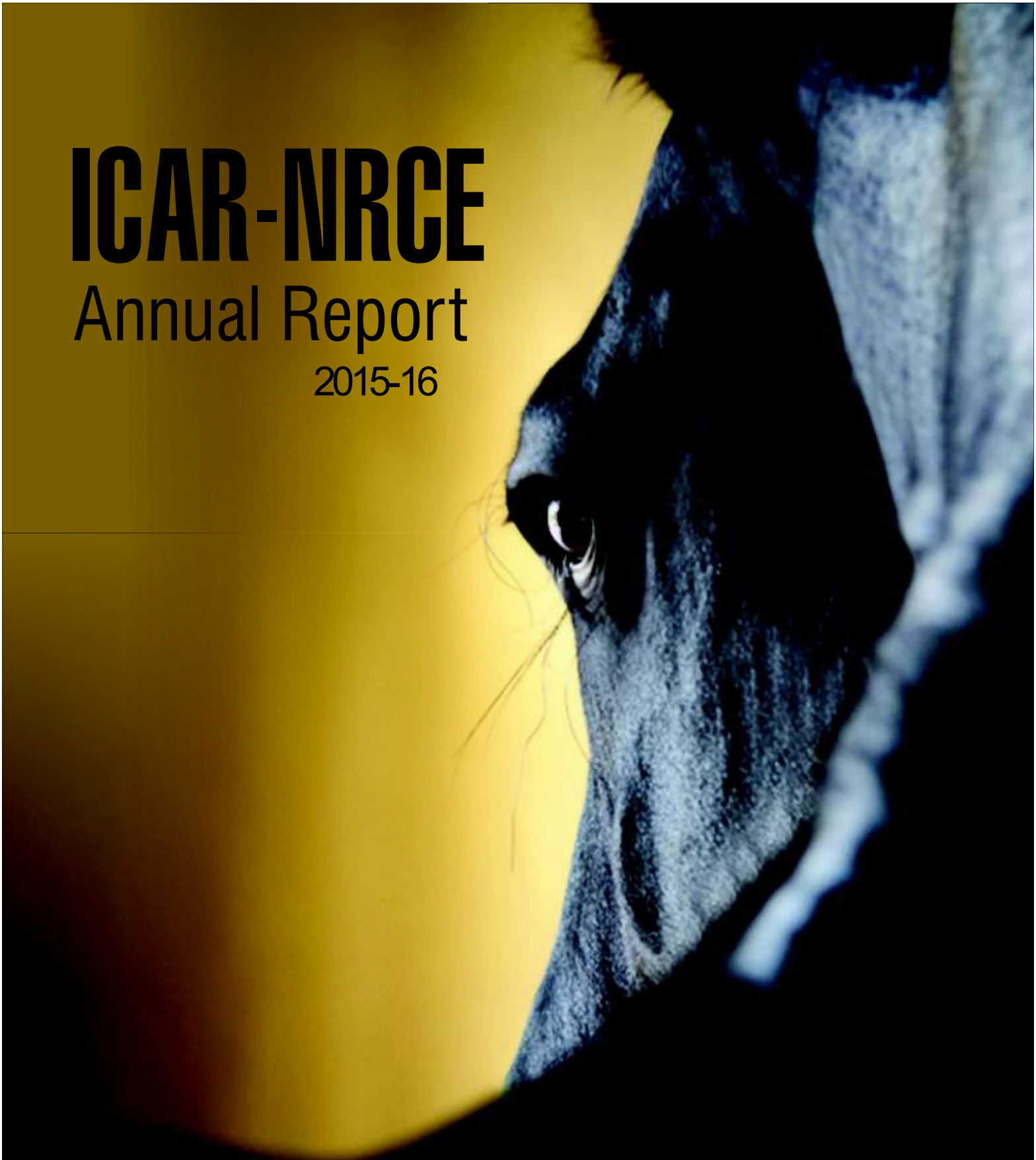
ICAR-NRCE

Annual Report

2015-16



भाकृअनुप-राष्ट्रीय अश्व अनुसंधान केन्द्र

ICAR-National Research Centre on Equines

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The achievements and activities of the Centre from April 2015 to March 2016 are presented in this Report. Mention of trademark, proprietary product or firm in the Report does not constitute an endorsement or rejection of other suitable products or firms.

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Index

Director's Foreword

With all humility and deep sense of pride for our institution, I wish to start the preface of this year's Annual Report by congratulating the vibrant and incredible team of scientists and staff at NRCE to have brought laurels to the institute by bagging coveted 'Sardar Patel Outstanding Institution Award – 2014' which was adorned on us by none other than Hon'ble Prime minister of India. All accolades for this go to the strong team work at NRCE encompassing through history of the Centre, which has its foundation built on hard work, perseverance, values and technical competence. The award is to be cherished for the challenging work done in the field of equine welfare through development of numerous technologies, vaccines, diagnostics and characterization of indigenous breeds, which have helped in improving equine health and production in the country.

Looking through the last year's progress report, I find we have many achievements in our kitty; prominent among which include the development of bacterial artificial chromosomes for EHV1, recombinant equine influenza virus, technique for differentiating neuropathogenic and non-neuropathogenic isolates of EHV1, development of new diagnostic assays for glanders and trypanosomosis, organizing two international workshops for delegates from SAARC countries on equine influenza and glanders under the banner of OIE, strengthening of repository of NCVTCC through accessioning of new viruses, bacteria and bacteriophages of economic importance and making the organization ISO 9001:2008 compliant.

During 2015-16, NRCE continued its surveillance and monitoring program for various viral, bacterial and parasitic diseases affecting equines. While equine infectious anaemia was not detected anywhere in the country, detection

of low titres for equine influenza in isolated cases requires further investigations as no active cases have been detected in India after 2009. Surveillance led to detection of sixty cases of glanders in UP, J&K, Punjab, Gujarat and Uttarakhand. The worrisome part is that in spite of the best efforts, glanders is spreading to new regions from its nidus in western UP. State of Gujarat through its effective monitoring and agreeing to NRCE's advice could manage to control the disease, and thus paved the way for other states to strictly adopt the follow up procedures once the disease is declared in the region. Development of diagnostics for emerging viral diseases is also picking up, and recombinant protein based diagnostics are on anvil for diseases like vesicular stomatitis and Venenzuelan equine encephalitis. The Centre has found a few promising drug molecules to combat equine piroplasmosis and further research on the toxicity trials for the drugs is in the process. Talking about diagnostics which is the frontier area at NRCE, the Centre has been able to develop a nanogold based lateral flow assay and recombinant flagellar protein by ELISA for diagnosis of trypanomosis. Centre has taken up new initiative under the Consortia Research Platform with the aim to improve already developed EHV1 vaccine in terms of better immune response and to develop faster diagnostics for EHV 1/4 and equine piroplasmosis. The Centre generated a revenue resource of over Rs 62.75 lakhs by providing consultancy services and diagnosis for the diseases and sale of farm produce.

The Centre has embarked on journey on important aspects of genetic characterization of horses and parentage testing for foals. These studies based on microsatellite markers will help in identifying true to breed marwari horses and testing parentage for which the equine industry sends the samples abroad. Post-thaw motility of the spermatozoa is a major concern for artificial insemination and through our continued research we have found that across various breeds dimethyl formamide at a concentration of 5% is an effective cryoprotectant. Load carrying by mules and donkeys is a vital animal welfare issue and NRCE, while working on this aspect could find that weight equivalent to 50% of the body weight did not alter the physiological profile in donkeys, and experiments with loading cart on mules deciphered a safe limit of 600N.

In its international endeavours to emerge as a leader in this part of the world, NRCE conducted two international trainings for the SAARC countries on important equine diseases viz. influenza and glanders. These diseases threaten the equine population with serious economic implications for the stakeholders. The Equine Influenza Laboratory and Glanders Laboratory, have this year completed the OIE twinning programs and are gearing up for getting an OIE referral status for the respective diseases. The 'Equine Piroplasma Laboratory' has already completed the OIE twinning project and is in the process of getting ISO17025 certification, a prerequisite to attain the referral status from OIE.

The National Centre for Veterinary Type Culture Collection has accessioned new bacteria, viruses, bacteriophages and recombinant clones, taking the total strength to 2939 accessions. The repository has rare bacteria such as *Lactococcus garvieae, Barrientosiimonas humi*; viruses viz. PPR, Pseudocowpox virus and Parapox virus and bacteriophages against *Pseudomonas spp.*, *Klebsiella pneumoniae* and other bacteria including a novel thermoresistant phage from river Ganga. Detection of antibiotic resistance genes (ARG) in bacteriophage DNA alarms the scientific community against the indiscriminate usage of antibiotics and phage mediated transfer of ARG's in environmental settings. I sincerely hope that these achievements will inspire us for further advances to come, promote national and international collaborations and ultimately contribute towards the improved equine health in India.

I gratefully acknowledge the kind guidance, moral support and continued encouragement from Dr Trilochan Mohapatra, Secretary DARE and Director General, ICAR, Dr H. Rahman, Deputy Director General (Animal Science), Dr Ashok Kumar, Assistant Director General (Animal Health), Principal Scientists and staff at ICAR headquarters. The commitment of the scientists and staff at NRCE needs commendation for their tireless efforts to make the Centre a vibrant hub for the research, which has been appropriately reflected in high rated peer reviewed International (50) and national (23) publications. The relentless efforts of the Editorial Board and Dr A.K. Gupta, Pr. Scientist, in compiling and editing the information across the year, to bring out this Annual Report deserve sincere thanks.

Jai Hind,

Btripathi

(B.N. Tripathi)

Executive Summary

National Research Centre on Equines was established under the aegis of Indian Council of Agricultural Research on November 26, 1985 at Hisar (Haryana). A sub-campus - Equine Production Campus (EPC) was established in 1989 at Bikaner (Rajasthan). The NRCE is mandated to undertake research on health and production management in equines, to act as national referral facility for diagnosis of equine diseases and to provide advisory and consultancy services for benefit of equine owners in the country. The Centre and its sub-campus have state-of-the art laboratories and facilities for undertaking research in areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry, biotechnology, genetics, breeding, reproduction, physiology and nutrition. The Centre has well maintained herd of Marwari, Zanskari & Manipuri horses and indigenous & exotic donkeys at Equine Production Campus, Bikaner.

During 2015, NRCE was conferred with the prestigeous "Sardar Patel Outstanding ICAR Institution Award" for excellence in research in last five years. In our research endeavours, we included work on developing a recombinant equine influenza virus employing reverse genetics approach. All the eight gene segments of H3N8 (Indian EIV strain) were cloned in pHW2000. The recombinant virus containing HA and NA gene segments of H3N8 and other six segments of H1N1 (wsn) was generated. With the objective to generate bacterial artificial chromosomes for EHV1 for mutagenesis studies and development of recombinant vaccine, mini F plasmid construct was co-transfected along with EHV1 viral DNA in RK13 cells and through homologous recombination recombinant virus with GFP was generated in the RK-13 cells. The construct was electroporated into *E. coli* cells to generate BACs. While characterizing pathogenic potential of EHV1 strains isolated from different outbreaks, it was observed that they differ due to single nucleotide polymorphisms (SNPs) in a few ORFs, significant being the ORF30 and ORF68. The ORF30 sequence analysis of 24 EHV1- positive isolates revealed that two of Indian samples from abortion cases (Delhi/2008 and Tohana/2007) had nucleotide substitution 'G' at position 2254 in ORF30 while the remaining 22 had 'A'2254 genotype, indicating that isolates with neuropathogenic potential are prevalent in India. Latency is one of the characteristic feature of all herpesviruses. To understand mechanism of latency in EHV 1 infection an *in vitro* system consisting of human lymphoblastoid cell line (LCL-2) co-cultured with Marmoset EBV- transfected B-cell Line (B-95) was developed. Cells persistently infected with EHV1 showed the expression of latency associated transcripts (LATs) by real-time PCR.

Working towards emergency preparedness and monitoring for exotic diseases, recombinant proteins were expressed in *E. coli* for development of diagnostics for equine viral diseases like Vesicular Stomatitis and Venezuelan Equine Encephlalitis. Surveillance of diseases is an important activity of the Centre for monitoring the disease situation in India. NRCE screened 5360 samples for equine infectious anaemia (EIA), 2660 serum samples for equine influenza, 1735 samples for EHV1, 1546 samples for JEV and 13980 samples for glanders and 1531 samples for trypanosomosis. All serum samples were negative for EIA. EHV1 and JEV showed seropositivity in 14.04% and 2.87% cases, respectively. Major concern during the year was the occurrence of glanders, which spread to newer areas. This year, 60 animals were detected positive from the states of J & K, UP, Punjab, Gujarat and Uttarakhand. *B. mallei* could be isolated from five biosamples.

NRCE has been developing and improving its disease diagnostic capabilities through continued research. The Centre initiated a project funded by OIE to validate tests developed by different OIE referral labs & NRCE for serological diagnosis of glanders in equids for the purpose of certifying freedom from infection status in individual animals for trade or movement. In this process we will be validating methods alternative to CFT viz. Western blot (WB) and ELISAs as per the OIE prescribed standards.

The institute has been the seat of excellence for haemoprotozoan diseases. In an effort to provide a field level test the Centre has successfully developed lateral flow assay (LFA) a which is nano gold based immunochromatographic test for detection of *Trypanosoma evansi* infection in animals. The test compared well with ELISA developed earlier by NRCE using whole cell lysate (WCL) antigen in preliminary analysis. Similarly, a recombinant flagellar protein based indirect ELISA was also developed for diagnosis of *Trypanosoma evansi* infection in equines with diagnostic specificity and specificity comparable to WCL - ELISA. The assay has also been validated at NRCE through inter-lab comparison and results of testing over 3000 field serum samples were highly encouraging.

Equine cytokines are not readily available to study their utility in diagnosis, vaccines and protection studies against various diseases. Recombinant cytokines of equines were generated and biological activity of five recombinant equine cytokines (IL-2, IL-4, IL-10, IL-18 and IFN-γ) was assessed. The study revealed that recombinant equine cytokines retained immuno-biological activity, hence they will serve as ready resource for studying antiviral and antibacterial effects as a therapeutic agent or vaccine adjuvant.

Drug discovery is an important aspect of research to combat the diseases which lead to huge economic losses. NRCE has been working on novel synthetic drug molecules against *Theileria equi*. IC50 concentrations of drug molecules were determined through *in vitro* cytotoxicity testing. O-Choline and berberine were found to be least cytotoxic - 8.7% and 13.6%. Organ toxicity trials in mouse model are being conducted for Imidocarb, Novobiocin and DMB drug molecules as well. Efficacy of Isometamidium chloride-loaded nanoparticles against *T. evansi* was adjudged and its toxicity on cell lines revealed a concentration-dependent cytotoxicity and genotoxicity. *In vitro* studies revealed that formulated drug nanoparticles were able to kill parasites and beyond that efficacy of nanoformulation was optimized in mice at different doses and found to be effective against *T. evansi*. Quinapyramine sulfate used for treatment of trypanosomosis in domestic animals was assessed for hepatic expression profile of metabolizing genes in rat which revealed that the metabolism is mainly through oxidation and it has greatest induction ability on flavin containing monooxygenase 1 followed by monoamine oxidase A and cytochrome P450 family enzymes.

The National Research Centre on Equines completed two International Twinning projects funded by OIE on Equine Influenza and Glanders. The basic objective of the projects was to build the capacity of the candidate laboratory (NRCE) so that they can act as OIE referral centres for the region. Under the project on glanders, multilocus sequence typing (MLST) and variable number tandem repeats (VNTR) typing of *Burkholderia mallei* field isolates was carried out. Under equine influenza project, Director alongwith two scientists and a technician visited Animal Health Trust, UK for a Laboratory Exchange Program. The scientists tested their monoclonal antibody based sandwich ELISA for detection of equine influenza antigen. The assay was working satisfactorily for detecting the influenza antigen in clinical samples. Further, HI assay was validated through testing of 30 samples sent to AHT. In both the Twinning Projects, International Workshops were conducted for the delegates from the SAARC countries in the month of February, 2016. Training on glanders was imparted by experts from NRCE and Friedrich Loeffler Institut, Jena, Germany and was attended by 15 delegates including 7 from SAARC countries. Training on equine influenza was imparted by experts from NRCE and AHT, UK and was attended by 16 delegates including 5 delegates from SAARC countries.

A nucleus herd of indigenous equines including Marwari, Manipuri, Zanskari horses, grey and white donkeys and the exotic Poitou donkeys is being maintained at equine production campus of ICAR-NRCE. Semen cryopreservation is a well established method of *ex situ* conservation, therefore semen from Marwari & Kathiawari horses, Manipuri & Zanskari ponies and exotic donkeys have been cryopreserved for AI purpose. Semen cryopreservation of elite stallions in field for the selective breeding of mares at our Centre has been initiated. In order to upgrade the quality of post thaw semen, our Centre keeps on performing research on cryopreservation of semen. In recent studies, dimethyl formamide (DMF) was found to be most suitable cryoprotectant for Marwari, Manipuri and Zanskari breed stallion's semen cryopreservation and in another study, addition of caffeine has improved significantly the post thaw motility of spermatozoa in Marwari horse and exotic donkeys.

In view of increasing demand from stakeholders the centre has initiated developing genetic markers for characterization of true to breed Marwari horses. At present, there is no commercial facility available in India for parentage testing and mostly breeders get their animals tested for parentage outside the country which is expensive. This necessitated NRCE to standardize the DNA markers based test for parentage authentication in horses, which is in the process of validation. Such DNA typing test will ease the equine breeders to rely on a government organization for parentage proofing of their foals. Development of a lateral flow assay (LFA) based kit for pregnancy diagnosis in horse mares in underway.

In order to fulfill the need of farmers regarding requirement of area specific concentrate mixture in horse populated region in India, NRCE has initiated a program for developing the concentrate mixture with combination of 3 energy supplements (oats, barley and maize), 3 protein supplements (groundnut cake, mustard cake and mung bean) and a filler (wheat bran) for preparing 9 iso-nitrogenous concentrate mixture for in vitro study. The combination barley + wheat bran + groundnut cake showed highest digestibility among the concentrate mixture combinations.

National Centre for Veterinary Type Culture Collection (NCVTCC) is ISO9001:2008 certified and has been mandated to act as a national repository of microorganisms of animal origin comprising veterinary, rumen and dairy microbes. The major activities include isolation, characterization, conservation, maintenance and distribution of these microbes for their utilization in animal health and production. This year a total of 383 microbes/ genomic DNA/clones were accessioned in the NCVTCC repository which includes 110 bacteria, 14 viruses, 44 bacteriophages, 45 clones, 57 genomic DNA, 74 rumen microbes and 39 dairy microbes.

These deposits including veterinary, rumen and dairy microbes are contributed by 19 network units apart from ICAR institutes and State Agricultural and Veterinary Universities. The microbial resources include viruses, bacteria, bacteriophages & clones; rumen microbes comprising anaerobic bacteria and fungi; and dairy microbes. The Centre is also maintaining 17 cell lines and primary cultures. The repository is currently represented with more than 70 genera of bacteria including some novel taxa and various families of viral pathogens. Since March 2015, the microbial cultures received at NCVTCC are processed under a streamlined ISO9001:2008 procedure.

Some of the prominent viral isolates deposited include Newcastle disease virus, PCV-2, fowlpox virus, CSFV, NDV and SPPV. Further, methodologies were developed to successfully purify a positive stranded RNA virus (FMDV) from the virus mixture containing a negative stranded RNA virus (PPRV). The scientists also investigated outbreaks and isolated viruses including poxvirus/parapox virus from camels and small ruminants and infectious bursal disease (IBD) virus from poultry. The molecular mechanism of interaction between host cell protein kinase and PPRV were studied and antiviral efficacy of SERCA inhibitors was evaluated. The bacterial repository

currently has 1037 economically/scientifically important bacterial isolates including 42 rare bacterial strains accessioned this year including *Lactococcus garvieae, Barrientosiimonas humi, Corynebacterium amycolatum* and *Actinobacillus equilli,* etc. Whole genome sequencing of two accessioned cultures i.e., *Pasteurella multocida ssp. multocida* and *Salmonella enterica bv. Gallinarum* was done and sequence data accessioned in NCBI. Under new initiatives, we performed enumerations and isolation of bacteria from 45 mule dung samples from hilly regions which are further being assessed for composting. We were also able to generate the expression-ready gateway Open Reading Frame (ORF) clone collection of equine influenza virus and provided the proof of their utility in recombinant protein production and protein-protein interaction studies in yeast-2-hybrid system based screening.

In process of strengthening repository of bacteriophages, phages against some of the prominent bacteria of zoonotic importance including *Pseudomonas sp., Klebsiella pneumoniae*, *Shigella sp., E. coli* etc. were isolated. A unique thermotolerant bacteriophage was also isolated from water from river Ganga. The presence of Antibiotic Resistant Gene (ARGs) in environmental bacteriophages was explored and important ARGs detected included - bla-TEM, Oxa-2, tetA and tetW. The presence of these ARGs in bacteriophage DNA provides an early warning system for future clinically relevant and emerging bacteria. NCVTCC has also initiated preservation of induced pluripotent stem (iPS) cells in its repository.

NRCE generated a revenue of Rs. 62.72 lakhs comprising Rs. 48.00 lakhs from contractual diagnostic services and sale of farm (Agricultural and Livestock) produce.

The Centre organized Equine Health camps and Kisan Goshthis and participated in various exhibitions to showcase its activities. NRCE participated in the Flagship programs instituted by government of India including 'Mera Gaon Mera Gaurav' and 'Swachh Bharat Abhiyan'. During the year, the institute conducted various activities such as Foundation Day, World Veterinary Day, National Science Day, Interactive Meet of farmers and Progressive Equine owners and Drawing Competition. Awards/honours were conferred on to scientists and students working at the Centre by various prestigious national Associations and Societies. Further, the scientists of the Centre published 73 research articles in indexed research journals of international (50) and national (23) repute, in addition to several book chapters, abstracts in compendium and large number of gene sequences to GenBank.

कार्यकारी सारांश

राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार की स्थापना 26 नवम्बर, 1985 को भारतीय कृषि अनुसंधान परिषद के तत्वावधान में की गई थी। इसका एक उप–कैम्पस अश्व प्रजनन संस्थान, बीकानेर, राजस्थान में, 1989 में स्थापित किया गया था। केन्द्र को घोडों के रोगों के निदान के लिए राष्ट्रीय रेफरल सुविधा, देश में घोड़े के मालिकों के लिए सलाह व परामर्श सुविधा प्रदान करने एवं घोड़ों में स्वास्थ्य और उत्पादन प्रबंधन पर शोध करने का भार सौंपा गया है। केन्द्र व उप–कैम्पस में घोड़ों पर अनुसंधान के लिए विषाणु एवं जीवाणु विज्ञान, परजीवी विज्ञान, विकृति विज्ञान, इम्यूनोलॉजी, चिकित्सा, जैव रसायन, जैव–प्रौद्योगिकी, आनुवंशिकी, प्रजनन, शरीर विज्ञान एवं पोषण विज्ञान की प्रयोगशालाएँ विकसित की गई हैं। केन्द्र में मारवाड़ी, जाँसकारी, मणिपुरी घोड़ों और स्वदेशी व विदेशी गर्दभों को संरक्षित किया गया है। वर्ष 2015 में केन्द्र को प्रतिष्ठित सरदार पटेल पुरस्कार से सुशोभित किया गया है। यह पुरस्कार केन्द्र में पिछले पाँच वर्शों के उत्कृष्ट अनुसंधान के लिए प्रदान किया गया है। इस वर्ष अश्व इम्फ्लूएंजा विषाणु का पुनः संयोजक विषाणु रिवर्स आनुवंशिकी द्वारा विकसित किया गया। इसके लिए भारतीय आनुवंशिकी के H3N8 के सभी 8 खण्डों को PHW 2000 में क्लोन किया गया। पुनः–संयोजक विषाणु बनाने के लिए H3N8 के HA-NA जीन एवं H1N1 के अन्य 6 खंडों का प्रयोग किया गया। EHV1 पर अनुसंधान करते हुए म्यूटाजेनेसिस के अध्ययन एवं पुनः संयोजक वैकसीन को विकसित करने हेतु जीवाणु कृत्रिम गुणसूत्रों को विकसित किया गया।

EHV1 की रोगजनक क्षमता कुछ ORF में भिन्नताओं के कारण EHV1 स्ट्रेन्स में भिन्न पाई गई है एवं यह देखा गया है कि एकल न्यूक्लियोटाईड बहुरूपताओं के कारण ऐसा सम्भव है। देश के 24 EHV1 आईसोलेट्स के ORF 30 अनुक्रम विश्लेषण में गर्भपात के ऊत्तक के नमूनों (दिल्ली / 2008, टोहाना / 2007) में 2254 स्थान पर न्यूक्लियोटाईड 'G' का प्रतिस्थापन उनकी न्यूरोपैथोजेनिक क्षमता दर्शाता है जबकि शेष 22 आईसोलेट्स में इस स्थान पर 'A' न्यूक्लियोटाईड पाया गया है। हर्पिस विषाणु में विलम्बता एक विशिष्ट लक्षण होता है। EHV1 में इसके अनुसंधान के लिए, संस्थान में इन वीट्रो मानव लिम्फोब्लास्टोइड सेल लाइन का सुसंस्करण मारमोसेट EBV सेल लाइन (BA-5) के साथ करके कोशिकाओं को EHV1 से निरंतर संक्रमित करने पर लेटेन्सी एसोसिऐटिड ट्रॉंस्क्रिप्ट्स की अभिव्यक्ति को दर्शाया गया है।

विदेशी रोगों की निगरानी एवं आपातकालीन तत्परता हेतु वेसीकुलर स्टोमेटाईटिस एवं VEE की पुनः संयोजक प्रोटीन व्यक्त करके इन रोगों के निदान के लिए केन्द्र परीक्षण विकसित कर रहा है। रोगों की निगरानी हमारे केन्द्र की एक महत्वपूर्ण गतिविधि है। गत वर्ष EIA के लिए 5360 नमूनो, अश्व फ्लू के लिए 2660 नमूनो, EHV1 के लिए 1735 नमूनों, JEV के लिए 1546 नमूनों एवं ग्लैण्डर्स के लिए 13,980 नमूनों की जाँच की गई। EIA के लिए सारे नमूने नकारात्मक रहे। EHV1 एवं JEV की सीरोपॉजिटिविटी, 14.4 प्रतिशत एवं 2.87 प्रतिशत क्रमशः पाई गई।

ग्लैण्डर्स रोग का संक्रमण नए क्षेत्रों में पाया जाना चिंता का विषय है। पिछले वर्श में इस बीमारी का संक्रमण 60 अश्वों में जम्मू–कश्मीर, उत्तर प्रदेश, पंजाब, गुजरात और उत्तराखंड में पाया गया। इसके जीवाणु बी. मेलियाई को पाँच नमूनों से पृथक किया गया।

केन्द्र ने गत वर्ष OIE द्वारा प्रायोजित एक परियोजना में कार्य शुरू किया जिसका मुख्य उद्देश्य ग्लैण्डर्स के लिए निदान की तकनीकों की मान्यता का पुष्टीकरण है। संस्थान ने ट्रिपैनोसोमा इवैन्साई के संक्रमण के निदान के लिए दो नई तकनीकों को विकसित किया जिसमें नैनोगोल्ड आधारित इम्यूनोक्रोमैटोग्राफिक परीक्षण एवं पुनः संयोजक फ्लैजेलर प्रोटीन पर आधारित एलाईसा शामिल हैं।

संस्थान ने घोड़े के संयोजक साइटोकाइन विकसित किए जिनमें IL-2, IL-4, IL-10, IL-18 , IFN- γ की जैविक गतिविधि का मूल्यांकन किया गया। इस अध्ययन से पता चला कि पुनः संयोजक साइटोकाइन में इम्यूनोजैविक गतिविधि बरकरार है, इसलिए वह टीका सहायक के रूप में विषाणु एवं जीवाणु रोधी अध्ययन के रूप में सक्षम है।

संस्थान कृत्रिम दवा अणुओं पर शोध करने में कार्यरत है। इसके तहत पाईरोप्लास्मोसिस पर कार्य करते हुए, वैज्ञानिकों के द्वारा दवा अणुओं की आईसी 50 साँद्रता का इनवीट्रो साईटोटॉक्सिसिटी द्वारा अवलोकन किया गया है। कोलीन एवं बार्बरीन दवा अणुओं को कोशिकाओं पर सबसे कम विषाक्त पाया गया। इसी क्रम में आईसोमेटामीडिय–मक्लोराइड से भरे नैनो कणों को सर्रा बीमारी के लिए प्रभावकारी पाया गया। सर्रा के लिए एक दवाई, क्वीनापाइरामीनसल्फेट के हिपेटिक एक्सप्रेशन प्रोफाइल का परीक्षण किया गया और पाया गया कि चय–अपचय मुख्यतः ऑक्सीडेशन के माध्यम से होता है।

केन्द्र में ओआईई द्वारा प्रायोजित दो अंतर्राष्ट्रीय ट्रेनिंग परियोजनाओं अश्व फ्लू एवं ग्लैण्डर्स पर शोध किया जा रहा है। इन परियोजनाओं का मूल उद्देश्य संस्थान की प्रयोगशालाओं की क्षमता का निर्माण करना है, जिससे वे OIE रेफरल केन्द्र के रूप में कार्य कर सकें। ग्लैण्डर्स परियोजना के अंतर्गत बी. मेलियाई का एमएलएसटी एवं वीएनटीआर द्वारा टाइपिंग किया गया। अश्व फ्लू परियोजना के तहत, निदेशक, दो वैज्ञानिक और एक तकनीशियन प्रयोगशाला विनिमय कार्यक्रम के अंतर्गत एनिमल हैल्थ ट्रस्ट (यू.के.) के दौरे पर गए। वैज्ञानिकों ने इन्फ्लूएंजा प्रतिजन का पता लगाने के लिए मोनोक्लोनल एंटीबॉडी आधारित सैण्डविच एलाईजा का परीक्षण किया। दोनों ट्विनिंग परियोजनाओं में फरवरी मास में दस दिन की एस.ए.ए.आर.सी. देशों के प्रतिनिधियों के लिए कार्यशाला आयोजित की गई। मारवाड़ी, मणिपुरी, जाँसकारी और ग्रे एवं सफेद गर्दभों तथा विदेशी पोइटू गर्दभों का एक नाभिक झुंड उत्पादन परिसर में रखा जा रहा है। वीर्य क्रायोप्रिजरवेशन एक्स सीटू कन्जरवेशन की एक स्थापित विधि है, इसलिए उपरोक्त नस्लों के वीर्य क्रायोप्रिजर्व किये जा रहे हैं। वीर्य की गुणवत्ता को उन्नत करने के लिए क्रायोप्रिजरवेशन करते समय डाई मिथाइल फोर्मामाइड के योग से मारवाड़ी, मणिपुरी और जांस्कारी अश्वों के वीर्य की गतिशीलता में सुधार पाया गया। हितधारकों की बढ़ती मांग को देखते हुए केन्द्र ने मारवाड़ी घोड़ों के लक्षण–वर्णन के आनुवंशिक मार्करों के विकास पर कार्य शुरू कर दिया है। वर्तमान में भारत वर्ष में अश्वों में पितृत्व प्रमाणीकरण सत्यापन की प्रक्रिया उपलब्ध नहीं है। इस विषय में केन्द्र ने डीएनए पर आधारित परीक्षण में सफलता पाई है ओर इसके मानकीकरण पर कार्य कर रहा है।

भारत में घोड़ों की आबादी वाले क्षेत्रों के लिए विशेष मिश्रण तैयार करने के लिए केन्द्र कार्य कर रहा है। साथ ही केन्द्र एक सारकृत मिश्रण विकसित कर रहा है जिसमें तीन ऊर्जा की खुराक (जई, जौ और मक्का) तीन प्रोटीन की खुराक (मूंगफली, सरसों और मूंग के केक) और एक पूरक (गेहूं की भूसी) संयोजित किए गए हैं। जौ, गेहूं की भूसी और मूंगफली केक के मिश्रण की पाचन शक्ति सबसे अधिक पाई गई है।

राष्ट्रीय वैटरीनरी टाईप कल्चर कलैक्शन केन्द्र ISO 9001, 2008 प्रमाणित होकर पशु चिकित्सा रूमन, डेयरी रोगाणुओं के राष्ट्रीय भंडार के रूप में कार्य कर रहा है। इस केन्द्र की मुख्य गतिविधियों में रोगाणुओं के अलगाव, लक्षण, जाँच, संरक्षण, रख–रखाव एवं वितरण शामिल हैं। इस वर्ष 383 रोगाणुओं/जिनोमिक डीएनए (110 जीवाणु, 14 विषाणु, 44 जीवाणु भोजी सहित 39 डेयरी व 74 रूमन रोगाणु) संरक्षित एवं परिग्रहित किए गए। ये रोगाणु 19 नेटवर्क इकाईयों के योगदान से संग्रहित किए गए। केन्द्र ने 17 सेल लाईन और प्राथमिक सेल कल्चर्स का रख–रखाव भी कर रखा है। वर्तमान में केन्द्र के पास 70 से अधिक जेनेरा के जीवाणु संरक्षित हैं।

संरक्षित विषाणुओं में न्यूकैसल रोग विषाणु, पीसीवी–2, फाउलपॉक्स विषाणु, सीएसएफवी, एनडीवी एवं एसपीपीवी शामिल हैं। वैज्ञानिकों ने उाउटब्रेक्स में बीमारियों के विषाणु जैसे कि पाक्स विषाणु, पैरापॉक्स विषाणु, आईबीडी विषाणु इत्यादि का अलगाव भी किया। मेजबान कोशिका प्रोटीन काइनेज तथा पीपीआरवी का अध्ययन करते हुए एसईआरसीए का एंटीवाईरल क्षमता का मूल्यांकन किया गया। जीवाणु रिपाजिटरी में वर्तमान में 1037 जीवाणु हैं जिनमें 42 दुर्लभ जीवाणुओं का परिग्रहण किया गया। इनमें प्रमुख लैक्टोकोक्स गार्वी, बेरिएंटोसीमोनास हूमी, कोराइनीबैक्टीरियम एमीकोलेटम् इत्यादि शामिल हैं। पास्चुरेला मल्टोसिडा सब स्पी. मल्टोसिडा एवं सालमोनेला एन्टेरिका (बी.वी.) गैलिनेरम् के पूरे जीनोम का विश्लेषण किया गया।

एक नई पहल के तहत पहाड़ी क्षेत्रों से 45 खच्चरों के गोबर के नमूनों से जीवाणुओं का पृथक्कीकरण किया गया। इन नमूनों का खाद के लिए मूल्यांकन किया जा रहा है। अश्व फ्लू विषाणु के ओआरएफ क्लोन विकसित किए गए और यीस्ट–2 संकर प्रणालि आधारित स्क्रीनिंग में पुनः संयोजित प्रोटीन के उत्पादन और प्रोटीन–इन्टरैक्शन में उनकी उपयोगिता को प्रमाणित किया गया है।

जीवाणु भोजियों के भण्डार को मजबूत बनाने की प्रक्रिया में हमने सूडोमोनास सहित जूनोटिक महत्व के प्रमुख जीवाणुओं के खिलाफ संग्रहण तैयार किया है। एक अद्वितीय, थरमोटोलरेंट जीवाणुभोजी भी गंगा नदी के पानी से पृथक किया गया। पर्यावरण में जीवाणु भोजियों में एआरजी की उपस्थिति का पता लगाया गया जिनमें bla-TEM, OXA-2, TET-2 और TET-W शामिल हैं। केन्द्र ने अपने भंडार में प्रेरित प्लूरिपोटेंट स्टेम सेल कोशिकाओं का संरक्षण भी आरम्भ कर दिया है।

केन्द्र ने जाँच और निदान के माध्यम से परामर्श और फसलों एवं पशुओं की बिक्री द्वारा 62.75 लाख रूपयों का राजस्व अर्जित किया। केन्द्र ने अश्व स्वास्थ्य शिविरों, किसान गोष्ठियों का आयोजन किया एवं अपनी गतिविधियों को प्रदर्शित करने हेतु विभिन्न प्रर्दशनियों में भाग लिया। संस्थान ने मेरा गाँव–मेरा गौरव और स्वच्छ भारत अभियान सहित भारत सरकार द्वारा स्थापित प्रमुख कार्यक्रम में भाग लिया। केन्द्र में स्थापना दिवस विश्व पशुचिकित्सा दिवस, राष्ट्रीय विज्ञान दिवस इत्यादि का आयोजन किया गया। केन्द्र के वैज्ञानिकों ने अनुक्रमित शोध पत्रिकाओं में 73 शोध लेख प्रकाशित किए जिनमें उच्च प्रभावी गुण के अंर्तराष्ट्रीय शोध पत्र (50) शामिल हैं। इसके अलावा वैज्ञानिकों ने किताबों में अध्याय, सम्मेलनों में सार और जीन अनुक्रम भी प्रकाशित किए।

Introduction

Our association with the odd toed ungulate mammal (horse) traverses us 6000 years down the history, when mechanized world was not even a distant dream. Alliance of human civilization with this companion animal made us learn the concepts of speed and power, which are backbone of mechanized world and it is on the back of this animal that we spread culture, tradition, languages and religion. In the present era, automation might have decreased the utility of animal power; still, we find this creature to have some unique anatomical and physiological features of great relevance, especially in the hilly and difficult terrains, where other means of transport are inaccessible. In order to improve the health, performance and production potential of equines in India, the Indian Council of Agricultural Research established National Research Centre on Equines (NRCE) on November 26, 1985 at Hisar (Haryana). The main campus of NRCE is located at Hisar (Haryana) which has state-of-the-art laboratories and facilities for undertaking research in areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry and biotechnology. Equine Production Campus (EPC), a sub-campus of NRCE was established in 1989 at Bikaner in Rajasthan to undertake research on equine production, genetics and breeding, reproduction, physiology and nutrition. The research activities are supported by centralized services such as animal and agriculture farms, experimental animal facility, BSL-III facility, ARIS cell, ATIC, library and Info-equine museum. The Centre has well maintained herd of Marwari, Zanskari and Manipuri horses and indigenous and exotic donkeys at Equine Production Campus, Bikaner. The Centre has strived hard since inception in a targeted manner to uplift the socio-economic status of the farmers and other stakeholders and focussed its efforts on infectious diseases confronting equines, surveillance and monitoring of equine diseases, development of diagnostics and vaccines for improving equine health and production. The Centre's high quality research has led to its recognition at national and international levels. The vision of the Centre is the enhanced utilization of equines for agricultural and transport purposes through equine development programmes in order to elevate socio-economic status of under privileged. The National Centre for Veterinary Type Culture Collection (NCVTCC) was established in the year 2005 at NRCE, Hisar, for collection and preservation of microbes of animal origin and veterinary importance. Presently, it is working through 19 network units spread throughout the country.

