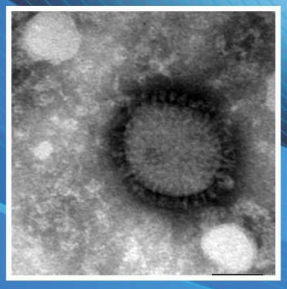
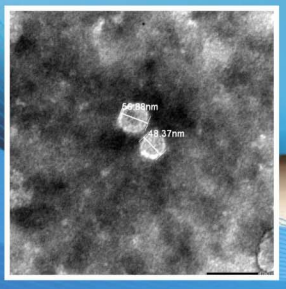
Annual Report 2014-15





भाकृअनुप
ICAR

ICAR-National Institute of High Security Animal Diseases, Bhopal

राष्ट्रीय उच्च सुरक्षा पशुरोग संस्थान
National Institute of High Security Animal Diseases

Annual Report
2014-15

ICAR-National Institute of
High Security Animal Diseases, Bhopal

Contents

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Preface

It gives me immense pleasure to release the first Annual Report of National Institute of High Security Animal Diseases, Bhopal, a premier institute under Indian Council of Agricultural Research working on exotic and emerging animal diseases since 8th August, 2014. Earlier, it was known as High Security Animal Disease Laboratory working as a regional station of Indian Veterinary Research Institute, Izatnagar.

The institute is known as an established BSL-3+ biocontainment facility having laboratories and animal experimentation units. Due to the significant contribution in providing laboratory diagnosis of highly pathogenic avian influenza (H5N1) as a national service, World Organization for Animal Health (OIE) recognized this institute as "OIE Reference Laboratory for Avian Influenza" in 2009. During the last decade or so, India faced several exotic/ emerging/ re-emerging diseases involving high risk pathogens like AIV H5N1, Pandemic Influenza H1N1-2009, Crimean Congo hemorrhagic fever, Nipah as well as fast spreading animal diseases like Porcine Reproductive and Respiratory Syndrome, Bovine Viral Diarrhoea, Border Disease, Malignant Catarrhal Fever etc. The diagnostic preparedness against these and many other diseases has been completed, for some more diseases it has been taken up, several aspects of host-pathogen interactions are being studied, diagnostic services are provided for the nation as well as to the neighboring countries. All the animals/ animal products imported into the country are tested for exotic/ emerging diseases at this institute and the results promptly communicated also generating significant revenue for the institute.

The institute has contributed in helping other national/ regional institutes/ laboratories in biosafety and biosecurity trainings of their manpower. NIHSAD has also extended the training facility for molecular and confirmatory diagnosis of emerging diseases as well as bio-risk management to SAARC countries and ASEAN member countries which has received appreciation at international level.

With limited manpower and enhanced responsibilities, this institute is continuing its journey with progress. We humbly request all the readers to point out shortcoming in this Annual Report 2014-15 so that next year they would be corrected. I thank my team members who took pains in preparing this report and just to mention a few - Dr. Sandeep Bhatia, Dr. S Nagarajan, Dr. Anamika Mishra , Dr. Atul Kumar Pateriya, Dr. Manoj Kumar and Shri S. K. Gupta and all the staff.

Bhopal
Dated : June 29, 2015

D. D. Kulkarni
Acting Director

EXECUTIVE SUMMARY

Carrying the march towards excellence and delivering to the animal health system of the country since year 2000, the popularly known "*bird flu laboratory*" of the nation was upgraded in August 2014 as **ICAR-National Institute of High Security Animal Diseases**. Always adding feathers in the cap of ICAR, the institute has come a long way to proclaim itself as an unique amalgamation of state of the art diagnostic & research capacity and engineering infrastructure. Today, the institute glimmers with 18 scientists, 15 technical, 7 administrative and 5 supporting staff. Though meagre in head-count, the institute covers vast area of delivery to the animal health system of the country right from being national referral facility for exotic and emerging diseases of animals, a national training hub for biosafety and biosecurity and an OIE Reference Laboratory for Avian Influenza. The research programs and activities of the institute are multifarious and include disease diagnosis, development of diagnostics and vaccines, studies on host-pathogen interactions, risk analysis, genomics approach to pathogen research, molecular epidemiology and environmental studies for pathogen survival etc.

Diagnostic vigilance and repository maintenance

The institute has a dedicated diagnostic team for avian influenza working 365 days in a year for delivering reliable laboratory diagnosis not only for emergency situations of highly pathogenic avian influenza outbreaks in the country but also for quarantine departments, surveillance samples from field, referral samples from Regional Disease Diagnostic Laboratories (RDDLs) and state diagnostic units, as well as samples from SAARC countries. The institute provides comprehensive technical help including diagnosis, virus characterization, control policies, biosafety guidelines to Department for Animal Husbandry, Dairying & Fisheries (DADF), Ministry of Agriculture, GoI for control of avian influenza in the country.

Avian Influenza

During the year 2014-15, a total of 52,665 samples (45980 morbid materials and 6685 serum) were received from various parts of the country for avian influenza diagnosis from which 25 morbid samples from four States/Union territories (17 from Kerala, 01 from Chandigarh, 01 from Odisha and 06 from Uttar Pradesh) have tested positive for H5N1. The other sub-types of avian influenza isolated from Kerala state included two H9N2, one H6N2 and four H3N8 viruses. Of the 5496 serum samples, 18 sera (14 from chicken and 4 from ducks) were positive for antibodies to avian influenza virus (subtype H5) and 20 sera were found positive for H9N2 AIV antibodies (17 from Kerala and 3 from Chandigarh).

On request from Bhutan and Nepal, AIV and PRRS diagnostic services were provided. Through OFFLU coordinated network for avian influenza, NIHSAD participated in OFFLU-2014 proficiency testing ring trial conducted in July-August, 2014. The results indicated that NIHSAD could identify correctly the status (positive or negative) and subtype of the positive samples and was placed third with a score of 42 among 9 OIE reference laboratories for Avian influenza.

Bovine Viral diarrhea

Testing of 387 diagnostic specimens revealed negative results for BVDV by virus isolation and/or RT-PCR, while BVDV neutralizing antibodies were detected in 35 Indian cattle, and 6 imported bovine sera by virus neutralization test. Testing of 713 serum samples collected from mithun, cattle, buffaloes, sheep and goats from NE region showed that five animals were positive for BVDV neutralizing antibodies indicating a low BVDV prevalence in this region.

Malignant Catarrhal fever

Malignant Catarrhal fever (MCF) is a fatal herpesvirus infection characterized primarily by high fever, swollen lymph nodes, neurological disorders and ocular lesion. A series of clinical cases of sheep-associated malignant catarrhal fever (SA-MCF) caused by ovine herpesvirus 2 (OvHv2) have been recorded in susceptible species in Karnataka, Tamil Nadu and Mizoram within a span of 2 years. Blood samples of pigs from Mizoram were confirmed for OvHV2 infection for the first time in India. Of the 179 blood samples of cattle, buffalo and sheep, 22 samples were positive for MCF. An occurrence of clinical MCF in one cattle samples in Mizoram, as well as one buffalo from Namakkal (NT) was diagnosed.

Porcine Reproductive and Respiratory Syndrome

A total of 495 porcine whole blood/sera samples received from North East India (comprising of sera samples from Meghalaya, Assam, Nagaland and Mizoram) were tested for PRRSV antibodies by in-house developed recombinant N-ELISA. Out of the 495 samples, 238 (48%) were positive for PRRSV antibodies. Two sera (one each from Mizoram and Meghalaya) out of 163 inoculated on PAM cultures were positive for virus isolation and were confirmed by immunoperoxidase monolayer assay on MARC-145 cells using PRRSV specific polyclonal serum. RT-PCR and nucleotide sequencing of ORF 7 of both the isolates showed that they belonged to PRRSV genotype-2.

Other viruses

In the year 2014-15, 196 nasal swabs received from different states of India were tested with RT-PCR against matrix, HA gene (H1N1) and were found to be negative. 162 sera samples from pigs from different parts of the country have been processed and tested with HI test and 43 samples were positive for antibodies against H1N1.

Crimean-congo haemorrhagic fever (CCHF) is an emerging zoonotic disease causing public health problems in Gujarat, Rajasthan & Uttar Pradesh states. During 2014-15, 142 post CCHF outbreak samples (serum/blood/ticks) of animals suspected for CCHF were received from these states for the detection of CCHFV and were found negative.

Schmallenberg virus (SBV) infection is another newly emerging disease of ruminants. The virus causes huge economic losses to livestock ruminants, especially in small ruminant production. As a first and preliminary report, the institute reported presence of antibodies to SBV in 38 randomly selected sera samples of cattle and buffaloes originating from Rajasthan and other parts of India.

West Nile virus (WNV) is a mosquito-borne zoonotic arbovirus belonging to the genus *Flavivirus* in the family *Flaviviridae*. It is found in temperate and tropical regions of the world and may be harbored by wild birds. Testing of 1848 samples from wild and peridomestic birds in 11 states demonstrated that all the samples were negative for West Nile virus by RT-PCR.

Testing for diseases / pathogens in imported products

NIHSAD is offering diagnostic services to Animal Quarantine and Certification Services (AQCS) centers for screening of imported livestock/birds and meat and meat products for various emerging and exotic animal diseases. During 2014-15, a total of 4746 samples of various categories of imported products received from quarantine stations were processed for screening of a number of exotic and emerging diseases.

Virus characterization and repository maintenance

Evolutionary Analysis of avian influenza viruses

During 2014-2015, outbreaks of H5N1 virus infection in poultry occurred in the states of Kerala, Uttar Pradesh, and Union Territory of Chandigarh. Besides poultry, H5N1 virus was isolated from dead crows from the State of Odisha. The viruses were characterized on molecular basis to be highly pathogenic and sensitive to neuraminidase drugs. The current phylogenetic analysis shows that H5N1 viruses isolated till early 2014 grouped with clade 2.3.2.1A, whereas the recent H5N1 viruses isolated from Kerala, Chandigarh and Uttar Pradesh grouped with a new clade 2.3.2.1 C, which has not been detected before in Indian poultry. Detailed phylogeny indicates role of migratory birds in the spread of H5N1 virus, although trade of poultry/poultry products cannot be ruled out.

Antiviral resistance in H5N1 viruses

Emergence of antiviral resistance among H5N1 avian influenza viruses is a major challenge in the preparedness for pandemic threat due to H5N1 viruses. During characterization of H5N1 viruses, genetic evidence of resistance to amantadine antiviral drug was

found in some of the viruses which was also confirmed by biological antiviral assays. A total of 46 H5N1 isolates form the year 2007 to 2014 have been screened for drug resistance for Oseltamivir and Zanamivir revealing drug resistance in 3 Indian isolates and decreased susceptibility for two isolates. These findings indicate emergence of antiviral resistance in avian influenza viruses isolated in India and underline the need to have a constant vigil on changing character of the virus.

Survivability factors of H5N1

Preen oil was found to increase survivability of avian influenza virus even at lower initial viral concentrations, particularly at lower temperatures. The present study establishes the role of preen oil present in the duck feathers in higher viral titres, and may be resulting in protecting the avian influenza virus present on the feathers from the effects of higher temperatures for a longer time.

Molecular characterization of newly identified BVDV-3 viruses

BVDV-3 (HoBi-like pestivirus) has been sporadically reported from naturally infected cattle, in selected countries in South America, Asia and Europe since 2006 but have not yet been identified in India. During a study on prevalence of bovine viral diarrhoea viruses (BVDV) between 2012 and 2013 in cattle (n=1049) in India, BVDV-3 viruses were indentified in 19 of the 20 pestivirus positive cattle blood samples. The phylogenetic analysis of the 5'-UTR sequence identified five clusters within the BVDV-3 virus clade and demonstrated that Indian BVDV-3 isolates were grouped into two distinct clusters separate from the previously reported BVDV-3 viruses from South America, Europe and Australia. IndBHA5309/12 group of viruses were found to be the most divergent HoBi-like strains reported so far. Additional phylogenetic analysis of full length N^{pro} gene sequences demonstrated that within the BVDV-3 virus clade, the two novel Indian BVDV-3 virus groups formed distinct phylogenetic groupings to each other and to all other previously reported BVDV-3 viruses with strong support (98-100%). Overall, three BVDV-3 virus lineages could be identified: the first lineage included previously reported BVDV-3 viruses from South America, Europe, Australia and South East Asia, the second lineage included IndABI15385/12 group of viruses and the third lineage encompassed IndBHA5309/12 group of viruses from India. The study has proposed that BVDV-3 viruses can be classified into three subtypes (a, b and c), subtype 'a' consisting of all the previously reported strains, subtype 'b' consisting of four Indian strains (Ind IndABI15385/12 group) and subtype 'c' encompassing fifteen Indian strains (IndBHA5309/12 group). This study has highlighted the global circulation and independent evolution of at least three groups of BVDV-3 viruses with two groups in India. The increasing reports of BVDV-3 viruses from cattle in the field suggest that natural infection of cattle with BVDV-3 virus may be more widespread than previously thought. Considering the growing evidence of occurrence of BVDV-3 in geographically distant cattle populations, these emerging viruses present considerable risk to the cattle health and management and BVD control programmes. The identification of BVDV-3 in Indian cattle emphasizes the need for continued monitoring besides determining the extent of economic losses it can cause in dairy farming.

Molecular characterization of PRRS Virus isolated from India

Genetic analysis of the complete ORF 5, ORF 7 and nsp2 coding regions showed that the Indian PRRSV isolate grouped with genotype-2 viruses reported from China, sharing high sequence identity (97 to 99%). With PRRSV-2 isolates from Asian countries including Bhutan, Laos, Vietnam, Thailand, Japan, the sequence homology ranged from 88 to 98%. With classical genotype-1 and genotype-2 isolates, the sequence identity was from 62 to 70% and 88 to 93% respectively. Analysis of deduced amino acid from ORF 5, ORF 7 and nsp2 (within ORF 1a) sequences showed the presence of 2, 1 and 14 unique amino acid changes, respectively, as compared to the PRRSV genotype-2 currently existing in various parts of the world.

Development of diagnostics and vaccines

Development of new and improved diagnostics for exotic and emerging diseases is an important part of the mandate of the institute. The institute continuously takes efforts in this direction and every year adds some new diagnostic or improvement of the existing diagnostic tests.

Diagnostic preparedness for upcoming pathogen threats

Outbreak of novel H7N9 avian influenza A virus (AIV) in China in March 2013 raised the concern for India due to common border. This was the first incidence when AIV H7N9 subtype was reported to cause mortality in human population. Diagnosis of presently circulating AIV H7N9 is challenging due to the absence of clinical sign in infected birds. Wide host range of this virus is evident by its isolation from chicken, duck, pigeon, environmental sample etc. To prevent such pandemic situation in India, as preparedness for immediate diagnosis of AI H7N9 subtype, molecular diagnostic tests were successfully optimized at the institute. Using reverse genetics H7N9 and H7N2 viruses were generated that can be used as reference in diagnosis and can be used as antigen for HI test.

RT-PCR ELISA for pestiviruses

During 2014-15, the institute developed a reverse transcription polymerase chain reaction ELISA (RT-PCR ELISA) for detection of ruminant pestiviruses and evaluated its diagnostic performance on clinical samples obtained from cattle, sheep and goats. The test has been optimized using in-house developed digoxygenin-labelled RT-PCR product standards obtained from pestivirus isolates and pestivirus infected animals. The assay had high analytical specificity, good reproducibility with 95.9% diagnostic sensitivity, 98.6% specificity and a strong agreement (97.5% concordance) with the reference RT-PCR test.

PCR-array based multiple pathogen diagnostic

The identification of infectious agents by detection of specific nucleic acid sequences of the infectious agents by PCR and realtime PCR is most preferred choice for diagnosis, identification, and quantitation due to greater sensitivity and specificity of these tests. The institute developed PCR arrays for the detection of 22 prioritized exotic and emerging viruses as per the mandate of the institute using Molecular beacon (MB) and SYBR Green realtime PCR chemistry. This technology offers the simultaneous detection of various pathogens by running a panel of real time PCR in different wells of a single PCR plate under uniform reaction and thermal conditions.

Development of AGID test kit for avian influenza

A kit for agar gel immunodiffusion (AGID) test for detection of avian influenza antibodies in chicken and other birds sera was developed and evaluated against reference test. A total of 2071 sera samples from various parts of the country were tested for AIV antibodies by AGID antigen and HI test. The sensitivity is 94.28% and specificity is 94.96% compared to HI test. The AGID antigen could detect all the subtype specific antisera raised. The intra-laboratory validation on lyophilized antigen was conducted successfully by three independent scientists.

Development and evaluation of neuraminidase DIVA marker vaccines against H5N1 avian influenza virus

For the control of avian influenza in poultry, India follows a policy of elimination of infected and susceptible avian population at the face of outbreak and does not recommend vaccination. However, as part of preparedness for emergency situations wherein biosecurity measures alone cannot control avian influenza, readiness with a suitable vaccine against highly pathogenic H5N1 influenza virus is required. A DIVA (differentiation of infected from vaccinated animals) marker H5N2 inactivated vaccine has been developed using the rgH5N2 virus (8+2 reassortant) generated through plasmid based reverse genetics. During 2014-15, the vaccine was tested in turkeys and ducks. The vaccine has already been tested in chickens in 2013-14. Development of a recombinant NA1 (rNA1) based indirect ELISA test has been initiated as DIVA test. For developing this test, the NA1 gene was expressed in 3 overlapping fragments designated as N1a, N1b and N1c. The primary molecular characterization and preliminary reactivity check of the three N1 fragments based on the reactivity with positive

serum and non-reactivity with negative serum has been conducted. Further optimization and specificity testing is underway to develop the DIVA test using N1a antigen.

Host- pathogen interactions

Transmission of H5N1 influenza virus from crows to chickens

The role of crows in the epidemiology of highly pathogenic avian influenza remains unclear although H5N1 virus had been isolated from dead crows. An experimental study carried out on Indian house crows (*Corvus splendens*) with H5N1 virus produced systemic disease leading to death in crows but virus excreted at a very low level. Highest virus concentration in brain leading to apoptosis with neurological signs indicated that the virus is neurotropic in nature and may be the factor for fatal outcome of the disease in crows. Absence of clinical signs, death and failure in detection of virus in swabs and tissues of chickens fed with oral and cloacal swabs of infected crows indicated that the excretion of H5N1 virus in crows by oral and cloacal routes is below the minimum threshold of chicken lethal dose. This might be one of the reasons for the absence of H5N1 virus infection in chickens in areas from where the H5N1 virus was isolated in crows.

Avian influenza virus in different avian hosts

In the wake of expanding host range of H5N1 observed in the outbreaks since 2011 in India, experimental infection and transmission studies of H5N1 in ducks and crows with different H5N1 isolates were done under the containment experimental animal facility of the institute. Significant differences in the pathogenecity and transmissibility of the H5N1 isolates of the closely related clade 2.2 and clade 2.3.2.1 of Asian lineage were observed. The isolates of both clades were transmitted to the contact birds, but the transmission rate and mortality of in contact birds varied between the different clades. Microarray analysis of global gene expression in ducks revealed that several immune related Gene ontology (GO) terms and immune pathways activated in response to viruses of the two clades were qualitatively similar but quantitatively different. The differential regulation of genes associated with cytokines stroms were found to be responsible for the differential severity of disease in ducks.

Viral Metagenome of duck gut

Viral metagenome is the genomes of all the viruses that inhabit a particular organism or environment. It gives an all inclusive profile of viruses inhabited and thus is an indicator of possible virus evolution due to cohabitation in a particular host. An initial unbiased measure of the viral diversity in the enteric tract of duck, which is one of the principal natural reservoir for influenza virus, was generated from the virome purified from the cloacal swabs of ducks. This first metagenomic analysis of viruses in ducks using second-generation sequencing, showed 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.

Qualitative Import Risk Analysis of Livestock and Livestock products for Notifiable Avian Influenza

A qualitative import risk analysis was conducted to assess the risk of introduction of Notifiable Avian Influenza (NAI) through import of certain livestock and livestock products, as listed in Gazette Notification S.O. 1663 (E), from countries reporting NAI. The main aim of import risk analysis was to provide India with an objective and defensible method of assessing the disease risks associated with the importation of animals or animal products for the avian influenza types H5 or H7 that may have potential threat for human involvement as these two types are considered as serious zoonotic infections. The release and exposure assessment for introduction of NAI through all the livestock and livestock products were found to be negligible except for import of wild birds where it was estimated to be of **low** probability. The probability of occurrence of NAI introduction through import of products mentioned and studied in this risk assessment was limited to negligible. Though the consequences of NAI introduction in the country are considered to be of high impact, the overall risk estimated for NAI through import of the products is "very low" which is below the accepted level of protection (ALOP) for India.

INTRODUCTION

National Institute of High Security Animal Diseases (NIHSAD) of Indian Council of Agricultural Research is an institute for research on exotic and emerging pathogens of animals. This institute was originally established in 2000 as High Security Animal Disease Laboratory (HSADL), a regional station of Indian Veterinary Research Institute (IVRI), Izatnagar. On 8th Aug 2014 it was elevated to the status of an independent institute and renamed as National Institute of High Security Animal Diseases (NIHSAD). The institute has contributed significantly towards the animal health scenario of the nation, by detecting many animal diseases of exotic origin at its port of entry and preventing them from entering the country. The fully functional bio-containment laboratory at the institute was dedicated to the Nation on June 23rd, 2000 and since then the lab has been functional round the clock with unmatched safety record. The institute has developed several national and international linkages on several platforms with its increasing role as a referral facility at national and regional level for diagnosis of various emerging infections animal diseases, an infrastructural model for bio-containment facilities (BSL-3 & BSL-4), a leading learning centre for bio-risk management and as an animal disease institution partnering with medical institutions for reduction of public health risks from zoonotic infections under one-health concept. The institute had relentlessly pursued the goal to achieve preparedness to diagnose exotic and emerging diseases at hours of need. With prioritization of diseases based on the disease status of the world and neighbouring nations, this institute has always been ready with tools and expertise to diagnose the exotic animal diseases. In view of India's achievement towards diagnosis and control of bird flu using its own expertise, facilities and resources world's apex body for animal health (OIE) recognized this institute as **OIE Reference Lab for Avian Influenza** in May 2009 in its 77th General Session held at OIE head quarters at Paris. This recognition could be achieved only after meticulous and unrelenting contributions of this institute's scientists and the timely and encouraging support of IVRI the then parent institute, ICAR and the Department of Animal Husbandry, Dairying and Fishery (DADF), Ministry of Agriculture Government of India.

The preparedness for providing the lab diagnostic services for exotic and emerging diseases of animals was initiated at this institute in 2001. On the basis of prioritization and as per the need and request from Quarantine Department under DADF, a few diseases viz., Bovine Viral Diarrhoea (BVD), Bovine Immunodeficiency Virus (BIV), Transmissible Gastroenteritis (TGE), Avian Influenza, Caprine Arthritis and Encephalitis (CAE), Rabbit Haemorrhagic Disease (RHD) and Malignant Catarrhal Fever (MCF) were taken up. Later, Avian influenza, Nipah, Swine influenza (H1N1), Bunyaviral infections including CCHF etc. were added to the list. Being the first bio-containment lab of the country, NIHSAD needs to work continuously to develop its R&D and HRD programme for providing diagnostic services, vigilance for unknown pathogens and training in biosafety and biosecurity for contributing towards the ultimate goal of food security through better animal health.

The institute has been created to address various challenges the livestock sector is facing today or going to face in near future. Emergence of new and unknown pathogens is one of the greatest challenges the country's agriculture is facing today. In livestock sector, this problem is threatening its prosperous growth and creating trade barriers for export of livestock /livestock products and is becoming a major factor of economic losses to the sector. With opening trade ways in globalized economy, the ability to manage and reduce health costs of farm animals shall be the key to success in a globally competitive scenario. The institute is poised to use new high throughput technologies and inventions coming from basic sciences like NGS and microarrays to open new gateways for rapid diagnosis of known and unknown pathogens to thwart the challenges and risk posed by emergence of pathogens. The changing pattern

in animal husbandry practices due to socio-economic changes is leading to altered host pathogen interaction. Intensification of animal production system in last few decades has led to greater contact between humans and animals resulting increase of animal pathogens adopting human beings by crossing species barriers with greater ease. The increasing risk of zoonotic infections is a new challenge which the livestock farmer has to deal with right from managing public health risk at farm level and food safety risk from his produce. The average farmer of the country is not well equipped to handle this problem and needs to be trained in area of farm level biosafety and biosecurity. With involvement of communities at rural, urban and even at industrial level, the institute is aiming at achieving direct linkages with grass root level so that the useful information in terms of either disease awareness, technology, diagnosis, public health related measures can be effectively passed on for broad aim of better sanitation and disease control in animal health sector.

INSTITUTES PROGRAMS

Major Programmes approved in XII Plan (2012-17)

1. **Development of diagnostics and vaccines using current and next generation technologies for exotic and emerging animal diseases (Flagship programme)**

Part-1: Diagnostics

- Development of nucleic acid-based diagnostics for exotic and emerging animal diseases.
- Development of Recombinant Protein and Monoclonal antibody based diagnostics.
- Establishment of multiplex diagnostic platforms (Microarray, Next Generation Sequencing, etc.) for simultaneous detection of multiple exotic pathogens.

Part-2: Vaccines

- Novel vaccine platforms–Nucleic acid-based vaccine, reverse genetics system, viral vectored vaccine and recombinant antigen based vaccine.
- Development of vaccines against major emerging viral disease threats to Indian livestock and poultry using newly developed vaccine platforms.

2. **Diagnostic vigilance and repository maintenance for exotic and emerging diseases**

- Providing referral lab diagnostic services for exotic/emerging diseases.
- Pathogen characterization and their epidemiology
- Development & maintenance of pathogen repository & serum bank with updated accession and retrieval system.
- Production of diagnostic reagents for in house use / supply on demand
- Development and refinement of lab info-management system
- Training in Bio-Risk Management (Biosafety, biosecurity and biocontainment) for regional and national level laboratory and field personnel.

3. **Host-pathogen interaction studies on exotic and emerging diseases of animals**

- Species specific host response to pathogens.
- Elucidation of inter/intra species transmission mode of pathogens
- Evaluation of intervention strategies for control of the pathogens
- Genomics and bioinformatics approach for host-pathogen-environment interaction
- Assessment of environmental persistence of pathogens
- Host genetic factors for disease resistance.

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, and 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.

OBJECTIVES

1. To carry out basic & applied research on exotic, emerging and re-emerging diseases of animals.
2. To develop competency for diagnosis & control of exotic/emerging diseases of animals.
3. To create & update repository and data-bank on exotic/emerging pathogens.
4. To develop skills in biorisk management & train manpower in the areas of biosafety, biosecurity and biocontainment.
5. To create National Centre of excellence in exotic/emerging disease research and policy advice on animal health.

