7	Dr. G Venkatesh	Member Secretary

Institutional Biosafety Committee (IBSC)

S.No.	Name	Designation
1	Dr. D. D. Kulkarni	Chairman
2	Dr. Himanshu Kumar	DBT Nominee
3	Dr. Atul Gupta, AMA, NIHSAD	Member
4	Dr. C. Tosh	Member
5	Dr. G. Venkatesh	Member
6	Dr. H.V. Murugkar, Biosafety officer	Member Secretary

Works Committee

S.No.	Name	Designation
1	Dr. D. D. Kulkarni, Principal Scientist	Chairman
2	Dr. N.Mishra, Principal Scientist	Member
3	Er. R.K. Kaushik, CTO (Inst.)	Member
4	Er. R.B. Srivastava, CTO (Civil)	Member
5	Senior Administrative Officer	Member
6	Assistant Finance & Account Officer	Member

Institute Joint Staff Council (IJSC)

S.No.	Name	Designation
(A)	**OFFICIAL SIDE**	
1	Dr. V. P. Singh, Director	Chairman
2	Dr. H.V. Murugkar, Principal Scientist	Member
3	Dr. Sandeep Bhatia, Principal Scientist	Member
4	Dr. (Mrs.) Anamika Mishra, Scientist	Member
5	Dr. A.K. Pateriya, Scientist	Member
6	Shri B.K. Kanchan, AF & AO	Member
7	Shri S.K. Gupta, SAO	Member Secretary
(B)	**STAFF SIDE**	
1	Shri B.K. Singh, Assistant	Member/Member Secy.
2	Mrs. Mehjabin Bilgrami, LDC	Member
3	Shri R.K. Shukla, STA	Member
4	Shri S.B. Somkuwar, STA	Member
5	Shri Sita Ram Imne, SSS	Member
6	Shri Sitai Prasad, SSS	Member

Research Projects

S. No.	Title of the project (Period)	PI & Co-I
	Institute Funded	
1.	Surveillance of exotic and emerging animal diseases in Indian and imported livestock & poultry and their products (April, 2009 – continuing)	**V. P. Singh** (w.e.f Sept. 2015) D.D. Kulkarni, H.V. Murugkar, C. Tosh, N. Mishra, A. A. Raut, S. Nagarajan, Richa Sood, G. Venkatesh, K. Rajukumar, A. Mishra, Manoj Kumar, Kh. Victoria Chanu, Atul K. Pateriya, D. Senthil Kumar, S. Kalaiyarasu, P. Gandhale, S. B. Sudhakar, Naveen Kumar, S. Gautam
2.	Evolutionary analysis of avian influenza viruses isolated in India (July 2014- June 2017)	**C. Tosh** S. Nagarajan, G. Venkatesh, Manoj Kumar
3.	Evaluation of diagnostic potential of monoclonal antibodies raised against H5N1 avian influenza virus (July 2014 June 2017)	**G. Venkatesh** C. Tosh, S. Nagarajan, Richa Sood, Kh. Victoria Chanu
4.	Development of chicken dendritic cell binding synthetic peptide for delivering avian influenza virus M2e antigen and elucidating associated immunological mechanism (April 2015– March 2018)	**S. Bhatia** S. Kalaiyarasu
5.	Pathobiology of Indian PRRSV in pigs (April 2015– March 2018)	**K. Rajukumar** Senthil Kumar D
6.	Investigation of Schmallenberg or related virus in Ruminants and Culicoides spp. in India (April 2015– March 2018)	**S. B. Sudhakar** P. Gandhale, A . A. Raut, D. D. Kulkarni
7.	Generation of artificial equine influenza virus through reverse genetics approach and to explore its potential as vaccine candidate through challenge studies in mice model ***(Inter-institutional project with NRCE, Hisar)*** (April 2015– March 2018)	**S. Bhatia** Richa Sood Naveen Kumar
8.	Cross-sectional study of Malignant Catarrhal Fever infection in domestic ruminants in Southern India **(Inter-institutional project with NIVEDI, Bengaluru)** **(**October, 2012- October 2015)	**Richa Sood** Kh. Victoria Chanu Manoj Kumar