Mandate of NRCE

- Basic and strategic research on equine health and production
- To provide advisory and consultancy services and capacity development

Objectives

- Generation of demand-driven technologies for equine health and production management.
- Capacity building for competitive equine power utilization in agricultural operations to serve the under privileged under changing environment & socio-economic scenario.

Major issues and thrust areas

- Achieving freedom from dreaded equine diseases through development of modern diagnostics and vaccines.
- Transfer of technology for superior mule and true-to-breed indigenous horse production in their home tracts.
- Artificial insemination and embryo transfer

technology with an aim to establish embryo bank of Marwari/Kathiawari horses to enhance export.

- Enhancing performance of working equids especially in arid, semi-arid and mountainous regions.
- Income generation through market intelligence activities.

MAJOR ACHIEVEMENTS

Diagnostics for equine diseases

The Centre has been recognized as National Referral Centre for diagnosis of important equine infectious diseases by Ministry of Agriculture, Government of India, Department of Animal Husbandry, Dairying & Fisheries. The Centre has developed and refined diagnostics against various equine diseases including immunodiagnostics and molecular diagnostics such as:

Equine herpes virus 1 (EHV1): A highly sensitive and specific neutralizing monoclonal antibody-based diagnostic kit namely **Equiherpes B-ELISA** was developed by the Centre for diagnosis of EHV-1 antibodies. Presently the kit is under the process of commercialization.

Equine herpes virus 4 (EHV4): A type-specific ELISA using EHV-1/4 recombinant glycoprotein G has been developed for differentiation of EHV-1 and EHV-4 infections. A multiplex PCR targeting glycoprotein C and G genes has also been developed for differentiation of EHV-1 and EHV-4 and is routinely used in the laboratory.

Equine rotavirus: A sandwich enzyme-linked immunosorbent assay (s-ELISA) was developed employing a monoclonal antibody (mAb) raised against VP6 of rotavirus, for detection of equine rotavirus (ERV) from faecal samples. This assay has been validated by two external laboratories using bovine, sheep and equine rotavirus samples and detects rotavirus infection among different animals. An RT-PCR using VP6 gene primers was also developed, which compared well with the s-ELISA.

Equine influenza virus (EIV): EIV is routinely diagnosed by haemagglutination inhibition (HI) assay. Recently, RT-PCR and real-time RT-PCR based assay targeting M gene were developed for typing and diagnosis of EIV. Additionally, development of monoclonal antibody based sandwich ELISA for antigenic detection is under progress.

***Theileria equi*:** For serodiagnosis of *T. equi*, a recombinant antigen based-ELISA has been developed using a truncated gene segment of a merozoite surface protein, EMA-2. The DSp and DSn of this assay in comparison to OIE-approved CI-ELISA kit were 0.97 and 0.96, respectively.

Trypanosomosis: An indirect ELISA has been standardized using whole cell lysate antigen of *Trypanosoma evansi*. RoTat 1.2 gene-specific PCR has also been standardized for sensitive detection of trypanosomal nucleic acid in the blood.

Japanese encephalitis virus (JEV): Serum neutralization test (SNT) and haemagglutination inhibition (HI) has been standardized for diagnosis of JE. Monoclonal antibodies against JEV have also been raised and are under trial for development of mAb-based capture ELISA.

Equine infectious anemia: Coggins test for EIA is routinely being used at the Centre. A recombinant protein from a synthetic gene of 26 kDa expressed in *E. coli* was evaluated for use in AGID/indirect ELISA in a pilot study for sero-diagnosis of EIA. The DSn and DSp for the assay were found to be 100% in comparison to Coggins test.

Equine viral arteritis: A standardized virus neutralization test is routinely used for serodiagnosis of EVA.

Small Animal models for understanding pathology and disease mechanisms

Equine influenza: ICAR-NRCE has developed a

novel BALB/c mouse model for studying pathology and pathogenesis of equine influenza virus. The model will help in understanding disease mechanisms and host-pathogen interaction, while simultaneously working for screening of vaccine candidates for their protective efficacy and immune response.

Equine herpes Virus 1: ICAR-NRCE has standardized the BALB/c mouse model for EHV1 for respiratory infection as well as for abortion studies. The abortion model has been utilized in Pathology Laboratory of NRCE for immuno-protective efficacy of inactivated EHV-1 vaccine. Also the respiratory model has been used for immune-prophylactic studies of recombinant proteins of EHV-1.

Vaccines and Immuno-biologicals developed by NRCE

EHV1 vaccine: An equine herpes virus 1 (EHV1) killed vaccine namely "EquiherpAbort" incorporating indigenous strain (Hisar-90-7) of EHV1 has been developed by the Centre. This killed vaccine has already undergone field trials in mares. The vaccine with a three dose schedule generates protective immune response in pregnant mares, which is comparable to that of commercially imported Pneumabort 'K' vaccine.

Update equine influenza vaccine: During 2008-09, India experienced another outbreak of equine influenza caused by antigenically and genetically divergent EIV strain and was significantly different from the previous (1987) isolate. Thus, the vaccine has been updated in 2010, incorporating the new strain {A/eq/Katra-Jammu.06/08 (H3N8)} responsible for EI outbreaks during 2008-09. The updated vaccine is safe and efficacious as evident by the protective immune response generated by the vaccine in field trials in equines.

***Salmonella* Abortus equi**: Improved bacterin and outer membrane protein-based vaccines have been developed for *Salmonella* Abortus equi.

Monoclonal antibodies: Monoclonal antibodies have been developed for diagnosis and characterization of equine herpes virus 1, equine rotavirus, equine influenza, Japanese encephalitis & *Trypanpsoma evansi*.

Kits for disease diagnosis: HERP kit & Equiherpes B-ELISA kit (for EHV1 diagnosis), recombinant protein based ELISA for the diagnosis of *Theileria equi* & COFEB kit for diagnosis of *Theileria equi* have been developed by the Centre.

Surveillance and monitoring of equine diseases in India

ICAR-NRCE is involved in nation-wide monitoring and sero-surveillance of important equine infectious diseases with a view to manage, control and eradicate diseases. Important achievements of the Centre in disease surveillance are:

- Information generated by institute about the status of African horse sickness (AHS) in the country helped in declaring India free of AHS in 2006 by Office International des Epizooties (OIE).
- Outbreaks of glanders in equines have been detected since 2006-07 and control measures are being adopted for preventing its further spread. After a brief pause for two years, the disease again emerged in December 2010, in Chandpur area of Bijnor (UP). From 2012 onwards, various states such as Chattisgarh, Himachal Pradesh, Uttarakhand, Andhra Pradesh, Haryana, Maharashtra, Punjab, J&K and Gujarat have reported incidence of glanders. A few of these states have been able to control the disease with the help of NRCE. Presumably the disease spreads to other states from U.P.
- NRCE diagnosed equine influenza (EI) in India in 2008 from Jammu region (July 2008) that subsequently affected equines in 13 different states. The biosecurity measures were implemented in collaboration with various state animal husbandry departments. No new cases

of EI have been reported from India since May 2009, however, low antibody titres in serum from some parts of the country with no apparent signs are a matter of worry and thus surveillance and monitoring for the disease continues.

- NRCE has continuously been screening equines for equine infectious anaemia from 1998. One mule was found to be seropositive during 2009-10 followed by a horse detected positive in 2011-12 in Haryana state.

Molecular characterization of equine pathogens

Equine influenza virus (EIV): HA genes of EIV isolates from 2008 outbreak (A/eq/Jammu-Katra/08, A/eq/Mysore/08 and A/eq/Ahmedabad/09) were cloned and sequenced, which were identical to the Clade 2 of American lineage of H3N8 subtype. Also, the genetic analysis and selection pressure of matrix (M) gene of the Indian isolates from 2008-09 outbreaks were studied and it was found that M1 and M2 proteins shared 98.41% and 99.54% homology with other Clade 2 viruses of Asian origin for M1 and M2 amino acid sequences, respectively. Phylogenetic analysis revealed clustering of Indian and Chinese isolates in a separate cluster designated as "Asian clade" for M gene.

Equine rotavirus (ERV): Sequencing of VP7 gene of ERV isolates indicated circulation of G10, G3 and G6 serotypes in India. Sequencing of outer surface proteins (VP4 and VP7) of equine rotaviruses for their genotyping and molecular epidemiology was done.

Japanese encephalitis virus (JEV): Whole genome sequencing has been done. Sequence analysis of E-gene of JEV isolated from an equine indicates genotype 3 was responsible for causing the disease in equines and that the equine JEV isolate clustered with Vellore group of JE isolates responsible for JEV in humans in India.

***In vitro* culture of *Trypanosoma evansi* and *Theileria equi*:** The Centre succeeded in *in-vitro* cultivation of bloodstream forms of *T. evansi* in artificial media by using specially formulated cell culture medium supplemented with 20% adult horse serum, however, *Theileria equi* has been successfully cultivated in microaerophilous stationary phase (MASP) culture from infected blood.

Biological resource Bank

NRCE has a strong biological resource base having numerous pathogens, recombinant clones, reference sera, equine sera, monoclonal antibody secreting hybridomas, etc.

- Pathogenic isolates (viruses, bacteria and parasites) of equine origin available with NRCE include EHV1 (14 isolates), EHV4 (14), equine rotavirus (29), equine influenza (11), Japanese encephalitis virus (2), West Nile virus (1), *Rhodococcus equi, Streptococcus equi, S. zooepidemicus, S. equisimilis, Burkholderia mallei*, *Salmonella* Abortus equi, *Enterobacter aerogenes*, *Escherichia coli, Staphylococcus aureus* and *Trypanosoma evansi*.
- ICAR-NRCE has a number of hybridomas secreting monoclonal antibodies against equine herpes virus 1, equine influenza, equine rotavirus, Japanese encephalitis virus and West Nile virus.
- ICAR-NRCE has a repository of more than 15,000 equine serum samples collected from different geographical locations in its Equine Serum Bank.
- ICAR-NRCE has a collection of recombinant plasmid clones with recombinant genes of pathogens including equine influenza virus, equine rotavirus, EHV1, EHV4, EI, JEV, EIAV, *R. equi*, *Burkholderia mallei*, *Trypanosoma evansi* and *Theileria equi.*

Indigenous breed characterization

Phenotypic characterization of Indigenous horse and pony breeds

Populations of equines in the country has drastically decreased. Six breeds namely, Marwari, Kathiawari, Spiti, Zanskari, Bhutia and Manipuri were characterized phenotypically on the basis of their biometric indices and coat colour and significant differences were observed. On the basis of their heights at wither, Kathiawari and Marwari breeds were grouped under "horse" while Zanskari, Manipuri, Bhutia and Spiti fell under "pony" breeds.

Genotypic characterization of Indian equine breeds

Genetic diversity analysis, population structure and relationship among six Indian horse (Kathiawari, Marwari) and pony breeds (Manipuri, Spiti, Zanskari and Bhutia), along with English Thoroughbred horses as an outgroup was carried out which indicated high genetic diversity in all India breeds except Spiti ponies. Individual assignment indicated admixture in all the breeds except Thoroughbred horses.

The neighbor-joining dendrogram using the allele sharing distance clearly defined clusters for most of the breeds; Indian horse and pony breeds clustered separately while Thoroughbred formed a separate out-group. The Bayesian analysis revealed three distinctive clusters of Indian horse and pony breeds with Kathiawari as the most prominent cluster in horse breed, second of Zanskari, Spiti and Manipuri ponies and third one having Bhutia and a sub-population of Marwari horses.

Establishment of nucleus herd

- Exotic Donkeys: Jennies and jacks of European breed (Poitou), imported from France through ODA, UK in 1990, are being maintained at EPC, Bikaner for the improvement of indigenous donkeys and production of superior mules.
- Marwari Horses: In an effort to conserve the true to breed equids, the Centre has also established a nucleus herd of Marwari horse at EPC, Bikaner.
- Indigenous donkey: The Centre has initiated the establishment of nucleus heard of small grey and large white donkeys found in India for conservation and improvement of donkeys.
- Equine sanctuary at EPC, Bikaner: ICAR-NRCE has initiated an *in-vivo* conservation programme in the form of developing an equine sanctuary at EPC, Bikaner. Under this 12 Zanskari ponies (eight mares & four stallions) were brought from Zanskar valley, Kargil, Ladakh, Jammu & Kashmir in November, 2009. In 2014, a total of 11 Manipuri ponies (seven mares & four stallions) were brought from Imphal, Manipur.

Improvement in production potential of equines

Semen cryopreservation and artificial insemination (AI): In order to conserve the germplasm of indigenous equine breeds, the technique for cryopreservation of semen of Marwari stallions and donkeys have been standardized. The technique of artificial insemination using frozen semen for production of superior quality Marwari horses, superior mules and donkeys has been perfected. The pure germplasm of endangered indigenous breeds of horses is being conserved using this technology.

Pregnancy diagnosis: An eCG based sandwich ELISA has been developed for detection of pregnancy between days 30 to 150 of gestation in mares. The kit is cost effective, horse specific and animal friendly. Pregnancy diagnosis between days 14 and 18 post-insemination has been achieved using ultrasonography in donkey and horse mares.

Donkey fibre has been used to produce carpets by mixing with sheep fibres in the ratio of 40:60 in association of CSWRI, Avikanagar.

Utilization of Animal Energy with enhanced System Efficiency

- Single animal drawn matching plough, seed drill (two furrow) and harness were designed and developed for donkeys and mules for performing various agricultural operations. Animal energy potential was utilized successfully in agricultural operations namely ploughing and sowing for different work hours without any adverse effect on the animals.
- Similarly, mules also used in different ploughing experiments indicated that these can also be used efficiently in agricultural operation as all resumed to normal physiological conditions by the next morning.

Sustainable utilization of Mule power for chaffing operation:

The mules were successfully used for chaff cutting operation to reduce women drudgery. Average output capacity of chopped bajra straw in rotary mode chaff cutter was 660 kg/ hour. Deployment of mules for operating a chaff cutter in rotary mode of operation is a viable option for sustainable utilization of equine power during idle hours.

Utilization of equine dung for preparation of vermicompost:

To overcome the problem of dung disposal, vermicompost is being prepared using equine dung in readymade vermibeds successfully and it is being applied in agricultural fields, lawns and plants.

Patents

- A highly sensitive kit for detection of antibodies against *Theileria equi* in serum of equids. Application No. 2763/DEL/2012 dated 06.09.2012
- Nano-drug delivery for quinapyramine sulphate. Application, No. 2560/DEL/2011, dated 06.09.2011. (NRCE, Hisar and GJUS &T, Hisar)
- Polynucleotide sequence, processes, composition and methods thereof- Application No. 1575/CHE/2010 and PCT/IB 2011/052475 (IIsc Bangalore and NRCE, Hisar)
- A recombinant haemagglutinin domain-containing protein for the detection and diagnosis of glanders and method of preparation thereof. Application No.1328/ DEL/2010 dated 08.06.2010. (DRDE Gwalior and NRCE, Hisar)

Services

NRCE provides following services to the farmers and equine breeders:

- The Centre provides disease diagnostic services for various infectious and non-infectious equine diseases to equine owners, breeders, state animal husbandry departments, police and army horses.
- Artificial insemination to augment the production of superior quality Marwari horses, mules and donkeys.
- Quality jacks and jennies are supplied to various states, breeding societies and farmers, for production of superior quality mules and donkeys.
- NRCE is providing health certification for movement of equines within and outside the country. This facility has helped in promotion of export of horses.
- Extension activities: The scientific and technical staff provides clinical and diagnostic (including pregnancy diagnosis) services and consultancy to the farmers on demand in the areas of equine health and production. Farmers are imparted trainings, supplied education materials for equine management, production and health and received feed back by organizing health camp, awareness and farmers meets on regular basis in different areas of the country.

National Centre for Veterinary Type Culture Collection

National Centre for Veterinary Type Culture Collection was established at NRCE by ICAR in 2005 as a national repository of animal microbes including veterinary dairy and rumen microbes with the following mandes.

Mandate

a. National repository of veterinary, diary and rumen microorganisms and their identification, characterization and documentation.
b. Distribution of microbes for teaching, research and development of new technologies

Aims & Objectives

a. Exploration and collection of microorganisms of animal origin/significance/relevance;
b. Central storage of animal microbes from existing culture collection centres, institutions and universities;
c. Characterization, documentation and digitization of microbial database of cultures of animal microbes;
d. Development of a National Microbial Gene Bank for conserving the biodiversity of animal microbes;
e. Conservation (both short-term and long-term) and utilization of microorganisms.

This microbial resource centre focuses on the acquisition, authentication, production, preservation, development and distribution of standard reference microorganisms, cell lines and other microbial resources for research in veterinary and life sciences.

Achievements during 2015-16

- Methodologies were developed to successfully purify a positive stranded RNA virus (FMDV) from the virus mixture containing a negative stranded RNA virus (PPRV).
- Carried out the genetic characterization of infectious bursal disease viruses (IBDV) from Haryana state.
- Isolation and identification of sheep poxvirus from outbreak in Jammu & Kashmir
- Pseudocowpox virus infection was confirmed in cattle from Udaipur, Rajasthan.
- A severe outbreak of *Parapoxvirus* infection in camel was confirmed, in Rajasthan.
- Rare strains of bacteria were accessioned including: *Campylobacter* spp., *Bacillus megaterium, Enterococcus casseliflavus, E. cecorum, Barrientosiimonas humi, Corynebacterium amycolatum, Enterococcus devriesei, E. hirae, E. faecium, Nocariopsis alba, Ignatzschineria larvae and Escherichia hermanii.*
- *Actinobacillus equuli* was isolated from infected foals: first instance of confirmatory diagnosis of *A. equuli* infection in foals in India.
- *Lactococus garviae* – an etiological agent of haemorhhagic septicaemia was isolated from poultry intestine.
- A total of 44 bacteriophage isolates against a variety of pathogenic bacteria were added to NCVTCC repository.
- A novel thermotolerant bacteriophage was isolated from Ganga river water against *Pseudomonas* spp.
- A novel siphoviridae phage was isolated against *Citrobacter sedlakii.*
- Antibiotic resistance genes were detected in bacteriophage DNA which indicated their role in ARG transmission.

So far, a total of 2939 cultures/clones have been deposited in the NCVTCC after authentication and conventional and molecular characterization including GC-FAME and sequence analysis of 16S rRNA & other genes. These microbes are being

contributed by 19 network units including veterinary (7), rumen (8), and dairy (4) network units, and other ICAR institutes and State Agricultural and Veterinary Universities. These cultures/clones include veterinary pathogens including viruses, bacteria, bacteriophages, clones, rumen microbes comprising anaerobic bacteria and fungi, and dairy microbes. Currently, the repository is represented by 170 virus isolates, 1037 bacteria, 76 bacteriophages, 511 clones, 280 genomic DNA etc. The Centre is also maintaining 17 cell lines and 3 primary cultures.

The flexible Gateway ORF library of equine influenza virus was generated and utility of such clones shown in production of recombinant proteins and protein-protein interaction studies employing yeast-two-hybrid system.

Staff position of NRCE and NCVTCC (as on 31.03.2015)

Name of the post	NRCE			VTCC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	1	1	0	-	-	-
Scientific	26	20	6	10	8 (Deputation :1)	2
Technical	25	24	1	1	-	1
Administrative	13	11	2	-	-	-
Supporting	22	19	3	-	-	-
Total	87	75	12	11	8	3

Major Landmarks

1985	NRCE established at Hisar with Prof. P. K. Uppal joining as Founder Director
1987	Outbreak of Equine Influenza in Northern India
1989	Sub Campus of NRCE established at Bikaner for research on production in equines
1989	Occurrence of Equine Infectious Anaemia in India
1990	Exotic donkey germplasm with Poitu blood introduced from France
1991	Artificial insemination (AI) initiated in equines using fresh extended liquid semen
1991	Early pregnancy diagnosis (15 days post insemination) using ultrasonography
1995	Ciq-ELISA developed for detection of circulating immune complexes in EIA-infected horses
1995	Development of field-oriented immune-stick ELISA kit for detection of EHV-1 latent infection in Throughbred horses
1995	Cryopreservation of Jack semen and technology of AI perfected using frozen semen with 40% conception rate
1996	Establishment of a nucleus herd of Marwari horses at Bikaner campus
1996	Crystal structure of mare milk lactoferrin deduced by crystallography
1996	New carpet fabric developed by blending of donkey and sheep hair (Assheep)
1997	Equine Influenza vaccine using indigenous isolate (A/Equi-2/Ludhiana/87) released
2001	Patent for complement fixation test based diagnostic (COFEB)
2003	An Indian patent granted to a diagnostic kit for forecasting EHV
2005	Mab-based sELISA for detection of animal rotaviruses
2005	Establishment of Veterinary Type Culture Collection, at NRCE, Hisar
2006	Collection and cryopreservation of stallion semen at farmer's door using mobile laboratory
2006	World Organization for Animal Health declared India free of African horse sickness
2006	Outbreaks of Glanders in equines
2008	Re-emergence of Equine Influenza after 1987
2008	Equine Herpes Virus-1 diagnosis kit released
2008	ELISA based pregnancy diagnosis kit (Pregmare kit) for pregnancy diagnosis in mares released
2009	Development of Equine Herpes Virus 1 vaccine
2009	A nucleus herd of Zanskari ponies established at Bikaner
2009	First loboratory confirmed Camelpox zoonosis in the world
2009	Japanese Encephalitis Virus isolated from equines in India
2009	Re-emergence of Glanders in Chhattisgarh
2009	Updation of Equine Influenza vaccine
2009	First isolation of *Bordetella bronchiseptica* from horse, *Staphlococcus hyicus* from pig, *Corynebacterium pseudotuberculosis* and *Corynebacterium bovis* from horse & Methicillin-resistant Coagulase Negative *Staphylococcus sciuri* from goats
2010	Equine sanctuary for conservation of indigenous breeds of horses and indigenous donkeys initiated
2010	A new clade designated as 'Asian Clade' of Equine Influenza Virus reported
2010	Award of OIE twining project on Equine Poroplasmosis between NRCPD, Japan and NRCE, India
2010	EIA-positive mule detected in Haldwani: Re-emergance of EIA after 1998
2010	Phenotypic characterization of all six indigenous equine breeds
2010	Re-emergence of glanders in Himachal Pradesh and Uttar Pradesh
2010	Standardization of AI using semen of Poitu donkeys & Marwari horses
2010	Zanskari stallion semen cryopreserved
2010	Started toll-free helpline no. 1800-180-1233 for advisory services to equine owners at NRCE Hisar

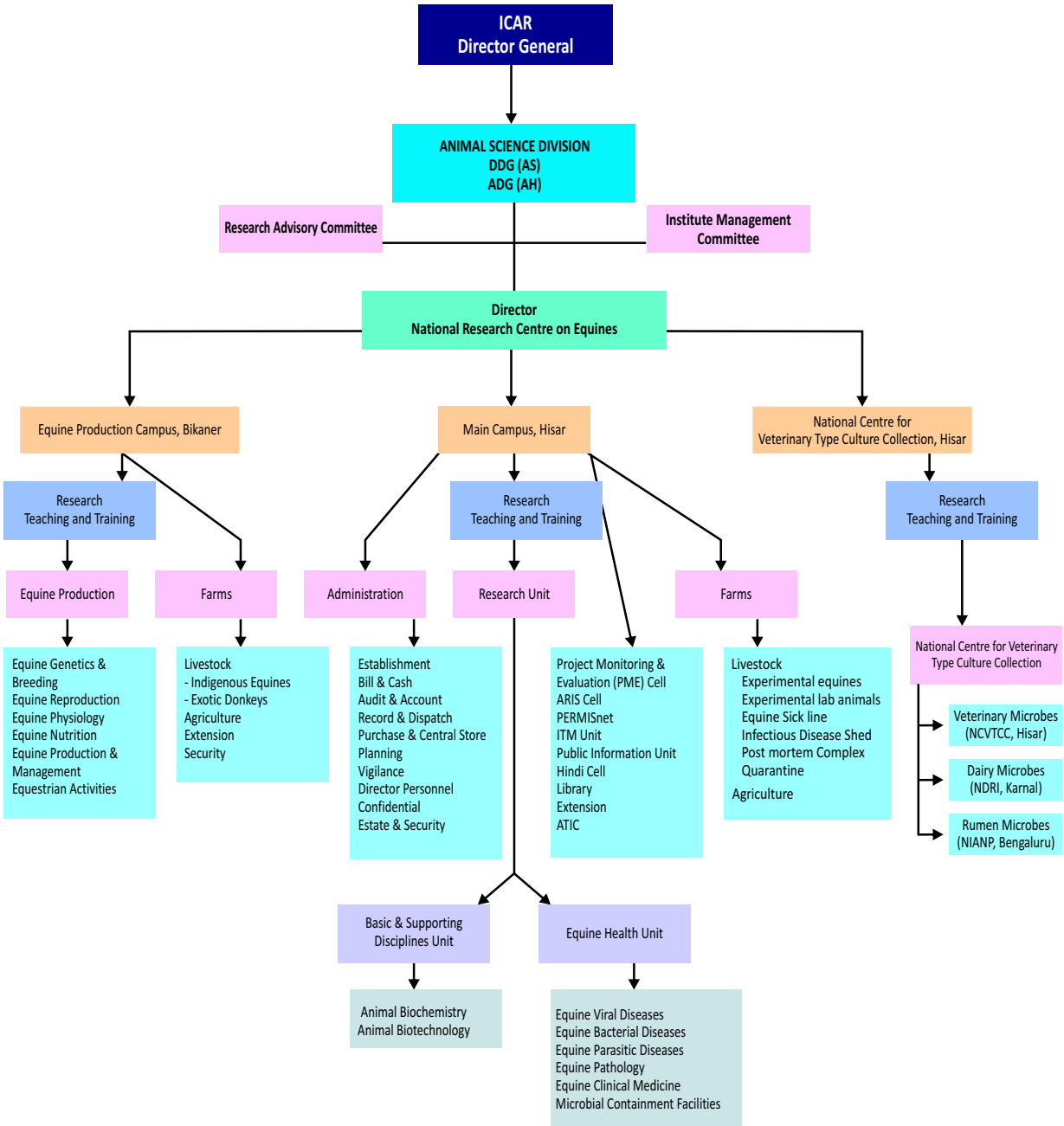
2011	First laboratory confirmed report on BPXV causing disease in Buffalo, human and cow in same time and space
2011	Whole genome sequencing of Indian strain of Japanese Encephalitis virus
2011	Whole genome sequencing of *Pasteurella multocida* B : 2 strain
2011	First isolation of *Trueperella pyogenes* from buffalo, *Enterococcus asini* from horse & *Exiguobacterium* spp. from pig and *Brevibacterium* spp. and *Brevibacillus* spp.from Equine
2011	Indigenous donkeys (Small grey & Large white) inducted in Equine Sanctuary at EPC, NRCE, Hisar
2012	MOU with NRDC for commercialization of technologies generated by NRCE
2012	OIE twinning proposals for Equine Influenza and Glanders with Animal Health Trust, UK and Friedrich Loeffler Institute, Germany initiated
2012	Re-emergence of Equine Infectious Anaemia in Thoroughbred Polo horse in Haryana
2012	Started toll-free helpline no. 1800-180-6225 for advisory services to equine owners at EPC Bikaner
2012	Isolation of *Rhodococcus equi* from double-humped camel of Leh & Ladakh
2012	Development of recombinant protein -based ELISA kits for Glanders and Equine Piroplasmosis
2012	Development of EIA virus p26 synthetic protein -based ELISA for diagnosis of Equine Infectious Anaemia
2012	Whole genome sequencing of *Bordetella bronchiseptica, Pasteurella multocida, Actinobacillus equuli, Salmonella gallinarum and EHV-1*
2012	Single donkey/mule use ploughs and double donkey/mule use ploughs developed
2012	Work-Rest-Cycle established for indigenous donkeys/mules for ploughing/sowing
2012	Technique for Vermi-composting using equine dung developed
2013	Microbial Containment Laboratory (BSL-3 facility), Phase 1 of VTCC Laboratory Complex, ATIC and Info-Equine Museum at NRCE dedicated to nation inaugurated by Dr S. Ayyappan, Secretary DARE and DG, ICAR
2013	Foundation stone of BSL-3 Facility of VTCC laid by Dr S. Ayyappan, Secretary DARE and DG ICAR
2013	First isolation of a *Nocardia otitidiscaviarum* from equine granulomatous pneumonia case and *Moraxella (Branhamella) ovis* from ovine keratoconjunctivitis in sheep
2014	First isolation of *Mannheimia varigena* from pneumonia in buffalo.
2014	Monoclonal raised against *T. evansi* for development of diagnostics.
2014	Recombinant protein based ELISA for diagnosis of *Burkholderia mallei*.
2014	Recombinant heat shock protein (HSP70) based ELISA for diagnosis of *Trypanosoma evansi* infection.
2015	Sardar Patel Outstanding ICAR Institution Award conferred on NRCE
2015	Two technologies viz. Equiherpabort vaccine (EHV1 vaccine) and *Theileria equi* antibody detection kit released by Hon'ble Minister of Agriculture on 18 February 2015 at Annual General Body Meeting of ICAR
2015	Whole genome sequencing of classical swine fever virus completed
2015	Novel thermotolerant bacteriophage isolated from Ganga river
2015	Bacterial artificial chromosome of EHV 1 developed
2015	Developed murine model for Equine Influenza
2016	Two international SAARC trainings were conducted on Equine Influenza and Glanders under OIE Twinning Programs

Summary of Expenditure & Revenue Generation

(₹ in lacs)

Summary of Expenditure		2014-15	2015-16
	Non-plan		
1.	Establishment charges including LSP/PF, wages, OTA	689.29	700.46
2.	Traveling allowances	3.98	3.99
3.	Others charges including equipments & recurring charges	326.77	374.65
4.	Works	0.00	0.00
	Total Non-Plan Expenditure	**1020.04**	**1079.10**
	Plan		
1.	Establishment charges including LSP/PF, wages, OTA	0.00	0.00
2.	Traveling allowances & HRD	17.45	12.00
3.	Others charges including equipments & recurring charges	451.85	614.70
4.	Works	150.36	54.71
	Total Plan Expenditure	**619.66**	**681.41**
	Total Expenditure (Plan & Non Plan)	**1639.70**	**1760.51**
Summary of Revenue Generation		(₹)	(₹)
1.	Sale of farm produce	736213.00	495338.00
2.	Sale of livestock	991160.00	977400.00
3.	Sale of publication and Advertisements	292435.00	2100.00
4.	License fee	173735.00	224020.00
5.	Interest on loans and advances	328585.00	257727.00
6.	Interest on short term deposits	2100470.00	1913175.00
7.	Income from internal resource generation	5242288.00	4802276.00
8.	Receipt from services	0.00	0.00
9.	Other misc. receipts	898309.00	1728818.00
Total Revenue		**10763195.00**	**10400854.00**

Organizational Set-up



Research Achievements

Generation of bacterial artificial chromosomes for EHV 1

Bacterial artificial chromosomes have become important tools for herpesvirus research and are being used for generation of mutants of EHV1. They are contributing significantly to decipher the molecular pathogenesis and reverse vaccinology. The infection due to EHV1 is endemic in India and NRCE has been working for its control through surveillance, developing newer and faster diagnostics and has also developed an inactivated vaccine using indigenous EHV1 isolate. With a larger objective to develop an attenuated vaccine and to undertake further research on functional aspects of EHV1, generation of BAC clone was taken ahead from previous year, wherein we had successfully developed a transfer vector by sequential cloning of flanking regions of gene 71 in pUC19 vector and subsequently sub-cloned it into the mini-F plasmid having the green fluorescent protein (GFP) gene. RK-13 cells were co-transfected using a polycationic reagent (for an event of homologous recombination) using various combinations of transfer mini F plasmids and EHV1 DNA/virus so as to generate the recombinant mini F Plasmid having the EHV1 DNA. Recombinant green fluorescing virus was observed 24 hrs post transfection in RK-13 cells in very low numbers admixed with wild type virus. The green fluorescing virus was amplified by series of limited dilutions in RK-13 cells (Fig. 1 A & B). The purified viral DNA construct in mini F plasmid was electroporated in DH10 *E. coli* as bacterial artificial chromosome. The BAC of EHV1 is being utilized further for the development of vaccine and for mutagenesis studies.