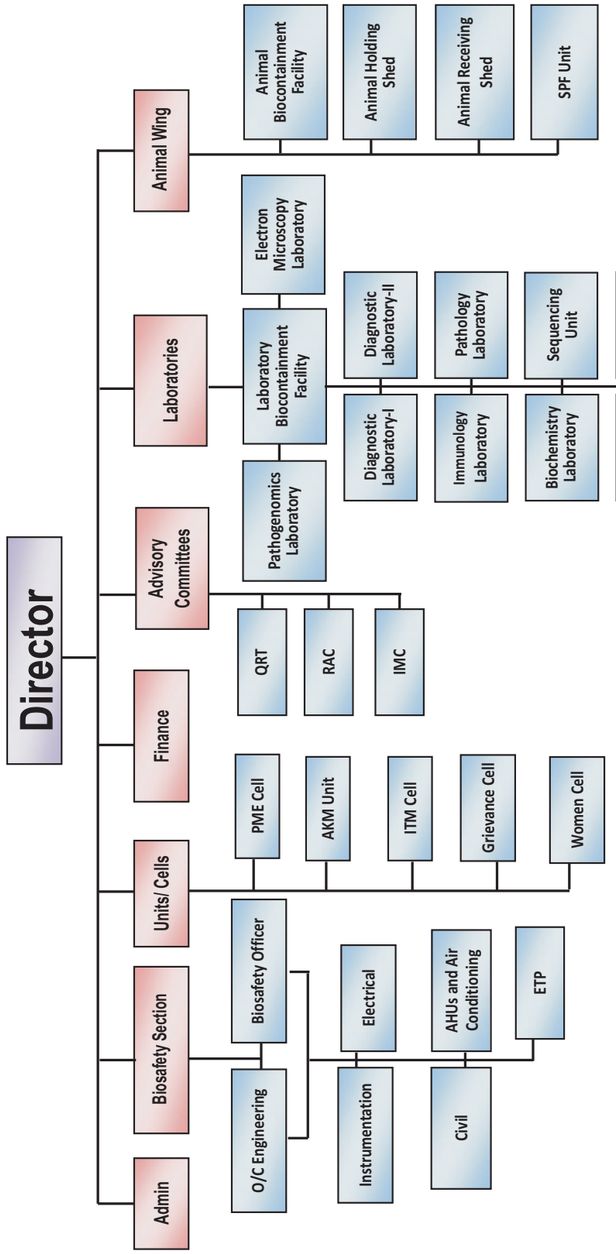
VISION STATEMENT

"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

Organizational Setup of NIHSAD



Expenditure Statement 2014-15

Financial statement showing receipts and expenditure of Institute (By AF&AO): *(Rs. In Lakhs)*

Sl.No.	Heads	Non-Plan up to 31.03.2015			Plan up to 31.03.2015		
		RE 2014-15	Release	Expenditure	RE 2014-15	Release	Expenditure
1	2	3	4	5	6	7	8
Grants for creation of Capital Assets (Capital)							
1	Works (Office buildings)	0.00	0.00	0.00	50.00	50.00	50.00
2	Equipments	5.00	5.00	4.52	218.00	218.00	217.64
3	Information Technology	0.00	0.00	0.00	0.00	0.00	0.00
4	Library Books and Journals	0.00	0.00	0.00	0.10	0.10	0.00
5	Vehicle & Vessels	0.00	0.00	0.00	0.00	0.00	0.00
6	Livestock	0.00	0.00	0.00	0.00	0.00	0.00
7	Furniture & Fixtures	2.00	2.00	0.00	6.50	6.50	6.48
8	Others	0.00	0.00	0.00	0.00	0.00	0.00
Total Capital (Grants for creation of capital Assets)		**7.00**	**7.00**	**4.52**	**274.60**	**274.60**	**274.12**
Grants in Aid-Salaries(Revenue)							
1	Establishment Expenses	0.00	0.00	0.00	0.00	0.00	0.00
	A. Salaries	0.00	0.00	0.00	0.00	0.00	0.00
	I. Establishment Charges	442.00	442.00	441.91	0.00	0.00	0.00
	ii. Wages	0.00	0.00	0.00	0.00	0.00	0.00
	iii. Overtime Allowance	0.10	0.10	0.03	0.00	0.00	0.00
Total Establishment Exp. (Grants in Aid -Salaries)		**442.10**	**442.10**	**441.94**	**0.00**	**0.00**	**0.00**
Grants in Aid-General (Revenue)							
1	**Pension & Other Retirement Benefits**	21.00	21.00	19.56	0.00	0.00	0.00
2	**Travelling Allowances**						
	A. Domestic TA/Transfer TA	3.50	3.50	3.50	5.50	5.50	5.50
	B. Foreign TA	0.00	0.00	0.00	0.00	0.00	0.00
	Total Travelling Allowances	**3.50**	**3.50**	**3.50**	**5.50**	**5.50**	**5.50**
	Research and Operational Expenses						
	A. Research Expenses	44.00	44.00	43.75	108.00	108.00	107.94
	B. Operational Expenses	36.00	36.00	35.56	3.00	3.00	2.80
	Total Research and Operational Expenses	**80.00**	**80.00**	**79.31**	**111.00**	**111.00**	**110.74**
	Administrative Expenses						
	A. Infrastructure	121.40	121.40	121.34	123.50	123.50	123.49
	B. Communication	2.25	2.25	2.18	0.00	0.00	0.00
	C.Repairs/Maintenance						
	i.Equipments, Vehicles & Vessels	45.00	45.00	44.91	27.00	27.00	26.80
	ii.Office building	3.00	3.00	2.96	0.00	0.00	0.00
	iii.Residential building	0.25	0.25	0.22	0.00	0.00	0.00
	iv.Minor Works	2.00	2.00	1.96	0.00	0.00	0.00
	D.Others (excluding TA)	21.00	21.00	21.00	102.50	102.50	102.35
	Total Administrative Expenses	**194.90**	**194.90**	**194.57**	**253.00**	**253.00**	**252.64**

Sl.No.	Heads	Non-Plan up to 31.03.2015			Plan up to 31.03.2015		
		RE 2014-15	Release	Expenditure	RE 2014-15	Release	Expenditure
1	2	3	4	5	6	7	8
Miscellaneous Expenses							
A. HRD		0.00	0.00	0.00	1.00	1.00	0.98
D. Guest House – Maintenance		1.00	1.00	0..99	0.00	0.00	0.00
E. Other Miscellaneous		0.50	0.50	0.50	0.00	0.00	0.00
	Total Miscellaneous Expenses	**1.50**	**1.50**	**1.49**	**1.00**	**1.00**	**0.98**
	Total Grants in Aid -General	**743.00**	**743.00**	**740.37**	**370.50**	**370.50**	**369.86**
	Grand Total : Capital + Revenue	**750.00**	**750.00**	**744.89**	**645.10**	**645.10**	**643.98**
B. Loans & Advances		3.50	3.50	2.87	0.00	0.00	0.00

Revenue Generation (By the Division / Section)

Sl. No.	Name of Head	Amount
1	Sale of Farm Produce	0.74
2	Income from Analytical Testing Fee	54.42
3	License Fee, Interest on Loans & Advances and Interest on short TDRs	41.88
4	Other Misc. Receipts	14.44
	Total Revenue Receipts	**111.48**

List of filled and unfilled technical and other posts

	Sanctioned	Filled	Unfilled
Scientific	21	20	01
Administrative	18	15	03
Technical	25	12	13
Total	**64**	**47**	**17**

Research

Institute Funded Projects

Service project : Surveillance of exotic and emerging animal diseases in Indian and imported livestock & poultry and their products

PI : Dr. D. D. Kulkarni

Co-PIs: H. V. Murugkar, C. Tosh, N. Mishra, A. A. Raut, K. Rajukumar, R. Sood, S. Nagarajan, G. Venkatesh, A. Mishra, A. K. Pateriya, Manoj Kumar, S. B. Sudhakar S. Kalaiyarasu, D. Senthilkumar, P.N. Gandhale, Kh. Victoria Chanu,

1. Avian Influenza

1.1 Surveillance of Indian poultry and imported samples

A total of 52,665samples (45980 morbid materials and 6685 serum) had been received from various parts of the country for avian influenza virus surveillance during this year. A total of 4020 samples were found unfit for processing mostly due to contamination, spillage, low quantity, non-identification of samples, non-confirmation of samples etc. The random samples, emergency samples and post operation surveillance programme (POSP) samples received during this year and their results are given in Table 1. The specimen-wise sample details and their results are given in Table 2. The species-wise samples received and their results are presented in Table 3. The state-wise samples received and their results are presented in Table 4 and 5.

Out of 45,980 morbid/swab/fecal samples tested, 25 samples from four States/Union Territories (17 from Kerala, 01 from Chandigarh, 01 from Odisha and 06 from Uttar Pradesh) have tested positive for H5N1 by RT-PCR, Real time RT-qPCR and virus isolation. The timeline of outbreaks of H5N1 HPAIV in India since its first detection in 2006 is depicted in Fig. 1. Out of these, 2 samples were from crow, one from turkey and rest from chicken (Table 3). Further, two H9N2, one H6N2 and four H3N8 viruses were isolated from samples received from Kerala State (Table 5). A total of 5496 random serum samples had been tested, 18 sera (14 from chicken and four from ducks) were positive for antibodies to avian influenza virus (subtype H5) (Table 4). The state-wise details of POSP samples received and their results are given in Table 6. Out of the five states from which samples have been received, 20 sera were found positive for H9N2 AIV antibodies (17 from Kerala and three from Chandigarh) and seven sera from Kerala State were found positive for H5N1 AIV antibodies (six from ducks and one from chicken) (Table 6).

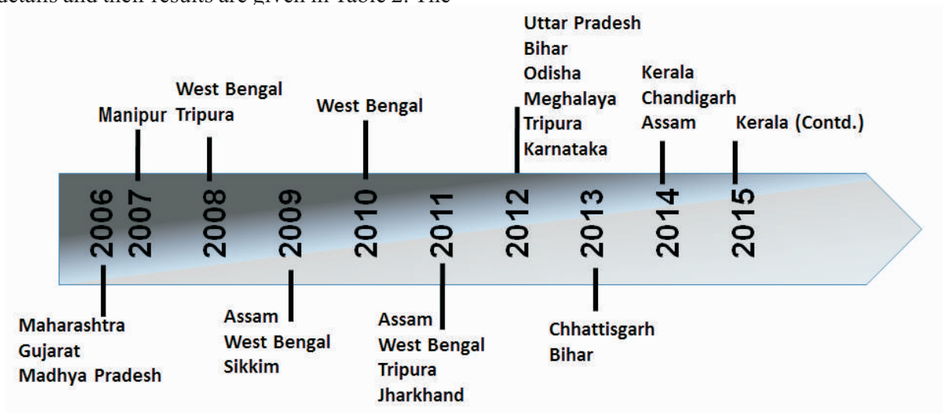


Figure 1. Timeline of outbreaks of H5N1 in Indian states

Table 1: Summary of samples received for avian influenza (2014-15)

Nature of samples	No. of samples*	Positive samples				
		H5N1	H9N2	H6N2	H3N8	NDV
Emergency Samples	669	24	2	1	4	21
Random Samples	49171	0	18	-	-	07
POSP	2825	07	20	-	-	0
Total Samples	**52665**	**31**	**40**	**1**	**4**	**28**
Unfit Samples	4020					

Note: *Includes serum, swabs, carcass and tissues.

Table 2: Specimen-wise samples received for avian influenza (2014-15)

Specimen material	Samples Received	Positive samples				
		H5N1	H9N2	H6N2	H3N8	NDV
Cloacal swabs/Feacal (POSP)	1454	0	0	0	0	0
Blood/Sera samples (Random and emergency)	5496	0	18	0	0	02
Blood/Sera samples (POSP)	1189	7	20	0	0	0
Oro-pharyngeal/Trachealswabs	5499	8	0	0	1	09
Cloacal swabs/Feacal	37564	1	1	1	3	16
Carcass/tissue/morbid samples (Emergency)	262	15	01	0	0	01
Oro-pharyngeal/Trachealswabs (POSP)	539	0	0	0	0	0
Environmental sample (water/soil)	662	0	0	0	0	0
Total	**52665**	**31**	**40**	**01**	**04**	**28**

Table 3: Species-wise samples received for avian influenza (2014-15)

Species/Sample types	Samples received	Positive samples				
		H5N1	H9N2	H6N2	H3N8	NDV
Chicken						
Blood/Sera samples (POSP)	746	1	3	0	0	0
Blood/Sera samples (Random and emergency)	5480	0	18	0	0	0
Oro-pharyngeal swabs/Tracheal/Nasal	5407	0	0	0	0	5
Cloacal swabs/Feacal	33942	0	0	0	1	4
Carcass/tissue/morbid samples (Emergency)	153	0	1	0	0	0
Oro-pharyngeal swabs/ Tracheal (POSP)	312	0	0	0	0	0
Cloacal swabs/Feacal (POSP)	662	0	0	0	0	0
Duck						
Blood/Sera samples (Random and Emergency)	16	0	0	0	0	2
Blood/sera samples(POSP)	443	6	17	0	0	0
Oro pharyngeal swabs/Tracheal/Nasal	85	8	0	0	1	4
Cloacal swabs/Feacal	210	1	0	1	2	12
Carcass/tissue/morbid samples (Emergency)	51	10	0	0	0	1
Oro-pharyngeal swabs/Tracheal/Nasal (POSP)	227	0	0	0	0	0
Cloacal swabs/Feacal (POSP)	435	0	0	0	0	0
Migratory Bird						
Oro-pharyngeal swabs/Tracheal	3	0	0	0	0	0
Cloacal swabs/Feacal	3044	0	0	0	0	0
Carcass/tissue/morbid samples (Emergency)	9	0	0	0	0	0
Wild Bird						
Oro-pharyngeal swabs/Tracheal	1	0	0	0	0	0
Cloacal swabs/Feacal	363	0	0	0	0	0
Carcass/tissue/morbid samples (Emergency)	7	0	0	0	0	0
Peacock						
Oro-pharyngeal swabs	1	0	0	0	0	0
Cloacal swabs	1	0	0	0	0	0
Carcass/tissue/morbid samples (Emergency)	7	0	0	0	0	0
Emu						
Carcass/tissue/morbid samples (Emergency)	5	0	0	0	0	0
Quail						
Oro-pharyngeal swabs	2	0	0	0	0	0
Turkey						
Carcass/tissue/morbid samples (Emergency)	1	1	0	0	0	0
Feaces	4	0	0	0	0	0
Crow						
Carcass/tissue/morbid samples (Emergency)	18	2	0	0	0	0
Goose						
Carcass/tissue/morbid samples (Emergency)	2	0	0	0	0	0
Eagle-Carcass						
Carcass/tissue/morbid samples (Emergency)	3	0	0	0	0	0
Pigeon						
Carcass/tissue/morbid samples (Emergency)	4	0	0	0	0	0
Species not declared						
Cloacal /Feacal (POSP) -	356	0	0	0	0	0
Tissues	2	2	0	0	0	0
Swab type not mentioned	1	0	1	0	0	0
Environmental Samples (water/soil)	662	0	0	0	0	0
Total	**52665**	**31**	**40**	**01**	**04**	**28**

* Tissue samples from Kerala.

Table 4: State-wise distribution of blood/sera samples received:(2014-2015)

State	Samples received	Positive by AGID	Subtyping by HI test
Assam	60	0	0
Gujarat	51	0	0
Kerala	15	06	H9N2 (Chicken)
Madhya Pradesh	780	0	0
Maharashtra	3079	03	H9N2 (Chicken)
Odisha	58	0	0
Punjab	79	01	H9N2 (Chicken)
Rajasthan	20	0	0
Uttar Pradesh	1068	0	0
Chandigarh	73	0	0
Uttarakhand	205	0	0
Tamil Nadu	08	04	H9N2 (04-Duck & 04-Chicken)
Total	**5496**	**09**	**18**

Table 5: State-wise distribution of tissue/swab samples received and their results (2014-2015)

State	No. of samples received	Positive samples
Assam	508	0
Goa	264	0
Gujarat	22363	0
Haryana	44	0
Himachal Pradesh	08	0
Kerala	196	17 (H5N1), 1 (H6N2), 4 (H3N8), 2 (H9N2), 19 (NDV)
Madhya Pradesh	7197	0
Maharashtra	6570	0
Odisha	34	1 (H5N1)
Punjab	79	0
Rajasthan	4392	0
Sikkim	68	0
Uttar Pradesh	2357	6 (H5N1)
Delhi	05	0
Chandigarh	173	1 (H5N1)
Mizoram	70	07 (NDV)
Uttarakhand	56	0
Tamil Nadu	16	0
Total	**44400**	

Table 6: State-wise distribution of POSP samples received: (2014-2015)

State	Serum			Swabs			Total
	Received	AGID	Subtyping by HI test	Received	Positive*	Subtype	
Kerala	1066	18	17-H9N2 (Chicken) 07-H5N1 (6 duck & 1 chicken)	1438	0	0	2504
Odisha	0	0	0	0	0	0	0
Uttar Pradesh	60	0	0	30	0	0	90
Punjab	04	0	0	32	0	0	36
Haryana	01	0	0	13	0	0	14
Chandigarh	58	03	03-H9N2 (chicken)	123	0	0	181
Total	**1189**	**21**	**27**	**1636**	**0**	**0**	**2825**

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

1.2 Detection and isolation of Influenza A virus H3N8 and H6N2 subtype in Indian poultry

During 2014-15, four H3N8 (three from duck and one from chicken samples) and one H6N2 (from a duck sample) Influenza A viruses were isolated from samples received/collected from Kerala. Virus isolation was carried out in 9-11 days embryonated SPF chicken eggs and confirmed to be H3 and H6 HA subtypes using subtype-specific anti-sera in haemagglutination inhibition assay. The HA and NA subtype of the isolates were confirmed by RT-PCR (Fig.2 and 3). Further, characterization including sequencing of the isolates is continuing. Detection of subtypes other than high pathogenic H5N1 is significant as they reveal the presence of low pathogenic subtypes circulating in the poultry population, which could play an important role in the evolution of highly pathogenic avian influenza viruses. Wild birds, particularly ducks, have been identified as high risk factor for the

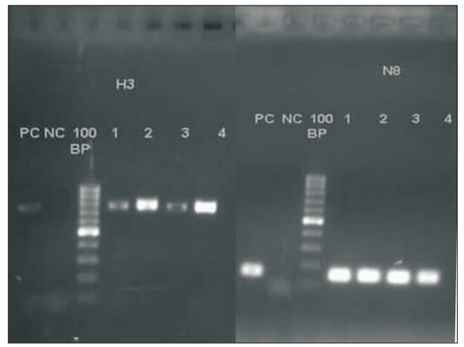


Figure 2. Agarose gel electrophoresis of RT-PCR amplified products. PC, positive control; NC, negative control; 100BP, 100bp DNA ladder. 1, 2, 3 & 4 are 722 bp HA gene amplified products (left panel), and 1, 2, 3 & 4 are 145 bp amplified products of NA gene (right panel)

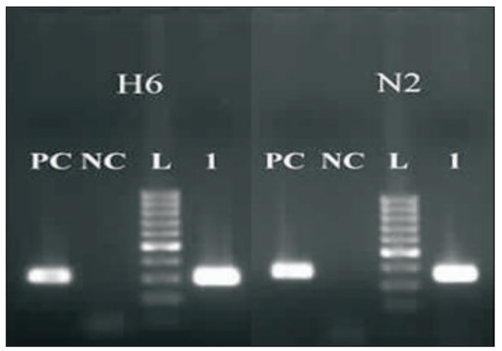


Figure 3. Agarose gel electrophoresis of RT-PCR amplified products. PC, positive control; NC, negative control; L, 100bp DNA ladder. 1, 302 bp HA gene amplified products (left panel), and 1, 362 bp amplified products of NA gene (right panel)

introduction of low pathogenic influenza A viruses into free range poultry. Therefore, there is a risk of introduction of influenza A viruses into Indian poultry underlining the significance of surveillance of avian influenza in wild birds.

1.3 Prevalence of amantadine resistance among avian influenza viruses (H5N1) in India

Emergence of antiviral resistance among H5N1 avian influenza viruses is a major challenge in the preparedness for pandemic threat due to H5N1 viruses. Genetic analysis with specific nucleotide substitutions in the transmembrane region if the Matrix 2 protein is one of the established methods of identification of drug resistance for the virus, to correlate the drug resistant genotype with a drug-resistant phenotype, a biological assay was also undertaken to test the resistance phenotype of a subset of 12 viruses to further validate the sequence reporting. The first identification of resistant virus was in the year 2010 where three viruses were identified with point mutation at position 31 where serine was replaced with Asparagine. Recently after a gap of 3 years a novel mutation was identified in two viruses in the transmembrane region valine replaced with Alanine at position 27. The IC_{50} (Inhibitory Concentration) calculated revealed more than 1000 times increased resistance to amantadine for viruses having both these mutations in comparison to the susceptible viruses. The ability of the virus to replicate in MDCK cells in the absence or presence of amantadine was also assessed. Phenotypic results from the biological assay of both sensitive and resistant viruses correlated 100% with virus genotypes identified by sequencing. The study identifies the correlation between in vitro antiviral assay and presence of established molecular markers of resistance, the retention of replicative capacity in the presence of amantadine hydrochloride by the resistant viruses and the emergence of resistant mutations against amantadine without selective drug pressure.

1.4 Preparedness for diagnosis of avian influenza

a. Generation of reassortant H7 virus

The gene sequence of HA and NA genes of LPAI H7N9 reported in China were selected from NCBI database and were chemically constructed through commercial services. The synthetic H7 HA gene was suitably modified through PCR using Hoffman primers and cloned in pHW2000 reverse genetics vector with Bsm B1 restriction enzyme site. Using the backbone plasmids of WSN/33 H1N1, one H7N9 virus and one H7N2 (using already available NA N2 clone). The reverse genetics based H7N9 and H7N2 viruses can be used as reference in diagnosis and can be used as antigen for HI test. The H7-specific serum was generated in chickens using H7N9 virus.

b. Diagnostic preparedness for AI H7H9 subtypes

Outbreak of novel H7N9 avian influenza A virus (AIV) in China in March 2013 raised the concern for India due to common border. This was the first incidence when AIV H7N9 subtype was reported to cause mortality in human population. Diagnosis of presently circulating AIV H7N9 is challenging due to the absence of clinical sign in infected birds. Wide host range of this virus is evident by its isolation from chicken, duck, pigeon, environmental sample etc. To prevent such pandemic situation in India, as preparedness for immediate diagnosis of AI H7N9 subtype, molecular diagnostic tests were successfully optimized at ICAR-NIHSAD. Positive control for real time RT-PCR was prepared from synthesized full length H7 HA gene. RNA (IVT-RNA) was transcribed in vitro from the synthetic H7 gene construct. Reaction conditions and thermal profile for conventional and TaqMan real-time RT-PCR were optimized using all the primers and probes recommended by WHO-FAO-OIE for detection of AIV H7N9. Clear sigmoid amplification curves could be obtained by TaqMan realtime RT-PCR assay. The optimized protocols were used for detection of AIV H7 subtypes in the panel of samples received for proficiency testing (OFFLU ring trial 2014). All coded samples containing H7 subtypes and negative samples within the panel were correctly identified. Presently this institute is fully prepared for the diagnosis of AIV H7N9 infection in case of national emergencies.

1.5. OFFLU activities

a. Diagnostic services to SAARC countries

16 samples from Bhutan and 02 samples from Nepal were received for testing against AIV, and all turned out to be negative to AIV by RT-PCR, realtime RT-qPCR and virus isolation.

b. Participation in Proficiency testing

The OFFLU proficiency test technical working group has developed an avian influenza (AI) ring trial panel consisting of a variety of inactivated viruses. Each laboratory will receive one panel for evaluation using molecular assays. Results are to be reported back to the technical working group for data compilation and statistical analysis. The sample panel comprised 15 blinded inactivated viral antigen samples (samples 1-15) for molecular characterization to be tested in generic AI real-time reverse-transcription PCR (RT-qPCR) assay(s) as established and validated in the participating laboratories. Any samples testing positive by the generic AI assay(s) were to be tested by H5 and H7 subtyping assays. The PT panel was prepared and sent by NVSL, USA. A total of 21 laboratories requested panels, among them nine out of the ten laboratories designated as international reference laboratories/centers of the OIE and/or the FAO. Eleven participants represented regional and national reference laboratories for diagnosis of AI in SE Asia, sub-Saharan Africa, and Central/South America. Six laboratories were located in the Americas while the remaining 15 were from Africa, Eurasia, and Australia.

The NIHSAD participated in OFFLU-2014 proficiency testing ring trial conducted in July-August, 2014. The results indicated that NIHSAD could identify correctly the status (positive or negative) and subtype of the positive samples correctly in all the 15 samples and the weighted qualitative results indicated that NIHSAD has been placed third with a score of 42 among the OIE reference laboratories for Avian influenza. The results are available in OFFLU website.

c. Participation in OFFLU teleconferences

Scientists from NIHSAD participated in teleconferences convened by OFFLU on 20.11.2014 and 15.12.2014 with discussion agenda including HPAI H5N8 Situation update, Diagnostics, potential role for wild birds, Experimental studies on H5N8 and vaccine- info if current vaccines are protective. These teleconferences are being organized to spread awareness and preparedness including diagnosis and vaccines about the emerging avian influenza threats to public health and poultry industry.

2. Bovine Viral Diarrhoea

2.1 Development and evaluation of a RT-PCR ELISA for simultaneous detection of BVDV-1, BVDV-2 and BDV in ruminants

Laboratory diagnosis of pestivirus infections is routinely based on virus isolation (VI), immunohistochemical (IHC) staining of skin biopsy specimens for viral antigen or antigen-capture ELISAs from serum, blood, and other tissues. However, these tests suffer from certain disadvantages. In contrast, molecular-based assays such as reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR are routinely used for detection of pestiviruses, since they are highly sensitive and faster. However, conventional RT-PCR is not ideal for handling large number of samples and most diagnostic laboratories at remote locations in the developing countries cannot afford the equipment, probes and expertise to perform the real-time RT-PCR based assays. Hence, a study was undertaken to develop a reverse transcription polymerase chain reaction ELISA (RT-PCR ELISA) for detection of ruminant pestiviruses and to evaluate its diagnostic performance on clinical samples obtained from cattle, sheep and goats.

The test has been optimized using in-house developed

digoxygenin-labeled RT-PCR product standards obtained from pestivirus isolates and pestivirus infected animals. The concentrations of probe pestpr-2 and DIG-labeled RT-PCR products have been optimized with three types of samples (culture supernatant from BVDV infected and uninfected cells, blood leukocytes from uninfected and BVDV-1 infected sheep and tissue samples from uninfected and BVDV-1 infected sheep. Of the two different conjugate substrate systems evaluated, anti-DIG AP-PNPP and anti-DIG POD-ABTS, the latter performed better and hence used in the test.

The RT-PCR ELISA showed no positivity in any of the known negative samples and no cross reactivity was found with the unrelated viruses tested suggesting that the assay was specific. The cut-off value was set at 0.3 based on analysis of negative controls (n=56).The detection limit of the assay was 10 TCID50/ml, similar to virus isolation and real-time RT-PCR but 10-fold higher than RT-PCR (Fig. 4). The assay had high analytical specificity along with a good reproducibility. When the assay was evaluated on the samples obtained from animals infected experimentally with BVDV and from the field using virus isolation as standard, it showed a high diagnostic sensitivity (95.9%) and specificity (98.6%) and there was strong agreement (97.5% concordance) between the two tests (Table 7). However, it displayed an increased diagnostic specificity and sensitivity over RT-PCR. Additionally, when a few samples (n=26) were tested by RT-PCR ELISA and real-time RT-PCR, 100% concordance was obtained between them.

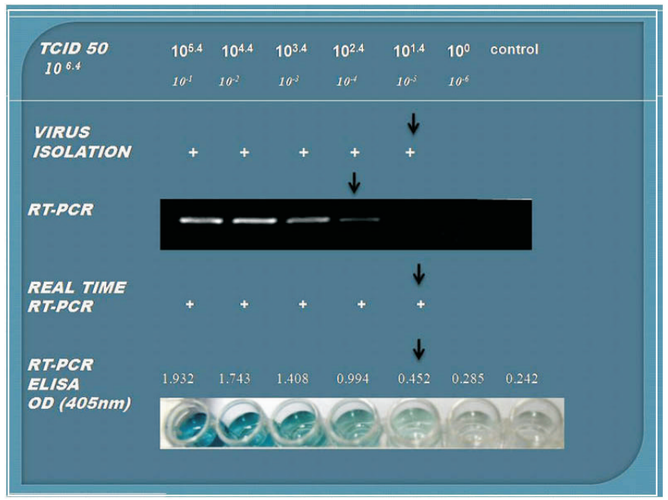


Figure 4: Sensitivity of RT-PCR ELISA, virus isolation, RT-PCR and real-time RT-PCR for detection of BVDV-1 and BVDV-2. ↓ : detection limit; +: positive result.

Table 7: Relative sensitivity and specificity of RT-PCR ELISA with virus isolation and RT-PCR.

RT-PCR ELISA	Virus Isolation			RT-PCR		
	Positive	Negative	Total	Positive	Negative	Total
Positive	47	01	48	39	08	47
Negative	02	71	73	0	74	74
Total	49	72	121	39	82	121
Relative Sensitivity =	95.9%			100%		
Relative Specificity =	98.6%			90.2%		
Concordance =	97.5%			93.3%		
k =	0.9			0.8		
95% CI =	0.9-1.0			0.7-0.9		

The results showed that RT-PCR ELISA can be a rapid, cost effective and alternative molecular diagnostic test for simultaneous detection of BVDV-1, BVDV-2 and BDV in ruminants in ordinary laboratory settings lacking real-time RT-PCR facility and has certain advantages. First, the test is rapid (one day), while virus isolation from clinical samples generally requires 10-14 days. Secondly, virus isolation requires maintenance and culture of cells and other infrastructural facilities that are not available in most of the diagnostic laboratories in developing countries. Thirdly, although both RT-PCR ELISA and real-time RT-PCR have two levels of specificity, RT-PCR ELISA does not require expensive equipment and uses relatively inexpensive capture probes. Additionally, it has the potential to be used as a screening assay for pestiviruses.

2.2 Serological evidence of BVDV in Indian cattle

Testing of 387 diagnostic specimens revealed negative results for BVDV by virus isolation and/or RT-PCR, while BVDV neutralizing antibodies were detected in 35 Indian cattle, and 6 imported bovine serum by virus neutralization test.

3. Malignant Catarrhal Fever

3.1 Emergence of naturally transmitted sheep associated malignant catarrhal fever in diverse ruminant species in India

Malignant Catarrhal fever (MCF) is a fatal herpesvirus infection, with a short and dramatic clinical course characterized primarily by high fever, severe depression, swollen lymph nodes, salivation, diarrhoea, dermatitis, neurological disorders and ocular lesions often leading to blindness. A series of clinical cases of sheep-associated malignant catarrhal fever (SA-MCF) have been recorded in susceptible species Karnataka, Tamil Nadu and Mizoram within a span of 2 years. The first case was recorded in a captive bison in the year 2013 with typical symptoms of SA-MCF which was later confirmed by OIE approved laboratory test. Subsequently, fatal clinical cases of SA-MCF were identified in cattle with symptoms of diarrhoea, respiratory distress, conjunctivitis, nasal discharges. Laboratory diagnosis from the samples of two ailing animals confirmed the detection of ovine herpesvirus-2 (OvHV-2) genome in the peripheral blood samples of cattle. Recently 4 buffalo samples from in and around the villages of Namakkal with typical symptoms of MCF were diagnosed by PCR (Fig 5) and histopathology revealed typical vasculitis and perivascular cuffing, (Fig 6 and Fig 7) The sheep blood samples collected subsequently from the neighbouring areas also showed presence of OvHV-2 genome indicating a nidus of infection in the state. Very recently one of the cattle samples submitted from Mizoram was diagnosed for MCF and ailing pig samples also submitted from the same place were diagnosed as positive indicating the far and wide spread of the disease (Fig 8 and Fig 9). The laboratory test results of positive samples were further confirmed by nucleotide sequencing of the OIE approved portion of tegument gene as well as complete ORF 8 region of the OvHV-2 genome. A diagnostic vigil for the disease as well as a control strategy needs to be formulated for the prevention of economic losses incurred by this fatal infection.