	ICAR funded schemes		Budget (in lakhs)
1.	Outreach project on zoonoses Avian Influenza part **(ICAR outreach program)** July 2008 onwards	**H.V. Murugkar** Manoj Kumar	Rs 68.55
2.	**CRP on Vaccine and Diagnostics** August, 2015 March, 2017 **Sub-Project 1:** Development of recombinant nucleoprotein based ELISA for Avian Influenza antibody detection	**S. Bhatia** A. K. Pateriya, Richa Sood, Naveen Kumar	Rs 40.25
	Sub-Project 2: Development of antibody based rapid test for detection of H5 Avian influenza in poultry	**G. Venkatesh** S. Nagarajan, D. Senthil Kumar, Richa Sood	
	Sub-Project 3: Development of DNA strip test for sub-typing of avian influenza viruses	**B. Sudhakar**, A. A. Raut, S. Nagarajan, A. K. Pateriya	
3.	Development and evaluation of neuraminidase DIVA Marker vaccines against highly pathogenic H5N1 avian influenza viruses in chickens **(National Fellow Project)** April 2011 – March 2016	**S. Bhatia** National Fellow	Rs 151.73
	Externally funded		
1.	Prevalence & molecular epidemiology of BVD in ruminants with special reference to Mithun in North East states of India **(DBT twinning project)** October 2013 - September 2016	**N. Mishra** K. Rajukumar S. Kalaiyarasu	Rs 21.58
2.	Deployment of nucleic acid and ELISA based diagnostic tests to determine incidence of porcine reproductive and respiratory syndrome (PRRS) in the North East and evaluation of the prospects of a potential candidate vaccine against the disease (**DBT twinning project)** Nov., 2012-May, 2016	**K. Rajukumar** D. Senthil Kumar	Rs 42.97
3.	Identification of the molecular basis of differential host responses to rapidly evolving Avian Influenza viruses in different avian species **(BBSRC-DBT)** February 2014 – January 2017	**A. Mishra** A.A. Raut H.V. Murugkar	Rs 111.63
4.	Advanced Animal Disease Diagnosis Management Centers in the North East (ADMaC) **(DBT-NER program)** April 2014 - March 2019	**D. D. Kulkarni** A. A. Raut K. Rajukumar A. K. Pateriya S B Sudhakar P N Gandhale	Rs 211.49
5.	Viral metagenomic profiling of native and migratory aquatic birds of North-Eastern states to unravel influenza virus ecology harbored in them **(DBT twinning project)** September 2014 – August 2017	**A.A. Raut** D.D. Kulkarni H.V. Murugkar A. Mishra	Rs 26.80
6	Synthetic peptide based diagnostic for highly pathogenic avian influenza **(DBT)** November 2014 – October 2017	**Kh. Victoria Chanu** Richa Sood Atul Pateriya	Rs 14.30

Personnel

Administration

1.	Dr. V. P. Singh	Director
2.	Shri S. K. Gupta	SAO
3.	Shri B. K. Kanchan	AF&AO
4.	Shri B. C. Kandpal	AAO

Scientist

S.No.	Name	Designation	Discipline
1	Dr. D.D. Kulkarni M.V.Sc., PhD	Principal Scientist	Veterinary Microbiology
2	Dr. H.V. Murugkar M.V.Sc., PhD	Principal Scientist	Veterinary Public Health
3	Dr. C. Tosh M.V.Sc., PhD	Principal Scientist	Veterinary Microbiology
4	Dr. N. Mishra M.V.Sc., PhD.	Principal Scientist	Veterinary Microbiology
5	Dr. Sandeep Bhatia M.V.Sc., PhD.	National Fellow	Veterinary Microbiology
6	Dr. Ashwin Ashok Raut M.V.Sc., PhD.	Sr. Scientist	Animal Biotechnology
7	Dr. K. Rajukumar M.V.Sc., PhD.	Sr. Scientist	Veterinary Pathology
8	Dr. (Mrs.) Richa Sood M.V.Sc., PhD.	Sr. Scientist	Veterinary Medicine
9	Dr. S. Nagarajan M.V.Sc., PhD.	Sr. Scientist	Animal Biotechnology
10	Dr. G. Venkatesh M.V.Sc., PhD.	Sr. Scientist	Animal Biotechnology
11	Dr. (Mrs.) Anamika Mishra, M.V.Sc., PhD.	Scientist	Animal Genetics and Breeding
12	Dr. Atul Pateriya M.Sc., PhD.	Scientist	Animal Biotechnology
13	Dr. Manoj Kumar M.V.Sc., PhD.	Scientist	Veterinary Pathology
14	Dr. S.B. Sudhakar M.V.Sc., PhD.	Scientist	Veterinary Microbiology
15	Dr. S. Kalaiyarasu M.V.Sc.	Scientist	Veterinary Microbiology