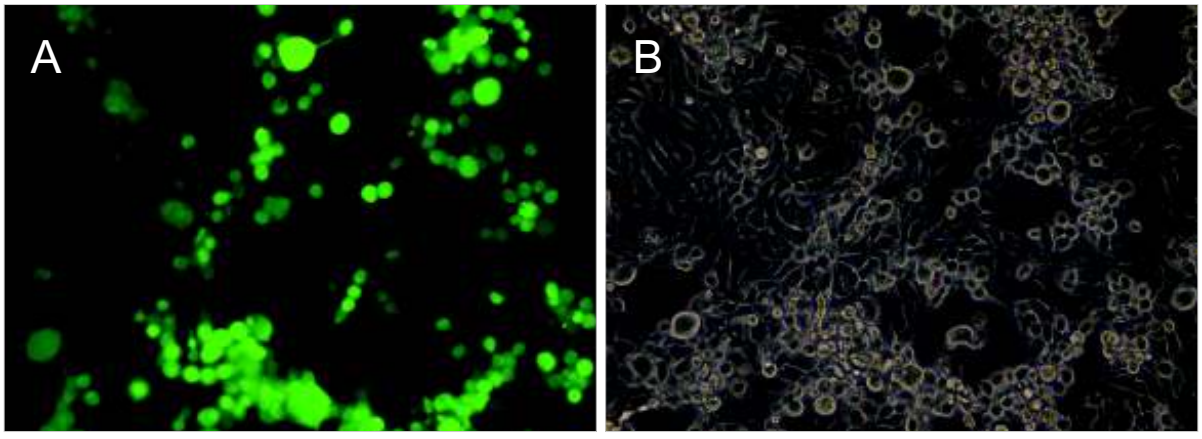


Fig. 1: CPE in RK-13 cells transfected with EHV1 virus having F mini plasmid sequence at 48 hrs, at passage 8. Green fluorescence is due to GFP gene in transfer plasmid indicating successful homologous recombination; (A) Fluorescent view (B) Light view

(Nitin Virmani, B. C. Bera and Pavulraj S.)

Equine Herpesvirus 1 genetic diversity in India

Equid herpesvirus 1 (EHV1) is enzootic in horse populations worldwide and causes a range of clinical signs including, respiratory disease in young horses, late-term abortion in pregnant mares, neonatal foal mortality and equid herpesvirus myeloencephalopathy (EHM) resulting in paresis/paralysis. EHV1 strains isolated from different outbreaks differ in their pathogenic potential due to single nucleotide polymorphisms (SNPs) in a few ORFs, significant being the ORF30 and ORF68. EHV1 strains causing EHM show single nucleotide polymorphism (A to G) at position 2254 in the EHV1 DNA polymerase gene. Further, EHV1 strains have been divided into 6-10 groups based on SNPs in the polymorphic region of ORF68. We analyzed genetic diversity among EHV1 isolates from India by sequence analysis of ORF30 and ORF68.

The ORF30 sequence analysis of 24 EHV1-positive isolates/ samples revealed that two of samples from abortion cases (Delhi/2008 and Tohana/2007) had nucleotide substitution 'G' at position 2254 of ORF30, while the 22 isolates had A2254 genotype (Fig. 2a).

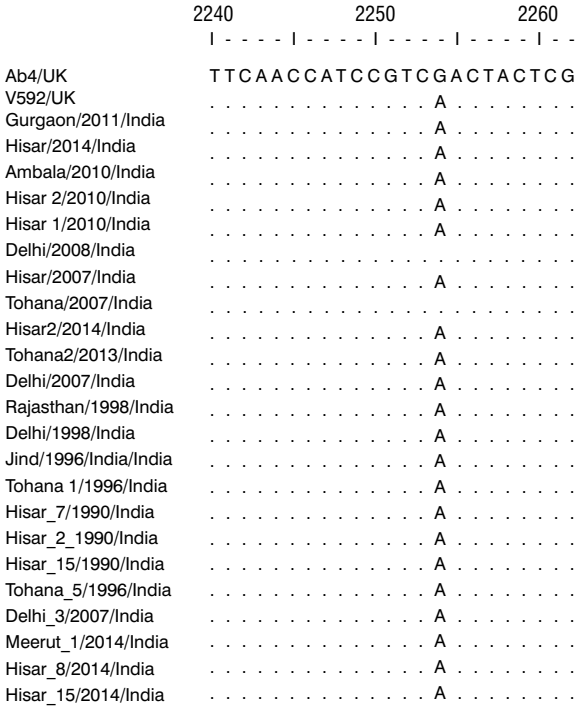


Fig. 2a: Sequence alignment of partial ORF30 sequences of 24 EHV1 isolates

Based on SNP variations in ORF68, four isolates (Delhi/1998, Tohana-2/2013, Hisar-2/2014 and Hisar-15/1990) were classified into group 4, while three EHV1 isolates (Jind/1996, Rajasthan/1998 and Delhi-3/2007) clustered into group 5 (Fig. 2b). Hisar-7/1990 has been used in the vaccine and after repeated passages in RK-13 cells, it has developed point mutations, leading to its drift from group 5 and forming a separate clade. The findings proved that genetically diverse strains of EHV1 are prevalent in

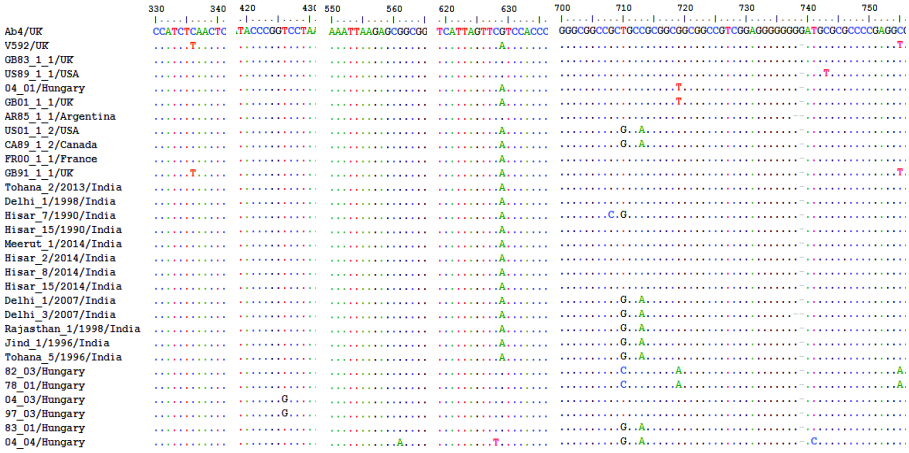


Fig. 2b: Sequence alignment of partial ORF68 sequences of Indian EHV1 isolates

(B. R. Gulati, Riyesh T. and Anagha G.)

Development of *in vitro* and *in vivo* model of persistent/latent EHV1 infection

Like other herpesviruses, EHV1 establishes lifelong persistent infection in recovered horses following infection. Our knowledge of EHV1 persistent infection is based on the various experiments made in living animals (mice, rabbits, horses, etc.). Such animal experimentation has certain limits in terms of costs, homogeny, ethical issues, etc.; so there is need to develop an *in vitro* system for study of EHV1 persistent infection. In the present study, persistent infection was induced in a human lymphoblastoid cell line (LCL-2) and Marmoset EBV- transfected B-cell Line (B-95) at a multiplicity of infection (MOI) of 0.3. Persistently infected cells were maintained and observed for 120 days. Samples of culture were taken at regular time interval to monitor virus production. Presence of virus in both the cells was confirmed by gB-based nested PCR,real-time PCR, immunoflorescence, and infection of RK13 cells. Both the cells showed production of virus as early as 3rd day and remained infected till 120 days. In the infected cells, virus was localized intra-cellularly in the nucleus, as observed by immunoflorescence at different intervals post-infection. The RK13 cells were co-cultured with EHV1 infected B-95 and LCL-2 cells at different intervals post-infecton. The RK13 cell monolayers were infected by co-cultivation, showing characteristic cytopathic effects (CPE), with virus titers of 4.1 and 2.9 TCID50/ml in B-95 and LCL-2, respectively. The persistently infected cells also produced excretory virion particles, as confirmed by infection of RK-13 with cell-free supernatant, yielding virus titers of 5.1 and 4.6 TCID50/ml in B-95 and LCL-2, respectively. Cells persistently infected with EHV1 also showed the expression of latency associated transcripts (LATs) by real-time PCR using primer-probe designed against ORF64 complementary region. The establishment of EHV1-persistently infected LCL-2 and B95 cells provides a useful in vitro model which may help in understanding the mechanism of herpesvirus latency.

(B. R. Gulati, Riyesh T. and Sharma H.)

Surveillance, monitoring and control of existing and emerging diseases of equines

Sero-surveillance was conducted on samples received/ collected from various States/ UTs of India, *viz.* Maharashtra, Rajasthan, Delhi, Haryana, Punjab, Chandigarh, Tamil Nadu, Uttar Pradesh, Karnataka, Andhra Pradesh, Chhatisgarh, Manipur, Pondichery, Uttarakhand, Gujarat, J&K and Assam for various equine diseases.

For EIA, 5306 serum samples from thoroughbred as well as indigenous equines were examined by Coggins test under S&M (1531), DI (253) and contractual service (3522) (Table 1) and none of them were found positive. Out of a total of 2660 serum samples (S&M-1531, DI- 1111, Contractual - 18) were tested for equine influenza from various states, 108 samples showed weak positive titres (reciprocal titre of 16-32) by haemagglutination inhibition assay. Last active case of equine influenza was detected in July, 2009 during 2008-09 epizootic. Surveillance for EHV1 on 1735 samples revealed an overall positivity in 230 serum samples. Antibodies against JEV were noticed in 46 serum samples out of 1546 samples examined by haemaggluitnation inhibition assay. All 72 equine serum samples tested for AHS were negative. The information regarding negative status of AHS in all States of the country was collected through all RDDLs and compiled data in form of a dossier was sent to DAHDF for onward submission to OIE for continued disease free status of country from AHS. Table 1 depicts overall incidents of important equine diseases tested at NRCE.

Glanders outbreaks are consistently occurring in the country and NRCE is providing diagnostic services to detect positive cases. In this process, serum samples (n=13980) were tested for glanders, which included S&M (1531), contractual service (4230) (Table 1) and DI (8219) (Table 2). Sixty serum samples from J&K (18), UP (22), Punjab (4), Gujarat (13), and Uttarakhand (3) were found to be positive under

Table 1: Prevalence of important diseases of equines

Disease	Contractual	S&M	DI	Total	% Positivity
EIA	3522	1531	253	5306	0
Glanders	4230	1531	8219 (60)	13980 (60)	0.43
EI	18 (8*)	1531 (91)	1111 (17)	2660 (116)	4.36
EHV1	26	1531 (215)	178 (15)	1735 (230)	13.26
JE	5	1531 (44)	10 (2)	1546 (46)	2.98
T. evansi	4 (0)	1531 (57)	449 (56)	1984 (113)	5.70
T. equi	45 (14)	1531 (442)	31 (19)	1607 (475)	29.56

Table 2: State-wise status of Glanders

State	Glanders
Himachal Pradesh	1193
Rajasthan	24
Haryana	1
UK	3/288
UP	22/589
J&K	18/4486
AP	--
Gujarat	13/1289
Punjab	4/195
MP	0/94
Maharashtra	0/60
Total	60/8219

disease investigation. *B.mallei* could be isolated from five biosamples (abscess, 4 & pus swab,1) from UP. Outbreaks of Glanders were seen in several states as mentioned above. The 1984 serum samples were tested for *T. evansi,* which included S&M (1531), DI (449) and contractual service (4). 113 serum samples were found positive for *T. evansi*.

Bacteriological analysis done on 196 samples, originating from Rajasthan, Haryana, U.P. and Gujarat yielded 57 isolates including *Streptococcus equi subsp. equi* (7), *Streptococcus equi subsp. zooepidemicus* (7), *E.coli* (29), β *hemolytic Streptococci* (9) and *B. mallei* (5) (Table 5). 98 swab samples from animal quarantine centres tested for CEM were found to be negative. Antibiotic sensitivity testing of clinical samples was also carried out and

Table 3: Isolates recovered and their origin (from 196 biosamples)

Organism	No.	Site	From
S.equi subsp. equi	7	Nasal swab (6), Abscess (1)	Rajasthan (7)
B. mallei	5	Abscess (4), Pus swab (1)	UP (5)
S. zooepidemicus	7	Nasal Swab (4), Lesion Swab (1), Pus swab (2)	UP (6), Rajasthan (1)
E. coli	29	Urinary Bladder (3), Stomach Tissue (4), Large Intestine (4), Small Intestine (4), Heart piece (4), Lymph Node (1), Tracheal Swab (1), Heart blood (1), Lung (1), Spleen (1), Brain (1), Liver (2), Caecum (1), Caecum content (1)	Rajasthan (25) Haryana (2) Gujrat (2)
β hemolytic *Streptococci*	9	Nasal swab (7), Wound swab (2)	Gujarat (6), UP (3)
Total	57	-	-

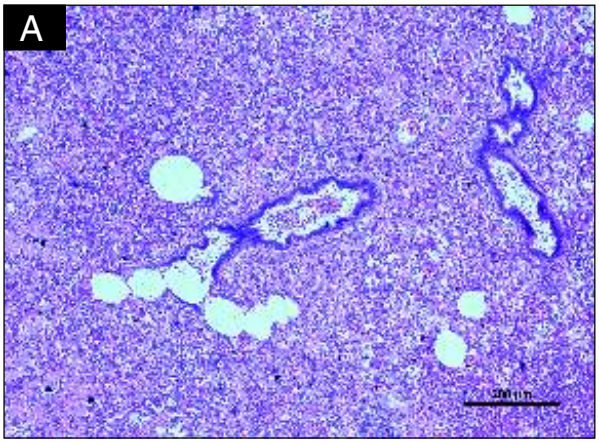


Fig. 3 (A): Lung: Broncho-interstitial pneumonia: Severe consolidation of pulmonary parenchyma with bronchiolar and interstitial infiltrations of neutrophils [10X]

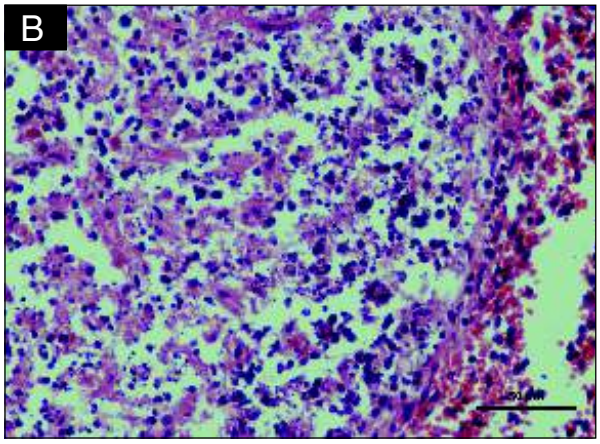


Fig. 3 (B): Spleen: Severe lymphocytonecrosis and depletion of lymphocytes from white pulp area in splenic parenchyma [40X]

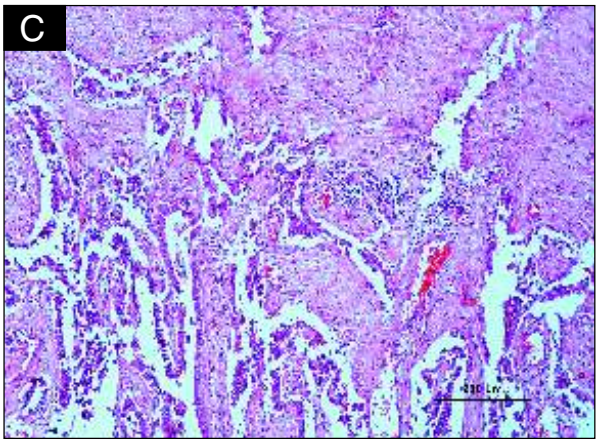


Fig. 3 (C): Metastatic adenocarcinoma : Proliferation of atypical glandular epithelium with neovascularisation, haemorrhage in between fibrous tissues, vascular congestion and moderate focal infiltration of lymphocytes [10X]

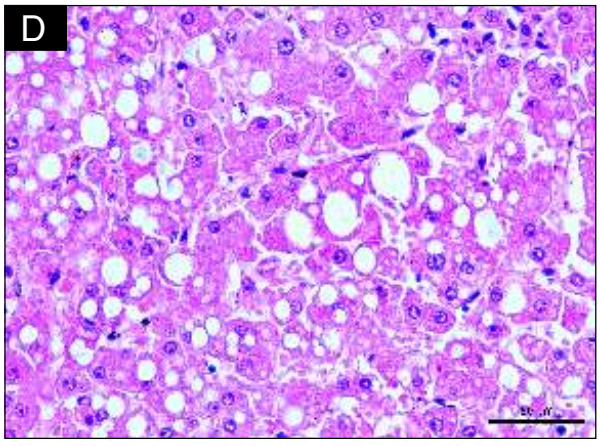


Fig. 3 (D): Liver - Paracentral fatty changes in hepatocytes [40X]

results were conveyed to various concerned quarters. 1531 serum samples tested negative for Brucellosis and *Salmonella* Abortus equi (H antigen) revealed no positive samples.

Important conditions recorded on samples received for histopathology/ morbid material received from the field included metastatic adenocarcinoma (1), suppurative bronchopneumonia and typhilitis (1), enteritis (3), fatty degeneration of liver (1), cardiomyopathy and bronchopneumonia (1), intestinal obstruction leading to acute enteritis (1), rupture of stomach (1), intestinal torsion at caeco-colic junction(1), etc (Fig. 3).

(S. K. Khurana, B. N. Tripathi, S. C. Yadav, B. R. Gulati, Rajender Kumar, N. Virmani, Sanjay Kumar, Sanjay Barua, Rajesh Vaid, Ramesh Dedar, H. Singha, Anju Manuja & Balvinder Manuja)

Hepatic gene expression profiling of rats reveals key enzymes involved in quinapyramine sulfate metabolism

An aminoquinaldine derivative, quinapyramine sulfate (QS) is the commonly used and effective drug for treatment of trypanosomosis in domestic animals. A drug which affects certain metabolic processes in the parasite often has a similar disruptive effect on the host cells also. Drug-metabolizing enzymes (DMEs) are important battery of proteins that are involved in drug metabolism, xenobiotic detoxification and drug-induced toxicity. There is no report on effects of QS on DMEs till date. Enzymatic induction is primarily mediated by increasing the transcription levels of drug metabolisms genes (DMGs), whereas down regulation of these genes is one of the mechanisms for enzymatic inhibition. Thus, assessing gene expression at the mRNA level is an important approach in identifying drug-induced effects on DMGs. In this study, we employed a high-throughput method, the Rat Drug Metabolism RT2 ProfilerTM PCR Array, to profile phase I drug metabolizing genes in liver following administration of QS to rats at the recommended therapeutic dose. Here we report for the first time that, in response to quinapyramine sulfate, hepatic expression profile of metabolizing genes in rat revealed that the metabolism is mainly through oxidation and it has greatest induction ability on flavin containing monooxygenase 1 followed by monoamine oxidase A and cytochrome P450 family enzymes. The possible interactions of differentially expressed genes of phase I metabolism are depicted in Fig. 4.

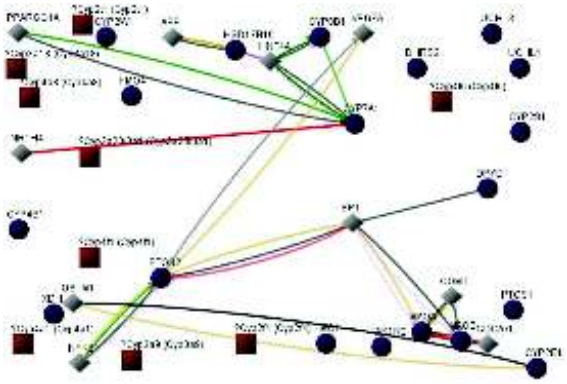


Fig. 4: Graphical representation of possible interactions of differentially expressed genes of phase I metabolism

(Balvinder Manuja, Anju Manuja, Rajender Kumar, S. C. Yadav)

Efficacy of Isometamidium chloride-loaded nanoparticles against *T. evansi*

We have synthesized, characterized and evaluated a nanoformulation of isometamidium hydrochloride. The nanoformulation was synthesized using biocompatible polymers as carrier to enhance the drug efficacy at lower doses, with sustained release of the drug while minimizing undesirable side effects. The toxicity of Isometamidium chloride-loaded nanoparticles on cell lines unveiled a concentration-dependent cytotoxicity and genotoxicity. Compatibility testing of the drug-loaded NPs by comparison with conventional drug revealed decreased hemolytic rate as well as decreased oxidative stress. Formulated drug nanoparticles were evaluated on cultured *T. evansi* parasites in vitro at different concentrations. Although the parasites were killed at 24 hrs but after 72 hrs, sufficient drug was released from nanoformulation and was able to clear the parasites at all the concentrations. Further to optimize the efficiency of the nanoformulation in vivo at different doses, frozen stabilates of the *T. evansi* were expanded in albino mice by injecting 1X104 trypanosomes intraperitoneally (i.p.). The parasitemia was monitored in blood smears of treated and untreated groups daily for a week and thereafter alternate days for a month. The nanoformulation has been found to be effective against *T. evansi* providing prolonged survival/removal of the parasite at reduced doses in in vitro and in vivo studies in rodent model using horse and cattle isolate of T. evansi.

(Anju Manuja, Rajender Kumar, Balvinder Kumar & S. C. Yadav)

Development of diagnostics for emergency preparedness and monitoring of emerging equine viral diseases

Recombinant proteins were expressed in *E. coli* for development of diagnostics for exotic equine viral diseases like Vesicular Stomatitis (VS) and Venezuelan Equine Encephlalitis (VEE). Five antigenic regions from VSV glycoprotein and twelve immunodominant regions from capsid and envelop proteins of VEEV were identified by Kolaskar & Tongaonkar antigenicity prediction (Fig. 5), Parker hydrophilicity prediction and BepiPred linear epitope prediction to design multiepitope constructs. These synthesized constructs were cloned into expression vector. The recombinant proteins were expressed in *E.coli* (Fig. 6). In addition 150 serum samples from different states were tested for Rift Valley Fever by ELISA. All these samples tested negative for serum antibodies.

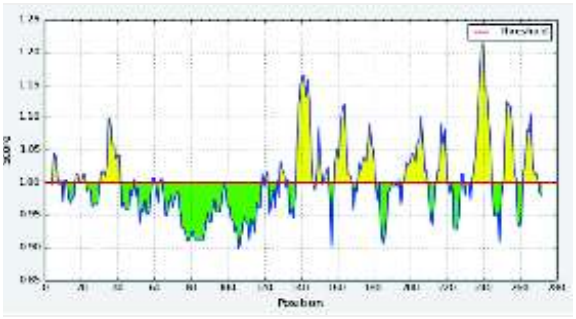


Fig. 5: Kolaskar & Tongaonkar antigenicity prediction for antigenic determinants for Venezuelan equine encephlitis virus Capsid

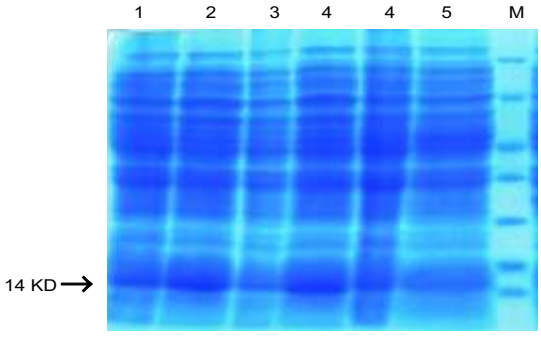


Fig. 6: SDS-PAGE depicting 14 kDa recombinant protein of VEEV. L 1,3,5: Control without induction; L 2,4,6: Induced with 1 mM IPTG, M-Marker.

(Balvinder Kumar, Harisankar Singha, Anju Manuja and Naveen Kumar)

Evaluation of *in vitro* growth inhibitory efficacy of some novel synthetic drug molecules against *Theileria equi* haemoprotozoa

Equine piroplasmosis is a vector-borne disease, caused by two intraerythrocytic protozoan parasites, *Theileria equi* (formerly called as *Babesia equi*) and *Babesia caballi*. These parasites can affect all equids - horse, donkey, mule, ass and zebras and are endemic in tropical and subtropical regions of the world, where suitable vector-tick population exist. Most of the apicomplexa groups of parasites possess unique organelle and metabolic pathways, which have been the drug targets. A total six drug molecules were selected which were specific targets for choline kinase, DOXP reductoisomearse, ATPase, lactate dehydrogenase, nucleic acid and protein synthesis. Imidocarbdipropionate was included as positive drug control, so as to analyse the IC50 concentrations of each drug molecules. Target specific DABCO33LV, FR0098, O-Choline, Berberine, Lumenfentrine and Eugenol were tested for in vitro growth inhibitory growth efficacy against *Theileria equi.* These drug molecules were tested at different concentrations - 10 µM, 25 µM, 50 µM, 100 µM, 200 µM and 400 µM, in triplicate and all the experiments were repeated. The IC50 values of these drug molecules were 590 µM, 124.50 µM, 62.50 µM, 4.63 µM, 28.45 µM and 45 µM, respectively. Cytotoxicity of these above drugs was tested atdifferent concentrations (1µm to 2000µm) on PBMC collected form a horse. O-Choline and berberine were least cytotoxic - 8.7% and 13.6%, respectively on horse PBMC.

Organ toxicity trials in mice model were undertaken with imidocarb, novobiocin and DMB drug molecules. These drugs were injected I/p route @ 1mg/kg bw., 200mg/kg and 900µg/kg. Serum samples were collected at 24 and 48 h interval and biochemically analysed for organ function tests. The analysis on mice group treated with imidocarb indicated slight deviation in organ function biomarkers at 24 h interval and biochemical values return to normal after 48 h interval. Liver function biomarkers (SGOT, SGPT) were elevated in mice (n=6) at 24 h and 48 h post inoculation of novobiocin, indicating toxic effect of the drug, further trial with this drug will be taken at lower dose rates.

(Sanjay Kumar, Rajender Kumar and A. K. Gupta)

Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines

Semen cryopreservation is a well established method of ex-situ conservation and breed propagation at a faster rate. A nucleus herd of indigenous equines including Marwari, Manipuri, Zanskari horses and donkeys besides the exotic Poitou donkeys is being maintained at Equine Production Campus of ICAR-NRCE. In addition to semen cryopreservation and AI in our farm equids, semen cryopreservation of elite stallions in field for the selective breeding of mares at our centre has been initiated to improve the desirable characters in their offspring and to avoid the inbreeding at the same time. Some of the salient achievements have been reported under following sub headings

On farm and field cryopreservation of semen from Marwari stallions for selective breeding

Eleven horses were identified from private equine breeding farms at Jodhpur and Nawalgarh, Rajasthan for semen collection and cryopreservation. Fresh semen characteristics were recorded and processed for cryopreservation (Fig. 7). Average volume of ejaculate, pH, total and progressive sperm motility were 81.90 ± 10.23 ml, 7.28 ± 0.02, 76.66 ± 2.56% and 71.19 ± 2.77%, respectively. The pre-freeze and post-thaw sperm motility was 60.23 ± 2.61 and 38.33 ± 2.05%, respectively. A total of 152 frozen semen doses (0.5 ml x 8 straws) were cryo-preserved under field conditions at Jodhpur and Nawalgarh. At EPC, Bikaner also, 20, 23, 12 frozen semen doses of Marwari, Zanskari and Manipuri horses were cryopreserved, respectively besides 18 doses of indigenous jacks for future use and conservation.

Eleven (64.47%) out of 17 Marwari mares inseminated with frozen semen in single or multiple estrous cycles became pregnant. The conception in Marwari mares was 64.47% (3.1 AI per conception and 1.52 AI per cycle). Similarly, 2 of 4 Manipuri and 4 of 6 Zanskari mares inseminated with frozen semen were pregnant. The conception in Manipuri and Zanskari mares was 50 and 83.33% with 2.5 and 3.8 AI per conception, respectively. The number of AI per cycle was 1.20 and 1.78 per cycle for Manipuri and Zanskari mare, respectively. Ultrasound guided pregnancy diagnosis was performed at day 15 and 35 post-ovulation in all the inseminated mares. No embryonic loss was observed in Marwari and Manipuri mares whereas one Zanskari mare lost the embryo. Biometry of eight Marwari foals was performed to the record growth and to investigate effect of selective breeding. A regular increase in total body weight, height and body length of these foals was observed. Weight increased from 36.40 ± 0.80 kg (day 0) to 175.33 ± 6.38 kg in 270 days (post natal) while height and length increased from 91.8 ± 0.60 and 66.2 ± 0.62 cm to 126 ± 0.88 and 112 ± 0.50 cm, respectively during the same tenures.

Fig. 7: Semen processing in an equine breeding farm, Balsamand, Jodhpur, (Rajasthan)

Services to equine breeders for reproductive issues in field mares

A total of 30 field mares were brought to EPC, Bikaner for seeking services with respect to estrous cycle stage, AI and pregnancy diagnosis. All the mares including 16 for estrous cycle stage, 7 for AI and 7 for pregnancy diagnosis were attended.

(S. K. Ravi, T. R. Talluri, J. Singh, R. A. Legha, Yash Pal and A. K. Gupta)

Endocrine, biochemical and gene expression profiling of reproductive states in Marwari mares

Onset of puberty in the fillies above 6 months of age (n=3) were monitored by the expression of behavioral signs of estrus at the first estrus. Age at puberty in Marwari fillies was 382.67 ± 20.73 days. The body weight at puberty was 193.33 ± 9.33kg. Mean length of estrus was: 11.57 ± 1.2 days and the mean inter-estrus period was 26.0 ± 2.86 days.

Hormones progesterone and estradiol 17 beta; and biochemicals, glucose and triglycerides were estimated in plasma of fillies and mares (cycling & pregnant mares). No significant difference could be observed in the level of progesterone and estradiol 17 beta in the fillies during onset of estrus and at 4 & 8 days post estrus though progesterone was low and estrogen higher at estrus. Progesterone was significantly high ($p<0.05$) in adult mares in diestrus stage than at the beginning and peak estrus. The levels of estradiol 17 beta did not show variation although higher values were found at peak estrus when pre-ovulatory follicle was present. Both Progesterone and estradiol 17 beta were higher at one month pre-partum and at partum than at one month post-partum. Fillies had an erratic hormonal pattern which was indicative of the developing ovarian cyclicity which was yet to attain complete maturity.

Significantly higher glucose and triglyceride levels were observed in the mares at parturition and at one month pre-partum than at one month post partum (Fig. 8). Significantly lower glucose and higher triglycerides were observed at estrus than at 10 days prior and 8 days post estrus in fillies. The higher triglyceride at parturition is indicative of the higher energy requirement in the animal system at this stage as triglycerides are mobilized to provide energy to the animal in stress.

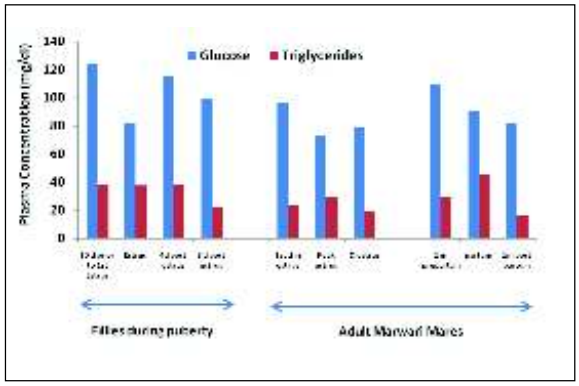


Fig. 8: Plasma glucose and triglycerides in the Marwari fillies and mares at different reproductive states.

(Vijay Kumar, R. K. Dedar and S. K. Ravi)

Composition and periodic changes in equine milk

Comparative analysis of milk composition of different animal species:

To assess the nutritive value of equine (mares and jennies) milk as compared to other milch animals, milk was collected from donkeys, mares, sheep, HF cows, camel and buffaloes. Milk composition of Marwari mares & exotic donkey mares was observed to be almost similar. Further, equine milk contained very low fat and protein as compared to that in sheep, HF cows, camel and buffalo milk but lactose content was at par (Table 4). There was significant variation in milk composition of various species studied.