3.2 Regular screening of MCF suspected samples -

179 blood samples from cattle, buffalo and sheep submitted from various parts of the country for diagnosis of MCF were tested and 22 samples were found positive by both outer PCR and nested PCR (Table 8). An occurrence of clinical MCF in one cattle samples in Mizoram, as well as one buffalo from Namakkal was diagnosed and was confirmed by nucleotide sequencing.

Table 8 : Detection of OvHV-2 infection in clinical cases of MCF and carrier species sheep (2014-15)

S. No.	Sample Type	place	Species	Samples tested by nested PCR	Positive samples
1	Tissues	Orissa Vet College	Cattle	14	0
2		KVK Thoothukudi	Cattle	05	0
4	Whole Blood	Orissa Vet College	Cattle	03	0
5		Orissa Vet College	Sheep	03	2
6		Mizoram	Pig	32	16
7	Nasal Swab	CADARD IVRI	Cattle	08	0
8	DNA Sample	CADARD IVRI	Cattle	41	0
9		NIVEDI	Cattle	73	4
		Total		**179**	**22**

3.3 Detection of OvHV-2 infection in pigs and buffaloes in India

Blood samples of pigs from Mizoram same region were confirmed for OvHV2 infection for the first time in India. The OvHV-2 tegument region amplified by PCR from pig sample as well as buffalo and were further confirmed by nucleotide sequencing.

3.4 Nucleotide sequencing and of OvHV-2

The complete glycoprotein gene amplified from positive case of cattle from Mizoram and buffalo from Namakkal were cloned and sequenced by primer walking. The gene

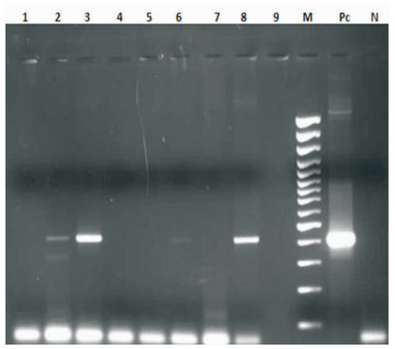


Figure 5. Amplification of the specific tegument gene fragment in samples tested for MCF in buffaloes from Tamil Nadu

encoding Tegument (423bp fragment from both was cloned in pGEMT easy vector and was sequenced by

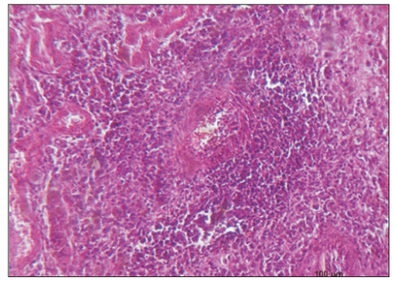


Figure 6. Kidney: Infiltration of mononuclear cells around tunica adventitia of blood vessel in Buffalo (Vasculitis)

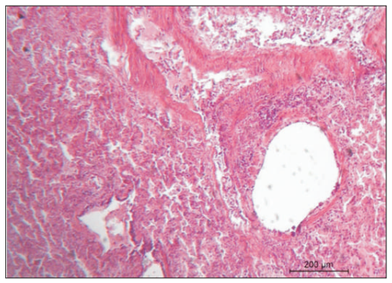


Figure 7. Lung: Bronchiolitis with cellular debris in lumen and thickening of alveolar septa and alveolar lumen filled with cell debris and complete loss of lung architecture in Buffalo (Pneumonia)

Sanger's Dideoxy method on an ABI automated sequencer. The nucleotide sequence of the 422 bp fragment of two pigs matched 99 per cent with OvHV-2 reference sequence (GenBank accession number AY839756.1) and other sequences reported earlier from India and elsewhere. Gen bank submission has been made. The tegument and the 2.8 kb glycoprotein gene sequences of buffalo have been submitted (Gen Bank accession number KF017577 and KR063167).

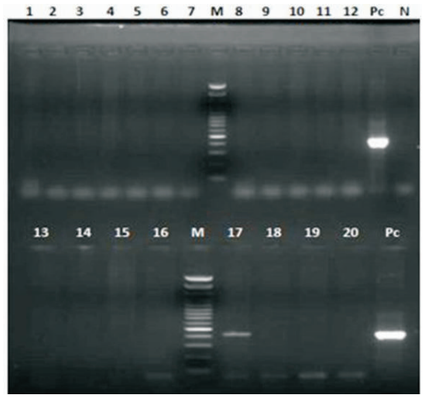


Figure 8. Amplification of the specific tegument gene fragment in samples tested for MCF in cattle from Mizoram. Lane no: 17 showing specific 423bp fragment amplified by OIE approved primers for outer PCR; PC: Positive control; N: Negative control; M: marker

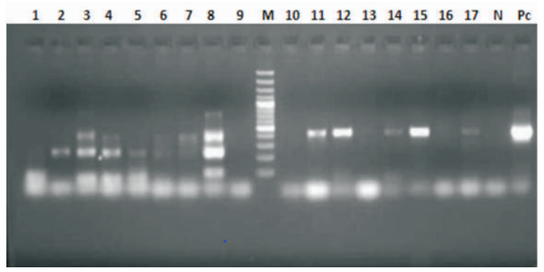


Figure 9. Amplification of the specific tegument gene fragment in samples tested for MCF in pigs from Mizoram
Lane no: 11,12,14,15 and 17 showing specific 423bp fragment amplified by OIE approved primers for outer PCR; Lane No: 2,3,4,5,and 8 showing specific 238 bp fragment amplified by nested PCR; PC: positive control; N: Negative control; M: marker.

4. Detection of PRRSV infection in north-eastern states of India

A total of 495 porcine whole blood/serum samples received from ICAR-research complex for NEH region, Barapani (comprising of serum samples from Meghalaya, Assam, Nagaland and Mizoram) and DI lab, Aizawl, Mizoram were tested by for in-house developed rNA ELISA.238 out of the 495 samples (48%) were positive for PRRSV antibodies. 163 serum samples were processed for virus isolation in PAM cells. Two serum samples (one each from Mizoram and Meghalaya) were positive for virus isolation and were confirmed by immunoperoxidase monolayer assay on MARC-145 cells using PRRSV specific polyclonal serum (Fig. 3). Cytopathic effect could be observed on infected PAM cells from 5th dpi during 2nd passage (Fig. 10 and 11). ORF 7 of both the isolates were amplified by RT-PCR and sequenced. Both these isolates belonged to genotype-2. Further characterization of the new isolates are under progress.

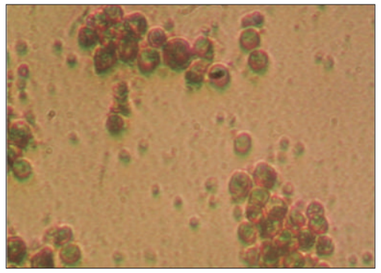


Figure 10. PRRS virus infected porcine pulmonary alveolar macrophages exhibiting CPE on 5th DPI

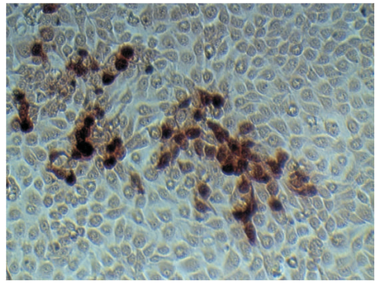


Figure 11. Confirmation of PRRS virus infection in MARC-145 cells by immunoperoxidase monolayer assay

5. Detection of West Nile Fever virus infection in wild birds

Testing of 1848 samples (oral swab: 13, cloacal swab: 1794, tissues from dead birds: 41) from wild and peridomestic birds in 11 states demonstrated that all the samples were negative for West Nile virus by RT-PCR.

6. Detection of Schmallenberg virus infection in Bovine spp.

Schmallenberg virus (SBV) infection is a newly emerging infectious disease of ruminants characterized by fever, inappetence, decreased milk production, loss of condition and diarrhea in cattle and abortion, stillbirths in sheep and goats. Thus, causes huge economic losses to livestock ruminants, especially in small ruminant production. This virus was first detected in early November 2011 by metagenomic analysis and virus isolation from infected cattle in Germany and now prevalent in most of the European countries. SBV spreads through Culicoides midges bites belongs to the Bunyaviridae family, genus Orthobunyavirus, Simbu serogroup is an enveloped, negative-sense, segmented, single-stranded RNA virus and is closely related to Akabane, Ainoa and Shamonda viruses and are widespread in Oceania, Australia, Africa, and Asia. Of the total 38 out of 40 number of bovine sera samples from Rajasthan and other states screened by SBV ELISA kit (ID screen competition multispecies cat #SBVC-4P/1114), 38 samples showed positive reactivity, however, SBV genome was not detected in any of the sample and until now no SBV cases have been reported from India. But other viruses from the Simbu serogroup have been reported from India on couple of occasions. These findings suggest that a very closely related virus to SBV may be already circulating in India, which needs to be supported by virus isolation. Further elaborate investigation and attempts to isolate virus needs to be undertaken.

7. Detection of Crimean Congo Haemorrhagic Fever

Total no of 142 post CCHF outbreak samples (serum/blood/ticks) of animals suspected for CCHF received from Gujarat, Rajasthan & Uttar Pradesh state for the detection of CCHFV (Table 9-12) were found to be negative.

Table 9: Post outbreak samples suspected for CCHF from Rajasthan state

Sl.No.	Animal sample ID	Nature of sample	Species	Test done	Result
1	C1-C23	Serum	cow	RT-qPCR	All samples negative for presence of CCHF virus genome
2	S1-S10	Serum	sheep	RT-qPCR	
3	G1-G10	Serum	goat	RT-qPCR	
4	Camel	Serum	camel	RT-qPCR	
5	Ticks	Ticks	cow	RT-qPCR	
6	Ticks	Ticks	sheep	RT-qPCR	
7	Ticks	Ticks	goat	RT-qPCR	
8	Ticks	Ticks	camel	RT-qPCR	
	Total No. of Samples - 48				

Table 10: Post outbreak samples suspected for CCHF from Gujarat state

Sl.No.	Animal sample ID	Nature of sample	Species	Test done	Result
1	Guj/AMR/CCHF/1-23	Serum	cow	RT-qPCR	All samples negative for presence of CCHF virus genome
2	Guj/AMR/CCHF/24-46	whole blood	cow	RT-qPCR	
3	Guj/AMR/CCHF/47	Ticks	pulled sample	RT-qPCR	
4	Guj/AMR/CCHF/48-53	serum	sheep	RT-qPCR	
5	Guj/AMR/CCHF/54-57	serum	goat	RT-qPCR	
6	Guj/AMR/CCHF/58-63	whole blood	sheep	RT-qPCR	
7	Guj/AMR/CCHF/64-67	whole blood	goat	RT-qPCR	
	Total No. of Samples - 67				

Table 11: Post outbreak samples suspected for CCHF from Patan, Gujarat

Sl.No.	Animal sample ID	Nature of sample	Species	Test done	Result
1	C1-C15	Serum	Buffalo	RT-qPCR	All samples negative for presence of CCHF virus genome
2	Ticks1-3	Ticks	Buffalo	RT-qPCR	
	Total no. of samples - 18				

Table 12: Post outbreak samples suspected for CCHF from Dist: Moradabad, UP

Sl.No.	Animal sample ID	Nature of sample	Species	Test done	Result
1	CC-15	Serum	Cow	RT-qPCR	All samples negative for presence of CCHF virus genome
2	CC-16	Serum	Cow	RT-qPCR	
3	CC-17	Serum	Buffalo	RT-qPCR	
4	CC-18	Serum	Cow	RT-qPCR	
5	CC-19	Serum	Buffalo	RT-qPCR	
6	CC-20	Serum	Buffalo	RT-qPCR	
7	CC-21	Ticks	Ticks	RT-qPCR	
8	CC-22	Ticks	Ticks	RT-qPCR	
9	CC-23	Ticks	Ticks	RT-qPCR	
	Total no. of samples - 9				

8. Report of outbreak of PPMV in Pigeon

During December to January 2015, heavy mortality of pigeons was reported in pigeons brought from local market in Bhopal for some other experimentation. The clinical signs among live birds included ocular and nasal discharge, dyspnea, bloody diarrhea, depression, balance problems, tremors, especially of the head and neck, weakness and partial or total paralysis and depend on factors like age, sex, immune status of the birds and environmental conditions. Post mortem lesions were mainly found in brain, liver, kidneys and spleen. Amongst various organs, kidneys were more frequently affected. Also there was high seroconversion among pigeons as indicated by heammaglutination assay. The tissue samples Viz. kidney, lungs, spleen, heart and liver were collected from dead pigeons and the virus was isolated in 9-11 days old embryonated chicken eggs from these tissue samples. Upon serological, molecular and pathological investigation the disease was confirmed as Pigeon Paramyxivirus-1 (PPMV-1). Further, the disease was confirmed by serological methods such as haemaglutination and haemaglutination inhibition assay and molecular technique like polymerase chain reaction using reported primers. In the presence of NDV antiserum, the inhibition of chicken red blood cells was observed. However using seven sets of reported primers the whole genome of NDV was amplified in seven segments by employing reverse transcriptase PCR and sequenced.

The report of PPMV was important due to high mortality in free birds and chances of spread of the disease to other birds including poultry. This evidence necessitates for the active surveillance of the disease in chickens as well as pigeons for successful control of the disease in the country. Likewise the detailed study of the virus-host interaction needs to be undertaken in order to identify role of pigeons in pathobiology of the virus and persistence of the virus in the environment.

9. Diagnostic services for Animal Quarantine and Certification Services:

a. Testing of German bulls imported to India in Feb. 2015 for BVD & Schmallenberg infection:

Blood and serum samples collected from 76 German HF bulls stationed at AQCS, Chennai and Kolkata in Feb. 2015 were tested for bovine viral diarrhoea and Schmellanberg virus. All the animals were found free of BVDV and Schmallenberg infection when tested by methods prescribed by the OIE and hence were released from the quarantine stations.

b. Testing of horses returning after participating in 17th Incheon Asian Games for Nipah and Hendra :

A total of 15 nos. of samples from 5 horses returning after participating in 17th Incheon Asian Games were received for diagnosis of Nipah and Hendra viruses. All samples were negative by RT-PCR (Table 13) for Nipah and Hendra virus infections.

Table 13: Testing of samples from horses imported horses returning after participating in 17th Incheon Asian Games.

Sl.No.	EFI registration no.	Name of Horse	Nature of sample	Test done	Result
1	2458	Ramases	Serum/blood/nasal swab	RT-PCR	All samples were negative for Nipah & Hendra viruses genome by specific RT-PCR assay
2	1351	Allegro	Serum/blood/nasal swab	RT-PCR	
3	3093	Laila Lordanos	Serum/blood/nasal swab	RT-PCR	
4	2263	Cantaro	Serum/blood/nasal swab	RT-PCR	
5	2291	Fleece Clover	Serum/blood/nasal swab	RT-PCR	
Total no of samples-15					

c. Testing of samples from imported livestock/birds, meat and meat products:

NIHSAD is offering diagnostic services to AQCS centers for screening of imported livestock/birds and meat and meat products for various emerging and exotic animal diseases. The sample-wise details received at NIHSAD and the diseases for which testing was carried out and the methodology used are given in Table 14 and 15.

Table 14: Specimen wise AQCS Samples received during (2014-15).

AQCS	Type of sample	Diseases tested								
		AI	BVD	MCF	CCHF	PRRS	NSD	CAE	RHD	RVF
Mumbai	Pet food	195	-	-	-	-	-	-	-	-
	SPF Egg	615	-	-	-	-	-	-	-	-
	Pork Meat	114	-	-	-	98	-	-	-	-
	Pig Bristles	3	-	-	-	-	-	-	-	-
	Turkey Meat	6	-	-	-	-	-	-	-	-
	Chicken Meat	15	-	-	-	-	-	-	-	-
	Duck Feather	1	-	-	-	-	-	-	-	-
	Duck Meat	4	-	-	-	-	-	-	-	-
	Lamb meat	-	-	33	-	-	33	33	-	-
	Bull semen	-	-	-	-	-	-	-	-	-
Chennai	Pet Food	796	-	-	-	-	-	-	-	-
	Cloacal Swab	2316	-	-	-	-	-	-	-	-
	Serum	14	40	-	-	-	-	-	-	-
	Rabbit serum	-	-	-	-	-	-	-	24	-
	Whole blood	-	40	40	-	-	-	-	-	-
Delhi	Pet Food	43	-	-	-	-	-	-	-	-
	Pork Meat	21	-	-	-	21	-	-	-	-
	Serum	420	18	-	13	5	-	-	-	15
	Pig Bristles	53	-	-	-	-	-	-	-	-
	Chicken Meat	3	-	-	-	-	-	-	-	-
	Duck Feather	1	-	-	-	-	-	-	-	-
	Shuttle Cock	5	-	-	-	-	-	-	-	-
	Lamb meat	1	-	-	-	-	10	10	-	-
	Bull semen	-	36	-	-	-	-	-	-	-
	Rabbit serum	-	-	-	-	-	-	-	4	-
	Whole blood	-	18	18	13	5	-	15	-	-
Bangalore	GP Serum	120	-	-	-	-	-	-	-	-
Kolkata	Whole blood	-	36	36	-	-	-	-	-	-
	serum	-	36	-	-	-	-	-	-	-
	TOTAL	**4746**	**206**	**127**	**26**	**129**	**43**	**43**	**28**	**15**

Table 15: Tests carried out for diagnosis of various diseases:

S. No.	Disease	Diagnostic test	
		Agent identification	Serology
1	Avian Influenza	1. Virus Isolation (VI) 2. RT PCR for Subtyping 3. Real Time RT -PCR	1. AGID (chicken) 2. HI test 3. ELISA (other than chicken)
2	Bovine Viral Diarrhea (BVD)/ Border Disease (BD)	1. Virus Isolation (VI) 2. RT -PCR 3. Real Time RT -PCR	1. Virus Neutralization Test (VNT) 2. Ab-ELISA
3	Porcine reproductive and respiratory syndrome (PRRS)	1. Virus Isolation (VI) 2. RT -PCR	1. ELISA
4	Rabbit Haemorrhagic Disease (RHD)	1. Antigen ELISA/ RT -PCR	1. Antibody ELISA
5	Nairobi Sheep Disease (NSD)	1. RT -PCR	
6	Rift Valley Fever (RVF)	1. RT -PCR	
7	Crimean–Congo haemorrhagic fever (CCHF)	1. Real Time RT -PCR	
8	West Nile Fever (WNF)	1. RT -PCR	
9	Caprine Arthritis Encephalitis (CAE)	1. Semi Nested PCR	
10	Malignant Catarrhal Fever (MCF)	1. Semi Nested PCR	
11	Swine Influenza (SI)	1. Virus Isolation (VI) 2. RT -PCR	1. HI test 2. ELISA
12	Aujeszky's disease (AD)	1. PCR	1. Antibody ELISA

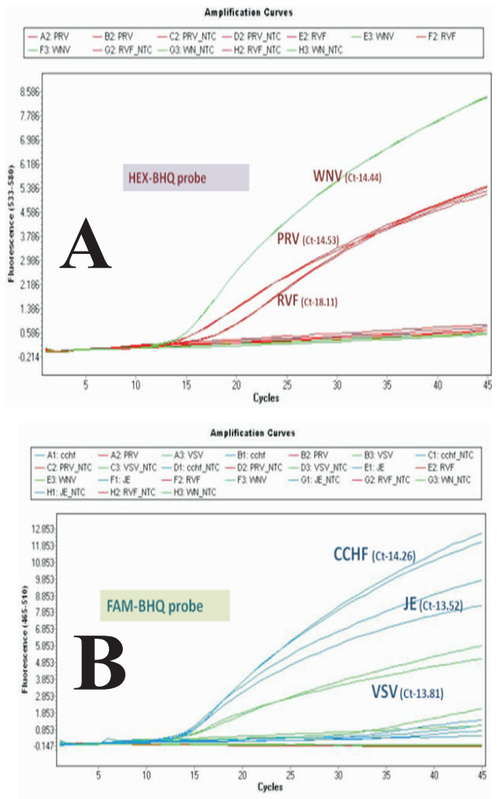
Project- Quest for a Universal Diagnostic PCR Array for multiple exotic and emerging viral diseases

PI: A. A. Raut, Co-PI: A. K Pateriya, A. Mishra, P. Gandhale

Development of Universal diagnostic PCR array for simultaneous detection of multiple exotic/emerging viruses.

The identification of infectious agents by molecular diagnostic methods is increasingly being used in clinical diagnostic laboratories. Among them, detection of specific nucleic acid sequences of the infectious agents by PCR and realtime PCR is most preferred choice for diagnosis, identification, and quantitation due to greater sensitivity and specificity of these tests. In the present study, PCR arrays which are now becoming popular for their ease, versatility, sensitivity etc. has been developed for the detection of 22 prioritized exotic and emerging viruses(as per the mandate of ICAR-NIHSAD) of multispecies, ruminants and swine origin using Molecular beacon (MB) and SYBR Green realtime PCR chemistry. This technology offers the simultaneous detection of various pathogens by running a panel of real time PCR in different wells of a single PCR plate under uniform reaction and thermal conditions.

In this study, primarily on the basis of earlier reports and available literature target gene/regions for each pathogen was selected and consensus sequence was determined using multiple sequence alignment to extend the scope of test. Accordingly,specific probes and primers for Molecular Beacon and SYBR green chemistry were designed and synthesized for diagnosis of ten multiple species diseases viz.Crimean-Congo haemorrhagic fever (CCHF), Rift Valley Fever (RVF), West Nile Fever (WNF), Vesicular stomatitis (SV), Japanese encephalitis (JE), Aujeszsky's disease, Ebola, Marburg, Eastern Equine Encephalitis (EEE), Hantaan**;** seven ruminant diseases viz Border Disease (BD), Bovine Viral Diarrhea (BVD), Caprine Arthritis Encephalitis (CAE), Enzootic Bovine Leucosis (EBL), Malignant Catarrhal Fever (MCF), Nairobi Sheep Disease (NSD), Schmallenberg virus infection; and six swine diseases viz. African swine fever (ASF), Nipah, Porcine reproductive and respiratory syndrome (PRRS), Swine Influenza (SI), Swine vesicular disease (SVD), Transmissible gastroenteritis (TGE). The positive control of the assay was designed by synthesizing a plasmid construct for each group of viruses containing target sequences in tandem with unique restriction site in between. The *in vitro* RNA (IVT-RNA) was synthesized as positive



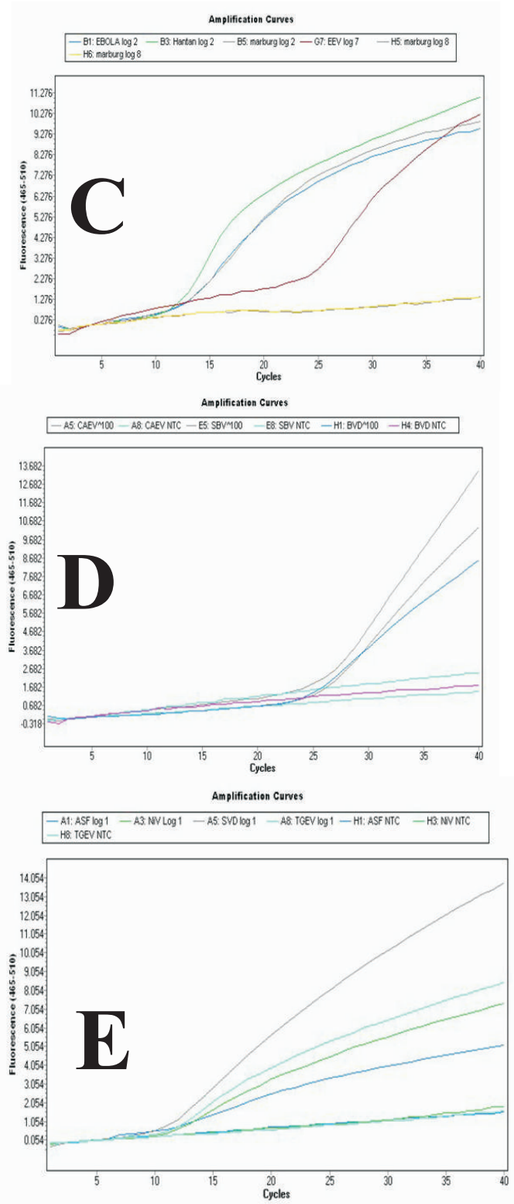


Figure 12. Amplification curves and melting curves of different pathogens under optimized universal reaction condition and thermal cycling condition of real time PCR array using Molecular Beacon Chemistry. A) CCHF, JE, VSV B) WNF, RVF, Aujeszskys C) Ebola, Marburg, Hantaan, EEE D) BVD, CAE, Schmallenberg E) ASF, Nipah, SVD, TGEV

control target RNA while optimizing the conditions for RNA viruses. The uniform reaction and thermal cycling conditions were optimized for simultaneous detection of all selected pathogen for both the MB and SYBR Green realtime PCR chemistries. The standard curves were established for each probe and primers set. The sensitivity (limit of detection) of the array using both the chemistries was assessed ranging from 200 to 10 copies with efficiency of 1.8 to 2.0 in Roche Light Cycler 480 II. The developed array can simultaneously detect 17 viruses using Molecular Beacon (MB) chemistry (Fig. 12) and 22 viruses using SYBR Green chemistry (Fig. 13).The proposed PCR array is rapid, cost effective and capable of detecting a panel of prioritized pathogens in a single operation. This array would be open to updating and addition to any number of new diseases.

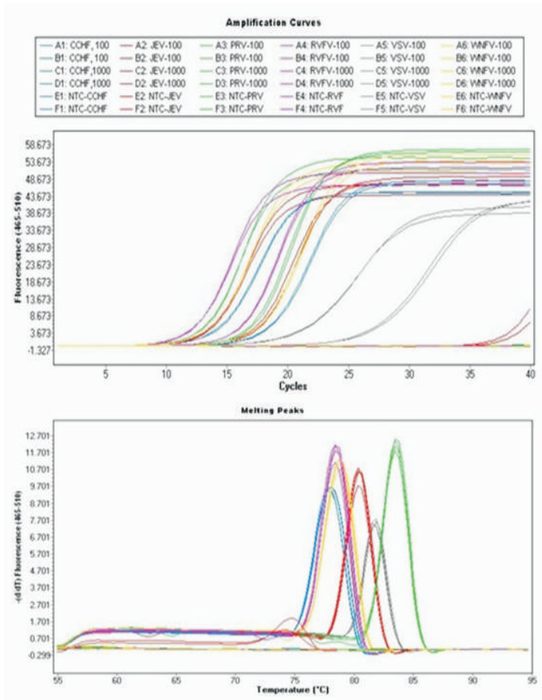


Figure 13. Amplification curves and melting curves of different pathogens under optimized universal reaction condition and thermal cycling condition of real time PCR array using SYBR Green chemistry.