16	Dr. D. Senthil Kumar M.V.Sc.	Scientist	Veterinary Pathology
17	Dr. Pradeep N Gandhale M.V.Sc., PhD.	Scientist	Veterinary Microbiology
18	Dr. (Mr.) Kh. Victoria Chanu, M.V.Sc., PhD.	Scientist	Animal Biochemistry
19	Dr. Naveen Kumar M.V.Sc., PhD.	Scientist	Veterinary Microbiology
20	Dr. Siddharth Gautam M.V.Sc.	Scientist	Veterinary Pathology

Technical

21	Shri R.K. Kaushik	CTO (Instrument)
22	Shri T.K. Ghosh	CTO (Electrical)
23	Shri R.B. Shrivastava	CTO (Civil)
25	Shri T.L. Bankar	ACTO
26	Shri Sunil Barange	Sr. T.O. (Lab.)
27	Shri Shambhu Dayal	Technical Officer
28	Shri R.K. Shukla	Sr. Tech. Asstt.
29	Shri Asanna Badge	Sr. Tech. Asstt.
30	Shri S. B. Somkuwar	Sr. Tech. Asstt.
31	Shri Mahesh Kumar	Sr. Tech. Asstt.
32	Shri Rakesh Kumar	Tech. Asstt.
34	Shri J.N. Meena	Tech. Asstt.
35	Shri Ram Lakhan	Tech. Asstt.
36	Shri Malkhan Singh	Tech. Asstt.
37	Shri R.R. Chouksey	Tech. Asstt.

Administration

38	Shri S.K. Gupta	Sr. Admin. Officer
39	Shri B.K. Kanchan	Asstt. Fin. A/Cs Officer
40	Shri B.C. Kandpal	Asstt. Admin. Officer
41	Shri B.K. Singh	Assistant
42	Shri Mansingh Hansda	Assistant
44	Shri K.S. Tantuway	UDC
45	Mrs. M. Bilgrami	UDC

Supporting

46	Shri Ram Prasad	SSS
47	Shri S.R. Imne	SSS
48	Shri Sitai Prasad	SSS
49	Shri Sita Ram	SSS

Joining /Transfers/Retirements/Promotions

- **Dr. V.P. Singh**, Joint Director (Academic) at ICAR-Indian Veterinary Research Institute, Izatnagar joined as Director, ICAR-National Institute of High Security Animal Diseases, Bhopal w.e.f. 09.09.2015.
- **Dr. Naveen Kumar**, Scientist (Veterinary Microbiology) joined at ICAR-National Institute of High Security Animal Diseases, Bhopal w.e.f. 10.04.2015.
- **Dr. Siddharth Gautam**, Scientist (Veterinary Pathology) joined at ICAR-National Institute of High Security Animal Diseases, Bhopal w.e.f. 10.04.2015.
- **Dr Kh Victoria Chanu,** Scientist (Animal Biochemistry) transferred to ICAR-DCFR, Bhimtal w.e.f. 10.03.2016.
- **Er. T.K. Ghosh**, Chief Technical Officer (Electrical) retired from ICAR-National Institute of High Security Animal Diseases, Bhopal w.e.f. 31.12.2015.
- **Er. R.B. Srivastav** Chief Technical Officer (Civil) retired from ICAR-National Institute of High Security Animal Diseases, Bhopal w.e.f. 31.1.2016
- **Mr. B.C. Kandpal**, Assistant at ICAR-National Institute of High Security Animal Diseases, Bhopal promoted to Assistant Administrative Officer w.e.f. 17.01.2015.
- **Mrs. M. Bilgrami**, Lower Divisional Clerk at ICAR-National Institute of High Security Animal Diseases, Bhopal promoted to Upper Divisional Clerk w.e.f. 17.01.2015.

हर कदम, हर डगर
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भारतीय कृषि अनुसंधान परिषद
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