Periodical change in acidity and pH in equid milk:

Shelf life of milk is a very important aspect as it helps in its storage, processing, packaging and supply. It is the actual time period in which it deteriorates to an unacceptable degree. Higher is the shelf life, better it is. Mare and donkey milk samples collected aseptically were incubated at 37°C for 24 hours and periodical change in acidity and pH in equine milk was studied at two hours interval regularly. Shelf life results indicated that mare and donkey milk was stable at 37°C up to 8h and 10h, respectively.

Table 4: Milk composition of equines and other animal species.

Species / Parameters	Fat %	SNF %	Protein %	Lactose %
Donkeys (12)	0.78±0.121c	7.60±0.06a	2.22±0.025b	4.44±0.034a
Mares (11)	1.09±0.087c	7.74±0.05ac	2.29±0.020b	4.5±0.029a
Sheep (5)	8.83±0.37b	8.85±0.52bc	3.39±0.148a	4.53±0.31a
HF Cows (14)	3.08±0.38a	8.27±0.098b	2.62±0.047b	4.64±0.083a
Camel (16)	4.12±0.28d	7.62±0.12a	2.59±0.0b	4.17±0.05a
Buffaloes (10)	8.72±0.45b	7.85±0.145c	3.15±0.043a	5.42±0.162b

(Yash Pal, S. Kumar, A. K. Mohanty and Anuradha Bhardwaj)

Characterization of donkeys of Rajasthan

Physical and biometric characteristics of adult donkey

In India, most of the donkeys are non-descript. Donkeys from three districts of Rajasthan - Barmer, Bikaner and Churu were selected to record physical, biometry & reproductive indices, feeding, management and health problems. Data was recorded from one week (8), 3 months (45), 6 months (36) & 1 year old foals (36); 1-2 years old (49) & 2-3 years old stock (105); adult female (252) & adult male (286) donkeys. These donkeys were of light brown, dark brown, grey/white and black colour. Light brown colour donkeys were predominant. Belly, inner surfaces of legs, ventral side of neck and inner sides of ears were generally of lighter shade or white in most of the donkeys. The white marking were around muzzle and eyes. Zebra marking were also seen on legs of few donkeys. Shoulder strip and dark outline markings were common in light and dark coloured donkeys. The manes was small as compared to horses. Mane was usually of darker shade than the rest of the body colour.

The mean body lengths of male and female donkeys were 97.52±0.34 and 97.73±0.44 cm, respectively. The heights at withers of adult male and female donkeys were 95.89±0.34 and 95.65±0.36 cm, while average heart girth were 104.87±0.46 and 104.06±0.44 cm, respectively. Average neck length was 34.35±0.19 and 34.53±0.24 cm in adult male and female donkeys while average face length was 46.65±0.31 and 46.47±0.32 cm, respectively. The forehead was slightly convex. The nasal bone was straight to slightly concave. The ears were straight and erect with slight lateral orientation. The average length of ears was 22.6±1.63 in male and 22.3±1.88 cm in female donkeys. The fore leg length and hind leg lengths in adult male donkeys were 65.22±0.34 and 72.28±0.29 cm while in female donkeys these values were 64.02±0.32 and 71.62±0.25 cm, respectively. The mean canon lengths for fore and hind limbs of adult donkeys were 18.80±0.10 and 27.56±0.12 cm, respectively whereas these values for female donkeys were 18.49±0.10 and 27.34±0.12 cm, respectively. The pastern length in male donkeys ranged between 6-9 cm and in female donkeys it ranged between 5.5-9.0 cm. The hoof circumferences of fore and hind limbs of male donkeys were 21.75±0.12 and 20.90±0.13 cm, while the hoof circumferences of fore and hind limbs of female donkeys were 21.7±1.34 and 20.9±1.26 cm, respectively.

Biometric characteristics of young donkey stock

The biometric measurements for the young stock of donkeys at different age groups have been presented in Fig. 9. The donkeys of age one week, 3 months, 6 months, 1 year, 1-2 years and 2-3 years had mean heights at withers as 65.62±0.97, 70.52±0.86, 79.17±1.35, 87.48±0.77, 91.18±1.07 and 93.07±0.62 cm, respectively.

The mean heart girths at these age groups were 59.25±0.98, 72.77±1.22, 83.97±1.56, 94.94±1.30, 98.25±1.39 and 100.11±0.77 cm, respectively. The body length increased with advancement of age from birth to 2-3 years of age. The body length increased from 51.62±0.75 (one week), to 62.55±1.09 (3

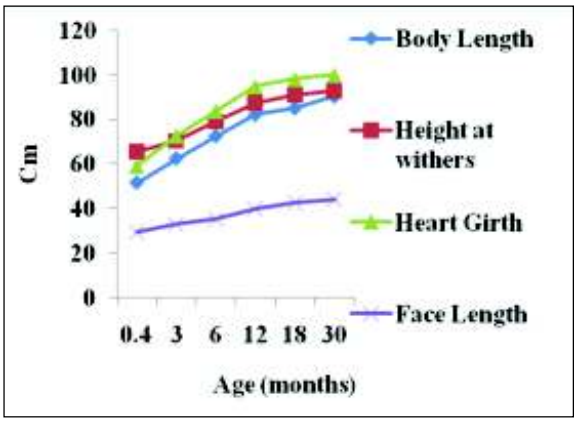


Fig. 9: Body measurements of young donkey stock

months) to 72.65±1.51 (6 months) to 82.31±0.99 (1year) to 85.20±1.01 (1-2 years) and 90.53±0.57 cm (2-3 years).

Reproductive characteristics

Main breeding season in donkeys extends from March to October. The age of puberty of the male and female donkeys is reported as 1.5-2.0 years. The age at first service is 2.0-2.5 years. No scientific breeding or artificial insemination is in practice. Natural mating practice is adopted and donkey owners hardly know about the pregnancy status of their donkey mares. Male and female donkeys are left loose in field when not in use so controlled breeding is not expected. The duration of estrus and estrus cycle are 4-10 & 19-25 days respectively. The age at first conception is 2-3 years. The gestation period is 12-12.5 months, service period (2-3 months) and foaling interval is between 15-18 months. The age at first foaling is between 3.0-4.0 years. The donkey mares are observed breedable up to 15 years.

Management and feeding Practices

In Rajasthan, donkeys are not only reared by Kumhar, Sansi & Muslim community but also by landless as well as small and marginal farmers irrespective of caste because, most of the farmers rear sheep herds to increase income. One to four donkeys are being maintained by the sheep herders to carry the luggage while grazing their sheep. Donkeys are not housed most of the time; they are left loose for grazing after work. They are allowed to graze in the waste lands near villages or the fields. No feed supplements were being provided to donkeys which were being reared by sheep herders. But, the people engaged in dairy business also maintain one male donkey to carry the cow dung for disposal. This donkey is housed and fed along with the other livestock. The donkeys rarely suffer from diseases. The major problem in donkeys is of colic. De-worming and vaccination are not provided to donkeys.

Donkeys are also used for pulling carts in urban and semi-urban areas. These donkeys are housed either in open space under trees or in sheds covered with thatched, tin roofs and raised both on grazing and stall feeding. Besides grazing, they are also supplemented with some amount of dry or green fodder. Some of the donkey keepers also provide some amount of concentrate and gur per day. The amount of concentrate is increased when they are employed for hard work. The dried fodder mostly consisted of crop residues of bajra and jowar straw, moth straw, groundnut straw, doob and other crops grown in the region. The concentrate mainly consists of locally available grains mainly bajra, wheat bran and barley. The water is provided twice/thrice a day. Salt is also being provided to the working donkeys.

(Yash Pal, A. K. Gupta and R. K. Dedar)

Parentage testing in horses

Parentage testing in horses has become a necessity for breeders because the buyers ask for authentic proof of parentage of foal before purchasing the animal. All the major horse breed registries throughout world have adopted parentage testing programs to assure horse pedigree integrity. Traditionally, in most laboratories, blood groups and protein polymorphisms have been used for parentage testing but these markers are age, sex and environment dependent and 40-60% reliable. Currently available microsatellite genotyping can greatly improve the success of parentage tests (up to 99.9%).

This necessitated the need of developing an in-house

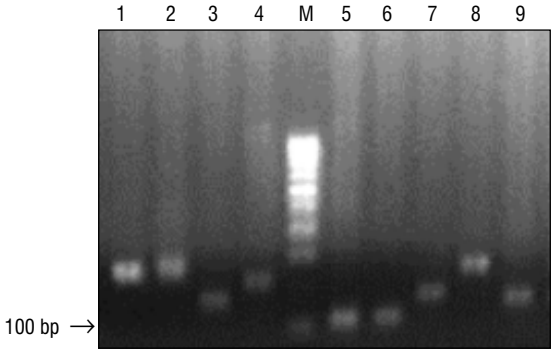


Fig. 10: Amplification of microsatellite markers.

facility for parentage testing in horses. NRCE has standardized the DNA markers based test for parentage authentication in horses which is in the process of validation. A battery of microsatellite markers has been optimized for the parentage testing in horses (Fig. 10). Genotyping of foal, mare and stallion's DNA at these microsatellites depicts the qualification of parents for parentage. If amplification fragment size of foal matches with that of parents, the parentage is confirmed. Such DNA typing test will ease the equine breeders to rely on a government organization for parentage proof of their foals.

(Mamta Chauhan, Anuradha Bhardwaj, Yash Pal, B. N. Tripathi and A. K. Gupta)

Rapid diagnostic test for pregnancy diagnosis in horse mares

Hyperimmune serum was raised against equine chorionic gonadotropin (eCG) as per standard dose schedule in two lab animals for its use in sandwich ELISA based Pregmare kit. Antibody titre ranged from 1:1600 to 1:6400 in ELISA. Work on development of monoclonal antibodies was also initiated by immunization of three BALB/c mice. Fusion was attempted with spleen cell of mice having maximum antibody titre but hybridoma developed were found to be quite low secretory. Titre was not good enough for further sub-cloning. Monoclonal antibody development work is under progress with purified eCG for preparation of LFA based kit for pregnancy diagnosis in horse mares.

(A. K. Gupta, Sanjay Kumar, Yash Pal and Sanjay Ravi)

Genetic characterization of true to breed Marwari horses

Marwari horse is really an owner's pride and a mark of grace, elegance and royalty among six registered indigenous horse breeds. For a long time, the equine stakeholders have been demanding characterization of Marwari horses for true to breed selection and germplasm conservation. Our earlier study with selected and limited number of Marwari horses has revealed their mixed population due to indiscriminate breeding. As a part of the study, 323 horses of Marwari breed were included from different areas of Hanumangarh, Nawalgarh, Jodhpur, Pali, Udaipur, Rajsmand (Rajasthan), Bhatinda (Punjab) and from EPC Bikaner and main campus, NRCE, Hisar. Animal selection was made on the basis of their phenotypic characters and important biometric indices were recorded. DNA has been isolated from the blood samples stored. Amplification conditions of microsatellites markers with 44 primers pairs through polymerase chain reaction have been standardized, out of which 35 microsatellite markers are selected for genotyping.

(Anuradha Bhardwaj, A. K. Gupta, Yash Pal, Mamta Chauhan and Vijay Kumar)

Increased utilization of animal energy with enhanced system efficiency

Study of pack load capacity of indigenous donkeys

Five male adult indigenous donkeys (9-12 years) were used to study their work performance with pack load of 50% and 66% of their live body weight. The donkeys were carried 50% and 66% pack load with a walking speed of 3.81-5 & 4-5 km/h to a mean distance of 17.5 &12 km respectively. Physiological responses (rectal temperature (RT); respiration rate (RR); & pulse rate (PR) when recorded immediately after work increased significantly but after 20 minutes of rest,

these indices declined to acceptable limits under 50% pack load but remained significantly higher ($p<0.05$) even after 20 min with 66% pack load (Fig. 12). Donkeys lost a mean body weight by 5.84 and 5.79% at 50 % and 66% pack loads respectively, post work. Moreover with 66% pack load, anal tone and gait were abnormal and changed, pain in the pastern and knee in the animals initiated after 10 km showing that the load was higher than the animal could comfortably carry on. This load is not recommended for work in donkeys.

Draughtability studies with mules

Three apparently healthy mules (9-11years) weighing between 350-400 kg were used for training to draught in loading car for pulling draft of 600N, 792N and 1000N during summer season. All physiological indices (RR, RT and PR) increased significantly after work. The pulse rate declined to within normal acceptable limit of 64 by 20 minutes of work at 600N draught load. With 792 N draft, even the well trained mule was not able to cover more than 10 km distance comfortably and its gait was also improper. Hemoglobin, packed cell volume, red blood cells and white blood cells increased after work. After 20 minutes of rest, the recovery of pulse rate to below 64 was seen in the animals with 600N draught load but not in case of 792 N draught load.

(R. A. Legha and Vijay Kumar)

Optimization of Interspecies Somatic Cell Nuclear Transfer (SCNT) technique for production of horse (*Equus caballus*) cloned embryos

Animal cloning is the most suited available assisted reproductive technique to increase the number of superior animals in the shortest possible time. The limiting factor for the application of SCNT in horses is the non-availability of oocytes from slaughter house, which precludes the use of SCNT in this species. Therefore, an attempt was made to explore the possibility of interspecies SCNT (iSCNT) using somatic cells/donor genome from horse and easily available recipient oocytes from buffalo for the production of cloned horse embryos. Skin tissue biopsies from 3 horses were taken and transported to ICAR-CIRB laboratory with in 24 h for isolation and culture of somatic cells (Fig. 11). Aliquots of cells at early passages (passage 2-3) were cryopreserved and were stored in liquid nitrogen for future use. At least 20 cryovials were frozen in liquid nitrogen.

Buffalo oocytes were collected from ovaries from a slaughter house. The oocytes were matured in a maturation medium. Then the oocytes were subjected for cumulus/zona removal and manual

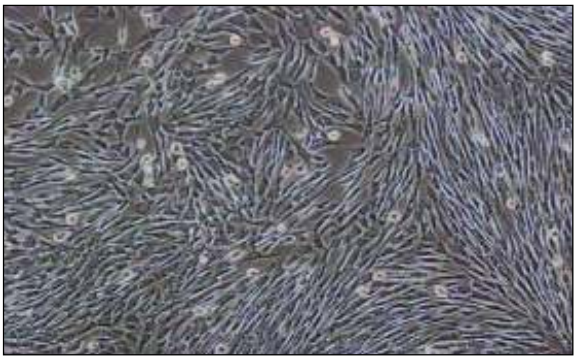


Fig. 11: Confluent equine somatic cells used for SCNT

enucleation followed by subsequent fusion with horse somatic cells and its activation and culture of produced embryos. With the cell line of adult mare and 3 months foal, we have conducted three fusion experiments by using isolated ooplasts from buffalo ovaries. In total, 153 buffalo oocytes were used and 53 interspecies horse embryos were constructed and maintained in the culture for 5 to 6 days. All cloned embryos had shown growth only till the 16 to 32 cell stage and further growth was arrested.

(T. R. Talluri, Naresh Seloker, Dharmendra Kumar, S. K. Ravi, Taruna Anand and P. S. Yadav)

Reproductive parameters of Zanskari stallions and production of foals through artificial insemination (AI)

Various reproductive parameters were recorded in respect of Zanskari mares for four consecutive breeding seasons (2010-2014). The length of the estrus cycle, duration of estrus and size of the follicle at ovulation were observed as 17.58 ± 0.56 (range 15-26) days, 5.76±1.02 (3-8) days & 36.24±3.72 (32.8 - 40.5) respectively. The values observed for gestation length, foal heat and foal birth weight were recorded as 326.11 ± 3.23 (314 - 342) days, 16.09 ± 3.84 (6 - 48) days and 22.88±0.88 (19-28) kg. The observed reproductive parameters in Zanskari mares are correlated with the observations recorded earlier for Marwari mares.

(T. R. Talluri, S. K. Ravi and J. Singh)

Evaluation of total mixed rations for maintenance horses

Preparation of area specific total mixed ration based on the availability of ingredient in that area for maintenance horses is the demand of the hour. It is easy to screen feeds by *in vitro* trials, which consume less time and is economical and more practical. Thus, feed ingredients were screened in every step by digestibility and gas production test by taking equine faecal liquor inoculums. Four each energy, 4 protein supplements, 10 local grasses, 4 locally available forages and 4 fillers were taken for preliminary step for TMR (total mixed ration) preparation. Among the energy supplements, maize had highest digestibility (74.32 %) while pearl millet had the lowest (56.49 %). In protein supplements, mustard seed cake (MSC) had the highest (74.36 %) and guar meal had the lowest (58.74 %) digestibility value. Lucerne showed highest digestibility (66.52 %) among forages and rice polish (75.25 %) among fillers.

Digestibility of feed implies the extent of nutrients useful for animal while, gas production shows the digestibility kinetics. By observing the kinetics of gas production and digestibility with 3 energy supplements (oats, barley and maize), 3 protein supplements (groundnut cake, mustard cake and mung bean) and a filler (wheat bran) were taken for preparing 9 iso-nitrogenous concentrate mixtures (crude protein, 15%) for *in vitro* study. These mixtures were subjected to *in vitro* digestibility and gas production test with equine faecal liquor. The combination barley + wheat bran + groundnut cake showed highest digestibility among the concentrate mixture combinations (DMD % - 67.88, OMD% - 69.57), while the minimum value was observed in oats grain + wheat bran + mung grain combination (DMD % - 59.62, OMD% - 60.60. Digestibility kinetics was kept in mind while selecting the ingredients for preparing the concentrate mixture. Comparing digestibility kinetics of gas production, 2 concentrate mixtures were selected for *in vivo* trial (Oats grain + Wheat bran + GN cake & Barley grain + Wheat bran + Mustard cake) along with suitable forage.

(P. A. Bala, R. K. Dedar and N. V. Patil)

Effect of feeding various combinations of dry roughages available in arid region of Rajasthan on growth and nutrient utilization in growing horses

Growth trial on young foals

A growth trial was undertaken for 165 days with young horses (6 month to 1 year old) by feeding them three different rations. Animals were divided in three different groups, weighing 167 (Control), 168.33 (T-1 group) and 167.67kg (T-2 group). Control group was fed with 50% grain mixture + 50% Sewan hay + 5kg green lucerne; T-1 group animals were fed with 50 % grain mixture + 50% groundnut haulm + 5kg green lucerne and T-2 group was fed with 50% grain mixture + 25% Sewan hay + 25% Groundnut haulm + 5kg green lucerne. No significant difference in growth rate in animal of different groups was observed indicating that depending upon the availability of dry roughages, any of these can be included in the diet safely. Feed intake of animals was 3.32, 3.07 and

3.33kg/100kg body weight and dry matter digestibility was 44.41, 44.87 and 45.59% for control, T-1 and T-2 groups, respectively.

Nutrient requirement for maintenance of large white indigenous donkeys

Four large white indigenous adult donkeys were chosen for studying the digestibility and nutrients intake by the donkeys. The animals were kept separately in individual stalls. The feeding trial was conducted for one month with five days of digestibility trial. The animals were fed with concentrate mixture (1.44 kg from Hafed), green oats (2kg) and sewan hay (4.5). Records were maintained for daily feed intake and left over. The average dry matter intake (DMI in kg) was 2.34±0.04, crude protein intake (CPI in g) was 458.83±20.26 and dry matter digestibility content (DM Digestibility (%) was 79.13±0.79 for these indigenous donkeys.

(R. A. Legha, P. A. Bala and N. V. Patil)

Research by students under the guidance of Scientists from NRCE

Name of the Student	Major Advisor at NRCE	Institution to which student belongs	Title of the dissertation / Research	Status
Ameya Gupte M.V.Sc. Student	Dr. B.R. Gulati Principal Scientist	IVRI, Izatnagar	Development of peptide ELISA for serodiagnosis of Equine Herpesvirus 1	Completed
Dr. Ramesh Kumar	Dr. Nitin Virmani Principal Scientist	LUVAS, Hisar	Pathological investigation and protective immunity of recombinant vaccines of equine influenza virus in BALB/c mice	Continuing
Suthar Anubhav Navinchandra	Dr. Sanjay Kumar Principal Scientist	IVRI, Izatnagar	In vitro evaluation of cytotoxic damage and antipiroplasmic activity of some novel drug molecules targeting phospholipid metabolism and heat shock protein of *Theileria equi.*	Completed
Sandeep Singh	Dr. Anju Manuja Senior Scientist	GJUS&T, Hisar	Synthesis, characterization & toxicological evaluation of isometamedium loaded nanoparticles.	Continuing
Sheetal Saini	Dr. H.S. Singha Principal Scientist	Chaudhary Devi Lal University	Expression of recombinant equine cytokines and analysis of their biological activities.	Completed
Dr. Saurabh Kant	Dr. Yashpal Sharma Principal Scientist	IVRI, Izatnagar	Effect of Caffeine as an additive in semen extender to improve frozen thawed semen quality of Marwari horses and Exotic Donkeys	Continuing
Dr. Tejpal	Dr. S. K. Ravi Scientist	RAJUVAS, Bikaner	Quality and Cryopreservability Testing of Equine Semen	Continuing
Dr. Yogesh Soni	Dr. Talluri Rao Scientist	RAJUVAS, Bikaner	Study on Cryopreservation of Stallion Semen using Glycerol and Dimethyl formamide Cryoprotectants	Continuing
Jaideep Ph.D. Student	Dr. S. C. Yadav Principal Scientist	GJUS&T, Hisar	Identification & characterization of infection specific antigen from proteome of *Trypanosoma evansi* & its applications in serodiagnosis	Completed
Ritesh Kumar Ph.D. Student	Dr. S. C. Yadav Principal Scientist	GJUS&T, Hisar	Studies on development of gold nano-particles based diagnostic method for *T. evansi* infection in animals	Continuing

National Centre for Veterinary Type Culture Collection (NCVTCC)

Culture Collection: At a Glance

National Centre for Veterinary Type Culture Collection (NCVTCC) has been mandated to act as a national repository of microorganisms of animal origin comprising veterinary, rumen and dairy microbes. The activities include isolation, characterization, conservation, maintenance and distribution of these microbes for their utilization in animal health and production.

Currently, a total of 2939microbial resources have been reposited in the repository after authentication, and conventional and molecular characterization. These microbial deposits are being contributed by 19 network units including veterinary (7), rumen (8), and dairy (4) network units, and other ICAR institutes and State Agricultural and Veterinary Universities. The microbial resources in the repository include veterinary microbes– viruses, bacteria, bacteriophages & clones; rumen microbes comprising anaerobic bacteria and fungi; and dairy microbes. The centre is also maintaining cell lines (17) and primary cell cultures (3). The repository represented with more than 70 genera of bacteria including some novel taxa and 13 families of viral pathogens. The following table reveals deposits during the year and total strength of NCVTCC.

Table 1. Present Status of the Microbial Repository

Microbial Resources	Upto March 2015	2015-16	Total Deposit
Veterinary Microbes			
Bacteria	927	110	1037
Virus	156	14	170
Bacteriophages	32	44	76
Phage library	27	-	27
Recombinant clones	466	45	511
Genomic DNA	223	57	280
Total	1831	270	2101
Rumen Microbes			
Anaerobic bacteria	142	74	216
Fungi/Yeast	107	-	107
Methanogenic Archeae	8	-	8
Total	257	74	331
Dairy Microbes			
Bacteria	462	39	507
Total	2576	383	2939

Preservation, maintenance and distribution of cell lines

A total of 17 different cell lines along with 3 primary cultures *viz.*, Vero, MDBK, MDCK, BHK21, RK13, HELA, PK15, HEP2, MRC5, NLBK, MA104, Equine lung, Porcine Stable, CEF, CEL, BRT, primary lamb testicle, primary goat kidney and primary goat testis are being maintained for isolation of different viruses in the repository. All cell lines are being routinely revived for assessing their viability and 75 vials of 5 different cell lines/primary cultures *viz.*, Chick embryo fibroblast, CEF (15 vials), Lamb testicle, LT (15 vials) , PK15 (15 vials), MDCK (15 vials) and Vero (15 vials) have been propagated & preserved in liquid nitrogen in the NCVTCC repository. Various cell lines / primary cultures *viz.*, Vero, RK13, BHK21, HELA, MDCK, PK15, Lamb testicle etc., were distributed to the scientific community in TANUVAS Chennai, COVAS Palampur, CMVL Meerut and COVAS, Guwahati.

Authentication of virus isolates for reposition

Six Newcastle disease virus (NDV) isolates deposited by Vaccine Research Centre– Viral Vaccine, TANUVAS, Chennai unit were processed for virus identification by PCR and propagated in embryonated chicken eggs for assessing the viability. Five out of six isolates were found to be positive for NDV by PCR targeting nucleoprotein gene of NDV. All the isolates were passaged in 10 day old embryonated chicken eggs and the allantoic fluid was authenticated by hemmagglutination test for detecting NDV. Methodologies were developed to successfully purify a positive stranded RNA virus (FMDV) from the virus mixture containing a negative stranded RNA virus (PPRV). The repository has been strengthened with the addition of virus isolates from different animal species *viz.*, bovine, ovine, camel, swine and poultry. A total of 19 virus isolates were identified/ authenticated by PCR amplification of virus-specific regions, of which 14 isolates - SPPV (1), NDV (7), PPRV (1), CSFV (2), FMD (1), FPV (1) and PCV2 (1) were successfully passaged in appropriate cell lines/ primary cultures for subsequent reposition in the repository (Table 2).

Table 2. Virus isolates successfully passaged in cell culture

Sr. No.	Virus	No. of isolates	Depositing institute
1	Newcastle disease	03	COVAS, Guwahati
2	Newcastle disease	03	TANUVAS, Chennai
3	PCV-2	01	
4	Fowlpox virus	01	IVRI, Bareilly
5	FMDV	01	NCVTCC, Hisar
6	CSFV	02	
7	NDV	01	
8	SPPV	01	
9	PPRV	01	
	Total	14	

Accessioning, reposition and distribution of viral isolates

The viral repository increased its collection to 170 with the addition of viral isolates from different animal species *viz.*, bovine, ovine, camel, swine and poultry. The authenticated viral isolates have been distributed to scientific community including – bovine herpes virus isolate (1 no.) to Navsari Agriculture University, Gujarat; bovine rotavirus isolate (1 no.) to HPKVV, Palampur and canine parvovirus isolate to IVRI, Izatnagar, Bareilly for research purpose.

Preservation of characterized viral isolates in the repository

A total of 584 vials of 11 characterized viral isolates including CSFV, NDV, CMLV, BPXV, PPRV, SPPV, PCV2, CSFV, FPV, CAV and CPV have been cryopreserved in the repository. 170 virus isolates are being maintained in -20°C and -70°C storage facility as well as in freeze dried form. The different virus isolates available in NCVTCC, Hisar is depicted in Fig.1.

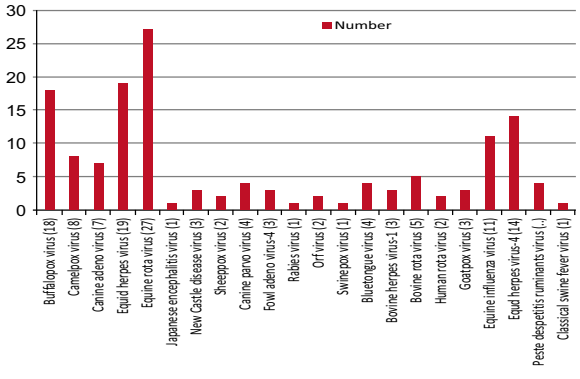


Fig.1. Various virus isolates available in NCVTCC repository: 22 different types of virus isolates are available in the repository.

Orf virus infection in camels in Rajasthan

A severe outbreak of pox infection in camel (Fig. 2) and small ruminants (sheep and goat) was observed in Udaipur, Rajasthan with a morbidity and mortality of 100% and 5%, respectively. Parapoxvirus infection was confirmed by PCR amplification of B2L gene (Fig 3). Similarly, Orf virus (ORFV) infection in sheep and goats was confirmed by PCR. Virus isolation of these poxviruses in primary goat testes cell is underway.

Fig. 2. Pox infection in camel

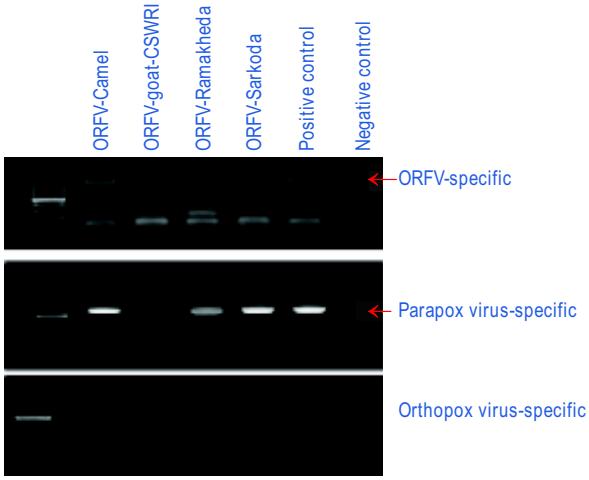


Fig. 3. PCR confirmation of Parapoxvirus infection in camel and Orf virus in sheep and goat

Isolation and identification of sheeppox virus from an outbreak in Jammu & Kashmir

A severe pox infection case was observed in sheep at Jammu and Kashmir (Fig. 4A). The scab sample collected was processed and sheeppox virus was confirmed by PCR amplification of p32 gene (Fig. 4B). Further the sample was passaged in LT cells for virus isolation and virus could be isolated at second blind passage (Fig. 4C).

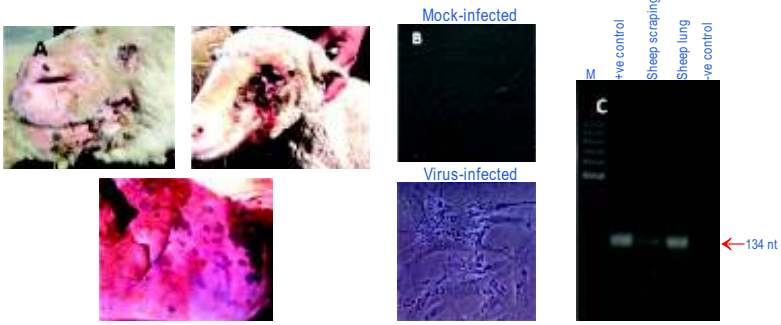


Fig 4. Panel A: Animals exhibiting pock lesions on mouth. B. PCR confirmation of sheeppox virus infection, C: Virus isolation in LT cells

Genetic characterization of Infectious bursal disease viruses (IBDV) from Haryana

Infectious bursal disease (IBD), also known as Gumboro disease, is caused by a virus which belongs to the genus Avibirnavirus of the family Birnaviridae. The virus primarily affects young birds and causes lymphoid depletion of the bursa resulting in significant depression of the humoral antibody response. Two serotypes (serotypes 1 and 2) of IBDV are recognised and "very virulent IBDV" strains of serotype 1 are now common and are causing serious disease in many countries including India. Bursa of Fabricius (n=10) of infected/dead birds collected from two commercial poultry farms (Rohtak district,

Haryana) were tested for IBDV by employing VP2 gene based PCR. The PCR amplification resulted in an expected amplicon of 580 bp in all the samples tested. Further to ascertain the genotype/strain of the viruses, PCR products were sequenced. Sequence and phylogenetic analysis revealed a nucleotide identity ranging from 98-99% with "very virulent IBDV" previously reported from India and neighbouring countries.

Pseudocowpox virus infection from Udaipur, Rajasthan

Pock-like lesions were observed on the hands of milkers and udder of cattle in a dairy farm at Udaipur, Rajasthan (Fig. 5). The samples collected were tested for the presence of Pseudocowpox virus infection by B2L gene based PCR and the Pseudocowpox virus genome could be amplified in 3 out of 4 samples tested.

Fig. 5. Pseudocowpox lesion in cattle and human

Evaluation of the stability of poxvirus isolates at various temperatures over different time intervals

The stability of the buffalopox virus isolates was tested at different temperatures *viz.* 42°C, 37°C, 4°C, -80°C and LN2 (-196°C) for various time period. At 42°C, the poxvirus was completely inactivated within 96 h (Fig.6a). A 100-fold reduction in the buffalopox virus titer was observed when it was incubated at 37°C for 7 days (Fig. 6b).

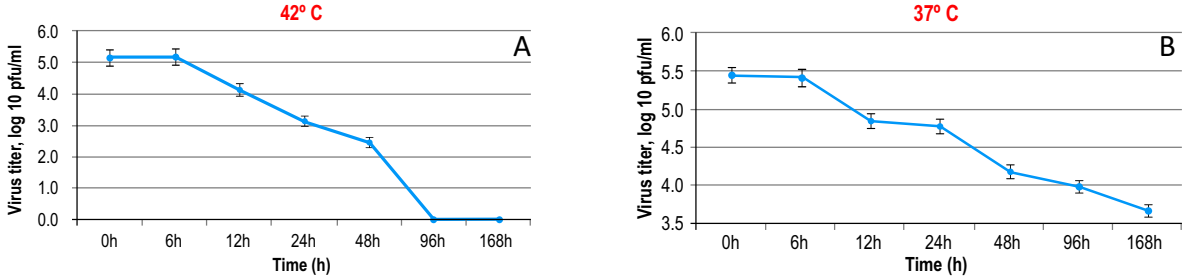


Fig. 6a & b. Thermostability of poxviruses

Identification, characterization and reposition of identified microbial cultures/isolates from the North East region

Authentication of Newcastle disease virus from Guwahati, Assam:

Three Newcastle disease virus isolates deposited by AAU, Khanapara under ADMaC project, were processed for virus identification by PCR and propagated in embryonated chicken eggs for assessing the viability. All three isolates were found positive for NDV by PCR targeting nucleoprotein gene of NDV. All the isolates were passaged in 10 day old embryonated chicken eggs and the allantoic fluid collected upon harvesting were tested by hemmagglutination test for detecting NDV. These viruses were also passaged in CEF and CPE was observed on 3rd day post infection. All the three isolates have been accessioned.

Authentication of fowlpox isolates:

Two fowlpox isolates deposited by AAU, Khanapara were processed for virus identification by PCR and propagated in embryonated chicken eggs for assessing viability. These isolates were positive for FPV by PCR. However, pock lesions were absent on CAM of SPF eggs even after three passages.

Authentication of duck plague virus:

One duck plague virus isolate deposited by Core lab was processed for virus identification by PCR and propagated in embryonated chicken eggs for assessing viability. The isolate was positive for DPV by PCR. However, pock lesions were not observed on CAM of SPF eggs even after three passages.