Project-Evolutionary analysis of avian influenza viruses isolated in India

PI: C. Tosh Co-PI: S. Nagarajan, G. Venkatesh, M. Kumar

Introduction of a novel reassortant H5N1 avian influenza virus into Indian poultry during 2014-2015

Avian influenza, particularly H5N1 subtype continues to threat poultry industry worldwide. During 2014-2015, outbreaks of H5N1 virus infection in poultry occurred in the States of Kerala, Uttar Pradesh, and Union Territory of Chandigarh. Besides poultry, H5N1 virus was isolated from dead crows from the State of Odisha. To characterize, genome sequence of the viruses isolated from chickens, ducks, turkey and crow were determined and analyzed. The viruses were characterized to be highly pathogenic, as they possessed multiple basic amino acids (arginine/lysine) at their Haemagglutinin (HA) cleavage site. Sequence analysis of the neuraminidase genes revealed that the viruses are sensitive to neuraminidase drugs. However, the matrix gene revealed Val-27-Ile substitution in one of the amantadine-binding sites (L26, V27, A30, S31 and G34) of M2 gene, but the significance of the change to confer drug resistance is uncertain. The viruses compared grouped into two distinct phylogenetic clades in the HA tree (Fig. 14). The viruses isolated till early 2014 grouped with clade 2.3.2.1A, whereas the recent H5N1 viruses isolated from Kerala, Chandigarh and Uttar Pradesh grouped with a new clade 2.3.2.1 C, which has not been detected before in India poultry. Within clade 2.3.2.1 C, the Indian viruses grouped closely with H5N1 viruses isolated from chicken, duck, myna and tiger in China and Vietnam during 2012-2013. In all other genes, except PB2, the viruses grouped with H5N1 viruses isolated from China and Vietnam. Whereas in the PB2 gene phylogeny, the Indian viruses grouped with H9N2 viruses isolated from chicken and quail in China, Tibet and Vietnam during 2007-2013 indicating that the H5N1 virus isolated in India are resossortants of H5N1 and H9N2 viruses (Fig. 15). Since the PB2 gene is related to H9N2 virus isolated in China, Tibet and Vietnam, the

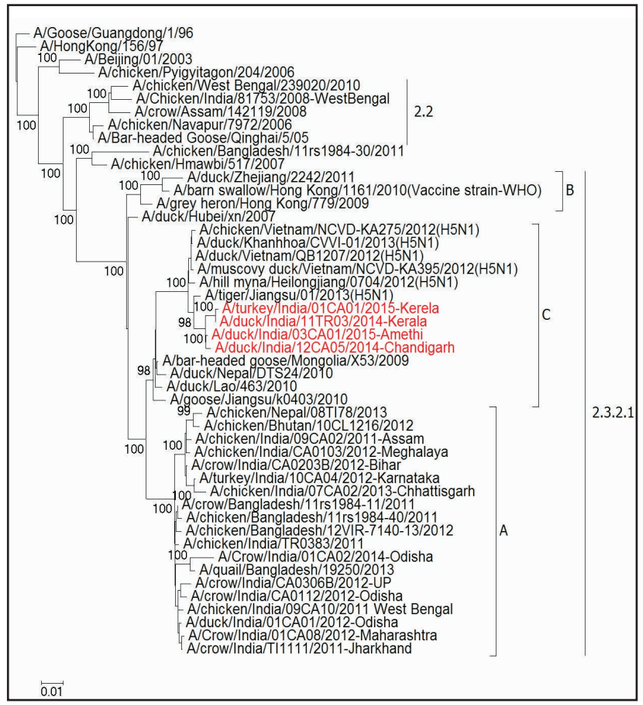


Figure 14. Phylogenetic analysis of H5N1 AIV (based on HA gene).

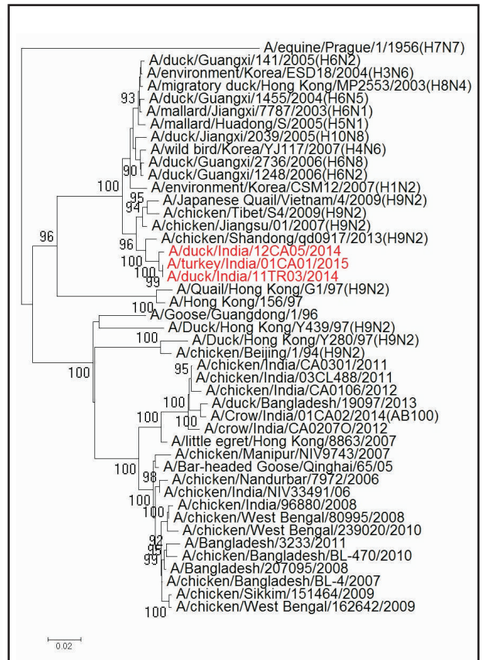


Figure 15. Phylogenetic analysis of H5N1 AIV (based on PB2 gene).

reassortment event might have occurred before introduction of the virus into Indian poultry. Role of migratory birds has been implicated in the spread of H5N1 virus. As India falls in one of the major flyways (Central Asian), the novel H5N1 virus might have been introduced to Indian poultry through migratory birds, although trade of poultry/poultry products cannot be ruled out. Therefore, surveillance in poultry, wild birds and peridomestic birds should be continued for early detection of virus to help control avian influenza.

Genetic diversity of avian influenza H9N2 viruses isolated from poultry in India during 2014

Infection of poultry with low pathogenic avian influenza (subtype H9N2) virus is geographically widespread and some regions endemic. Human infections with H9N2 virus has also been report in Asian countries thereby revealing its pandemic potential. Studies revealed that the H9N2 virus has contributed gene segments for the emergence of novel H5N1 virus in Hong Kong in 1997 and H7N9 virus in China in 2013 resulting in transmission virus from birds to humans leading, in many occasions to death. In this report, three H9N2 viruses isolated from poultry farm the States of Odisha and Kerala during 2014 were characterized. The nucleotide sequence of the haemagglutinin (HA) gene of the viruses isolated from chickens were determined and analyzed. The HA genes of the two Odisha isolates possessed single basic amino acid at the HA cleavage region indicating low pathogenic avian influenza, whereas the Kerala isolate possessed two basic amino acids at the same region (KSKR/GLF). To ascertain pathogenicity of Kerala isolate, intravenous pathogenicity test in chickens needs to be carried out. In the phylogenetic analysis, the H9N2 isolates from Kerala and Odisha formed two distinct groups within

G1-like sublineage of H9 viruses (Fig. 16). The Odisha virus grouped with H9N2 viruses isolated from chickens in West Bengal, Haryana and Chhattisgarh and Nepal during 2009-2013, whereas the virus isolated from Kerala grouped with H9N2 virus isolated in Tamilnadu during 2010. Emergence of genetic diversity highlights the need for surveillance to control the avian influenza.

Figure 16. Phylogenetic analysis of H9N2 AIV (based on HA gene).

Project- Development and evaluation of referral group specific and subtype specific diagnostic reagents for avian influenza

PI: S. Nagarajan Co-PI: C. Tosh, G. Venkatesh K. Rajukumar, Manoj Kumar, Richa Sood D. Senthil Kumar

Development of Agar Gel immunodiffusion test kit for avian influenza:

A total of 2071 (813 in 2013-2014) sera samples from various parts of the country were tested for AIV by AGID antigen and HI test. The sensitivity is 94.28% and specificity is 94.96% compared to HI test. All the sera (84) of SPF birds were negative by AGID and HI tests. Experimental samples (1-60 DPI) – Detection by AGID starts on 6 DPI and continue up to 60 DPI (period tested). Sensitivity from 6-28 DPI is 97.37%. 29-60 DPI – 73.55%. Specificity is 100%. Stability of AGID antigen at -70°C – up to 6 months. Antisera against H1, H3, H4, H5, H9 and H11 AIV raised using inactivated whole virus antigen. The AGID antigen could detect all the subtype specific antisera raised. Lyophilization of AGID antigen prepared was done and the intra-laboratory validation on lyophilized antigen was carried out with blinded panel of sera by three independent scientists. The analysis of the intra-laboratory results validated the Standard Operating Protocol.

Recombinant expression of neuraminidase protein of avian influenza virus and its characterization

Recombinant neuraminidase (NA) protein of avian influenza virus isolate (A/chicken/Nandurbar/India/7972/2006) has been expressed in pET28a **(+)** vector. After purification, the recombinant NA N1 (rNA1) protein was found localized in inclusion bodies fraction. To obtain a high purity, the inclusion bodies fraction was solubilized in binding buffer with Urea (6M) and purified using Ni-NTA agarose. The purified rNA protein (50KDa) was used for development of an indirect ELISA to screen sera samples for antibodies to NA (N1) types. Sample to positive (Sp) ratio value of 0.570 was found to be the most appropriate cut off (95% specificity, and 88.63% sensitivity). Comparatively less sensitivity of the test (88.63%) can further be assessed by including more number of experimental samples. In future, the test will be useful for differentiating between infected and vaccinated population to evaluate the success of vaccination program.

Project- Evaluation of diagnostic potential of monoclonal antibodies raised against H5N1 avian influenza virus

PI: G. Venkatesh Co-PI: C.Tosh, S. Nagarajan, R. Sood, Kh.Victoria Chanu

Characterization of haemagglutinin (HA) specific MAbs

Four cryopreserved HA specific MAb producing hybridoma clones could be successfully revived. The testing of the supernatant confirmed the production of the MAb. Using the hybridoma clone cells the ascites fluid were produced. The characterization of the MAbs by neutralization assay, Western blotting and haemagglutinin assay confirmed that the MAbs were directed against the HA protein of the H5N1 Avian influenza virus. The MAbs showed a neutralization index of more than 1:256 and HI titre of more than 2^7. The MAbs were purified by affinity column chromatography. To elucidate the Mab binding sites the MAb resistant mutants (MARM) against these four MAbs were selected. The selection of MAR mutants were confirmed by HI, VNT, Western blotting and IPMA. The HA gene of each of the MARM virus was amplified and sequenced.

Project- Identification of neuraminidase inhibitor drug resistance in H5N1 viruses

PI: R. Sood Co-PI: A. Mishra, A.K. Pateriya, Kh. Victoria Chanu

Identification of neuraminidase inhibitor drug resistance by enzymatic colorimetric assay (MUNANA) in the HPAI isolates from NIHSAD repository

Colorimetric enzymatic assay MUNANA was standardized and optimized for identification of drug resistance against currently approved FDA drugs, Oseltamivir and Zanamivir Neuraminidase inhibitors and has been established in the laboratory for fast and efficient screening of drug resistance in the currently circulating influenza viruses in the country. The protocol for virus inactivation with minimal loss of NA activity and use of this inactivated virus in the assay was optimized. A total of 46 isolates form the year 2007 to 2014 have been screened for drug resistance for Oseltamivir and Zanamivir revealing drug resistance in 3 Indian isolates with known mutation markers E119A and N294S and decreased susceptibility for two isolates A/Chicken/West Bengal/142121/2008 and A/chicken/ West Bengal/155505/2009 having Isoleucine at position 117 replaced with valine and threonine respectively.

Project- Cross-sectional study of Malignant Catarrhal Fever infection in domestic ruminants in Southern India

PI: R. Sood Co-PI: M. Kumar, Kh. Victoria Chanu

Cross-sectional study indicates large part of sheep population in Southern and Central India is infected with ovine herpesvirus-2.

In a cross-sectional study, prevalence of ovine herpesvirus-2 (OvHV-2) infection was estimated in sheep population of two states; Maharashtra and Telangana (earlier part of Andhra Pradesh). Based on epidemiological method, Random stratified sampling, whole blood samples of sheep were collected from identified sheep-dense districts of these states. The samples were tested for presence of OvHV-2 genome by OIE recommended hemi-nested polymerase chain reaction (PCR) test. Sheep density plays a considerable role in transmission of Ovine herpes virus-2 (OvHV-2), the villages were classified into three different strata based on sheep density and proportionate samples were drawn randomly. In Maharashtra from a total of 553 samples collected from sheep dense areas which included in 17 taluks of nine districts Maharashtra (Fig. 17) a high prevalence of 55.6% was identified (Table 16). In Telengana

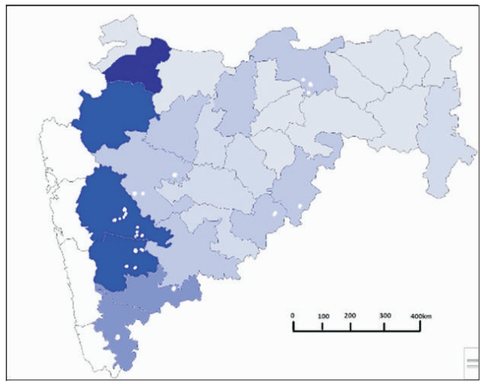


Figure17. Sample distribution of sheep samples collected from Maharashtra State

Table 16: Prevalence of OVHv$_2$ infection in sheep population of Maharashtra State

District	Taluk	Village	True Prevalence (%)	95% CI
Kolhapur	Bhudargad	Appachiwadi	50	15.35-84.65
	Panhala	Yavaluj	21.43	6.23-36.63
Satara	Man	Bijavadi	66.67	39.99-93.34
		Takewadi	64.29	46.54-82.03
	Phaltan	Mirewadi	64.29	46.54-82.03
Ahmadnagar	Parner	Ganjibhoyare	40.91	20.36-61.19
		Hinjangaon	60.71	42.62-78.80
		Ranjangaon masjid	42.86	24.53-61.19
		Sangvi surya	71.43	37.96-100.00
	Pathardi	Sakegaon	60.71	42.62-78.80
Solapur	Malshiras	Kothale	12	0.00-24.74
	Mangalwedha	Lonar	13.79	1.24-26.34
Sangli	Jat	Motewadi	44.44	11.98-76.91
Pune	Baramati	Baburdi	96.15	88.76-100.00
		Magarwadi	68.97	52.13-85.80
		Pandare	69.23	51.49-86.97
	Shirur	Shikrapur	22.22	3.02-41.43
		Talegaon dumdere	45.45	16.03-74.88
	Daund	Pimpalachiwadi	90.91	78.90-100.00
	Haveli	Holkarwadi	60	35.21-84.79
Amravati	NandgaonKh	Rohana	82.14	67.96-96.33
	Amravati	Wadad Pusad	37.5	20.73-54.27
Latur	Udgir	Halli Handarguli	89.66	78.57-100.00
Nanded	Biloli	Chitmogra	51.85	33.00-70-70

sheep density in varies from 10-225 sheep per square km, with highest sheep density at Mahaboob nagar. (Fig. 18). Accordingly, 260 blood samples were collected from four districts and 11 mondals. The results are shown in the Table 17 given below. The overall prevalence was 43.03%. The nucleotide sequence of the 422 bp DNA fragment, amplified in PCR, matched 99% with OvHV-2 reference sequence and other sequences reported from India. Grouping of OvHV-2 sequences obtained from Karnataka with those from Andhra Pradesh, Tamil Nadu and Jammu and Kashmir in the neighbour joining tree indicated a close relationship among the OvHV-2 viruses circulating in India. There is need to initiate immediate control measures to prevent losses.

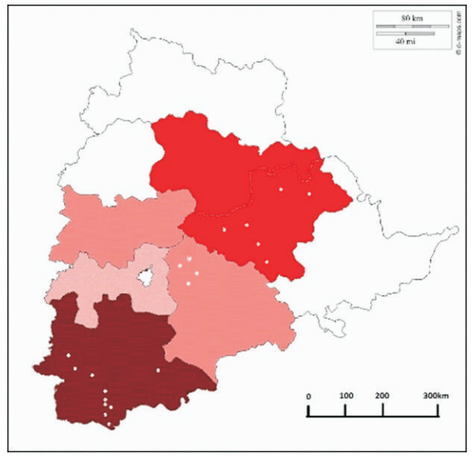


Figure18. Sample distribution of sheep samples collected from Telengana State

Table 17: Prevalence of OvHV-2 infection in sheep population of Telangana state

SI No.	Name of District	Mondal	Village	True Prevalence (%)	95% Confidence interval
1	Warangal	Ghanpur	Ippagudem	17.65	0.0035.77
		Sonakpally	Thanedarpalle	11.76	0.00- 27.08
		Thorrur	Gurthur	25.53	3.37- 43.69
2	Nalgonda	Bibnagar	Jameelapet	88.24	72.92- 100.00
		Bhongir	Bandasomaram	70.59	48.93-92.25
3	Mahabubnagar	Chinnachintakunta	Kurumurthy	42.14	33.96- 50.32
		Kollapur	jawaipally	58.82	35.43- 82.22
		Makthal	Jaklar	66.67	44.89- 88.44
		Pangal	Pangal	23.53	3.37- 43.69
		Peddamandadi	Manigilla	70.59	48.93-92.25
		Telkapalle	Gouraram	11.11	0.00- 25.63
		Veepangandla	Saginepally	66.67	44.89- 88.44
4	Kurnool	kurnool	konidela	35.00	14.10- 55.90

Project- Identification of transcriptomics variations of duck and crow in response to evolving Avian influenza virus(H5N1)

PI: A. Mishra, Co-PI: D.D. Kulkarni, A. A. Raut, R. Sood

Genome wide gene expression pattern underlying differential host response to different H5N1 avian influenza virus isolates in ducks revealed

The comparisons of molecular pathogenesis of different pathotypes of H5N1 viruses in ducks are necessary to determine commonalities and differences in the host response shall be helpful to understand the molecular basis of different outcomes of H5N1 infection in ducks. The genome wide host gene expression of lung tissues infected with A/duck/India /02CA10/ 2011(AD2011) H5N1 virus and A/duck /Tripura/ 103597/ 2008 (AD2008) H5N1 virus in ducks was analysed using microarray. Duck specific 8x60k microarray chip (AMADID G4102A_059612) was designed using recently published genome sequence of Anas platyrhynchos available in the NCBI database. Comparative analysis of differentially expressed genes revealed that 688 genes were commonly expressed, 877 and 1556 genes are uniquely expressed to infection with AD2011 and AD2008 virus isolate, respectively (Fig. 19). The up-regulation of cytokines genes OAS, IL1B, IL17, IFITM2, CCL4, CXCR4, STAT3, TGFB1 and TGFB2 in the lungs tissues may cause high mortality in

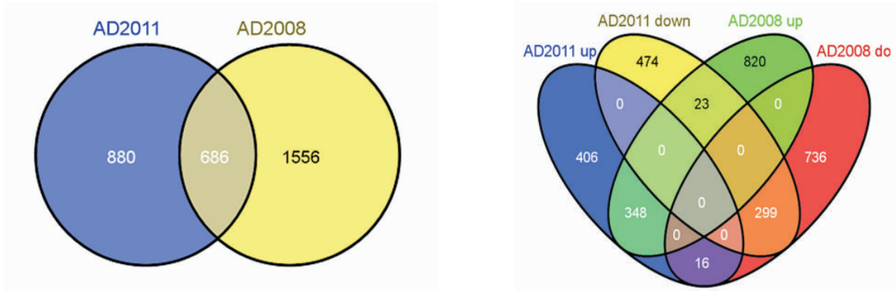


Figure 19: Comparative analysis of gene expression changes between AD2011 and AD2008 infection condition at 5dpi. A. Venn-diagram showing the comparison of genes list between AD2011 and AD2008 infection condition. B. Venn-diagram showing the number of differentially expressed genes during AD2011 and AD2008 infection in duck lungs.

ducks infected with AD2011 virus. The expression of important antiviral immune genes IFIT5, IFITM5, RSAD2, EIF2AK2 (PKR), Mx, β-defensins, TRIM23 and SLC16A3 in AD2008 infection, but not in AD2011 infection, fine-tunes the host innate immune responses and prevent cytokines storms and tissue damage. Several immune related Gene ontology (GO) terms and immune pathways activated were qualitatively similar but quantitatively different during infection of these two viruses (Fig. 20). The differential regulation of genes associated with cytokines storms are responsible for the severity of disease caused by the AD2011 infection compare to AD2008 in ducks.

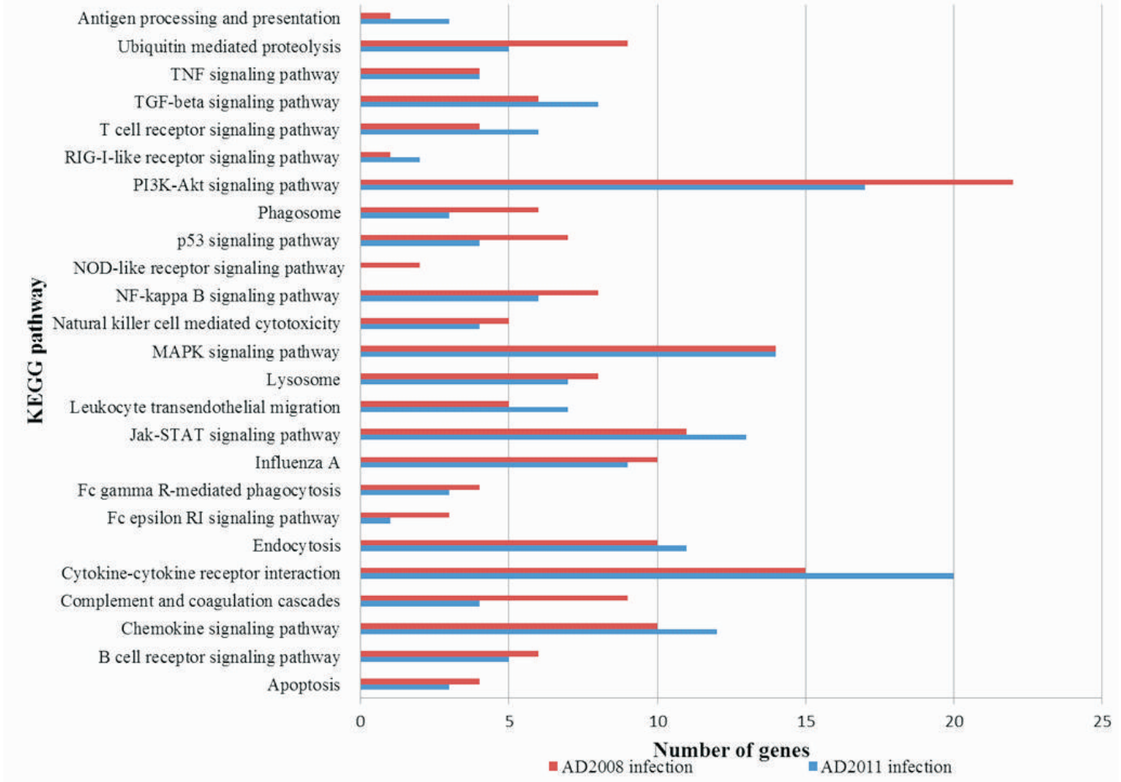


Figure 20. KEGG pathways differentially enriched in response to AD2008 and AD2011 infection in ducks.

Microarray analysis of host gene expression during HPAIV H5N1 and LPAIV H9N2 infection in chicken

Global immune response of chicken experimentally infected with HPAI H5N1 (A/duck/India/02CA10/2011) and LPAI H9N2 (A/duck/India/249800/2010) viruses was studied using microarray to identify crucial host genetic components responsive to these infection. A custom designed 8X60K microarray chip developed by modification of the commercially available 45K chicken chip was used on Agilent platform. Total RNA isolated from the infected lung tissue was subjected ti Microarray analysis against non infected lungs as control. Genes upregulated in HPAI H5N1 reveled enrichment of Influenza A pathway, Jak-STAT signaling pathway, NOD-like receptor signaling pathway, p53 signaling pathway, RIG-I-like receptor signaling pathway, Toll-like receptor signaling pathway, TNF signaling pathway etc (Fig. 21). HPAI H5N1 down regulated genes enriched the Cytokine-cytokine receptor interaction, Jak-STAT signaling pathway, MAPK signaling pathway, PI3K-Akt signaling pathway etc (Fig. 21). However, it was found that the number of genes was quantitatively different between these two genes list. The cellular pathways TLR pathway, RIG-I pathway, Jak-STAT signaling pathway, NF-kappa B signaling pathway, Cytokine-cytokine receptor interaction, MAPK signaling pathway, TGF-beta signaling pathway, Chemokine signaling pathway etc (Fig. 22) are activated in response to LPAIV in chickens confirming an innate immune response at the transcriptional level. This study indicated the relationship between host antiviral genes and their roles in pathogenesis in chickens infected with HPAIV. HPAI H5N1 virus infection in chickens triggers the intense induction of genes relevant to IFN, inflammatory, and innate immune responses. The intense host antiviral response is however unsuccessful in controlling the rapidly progressing infection and is thus consistent with the high mortality in chickens. In contrast, the expression levels of most of the immune genes remains unchanged in the lungs of LPAI H9N2 virus-infected chickens. These microarray dataset provide further insight into the potential molecular mechanisms triggered in chickens in response to AIV infection and reveals the molecular basis of differential response of chicken to the different pathotypes of AIV.

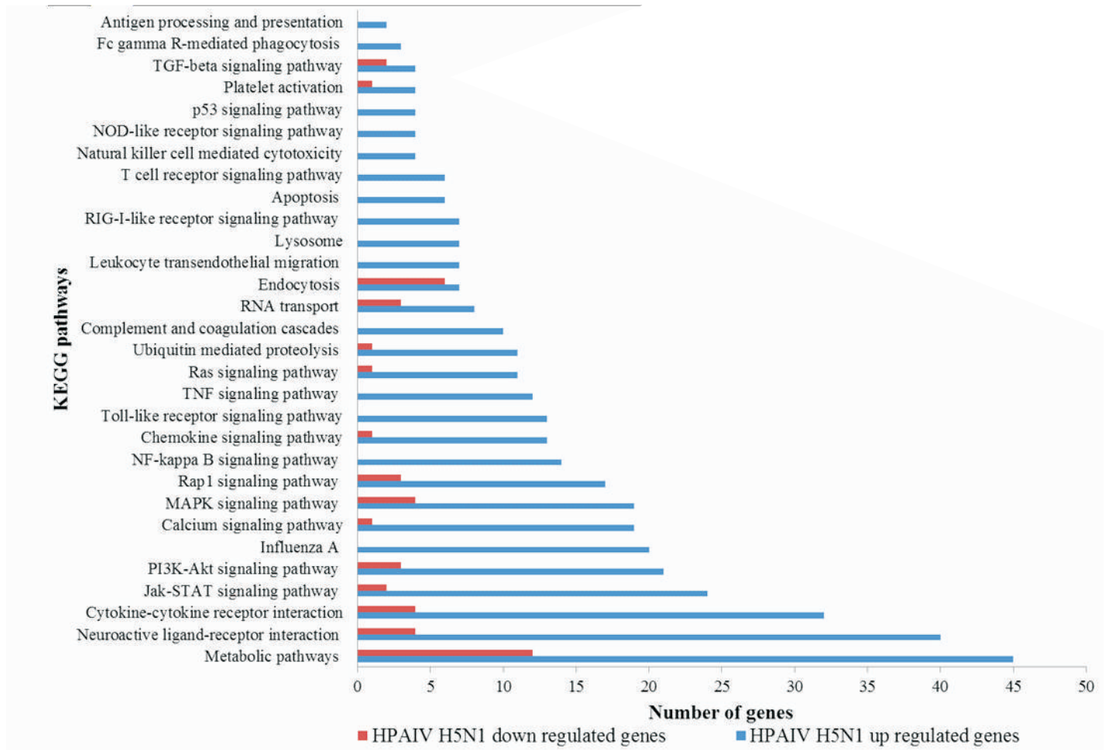


Figure 21. KEGG pathways activated in response to HPAIV H5N1 infection. Up regulated and down regulated genes differentially enriched in various pathways are shown.

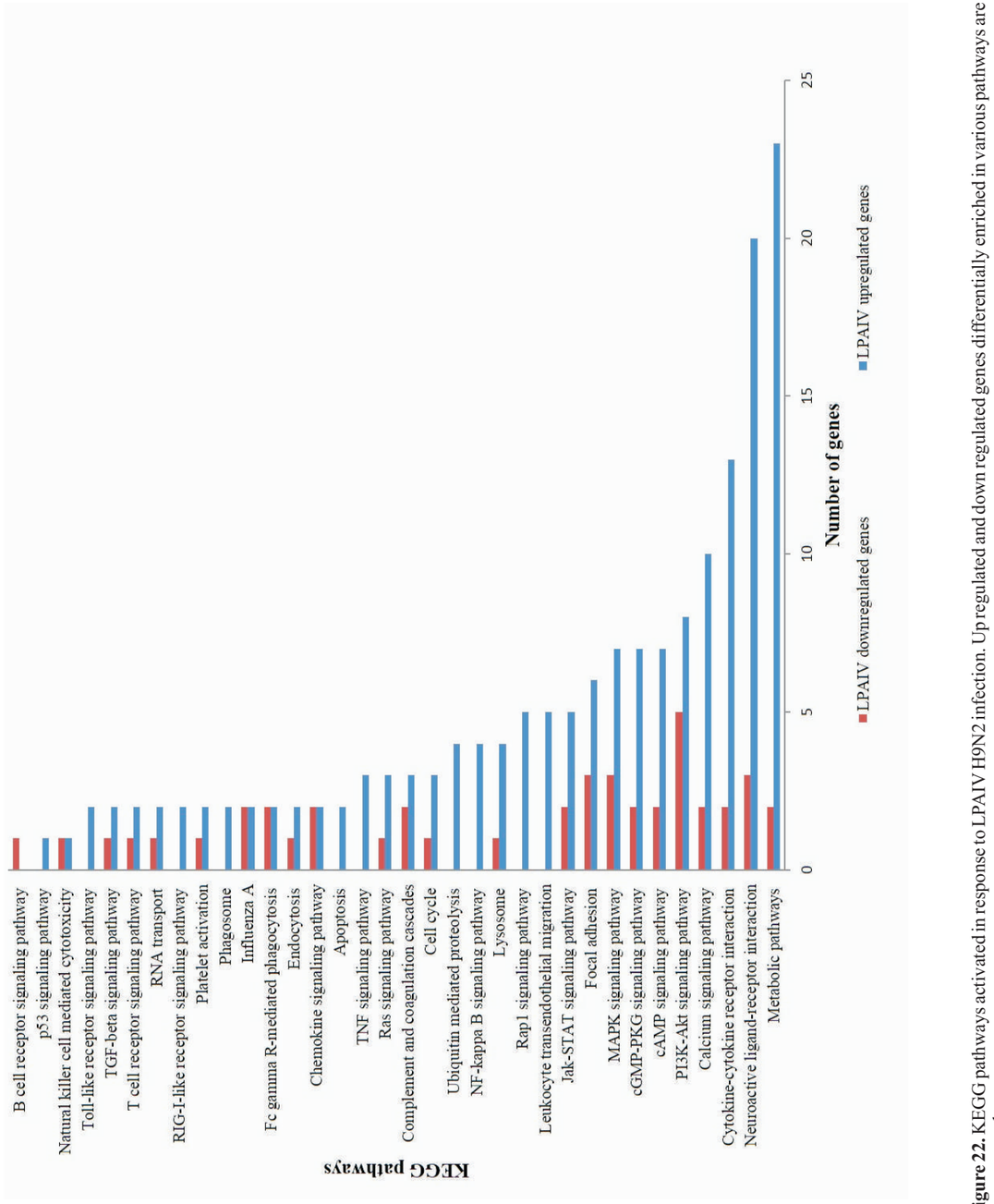


Figure 22. KEGG pathways activated in response to LPAIV H9N2 infection. Up regulated and down regulated genes differentially enriched in various pathways are shown.