(Sanjay Barua, Naveen Kumar, Riyesh T, B. C. Bera & Taruna Anand)

Accession of bacterial isolates at NCVTCC

At present, a total of 1037 economically/scientifically important bacterial isolates are accessioned and stored at -80°C in NCVTCC. The bacterial cultures received at NCVTCC are processed under a streamlined ISO9001:2008 procedure. During 2015-16, 110 bacterial cultures were accessioned out of 272 cultures received from various quarters. The accessioned isolates include *Pseudomonas aeruginosa*, Bacillus spp, *Staphylococcus aureus*, *Enterococus* spp. from CSWRI, Avikanagar; *Escherichia coli* cultures from SKUAST, Jammu and IVRI, Izatnagar and *Staphylococcus* spp. and *E. coli* submitted by TANUVAS, *Pasteurella multocida* strains and others from AAU, Khanapara and *P. multocida* from NRDDL, Jalandhar. Three cultures of *Burkholderia mallei* were also accessioned from NRCE, Hisar. Bacterial culture deposits were received from various Network Units, Veterinary Colleges and ICAR Institutes apart from isolations from bacteriology laboratory of NCVTCC, Hisar. Further, 37 authenticated cultures are preserved in repository and are ready to be accessioned after receipt of bacterial "Accession Forms" from depositors. For the cultures preserved in NCVTCC, the phenotypic/genotypic information including microscopic cell-morphology photographs, 16S rRNA and other specific gene sequences and clones of isolates have been generated.

Two accessioned cultures i.e., *Pasteurella multocida* ssp. *multocida* and *Salmonella enterica* bv. Gallinarum, whose whole genome sequences have been accessioned in NCBI, are additionally annotated at the Pathosystems Resource Integration Center with Pasteurella multocida PATRIC Genome ID 1161102.3.

The seed culture collection was started a few years back as parallel repository of accessioned bacterial cultures. It is supposed to source bacteria for use and distribution without disturbing the integrity of original collection.

Rare strains of bacteria among cultures isolated and accessioned by NCVTCC

In order to increase the microbial genomic resource diversity of culture collection, the rare strains of bacteria have been isolated, identified and characterized from diverse animal backgrounds. The 42 accessioned strains belong to 16 different genera and 3 phyla i.e., *Proteobacteria, Actinobacteria* and *Firmicutes*. The strains have been isolated from diverse host species such as pig, buffalo, poultry, horse, donkey, mice and human. Investigations on isolates from poultry intestines led to identifications of strains of *Campylobacter* spp., *Bacillus megaterium*, *B. cereus*, *Enterococcus casseliflavus*, *E. cecorum* and *Lactococcus garvieae*. Rare strains of *Barrientosiimonas humi* and *Corynebacterium amycolatum* have been isolated from mare milk. *Actinobacterium Barrientosiimonas humi* is a recently classified member of a small family *Dermacoccaceae*, which was first time reported from Barrientos Island of Antarctica in 2013. *Corynebacterium amycolatum* from mare milk is significant in view of its status as an opportunistic infectious agent in nosocomial settings. It has been reported from cattle mastitic milk. One human pus isolate was identified as *Bacillus altitudinis*. Many strains of alpha-haemolytic members of family Enterococcaceaee as *Enterococcus devriesei*, *E. hirae*, *E. faecium* were identified from pigs and mice.

Fifteen bacterial strains of equine origin have been isolated from pathological specimens like aborted fetus, swabs and few environmental samples. These cultures further have been further identified and accessioned in the repository (Table 3). They represent, with few exceptions like *Rhodococcus equi* and

Actinobacillus equilli, such microbial species which have been rarely reported from equines. Many of them have been isolated from human clinical sources, like *Pseudomonas argentinensis* from human skin and soil. The isolation of *Comamonas kerstersii* from a case of a horse which died of colic is interesting as the isolation was done from horse intestinal lesions. This is significant in view of reports of isolation of *C. kerstersii* from human intestinal perforation and intra-abdominal infections. Other isolates include Nocariopsis alba, *Rhodococcus rhodochrous*, *Ignatzschineria* larvae, *Achromobacter* sediminum and *Escherichia hermanii* have been reported as agents of opportunistic infections in humans.

Table 3. Different taxa of bacteria isolated from equine pathological samples and environment

S.No.	Taxa	Strain	Source	Literature reports of source
1	*Pseudomonas argentinensis*	Eq31A	Fetal liver	Soil, human skin
2	*Actinobacillus equilli*	Eq163F	Foal joint ill	Foals, pigs
3	*Rhodococcus rhodochrous*	D2AB	Donkey dung	Soil, human eyes
4	*Barrientosiimonas humi*	Fo52A	Mare milk	Soil
5	*Corynebacterium amycolatum*	Fo52C	Mare milk	Cattle mastitis
6	*Shigella flexneri*	Eq54	Mare vaginal swab	Diarrhoea-humans, animals
7	*Shigella flexneri*	Eq62Y	Mare vaginal swab	Diarrhoea- humans, animals
8	*Comamonas kerstersii*	Eq52C	Colic Horse Intestine lesion	Human intra-abdominal infections
9	*Ignatzschineria larvae*	Eq63C	Horse Heart vegetative growth	Myiasis, Necrotizing wounds
10	*Achromobacter sediminum* (*Verticia sediminum* sp. nov.)	Eq63D system	Horse Kidney	Marine origin, respiratory
11	*Aeromonas media*	Eq68	Vermicompost	Water, rare clinical
12	*Escherichia hermanii*	Eq170A	Mare nasal discharge	Clinical
13	*Corynebacterium lipofiloflavum*	Eq117C	Foal nasal sample	Contaminants, opportunistic
14	*Nocardiopsis alba*	Eq135	Nasal sample	Air, honey bee gut
15	*Rhodococcus equi*	EO2D	Horse dung	Gut, foals

Reports of *Actinobacillus equuli* ssp. *equuli* infections in foals in India

Actinobacillus equuli subsp. *equuli*, is found in the oral cavity and alimentary tract of horses. It is a Gram-negative rod of the family *Pasteurellaceae*, and is associated with "sleepy foal disease", a disease characterized by acute septicaemia of foals generally related to failure of maternal immunoglobulin immunity. If the foal survives bacteraemia or septicaemia, the disease becomes chronic in the form of "joint ill". We report isolation, purification, characterization and molecular identification by 16S rDNA PCR, cloning, sequencing and phylogenetic analysis of 3 strains of *Actinobacillus equuli* from cases of foal pneumonia and foal polyarthritis and to our knowledge, this is the first instance of confirmatory diagnosis of *A. equulli* infection in foals in India. The foal was dull, depressed and had a wound on its leg.

Isolation and accessioning of *Lactococcus garvieae* from poultry intestine

During the processing of poultry intestinal content samples obtained from retail shop for isolation of *Campylobacter* sp., we detected many strains of small alpha-haemolytic colonies on Sheep Blood Agar. Cloning and sequencing of ribosomal gene led to the identification of bacteria as *Lactococcus garvieae* (Fig. 7), which is the etiological agent of Lactococcosis or Haemorhhagic Septicaemia of Rainbow trout and number of freshwater and marine aquaculture species. This may be probably the first report of isolation and genotypic identification of *L. garvieae* from poultry in India.

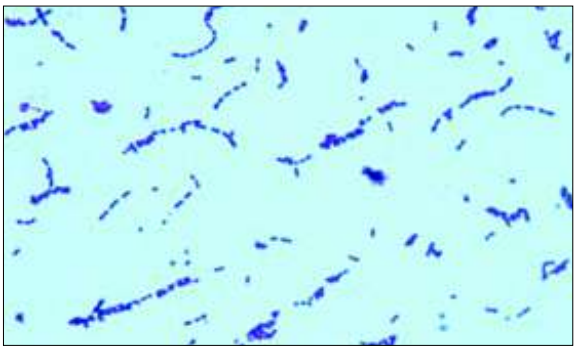


Fig. 7. Lactococus garviae as Gram-positive cocci in small chains

(R.K. Vaid, Taruna Anand, T. Riyesh and B.C. Bera)

Expression-ready gateway open reading frame (ORF) clone collection of equine influenza virus and proof of their utility in recombinant protein production and protein-protein interaction studies

A collection of ORFs clones of Equine Influenza Virus (EIV) from Indian epizootic was generated in a versatile format for proteome analysis. The ORFs in each clone was represented by coding sequences only without stop codon to allow expression of amino -terminal as well as carboxy-terminal fusion proteins.

Construction of ORF clone collection of equine influenza virus : The gateway clone collection of EIV-ORFs was generated using the Gateway® cloning system employing homologous recombination based cloning strategy. The ORFs of EIV amplified in two rounds of PCRs for incorporation of complete attB recombina-tion sites of lambda phage. The five copies of each clone were preserved as glycerol stocks for long-term storage in the NCVTCC repository.

Expression of recombinant tagged proteins in prokaryotic expression system

The utility of developed entry clones in downstream protein production application was tested to demonstrate expression of recombinant proteins of 4 ORFs. For this, ORFs were sub-cloned into different destination vectors through a LR recombination reaction, which recombines the attL-sites on the entry clones with the attR sites on the destination vectors. Four entry clones of NA, M1, NS1 & NP - ORFs were shuttled into the prokaryotic expression vector-pDEST17 designed to make a fusion protein with an N-terminal His-Tag. We successfully expressed these three recombinant proteins in BL21 (DE3) expression strain of *E. coli* upon induction with IPTG and detected specific bands of expressed His-tagged rproteins in SDS-PAGE (Fig. 8).

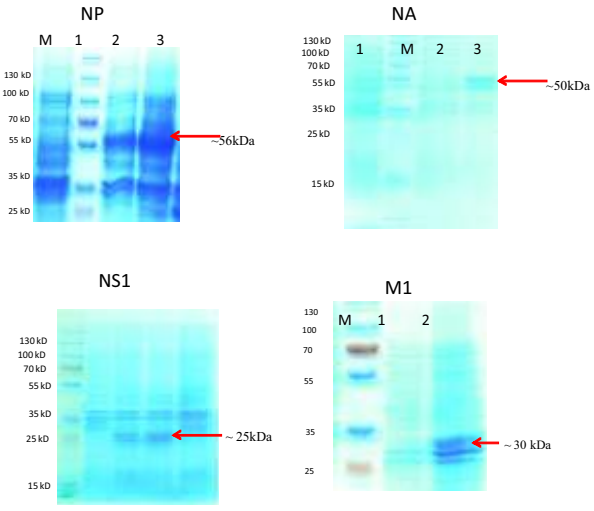


Fig. 8. Expression of recombinant His-tagged proteins of EIV

Yeast-two-hybrid screening for protein-protein interaction

To demonstrate the use of generated gateway clone collection in protein-protein interaction studies, we performed yeast-2-hybrid system based screening for protein-protein interactions among polymerase as well as NP proteins. Four entry clones (PB2, PB1, PA & NP) were shuttled into Gateway-compatible Y2H destination vectors (pDEST22 & pDEST32) and interaction studied in MaV203 yeast strain. The strong interactions among polymerase proteins and between polymerase & NP proteins were screed in auxotrophic selection marker. As influenza viruses are prone to fast evolution leading to generation of new pathogenic strains, such studies will help in continuous systemic study to find out the cellular mechanism responsible for hijacking host immune system.

(B.C. Bera, Taruna Anand, Nitin Virmani, Sanjay Barua and Riyesh T.)

Accessioning of bacteriophages in the repository

Phages are abundant and can be found in all environments where bacteria exist. An immense diversity of phages exists in the environment and it has been estimated that the phages out number bacterial numbers by an estimated tenfold. However, only a very small fraction of environmental phages have been properly characterized and novel phages are being discovered day by day. The bacteriophages from diverse environments including animal farms, village ponds, sewage, farm yard slurry have been isolated, characterized and cryopreserved in the form of a repository at NCVTCC, Hisar. The bacteriophage repository has been strengthened with addition of 44 bacteriophages against various hosts during current year (Fig. 9).

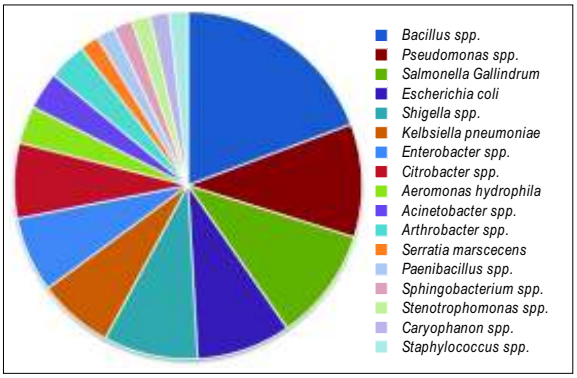


Fig. 9. Bacteriophages isolated against differsified host bacteria

Bacteriophages were isolated against *Pseudomonas* sp., *Klebsiella pneumoniae*, *Citrobacter sedlakii*, *Serratia marscecens*, *Enterobacter* sp., *Shigella* sp., *Escherichia coli*, *Acinetobacter* sp., etc. The bacteriophages were bulk cultured and concentrated using PEG and preserved in phage repository after titre determination.

A novel thermotolerant bacteriophage from river Ganga-isolation and characterization

Interestingly, thermophilic phages are of great significance as they can serve as model systems for understanding the survival of life at extreme temperatures. They can influence many biogeochemical and ecological processes as they directly affect bacterial diversity and density, species distribution and mediate genetic transfers. A bacteriophage isolated against Gram's negative host bacteria was identified as *Pseudomonas* spp. producing ~2-3 mm, clear and circular, plaques on nutrient agar in the presence of host bacteria. Upon

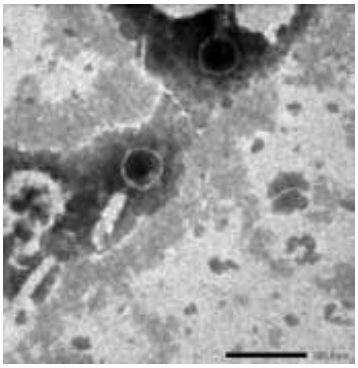


Fig. 10. TEM of thermophilic bacteriophage of *Myoviridae* family isolated from Ganga.

visualization by transmission electron microscopy (Fig. 10), the phage was found to be a member of the family *Myoviridae* with head of icosahedral symmetry. The phage BPA43 was tested for its thermal stability and found to be active over a range of temperatures from 4°C to 80°C. The unique phage was found to be stable between pH of 4 to 13. This is the first ever isolation of a thermophilic bacteriophage from Ganga water where both the host (*Pseudomonas* spp.) and it's predator (lytic phage) were isolated from the same source.

Antibiotic resistance gene carriage in environmental bacteriophages

The ecosystem is continuously exposed to a wide variety of antimicrobials through wastewater treatment plant effluents, agricultural runoff, animal related and anthropogenic activities, which may contribute to the emergence and spread of antibiotic resistance genes (ARGs) and transduction is a significant mechanism of horizontal gene transfer in naturally occurring environments. We explored the presence of ARGs in environmental bacteriophages and found the presence of ARGs in bacteriophage DNA. Among detected ARGs, phage isolates from soil samples were most versatile. Also, interestingly the phage isolates from organized farms showed presence of more varied ARGs as many of them were observed in one or the other phage DNA in comparison to bacteriophages isolated from unorganized zones.

Out of different ARGs tested, those which were detected in phage DNA include bla-TEM (Fig. 11), Oxa-2, tetA, tetW, Int1, Int2 and Int3.

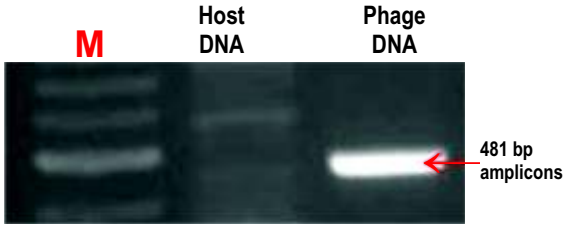


Fig. 11. Presence of bla-TEM in VTCCBPA17 phage (against *Stenotrophomonas*)

Among detected ARGs, phage isolates from soil samples were most versatile; the abundance of bla-TEM was highest followed by Int1, Int2, TetA, Int3, tetW and Oxa-2 (Fig. 12). Also, interestingly the phage isolates from organized farms showed more varied ARGs. bla-TEM was detected significantly in phage isolates from organized farms as compared to unorganized zone isolates.

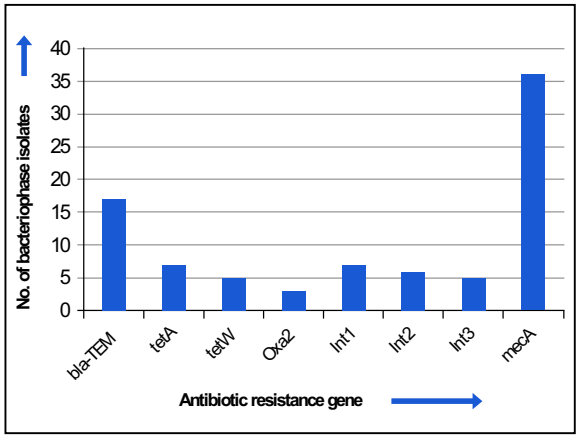


Fig. 12. Prevalence of antibiotic resistance genes in bacteriophage isolates

The study points towards excessive use of antibiotics and reflects the role of phages in dissemination of ARGs in environmental reservoirs, which may provide an early warning system for future clinically relevant resistance mechanisms.

Isolation of bacteriophages from the carcass disposal site

The phage diversity of the soils of equine cadaver disposal site was assessed and monitored for the soil microbial flora so as to gain an insight into the issue of animal-soil transmission of microbes and to assess the role of phages in biological dynamics of manure thus formed over years. We were able to isolate and purify microbes and the corresponding phages from the same soil samples. The bacterial isolates thus obtained were identified and corresponding bacteriophages were characterized partially. When these purified hosts were used to enrich naturally occurring phages from cadaver affected soil,

bacteriophages were detected by spot test. These host bacteria were identified as *Arthrobacter creatinolyticus*, *Bacillus pichynoti*, *Bacillus cereus* and *Caryophanon* spp. on the basis of 16s rRNA sequence analysis. Morphologically, bacteriophage - VTCCBPA38 against *Bacillus cereus* belonged to family *Myoviridae* and had an isometric head with visible base plate. The bacteriophage against Caryophanon spp. also belonged to family *Myoviridae* and had a comparatively smaller head. The phages have been bulk cultured and preserved in the NCVTCC repository.

Unique bacteriophage belonging to family *Siphoviridae* isolated against *Citrobacter sedlakii*

Citrobacter sedlakii is a Gram negative bacteria found in water, soil, food, and the intestinal tracts of animals and human beings. A bacteriophage was isolated against *C. sedlakii*, which showed unique characteristics and a TEM assessment identified it as a member of family *Siphoviridae* (Fig. 13).

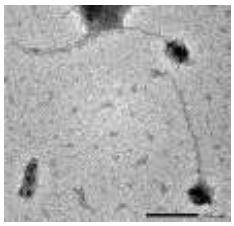


Fig. 13. TEM of thermophilic bacteriophage of *Siphoviridae* family isolated against *Citrobacter sedlakii*

(Taruna Anand, B.C. Bera, R.K. Vaid, Sanjay Barua and Riyesh T.)

Inter-Institutional and Externally Funded Projects

OIE Twinning Laboratory Project on Glanders (OIE funded)

The World Organisation for Animal Health (OIE), Paris, France funded twinning project on Glanders which was successfully completed in collaboration with Friedrich Loeffler Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany. In this project, capacity building for the glanders laboratory of ICAR-NRCE, India was envisaged by the parent laboratory. The long-term objective of ICAR-NRCE was to become OIE Reference Laboratory for SAARC regions. Two major activities were done under the project including molecular typing of *Burkholderia mallei* field isolates and international workshop on 'Surveillance and Diagnosis of Glanders' for SAARC countries.

Table 1. Multilocus sequence typing (MLST) and variable number of tandem repeats (VNTR) typing of *Burkholderia mallei* field isolates (using *B. cepacia* complex primers)

B.mallei isolates	*atpD*	*gltB*	*gyrB*	*recA*	*lepA*	*phaC*	*trpB*	ST
ATCC23344	95	117	128	46	32	34	43	734
Bogor	95	117	128	46	32	34	43	734
Zagreb	95	117	128	46	32	34	43	734
Mukteswar	95	117	128	46	32	34	43	734
India_3076	95	117	128	46	32	34	43	734
India_3081	95	117	128	46	32	34	43	734
India_3324	95	117	128	46	32	34	43	734
India_3478	95	117	128	46	32	34	43	734
India_3595	95	117	128	46	32	34	43	734

Multilocus sequence typing (MLST) and variable number of tandem repeats (VNTR) typing techniques were used for genetic characterization of *Burkholderia mallei* field isolates. MLST typing was performed using *Burkholderia cepacia* complex MLST scheme (http://pubmlst.org/bcc/info/protocol.shtml) and *Burkholderia pseudomallei* MLST scheme (http://pubmlst.org/bpseudomallei/info/Bpseudomallei_primers.shtml). The *Burkholderia cepacia* complex MLST scheme uses fragments of the following seven house-keeping genes: ATP synthase beta chain (atpD), Glutamate synthase large subunit (gltB), DNA gyrase subunit B (gyrB), Recombinase A (recA), GTP binding protein (lepA), Acetoacetyl-CoA reductase (phaC), tryptophan synthase subunit B (trpB). On the contrary, *B. pseudomallei* scheme uses different house-keeping genes (Table 1). For typing, internal fragments of seven housekeeping genes were PCR amplified, sequenced, assembled and high-quality double-stranded sequence data were used for analysis. For each locus, every unique sequence was assigned a distinct allele number, and each sequence type (ST) was defined by the allelic profile corresponding to the alleles at the seven loci. MLST

typing of five *B. mallei* isolates revealed that they belong to ST-734 and ST-40 according to *B. cepacia* and *B. pseudomallei* MLST scheme, respectively.

VNTRs allow superior discrimination between closely related isolates. For genetic characterization, 21 loci from a previously described multiple-locus VNTR analysis (MLVA) system were used to genotype five *B. mallei* isolates. The degree of VNTR variability was assessed by the number of repeat sequences observed and it was found that Indian *B. mallei* isolates differ in number of tandem repeats in 8 loci as compared to other isolates of different geographical origin. However, in-depth analysis in terms of frequency, location, duplicate nature and mutation rate analysis of tandemly repeated regions within the *B. mallei* genome could prove to be an important tool for fine-scale epidemiological study of this pathogen.

International workshop for SAARC countries

A 10 days international workshop on 'Surveillance and Diagnosis of Glanders' was organized from 8th-17th February, 2016 at ICAR-NRCE. Seven International participants from various SAARC countries viz., Sri Lanka, Nepal, Pakistan, Bhutan, Bangladesh, Afghanistan and Iran and 15 Veterinary Officer from various states attended the workshop. The workshop was organized in association with FLI, Federal Research Institute for Animal Health, Institute of Bacterial Infections and Zoonoses, Jena, Germany (Parent laboratory). Aim of the workshop was to provide hands on training in the area of serological diagnosis of glanders such as complement fixation test and indirect ELISA and to build a network among glanders endemic countries for its proper surveillance.

During the workshop three scientists from parent laboratory Prof. H. Neubauer, Dr Mandy Elschner and Dr Falk Melzer visited NRCE, Hisar and expressed their satisfaction on the working and progress of the candidate lab. To apply for OIE-reference laboratory status, future emphasis needs to be given for obtaining laboratory accreditation based on ISO17025:2005 and easy exchange system for sharing of biologicals between laboratories.

(Harisankar Singha)

OIE Twinning Project on Equine Influenza between ICAR-NRCE, India and Animal Health Trust, UK (OIE Funded)

An OIE twinning project was commissioned between Animal Health Trust, New Market, UK and National Research Centre on Equines. The basic objective of this project was to build capacity and to develop and validate laboratory diagnostic methods based on the OIE Standards. The ultimate aim of the project was to develop NRCE as referral laboratory for equine influenza for SAARC countries. For capacity building, Laboratory Exchange Program was executed at the Animal Health Trust, UK (AHT) from 17th July, 2015 till 13th August, 2015. Director NRCE - Dr. B.N. Tripathi, Scientists - Dr. Nitin Virmani and Dr. B.C. Bera and Sh. Mukesh Chand (STA), visited OIE referral laboratory, AHT, UK. During the visit, Director NRCE participated in series of meetings with CEO Mark Vaudin, Dr Debra Elton and other scientists, while Sh. Mukesh got familiarized with working in the OIE referral laboratory and learnt various procedures including record keeping.

NRCE developed mAbs based sandwich ELISA, which was already tested in house and known to react with equine influenza virus isolates. The assay was extensively tested across various lineages of the virus followed by detailed investigations for the detection of equine influenza virus using clinical samples as well as nasal swabs spiked with EIVs from various clades available at AHT. Our assay was found to successfully detect the EIVs in clinical samples as well as in spiked nasal swabs, which indicates that sELISA developed at NRCE could be an efficient diagnostic assay for detection of EIV in field samples.

Validation and proficiency testing of HI assay employed at NRCE was carried out at AHT and results for HI assay in blinded study compared well to those obtained at NRCE with clade 2 virus. To validate the

process being employed at NRCE for HI test for detection of the titre in serum samples, a panel of 30 positive as well as negative serum samples were sent to AHT. AHT tested the serum samples by HI test and the results concurred with those obtained at NRCE.

Hands-on training was received for whole genome sequencing of the EIV isolates available at AHT using Illumina-MiSeq platform. Various sequencing steps including isolation of RNA, fractionation of RNA, library preparation, checking the quality of the library, sequencing reaction preparation, loading of samples into sequencing chip and running of sequencing in MiSeq equipment were performed. Upon sequencing, the quality of sequence data was checked in the MiSeq software. The alignment of the sequence read with reference sequence was carried out using "bwa" program of "samba64" server. The reads were aligned into contigs and complete sequence of the EIV genome was deduced.

An International workshop on 'Surveillance and diagnostics for equine influenza' was organized at the Centre for delegates from SAARC nations under 'OIE twinning project on Equine influenza' from 16th-25th Feb., 2016. The workshop was attended by 16 delegates: one each from five SAARC countries *viz.* Sri Lanka, Bangladesh, Bhutan, Nepal, Afghanistan and 11 delegates from India including Jammu and Kashmir (1), Himachal Pradesh (1), Punjab (1), Rajasthan (1), Uttarakhand (2), Uttar pradesh (2), Gujarat (1), Maharashtra (1) and Animal Quarantine Station, Mumbai (1). The training was provided by Experts from NRCE, India (Dr. Nitin Virmani, Dr. B.C. Bera) and Animal Health Trust, UK (Dr. Debra Elton and Elizabeth Medcalf). The 'Hands On' training was imparted to participants, who were trained in various techniques required for isolation of equine influenza viruses in embryonated eggs/MDCK cells, biosafety and biosecurity, virus titration through haemgglutination, haemagglutination inhibition assay, antigenic characterization of EIV by HI assay using ferret antisera, monoclonal antibody based sandwich ELISA for EI antigen detection, single step RT-PCR for diagnosis of EI infection, two step subtyping RT-PCR for HA/NA genes of EIV (H3N8), TaqMan Probe based qRT-PCR for diagnosis of EI infection etc.

(Nitin Virmani, B.C. Bera, R.K. Vaid and B.N. Tripathi)

Validation study of a Western blot technique and ELISAs for serological diagnosis of glanders in equids for the purpose of certifying freedom from infection in individual animals for trade or movement (OIE funded)

The Complement Fixation test (CFT) is an OIE prescribed sero-diagnostic method for glanders for international trade. However, its sensitivity and specificity has been questioned by various stakeholders. False-negative glanders test results may lead to the introduction of the infectious agent into a glanders free-region and false-positive test results may lead to unnecessary restrictions on international trade of animals leading to financial losses for owners and the equine industry. Alternative methods to CFT such as Western blot (WB) and ELISAs have been developed, but have not been fully validated to date according to the principles and methods of validation of diagnostic assays for infectious diseases. This project aims at further development and validation of alternative methods like indirect ELISA including recombinant ELISA already developed in several laboratories worldwide. OIE-reference laboratory on Glanders at the Institute of Bacterial Infections and Zoonoses, FLI, Jena, Germany will co-ordinate the overall activity of the project. ICAR-National Research Centre on Equines, Hisar and other five institutes around the globe are participating as project partners.

The WB technique developed by the OIE-reference laboratory, Germany and iELISAs developed by the project partners - EU Reference Laboratory for Equine diseases, France and the Universidade Federal Rural de Pernambuco, Departamento de Medicina Veterinária, Brazil using semi-purified *B. mallei* antigenic fractions based ELISA as well as the recombinant protein based-ELISAs developed by the ICAR-NRCE,

Hisar, will be validated in OIERL, Germany according to OIE principles and methods of validation of diagnostic assays. Other project partners from Pakistan and Brazil will contribute by providing large sample collections. The validation will include the analytical performance characteristics; repeatability, analytical specificity including exclusivity.

(Harisankar Singha and B. N. Tripathi)

Development of a recombinant flagellar protein based indirect ELISA for diagnosis of *Trypanosoma evansi* infection in equines (National Fellow Scheme)

Expression and purification of recombinant flagellar protein

The flagellar gene fragment (657 bp) of *T. evansi* was PCR amplified, amplicons purified and cloned into pQE30 vector. The desired clones were grown in small cultures and were induced with 1 mM IPTG. Expression of recombinant protein was confirmed by SDS-PAGE. The recombinant protein was purified by affinity chromatography (IMAC) using Ni-NTA resin under denaturation condition (Fig. 1a &1b) and elutes showing high purity and concentration were pooled together and concentration of the purified recombinant protein were preserved.

Following SDS-PAGE, the recombinant FL 657 protein was subjected to immunoblot analysis. A strong immunoreactive band of ~25 kDa was observed in corresponding position with known positive serum samples of equids. Further, the blot assay was applied on experimentally infected donkey serum samples from day 0 to 156 post infection and field serum samples demonstrated the presence of immunoreactive band at 25 kDa position in positive samples. The blot assay detected antibodies in experimentally infected donkey serum samples from 10th d.p.i.

Standardization of indirect ELISA using recombinant antigen

The ELISA was standardized using the recombinant protein by chequerboard analysis. After standardization, the test was employed on pooled experimental donkey serum samples from 0 to 192 days post infection as well as on *T. evansi* positive equine serum samples from field. The assay detected antibodies in serum samples from 14th d.p.i. (Fig. 1).

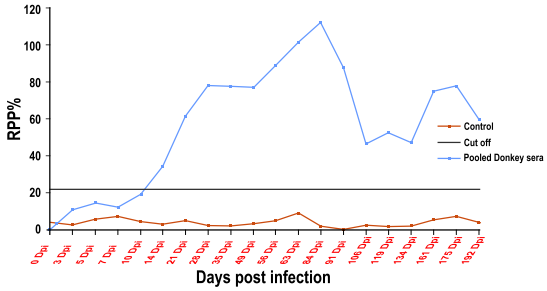


Fig. 1. Indirect ELISA using recombinant antigen

The diagnostic sensitivity (DSe) and specificity (DSp) of r-ELISA kit were worked out *vis-à-vis* WCL ELISA (an OIE recommended assay). We assumed that the most likely value is 99% for both DSe and DSp, with a desired confidence of 95% for both estimates and the desired error margin was set at 2%. A total of 200 serum samples were selected which included 100 positive and 100 negative serum samples in WCL-ELISA. The diagnostic specificity and sensivity of r-ELISA for detecting antibodies against *T. evansi* were found to be 0.92 (0.82-0.95) and 0.98 (0.96-0.99) in relation to WCL- ELISA, respectively based on estimated DSe and DSp of 99% with 2% error margin in estimates at 95 % confidence interval.

Internal Lab Validation

For in-house inter-lab comparison, the assay was performed in five different laboratories. The results of four laboratories were in 100% agreement with the results of National Fellow Laboratory; however, the result of one laboratory differed (90.9% agreement). Further, process of external lab validation of this assay is in progress. After inter-lab validation, the assay was applied on field serum samples, collected from equines inhabiting different geographical regions of the country. A total of 3000 serum samples were

tested and results were comparable with WCL-based ELISA.

To test the stability of the recombinant antigen, a number of modules were coated with recombinant antigen and blocked with two different blocking buffers and stored at 4°C. The ELISA was performed using coated modules at different intervals up to 180 days. The stability of the antigen in both blocking buffers was found to be almost similar and worked well up to 180 days.

(Rajender Kumar)

Generation of reverse genetics based recombinant equine influenza virus

The H3N8 subtype of Influenza A virus causes frequent outbreaks in equines worldwide leading to huge economic losses. The segmented nature of the genome and error prone polymerase enzyme of the virus leads to point mutations and re-assortment resulting into generation of the escape mutants and rendering vaccines ineffective. Reverse genetics technique has been exploited in developing new vaccine as well as to decipher the mechanisms of the host pathogen interaction, disease pathogenesis and cellular dynamics strategies. Current work pertains to generation of a recombinant equine influenza virus using this platform. MDCK cell-culture adapted EIV strain A/equine/Jammu-Katra/2008/H3N8 was used for viral RNA extraction and cDNA synthesis for the amplification of all gene segments. The eight gene segments of this EIV strain were successfully amplified with suitable restriction site (BsmB1) for all gene segments of EIV. Individual gene segments of EIV were cloned in pHW2000 reverse genetics vector obtained from St. Jude's Hospital, USA under MTA. All eight gene segments of EIV namely PA, PB1, PB2, HA, NA, NS, NP and M were successfully cloned into pHW

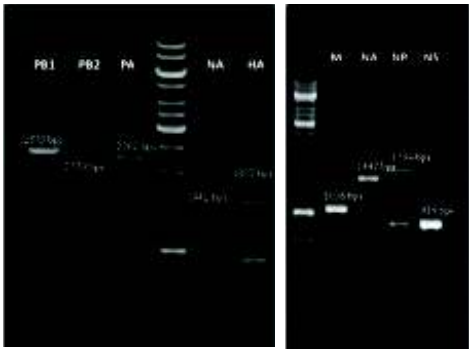


Fig. 2. a & b: PCR amplification of eight gene segments of EIV cloned in pHW 2000 vector

2000 vector (Fig. 2a & 2b). The generated clones were verified by sequencing of the recombinant plasmids and BLAST, NCBI homology analysis of the sequences.

Further, for generation of recombinant virus, back bone of six cloned plasmids (except HA and NA) of H1N1 (wsn) and two clones from H3N8 (HA and NA genes) were transfected in co-cultures of T293 cell line and MDCK cells. This rescued recombinant virus is to be further assessed for virus kinetics and in vivo studies in mouse model.

(Nitin Virmani, Sandeep Bhatia, B.C. Bera, Naveen Yadav, Taruna Anand and Richa Sood)

Eukaryotic expression of important equine cytokines and analysis of their biological activities (DST funded)

Assessment of biological activity of recombinant horse cytokines

Cytokines play important roles in determining the immune response against infections. Cytokines of equine origin are not readily available to study their utility in diagnosis, vaccines and protection studies against various diseases. In this study recombinant cytokines of equines were generated and biological activities of five recombinant equine cytokines (IL-2, IL-4, IL-10, IL-18 and IFN-γ) were assessed in terms of their ability of inducing cell proliferation and cytokine secretion in the equine peripheral blood mononuclear cells (PBMC). The stimulation index (SI) was calculated as the ratio between optical density values of stimulated cells to un-stimulated cells. Recombinant equine cytokines: IL-2, IL-4, IL-10, IL-18

and IFN-γ induced significant cell proliferation (SI>1) at 250 ng/ml concentration. The level of proliferation induced by these recombinant proteins were comparable and higher to that induced by mitogens-Concanavalin A and PHA (Fig. 3).

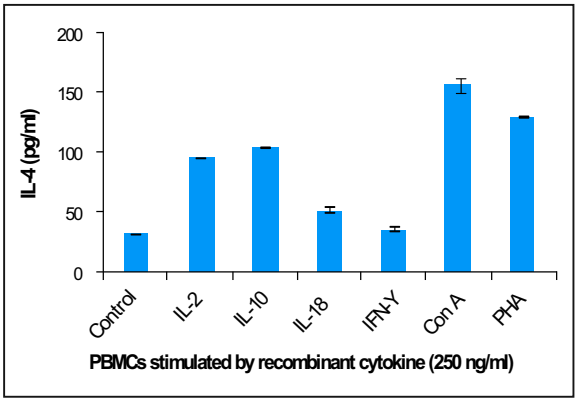


Fig. 3. Stimulation index of lymphocyte proliferation assay at different concentration of recombinant IFN-γ and IL-10.

Level of various cytokines in stimulated PBMCs were quantified. ELISA data revealed that recombinant IL-10 acts as potent inducer of IL-4 (134 pg/ml), whereas recombinant IFN-γ and IL-18 were poor inducers of IL-4. On the other hand, all of the recombinant cytokines used in the study were equally able to induce pro-inflammatory cytokine - TNF-α.

The qPCR assays were performed to assess the transcription of eight cytokine genes (IL-2, IL-4, IL-10, IL-18, IFN-γ, IL-6, TNF-α and IL-12p35) in PBMCs stimulated with recombinant cytokines. Recombinant IL-18 favorably augments the production of IFN-γ followed by IL-2 and IL-6. Highest copy number of IL-10 transcript was observed in recombinant IL-4 stimulated cells

The study revealed that recombinant equine cytokines retain immuno-biological activity, hence these equine cytokines will serve as ready resource for studying antiviral and antibacterial effects as a therapeutic agent or vaccine adjuvant. Future studies in the generation of monoclonal antibodies against these recombinant cytokines will be helpful for development of enzyme immunoassays for detection of equine cytokines in biological fluids.

(Harisankar Singha and Sheetal Saini)

Development of Nano gold based immunochromatography / immuno dot blot assay for detection of *Trypanosoma evansi* infection in animals (DST funded)

The aim of the project is to develop gold nanoparticles based point-of-care (POC) test for effective diagnosis of *Trypananosoma evansi* infection in equines. In this test, the analyate in sample binds with labelled antibody, which is then captured by a second antibody or antigen immobilized on membrane, thus effectively concentrating coloured particles to produce coloured test line. A control line is also included by immobilization of anti-species antibodies.

Synthesis and characterization of nano gold particles

The colloidal gold nanoparticles were prepared by the citrate reduction method and measured by particle size analyzer. The synthesized gold nanoparticles (Fig. 4 & 5) were stable (zeta potential - 41.1 mV). The UV-vis measurement of the synthesized gold nanoparticles showed the characteristic absorbance maximum at λmax ~ 518 and spherical shape of size 12-15 nm as characterized by TEM.

Anti-horse IgG conjugate with serum raised in rabbits was prepared using characterized gold nanoparticles and characterized. The dilution of reference positive,

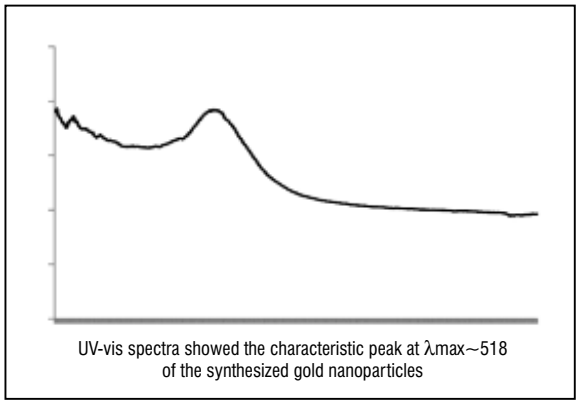


Fig. 4: UV absorb spectra of gold nanoparticles

negative serum samples and conjugate were optimized for further use in LFA.

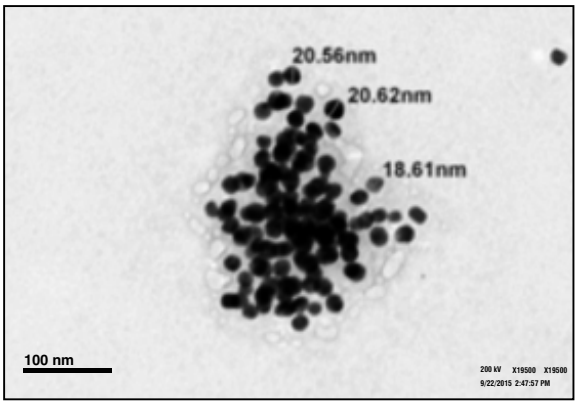


Fig. 5: TEM image of gold nanoparticles

The antigen concentration (test line) and IgG (control line) were optimised. Initially, the serum samples of experimentally infected ponies (n=6) with *T. evansi* (reference serum) along with healthy controls were subjected separately from sample pad, followed by colloidal gold conjugate. It was observed that all the infected ponies serum samples reacted sharply at both positions *i.e.* test line and control line within 5-10 minutes showing 100% sensitivity (Fig. 6). Serial serum samples from experimentally infected ponies (n= 6) showed positive results from 10-14 DPI in the assay. The assay was also carried out on 88 sera collected from field equines (58 seropositive & 33 seronegative as tested by WCL ELISA) for comparison (Table 2).

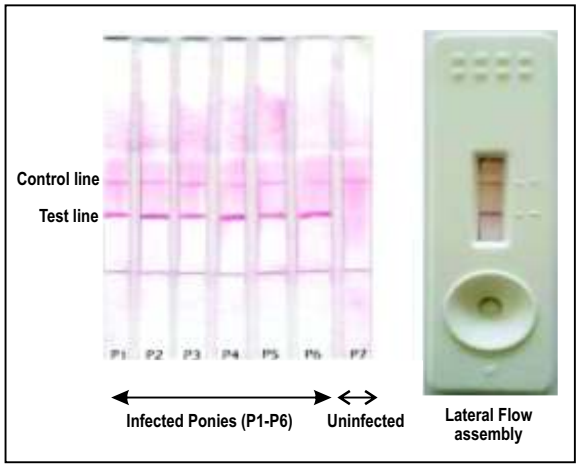


Fig. 6. Lateral flow assay using exp. infected/uninfected ponies in pooled reference serum samples (28 DPI)

Table 2. Comparision of sensitivity and specificity of seropositive cases of *T. evansi* tested by ELISA *vis-à-vis* immuno chromatographic test strip (ICT) test

No. of samples	ELISA (Whole cell lysate antigen)			Immunochromatographic test strip (ICT) test			
	Positive	Negative	Positive Rate (%)	Positive	Negative	Sensitivity	Specificity
55	55	0	100	53	2	96.3 %	--
33	0	33	0	2	31	--	95.1%

LFA did not cross react with serum samples (10 each) positive for *T. equi*, EHV 1, Equine influenza and glanders (*B. mallei*). Preliminary results revealed that there was no significant difference in sensitivity and specificity between LFA and ELISA, but more number of samples need to be tested. This test can be adopted for quick screening of suspected cases of trypanosomosis during outbreak as well for point - of - care diagnosis and is first ever successful attempt for development of LFA against parasitic infection in livestock.

(S.C. Yadav, N. Dilbaghi, A.K. Gupta and Sandeep Kumar)

CRP on vaccine and diagnostics

Development of improved diagnostics for equine herpesviruses (EHV1/4)

Quantitative real-time PCR has been standardized for detection and SNP typing of EHV1 and the assay has been validated using infected equine samples.

For gG-based ELISA, recombinant gG protein for EHV-1 and 4 was expressed in prokaryotic system and bulk purified. A total of 659 serum samples have been screened by gG ELISA for EHV-1/4. 42 serum samples (6.37%) were found positive for EHV 1 while 425 samples (64.49%) were positive for EHV 4. ELISA plates coated with recombinant protein for EHV 1 and 4 have been stored at 40°C and are being tested at weekly interval for their performance for the ELISA with same set of serum samples. The OD's are being recorded so as to compare the results and bring the assay to the kit format.

For development of peptide-based ELISA, the linear antigenic peptides have been designed against gE and gC of EHV1, synthesized and evaluated for their efficacy in diagnosis of EHV1 Infection. An ELISA has been standardized for detection of EHV1 using one of the peptide, which has good sensitivity and specificity.

(B.R. Gulati and Nitin Virmani)

Development of refined vaccine for equine herpesvirus 1 infection

EHV 1 virus (H-90-7) was bulk cultured, purified through sucrose gradient following rate-zonal centrifugation, titrated (10 9.25 TCID50/ml) and inactivated with formalin. Vaccine was formulated with inactivated EHV1 vaccine using two novel adjuvants with specific aim to strengthen the cell mediated immune responses. BALB/c mice (n=180) are being utilized for the adjudging the protective efficacy of the vaccine. Mice were divided into 5 groups: Group 1 and 2 mice are being immunized with vaccine having two novel adjuvants, respectively, while group 3 mice act as control and have been vaccinated with inactivated vaccine initially developed by NRCE. Group 4 mice act as mock vaccinated challenge control while group 5 mice serve as unimmunized and unchallenged group. The mice are to be monitored through humoral and cell mediated immune responses and protective efficacy of the vaccine will be adjudged through histopathology, immunohistochemistry, virus isolation, qPCR and immune responses.

(Nitin Virmani, B.R. Gulati and B.C. Bera)

Development of rapid diagnostic (LFA) for equine piroplasmosis

Equine piroplasmosis, a tick-transmitted protozoan disease caused by *Theileria equi* and/or *Babesia caballi*, is endemic in India and a major stumbling block in international movement of the infected horses. Two types of immunodominant merozoite surface proteins *viz.*, equi merozoite antigen (EMA) -1 (34 kDa) and EMA-2 (30 kDa) have been identified in *T. equi* belonging to major piroplasm surface protein (MPSP) family which are conserved among the genus Theileria. These EMAs have been utilized as diagnostic antigens in many ELISAs. In a previous study we developed ELISA using expressed EMA-2 protein and have initiated work on nano-particle based lateral flow assay for rapid diagnosis of *Theileria equi* antibodies using recombinant protein. *Theileria equi* EMA-2 protein (231AA) was expressed in pGEX 4T-1 expression vector. The GST tagged recombinant EMA-2 protein was purified. EMA-2 recombinant protein (TE/tEMA-2-GST), *T. equi* infected horse's RBCs [in vitro (MASP technique) cultured *T. equi* infected horse RBCs], normal horse RBCs,and GST protein were subjected to SDS-PAGE (10% poly-acrylamide gel) analysis (Fig. 7) and further immunoblotted using *T. equi* positive serum which reacted with this recombinant expressed protein and

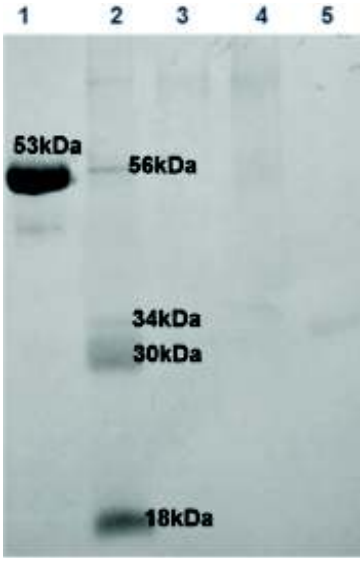


Fig 7. Reactivity of EMA-2-GST and *T. equi* infected RBCs to serum from *T. equi*-infected horse by Western blot analysis

Lane 1: EMA-2 recombinant protein

Lane 2 : *T. equi* native antigen

Lane 3: *Babesia caballi* native antigen

Lane 4: Normal Horse RBC lysate

Lane 5 : GST only antigen

T. equi infected horse RBCs only and not with normal and or GST only protein.

Hyper immune serum was raised against recombinant EMA-2 antigen and IgG were purified on Protein A column. The purified anti-EMA-2t IgG were validated by IFAT analysis and adjusted to 1mg/ml for further use in LFA experiment.

(Sanjay Kumar and Rajendra Kumar)

Advanced Animal Disease Diagnosis and Management Consortium (ADMaC) (DBT funded)

Investigations on Japanese Encephalitis virus infection

A total of 195 pig serum samples collected/received from pigs out of which, 34 (17.4%) were tested positive for JEV antibodies. A nested RT-PCR for JEV was standardized and the method along with reference materials was transferred to the Core Lab at Guwahati. The HI antigen and the positive control serum for 1000 sample testing was supplied to the Core Laboratory, along with SOPs.

Investigations for H3N8 influenza in pigs

Screening of 44 serum samples for H3N8 influenza virus serologically by HI assay revealed negative status for antibodies. RT-PCR for matrix gene on 40 samples for Influenza A viruses also revealed negative status.

Investigations for *T. evansi* infection

Serum samples (n=52) from cattle from districts Jagiroad, Guwahati and Dhubri, Assam, were screened for *T. evansi* (two samples were confirmed positive by wet blood examination). Out of 52 samples examined 5 (9.61 %) cattle were positive for *T. evansi* infection. Further an experiment was carried to evaluate the shelf life of WCL antigen and HSP70 full length C terminal (27 kDa) recombinant protein on pre coated ELISA plates. The antigens were coated on ELISA plates, kept at 4°C and tested with reference positive and negative serum samples at regular monthly intervals. The WCL and recombinant antigens have been observed stable on ELISA plates up to seven months till now, further evaluation is in progress.

Repository development for microbes from NER

Three Newcastle disease virus isolates deposited by Core lab were processed for virus identification by PCR and propagated in embryonated chicken eggs for assessing the viability. All the three isolates have been accessioned in NCVTCC. One duck plague virus isolate and two fowlpox isolates were processed for virus identification by PCR and propagated in embryonated chicken eggs for assessing viability. The respective isolates were positive for DPV and FPV by PCR however, pock lesions were not observed on CAM of SPF eggs even after three passages. Similar was the case of one pigeonpox isolate deposited by Core lab. Tissue samples (n=10) from pigs were tested for PCV-2 and CSFV also. A total of 69 bacterial isolates were also received from core lab out of which 18 have been accessioned. Additionally the samples of sewage, soil and fecal materials from poultry and piggery farms were collected from North-East region and were processed for bacteria and bacteriophage isolations. A total of 20 bacteria and two bacteriophages were isolated. These phages have been bulk cultured and concentrated using PEG, subjected to chloroform based purification and were preserved at -80°C in the VTCC repository. A training was imparted to 5 participants from NER on "Demonstration of In-house diagnostic tests developed by NRCE for detection of viral and protozoan diseases and development of repository of veterinary microbes" from 29th Feb-05th March 2016.

(B. N. Tripathi, Nitin Virman, Sanjay barua, S.C. Yadav, B.R. Gulati, Rajender Kumar, R.K. Vaid, Taruna Anand, B.C. Bera and Riyesh T.)

CRP on Agrobiodiversity

Viability checking of 232 microbial cultures (20 viruses and 212 bacteria) has been carried out during the period. A total of 114 Veterinary microbes, (bacteria (89), bacteriophages (7), genomic DNA (10) and clones (8) have been authenticated and preserved in the repository. The accessioned bacterial isolates include *E. marmotae*, *E. fergusonii*, *E. coli*, *Klebsiella* spp., *Aeromonas hydrophila*, *Pantoea aglomerans*, *Acrhomobacter* spp., *Pseudomonas* spp., *Vibrio* spp., *Seratia* spp., *Rahnella* aquaticus from, pig, deer, elephant, poultry, duck and dog. Eight clones of 16S rRNA gene of different bacteria namely *Aeromonas hydrophila* (from pig), *Aeromonas hydrophila* (from prawn), *E. coli* (from cattle), *E. coli* (from pig), *E. coli* (from deer), *E. coli* (from Duck), *Staphylococcus* spp. (from Elephant), *Aeromonas caviae* (Prawn) received from NER region, were generated and preserved in the repository. Also, the genomic DNA of the 10 bacteria received from NER region have been isolated and preserved in the repository. A total of 20 serum samples (16 bovine and 4 porcine) and 10 pig tissue samples were collected from Meghalaya for virus isolation and are being processed in cell culture for virus isolation. For the purpose of bacteriophage isolation against naturally occurring bacteria, the samples viz., sewage, soil and fecal materials from poultry and piggery farms were collected from North-East region and processed for bacteria isolations. A total of 40 bacteria were isolated which were purified, preserved and used for enrichment to isolate bacteriophages from original samples and seven phages have been isolated including against *Bacillus* spp., *Shigella* spp., *Caryophanon* spp., *Stenotrophomonas* spp. These phages have been bulk cultured and concentrated using PEG. The phage concentrates have been preserved at -80°C and accessioned in the NCVTCC repository.

(Sanjay Barua, R.K. Vaid, Naveen Kumar, Taruna Anand, B.C. Bera & Riyesh T)

Molecular mechanism of interaction between SERCA and PPR virus (DST funded)

A library of small molecule chemical inhibitors was screened against PPR virus. Inhibitors of Sarco-endoplasmic reticulum calcium ATPAse (SERCA) was shown to inhibit PPRV replication suggesting SERCA is critically required for PPRV replication or that the PPRV misuses SERCA for its effective replication (Fig. 8). The possible mechanism how SERCA interact with PPRV is underway.

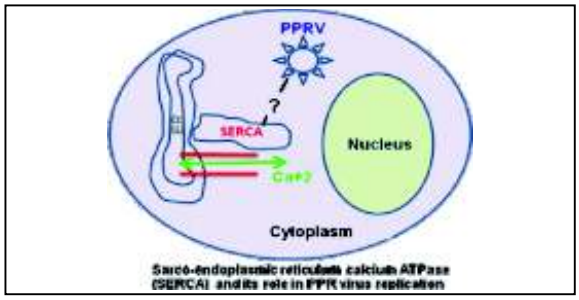


Fig. 8. Diagram showing possible interaction of SERCA & PPRV replication

For determination of the cytotoxicity concentration 50 (CC50) of Sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) inhibitors, three-fold serial dilutions (in serum free MEM) of SERCA inhibitors were incubated with Vero cells for 96 h and cytotoxic concentration was determined by MTT assay (Fig. 9).

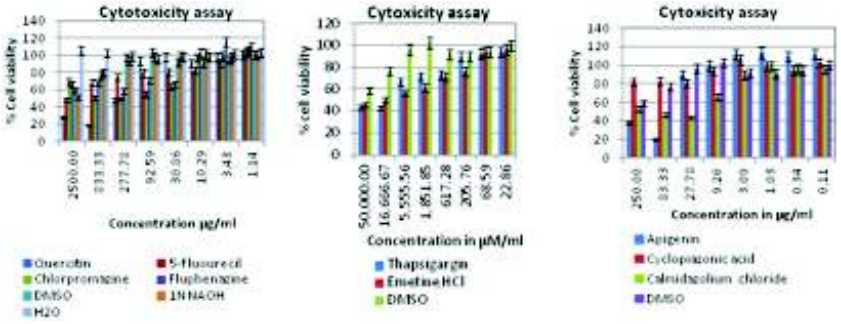


Fig. 9. Cytotoxicity concentration of SERCA inhibitors by MTT assay

Highest non-cytotoxic concentration of each inhibitor was used to evaluate its antiviral efficacy against PPRV in Vero cells. As compared to the vehicle control (0.1% DMOS or 01.% 1M NaOH), a significant reduction in PPRV production in Vero cells was observed in SERCA inhibitor treated cells (Fig. 10) suggesting SERCA is critically required for PPRV replication.

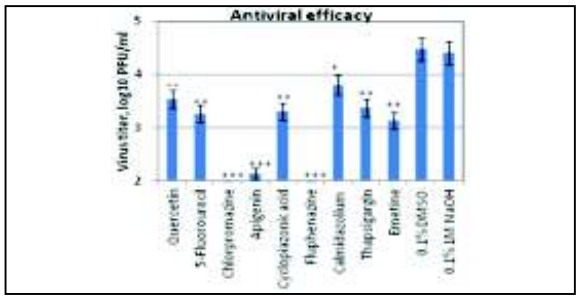


Fig. 10. Antiviral efficacy of kinase inhibitors against PPRV; ***=P<0.001, **=P<0.01, *=P<0.05

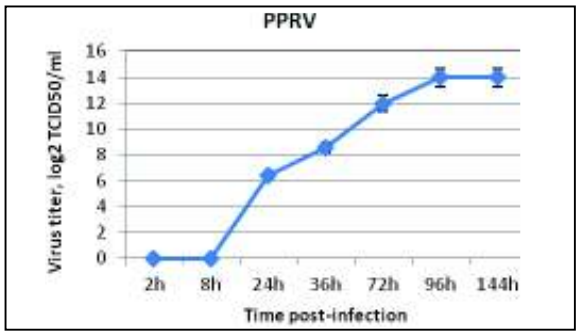


Fig. 11: PPRV life cycle

Determination of PPRV life cycle

In order to examine which step(s) of PPRV life cycle are affected by SERCA inhibitor, we first carried out a time course experiment to evaluate the life cycle of PPRV in Vero cells. As compared to 2 hours post-infection (hpi) and 8 hpi, there was a sharp rise in viral titer in supernatants at 24 hpi or later, which indicated that new progeny virus particles started to be released from the infected cells somewhere around 28 hpi as one full cycle of the viral replication had completed (Fig. 11).

Study on the dynamics of PPRV quasispecies heterogeneity (understanding mechanisms underlying mutations)

Sequential passage of PPRV was performed in the presence/absence of SERCA inhibitors. At each passage, confluent monolayer of Vero cells were infected with PPRV, washed 5 times with PBS before a fresh aliquot of DMEM was added and incubated for 96 hr -120 hr or until the appearance of cytopathic effect (CPE) in ≥75% cells. The virus released in the supernatant of passage 1 was used in the second round of infection upto 24 passages. RNA extraction and cDNA synthesis was carried out at every 5th passage. Fusion (F) gene has been amplified from the cDNA (every 5th passage) and is being subjected for nucleotide sequencing.

(Naveen Kumar and Sanjay Barua)

Feasibility studies on biogas and compost production from mule dung in hilly regions (DST funded)

The project was initiated in November, 2015 in collaboration with CSIR-National Environmental Engineering Research Institute, Nagpur, with the broad objective of studying biological characterization and pathogenic potential of mule dung obtained from hilly regions and to assess vermicomposting and biomethane gas production potential of mule dung with the objective to find eco-friendly means of mule dung disposal with bioenergy production. Forty five mule dung samples were collected aseptically and brought to the laboratories and were subjected to bacteriological enumeration by decimal dilution plating method. Bacteriological counts of dilutions of sample in PBS have been obtained employing different selective media viz, McConkey lactose Agar (MLA) for enteric microbes, Mannitol Salt Agar (for *Staphylococci*), YPD (for yeast and fungi); Sheep Blood Agar (for pathogens) and NANAT medium (for *Rhodococci* and Coryneforms). A total of 354 bacterial isolates have been purified and preserved. Parasitological analysis has revealed 25% samples to be positive for strongyle infection. For composting, a bed of dung from 3 mules was prepared for processing, with a parallel bed of dung from 1 horse and vermicompost obtained after 2-3 months would be processed by proximate analysis; organic matter, N, P, K and other trace elements.

(B.N. Tripathi, R.K. Vaid and R.A. Legha)

Generation of induced pluripotent stem (iPS) cells from buffalo fetal fibroblasts through non-viral approaches (DBT funded)

Most of the established iPS cell lines have been produced from viral approaches which ultimately integrate in the host genome and increase the risk of insertional mutagenesis. Hence iPS cells generated from viral approach make them unfit for therapeutic and regenerative purpose. Under the current project which has been initiated in collaboration with Central Institute for Research on Buffaloes, the generation and further preservation of iPS cells without viral gene transduction is envisaged which would be preferable for regenerative medicine. The project was recently initiated in the month of March. The primary colonies of iPS cells were generated (Fig. 12) and are being passaged further.

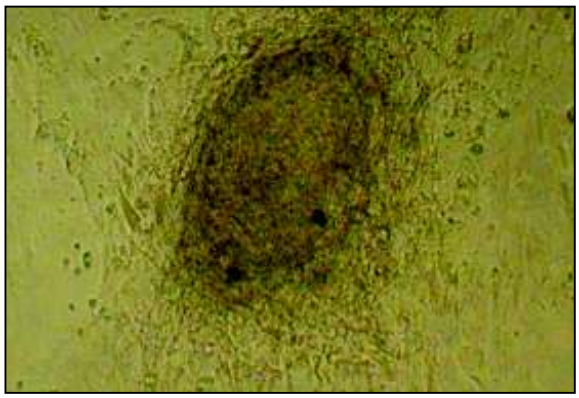


Fig. 12. Buffalo iPS cells-like colony after first passage

(Dharmendra Kumar, Naresh Selokar, P.S. Yadav, Taruna Anand, B.C. Bera and Nitin Virmani)

All India Network Programme on Neonatal Mortality In Farm Animals

A total of 126 samples were collected from neonate foals reared at different organized stud farms. Seventeen PM samples from foals died at these stud farms were also collected and histopathological examination revealed bronchopneumonia, anoxia, enteritis, and pericarditis as a cause of death in these foals.

Septicaemia due to pathological *E. coli* was ascertained in bacteriological samples collected from one of the stud farm. These foals were successfully treated with kanamycin antibiotic and it was included in package-of-practice. *Rhodococcus equi* was isolated from nasal swabs collected from another stud farm and it was prevalent at large scale in this farm. Intravenous injection of ezythromycin and oral rhiphimpicin was suggested as line of treatment. High levels of GGT (162.2 IU/l), BUN (73.5 mg/dl), were observed in foals (n=26) suffering from *R. equi,* indicating liver and kidney damage due to this bacterial infection.

It was observed that foals at the time of birth were naive and acquired passive EHV1 immunity from their mares through colostrum within 24 hrs of birth. A total of 94 foals below 6 months of age, including Tohana, Haryana (42), Hisar, Haryana (9) and Hapur, Uttar Pradesh (43) were tested for antibodies to EHV1 by VNT and 17 (18.1%) developed antibodies to EHV1. Tissue/blood samples of abortion/neonatal foal mortality (n =15), collected from breeding farms at Tohana, Haryana (2), Hisar, Haryana (9) and Hapur, Uttar Pradesh (4), were tested by nested PCR & virus isolation for EHV1 infection. Two cases of abortions and still birth were detected positive for EHV1 infection by nested PCR.

A total of 30 stool samples from diarrhoeic foals, collected from breeding farms at Tohana, Haryana (18), and Hapur, Uttar Pradesh (12) were tested for equine rotavirus infection by a mAB-based sELISA. A total of 12 samples were found positive for rotavirus infection. Equine rotavirus was isolated from 2 positive samples from Tohana in MA104 cell lines. The typing of equine rotavirus isolates was done by RT-PCR and both isolates belonged to G10P11. All the samples were found negative for Crytptosporidium upon ZN staining technique.

No case of failure of passive transfer (FPT) of immunity was detected in neonate foals. A total 42 foals were investigated for blood IgG concentration, which is > 800 mg/dl

(Sanjay Kumar, Ramesh Dedar, B. R. Gulati, Nitin Virmani and S. K. Khurana)

Consultancy and commercialization of technologies

Consultancy

NRCE is a National Referral Centre of Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture (Govt. of India) to conduct testing for health certification and to provide consultancy and diagnostic services for various equine diseases to the stake-holders. Samples received from state and regional disease diagnostic labs, regional animal quarantine and certification stations, polo associations, Equestrian Federation of India, field veterinarians and equine owners are tested for various diseases. The results and the expert technical advice are provided to the equine owners. The centre plays a vital role by informing the State and Central Government Animal Husbandry authorities to initiate containment and control measures with notification of equine diseases.

During the period under report, diagnostic services were provided to various stakeholders for EIA, glanders, equine influenza, EHV-1, EVA, CEM, Theileria equi, Trypanosoma evansi, Trypanosoma equiperdum, Babesia equi, Salmonella abortus-equi, and African horse sickness. A total of 5306 serum samples from thoroughbred as well as indigenous equines were examined by Coggins test for EIA under S&M (1531), disease investigation (253) and contractual service (3522), none was positive. Similarly a total of 13980 serum samples were tested for glanders, which included S&M (1531), disease investigation (8219) and contractual service (4230). Thirty six serum samples from J&K (18), UP (22), Punjab (4), Gujarat (13), and Uttarakhand (3) were found positive for glanders. Five biosamples (abscess-4 & pus swab-1) samples from UP were also found positive for B.mallei culturally. Outbreaks of Glanders were seen in several States as mentioned above. Testing of 2660 under S&M and DI (including 1531 under S&M and 1111 under DI) and 18 samples under contractual service from various states for screening for equine influenza (H3N8) antibodies employing Haemagglutination inhibition assay which revealed seropositivity in 108 samples (91samples under S&M and 17 samples under DI). 72 equine serum samples tested negative for AHS. The information regarding negative status of AHS in all States of the country was collected through all RDDLs, this was compiled in the form of a dossier and sent to DAHDF for onward submission to OIE for continued disease free status of country from AHS. Overall the Centre generated a revenue of Rs 47.00 lakhs through testing of samples.

Commercialization of technologies

National Research Centre on Equines, Hisar is actively involved in research on equine health and production since its inception. Many diagnostics kits, vaccines and packages of practices have been developed by the dedicated team of NRCE scientists for stakeholders and these technologies are ready for transfer and commercialization.

Name of Patent (Technology) Application and its Inventor

S.No.	Name of Patent Application	Name of the Inventor
1	A recombinant haemagglutinin domain-containing protein for the detection and diagnosis of glanders and method of preparation thereof. Application No.1328/DEL/2010 dated 08.06.2010. (DRDE Gwalior and ICAR-NRCE, Hisar)	Subodh Kumar, Shailendra Kumar Verma, Praveen Malik, Sonia, Harishankar Singha, Ganga Prasad Rai and Rajagopalan Vijayaraghavan
2	Polynucleotide sequence, processes, composition and methods thereof- Application No. 1575/CHE/2010 and PCT/IB 2011/052475 (Ilsc Bangalore and ICAR-NRCE, Hisar)	Utpal Tatu, Rani Pallavi, Suresh Chandra Yadav, Raj Kumar Singh and Rajender Kumar
3	Nano-drug delivery for quinapyramine sulphate Application, No.2560/DEL/2011, dated 06.09.2011. (ICAR-NRCE, Hisar and GJUS &T, Hisar)	Anju Manuja, Neeraj Dilbaghi, Sandeep Kumar, Harmanmeet Kaur, Gaurav Bhanjana, Rajender Kumar, Balvinder Kumar and S.C. Yadav
4	A highly sensitive kit for detection of antibodies against *Theileria equi* in serum of equids. Application No. 2763/DEL/2012 dated 06.09.2012	Sanjay Kumar, Rajender Kumar, Ashok Kumar Gupta, Suresh Chandra Yadav
5	Recombinant TssA protein for detection of antibodies against *Burkoholderia mallei* in Equines. Application No.3610/DEL/2015 (DRDO and ICAR-NRCE, Hisar)	Hari Shankar Singha, Praveen Malik, Sachin Kumar Goyal, S.K. Khurana and R.K. Singh
6	Recombinant Hcp1 protein for detection of antibodies against *Burkoholderia mallei* in Equines. Application No.4120/DEL/2015 (DRDO and ICAR-NRCE, Hisar)	Hari Shankar Singha, Praveen Malik, Sachin Kumar Goyal, S.K. Khurana and R.K. Singh

List of technologies for commercialization under MoA with National Research Development Corporation, New Delhi

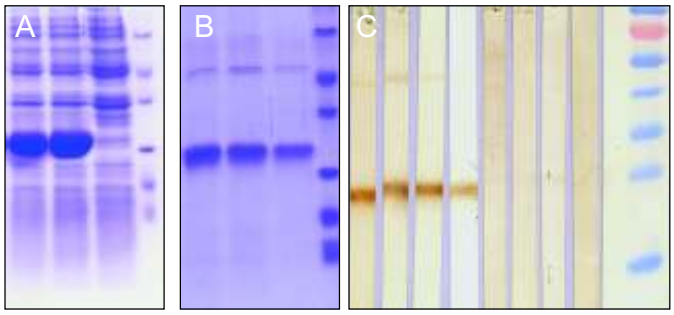
1.	A pregnancy diagnostic kit for equine, based on detection of eCG by ELISA.
2.	Monoclonal antibody based blocking ELISA for detection of EHV-1 infection.
3.	Monoclonal antibody based ELISA for diagnosis of rota virus infection in equines.
4.	Recombinant antigen based ELISA kit for diagnosis of *Theileria equi* infection in equines.
5.	Updated Equine Influenza Vaccine.
6.	Equine Herpes Virus-1 vaccine.
7.	Recombinant protein based ELISA for diagnosis of EIA.
8.	Recombinant protein based ELISA for differentiation of EHV-1 and EHV-4 infections.