Externally Funded Projects

Project-Outreach programme on zoonotic Diseases (Avian Influenza)

PI: H.V. Murugkar Co-PI: M. Kumar

Preen oil increases avian influenza virus survivability in duck feathers

Wild waterfowls and particularly ducks, constitute the natural reservoir of all subtypes of avian influenza viruses (AIV). Viruses are shed in large amounts in the faeces and can spread between birds by the faecal–oral route and this shedding can result in the release of the virus in the environmental system. In addition, tissues derived from infected birds can become a source of environmental contamination. Feathers easily drop off the body, and infected feathers have the potential to cause environmental contamination. Even though growing feathers are living and detection of H5N1 HPAI virus inside duck calamuses is because of viremia or virus replication, mmature feathers, are also known to be responsible for the persistence of the virus in the environment. Preen oil, the oily substance produced by a large number of waterfowls is used by these birds to waterproof the plumage and this oil has been reported to have the ability to absorb fine particles including microorganisms present in the water. This capacity to absorb microbes from the infected water sources and may result in contamination of duck plumage.

The objective of present work was to study the persistence of H5N1 avian influenza virus at different temperatures in the presence of preen oil in duck feathers and compare the same with the feathers that are artificially or naturally devoid of preen oil. The feathers samples from duck, duck feather after extracting preen oil, emu feather and duckling feathers were spiked with the H5N1 virus at two concentrations and stored at 37°C, 25°C and 10°C temperatures. The feather samples were tested for their survivability by virus isolation in embryonated chicken eggs and RNA quantification by Real time Reverse Transcriptase PCR. Mean persistence of H5N1 AIV in duck feathers containing preen oil at 10^4 EID_{50} and 10^6 EID_{50} spiked viral concentrations was 19.8 and 29.7 daysat 37°C; 13.7 and 44.7 days at 25°C and 55.8 and 73.3 days at10°C, respectively. In case of the treated duck feathers after extracting the preen oil at 10^4 EID_{50} and 10^6 EID_{50} spiked viral concentrations, the mean viral persistence observed was 9.7 and 13 daysat 37°C; 16 and 19 days at 25°C and 35 and 45 days at10°C, respectively. In emu feathers, the mean viral persistence observed at 10^4 EID_{50} and 10^6 EID_{50} spiked viral concentrations was 5 and 7 daysat 37°C; 13.7 and 17 days at 25°C and 29.2 and 40 days at10°C, respectively. In case of the feathers from day-old ducklings, at 10^4 EID_{50} and 10^6 EID_{50} spiked viral concentrations, the mean viral persistence observed was 7 and 8.7 daysat 37°C; 21 and 17.7 days at 25°C and 31.7 and 55 days at10°C, respectively (Fig 23 & 24). RNA quantity was found to be higher and the RNA could be detected for longer periods as compared to virus isolation in all the feather samples. It was highest in duck

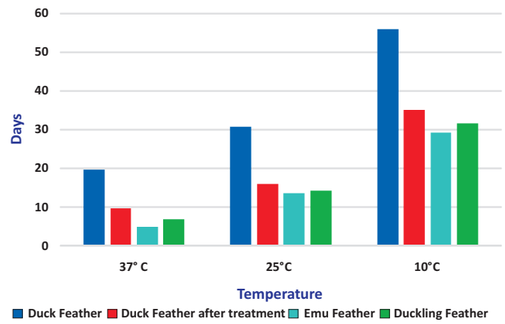


Figure 23. Persistence of HPAI virus with 10^4 EID_{50} at various temperatures

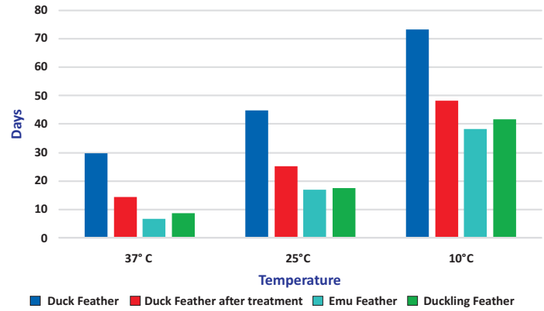


Figure 24. Persistence of HPAI virus with 10^6 EID_{50} at various temperatures

feathers indicating that the preen oil has the capacity to adsorb more virus into surface of feather. Preen oil was found to increase survivability of avian influenza virus even at lower initial viral concentrations, particularly at lower temperatures. The present study establishes the role of preen oil present in the duck feathers in higher viral titres, and may be resulting in protecting the avian influenza virus present on the feathers from the effects of higher temperatures for a longer time.

Experimental transmission of H5N1 influenza virus from crows to chickens

The role of crows in the epidemiology of highly pathogenic avian influenza remains unclear although H5N1 virus had been isolated from dead crows. An experimental infection was carried out on Indian house crows (*Corvus splendens*) to determine their susceptibility to infection, clinical signs, pattern of virus shedding and viral tissue tropism. Eight adult crows were inoculated with 10^6 EID_{50} of H5N1 virus isolated from a dead crow (A/crow/India/01TR01/2012) via intranasal route, and monitored daily until 14th day post inoculation (dpi) or death which ever was earlier. Dullness, reduced feed intake and neurological signs were observed in infected crows. Oral and cloacal swabs were collected up to 14 dpi.

To determine the virus tissue tropism, three crows were sacrificed on 3 dpi and organs/tissues (brain, trachea, lungs, heart, liver, spleen, small intestine, pancreas, large intestine and kidneys) were collected. Of the 5 remaining crows, 4 died during 6-7 dpi and one crow survived till 14th dpi and was euthanized. The crow which survived the infection seroconverted against H5N1 virus. Virus was re-isolated from all the organs from the dead crows. Mild to moderate diffuse hemorrhage on the skull with congested meningeal vessels and patchy reddish multiple foci on pancreas were prominent in dead crows.

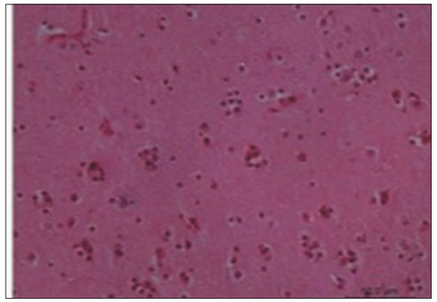


Figure 25. Brain-Neuronal degeneration and neuronophagia. (Bar: 50µm)

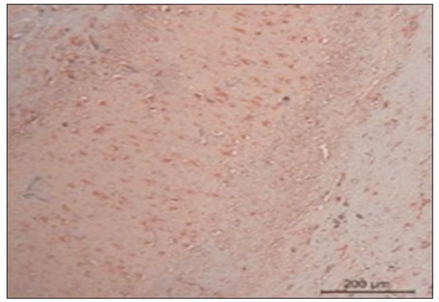


Figure 26. Brain-Viral antigen in cerebellum intracytoplasmic staining in neurons glial cells and capillary endothelium (Bar: 200 µm)

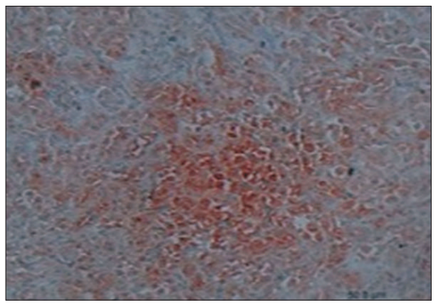


Figure 27. Kidney- Viral antigen in proximal convoluted tubules (Bar: 50 µm)

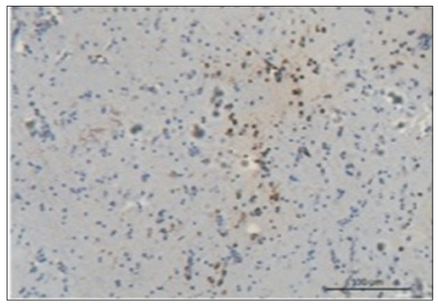


Figure 28. Brain- Apoptotic neurons And glial cells (TUNEL assay).

Brain showed neuronal degeneration along with neuronophagia (Fig.25). Malacic foci were also observed in the cerebral cortex. Pancreas showed necrosis of acinar cells. Kidney had interstitial congestion and degenerative changes in proximal convoluted tubules. Viral antigen was demonstrated by immunoperoxidase test in brain, trachea, lungs, pancreas, liver and kidneys (Figs.26 & 27). TdT-mediated dUTP Nick-End Labeling (TUNEL) assay for apoptosis showed positive signals in brain, lungs, pancreas and kidney (Fig.28). Virus was shed from oral and cloacal swabs. Virus could be detected by real time quantitative RT-PCR (RT-qPCR) from 24h and 4th dpi to till death from oral and cloacal swabs, respectively. The mean concentration in oral and cloacal swabs was 2.31 x 10^7 and 1.55 x 10^6 copies of viral RNA, respectively.

To determine the role of crows in transmitting the virus to domesticated poultry, two groups of SPF chickens, eight in each group, were fed separately with suspension of pooled RT-qPCR positive oral and cloacal swabs collected from crows intranasally infected with H5N1 virus. The infectivity titre of the swabs were low ($10^{1.35}$ and $10^{1.02}$ EID_{50} for oral and cloacal swabs, respectively). No clinical signs or death were seen in any of the infected chickens. Virus isolation and detection of viral RNA could not be made in any of the swabs or tissues collected from chickens.

In conclusion, the study showed that H5N1 influenza virus produced systemic disease leading to death in crows but excreted at a very low level. Highest virus concentration in brain leading to apoptosis with neurological signs indicated that the virus is neurotropic in nature and may be the factor for fatal outcome of the disease in crows. Absence of clinical signs, death and failure in detection of virus in swabs and tissues of chickens fed with oral and cloacal swabs of infected crows indicated that the excretion of H5N1 virus in crows by oral and cloacal routes is below the minimum threshold of chicken lethal dose. This might be one of the reasons for the absence of H5N1 virus infection in chickens in areas from where the H5N1 virus was isolated in crows.

Project- Development and evaluation of neuraminidase DIVA marker vaccine (National Fellow Project)

PI: S. Bhatia

Cloning and expression of NA gene of H5N1 for development of recombinant NA1 (rNA1) based indirect ELISA for the DIVA test

Development of recombinant NA1 (rNA1) based indirect ELISA was attempted as DIVA test. For this study, a library of rNA1 protein was developed by making overlapping fragments. The field virus isolate of H5N1 (10002) was amplified in SPF eggs and the NA gene was amplified using self designed primers for the development of the overlapping fragments. The NA1 gene was divided into 3 overlapping fragments designated as N1a, N1b and N1c. After cloning into the expression vector pET28a (Novagen), the three fragments of N1 were expressed in bacterial host *E. coli* Rosetta cells. The three fragments were amplified using respective primers as N1a (660 bp), N1b (714 bp) and N1c (663 bp) as shown in Fig. 29. Purified genes were ligated to pET28 (a) expression vector. The above ligation mixture was used to transform the competent *E.coli* Rosetta cells. The positive clones were selected and were used for induction for expression of recombinant proteins in *E. Coli* Rosetta cells. The

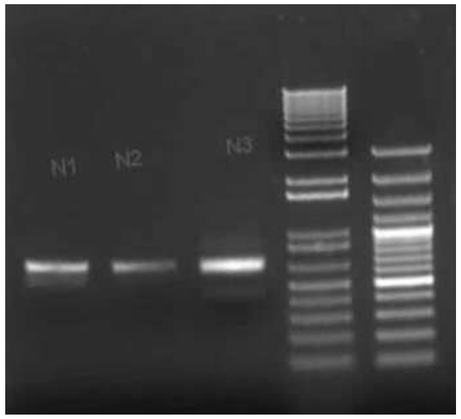


Figure 29. PCR amplification of NA1a, NA1b and NA1c

expressed NA1 proteins (N1a, N1b & N1c) were purified using Ni-NTA column chromatography. The confirmation purification and characterization of the recombinant protein was done by using SDS PAGE (Fig. 30) and Western blot analysis (Fig. 31). In the Western blot, all the three N1 proteins reacted with H5N1 specific serum.

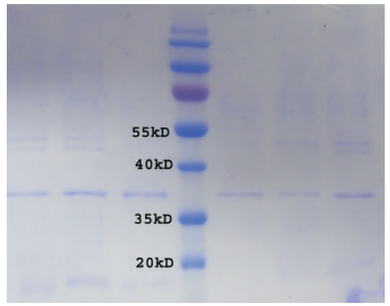


Figure 30. Purification of rNA1 (N1a, N1b & N1c) using column chromatography

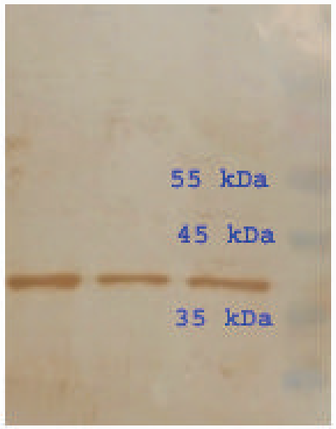


Figure31. Characterization of rNA1 (N1a, N1b & N1c) fragments using western blot assay

The preliminary reactivity check for the three N1 fragments was done in indirect ELISA. Based on the reactivity with positive serum and non-reactivity with negative serum, the fragment rN1a and N1b were tested for application in indirect ELISA based DIVA test. Optimization of antigen concentration with fixed dilution (1:200) of test serum was done by indirect ELISA with varying concentration of antigen. At a dilution of 1:100 of rN1a, the maximum P/N ratio (51.62) was achieved. Further optimization and specificity testing is underway to develop the DIVA test using N1a antigen.

Evaluation of protective efficacy of rgH5N2 vaccine (clade 2.2) in turkeys and ducks

In this trial, 21 specific pathogen free turkey birds of 6 weeks age were used. 11 SPF turkey birds were immunized with 0.5 ml of emulsified rgH5N2 vaccine (clade 2.2) containing 1075HAunits of virus per dose in thigh muscles. 10 birds were sham vaccinated with negative SPF egg allantoic fluid. Sera samples were collected pre-immunization and on weekly basis post immunization, and performed. Haemagglutination inhibition (HI) test as recommended by OIE for the assessment of immune response. At 21 days post booster, thevaccinated turkey showed mean titre of $2^{11.3}$ (Fig. 32). The day old ducklings were reared in the SPF unit of the institute. The birds were shifted in animal containment facility before start of the experiment. The rgH5N2 vaccine was administered via intramuscular route @ 0.5 ml / bird in 12 ducks and 12 ducks were kept as control.

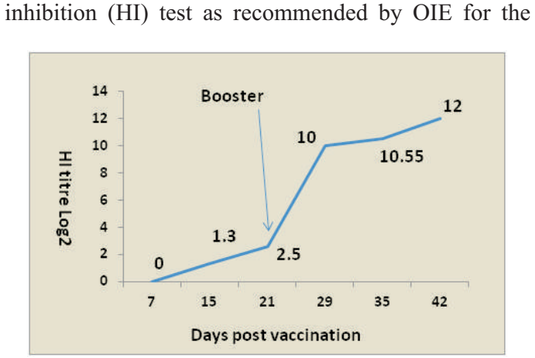


Figure 32. Serum HI titres in turkeys (a) and ducks (b) vaccinated with rgH5N2 vaccine

Project - 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 with ICAR-NEH)

PI: K. Rajukumar Co-PI: D. Senthil Kumar

Molecular characterization of Porcine Reproductive and Respiratory Syndrome Virus isolated from India reveals unique mutations in ORF 5, ORF 7 and nsp2 coding regions

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) (Ind-297221) isolated from Mizoram PRRS outbreak was characterized. Various parts of the Indian PRRSV genome were amplified by one step RT-PCR and sequenced. Overlapping sequences were assembled to get the whole genome sequence of the isolate (15326 kb). Genetic analysis of the complete ORF 5, ORF 7 and nsp2 coding regions of Ind-297221 was carried out. In the ORF-5 region, PRRSV-Ind-297221 shared highest sequence identity (99%) with post-2006 genotype 2 PRRSV reported from China and Vietnam. With pre-2006 Chinese PRRSV genotype-2 isolates and isolates from south east Asian countries including Laos, Cambodia and Thailand, the sequence identity ranged from 94 to 98%. The sequence homologies with the classical North American genotype-2 isolate (VR-2332) and European genotype-1 isolate (LV) were 88% and 62%, respectively. Two unique non-synonymous nucleotide (nt) mutations were observed at nt positions 17 and 32, corresponding to L6S and C11Y in the deduced amino acid sequence (genotype 2 numbering). Genetic analysis in the complete ORF-7 gene showed that the sequence homology with other PRRSV isolates from China (post 2006), other Asian countries, pre-2006 Chinese genotype 2 isolates, classical PRRSV2 and PRRSV1 isolates ranged from 97-99%, 88-98%, 95-97%, 93% and 70%, respectively. Two unique mutations, at nt positions 210 (synonymous) and 263 (non-synonymous), were observed. The latter corresponded to N88S in the deduced amino acid sequence (genotype 2 numbering). In the nsp-2 coding region, the sequence identity with other genotype-2 viruses having the 30 amino acid deletion circulating in China and south-east Asian countries, ranged between 92 and 98%. With classical genotype 2 isolates (CH-1a, VR-2332) the identity was between 82 to 94%. Analysis of the deduced amino acid sequence derived from the nsp2 coding nucleotide sequence showed 14 unique amino acid changes as compared to the PRRSV genotype-2 currently existing in various parts of the world. Understanding the significance of these mutations requires further study.

Project - Prevalence and molecular epidemiology of BVD in ruminants with special reference to Mithun in North East states of India (DBT – twinning)

PI: N.Mishra Co-PI: K. Rajukumar and S. Kalaiyarasu

Evidence of low BVD prevalence in the North Eastern States of India

Testing of 713 serum samples collected from mithun, cattle, buffaloes, sheep and goats from NE region showed that five animals were positive for BVDV neutralizing antibodies indicating a low BVDV prevalence in this region. However, cross neutralization test of VNT positive serum samples provided evidence of both BVDV-1 and BVDV-2 infection. Testing of PBL and tissues (106) by virus isolation and RT-PCR yielded negative results for BVDV. Pigs (14) suspected for CSF and BVD co-infection were found negative for BVDV-1 and BVDV-2.

Project-Identification of the molecular basis of differential host responses to rapidly evolving Avian Influenza viruses in different avian species (BBSRC -DBTfunded)

PI: A. Mishra Co-PI: A. A. Raut, H.V. Murugkar

International collaborative project with Roslin Institute, University of Edinburgh, UK

UK Team- Prof David W. Burt(PI), Prof Paul Digard, Dr Lonneke Vervelde

Avian Influenza Virus H5N1 Clades 2.2 and 2.3.2.1 differ in their pathogenicity in ducks and crows.

Experimental inoculation with H5N1 isolates A/duck/Tripura/103597/2008 (Clade 2.2) and A/duck/India/02CA10/ 2011/Agartala (Clade 2.3.2.1) in two different groups of ducks, and with isolates A/crow/Assam/142119/2008 (Clade 2.2) and A/crow/100011/2012 (Clade 2.3.2.1) in two different groups of crows was done by intranasal route. Chicken were placed in contact with the inoculated ducks and crows were placed in contact with the inoculated crows 18 hours post infection (hpi). Observations were taken in order to determine the difference in pathogenicity of H5N1 clades 2.2 and 2.3.2.1, in terms of clinical signs, mortality, virus shedding, virus transmission and virus distribution in tissues.

No mortality was seen with clade 2.2 infection while 33% mortality was observed in case of infection with clade 2.3.2.1 in ducks. However, there was 100% mortality in the contact chickens of both groups with significant difference in Mean Death Time (MDT) of 9 days with clade 2.2 and 3 days with clade 2.3.2.1. Oropharyngeal and cloacal route of viral shedding pattern of clade 2.3.2.1 shows marked difference from clade 2.2. Interestingly, there was no cloacal shedding in ducks infected with clade 2.2. There was 100 % mortality in crows infected with either clades of viruses. No mortality was recorded even upto 14 days in the crow group in contact with the clade 2.2 innoculated crows, though cloacal shedding was observed. On the other hand there was 100% mortality in the in-contact group in case of clade 2.3.2.1 infection.

Thus, both in ducks as well as crows there were differences in the pathogenecity and transmissibility of the H5N1 isolates of the closely related clade 2.2 and clade 2.3.2.1 of Asian lineage**.** All the virus isolates were transmitted to the contact birds, but the transmission rate and mortality of in contact birds varied between the different clades (Table18). The virus load in different tissues between these two clades with in each of the avian species also showed significant differences (Fig. 33).

Table 18: Mean Death Time and Mortality in infected and in-contact birds of Ducks, Crows with H5N1 (clade 2.2) and H5N1 (clade 2.3.2.1)

Experimentally Challenged Species	In contact species	Virus isolate	Clade Classification	No of deaths/No challenged	MDT (days) Challenged species	No of deaths/No In-contact	MDT (days) Ineontact species
Duck	**Chicken**	**A/duck/Tripura/103597/2008**	2.2	0/6	-	6/6	9
		A/duck/India/02CA10/2011/A gartala	2.3.2.1	2/6	10	6/6	3
Crow	**Crow**	**A/crow/Assam/142119/2008**	2.2	4/4	7	0/2	-
		/A/crow/100011/2012	2.3.2.1	4/4	7	2/2	8

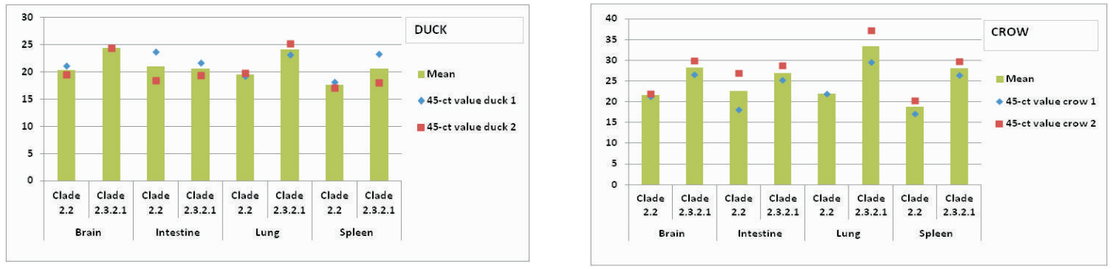


Figure 33. Virus Load in different organs of ducks and crows experimentally infected with H5N1 (clade 2.2) and H5N1 (clade 2.3.2.1) (Scatter plot of 45-Ct values of viral RNA from individual birds). Three Ducks and Three Crows were inoculated with H5N1 Clade 2.2 or Clade 2.3.2.1 and the presence of viral RNA in organs was examined at 5 days post inoculation (d.p.i.) for ducks and 4 d.p.i. for crows. The height of bar represents the mean of three individual birds. Triangles and squares represent the Ct-value of individual birds.

Project- Establishment of Advanced Animal Disease Diagnosis and Service Management Centers in the North East (AADSMC) (DBT funded)

PI: D.D.Kulkarni

Subprojects 1: Development of Multiplex diagnostic assay for all prioritized BSL-III/IV pathogens under NE-ADSAHD.

Co-PI: A. A. Raut , S.B. Sudhakar, P. N. Gandhale

Expression of recombinant nucleoproteins of Ebola, Middle East Respiratory Syndrome (MERS-CoV), Lyssa (Khujand) & Nipah viruses:

The nucleotide sequence of complete CDS of nucleoprotein gene of Ebola (Reston), MERS-CoV, Lyssa (Khujand) was retrieved from NCBI genbank database. The sequence was codon optimized for expression in *E. coli* prokaryotic expression host system. The codon optimized gene sequence was chemically synthesized from commercial gene synthesis service provider and placed in frame in pET-32a vector. The Nipah virus nucleoprotein expression plasmid (pET-30-Nipah) was obtained from Dr Paul Kitching, Canadian Good Inspection Agency, Canada.

Expression plasmid constructs of Ebola (Reston), MERS-CoV, Lyssa (Khujand) nucleoprotein (NP) CDSs in pET32a+ and Nipah Virus (NiV) nucleoprotein CDS in pET30a 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, which was then 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 OD600 of 0.6 was achieved. The cells were induced by 1mM IPTG for 6hrs at 30˚C. The rNP expression was checked by SDS-PAGE and coomassie staining. Protein lanes were compared with respective uninduced lanes and specific bands of sufficient intensity around the expected sizes were evident. The western blot analysis was conducted using anti-His antibody that reveled the expression of recombinant proteins of expected size (Fig. 34).

Subprojects 2: Development of quantitative multiplex real-time RT-PCR for the simultaneous typing and subtyping of Influenza type A viruses.

Co-PI: Atul Kumar Pateriya

Multiplex compatible functional probe & primers for simultaneous typing and subtyping (H5/N1) of Influenza A virus

In order to develop a multiplex RTqPCR for simultaneous typing and subtyping (H5/N1) of avian

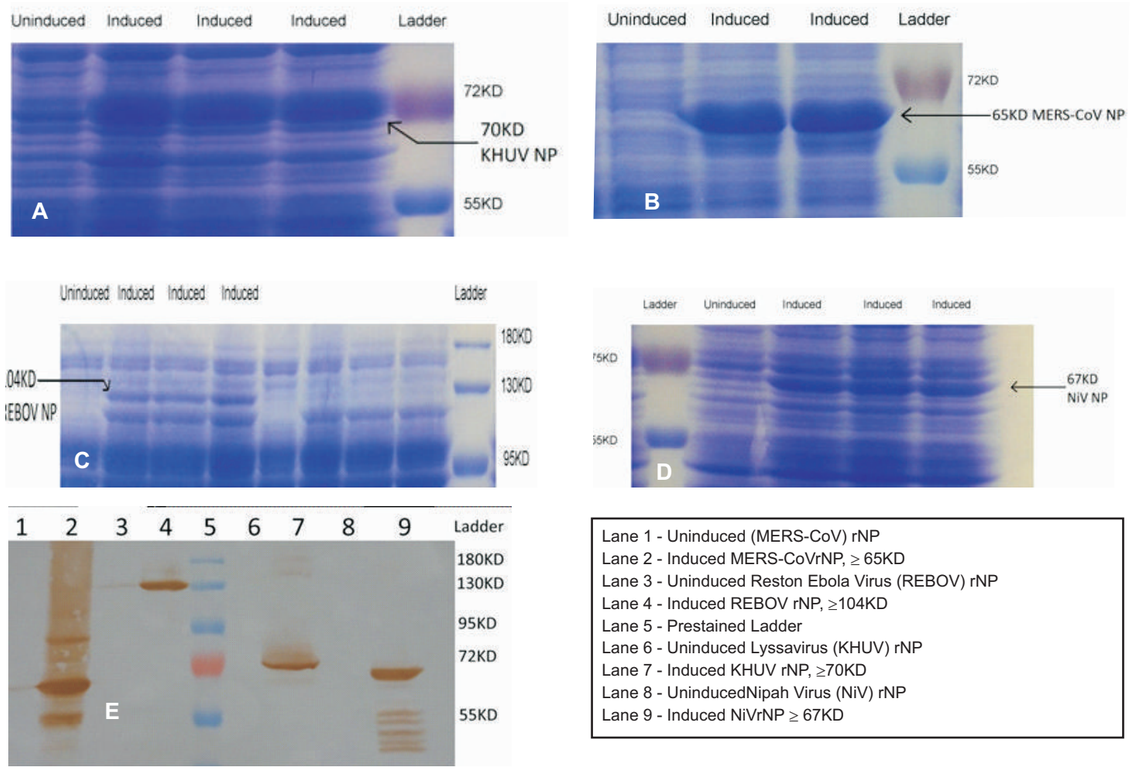


Figure 34. SDS-PAGE and Western Blot analysis (With anti-His antibody) of Total Cell Protein (TCP) of BL21 (DE3) PlysS with pET32a+ Nucleoprotein gene constructs induced by 1mM IPTG. (A) Khujand Virus nucleoprotein (Lyssavirus) (B) Middle East Respiratory Syndrome Coronavirus nucleoprotein (C) Reston Ebola Virus nucleoprotein (D) Nipah Virus nucleoprotein. (E) Western blot of MERS, Ebola (Reston), Lyssa (Khujand) and Nipah virus nucleoprotein.

influenza A viruses, sequences for matrix, nucleoprotein, haemagglutinin (H5) and neuraminidase (N1) genes were downloaded from NCBI database and aligned in clustal W software. As the source of AI virus for most of the Indian outbreaks is assumed to be circulating stain/mutant strain from neighboring countries therefore special preference was given to sequences from SAARC countries. For all the four genes, consensus sequences were determined using MEGA 5.0 software to select the representative sequence for the designing of probe and primers. Novel target regions (unreported) within these genes were identified on the basis of multiple sequence alignment and comparative analysis of existing literature. These regions were targeted to design the multiplex compatible primer and probe in Beacon Designer 8 software using multiplex probe/primer designing feature. All the multiplex compatible probes were primarily integrated and synthesized with 5'FAM and 3'BHQ1 reporter and quencher to evaluate their functionality in wet experiment. On assessment, all the probe and primers sets produced clear sigmoid amplification curve with high fluorescence in taqman RT-qPCR assay with RNA from reference virus (Fig. 35).