Technology Developed and Assessed

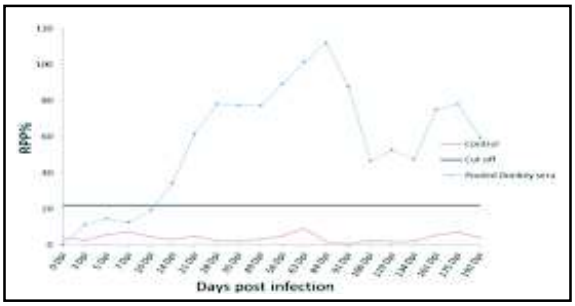
Recombinant flagellar protein based ELISA for diagnosis of surra in equines

Surra is one of the most important diseases of equines caused by *Trypanosoma evansi*, a haemoprotozoan parasite transmitted mechanically by biting flies. The disease is prevalent in all agro-climatic parts of India with more endemic index in states of Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Punjab, Rajasthan, Uttarakhand and Uttar Pradesh. The routine diagnosis of surra through conventional parasitological methods are generally not very sensitive to detect chronic and sub-clinical forms of disease. Presently, whole cell lysate (WCL) antigen based serological methods (CATT, LATEX, IFAT and ELISA) are being used which have limitation of uniformity and required laboratory animals for preparation of antigen. To address this problem a recombinant antigen based ELISA has been developed for sensitive detection of antibodies in serum of equines against *T. evansi* infection.

A flagellar gene fragment of 657 bp size was chosen for development of diagnostic assay for detection of *T. evansi*. The 657 bp flagellar region from indian strain (T.ev-India-NRCE-Horse1/Hisar/Haryana) was cloned in pQE30 vector, expressed and purified recombinant protein. The purified recombinant protein was used in ELISA for detection of specific antibodies against *T. evansi* parasite in equine serum. Recombinant antigen coated, blocked and dried plates are ready for use and stored at 4°C for further use in ELISA. Other components were also stabilized for further process of ELISA and this assay was transformed in the form of a diagnostic kit. The optimum cut-off value of ELISA was determined as 22 (RPP%) using known negative and known positive samples. The diagnostic sensitivity and specificity of r-ELISA for detecting antibodies against T. evansi was calculated 0.92 (0.82-0.95) and 0.98 (0.96-0.99) in relation to WCL-ELISA.



A : Expression of flagellar recombinant protein of T.evansi; B: Purification of recombinant flagellar protein of T. evansi
C : Western blot analysis of recombinant flagellar protein



Recombinant flagellar protein based ELISA showing detection of antibodies from 14th d.p.i onwards in serum samples of experimentally *T.evansi* infected equines.

Field application of r-ELISA

The assay was applied on 2638 field serum samples, collected from equines of different geographical regions of the country, detected 207 (7.84%) samples positive for *T. evansi* antibodies in comparison to WCL ELISA which detected 232 (8.79%) positive samples.

This assay overcomes the bottleneck faced due to lack of uniformity, batch to batch antigenic variation and requirement of laboratory animals for antigen preparation - in routinely used WCL antigen based - ELISA.

(Rajender Kumar)

Training and Capacity Building

International Workshop on 'Surveillance and diagnostics for Equine Influenza' for delegates from SAARC countries under OIE twinning project

16-25 Feb, 2016

Collaborating institutes : ICAR National Research Centre on Equines, Hisar, Haryana, India & Animal Health Trust, Newmarket, UK

NRCE organized international workshop for delegates from SAARC countries under 'OIE Twinning Project on Equine Influenza' from 16-25th February, 2016. The workshop was attended by 16 delegates: one each from five SAARC countries *viz.* Sri Lanka, Bangladesh, Bhutan, Nepal, Afghanistan and 11 delegates from India including Jammu and Kashmir (1), Himachal Pradesh (1), Punjab (1), Rajasthan (1), Uttarakhand (2), Uttar pradesh (2), Gujarat (1), Maharashtra (1) and Animal Quarantine Station, Mumbai (1).

Group photo of SAARC training on Equine Influenza - Director, Scientists from NRCE and AHT, UK with delegates from SAARC countries and India

The training was provided by experts from NRCE, India (Dr Nitin Virmani, Dr B.C. Bera) and Animal Health Trust, UK (Dr Debra Elton and Elizabeth Medcalf). The 'Hands On' training had major emphasis on learning by doing. During the inaugural session Dr. B.N. Tripathi, Director, NRCE gave an overview of the research activities of the institute and Indian scenario of Equine influenza. A training manual was released during the inauguration of the workshop.

Delegates performing various diagnostic tests for equine influenza

Interaction between the Scientist from NRCE and international expert from AHT, UK

Field visit to Hanumangarh, Scientist demonstrating collection of samples from horses to the delegates

Participants were trained in various techniques required for isolation of equine influenza viruses in embryonated eggs/ MDCK cells, biosafety and biosecurity, virus titration through haemgglutination, haemagglutination inhibition assay, antigenic characterization of EIV by HI assay using ferret antisera, monoclonal antibody based sandwich ELISA for EI antigen detection, single step RT-PCR for diagnosis of EI infection, two step subtyping RT-PCR for HA/NA genes of EIV (H3N8) and TaqMan probe based qRT-PCR for diagnosis of EI infection. A series of lectures were delivered on practical aspects of equine influenza.

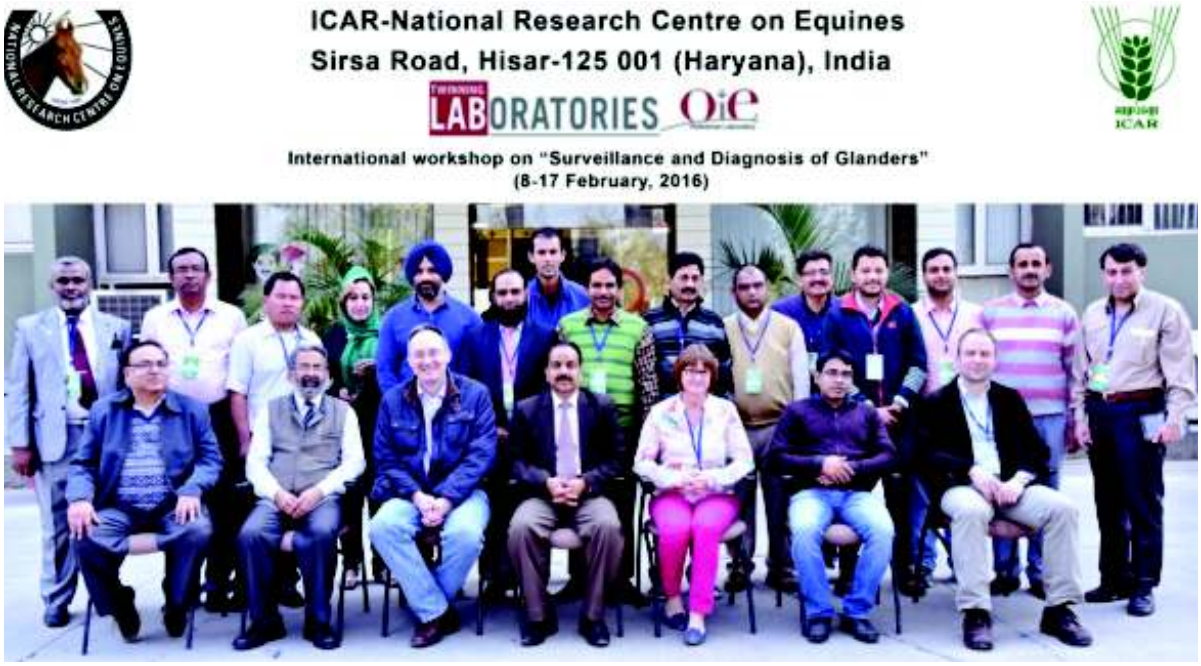
The delegates were also taken for one day field visit to Hanumangarh - to have real time feeling of our extension work, liaisoning with stakeholders, sample collection and developing a network across the country. This part was important for the participants as they could actually visualize and practically collect samples from horses in the field.

International workshop on 'Surveillance and diagnosis of Glanders' for delegates from SAARC nations under OIE twinning project on Glanders

8-17 Feb, 2016

Collaborating institutes : ICAR-National Research Centre on Equines, Hisar, Haryana, India & Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Institute of bacterial Infections and Zoonoses, Jena, Germany

An International workshop on 'Surveillance and diagnosis of Glanders' was organized **for delegates from SAARC nations under 'OIE twinning project on Glanders'** from 8-17th Feb, 2016. The workshop was attended by 15 delegates: one each from six SAARC countries *viz.* Sri Lanka, Bangladesh, Bhutan, Nepal, Pakistan and Afghanistan and one from Iran and 8 delegates from India including Jammu and Kashmir (2), Himachal Pradesh (1), Punjab (1), Uttarakhand (1), Uttar Pradesh (3).



Group photo of SAARC training on Glanders - Director, Scientist from NRCE and FLI, Germany with delegates from SAARC countries and India

The training was provided by Experts from NRCE, India (Dr Harisankar Singha, Dr S. K. Khurana) invited experts from CCSNIAH, India (Dr Praveen Malik) and FLI, Germany (Dr Mandy Elschner and Falk Melzer). Aim of the workshop was to provide hands on training in the area of serological diagnosis of glanders like complement fixation test and indirect ELISA and to build network among glanders endemic countries for surveillance of glanders. During the inaugural session Dr. B.N. Tripathi, Director, NRCE gave an overview of the research and activities of the institute and Indian scenario of glanders. Training manual and a video documentary on glanders was released during the workshop.

Participants were trained in various techniques required for diagnosis of glanders, complement fixation test (CFT), enzyme linked immunosorbent assay (ELISA) and PCR for diagnosis of *B. mallei* infection. Further the participants were detailed through lectures on various aspects of glanders, transmission patterns, quality control & GLP in the laboratory, biosafety and biosecurity, control & management of glanders at the face of outbreak.

Delegates from SAARC countries and various states of India conducting assays during the training

Training Imparted to NER candidates under DBT sponsored ADMaC project

A training was imparted to 5 participants (research fellows) from North East Region on "Demonstration of In-house diagnostic tests developed by NRCE for detection of viral and protozoan diseases and development of repository of veterinary microbes" from February 29 - March 05, 2016 under the DBT - NER project on Advanced Animal disease Diagnosis and Management Consortium (ADMaC). Training was imparted on diagnostic assays developed by NRCE for Equine Influenza, Trypanosomosis, Japanese Encephalitis repositioning and storage of cultures. "Hands On" training was provided to participants on assays such as haemagglutination inhibition assay, RT-PCR, qRT-PCR, cultivation and harvesting of viruses in embryonated eggs for EI; VNT & ELISA for JE, ELISA for antibody detection for Trypanosoma; cultivation and harvesting of viruses in cell culture, , repositioning of the viruses & bacteria for the culture collection. A training manual was released and reagents including kits, cell lines, etc. were provided to the Core Lab.

Group photo of trainees and faculty during training under ADMaC project

Workshop/Seminars/Farmer's day organized at the Centre

- Organized farmer's day on the occasion of Foundation day of Equine Production Campus on September 28, 2015.
- A training on 'Virus Neutralization and molecular assays for Japanese Encephalitis' imparted to a Scientist from IVRI, Izatnagar from June 28 - July 8, 2015.
- ICAR-NRCE participated in an exhibition to disseminate the information and technology to farmers organized on February 27th, 2016 by RAJUVAS and ATMA.

Capacity building through national and international trainings

International trainings, meetings and visits abroad

1. Dr B. N. Tripathi visited Animal Health Trust, UK under the OIE Twinning Laboratory project on "Equine influenza" from July 16-24, 2015.
2. Dr Nitin Virmani attended "Expert Surveillance Panel" meeting on Equine Influenza for vaccine strain selection organized by OIE held at head quarters in Paris, France on a formal invitation from Chairman of the ESP group on Equine Influenza, Dr Ann Cullinane on March 1, 2016.
3. Dr Nitin Virmani attended 3rd International Symposium on Neglected Influenza Viruses, The Georgia Center at the University of Georgia, Athens, Georgia, USA from April, 15 -17, 2015.
4. Dr Nitin Virmani attended program for the capacity building and training in the area of next generation sequencing, validation of assays developed by our laboratory, ISO 17025 certification training at Animal Health Trust, UK under the OIE Twinning Laboratory project on "Equine influenza" from July 17, 2015 to August 14, 2015.
5. Dr B. C. Bera attended program for the capacity building and training in the area of next generation sequencing, validation of assays developed by our laboratory, ISO 17025 certification training at Animal Health Trust, UK under the OIE Twinning Laboratory project on "Equine influenza" from July 17, 2015 to August 14, 2015.
6. Sh Mukesh Chand attended capacity building training in the area of diagnosis and validation of assays developed by our laboratory at Animal Health Trust, UK under the OIE Twinning Laboratory project on "Equine influenza" from July 16-21, 2015.

Participation in trainings

1. Dr Sanjay Kumar participated in Workshop of Nodal Officers of ICAR on Research Data Repository for Knowledge Management during August 04-05, 2015 at New Delhi.
2. Dr Sanjay Kumar participated in Workshop of HRD Nodal Officer at NAARM, Hyderabadfrom 10-12th Feb., 2016.
3. Dr Sanjay Kumar participated in Drug discovery technology: a molecular modelling approach, at Department of Bio And Nano Technology, Guru Jambheshwar University of Science And Technology Hisar-125001, Haryana from 28-30 March, 2016.
4. Dr P. A. Bala attended Winter School Training on "Livestock and Climate change: Challenges and Ways ahead for Sustainable Production" at NIANP, Bangalore.
5. Naveen Kumar attended capacity building workshop on Biorisk management, National Center for Disease Control, New Delhi in collaboration with CDC, Atlanta, USA held at New Delhi, May 5-9, 2015.

Expert lectures/lead papers delivered:

- A. K. Gupta delivered an expert lecture on "Breed characteristics and DNA based evaluation of Marwari horses" in Marwari Horse Judge Clinic, Balsamand Lake Palace, Jodhpur July 18 to 19, 2015.
- B.R.Gulati delivered a lecture on "Risk analysis and biosafety practices in research laboratories" in training Course "DNA based diagnostics and Cell culture techniques", Department of Animal Biotechnology, LUVAS, Hisar, 7-27 July 2015.

- B.R.Gulati delivered a lecture on "Concepts and procedures in establishment and maintenance of biosafe laboratories for veterinary pathogens" for 28th CAFT Course on "Development of Validated Diagnostic Assay and Accreditation of Diagnostic Laboroaties", Department of Veterinary Microbiology, LUVAS, Hisar from 3-23 February 2016 (ON 12 Feb 16)
- Khurana SK presented a lead paper on "An overview of impact of climate change on vector-borne zoonotic diseases" at 3rd IAVNAW Conference, COVAS, CSKHPKV, Palampur, India, November 4-5, 2015. pp 4-5.
- Kumar, N. delivered a talk on "Host-targeting antiviral agents" during XXII Annual Convention of ISVIB and National Symposium on Immunomics and proteogenomics in livestock health and productivity, held from 17-19 Dec 2015 at ICAR-National Research Centre on Equines, Hisar.
- Kumar R presented a lead paper on "Recent advances in diagnosis of trypanosomosis in animals using immunological and molecular approaches" during XII Annual Convention (VIBCON-2015) & National Symposium on "Immunomics and Proteogenomics in Livestock Health & Productivity." organized by ICAR-National Research Centre on Equines, Hisar, December 17-19, 2015, Pp. 88-89.
- Kumar, S delivered a talk on "Equine piroplasmosis - an insight into interacting molecules and novel drug targets" during XXII Annual Convention & National Symposium on Immunomics and Proteogenomics in Livestock health and productivity, Organized by Indian Society for Veterinary Immunology and Biotechnology, at NRCE, Hisar from December 17-19, 2015.
- T.R.Talluri was invited as a speaker to present lead paper on "Advances and applications of assisted reproductive technologies in animal reproduction" at XXXI Annual Convention of The Indian Society for Study of Animal Reproduction (ISSAR) on "Current challenges and opportunities in animal reproduction" held from 3rd to 5th December, 2015 at Veterinary College, Hebbal, Bengaluru-560024.
- T.R.Talluri invited as a speaker to present lead paper on "Approaches for derivation of Induced Pluripotent Stem cells from Cattle" at International livestock Conference & Expo 23rd annual Convention, ISAPM-2016 from 28-31, Hyderabad.
- Tripathi BN presented a talk on "Equine Zoonoses: A bird's eye view" during 1st Annual Conference of SRL & NAWAR and National Symposium on "Concepts in Zoonoses & Health in New Millenium" Nagpur, India, October 19-20, pp
- Vaid R K delievered lead paper on 'Exploration of cultural bacterial biodiversity and discovery of unusual animal pathogens' during XIIth Annual Convention and National Symposium on "Immunomics and Proteogenomics in Livestock Health and Productivity" Dec 17-19, 2015. NRCE, Hisar
- Virmani Nitin delivered an invited talk on "An overview of Equine Influenza and Indian scenario" at a one day National Workshop on "Influenza: Risk Factors, Massive Impact and Uncertain Future" organized by ICAR-IVRI, Izatnagar on 19thOctober, 2015.
- Virmani Nitin delivered lead paper on Equine influenza virus: An insight into evolution and interspecies transmission during XII Annual convention and National Symposium on "Immunomics and Proteogenomics in Livestock Health & Productivity". held at National Research Centre on Equines, Sirsa Road, Hisar 125 001 from 17-19 December 2015
- Yashpal Sharma delivered a lecture on "Application of Ultrasound in Equines" during Training programme on 'Ultrasonography in Large Animal Reproduction for Fertility Augmentation" held at CIRB, Hisar during 22-27 June, 2015.

RAC, IRC and IMC Meetings

XVIII Research Advisory Committee (RAC) meeting (2014-15)

XVIII Research advisory committee (RAC) meeting for the year 2014-15 was held at NRCE, Hisar under the chairmanship of Dr R.N. Srinivas Gowda, Ex - Vice Chancellor, Karnataka Veterinary Animal and Fisheries Sciences University, Karnataka on 9th April, 2015. The RAC members in the meeting included Dr S.K. Srivastava (Ex-Head, Veterinary Microbiology Division, IVRI, Izatnagar); Dr V.D. Sharma (Prof. & Dean - Life Science, Sai Institute of Paramedical & Allied Science, Dehradoon); Dr J.R. Rao (Emeritus Scientist, National Academy of Agricultural Research Management, Hyderabad); Dr K.C. Varshney (Prof & Head, Department of Veterinary Pathology, Rajiv Gandhi College of Veterinary & Animal Sciences, Puducherry); Prof. Gaya Prasad (ADG, AH) and Dr B.N. Tripathi (Director, NRCE). The RAC assessed the achievements of ongoing projects including externally funded projects in the areas of equine health, production, extension and National Centre for Veterinary Type Culture Collection. Dr B.N. Tripathi presented an overview of the institute activities under taken during 2014-15. New proposals were also presented in the meeting. The chairman RAC emphasized for formulation of research projects on need based problems being faced by stakeholders and field veterinarians. The Chairman also expressed concern over the declining equine population and emphasized to develop strategies to overcome the problem of declining equine population. RAC recommended exploring the constituents of the donkey milk responsible for therapeutic and commercial value, to look into the therapeutic potential of the bacteriophages, to explore the utility of equine recombinant cytokines as immune-modulator and to develop diagnostic markers useful in future therapeutics. RAC also stressed on developing specific and sensitive diagnostics for detection of encephalitis in equines resulting due to multi-etiological origins; to address the problem of infertility and emerging problem of reproductive syndrome in equines; to explore possibility of implementation of web based model server developed in IASRI for true breed selection; to include equine breeds present in the southern part of the country for genetic characterization and to develop a comprehensive identification/typing scheme for microbes at NCVTCC.

RAC meeting in progress

Annual Institute Research Committee (IRC) meeting

The annual meeting of Institute Research Committee (IRC) for the year 2014-15 was held under the chairmanship of Dr B.N. Tripathi, Director, NRCE, Hisar on 26th-27th May, 2015. The meeting was conducted to evaluate the research projects in the areas of equine health, production, NCVTCC and extension. All the In-charges of the respective units - BSDU, Equine health, Equine Production Campus (Bikaner) and NCVTCC presented the overall progress of research work followed by individual project presentation by respective PI's. New research proposals were also

IRC meeting in progress

presented. The chairman, IRC advised all the scientists to apply for external funding and raised concern over publishing at least two papers per year per scientist in peer reviewed journals. Chairman emphasized on the development of more user friendly technologies for combating emerging and re-emerging diseases, utilization of donkey milk for cosmetic purpose and development of more practical oriented technologies for utilization and conservation of equines.

Half yearly meeting of Institute Research Committee (IRC)

Half yearly meeting of Institute Research Committee (IRC) for the year 2015-16 was conducted on 30th November & 1st December, 2015. Dr B. N. Tripathi chaired the meeting. Research progress was presented and the chairman raised concern over addressing the infertility in mares and stressed on preserving the microbial cultures in the freeze dried form in NCVTCC repository. Dr. S. S. Paul (Principal Scientist, CIRB, Animal Nutrition) joined the meeting as an expert and provided suggestions regarding preparation of area specific mineral mixture and emphasized upon analysis of ingredients in pre-prepared mineral mixture.

NCVTCC Annual Scientific Review meet (2014-15)

The sixth annual scientific review meet of NCVTCC was held at National Agricultural Science Centre, New Delhi on 18th November, 2015. The meeting was chaired by Prof. Gaya Prasad, Assistant Director General (Animal Health). Dr B.N. Tripathi, PC & Director, NRCE coordinated the meet and Dr Jyoti Misri (Principal Scientist, ICAR) and Nodal Officers/PIs/Co-PIs from different Network units attended the meet. Dr. Sanjay Barua, welcomed the participants and apprised the members about the progress made by the repository over the years. Dr. B.N. Tripathi initiated the meet by presenting an overview of NCVTCC network programme including salient research achievement of the network during the year and action taken on previous recommendations. Subsequently, Nodal Officers of all network units presented the achievements made during the year. Prof. Gaya Prasad, ADG (AH) addressed the gathering and said that preservation of microbes was very essential and ICAR is convinced that this program is very important. He emphasized that all units should submit unique and well characterized microbes to NCVTCC repository. During the meet, Prof. K.M.L. Pathak (DDG, AS), also interacted with the gathering and encouraged scientists of each unit to deposit more cultures.

NCVTCC Annual Scientific Review meeting in progress

XIX Research Advisory Committee (RAC) meeting (2015-16)

XIX meeting of Research Advisory Committee for the year 2015-16 was held at NRCE, Hisar on 10th March, 2016 under the chairmanship of Dr. R.N. Srinivas Gowda (Ex-Vice Chancellor, KVAFSU, Bangalore) for appraisal of research achievements of ongoing projects and to consider new research proposals. The RAC members participating in the meeting included Dr V. D. Sharma, Dr J. R. Rao, Dr K. C. Varshney, Dr Ashok Kumar (ADG, AH), Sh. Gajender Pal Singh Posana, (Progressive farmer from Jodhpur), Sh.

Ranjeet Pawar (AT & PO, Baramati) and Dr B. N. Tripathi (Director, NRCE). RAC recommended that the research emphasis should be on the development of effective vaccines against important viral (Equine influenza & EHV 1) and bacterial (*Rhodococcus equi*) diseases of equines. Further, it was emphasised that linkages with stakeholders like donkey & horse owner societies, private equine breeders, SAU's and Turf Authorities of India, etc., need further strengthening. Other recommendations of RAC included-development of package of practice for management of Rhodococcosis and other clinical conditions such as colic, lameness, bog spavin, etc.; formulation of region specific feeding schedule for equines using local resources after completing necessary nutritional studies; strengthening of bacteriophage repository including phage typing and establishment of automated accessioning system in NCVTCC in view of its future requirements.

37th Institute Management Committee (IMC) meeting

The meeting of the Institute Management Committee (IMC) was held on 1st March, 2016 under the Chairmanship of Dr. B. N. Tripathi (Director, ICAR-NRCE). The members attending the meeting included Dr Ashok Kumar (ADG, AH), Dr Gurdial Singh (Dean, College of Veterinary Science, LUVAS, Hisar); Dr Ashutosh Kumar (Head, CSWRI, Bikaner); Dr P. S. Yadav (Pr. Scientist, CIRB, Hisar); Dr Nitin Virmani (Pr. Scientist, NRCE, Hisar); Shri Gajendra Pal Singh Posana (Jaipur, Rajasthan), Sh. A. K. Sidharth (F & A.O., CSSRI, Karnal) and Mr. Ashok Barapatre (Administrative Officer, NRCE, Hisar) acting as Member Secretary. Discussions were held about the issues of previous meeting and current issues of the Centre. The IMC confirmed and adopted the proceedings of the 36th meeting of the Centre. Various agenda items *viz.*

IMC meeting in progress

preparation of open paddocks for equines at Livestock Farm, NRCE, Hisar, creating hostel facility in house nos. 1 & 2 in main campus, Hisar, converting 35 acre of farm land suitable for farming, purchase of various laboratory and feed related equipments, fixation of rates of equine diagnostic vaccines, etc., were discussed and approved.

Workshop, Seminar and Institutional Activities

Foundation Day

31st Foundation day of the Centre was organized on 26th November, 2015. Prof. P. K. Uppal (Advisor to Punjab Government Animal Husbandry), was Chief Guest on occasion while, Brigadier S. S. Kashyap, (Commandant, EBS, Hisar) and Dr Rameshwar Singh (DDG, DKMA, ICAR, New Delhi) presided the function as Guests of Honour. On this occasion, Interactive meet with progressive farmers, drawing competition for students and plantation of trees in NRCE campus were organized. Prof. P. K. Uppal appreciated research activities of NRCE and said that NRCE has made its mark of International level and extends all its activities to the stakeholders.

Prize distribution to the students

Address by Chief Guest

Organisation of Interactive meet with progressive equine owners on ICAR-NRCE Foundation Day

The Centre organized an interactive meet with progressive farmers on 26th November, 2015 to celebrate the Foundation Day. Equine owners from Haryana participated in the meet. The farmers had a close interaction with the scientists on the various aspects of animal health, management and welfare. The problems faced by the equine owners in equine husbandry were discussed at stretch with special reference to animal health and animal keeping and important information was disseminated to the farmers regarding feeding, artificial insemination and disease prevention. Farmers listened advices from the scientist very keenly. Director, NRCE addressed the farmers and encouraged them to follow good husbandry practices for better health of equines.

Organization of Drawing Competition on the topic "Horse as a companion animal"

On the eve of Foundation day of NRCE, a Drawing Competition was organized on 24th November, 2015 in the lawns of ATIC for school childrens. The theme of the competition was "Horse as Companion Animal". A total of 42 students from eight schools of Hisar participated in their respective categories: *viz.* below class V, class VI - VIII and class IX and above. The paintings were judged by external judges and first prize was bagged by Paramveer of class-V from Aryan public school, Sahil of class-VIII from Sukh Ram Memorial Public School and Vaishnavi Sharma of class-XI from O. P. Jindal Modern School. The prizes were distributed to the winners by Prof. P. K. Uppal, who was the Chief guest on this occasion.

Swachh Bharat Abhiyan at ICAR-NRCE

एक कदम स्वच्छता की ओर

Swachh Bharat Mission activities were initiated at ICAR-National Research Centre on Equines, Hisar during the year 2015-16. All the Scientists/ Officers/ staff members joined the campaign and participated in various activities and involved themselves in cleaning lawns, NRCE main entry gate area, NRCE approach road and building premises, workplace, laboratories, etc. This activity was carried out on regular basis every week. Total 22 sanitation drives were organized during the year 2015. All the NRCE-staff members participated in these activities and planned activities were worked out, which eventually helped cleaning of NRCE campus as a whole. There is a group leader for individual weekly activity (for two hours each), who plans and executes this campaign for that particular day. This campaign helped in cleaning of entire campus of NRCE and NCVTCC. This year staff weeded out old office records and made arrangements for proper upkeep of the office files thereafter.

NRCE also auctioned old condemn equipment's, furnitures, waste materials kept in the central store and different laboratories, etc. This activity generated revenue as well. Through this drive we were able to sensitize our staff members about cleanliness and sanitation. We also educated the equine owners about the importance of Swachhta through our regular health camps and other activities (Gosthis) meant for the farmers. An adjoining village Shamsukh was also adopted for implementing cleanliness drive as a part of Swachh Bharat Abhiyan and sensitizing the villagers with respect to sanitation. This activity was an additional drive besides routine weekly cleaning activity in the premises of the NRCE. A Swachh Bharat Logo has also been adopted for this mission in particular.

Swachh Bharat Abhiyan at ICAR-NRCE premises

Annual Convention of ISVIB (VIBCON-2015) at NRCE

ICAR-National Research Centre on Equines, Hisar (including National Conference for Veterinary Type Culture Collection) in collaboration with The Indian Society for Veterinary Immunology and Biotechnology (ISVIB) organized the XXII Annual Convention of Indian Society for Veterinary Immunology and Biotechnology (VIBCON-2015) and National Symposium on "Immunomics and Proteogenomics in Livestock Health & Productivity" during 17-19 December, 2015 under the Chairmanship of Dr. B.N. Tripathi. Dr. B.R. Gulati acted as the Organizing Secretary along with Dr. Sanjay Barua as the Co-Organizing Secretary. The Convention provided an excellent occasion for Scientists, academicians, students and industrialists pursuing research and development in diverse areas of Veterinary Immunology and Biotechnology for sharing their scientific data and interacting with renowned scientists in the country.

Dr K. M. L. Pathak, DDG (Animal Sciences) was the chief guest in the inaugural session. Addressing the delegates, Dr Pathak highlighted the use of immunological and biotechnological tools for augmenting livestock productivity and germplasm conservation. Dr M.P. Yadav, Former Vice Chancellor, SBBPUST, Meerut was the guest of honour on the occasion. Dr J.M. Kataria, Director, ICAR-CARI, Izatnagar was conferred the Dr Masillamony Oration

Award for his lifetime achievement in the area of avian research. Dr B.N. Tripathi, Director, ICAR-NRCE and Dr Parimal Roy, Professor, TANUVAS, Chennai were bestowed with the ISVIB Fellowship.

The conference was attended by 182 delegates from 20 different states of India, representing institutes of ICAR, DBT, ICMR, DST, State Agricultural Universities. In the conference, 209 research papers were presented during 3 days in 14 different sessions, Plenary session, 10 oral sessions and 2 poster sessions. Dr Anjali Somal from IVRI and Dr Adarsh Mishra from TANUVAS Chennai were conferred the Young Scientist Award for their outstanding research.

Dr Mamta Pandey from GADVASU, Ludhiana was conferred with the Women Scientist award. The Mid career award was given to Dr Yashpal Malik and Dr K.G. Tirumurugaan.

Addressing the valedictory function, Dr Gaya Prasad highlighted the need of research collaborations between human, veterinary & wild life scientists for understanding the emerging and re-emerging zoonotic diseases. Dr Ashok Kumar, ADG (Animal Health) emphasized the need for enhancing bio-security measures to avoid spread of transmission of pathogens.

Inaugural session of VIBCON-2015. Dr K.M.L. Pathak Vice Chancellor, Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya, Mathura, U.P. and Ex-DDG (AS), ICAR giving a talk during the session

World Veterinary Day Celebration

World Veterinary Day was celebrated at ICAR-NRCE, Hisar on April 25, 2015. Brigadier S. S. Kashyap, Commandant, Equine Breeding Stud, Hisar was present as Chief Guest of the programme. Students from Thakur Das Bhargav Sr. Secondary Model School attended the function.

On the occasion, lectures were given by Dr Nitin Virmani, Principal Scientist, NRCE on the theme topic "Survival with the flu" and Dr B. R. Gulati, Principal Scientist, on the topic "Vector-borne diseases with zoonotic potential in India." Chief Guest of the function, Brigadier S. S. Kashyap, Commandant Equine Breeding Stud, Hisar briefed about veterinary profession and activities of equine breeding stud. He also motivated students for career options in veterinary science. The students visited Info-equine museum at Agricultural Technology Information Centre (ATIC) of the Centre.

Address by Chief Guest

1st Annual Review Meeting of All India Network Programme on Neonatal Mortality in Farm Animals (AINP-NM) at ICAR-National Research Centre on Equines, Hisar

ICAR-NRCE organized 1st Annual Review Meeting of the AINP-NM on 22nd September, 2016. ICAR started this platform in this five year plan with the sole objective to find reasons of neonate mortality in farm animals and make strategies for its control. This programme has seven participant institutes and ICAR-Indian Veterinary Research Institute is the coordinating centre. The other institutes are - CIRG, Makhdoom; College of Veterinary & Animal Sciences, palampur; College of Veterinary Sciences, LUVAS, Hisar; ICAR-NRC on Pigs, Guwahati; ICAR-CSWRI, Avikanagar and ICAR-NRCE, Hisar. Dr Gaya Prasad, ADG (AH) chaired the session and meeting was coordinated by Dr R. K. Agarwal (Acting Project Coordinator), Head Department of Bacteriology and Mycology, IVRI, Bareilly. Dr Jyoti Mishri, Principal Scientist, ICAR New Delhi was also present in this meeting. Dr. B.N. Tripathi, Director, ICAR-NRCE, Hisar and Dr Sanjay Kumar, Principal Scientist, NRCE, Hisar

1st Annual Review Meeting of AINP-NM in progress

organized this meeting. This review meeting was attended by the entire PI and Co-PI of the project and reports were presented and achievements were discussed. Technical program for the next year (2016-17) was also finalized. The coordinators were satisfied with the progress of the research work done under this platform.

One Day Workshop on Glanders organized at ICAR-NRCE

ICAR-National Research Centre on Equines, Hisar organized "One Day Workshop on Glanders" on 7th October, 2015. The workshop was held under the chairmanship of Dr Suresh Honnappagol, Animal Husbandry Commissioner, Govt. of India. Maj Gen (Dr) Shri Kant, Vice-Chancellor, LUVAS, Hisar and Dr Inderjeet, Director, ICAR-CIRB, Hisar were also present as the Guest of Honour on the occasion. Dr B.N. Tripathi, Director, ICAR-NRCE welcomed the participants to the workshop and presented the overview of Glanders in India. Maj Gen (Dr) Shri Kant, Vice-Chancellor, LUVAS, Hisar shared his experience for controlling the Glanders in army horses at RVC. Chief Guest, Dr Suresh Honnappagol stressed on joint efforts by State Animal Husbandry Department in collaborative manner with NRCE, NGOs and

One day workshop on Glanders at ICAR-NRCE

Department of Animal Husbandry, Dairying and Fisheries for eradicating Glanders in India. He emphasized on more intense surveillance and monitoring for Glanders in affected states. On this occasion the compendium on Glanders was also released.