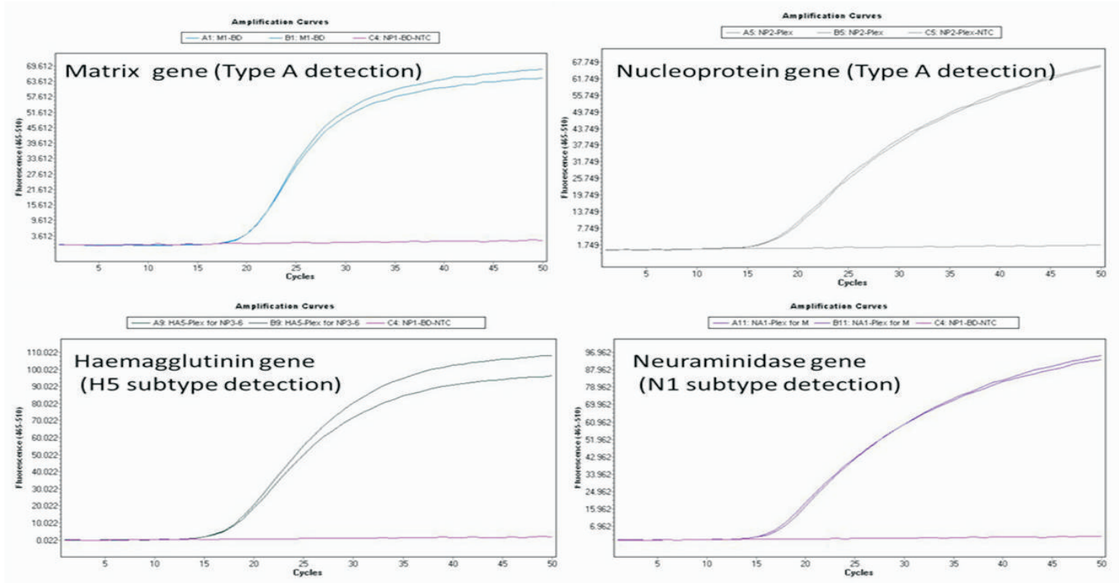


Figure 35. Amplification curve for the probe/primers designed for matrix, nucleoprotein, haemagglutinin (H5) and neuraminidase (N1) genes.

Project-Viral metagenomic profiling of native and migratory aquatic birds of North-Eastern states to unravel influenza virus ecology harbored in them (DBT-Twinning)

PI: A. A. Raut, Co-PI: D.D. Kulkarni, H.V. Murugkar, A. Mishra

Viral Metagenome analysis captures a snapshot of diversity of virome in duck gut

Metagenome refers to the genomic contents of the entire microbial community and virome is a subset of metagenome. Viral metagenome is the genomes of all the viruses that inhabit a particular organism or environment. As the domestic duck *(Anas platyrhynchos)* is one of the economically important waterfowls and also serve as the principal natural reservoir for influenza A viruses, effective prediction of future viral epidemic or pandemic requires an in-depth understanding of its virome. To obtain an initial unbiased measure of the viral diversity in the enteric tract of ducks, we purified total gut virome from cloacal swabs collected from the flock of 23 ducks inhabiting Chota Talab, Bhopal, a water-body visited by migratory birds. The total viral RNA as well as DNA was isolated and sequenced on illumina MiSeq platform. In total 7,455,180 reads with average length of 146 bases were generated and 7,354,300 reads were *de novo* assembled into 24,945 contigs with an average length of 220 bases and the remaining 1,00, 880 reads were kept as singletons. The virome was identified by sequence similarity comparisons (BLASTx E score, $<10^{-3}$) to known viruses (viral reference database). While the majority of sequences showed homology with bacteriphages, the non-bacteriphage virus hits were 2949. These non-bacteriophage virus hits when classified hostwise, were distributed in hosts ranging from plants, algae, invertibrates,vertebrates and environment (Fig. 36). This distribution reflects the diet and habitation of ducks. On familywise classification of the hits in nonbacteriphage virus, they grouped into the virus families namely Mimiviridae, poxviridae, baculoviridae, *Herpesviridae, Papillomaviridae,*

Retroviridae, Circoviridae, Picornaviridae, Parvovirinae etc. (Fig. 37).

Amongst the previosly uncharcterised duck viruses, sequences related to members of papillomaviruses family were identified. BLASTx searches showed that these sequences shared 43% to 68% amino acid identity to *Francolinus leucoscepus* papillomavirus 1 early protein (E1) and late protein sequences (L1 and L2). We provisionally named this virus as duck papillomavirus. It was phylogetically related to Francolinus leucoscepus papillomavirus-1 in the Dyoepsilon papillomavirus genus (Fig. 38). Further, we characterized partial genome of other previously uncharacterized duck viruses, including duck picornaviruses, duck ciroviruses, duck parvoviruses and other viruses. This initial characterization of the duck virome, the first metagenomic analysis of viruses in ducks using second-generation sequencing, therefore showed the presence of previously unidentified viral species infecting vertebrates, insects, plants or bacteria and expands the spectrum of viruses harbored by ducks in the gut.

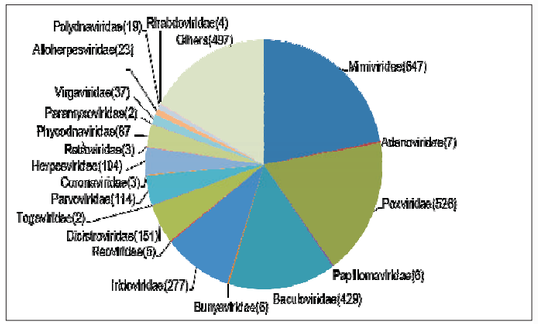


Figure 36. Family-wise distribution of non-bacteriophage viruses identified in duck gut viral metagenome

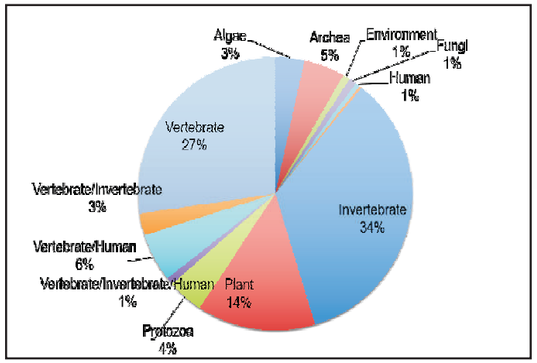


Figure 37. Host-wise distribution of non-bacteriophage viruses identified in duck gut viral metagenome

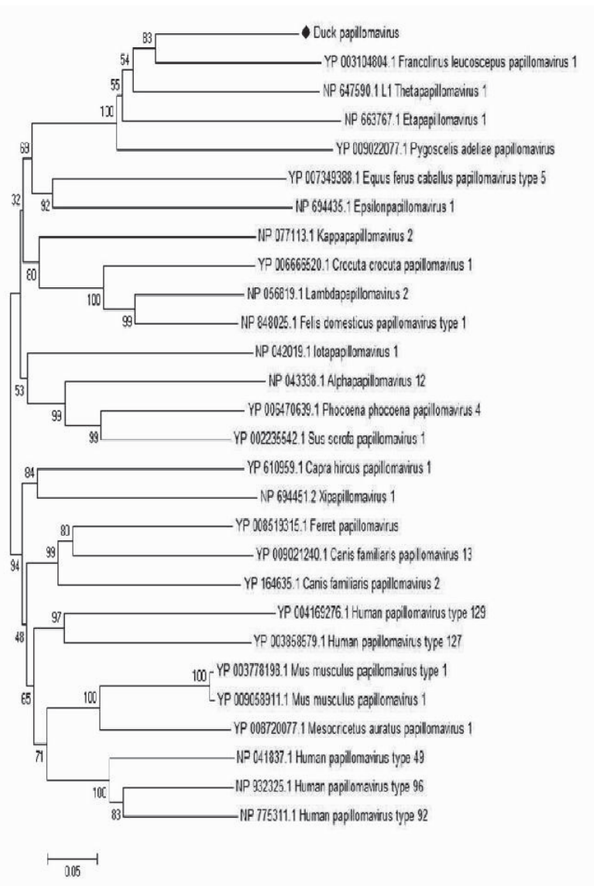


Figure 38. Phylogenetic analysis of duck papillomavirus with other representative papillomavirus species based on translated partial amino acid sequences of L1 gene (ORF).

Project- Synthetic peptide based diagnostic for highly pathogenic avian influenza

PI: Kh. Victoria Chanu Co-PI: R. Sood, A. K. Pateriya

Designing and synthesis of peptides for identification of immunologically dominant epitopes in the HA protein of H5N1 AIV

Haemagglutinin (HA) sequences of H5N1 reported from India since 2006 to 2013 were aligned using MEGA 6.06. The isolates included in the alignment were of both the clades, 2.2 and 2.3.2.1. Some conserved amino acid sequences were observed in HA2 subunit in all HA protein analyzed. Amino acids at 347 to 370, 372 to 388, 415 to 461 and 513 to 528 were found to be conserved in all the HA sequences of H5N1 reported till date from India. H5 sequences were also compared with HA sequences of other subtypes (H1, H3, H4, H9 and H11). The conserved regions of H5 showed dissimilarities at many amino acids with other subtypes. Considering all similarities and dissimilarities in amino acid sequences, an HA sequence of clade 2.3.2.1, (A/duck/02CA10/2011(H5N1)), was randomly selected and analyzed with PROTEAN of DNA star for positive antigenic index, hydrophilicity and surface probability. Based on the predictions by PROTEAN and the conserved sequences, six peptides, MV-14, DV-14, RA-15, HT-16, EM-16, GS-20 were designed. Out of these, two peptides MV-14 and DV-14 have been synthesized in linear format and purified by RP-HPLC using C18 column. The peptides were also characterized by ESI-MS and confirmed for its mass.

Contract Research project

Project- Prevalence of bovine viral diarrhea virus (BVDV) types and subtypes in cattle in organized dairy farms in India

PI: N. Mishra Co-PI: K. Rajukumar, A.K. Pateriya, M. Kumar

Genetic characterization of newly identified BVDV-3 (HoBi-like) viruses in Indian cattle reveals that there is global circulation and independent evolution of at least three groups of BVDV-3 viruses with circulation of two novel and divergent groups in India (Pfizer funded)

BVDV-3 (HoBi-like pestiviruses) have been sporadically reported from naturally infected cattle, in selected countries in South America, Asia and Europe since 2006. Despite reports of BVDV-1 and BVDV-2, BVDV-3 viruses have not yet been identified in India. Here we report the molecular characterization of BVDV-3 viruses, identified during a study on prevalence of bovine viral diarrhoea viruses (BVDV) in cattle (n=1049) in India between 2012 and 2013. Through real-time RT-PCR, virus isolation and sequencing of the partial 5'-UTR and entire N^{pro} gene region, we confirmed the presence of BVDV-3 viruses in 19 and BVDV-1b in one of the 20 pestivirus positive cattle blood samples. The majority of animals from which BVDV-3 viruses were detected in this study, had history of reproductive disease, respiratory disease or diarrhoea, while some animals were apparently healthy.

The phylogenetic analysis (ML) using 239 nt of the 5'-UTR sequence identified five clusters within the BVDV-3 virus clade and demonstrated that Indian BVDV-3 isolates were grouped into two distinct clusters, one cluster containing all isolates from a farm in Chhattisgarh state and another cluster consisting of all remaining isolates from two farms in Maharashtra state and from the farm in Punjab state. All the previously reported BVDV-3 viruses from South America, Europe and Australia were grouped in a separate cluster. The Thai strain Th/04_Khonkaen and Bangladesh strain BGD/ZS5 were grouped into the fourth cluster, while the strains BGD/ZS1 and BGD/ZS3 from Bangladesh formed the fifth cluster. Surprisingly, IndBHA5309/12 group of viruses were the most divergent HoBi-like strains reported so far.

Additional phylogenetic analysis was carried out for full length N^{pro} gene sequences and for the concatenated datasets of 5'-UTR and N^{pro}. Since N^{pro} gene sequences of BVDV-3 viruses from Bangladesh have not yet been

determined, they could not be included in this analysis. The results (Fig. 39) demonstrate that within the BVDV-3 virus clade, the two novel Indian BVDV-3 virus groups formed distinct phylogenetic groupings to each other and to all other previously reported BVDV-3 viruses with strong support (98-100%). Overall, three BVDV-3 virus lineages could be identified: the first lineage included previously reported BVDV-3 viruses from South America, Europe, Australia and South East Asia, the second lineage included IndABI15385/12 group of viruses and the third lineage encompassed IndBHA5309/12 group of viruses from India. The nucleotide sequence identity between the two Indian lineages was 79.6%, while the predicted amino acid sequence identity was 83.9%.

Till date, all the previously reported BVDV-3 viruses except the Thai and Bangladesh strains are very closely related genetically. Based on the results of this study, and the subtype assignment criteria, we propose that BVDV-3 viruses can be classified into three subtypes (a, b and c), subtype 'a' consisting of all the previously reported strains including the strain Th/04_Khonkaen, subtype 'b' consisting of four Indian strains (Ind IndABI15385/12 group) and subtype 'c' encompassing fifteen Indian strains (IndBHA5309/12 group).

This study extends our knowledge on the epidemiology and genetic heterogeneity among the BVDV-3 viruses, highlighting the global circulation and independent evolution of at least three groups of BVDV-3 viruses with two groups in India. The increasing reports of BVDV-3 viruses from cattle in the field suggest that natural infection of cattle with BVDV-3 virus may be more widespread than previously thought. Considering the growing evidence of occurrence of BVDV-3 in geographically distant cattle populations, these emerging viruses present considerable risk to the cattle health and management and BVD control programmes. The identification of BVDV-3 in Indian cattle emphasizes the need for continued monitoring besides determining the extent of economic losses it can cause in dairy farming.

Student's Research

Students Name	Guide	Degree & Subject	Title
Dr. Athira C.K.	Dr. H.V. Murugkar	M.V.Sc., Veterinary Public Health	Role of preen oil in persistence of H5N1 avian influenza virus in duck feathers
Dr. Aron Jacob	Dr. Richa Sood	M.V.Sc., Veterinary Medicine	Study of Amantadine Resistance among Influenza A Viruses Isolated in India by Antiviral Assay and Molecular Characterization of Matrix Gene
Dr Mohammed Fawaz P.	Dr. Ashwin Ashok Raut	M.V.Sc., Animal Biotechnology	Metagenomic profiling of gut virome of domestic ducks *(Anas platyrhynchos)*
Dr Avneesh Kumar	Dr Anamika Mishra	M.V.Sc., Animal Genetics and Breeding	Identification of genome-wide host response to highly pathogenic avian influenza (H5N1) clade 2.2 virus and comparison with that of clade 2.3.2 virus in ducks
Dr. Ajai Lawrence D'Silva	Dr. S. Nagarajan	M. V. Sc. Animal Biotechnology	Prokaryotic expression of haemagglutinin gene of H7 subtyp e avian influenza virus and evaluation of its diagnostic potential
Dr. Nongthombam Boby	Dr. G. Venkatesh	M.V.Sc. Animal Biotechnology	Epitope mapping of Avian Influenza Virus Haemagglutinin specific monoclonal antibodies through Mab resistant mutants

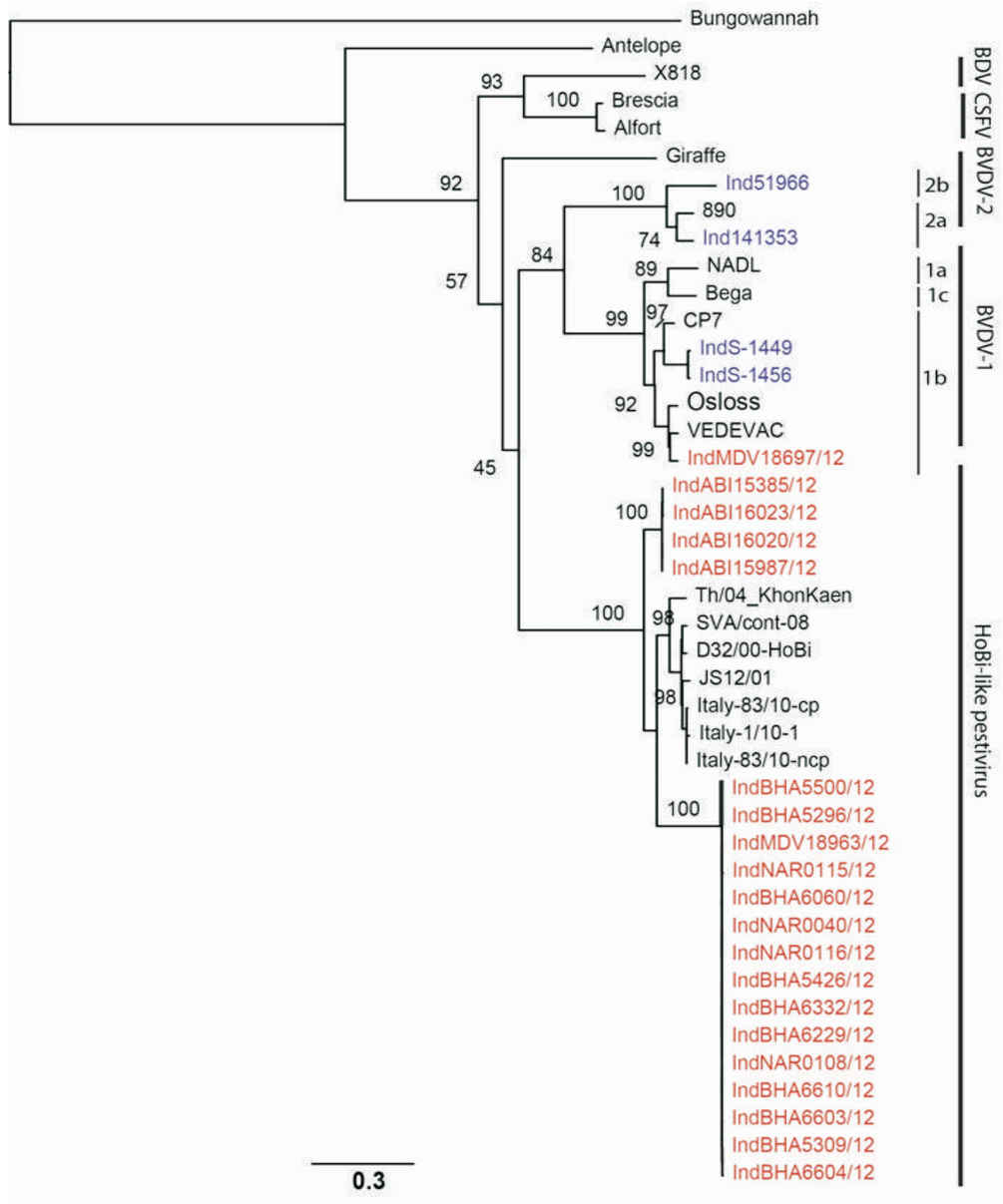


Figure 39. Phylogenetic tree based on the combined datasets of 5'-UTR and Npro sequences of HoBi-like pestiviruses analyzed in this study and other pestiviruses. The maximum likelihood tree was generated using concatenated datasets of 5'-UTR and Npro under the GTR+gamma substitution model in RAXML (Stamatakis et al., 2006). Numbers indicate the percentage of 1000 bootstrap replicates that support each phylogenetic branch.

Publications

Research articles

1. Behera P, Nagarajan S, Murugkar HV, Kalaiyarasu S, Prakash A, Gothalwal R, Dubey SC, Kulkarni DD, Tosh C (2015). siRNAs targeting PB2 and NP genes potentially inhibit the replication of the highly pathogenic H5N1 avian influenza virus. *J.Biosci.*40(2): 233-240.
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20. Venkatesh G, Vanamayya PR, Sharma N, Kulkarni DD and Dubey SC (2014). Expression and Characterization of truncated Non Structural Protein NS1 of Porcine Parvovirus in E.coli. *J. Pure Appl. Microbiol.* 9(1): 397-403.
21. Vijayakumar P, Raut AA, Kumar P, Sharma D, Mishra A (2014). De novo assembly and analysis of crow lungs transcriptome. Genome. 2014 Sep;57(9):499-506. doi: 10.1139/gen-2014-0122. Epub 2015 Jan 11.
22. Vijaykumar P, Mishra A, Ranaware P, Kulkarni DD, Burt D, Raut AA (2015). Analysis of the crow lung transcriptome in response to infection with Highly Pathogenic H5N1 Avian Influenza Virus. *Gene.* 2015 Mar 15;559(1):77-85. doi: 10.1016/ j.gene. 2015.01.016. Epub 2015 Jan 12.

Technical / invited papers

1. Kulkarni DD (2014). High Risk/exotic/emerging pathogens in India. Talk delivered at College of Veterinary & Animal Sciences, MAFSU, Parbhani (Maharashtra) on 17.11.2014.
2. Kulkarni DD (2014). Issues involved in systematic surveillance for Avian Influenza. Presentation at Influenza Surveillance Meeting at National Institute of Virology, Pune on 5.12.2014.
3. Kulkarni DD (2015). Biosafety and biosecurity under field conditions Lecture delivered at ADSAHD Training on 2.03.2015.
4. Kulkarni DD (2015) Biosafety for society. Talk delivered at Joint ASM-SBS-ICAR Workshop during Biosafety Awareness Program at ICAR-NIHSAD, Bhopal on 13.03.2015.
5. Kulkarni DD (2015). Role of NIHSAD in exotic and emerging diseases. Paper presented at "National conference on emerging and re-emerging viral outbreaks in India-clinical challenges and management" 20-22 January 2015 at CSIR-IICT-CCMB Auditorium, Hyderabad.
6. Kulkarni DD and Rana RS (2014). National Strategies for Controlling Avian Influenza Viruses- India. Paper presented at OIE Regional Workshop on Enhancing Influenza A viruses National Surveillance Systems, Tokyo, 26-28 August 2014.
7. Mishra N (2015). Invited lecture on 'Issues on BVD'. Workshop on revisiting minimum standards for bovine frozen semen production held by DADF & NDDB at NDDB, Anand, 12-13 March, 2015.
8. Mishra N (2014). Genetic and antigenic diversity of bovine viral diarrhoea viruses: Implications for diagnosis and management. Full invited paper, Veterinary pathology congress compendium 2013, p. 85-88. Bhubaneswar, 21-23 Nov.
9. Mishra N (2014). Invited lecture on 'epidemiology and diagnosis of BVD: Indian perspective'. Technical workshop on IBR and BVD control held by DADF at NDDB, Anand, 12th April, 2014.
10. Murugkar HV and Nagarajan S (2014). "Avian Influenza: is it challenging our competency?" XIII Annual Conference of Indian Association of Veterinary Public Health Specialists (IAVPHS) and National Symposium on Scafety of foods of animal origin for domestic and export markets: Legal perspectives from 10-12 Feb.2015 at Veterinary College, KVAFSU, Bengaluru. pp156-160.
11. Nagarajan S (2014) "Avian influenza – Surveillance and biosafety from the field veterinarian's perspective" at the one day ASCAD sponsored seminar on "Avian influenza awareness and biosecurity measures in poultry farming" Organized by Tamil Nadu Veterinary and Animal Sciences University at Namakkal, Tamil Nadu on 25.07.2014.
12. Nagarajan S (2014). Action Plan and Surveillance for avian influenza in India. Proceedings of the one day ASCAD sponsored seminar on "Avian influenza awareness and biosecurity measures in poultry farming" organized by Tamil Nadu Veterinary and Animal Sciences University at Namakkal, Tamil Nadu on 25.07.2014. pp 26-28.
13. Nagarajan S (2014). Collection and packaging of suspected samples for AI diagnosis. Proceedings of the one day ASCAD sponsored seminar on "Avian influenza awareness and biosecurity measures in poultry farming" organized by Tamil Nadu Veterinary and Animal Sciences University at Namakkal, Tamil Nadu on 25.07.2014. pp 19-21.
14. Nagarajan S (2014). Crop nutrient management and animal management in a mixed farming system. Training manual of the Department of Agriculture and Co-operation, Ministry of Agriculture, GOI sponsored Model Training Course on "Best nutrient management practices (BNMPs) for major crops and cropping systems of India" organized by ICAR-Indian Institute of Soil Sciences from 07-17, October, 2014. pp 192-199.
15. Nagarajan S (2014). Diagnosis of avian influenza. Proceedings of the one day ASCAD sponsored seminar on "Avian influenza awareness and biosecurity measures in poultry farming" organized by Tamil Nadu Veterinary and Animal Sciences University at Namakkal, Tamil Nadu on 25.07.2014. pp 22-25.

16. Nagarajan S (2014). Legal framework for avian influenza control measures. Proceedings of the one day ASCAD sponsored seminar on "Avian influenza awareness and biosecurity measures in poultry farming" organized by Tamil Nadu Veterinary and Animal Sciences University at Namakkal, Tamil Nadu on 25.07.2014. pp 29-31.
17. Nagarajan S and Kumar M (2014). Biosafety Precautions for field veterinarians during avian influenza outbreak investigation ASCAD sponsored seminar on Avian influenza awareness and Biosecurity measures in poultry farming. Organized by Central University Laboratory, Poultry Disease Diagnosis and Surveillance Laboratory, Centre for Animal Health Studies, TANUVAS, Chennai, pp 32-35.
18. Rajukumar K (2014). Invited lecture on 'HSADL experience: Acute Bovine Viral Diarrhoea Virus Infection in ruminants' presented in Technical workshop on IBR and BVD control held at NDDB, Anand, 12th April, 2014.
19. Rajukumar K (2015). Diagnostic Electron Microscopy in Identification of Viruses during unknown disease outbreaks invited lecture delivered in National conference on emerging and re-emerging viral outbreaks in India - clinical challenges and management, organized by Osmania University at CSIR- IICT- CCMB, Hyderabad, Jan 20-22, 2015.
20. Rajukumar K and Kumar M (2014). Scanning and Transmission Electron microscopy for ultra-structural studies. Invited paper in ICAR short course on "Advances in nutrient dynamics in soil-plant atmosphere system for improving nutrient use efficiency" held at ICAR-IISS, Bhopal during Sep 02-11, 2014. pp 107-112.
21. Rajukumar K, Murugkar HV and Kulkarni DD (2014). Biorisk management in laboratories handling highly pathogenic microorganisms, invited lecture delivered in International Conference on Host-Pathogen Interactions (ICHPI) Organized by NIAB, Hyderabad, July 12-15, 2014.
22. Sood, R. (2015). Surveillance and Diagnosis for Highly Pathogenic Avian influenza in Wild Birds. XXXIII Annual Convention of Indian society of Veterinary Medicine and National Symposium on: New Dimensions in Veterinary Medicine: Technological Advances, One Health Concept and Animal Welfare Concerns. University Veterinary Hospital & TVCC College of Veterinary & Animal Sciences Campus Mannuthy, Thrissur, Kerala, 22nd -24th Jan, 2015 pp328-332.
23. Tosh C and Murugkar HV (2014).Laboratory Biosfety, Presented in the International School on "One Health: An integrated view on Infectious diseases, food safety and zoonoses" at School of Public Health and Zoonoses, GADVASU, Ludhiana, 3-7 November, 2014.
24. Tosh C and Kulkarni DD (2014). Nipah virus infection: Current status, preparedness for prevention and control." Presented in the International School on "One Health: An integrated view on Infectious diseases, food safety and zoonoses" at School of Public Health and Zoonoses, GADVASU, Ludhiana, 3-7 November, 2014.
25. Tosh C and Kulkarni DD (2014). Use of molecular phylogeny to study the evolution of highly pathogenic avian influenza H5N1 virus. Presented in workshop on "Exploration of Molecular Phylogeny and DNA Sequence Analyses for Taxonomic, Biological Diversity and Disease Parental Association Findings," MPCST, Bhopal, 26-28 June, 2014.
26. Tosh C, Nagarajan S, Kulkarni DD (2014). Reassortments in avian influenza viruses, invited paper presented at Indian Virological Society- XXIII National conference on "Recent trends in virology research in OMICS Era", Tamilnadu Agricultural University, Coimbatore, Tamilnadu, December 18-20, 2014.
27. Venkatesh G and Nagarajan S (2014). Transboundary Animal Diseases: A SAARC Perspective. SAARC Agri News. Vol 8(2):4-5.
28. Mishra N (2015). Requirement for setting-up of BSL-4 laboratories. Lecture delivered in training on Laboratory biosafety and biosecurity for handling transboundary animal pathogens under NE-ADSAHD project, Feb 28. 2015.
29. Nagarajan S (2015). Role of animals in emergence and resurgence of zoonotic pathogens. Invited lecture delivered at the Continuous Medical Education programme held at AIIMS, Bhopal on 09.01.2015.

Abstracts/posters presented in conferences/ symposia

1. Aron Jacob, Richa Sood, Kh Victoria Chanu, R Khandia, AK Pateriya and S Bhatia (2014). Amantadine resistance among HPAI H5N1 isolated from India: A cause of Concern. In: XXVIII Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists, Specialists in Infectious Diseases and International conference on "Challenges and Opportunities in Animal Health in the Face of Globalization and Climate Change" held at UP Pandit Deen Dayal Upadhaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, (DUVASU), Mathura, UP from 30th Oct. -1st Nov 2014.
2. Athira CK, Arunraj MR, Murugkar HV, Kumar M, Nagarajan S, Tosh C, Bhatia S, Rajukumar K and Ashok Kumar (2015). Evaluation of Elution Methods for Recovery of Highly Pathogenic Avian Influenza (H5N1) Virus from Infected Duck Feathers. XIII Annual Conference of Indian Association of Veterinary Public Health Specialists (IAVPHS) and National Symposium on Safety of foods of animal origin for domestic and export markets: Legal perspectives from 10-12 Feb.2015 at Veterinary College, KVAFSU, Bengaluru, pp 98.
3. Dash S, Kumar M, Kataria JM, Murugkar HV, Tosh C, Kulkarni DD, Nagarajan S (2014). Characterization of pathogenicity and infectivity of H9N2 avian influenza virus in chickens, paper resented at Indian Virological Society- XXIII National conference on "Recent trends in virology research in OMICS Era", Tamilnadu Agricultural University, Coimbatore, Tamilnadu, December 18-20, 2014 pp 261.
4. Kh Victoria Chanu, R Sood, M Kumar, S Bhatia, R Khandia, G Jahagirdar and DD Kulkarni (2014). Malignant Catarrhal Fever: An Emerging Infection of Ruminants in India. In : "XII National Convention of Indian Association of Women Veterinarians and National Seminar on Livestock Breeding Strategies for Productivity Enhancement Towards Rural Prosperity" organized by RBRU, Coll. Of Veterinary Science and Animal Husbandary, Anand Agriculture University, Anand, Gujarat. 26- 28 August 2014. LP06.
5. Kumar M, Dash SK, Nagarajan S, Tosh C, Murugkar HV (2014). Effect of co-infection of H9N2 and H5N1 viruses on the survivability of chickens at different time intervals. Poster presentation in: International conference on Host-Pathogen Interactions, National Institute of Animal Biotechnology, Hyderabad, India, July 12-15, 2014 pp 71-72.
6. Nagarajan S, Tosh C, Murugkar HV, Kumar M, Sridevi R, Venkatesh G, Sood R, Senthil Kumar D, Tripathi S, Syed Z, Jain R, Behera P, S Shukla S, Vaid N, Mishra A, Kataria JM, Kulkarni DD (2014). Emergence of Reassortant H5N1 subtype avian influenza viruses with PB1 gene of endemic H9N2 subtype with low mice pathogenicity in India. Oral presentation In: XXI Annual Convention of Indian Society for Veterinary Immunology and Biotechnology, and International symposium on livestock diseases affecting livelihood options and global trade–strategies and solutions, TANVASU, Chennai, Tamilnadu, India, 17-19 July, 2014.
7. Nagarajan S, Ramaswamy V, Jain R, Rajukumar K and Pradhan HK (2014). Development of an indirect ELISA with recombinant nucleoprotein for diagnosis of influenza A. Paper presented at XXIII National Conference on Recent trends in virology in the Omics era organized by Indian Virological Society (IVS), VIROCON 2014 from 18.12.2014 to 20.12.2014.
8. Rajukumar K, Pramod KK, Karikalan M, Santosh Kumar, Rajesh K (2014). Effect of acute Bovine Viral Diarrhoea Virus type 1 infection on antigen presenting cells and cytokines in experimentally infected sheep in International Conference on Host-Pathogen Interactions (ICHPI) Organized by NIAB, Hyderabad, July 12-15, 2014, pp-68.
9. Rajukumar K, Mishra N, Kalaiyarasu S, Kumar M, Muthu Chelvan D, Pathak R and Dubey SC (2014). Effect of acute bovine viral diarrhoea infection on antibody response to Peste des Petits Ruminants in sheep, presented in 31st Annual Conference of IAVP and National Symposium on Climate change on Pathobiology of Diseases of Animals, Poultry and Fish at CoVSc, AAU, Anand, 13-15th Nov. 2014, pp 115.
10. R Sood, Kh Victoria Chanu, S Bhatia, C Tosh, S Nagarajan, AK Pateriya, R Khandia and DD Kulkarni (2014). *In-vitro* characterization of drug resistance in Highly Pathogenic Avian influenza virus in India. In: XII National Convention of Indian Association of Women Veterinarians and National Seminar on 'Livestock Breeding Strategies for Productivity Enhancement Towards Rural Prosperity' organized by RBRU, Coll. Of veterinary Science and Animal Husbandary, Anand Agriculture University, Anand, Gujarat. 26- 28 august 2014. ADT 17.
11. R Sood, Kh Victoria Chanu, D Hemadri, Premkrishnan GN, G Jahagirdar, N Mawale, M Kumar and S. Bhatia (2015). Cross-sectional study indicates large part of sheep population in Southern and Central India is infected with ovine herpesvirus-2. XXXIII Annual Convention of Indian Society of Veterinary Medicine and National Symposium on: New Dimensions in Veterinary Medicine: Technological Advances, One Health Concept and Animal Welfare Concerns. University Veterinary Hospital & TVCC College of Veterinary & Animal Sciences Campus Mannuthy, Thrissur, Kerala, 22nd -24th Jan, 2015 Farm Animal Infectious Diseases.5.14.
12. Senthil Kumar D, Kulkarni DD, Venkatesh G, Tosh C, Priyanka Patel (2014). Recombinant nucleocapsid protein based diagnostic ELISA to detect nipah viral infections in pigs. Poster presentation at International Conference on Host Pathogen Interaction. July 12-15, 2014 at NIAB, Hyderabad.
13. Tosh C, Nagarajan S, Murugkar HV, Kumar M, Shukla S, Tripathi S, Ved N, Mishra A, Kulkarni DD (2014). Isolation and genetic characterization of avian influenza (subtype H5N1) viruses isolated from chicken, duck and crow in Odisha, 2012 and 2014, Oral presentation at 2nd Annual conference and National Symposium on "Integrating poultry health and food safety" of Association of Avian Health Professionals, Pune, Maharashtra, 7-8 November, 2014.

14. Venkatesh G, Yisehak Redda, Kalaiyarasu S, Bhatia S, Senthil Kumar D, Nagarajan S, Pillai A and Kulkarni DD (2014). Use of Recombinant Haemagglutinin (ha) Protein Expressed in E.coli for Detection of AIV HA H5 antibodies in chicken. Oral presentation at the XXI Annual Convention of Indian Society for Veterinary Immunology and Biotechnology and International Symposium on "Livestock diseases affecting livelihood options and global trade- strategies and solutions" held at Madras Veterinary College, Chennai from 17.07.2014 to 19.07.2014.

15. Vijayakumar P, Raut AA, Ranaware PB, Kolte AP, Kulkarni DD , Mishra A, "Transcriptome analysis of Highly Pathogenic H5N1 Avian Influenza Virus infected crow lungs using Next Generation Sequencing" at International Conference on Host Pathogen Interaction. July 12-15, 2014 at NIAB, Hyderabad Oral Presentation:

NCBI: GenBank submissions

- Acc. No. KM668213: Rajukumar K, Kumar M, Senthilkumar D, Sen A, Pathak R, Shrivastava D, Tosh C and Kulkarni DD (2014). Genetic Characterization of Porcine Reproductive and Respiratory Syndrome Virus isolated from India - PRRSV isolate Ind-297221 proteinase-non-structural protein, glycosylated envelope protein GP5, and matrix protein M genes, complete cds.
- Acc. No. KJ661337: Rajukumar K, Kumar M, Senthilkumar D, Sen A, Das S, Pathak R, Tosh C and Kulkarni DD (2014). Genetic Characterization of Porcine Reproductive and Respiratory Syndrome Virus isolated from India - Porcine reproductive and respiratory syndrome virus nucleocapsid protein N gene, complete cds.
- Acc. No. KP663380**:** Rajukumar K, Kumar M, Senthilkumar D, Sen A, Das S, Pathak R, Tosh C and Kulkarni DD (2014). Genetic Characterization of Porcine Reproductive and Respiratory Syndrome Virus isolated from India - PRRSV RNA-dependent RNA polymerase gene, partial cds; and envelope protein GP2, envelope protein E, envelope protein GP3, and envelope protein GP4 genes, complete cds.
- Acc. No. from KM201299 to KM201318: Mishra N, Rajukumar K,Pateriya A, Kumar M, Dubey P, Behera SP, Verma A, Bhardwaj P, Kulkarni DD, Vijaykrishna D and Reddy ND(2014). Identification and molecular characterization of novel and divergent HoBi-like pestiviruses from naturally infected cattle in India, 5' UTR sequences of twenty Bovine viral diarrhea virus 3 isolates.
- Acc. No. from KM261863 to KM261882**:** Mishra N, Rajukumar K, Pateriya A, Kumar M, Dubey P, Behera SP, Verma A, Bhardwaj P, Kulkarni DD, Vijaykrishna D and Reddy ND (2014). Identification and molecular characterization of novel and divergent HoBi-like pestiviruses from naturally infected cattle in India, Npro sequences of twenty Bovine viral diarrhea virus 3 isolates
- Acc. No. KR021382- KR021389: Tosh C, Agarwal S, Kumar M, Nagarajan S, Mishra A, Shukla S, Singh B, Mishra P, Dubey P, Tripathi S, Murugkar HV, Kulkarni DD (2015). Genetic characterization of Influenza A/H5N1 virus isolated from duck in 2014, India (Eight nucleotide sequences of avian influenza H5N1 virus isolate A/duck/India/12CA05/2014.
- Acc. No. KP674452-KP674459: Tosh C, Shukal S, Nagarajan S, Kumar M, Murugkar HV, Tripathi S, Kulkarni DD (2014). Genetic characterization of Influenza A H5N1 virus isolated from crow, 2014 (Eight nucleotide sequences of avian influenza H5N1 virus isolate A/Crow/India/01CA02/2014.
- Acc. No. KJ643869-KJ643900: Nagarajan S, Syed Z, Tosh C, Murugkar HV, Manoj Kumar, Kulkarni DD (2014). Emergence of reassortant H5N1 subtype avian influenza virus with PB1 gene of endemic H9N2 subtype with low mice pathogenicity in South Asia. Thirty two nucleotide sequences (eight genes of each virus) of Avian influenza H5N1 viruses A/crow/India/11CA01/ 2011(H5N1), A/crow/ India/ 11CL01/ 2011 (H5N1), A/ chicken/ India/ 01CA03/2012 (H5N1) and A/ chicken/ India/ 01CA06/ 2012 (H5N1).
- Acc. No. KM110962- KM110976: Tosh C, Nagarajan S, Murugkar HV, Kumar M, Tripathi S, Syed Z, Behera P, Jain R, Dubey SC, Kulkarni DD (2014). Genetic analysis of Avian influenza H5N1 viruses isolated from India. Fifteen nucleotide sequences of Avian influenza H5N1 viruses A/Crow/ India/11TI07/ 2011 (H5N1), A/ chicken/ India/ 03CA02/ 2013 (H5N1), A/ Chicken/ India/ 04MO03/ 2012 (H5N1), A/ Chicken/ India/ 04MO04/ 2012 (H5N1).
- Acc. No. MK386909-MK386920.Tosh C, Nagarajan S, Murugkar HV, Kumar M, Jain R, Tripathi S, Syed Z, Behera P, Dubey SC, Kulkarni DD (2014). Genetic characterization of H5N1 avian influenza viruses isolated in India. Twelve nucleotide sequences of Avian influenza H5N1 viruses A/chicken/India/ 09CA01/2011(H5N1), A/chicken/ India/ 09CA02/2011(H5N1), A/chicken/ India/ 09CA09/2011 (H5N1) and A/Chicken/India/ 09CA10/ 2011(H5N1).
- Acc. No. MK386921-MK386928: Tosh C, Nagarajan S, Murugkar HV, Kumar M, Jain R, Tripathi S, Syed Z, Behera P, Shukla S, Kulkarni DD (2014). Genetic characterization of H5N1 avian influenza viruses isolated in India. Eight nucleotide sequences of Avian influenza H5N1 viruses A/duck/India/ 01CA01/2012 (H5N1) and A/chicken/ India/ 01CA01/ 2012 (H5N1).
- Acc. No. KM872062- KM872069 Das BR, Tosh C, Kumar M, Nagarajan S, Murugkar HV, Shukla S, Tripathi S, Mishra A, Kulkarni DD (2014). Isolation and Genetic Characterization of Influenza A (Subtype H5N1) Virus from Crows in India. Eight nucleotide sequences of Avian influenza H5N1 virus A/crow/ India/ 01TR01/ 2012 (H5N1).
- Acc. No. KJ417965 Kulkarni DD, Venkatesh G, Senthil Kumar D, Gupta V and Patel P (2014). Influenza A virus (A/swine/India/220966/2009 (H1N1)) segment 6 neuraminidase (NA) gene, complete cds.
- Accession No. KJ867526: Sood R, Jahagirdar GM, Khandia R, Chanu KV, Bhatia S and Kulkarni DD (2014). Identification of co-infection of ovine herpes virus 2 and caprine herpes virus infection in domestic goats in India.
- Accession No. KJ939434: Jacob A, Sood R, Bhatia S, Chanu KV, Khandia R, Tosh C, Nagarajan S, Jahagirdar G, Bhat S, Dimri U and Kulkarni DD (2014). Identification of Amantadine resistance mutation marker among Influenza A viruses (H5N1) in India.
- Accession No: KR063167: Sood R, Venkatesakumar E, Khandia R, Bhatia S, Chanu KV, Jahagirdar GM (2015). Detection of ovine herpesvirus 2 infection in buffaloes in India.
- Accession No KR092145-46: Sood R, Pillai, AS, Chanu KV, Mawale, N, Ralte EL Rajukumar K Kumar M Pateriya, A. K. Bhatia S Kulkarni, DD. (2015). Identification of ovine herpes virus 2 infection in pigs in India.
- Accession No KR092147: Sood R, Pillai AS, Chanu KV, Mawale N, Kumar M, Pateriya AK, Bhatia S, Kulkarni, DD (2015). Identification of ovine herpes virus 2 infection in sheep in Maharastra state in India.

NCBI: BIOPROJECT DATABASE SUBMISSION

Authors - A. Mishra, A.A. Raut, P. Vijay Kumar

1. Acc. No. PRJNA225507 [Title: Establishment of lung transcriptome of non-infected and highly pathogenic avian influenza virus (HPAIV) infected crow using next generation sequencing (NGS)].
2. Acc. No. PRJNA273455 [Title: Genome wide host gene expression analysis in chicken lungs infected with avian influenza viruses].
3. Acc. No. PRJNA273456 [Title: Genome-wide host gene expression analysis in duck lungs infected with high and low pathogenic H5N1 avian influenza virus].

NCBI: Sequence read Archive (SRA) Database Submission

Authors - A. Mishra, A.A. Raut, P. Vijay Kumar

1. Acc. No. SRX372694 [(LS454 (454 GS FLX Titanium) run: 859,241 spots, 326M bases, 206.1Mb downloads)]
2. Acc. No. SRX371713 [(LS454 (454 GS FLX Titanium) run: 651,062 spots, 255.2M bases, 152.5Mb downloads)]

NCBI: Gene Expression Omnibus (GEO) Database Submission

Authors - A. Mishra, A.A. Raut, D.D. Kulkarni, P. Ranaware

1. Acc. No. GSE65230 [Duck Gene Expression profile by Array] (Microarray data of 8 samples Accession No GSM159070-77).
2. Acc. No. GSE65231 [Chicken Gene Expression profile by Array] (Microarray data of 8 samples Accession No GSM159078-85).

Book Chapters

1. Tosh C and Nagarajan S (2014). Avian Influenza. in Zoonoses: Viral, Rickettsial and Prion Diseases Chapter 2 (Ed. S. R. Garg). Dayal Publishing House, New Delhi. pp. 8-34 (ISBN: 978-93-5124-272-7).
2. Mishra N (2014). West Nile Virus Infection. in Zoonoses: Viral, Rickettsial and Prion Diseases Chapter 5 (Ed. S. R. Garg). Dayal Publishing House, New Delhi. pp. 68-87 (ISBN: 978-93-5124-272-7).

Training Manuals

1. Rajukumar K, Pateriya AK and Kulkarni DD (2015). Training manual on 'Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Pathogens', training programme under DBT-NER-ADSAHD scheme organized at NIHSAD, Bhopal from 25th February to 3rd March, 2015.

Popular Articles

1. Kalaiyarasu S, Venkatesh G, Kumar M, Senthilkumar D (2014). TLR ligands-Novel adjuvants for poultry vaccines. *Poultry World*
2. Kumar M, Senthilkumar D, Kalaiyarasu S, Nagarajan S, Rajukumar K (2014). Innate immunity against viral infections. *Livestock line,* June, pp 24-27.
3. Kulkarni DD (2014). BSL-4 for high risks animal pathogens. Indian Farming. 64(9): 89-90 (Special Issue December 2014, on the occasion of 125 years of IVRI)
4. Kulkarni DD (2014) High Security Animal Disease Laboratory, IVRI Campus Bhopal in Hindi. Rajbhasha Smarika, IVRI 125 Years completion (1889-2014), Special Issue, Pp. 46-49.

Training, Conferences & Symposia

Training on 'Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Pathogens'

Scheme/ funding: DBT-NER-ADSAHD scheme

Participants: Five participants from NE region

Training on 'Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Pathogens', under DBT-NER-ADSAHD scheme organized at NIHSAD, Bhopal from 25th February to 3rd March, 2015

Training on 'Laboratory biosafety and Molecular diagnostic techniques'

Participants: Six post graduate students from CIFE, Mumbai

Training on laboratory diagnosis of BVD

Participants: Scientists of NDDB, Hyderabad.

Training on laboratory diagnosis of BVD to scientists of NDDB, Hyderabad. Dr. S. K. Rana, Senior Scientist and Dr. M. K. Poonanna, Scientist, NDDB participated. The course included molecular techniques of BVDV diagnosis such as one-step RT-PCR and real time RT-PCR for genome detection and differential RT-PCR and real-time RT-PCR for differentiation of BVDV-1, BVDV-2 and BVDV-3 in clinical samples.

Training on laboratory diagnosis of BVD

Participants:Scientist and RA of ICAR-NRC on Mithun, Nagaland.

Training on laboratory diagnosis of BVD for scientist and RA of ICAR-NRC on Mithun, Nagaland. Dr. Vidya Singh, Scientist and one Research Associate from NRC on Mithun participated in the training. The training included OIE recommended diagnostic tests such as virus isolation and identification through immunoperoxidase test and BVDV antigen ELISA for antigen detection, virus neutralization test and BVDV antibody ELISA for antibody detection, one-step RT-PCR and real time RT-PCR for genome detection and differentiation of BVDV-1, BVDV-2 and BVDV-3 by real-time RT-PCR.

SBS-ASM-ICAR Biosafety Awareness Programme including workshops on "Culture of Responsibility, Pathogen Inventory Management and Safety is the Rule: Fundamentals of working with Biosafety Cabinets"

A 'Biosafety Awareness Programme' including workshop on "Culture of Responsibility", "Pathogen Inventory Management" and "Safety is the Rule: Fundamentals of working with Biosafety Cabinets" was organized at ICAR-NIHSAD on March 13-14, 2015 in collaboration with Society for Biosafety and American Society of Microbiology, Washington, USA. This introductory course was designed for young microbiologists/ pathologists/public health professionals/ biotechnologists/ biologists/ dealing with disease agents/recombinant DNA etc and academicians in teaching. The Culture of Responsibility programme was conducted by Dr. Natasha Griffith, Director of High Containment Facilities at the University of California in Los Angeles and comprised of three modules on Biological Safety, Biosecurity and Responsible Conduct of Research. In addition to the culture of responsibility programme, workshops on working with the biosafety cabinets and Pathogen Inventory Management were also conducted. The participants were also made aware of the current biosafety regulations being implemented in the country during the programme.

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

S. No.	Name of the Symposium/Seminar/ Workshop	Date and venue	Scientists
1	OFFLU-STAR-IDAZ consultation on a global animal influenza research agenda	8th to 9th April, 2014, OIE HQs, Paris, France	C Tosh
2	Technical workshop on IBR and BVD control held by DADF	12th April, 2014, NDDB, Anand	K Rajukumar, N Mishra
3	Edinburgh India Institute Inaugural conference -"Innovative Engagement for Sustainable Development: the Edinburgh - India Story"	15th to 16th May, 2014, University Of Edinburgh UK	A Mishra, AA Raut
4	International Conference on Host-Pathogen Interactions (ICHPI)	12th to 15th July, 2014, National Institute of Animal Biotechnology, Hyderabad	K Rajukumar, M kumar D Senthilkumar
5	XXI Annual Convention of ISVIB and International symposium on "Livestock Diseases Affecting Livelihood options and global trade-strategies and solutions"	17th to 19th July, 2014, TANUVAS, Chennai	S Naragajan G Venkatesh
6	One day ASCAD sponsored seminar on "Avian influenza awareness and biosecurity measures in poultry farming"	25th July, 2014, Organized by TANUVAS at Namakkal Tamil Nadu	S Naragajan
7	Biosecurity and UNSCR 1540 Workshop at Participated in panel discussion session on Miti gating the Bioterrorism Threat by Applying P *athogen and Laboratory Security Measures at Public and Private Institutions* session.	21st to 22nd August, 2014, ICGEB - New Delhi	HV Murugkar
8	Third Global Conference of OIE Reference Centres - Challenges an d expectations for the future	14th to 16th October, 2014, Incheon (Seoul), Korea (Rep. of)	C Tosh
9	2nd Annual conference and National Symposium on "Integrating poultry health and food safety," Association of Avian Health Professionals	7th to 8th November, 2014, Pune, Maharashtra	C Tosh
10.	XII National Convention of Indian Association of Women Veterinarians and National Seminar on Livestock Breeding Strategies for Productivity Enhancement Towards Rural Prosperity"	RBRU, Coll. of Vety. Sci, AAU, Anand, Gujarat. 26- 28 August 2014	Dr. Richa Sood Dr. Kh. Victoria Chanu
11.	XXXIII Annual Convention of Indian Society of Veterinary Medicine and National Symposium on: New Dimensions in Veterinary Medicine: Technological Advances, One Health Concept and Animal Welfare Concerns	College of Veterinary & Animal Sciences Campus Mannuthy, Thrissur, Kerala 22nd -24th Jan 2015	Dr. Richa Sood
12	31st Annual Conference of IAVP and National Symposium on Climate change on Pathobiology of Diseases of Animals, Poultry and Fish	13th to 15th November, 2014, CoVSc, AAU, Anand	K Rajukumar
13	Participated as a speaker in the ASM Panel discussion on "Safe, Secure and Sustainable Laboratory Design" organized at ASM Session at MICROCON 2014 organised by Indian Association of Medical Microbiologists.	17th November, 2014, Birla Convention Centre, Jaipur	HV Murugkar
14	Training course on "Laboratory Quality Management System and Internal Audit as per ISO/IEC 17025:2005"	24th to 27th November, 2014, Indian Institute of Quality Management, Jaipur	S Naragajan, A K Pateriya
15	XXIII National Conference on Recent trends in virology in the Omics era	18th to 20th December, 2014, organized by Indian Virological Society 'VIROCON 2014	S Naragajan
16	National conference on emerging and re - emerging viral outbreaks in In dia - clinical challenges and management, organized by Osmania University, Hyderabad	20th to 22nd January, 2015, CSIR- IICT-CCMB, Hyderabad	K Rajukumar
17	12th Agricultural Science Congress on "Sustainable Livelihood Security for Smallholder Farmers"	3rd to 6th February, 2015, NDRI, Kannal, Haryana	C Tosh
18	XIII Annual Conference of Indian Association of Veterinary Public Health Specialists (IAVPHS) and National Symposium on Safety of foods of animal origin for domestic and export markets: Legal perspectives	10th to 12th February 2015, Veterinary College, KVAFSU, Bengaluru	HV Murugkar
19	Workshop on revisiting minimum standards for bovine frozen semen production held by DADF & NDDB	12th to 13th March, 2015, NDDB, Anand	N Mishra
20	SBS-ASM-ICAR Biosafety Awareness Programme including workshops on "Culture of Responsibility, Pathogen Inventory Management and Safety is the Rule: Fundamentals of working with Biosafety Cabinets"	13th to 14th March, 2015, ICAR- National Institute of High Security Animal Diseases	All Scientists of NIHSAD
21	Continuous Medical Education Program	9th Jan., 2015 AIIMS,	S Naragajan

Awards and Recognition

Awards

- Best poster award on "Peripheral blood mononuclear cells from field cattle immune to BVDV are permissive to BVDV in vitro" by Gupta V, Mishra N, Pateriya A, Behera SP, Rajukumar K, in XXVIII Annual Convention and International conf on challenges and opportunities in Animal Health held at DUVASU, Mathura, 30th Oct -1st Nov. 2014.
- Best Research Paper Award for article published in Journal, Veterinary Research International for paper titled "Genetic diversification of highly pathogenic avian influenza (HPAI) H5N1 virus in experimentally infected domestic ducks. (2013) Ranaware PB, Kulkarni DD, Mishra A, Pateriya A, Gandhale PN, Kumar VP, Tosh C, Murugkar HV, Raut AA.1(2): pp 33-40.

Scientists	Recognitions
A.A. Raut	Expert Member of Joint Working Group on Zoonosis of Department of Health Research, Nominated expert in ICMR Standing committees on Crimean Congo Haemorrhagic Fever, Kyasanur Forest Disease and Ebola. and Technical Expert in Committee for Establishment of Clean Air Animal Facility at IISER, Bhopal.
	Member, Govt, of India delegation (laboratory team) to Germany on diagnosis of Schmallenberg virus infections in HF bulls intended for export to India.
C. Tosh	Member, Institute Management Committee, National Institute of Biotic Stress Management, Raipur, Chhattisgarh.
G. Venkatesh	Member, Institute Biosafety Committee, All India Institute of Medical Sciences, Bhopal.
H.V. Murugkar	CPCSEA nominee, IAEC of (i) CSRD Peoples Group, Bhopal (ii) Aurobindo Medical College, Indore (iii) Chirayu Medical College, Bhopal
K Rajukumar	Member, expert committee meeting on PRRS situation review and sampling strategies for NE region organized at Directorate of Animal Husbandary, Aizawl, Mizoram.
N Mishra	Member, Govt, of India delegation (laboratory team) to Germany on diagnosis of BVDV infections in HF bulls intended for export to India.
	Member, committee of experts constituted by DADF, Govt. of India on inclusion of IBR, BVD in MSP for semen stations, 2014, Member, Madhya Pradesh State executive committee, under National Livestock Mission, 2014, Member, committee of experts constituted by DADF, Govt. of India on Biosafety and Animal Health standards in semen stations and quarantine stations, March, 2015, DBT Nominee, IBSC, IISER, Bhopal and Member, ITMC, ICAR-Indian Institute of Soil Science.
	Sectional Editor, Veterinary Virology in the journal Virus Disease
Richa Sood	CPCSEA nominee, IAEC of (i) Sagar Institute of Pharmaceutical Sciences, Sagar (ii) Rishiraj College of Pharmacy, Indore and Member IAEC, J.L.N. Cancer Hospital and Research Centre, Bhopal.
	Expert in Review Panel for projects, M.P. Council of Science and Technology, Bhopal
S. Bhatia	Member, Institute Management Committee, PD-FMD, Mukteshwar and CARI, Izatnagar.
S. Nagarajan	External member of high level committee for the execution of Anthrax Spore vaccine GMP Project and Poultry Disease Diagnostic Laboratory, Palladam GLP Project through Tamil Nadu Medical Service Corporation (TNMSC) in Tamil Nadu.

Meetings

Research Advisory Committee

Sl.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. Gaya Prasad, ADG(AH) Indian Council of Agricultural Research New Delhi	Member
3	Dr. D.T. Mourya, Director, NIV, Pune.	Member
4	Dr. Rajesh Chandra, Prof. & Head, Veterinary Microbiology, COVS, AIZWAL, CAU.	Member
5	Dr. R. Venkataramanan, Joint Director, IVRI, Bangalore.	Member
6	Dr. G.K. Sharma, Head Animal Health, NDDB, Anand	Member
7	Dr. D.D. Kulkarni, Director, NIHSAD, Bhopal	Member
8	Dr. H.V. Murugkar, Principal Scientist, NIHSAD, Bhopal	Member Secretary

Highlights of RAC recommendations

The first meeting of Research Advisory Committee (RAC) of National Institute of High Security Animal Diseases (NIHSAD), Bhopal was held on 30.10.2014 under the chairmanship of 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. The other members Dr. Gaya Prasad, Dr. D.T. Mourya, Dr. G.K. Sharma, Dr. Rajesh Chandra were also present in the meeting.

Dr. D. D. Kulkarni, Acting Director, NIHSAD welcomed the Chairman and other members. After a brief introduction by all the members and invitees, Dr. Kulkarni presented the institute profile, major research accomplishments and the status of various research projects (completed and being undertaken presently). He listed out the diagnostic facilities available presently at the institute against the following diseases- Influenza A infections incl. AIV, BVD, Border disease, MCF, CCHF, RVF, NSD, Nipah, PRRS, WNF, TGE, Pseudorabies, Bovine immunodeficiency virus, RHD and CAE. In view of the recommendations from ICAR and DADF, the diagnostic preparedness against various exotic and zoonotic diseases such as Schmallenberg, KFD, Hendra, Ebola and Hantan are proposed to be taken up. He also presented the proposed research programmes and activities as approved in the XII Plan.

Dr. Gaya Prasad, ADG (AH) initiated the discussion by expressing ICAR views on NIHSAD and suggested that being a premier institute, NIHSAD should take up basic research leading to high impact reporting and not just be satisfied with developing diagnostic preparedness. Acquiring technological competence for the cutting edge technologies should be a constant priority of all scientists. He indicated that specific programmes should be developed with clear targets and deliverables during the current plan. Under activities of the programmes proposed in XII plan, it was suggested that these should be spelt out more specifically. Dr. M. S. Oberoi,

Chairman of RAC, remarked that the research findings should culminate into new information or deliverable product or specific recommendations for the implementing agencies such as DADF, so that these can be applied in specific control measures for the diseases at field level. He suggested to take up study on the prevalence of MERS in camels and Ebola in Pigs and bats. Dr. D.T. Mourya suggested that in view of a large number of zoonotic infections emerging in the recent past, NIHSAD should collaborate with other institutions such as National Institute of Virology for validation of tests and the survey of animal populations for major zoonotic diseases such as CCHF as part of "One health" initiative so that a holistic picture can be obtained regarding the prevalence of such diseases both in human and animal populations. Dr. G. K. Sharma suggested that NIHSAD should look into the requirements of Animal Health Certificate issued by Government of India for import of animals and their products and should work at developing diagnostic capabilities against all the diseases listed in the certificate. He specifically suggested exploring the possibility of adding the diagnostic facilities for bovine enzootic leukosis, lumpy skin disease, vesicular stomatitis and bovine spongiform encephalopathy. Individual scientists who are the Principal investigators of the ongoing and newly approved institute and externally funded projects have presented the outline of their projects including expected outcome/deliverables and progress made so far.

The RAC opined that in addition to the work being carried out on avian influenza, NIHSAD should diversify the diseases and prioritize working on other diseases according to their importance and strive for becoming an OIE collaborating center in specialized areas. They suggested that work on diseases such as CCHF, MCF, PRRS, Nipah and Hendra virus infections should be extended. The RAC suggested that concerted efforts should be made to get the NIHSAD laboratories accredited with ISO:9000 (2008) and ISO:17025 certification since it is one of the important requirements for continuation of the laboratory as an OIE referral laboratory for avian influenza. It was also suggested that following the ISO:17025 accreditation, NIHSAD should apply for OIE status for more number of diseases.

The following important recommendations were made by the RAC-

1. RAC appreciated the research contributions of NIHSAD made in the last 15 years.
2. To have a wider perspective in research on exotic/ emerging diseases, NIHSAD should take up work on exotic diseases other than Avian Influenza.
3. The research findings should culminate into new information or deliverable products or specific recommendations for the implementing agencies
4. NIHSAD may also create the diagnostic facilities on diseases in relation to GoI-DADF certification for import of livestock/ poultry and their products.
5. The process of test validations and patent filing should be given priority.
6. A mechanism may be developed to store all the biological material like virus strains, recombinant clones, MAb clone etc in duplicate to avoid loss of valuable material in case of breach in storage conditions.
7. A focused programme on active surveillance on avian influenza in North East region (Assam and Tripura) and West Bengal should be formulated in collaboration with DADF and concerned state governments.

RAC meeting in progress

Institute Management Committee

Sl. No.	Name	Designation
1	Dr. D.D. Kulkarni, Director, NIHSAD	Chairman
2	Prof. Gaya Prasad, 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/NF, NIHSAD	Member
6	Dr. H.V. Murugkar, PS, NIHSAD	Member
7	Shri S.K. Gupta, SAO, NIHSAD	Member Secretary

IMC Meeting held on 29th Oct. 2014

Meeting on IPR issues and commercialization of technologies and a lecture by Dr. R. P. Singh, Principal Scientist & Incharge, ITMU, IVRI, Izatnagar.

Institute Technology Management Committee (ITMC)

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

Gender Sensitization Committee

Sl. 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 Non Govt. Organization
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)

Sl. 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)

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

Institute Joint Staff Council (IJSC)

Sl. No.	Name	Designation
(A)	**OFFICIAL SIDE**	Chairman
1	Dr. D.D. Kulkarni, Director, NIHSAD	Member
2	Dr. H.V. Murugkar, Principal Scientist	Member
3	Dr. Sandeep Bhatia, Principal Scientist/National Fellow	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, Sr. Admin. Officer	Member Secretary
(B)	**STAFF SIDE**	
	Administrative Category:	
1	Shri B.K. Singh, Assistant	Member/Member Secy. (Staff Side)
2	Mrs. Mehjabin Bilgrami, LDC	Member
	Technical Category:	
1	Shri R.K. Shukla, STA	Member
2	Shri S.B. Somkunwar	Member
	Supporting Category:	
1	Shri Sita Ram Imne, SSS	Member
2	Shri Sitai Prasad, SSS	Member

Distinguished Visitors

Shri Babubhai Bokhiria, Hon'ble Minister of Animal Husbandry & Dairying, Gujarat state interacting with scientists of NIHSAD

Dr. Feng Li, Professor, Veterinary and Biomedical Sciences Department,South Dakota State University, Brookings, SD, USA, he Interacted with Scientists and delivered a lecture on Influenza Drug resistance and Novel Influenza C-like Virus Emerging in the United States

Dr. David E. Swayne, Georgia, USA on visit to outside facilities of NIHSAD with Dr. D. D. Kulkarni

Prof. David W. Burt, Roslin Institute, University of Edinburgh, UK with NIHSAD Scientists

Date	Name of the visitor
27.06.14	Dr. Ameeta Kushwaha, Advisor, DBT, Govt. of Madhya Pradesh,
28.06.14	Dr. Amresh Kumar, Ex-Dean, G. B. Pant University, Pantnagar
03.07.14	Dr. Ashok Kumar Varshdaye, Arogya Bharti, Bhopal
29.10.14	Dr. M. S. Oberoi, Fomer FAO Expert & Chairman RAC, NIHSAD
29.10.14	Dr. G. K. Sharma, NDDB
29.10.14	Dr. Gaya Prasad, ADG (AH), ICAR
29.10.14	Dr. D. T. Mourya, Director, NIV, Pune
30.1014	Dr. Rajesh Chandra, Head of Department
07.11.14	Raghav Chandra, IAS, Additional Secretary cum Financial Advisor, Ministry of Agriculture, DADF GoI, New Delhi
21.11.14	Dr. David E. Swayne, Director, South East Poultry Research Lab, Athens, Georgia, USA
22.11.14	Shri Babubhai Bokhiria, Hon'ble AHD Minister Gujarat State.
11.02.15	Prof. David W. Burt, Chair Comparative Genomics, Roslin Institute, University of Edinburgh,UK
16.04.14	Feng Li, Professor, Veterinary and Biomedical Sciences Department, South Dakota State University, Brookings, SD, USA

Other Activities

Games and Sports Activities

Atul Kumar Pateriya, I/c Games and Sports

For the first time, after getting independent status, ICAR-NIHSAD, Bhopal participated in ICAR ZONAL TOURNAMENT (CENTRAL ZONE)-2014 organized at National Bureau of Soil Survey and Land Use Planning (NBSS & LUP), Nagpur from 16-20 September, 2014. A contingent of 11 staff members participated in various individual and team events under the leadership of Dr. A. K. Pateriya (chief-de-mission) and Mr. R. K. Shukla (Team manager).

Volleyball court for both smashing and shooting has been developed in NIHSAD campus

Rajbhasha Fortnight (Hindi Pakhwada) was organized between 8-29 Sept., 2014 in which all the staff and students actively participated. Prize Distribution Ceremony in progress.

Musical chair competition, Sac race and Lemon spoon race was organized on the occasion of republic day (26th Jan, 2015)

Activities of Staff welfare association

Staff welfare association (SWA) celebrated both national festivals on Independence Day (15th Aug, 2014) and Republic Day (26th Jan, 2015). Director, NIHSAD, hoisted the National Flag of India. Later, he addressed the achievements, upcoming activities and future challenges of this institute. The program was followed by distribution of sweets from SWA side.

Independent National status of NIHSAD after delinking from IVRI was also celebrated by SWA. Director, NIHSAD thanked ICAR, DARE, IVRI and other officials for recognizing the national importance of this institute. IVRI was remembered and realized as Mother Institute of this campus by the Director.

SWA also celebrated the New Year on 01st Jan 2015. On the occasion, Director, NIHSAD addressed and exchanged his feeling with the staff members.

The initiative for "Swachh Bharat Abhiyan" was taken by SWA by cleaning the NIHSAD campus on 02nd Oct, 2014.

Mr. G.V.S.N. Murty being felicitated by Dr. D.D. Kulkarni, Director, NIHSAD on his retirement day for the services rendered by him in the engineering section.

SWA gave big farewell and wished happy and healthy life to Mr. Shiv Om Agrawal and Mr. GVSN Murty on the occasion of their successful retirement from this institute.

Students of Jawahar NavodayaVidyalaya, Bhopal attending the lecture by Dr. H. V. Murugkar, Principal Scientist & Biosafety Officer

Celebration of Republic day 2015 at ICAR-NIHSAD campus

Staff of Disaster Management Institute, Govt. of MP, Bhopal visiting NIHSAD Campus

List of Scientific, Administrative, Technical and Supportive Staff

S. No.	Name	Designation	Qualification
1	Dr. D. D. Kulkarni	Principal Scientist and Acting Joint Director	M.V. Sc., Ph.D. (Veterinary Microbiology)
2	Dr. H. V. Murugkar	Principal Scientist	M.V.Sc. (Food Hygiene & V.P.H.) Ph.D. (Veterinary Microbiology)
3	Dr. C. Tosh	Principal Scientist	M.V.Sc., Ph.D. (Veterinary Virology)
4	Dr. N. Mishra	Principal Scientist	M.V.Sc., Ph.D. (Veterinary Virology)
5	Dr. Sandeep Bhatia	National Fellow	M.V.Sc. (Veterinary Immunology) Ph.D. (Microbiology & Immunology)
6	Dr. Ashwin Ashok Raut	Sr. Scientist	M.V.Sc., Ph.D. (Animal Biotechnology)
7	Dr. K. Rajukumar	Sr. Scientist	M.V.Sc., Ph.D. (Veterinary Pathology)
8	Dr. (Mrs.) Richa Sood	Sr. Scientist	M.V.Sc., Ph.D. (Veterinary Medicine)
9	Dr. S. Nagarajan	Sr. Scientist	M.V.Sc., Ph.D. (Animal Biotechnology)
10	Dr. G. Venkatesh	Sr. Scientist	M.V.Sc., Ph.D. (Animal Biotechnology)
11	Dr.(Mrs.) Anamika Mishra	Scientist	M.V.Sc., Ph.D. (Animal Genetics and Breeding)
12	Dr. Atul Kumar Pateriya	Scientist	M.Sc., Ph.D. (Biotechnology)
13	Dr. Manoj Kumar	Scientist	M.V.Sc., Ph.D. (Veterinary Pathology)
14	Dr. S.B. Sudhakar	Scientist	M.V.Sc. (Vety. Microbiology) Ph.D. (Avian Diseases)
15	Dr. S. Kalaiyarasu	Scientist	M.V.Sc. (Veterinary Microbiology)
16	Dr. Pradeep N Gandhale	Scientist	M.V. Sc., Ph.D. (Veterinary Virology)
17	Dr. D. Senthil Kumar	Scientist	M.V.Sc. (Veterinary Pathology)
18.	Dr. (Ms.) Khangembam Victoria Chanu	Scientist	M.V.Sc., Ph.D. (Animal Biochemistry)
		Technical Staff	
19.	Shri. R. K. Kaushik	CTO (Instrument)	Post Diploma (Electronics & Control) AMIE (Elect. & Communication Engg.)
20.	Shri. T. K. Ghosh	CTO (Electrical)	B.E. (Electrical), M.Tech., MBA,MIE
21.	Shri. R. B. Srivastava	CTO (Civil)	B.E. (Civil), M.I.E. (Civil)
22.	Shri. Rajeev Kumar	CTO (Mech. Engr.)	B.E. (Mechanical Engineering)
23.	Shri T. L. Bankar	ACTO	M.L.I.Sc., B.Ed., Diploma in Agril.
25.	Shri Sunil Barange	Sr. TO (Lab.)	B.Sc., PG Diploma in Clinical Pathology, M.Sc. (Zoology), M.Phil
24.	Shri Shambhu Dayal	Technical Officer	ITI (Fitter)
26	Shri R.K. Shukla	Sr. Tech. Asstt.	M.Sc. (IT), M.Lib.I.Sc., M.Phil
27	Shri Asanna Badge	Sr. Tech. Asstt.	B.E.(Elect.)
28	Shri Som Kumar	Sr. Tech. Asstt.	B.A. (Photography)
29	Shri Mahesh Kumar	Sr. Tech. Asstt.	ITI Lineman
30	Shri Rakesh Kumar	Tech. Asstt.	ITI Welder
31	Shri GVSN Murthy	Tech. Asstt.	Retired on 31-12-2014
32	Shri J.N. Meena	Tech. Asstt.	ITI (Boiler)
33	Shri Ram Lakhan	Tech. Asstt.	ITI Carpenter
34	Shri Malkhan Singh	Tech. Asstt.	Driver
35	Shri R.R. Chouksey	Tech. Asstt.	Driver
		Administration	
36	Shri S.K. Gupta	Sr. Admin. officer	B.Sc.
37	Shri B.K. Kanchan	Asstt.Fin.A/Cs officer	M.Com
38	Shri B.C. Kandpal	Asstt. Admin. Officer	B.A.
39	Shri B.K. Singh	Assistant	B.Sc.,M.A.
40	Shri Mansingh Hansda	Assistant	B.Sc.
41	Shri S.O. Agarwal	G.M. (Canteen)	Retired on 30-04-2014
42	Shri K.S. Tantuway	UDC	Inter
43	Mrs. M. Bilgrami	UDC	B.A.
		Supporting	
44	Shri Ram Prasad	SSS	
45	Shri S.R. Imne	SSS	
46	Shri Sitai Prasad	SSS	
47	Shri Sita Ram	SSS	

Appointments, Promotions, Transfers, Superannuation etc.

Sl. No.		Name of Officer/OfficialPurpose
1	Dr. Sandeep Bhatia	Promoted to the grade Principal Scientist
2	Dr. S.B. Sudhakar, Scientist	05.01.2015 (FN) Transferred from Mukteshwar. Promoted to the next higher grade pay of Rs. 7000/= w.e.f. 10.02.2013
3	Dr. S. Kalaiyarasu, Scientist	Promoted to the next higher grade pay of Rs. 7000/= w.e.f. 10.02.2014
4	Dr. Senthil Kumar D., Scientist	Promoted to the next higher grade pay of Rs. 7000/= w.e.f. 23.06.2014
5	Dr. Atul Kumar Pateriya, Scientist	Promoted to the next higher grade pay of Rs. 7000/= w.e.f. 11.12.2013 order issued on 22.01.2015
6	Shri Rajeev Kumar, CTO	Transferred to IVRI, Izzatnagar (UP) and retrieved of his duties in the (AN) of 31.01.2015
7	Shri S.O. Agarwal, GM (Canteen)	Retired from Council's service on 30.04.2015
8	Shri G.V.S.N. Murthy TA (wireman)	Retired form Council's service on 31.12.2014
9	Dr. S. Nagarajan	Promoted to the next higher grade pay of Rs. 9000/= w.e.f. 10.02.2014
10	Dr. Richa Sood	Promoted to the next higher grade pay of Rs. 9000/= w.e.f. 01.12.2013
11	Dr. G. Venkatesh	Promoted to the next higher grade pay of Rs. 9000/= w.e.f. 11.02.2014
12	Dr. K. Rajukumar	Promoted to the next higher grade pay of Rs. 9000/= w.e.f. 24.11.2013
13	Shri B.C. Kandpal	Promoted to AAO, w.e.f. 17.01.2015
14	Ms. Mehajabin Bilgrami	Promoted to UDC, w.e.f. 17.01.15

Animal Wing

Officer-in-charge : Dr Richa Sood, Sr. Scientist

Receiving Shed

This is an enclosed housing facility to keep newly procured animals in quarantine for 28 days. During this period the animals are acclimatized to the new environment and simultaneously they are also examined for health status and any pre-existing infectious disease. Special requirements like sexing of animals are also carried out here only. There are a total of 12 rooms for large animals, small animals, and laboratory animals along with store and office working lab and small clinic room. During the current year 56 crows, 10 guinea pigs, 143 pigeons, 200 turkey 40 geese and 150 mice were housed before they were taken in for experimentation.

Entry of Receiving Shed

Geese in Receiving Shed

Holding Shed

Animals from receiving shed are shifted to the holding shed if they are found fit after the completion of quarantine period until commencement of experimental trials. This shed comprises of 16 animal rooms with feed store, laboratory, clinical lab and office room. Birds housed in the shed are used for regular supply of blood for carrying out various tests like Haemagglutinatin and Haemagglutination inhibition for diagnostic as research purpose.

Entry of Holding Shed

Containment Animal Wing

The Animal wing portion of the containment laboratory is a biosafety level 3+ containment facility with graded negative pressure columns from -5 mm to -20 mm Water Column. Four sets of showers are provided at the entry of the animal wing containment area. The animal wing comprises of four large animals rooms, three small animal rooms, and three rooms designated for laboratory animals. The animal rooms are supplied with filtered air through Air Handling Units (AHU) and air is exhausted through HEPA filters.

A separate isolator room with three isolators (Class III cabinets) has been designated for testing the birds/laboratory animals with highly infectious agents or recombinant organisms. The entry to each animal room is from a common corridor which is designated as clean corridor. The central portion of the animal wing is the post mortem (PM) area which has one way opening from all the animal rooms into the dirty corridor, which leads to the open PM area. The movement of cages from PM area to the clean corridor area is through two large dunk tanks. There are two feed/ fodder storage rooms one each for large animal and small animal rooms. A Pass-Through Cabinet is the barrier between the laboratory wing and the animal wing. Besides this the containment shed also holds an office room, a store and a small canteen.

During 2014-15 various animal experimentations were conducted which included challenge studies for H5N2 vaccine in chicken, turkeys and ducks under National Fellow Project, study of differential host responses to H5N1 infection in ducks, turkeys chicken, geese and pigeons under BBSRC-DBT project, immunization and ascites production in Balb/c mice under Monoclonal antibody project, experimental transmission studies on H5N1 virus in H9N2 infected chickens and various other studies conducted by M.V.Sc. and Ph.D. students.

Clean Corridor of Animal Wing

Turkeys under experiment in Isolator (BSC-III)

Biosafety Engineering Section

Officer-in-charge : Dr N. Mishra, Principal Scientist
Associated Officers: Er. T. K. Gosh, Chief Technical Officer (Electrical)
Er. R. B. Srivastava, Chief Technical Officer (Civil)
Er. R. K. Kaushik, Chief Technical Officer (Instrmn.)

The Biosafety Engineering Section of NIHSAD forms the major backbone for non-stop functioning of the BSL-3 laboratory and animal facility. Besides the air handling units and effluent treatment plant the other supporting facilities for functioning of the laboratory/ animal wing are steam raising plant (boilers) for sterilization, de-mineralization plant, soft water plant, air conditioning plant, air compressors, a 33 KV electrical substation along with DG sets, and engineering workshop etc. Some of the important maintenance works and new initiatives taken up during the year 2014-15 in different units, are as listed below-

1. ***Civil Unit***
   - Designing of draft layout of construction of new ABSL-4 and ABSL-2 laboratories through NDDB, Anand.
   - Approval of extended guest house construction through CPWD.
   - Renewal of authorization of the M.P. State Pollution Control Board, Bhopal for Air and Water till 2019.
   - Water supply connection from Narmada water from BMC.
   - Repair of damaged RR masonry outer boundary wall of the institute.
2. ***Electrical Unit:***
   - Operation and Maintenance of 33 KV, 1000 KVA substation (500KVAx2), DG sets (500 KVAx1

and 300KVAx2), UPS (40 KVA, three phase) and other electrical installations.

- Replacement of two Metal Oxide 33 KV, 40 KA, Lightening Arrestor at incoming H- Pole DP Structure.
- Calibration of Protective relays, Main HT & LT System of substation & control room
- Replacement of 630 A, 33 KV, 3 Pole, rotating Arm, Gang operated Isolator at substation.

3. ***HVAC/AHU and Instrument Unit***

- Operation and maintenance of HVAC system comprising of 23 air handling units (AHU) with 97 HEPA filters fitted in 92 filter housings (Lab-58, animal wing -25, ETP and others- 09) and AC plant.
- Validation of HEPA filters twice and biosafety cabinets (class II B1) once.
- Formalin fumigation and DOP testing of filter housings.
- Fabrication of indigenous HEPA filters and pre-filters of SPF unit.
- Fabrication of silicon rubber heaters for SPF Isolators and repairing of various laboratory equipments.

4. ***Effluent Treatment Plant Unit***

- Maintenance of liquid waste sterilization plant (ETP), barrier autoclaves and ETO sterilizer.
- Replacement of leaking sterilizers of ETP with new sterilizers and calibration of 54 instruments of ETP.

5. ***Boiler Unit***

- Maintenance of two IEC make boilers (1500 kg/hr), two air compressors etc.

Electron Microscope Unit

Officer-in-charge : Dr. K. Rajukumar, Sr. Scientist
Associated Scientist : Dr. Manoj Kumar, Scientist
Associated Tech. Officer : Mr. Sunil Barange

The electron microscopy (EM) unit which functioning in a separate building in NIHSAD, is equipped with transmission electron microscope (JEOL, JEM-1400, Japan) along with its accessories including vacuum pump, air compressor, chiller and two UPS (10kVa and 15kVa). For sample processing, the unit has an ultramicrotome (Leica Ultracut UCT), glass knife maker (Leica EMKMR2), incubator (Jeiotech) amongst other minor equipments.

In the EM unit, a total of 67 samples comprising of 25 nanoparticle/ microemulsion/ bacteriophage samples, 11 tissue samples submitted by external users and 31 negatively stained grids containing virus samples including PRRSV and AIV of field as well as reverse genetics origin from NIHSAD were processed and viewed under TEM. The revenue generated through external user samples during 2014-15 was Rs. 42,932. Sample processing techniques for electron microscopy and viewing were demonstrated to participants of various training programmes organized at NIHSAD, Bhopal as well as to participants of a short course on nanotechnology organized by Indian Institute of Soil Sciences, Bhopal.

Agricultural Knowledge Management Unit (AKMU)

Officer-in-charge : Dr. K. Rajukumar, Sr. Scientist
Associated officers : Dr. Manoj Kumar, Scientist
Dr. S. Kalaiyarasu, Scientist (In-charge computer cell and nodal officer for MIS-FMS)
Dr. Pradeep Gandhale, Scientist

AKMU was constituted on 15.09.2014 after NIHSAD was declared by ICAR to function as an independent national institute. The major activities included commissioning of 100Mbps high speed internet connection of National Knowledge Network and its maintenance to provide internet and email services, maintenance of main server, desktop computers, Wi-Fi and LAN infrastructure of the Institute. AKMU activities also include maintenance and periodical updation of the contents of Institute website (www.nihsad.nic.in) and implementation of management information system including financial management system (MIS-FMS) for efficient administration at NIHSAD.

Specific Pathogen Free Unit (SPF Unit)

Officer-in-charge: Dr Anamika Mishra, Scientist
Associated officer: Dr Pradeep N. Gandhle, Scientist

The SPF Unit was set up in the campus of NIHSAD in the year 2009. This is an unique resource facility of the institute. The unit is located in an independent building and consists of two super isolators. It also has egg incubators and isolator hatchers. This unit has the capacity to maintain 120 adult chickens as two flocks of 60 each in each of the isolators.

The SPF flock in the SPF unit is generated by hatching fertile SPF eggs procured from commercial source. The flock is maintained as a pen mating flock with 50-55 females and 5-6 males in positive pressure super

isolators. The flock is screened regularly for 23 listed avian pathogens to verify the SPF status of the flock. The eggs produced from this flock are used for research and diagnosis of avian influenza at NIHSAD. The eggs are also used for producing small batches of SPF chicken (25-35) to be used for various purposes like IVPI, antisera raising and challenge experiments etc.

The flock in the two super isolators are maintained at 20-30 week apart in age so as to have continuous supply of SPF eggs. Each flock is maintained upto 72-76 weeks in order to have quality egg production in optimal numbers. The senile flock is then dissolved and replaced with a fresh flock. The flocks are fed with autoclaved crumb feed and supplemented with minerals, vitamins and calcium in filtered drinking water.

List of Research Projects / Service Projects:

S. No.	Title of the Project Date of Start-Date of Completion	Name of the PI and associates
1	Surveillance of exotic and emerging animal diseases in Indian and imported livestock & poultry and their products Project File No. ANSCNIHS ADSIL 200900100002 **April, 2009 - Continuing**	**PI- D. D. Kulkarni** Co-I -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. N. Gandhale
2	Evolutionary analysis of avian influenza viruses isolate in India Project File No. ANSCNIHSADSIL201400300010 **July 2014 - June 2017**	**PI - C. Tosh** Co-I- S. Nagarajan, G. Venkatesh, Manoj Kumar
3	Evaluation of diagnostic potential of monoclonal antibodies raised against H5N1 avian influenza virus Project File No. ANSCNIHSADSIL201400400011 **July 2014 - June 2017**	**PI - G. Venkatesh** Co-I- C.Tosh, S. Nagarajan, Richa Sood, Kh. Victoria Chanu
4	Identification of Neuraminidase Inhibitor Drug Resistance in H5N1 Viruses Isolated from India Project File No. ANSCNIHSADSIL201200100004 **May 2012 - March 2015**	**PI-Richa Sood** Co-I-A. Mishra , A. K. Pateriya, Kh. Victoria Chanu
5	Cross-sectional study of Malignant Catarrhal Fever infection in domestic ruminants in Southern India **(Inter-institutional project with NIVEDI, Bengaluru)** Project File No. ANSCNIHSADCIL201200200005 **Oct. 2012- Oct. 2015**	**PI-Richa Sood** Co-I- Manoj Kumar Kh. Victoria Chanu
	Externally Funded Projects	
6	Outreach programme on zoonotic diseases (Avian Influenza part) (ICAR funded) Project File No. ANSCNIHSADCOP200800100001 **July 2008 onwards**	**PI- H.V.Murugkar** Co-I- Manoj Kumar
7	Development and evaluation of neuraminidase DIVA Marker vaccines against highly pathogenic H5N1 avian influenza viruses in chickens (ICAR funded) Project File No. ANSCNIHSADSOL201100100003 **08.04.11-31.03.2016**	**PI- S. Bhatia, National Fellow**
8	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 in collaboration with ICAR-NEH region, Barapani) Project File No. ANSCNIHSADCOP201200300006 **Nov.2012 - Nov.2015**	**PI- K. Rajukumar** Co-I- D. Senthil Kumar
9	Prevalence & molecular epidemiology of BVD in ruminants with special reference to Mithun in North East states of India (DBT - twinning) Project File No. ANSCNIHSADCOP201300100007 **Oct.2013- Oct.2016**	**PI- Niranjan Mishra** Co-I- K. Rajukumar, S. Kalaiyarasu
10	Identification of the molecular basis of differential host responses to rapidly evolving Avian Influenza viruses in different avian species (BBSRC-DBT funded) Project File No. ANSCNIHSADSOL201400100008 **Feb. 2014 - March 2017**	**PI: A. Mishra** Co-I- A.A. Raut, H.V. Murugkar
11	Establishment of Advanced Animal Disease Diagnosis and Service Management Centers in the North East (AADSMC) (DBTfunded) Project File No. ANSCNIHSADCOP201400200009 **April 2014 - March 2019**	**PI: D. D. Kulkarni** Co-I- A. A. Raut, K. Rajukumar, A. K. Pateriya
12	Viral metagenomic profiling of native and migratory aquatic birds of North-Eastern states to unravel influenza virus ecology harbored in them (DBT-Twinning) Project File No. ANSCNIHSADCOP201400500012 **Sept. 2014 - Aug. 2017**	**PI: A. A. Raut** Co-I- D.D. Kulkarni, H.V. Murugkar, A. Mishra
13	Synthetic peptide based diagnostic for highly pathogenic avian influenza Project File No. ANSCNIHSADCOP201400600013 **Dec. 2014 - Nov. 2017**	**PI: Kh. Victoria Chanu** Co-I- Atul K. Pateriya, Richa Sood

NOTES

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