Vigilance Awareness Week at ICAR-NRCE from 26th to 31st October, 2015

The Vigilance Awareness Week was celebrated from 26 - 31st October, 2015. The theme for the Vigilance Awareness was "Preventive Vigilance as a Tool of Good Governance". Dr B. N. Tripathi, Director, ICAR-NRCE emphasized during his talk on changing ourselves and the system to prevent any misconducts and corruption. Vigilance awareness week was celebrated with the purpose of spreading awareness amongst officials about the ill effects of corruption.

Staff of ICAR-NRCE taking oath during vigilance awareness week

हिन्दी सप्ताह का आयोजन

राष्ट्रीय अश्व अनुसंधान केन्द्र में हिन्दी के प्रचार-प्रसार को प्रोत्साहित करने के लिए हिन्दी सप्ताह का आयोजन 23 से 30 सितम्बर, 2015 तक किया गया। इस दौरान विभिन्न प्रतियोगिताओं- निबंध प्रतियोगिता (महिलाओं के लिए), हिन्दी परिच्छेद अनुवाद प्रतियोगिता, हिन्दी कविता पाठ प्रतियोगिता (बच्चों के लिए), हिन्दी सुलेख प्रतियोगिता, हिन्दी प्रश्नोत्तरी प्रतियोगिता व रा.अ.अनु. केन्द्र व हिसार स्थित केन्द्रीय कार्यालयों के कर्मचारियों की कविता पाठ प्रतियोगिता का आयोजन किया गया तथा विजेताओं को पुरस्कार वितरित किए गए।

हिन्दी सप्ताह का समापन समारोह 30 सितम्बर, 2015 को मनाया गया जिसमें बतौर मुख्य अतिथि डॉ. नागेन्द्र शर्मा, पूर्व कुलपति, शेर-ए-कश्मीर पशु विज्ञान एवं प्रौद्योगिकी विश्वविद्यालय, जम्मू को आमंत्रित किया गया तथा विशिष्ट अतिथि के रूप में श्री अनिल कुमार राव, आई.जी., हिसार रेंज एवं डॉ. रमेश कुमार सेठी, पूर्व निदेशक, केन्द्रीय भैंस अनुसंधान संस्थान को आमंत्रित किया गया। कार्यक्रम का शुभारंभ मुख्य अतिथि तथा विशिष्ट अतिथियों द्वारा केन्द्र परिसर में पौधारोपण करके किया गया।

समापन समारोह के दिन केन्द्रीय कर्मचारियों के लिए कविता पाठ प्रतियोगिता करवाई गई एवं विजेताओं को पुरस्कार दिए गए। मुख्य अतिथि डॉ. नागेन्द्र शर्मा जी ने हिन्दी के उत्थान एवं हिन्दी की महता पर सम्बोधन दिया। विशिष्ट अतिथि श्री अनिल कुमार राव जी ने दैनिक कार्यों में हिन्दी के प्रयोग पर बल दिया। इस दौरान केन्द्र के निदेशक महोदय डॉ. भूपेन्द्र नाथ त्रिपाठी ने संस्थान के कर्मिकों को अधिकाधिक हिन्दी में कार्य करने के लिए प्रेरित किया।

हिन्दी सप्ताह समापन समारोह के मुख्य अतिथि कर्मचारियों को संबोधित करते हुए।

Mera Gaon Mera Gaurav

An innovative initiative "Mera Gaon Mera Gaurav" programme was launched by the Hon'ble Prime Minister on 87th Foundation day of ICAR at Patna on 25th July, 2015 with an aim to keep about 20,000 scientists of ICAR and AUs in direct touch with adopted villages. The main objective of the programme is to provide information on technical and other related aspects in a time frame to farmers.

All the scientists of the Centre were grouped in teams of four scientists to initially carry out Bench Mark Survey of 4 to 5 villages within 50 to 100 Km radius from their working place. Thirty one villages were selected in Hisar and Bikaner districts. Till December, 2015, bench mark survey of about 25 villages was completed by the teams altogether. Each group had been advised to visit selected villages regularly, to conduct Gosthis, initiate mobile advisory service, give literature support to the farmers and diagnose major problems.

Different activities during "Mera Gaon Mera Gaurav" programme

Exposure visit of farmers/ Educational tours of students

During 2015-16, visitors from various places including farmers, students from SAU's and schools visited NRCE, info-equine museum at ATIC (NRCE) and EPC, Bikaner campus. The visitors were briefed about research, extension and field activities of ICAR-NRCE.

S.No.	Details of Visitors	Date	No. of Visitors
1.	Exposure visit of farmers from Project Director, Agricultural Technology Management Agency (PD-ATMA), Rajasthan	15.05.2015	30
2.	Education Tour of students from Govt. College for Girls, Hisar	12.10.2015	75
3.	Exposure visit of farmers from Project Director, Agricultural Technology Management Agency (PD-ATMA), Rajasthan	07.11.2015	38
4.	Study tour of students from Centre for Conservation of Animal Diversity, Rajasthan Univ. of Veterinary and Animal Sciences (RAJUVAS), Rajasthan	20.12.2015	50
5.	Exposure visit of farmers from Project Director, Agricultural Technology Management Agency (PD-ATMA), Rajasthan	23.12.2015	42

S.No.	Details of Visitors	Date	No. of Visitors
6.	Study tour of students from Centre for Conservation of Animal Diversity, Rajasthan Univ. of Veterinary and Animal Sciences (RAJUVAS), Rajasthan	24.12.2015	55
7.	Studey Tour of students from Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan	08.01.2016	30
8.	Studey Tour of students from Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan	09.01.2016	45
9.	Studey Tour of students from Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan	14.01.2016	30
10.	Visit of trainees of ultrasonography from Central Institute for Research on Buffaloes, Hisar	16.03.2016	17

Services/consultancy provided to equine owners at ATIC

Agricultural Technology Information Centre (ATIC) at ICAR-NRCE, Hisar provides various services for the benefit of equines. In the year 2015-16, 17 cases of artificial insemination and 23 cases of pregnancy diagnosis / gynaecological examination and consultancy were provided to the equine owners at NRCE, Hisar. In addition, 385 consultancies were given to the equine owners for diseases, fertility problems, nutrition related and pregnancy diagnosis related issues through Kisan Call Centre at Toll Free number.

ICAR-NRCE's participation in exhibitions and fairs

ICAR-NRCE, Hisar participated in Agriculture Exibition at Piprakothi, East Champaran district Bihar on 20th August, 2015; in animal fair at ICAR-Central Institute for Research on Buffaloes, Hisar on 1st February, 2016, in an exhibition at College of Veterinary and Animal Sciences, RAJUVAS, Bikaner on 27th February, 2016 and in Krishi Unnati Mela at Pusa, New Delhi on 19th - 21st March, 2016.

Visit of Hon'ble Union Minister of Agriculture, GOI, Shri Radha Mohan Singh in Krishi Mela

Equine Health Camps and Kisan Goshtis

A total of 11 awareness camps, equine health camps, interactive meet with equine owners and Kisan Goshtis were organized by the scientists of NRCE, Hisar and EPC campus, Bikaner. During equine health camps, the animals were observed and treated for various ailments like wounds and injuries, parasitic infections, colic, etc. Deworming tablets and other medicines were provided to the equine owners for the benefit of equines. During the camps, AI with frozen Marwari horse semen and pregnancy diagnosis was also performed by trained staff of ICAR-NRCE.

S.No.	Place	Date	No. of equines/ Farmers benefited
1.	Dingsari village, Bikaner	May12, 2015	-
2.	Siwani, Bhiwani, Haryana	May 29, 2015	16
3.	Kaimri, Hisar, Haryana	June 30, 2015	15
4.	Rajli, Hisar, Haryana	July 16, 2015	31
5.	Pawta Chowk, Jodhpur, Rajasthan	August 21, 2015	-
6.	Singhana, Jind, Haryana	August 31, 2015	22
7.	Kachla, Badaun, UP	September, 09, 2015	44
8.	Nahianwala, Bhatinda, Punjab	October 26, 2015	13
9.	Umra, Hisar, Haryana	December 26, 2015	13
10.	Shamsukh, Hisar, Haryana	December 29, 2015	35
11.	Umra, Hisar, Haryana	January 6, 2016	10

An equine health camp at village Dingsari, Bikaner

An equine health camp near Powta Chowk, Jodhpur

International Yoga Day Celebration

International Yoga day was celebrated in the lawns of ATIC at NRCE on June 21st, 2015. The staff members including scientists and other staff members participated in the yoga session organized in the early morning from 6 - 7 a.m. The staff was benefited by performing various aasanas, pranayama and meditation performed during the session.

केन्द्र में आयोजित हिन्दी कार्यशालाओं का विवरण

राजभाषा कार्यान्वयन समिति के द्वारा हिन्दी कार्यशाला (विषय "वैज्ञानिकों के लिए हिन्दी का सरलीकरण") को दिनांक 03.09.2015 को केन्द्र के सभागार में आयोजित किया गया जिसमें केन्द्र के वैज्ञानिकों एवं अधिकारीगणों ने हिन्दी के सरल व सहज स्वरूप को समझा व सराहा। इस कार्यशाला को डॉ. वंदना पांडे, प्रोफेसर, गुरु जम्भेश्वर विश्वविद्यालय, हिसार द्वारा सम्बोधित किया गया। इस कार्यशाला में डॉ. वंदना पांडे ने हिन्दी के सरल व सहज रूप को बहुत ही उत्कृष्ट तरीके से वैज्ञानिकों के समक्ष रखा व उन्हें दैनिक प्रयोग में आने वाली शब्दावली से अवगत करवाया। उन्होंने हिन्दी के उत्थान तथा विकास का आहवान किया तथा वैज्ञानिकों व अधिकारियों को अधिकाधिक कार्य हिन्दी में करने के लिए प्रेरित किया।

हिन्दी की दूसरी एवं तीसरी कार्यशाला 4 दिसम्बर, 2015 एवं 03.03.2016 को करवाई गई जिसमें प्रशासनिक विभाग एवं अनुबंधित कर्मचारियों के लिए हिन्दी टंकण का प्रशिक्षण दिया गया। हिन्दी अधिकारी डॉ. अनुराधा भारद्वाज के निरीक्षण में प्रशासनिक अधिकारी द्वारा 11 कर्मचारियों को हिन्दी टंकण का अभ्यास करवाया गया। इस कार्यशाला से प्रतिभागियों को हिन्दी में कार्य करने के लिए प्रोत्साहित किया गया तथा हिन्दी के प्रयोग में आने वाली कठिनाइयों को दूर करने के प्रयास किए गए।

Infrastructure and Developmental Activities

Agriculture Farm Production

Production of crops

The agricultural land (about 112 acres) was rotationally cultivated for growing different crops. A total of 1005 Qt. green and 207 Qt. dry fodder were produced and used for feeding animals at Hisar campus (Table 1). During the year, 122.95 Qt. oat grains were produced in the agriculture farm, out of which 121.65 Qts were supplied for feeding to animals at EPC, Bikaner and 1.30 Qts were used as seed for sowing of green fodder in the farm at Hisar. The effort put in this activity not only resulted in self sufficiency of the Centre in terms of fodder requirement for animal feeding, but also yielded surplus crops for resource generation.

Table 1. Production of Green Fodder

Crop	Area (Acre)	Production (Qt.)
Oat+ Berseem	7.0	305.0
Berseem		39.0
Sorghum sudan grass + Cowpea	9.0	249.5
Sorghum sudan grass		203.0
Lucern	1.5	159.5
Cowpea	1.0	49.0

Landscape and plantation work

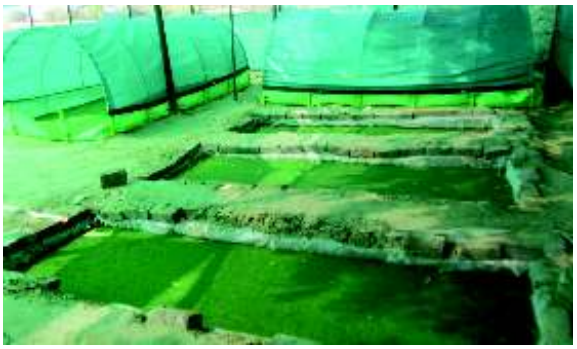
Activities were undertaken to improve the environmental condition of the campus. Different species of flowers, ornamental and shady plants were planted in the campus. The plantation around animal sheds and farm area will provide shelter to animals and farm people.

Resource generation

A sum of Rs. 4,70,838/- (Rs. Four lakh, seventy thousand eight hundred thirty eight only) was generated through sale of 125.33 Qt. mustard grain.

Agriculture farm production at EPC Bikaner

Various crops including kharif, rabi and annual crops were produced at the Centre for animal feeding. Kharif crops (sorghum, guar and groundnut) were cultivated on 29.50 acres; rabi crops (lucerne, oats & barley) on 28.50 acres and annual crops on 17.40 acres. A total of 2354.13 Qts of green fodder, 140.80 Qt. of dry fodder, 49.60 Qt. grain and 587.00 kg azolla were produced at the agriculture farm of EPC, Bikaner for the animals.



Azolla production at EPC, Bikaner

Livestock strength at ICAR-NRCE, Hisar and EPC, Bikaner

Various breeds of equines are being maintained in the nucleus herds at both Hisar and Bikaner campuses. Equine species which are being maintained includes - Marwari horses, Zanskari & Manipuri ponies and exotic & indigenous donkeys. The total herd strength is 115 animals including 87 animals at Bikaner campus and 28 animals at Hisar campus (Table 2). The stallions at Bikaner campus are primarily used for collection and cryopreservation of semen for artificial insemination.

Table 2. Equine herd strength at Equine Production Campus, Bikaner (2015-16)

Category	Marwari Horse		Pony				Donkey				Mule		Total
			Zanskari		Manipuri		Poitou		Indigenous				
	M	F	M	F	M	F	M	F	M	F	M	F	
Stock as on 01.04.2015	18	30	06	07	06	07	10	11	08	08	02	01	114
Birth during the year	03	05	01	01	01	01	–	03	–	–	01	–	16
Purchase during the year	–	–	–	–	–	–	–	–	–	–	–	–	–
Death during the year	01	01	–	01	01	–	01	–	01	01	–	–	07
Auctioned during the year	12	16	02	–	01	02	–	–	02	01	–	–	36
Balance as on 31.03.2016	08	18	05	07	05	06	09	14	05	06	03	01	87

Herd Strength at NRCE Main Campus, Hisar

The present herd strength of NRCE main campus is 28 animals, which include 21 Marwari horses, 2 ponies, 4 Poitou exotic donkeys (Table 3).

Table 3. Equine Herd Strength at NRCE campus, Hisar (2015-16)

S. No.	Kind of Animal		Opening Balance as on 01.04.2015	Birth	Death	Auctioned	Closing Balance as on 31.03.2016
1.	Horses	Mares	11	-	-	01	15
		Fillies	05*	-	-	-	01
		Colts	03	-	-	01	02
		Foals	01**	03	-	-	03
		Total	20	03	NIL	02	21
2.	Donkeys	Male	01	-	-	-	01
		Female	02	-	-	-	02
		Foals	-	01	-	-	01
		Filly	01	-	-	-	01
		Total	04	01	NIL	NIL	05
3.	Mules	Total	01	NIL	NIL	01	NIL
4.	Pony	Total	02	NIL	NIL	NIL	02
Grand Total			**27**	**04**	**NIL**	**04**	**28**

*Fillies matured to adult mares
**Matured to filly

Cryopreserved semen bank at EPC, Bikaner

Frozen semen bank of Marwari, Zanskari and Manipuri horses is being maintained at EPC, Bikaner. Good quality semen doses are cryopreserved every year for use in artificial insemination. During current year, a total of 55 semen doses of Marwari (20 doses), Zanskari (23 doses) and Manipuri (12 doses) horses were cryopreserved. Besides, 18 doses of indigenous jacks were also stored in the frozen semen bank for future use and conservation.

Resource generation

A sum of ₹ 8,86,400/- (Rs. Eight lakh, eighty six thousand four hundred only) was generated through the auction of farm equines (36 Nos.) from EPC, Bikaner.

Infrastructure development at NCVTCC

The developmental activities including furnishing of new phase building and animal house construction at National Centre for Veterinary Type Culture Collection was undertaken. The construction of second phase of the laboratory building has been completed, which will further strengthen the laboratory facilities of the repository (Fig. 4 & 5). The internal and external furnishing of the 1st and IInd phase buildings, respectively is being taken up through CPWD.

Fig. 4. NCVTCC IInd phase building

Fig. 5. Small Animal House, NCVTCC

On-Going
Research Projects (2015-16)

Equine Health

S.No.	Title	Team	From	To	PIMS Code
1.	Surveillance, monitoring and control of emerging and existing diseases of equines	S.K. Khurana*, S.C. Yadav, B.R. Gulati, Rajender Kumar, Sanjay Kumar, N. Virmani, Sanjay barua, Rajesh Vaid, Ramesh Dedar, H. Singha, Anju Manuja, Balvinder Kumar and B.N. Tripathi	April, 1995	Continuous Service Project	IXX00257
2.	Evaluation of in vitro growth inhibitory efficacy of some novel synthetic drug molecules against *Theileria equi* haemoprotozoa	Sanjay Kumar*, Rajender Kumar and A.K. Gupta	Nov., 2013	March, 2017	IXX10288
3.	Investigations on Neuropathogenic and Non–neuropathogenic Variants of Equine Herpes Virus 1 and associated Latency among Equines in India	B. R. Gulati*, Nitin Virmani and Riyesh T.	Sept., 2013	March, 2017	IXX10275
4.	Pathology of EHV 1 infection in BALB/c mice post-immunization with glycoprotein (gB, gD & gM) and bacterial artificial chromosome construct of EHV 1	Nitin Virmani*, B.R. Gulati and B.C. Bera	Oct., 2013	March, 2017	IXX10287
5.	Development of diagnostics for emergency preparedness and monitoring of emerging equine viral diseases	Balvinder Kumar*, H.S. Singha and Anju Manuja	April, 2014	March, 2017	IXX10853

*Principal Investigator

Equine Production

S.No.	Title	Team	From	To	PIMS Code
1.	Endocrine, biochemical and gene expression profiling of reproductive states in Marwari Mares	Vijay Kumar*, Sanjya Kr. Ravi, R.K. Dedar and Raghvendra Singh	Oct., 2012	March, 2016	IXX09663
2.	Evaluation of total mixed rations for maintenance horses	P.A. Bala*, R.K. Dedar and N.V. Patil	March, 2014	Feb., 2017	IXX11646
3.	Cryopreservation of semen, artifical insemination and pregnancy diagnosis in equines (Service Project)	S.K. Ravi*, T.R. Talluri, J. Singh, R.A. Legha, Yash Pal and A.K. Gupta	April, 2015	March, 2018	IXX11844
4.	Optimization of interspecies somatic cell nuclear transfer technique for production of horse (*Equus caballus*) cloned embryos	NRCE : T.R. Talluri*, Sanjay Kumar Ravi and Taruna Anand CIRB : Naresh Selokar*, Dharmendra Kumar and PS Yadav	Nov., 2015	Feb., 2018	IXX12608
5.	Characterization of donkey milk with emphasis on important milk proteins	Yash Pal*, R.A. Legha, Anuradha Bhardwaj Sanjay Kumar & A.K. Mohanty	Oct., 2012	Sept., 2016	IXX07761

S.No.	Title	Team	From	To	PIMS Code
6.	Development of rapid diagnostic test for pregnancy diagnosis in horse mares	A.K. Gupta*, Yash Pal, Sanjay Kumar and Sanjay Kumar Ravi	Jan., 2015	June, 2017	IXX11645
7.	Genetic characterization of Marwari horses for selection of true to breed animals	Anuradha Bhardwaj*, A.K. Gupta, Yash Pal, Mamta Chauhan and Vijay Kumar	July, 2015	June, 2018	IXX12220
8.	Development of DNA typing facility for parentage testing in horses	Mamta Chauhan*, Anuradha Bhardwaj, Yash Pal, B.N. Tripathi and A.K. Gupta	Oct., 2015	March, 2017	IXX12431

*Principal Investigator

Externally Funded Projects

S.No.	Title	Team	Location	Project Budjet Sanctd. from	Date of Start	Date of Completion	PIMS Code
1.	National Fellow Scheme-Development of sensitive and specific diagnostic assays for detection of *Trypanosoma evansi* infection in animals using modern molecular tools	Rajender Kumar*	NRCE	ICAR	April, 2011	April, 2016	OXX01431
2.	All India Coordinated Research Project on Utilization of Animal Energy with enhanced system efficiency (AICRP on UAE)	R.A. Legha, Yash Pal and Vijay Kumar	EPC Bikaner	ICAR	July, 2009	March, 2017	OXX00486
3.	Characterization of donkeys of Rajasthan Network Project from NBAGR, Karnal	Yashpal*, A.K. Gupta and R.K. Dedar	NRCE	NBAGR	April, 2014	Sept., 2016	OXX02851
4.	Synthesis, characterization and evaluation of drug loaded nano-formulation against *Trypanosoma evansi* in animal model	Anju Manuja*, Neeraja and Dilbaghi, Sandeep Kumar, Rajender Kumar, Balvinder Kumar and S.C. Yadav	NRCE	DST	March, 2012	Sept., 2015	OXX01526
5.	Eukaryotic expression of important equine cytokines and analysis of their biological activities	H. S. Singha*	NRCE	SERB	Jan., 2013	Dec., 2015	OXX02228
6.	DBT-NER Centre for Advanced Animal Diagnostics and Services on Animal Health and Diseases	B.N. Tripathi*, Sanjay Barua, Nitin Virmani, S.C. Yadav, B.R. Gulati, Rajender Kumar, R.K. Vaid, B.C. Bera, Taruna Anand and Riyesh T.			Sept., 2013	April, 2019	
7.	OIE Twining program for Glanders	H.S. Singha* and B.N. Tripathi	NRCE	OIE Twinning	July, 2012	March, 2016	OXX02428
8.	Twining program for Equine Influenza	Nitin Virmani*, R.K. Vaid, B.C. Bera and B.N. Tripathi	NRCE	OIE Twinning	Oct., 2012	Sept., 2015	OXX02429

S.No.	Title	Team	Location	Project Budjet Sanctd. from	Date of Start	Date of Completion	PIMS Code
9.	Development of nano gold based immuno- chromatography/immune dot blot assay for detection of *Trypnosoma evansi*	Neeraj Dilbaghi*, S.C. Yadav, Sandeep Kumar and A.K. Gupta	NRCE	DST	March, 2014	March, 2016	OXX02873
10.	All India Network Programme on Neonatal Mortality in Farm Animal	Sanjay Kumar*, Ramesh Dedar, B.R. Gulati, Nitin Virmani and S.K. Khurana	NRCE	ICAR	Jan., 2015	March, 2017	OXX03934
11.	CRP on Vaccines and Diagnositics	B.R. Gulati*, Component-1 (B.R. Gulati & Nitin Virmani) Component-II (Nitin Virmani, B.R Gulati & B.C. Bera) Component -III (Sanjay Kumar & Rajender Kumar)	NRCE	ICAR	May, 2015	March, 2017	OXX03182
12.	CRP on Agrobiodiversity (Sub Project & Management of NCVTCC)	Sanjay Barua*, R.K. Vaid, Naveen Kumar, Taruna Anand, B.C. Bera and Riyesh T.	NRCE	ICAR	August, 2015	July, 2017	OXX03183
13.	Targetting a host cell protein kinase for development of antiviral therapeutics against PPR virus	Naveen Kumar* and Sanjay Barua	NRCE	SERB, DST	August, 2015	March, 2018	OXX03186
14.	Generation of reverse genetics based equine influenza virus and explore its potential as vaccine candidate through challenge studies in mice model	NRCE : Nitin Virmani NIHSAD, Bhopal : Dr Sandeep Bhatia, National Fellow & PS, Richa Sood, Naveen Yadav NCVTCC: B.C. Bera, Taruna Anand	NRCE		April, 2015	March, 2018	OXX12259
15.	Pathogenicity and immunogenicity of recombinant mutant equine herpes virus-I (in tissue explants and murine model and their potential as vaccine candidate(s)	Nitin Virmani, B.C. Bera and Taruna Anand	NRCE	ICAR	Jan., 2016	March, 2017	OXX03412
16.	Feasibility studies on bio-gas and compost production from mule dung in hilly regions in India	B.N. Tripathi*, R.K.Vaid and R.A. Legha	NRCE	DST	Nov., 2015	March, 2017	OXX03413
17.	Validation study of a western blot (WB) technique and ELISAs for serological diagnosis of glanders in equids for the purpose of certifying freedom from infection in individual animals for trade or movement	H.S. Singha*	FLI FRIAH Germany	DST	Dec., 2015	March, 2017	OXX03410
18.	Generation of induced pluripotent stem (iPS) cells from buffalo fetal fibroblasts through non-viral approaches	CIRB: Dharmendra Kumar, Naresh Lalaji Selokar and P. S. Yadav NRCE/NCVTCC: Taruna Anand, B.C. Bera and Nitin Virmani	CIRB + NRCE	DBT	Feb., 2016	July, 2017	OXX03592

NCVTCC

S.No.	Title	Team	From	To	PIMS Code
1.	Development of protein expression clone repository of virulence associated genes of zoonotic buffalopox and equine influenza viruses	B.C. Bera*, Sanjay Barua, Nitin Virmani, Taruna Anand and Riyesh T.	Jan., 2012	March, 2016	IXX07760
2.	Development of bacteriophage repository	Taruna Anand*, R.K. Vaid, Sanjay Barua and B.C. Bera	Oct., 2013	Oct., 2016	IXX10698
3.	Authentication and accessioning of viruses of animal origin	Sanjay Barua*, Naveen Kumar, B.C. Bera, Riyesh T. and Taruna Anand	May, 2015	Service Project	IXX11882
4.	Isolation, characterization and Development of repository of poxviruses of caprine, ovine and bovine origin	Sanjay Barua*, Naveen Kumar, B.C. Bera and Riyesh T.	May, 2015	April, 2018	IXX11883
5.	Phenotypic and genotypic authentication and preservation of network bacterial isolates	R.K. Vaid* Taruna Anand, B.C. Bera and Riyesh T.	June 2015	Service Project	IXX12436
6.	Prevalence studies for porcine respiratory viruses and development of their repository	B.C. Bera*, Sanjay barua, Taruna Anand and Nitin Virmani	Jan., 2016	Dec., 2018	IXX12436

*Principal Investigator

Research

Publications

Research Articles

International

1. Anand, T., Vaid, R.K., Bera, B.C., Barua, S., Riyesh, T., Virmani, N., Yadav, N. and Malik, P. 2015. Isolation and characterization of a bacteriophage with broad host range displaying potential in preventing bovine diarrhoea. *Virus Genes,* 51 (2): 315-321.
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Popular articles /leaflet

1. Bhardwaj, A., Pal, Y. and Gupta, A.K. 2015. Ashava Genome: ek avlokan. Pashudhan Prakash, VI: Pp 44-47.
2. Pal, Y., Legha, R.A., Sharma, P. and Bhardwaj, A. 2016. Khachchar utpadan me Rashtriya Ashav Anusandhan Kendra ka yogdaan. Pashudhan Prakash (Accepted).

Books / Technical bulletin published

1. Khurana, S.K., Singha, H. and Tripathi B.N. 2015. Compendium of one day workshop on glanders. Published by ICAR-NRC on Equines, Hisar, October 7, 2015. Pp 1-83.
2. Ravi, S.K., Legha, R.A., Talluri, T.R., Dedar, R.K., Sharma, R.C., Pal, Y., Tripathi, B.N. and Singh, R.K. 2015. Cryopreservation of horse and donkey semen. Published by Director, ICAR - National Research Centre on Equines, Sirsa Road, Hisar, Haryana.
3. Tripathi, B.N., Virmani, N., Barua S., Yadav, S.C., Gulati B.R., Rajender Kumar, Vaid, R.K., Anand T., Bera, B.C. and Riyesh T. published Training manual on Demonstration of In-house diagnostic tests developed by NRCE for detection of viral and protozoan diseases and development of repository of veterinary microbes', organized under DBT-NER project on Advanced animal disease diagnosis and management consortium (ADMaC), Published by ICAR - National Research Centre on Equines, Sirsa Road, Hisar, Haryana from February 29 - March 5,

2016.

4. Singha, H., Malik, P., Khurana, S.K, Singh, R.K, Tripathi, B.N., Neubauer, H., Elschner, M. and Melzer, F. 2016. Technical Bulletin for International workshop on "Surveillance and diagnosis of equine glanders" for SAARC countries organized under OIE twinning program on Equine glanders, Published by National Research Centre on Equines, Sirsa Road, Hisar, Haryana, India.
5. Virmani N, Bera B. C, Vaid, R. K, Singh, R.K, Tripathi B.N. 2016. Technical Bulletin of 10 days international workshop on "Surveillance and Diagnostics for Equine Influenza" for Soutn Asian Association for Regional Cooperation (SAARC) country participants, Edited by Taruna Anand. Published by ICAR-NRC on Equines, Hisar, February 16- 25, 2016. Pp 1-80.

Chapter in Book/Compendium/ Technical bulletin

6. Balhara, A.K., Nayan, V. and Bhardwaj, A. 2016. Advances in development of pregnancy diagnostics: Strategies for development of field usable cow side pregnancy diagnostic tests. In compendium of CAFT Course on "Development of Validated Diagnostic Assay and Accreditation of Diagnostic Laboroaties", Department of Veterinary Microbiology, LUVAS, Hisar, February 3-23, 2016.
7. Gulati B.R. 2016. Concepts and procedures in establishment and maintenance of biosafe laboratories for veterinary pathogens. In compendium of CAFT Course on "Development of Validated Diagnostic Assay and Accreditation of Diagnostic Laboratories", Department of Veterinary Microbiology, LUVAS, Hisar, February 3-23, 2016.
8. Gupta, A.K., Pal, Y., Bhardwaj, A., Kumar, S., Legha, R.A. and Tripathi, B.N. 2016. Breed characteristics and DNA based evaluation of Marwari horses. In, Souvenir of All India Marwari Horse Society, Pp 38-44.
9. Legha, R.A. 2015. Cryopreservation techniques of Equine semen. In, IMV Technologies, News Letter.
10. Nayan, V., Bhardwaj, A., Balhara, A.K., Jerome, A. and Sharma, R.K.. 2016. Potential applications and prospects of nanotechnology for Animal reproductive biotechnologies. In, MTC compendium on Ultrasonography in large animal reproduction for fertility augmentation. CIRB, February, 2016.
11. Prasad, M., Ranjan, K., Deara, A., Dahiya, S., Khurana, S.K., Deswal, S. and Prasad, G. 2016. Rotavirus Genomic Diversity in India Using Molecular and Computer Tools. In, Vistas in Computer Aided Agri-Bio-Engineering Technologies, 1st ed. by R.K. Behl, Bhatia, R., Singh, B., Hasija, M., Khatkar, V., Deswal, S., 01/2016; AGROBIOS (International), India.
12. Ravi, S.K., Talluri, T.R., Legha, R.A. and Tripathi, B.N. 2016. Artificial Insemination in Horses: An Indian Perspective. In, All India Marwari Horse Society Souvenir. Pp 68-72.
13. Singh, J., Talluri, T.R., Legha, R.A. and Tripathi, B.N. 2016. Equine Management and Feeding. In, All India Marwari Horse Society Souvenir. Pp 87-91.
14. Virmani Nitin. 2015. An overview of Equine Influenza and Indian scenario. Published in "Influenza: Risk Factors, Massive Impact and Uncertain Future" ICAR-IVRI, Izatnagar on 19 October, 2015.
15. Yadav S.C. Kumar, R., Tehri, K. and Dayal, R.K. 2015. Diagnostic techniques for *Trypanosoma evansi:* A laboratory manual. Published by ICAR-NRC on Equines, Hisar, February 16- 25, 2016.

Participation in Training,
Workshop, Conferences and Symposia

International trainings, meetings and visits abroad

1. Dr B. N. Tripathi visited Animal Health Trust, UK under the OIE Twinning Laboratory project on "Equine influenza" from July 16-24, 2015.
2. Dr Nitin Virmani delivered invited lecture in '3rd International Symposium on Neglected Influenza Viruses', held at 'The Georgia Center' in University of Georgia, Athens, Georgia, USA from April, 15 -17, 2015.
3. Dr Nitin Virmani participated in capacity building and training program in the area of next generation sequencing, validation of assays developed by our laboratory, ISO 17025 certification training at Animal Health Trust, UK under the OIE Twinning Laboratory project on "Equine influenza" from July 17, 2015 to August 14, 2015.
4. Dr B. C. Bera participated in capacity building and training program in the area of next generation sequencing, validation of assays developed by our laboratory, ISO 17025 certification training at Animal Health Trust, UK under the OIE Twinning Laboratory project on "Equine influenza" from July 17, 2015 to August 14, 2015.
5. Sh Mukesh Chand attended capacity building training in the area of diagnosis and validation of assays developed by our laboratory at Animal Health Trust, UK under the OIE Twinning Laboratory project on "Equine influenza" from July 16-21, 2015.
6. Dr Nitin Virmani attended "Expert Surveillance Panel" meeting on Equine Influenza for vaccine strain selection organized by OIE held at head quarters in Paris, France through Skype on a formal invitation from Chairman of the ESP group on Equine Influenza, Dr Ann Cullinane on March 1, 2016.

Participation in trainings

1. Naveen Kumar attended capacity building workshop on Biorisk management, National Center for Disease Control, New Delhi in collaboration with CDC, Atlanta, USA held at New Delhi, May 5-9, 2015.
2. P. A. Bala attended winter school training on "Livestock and Climate change: Challenges and aays ahead for Sustainable Production" at NIANP, Bangalore from

Participation in Conference/Workshop/Symposia/ Meetings/Farmer's Day

1. A.K. Gupta delivered expert lecture on "Breed characteristics and DNA based evaluation of Marwari horses" in Marwari Horse Judge Clinic, Balsamand Lake Palace, Jodhpur July 18 to 19, 2015.
2. A.K. Gupta, S. C. Yadav, Yash Pal, S.K. Khurana, Rajender Kumar, Nitin Virmani, R.A. Legha, Sanjay Kumar, R.K.Vaid, Balwinder Kumar, Anju Manuja, Mamta Chauhan, Vijay Kumar, Naveen Kumar, Taruna Anand, Harisankar Singha, Anuradha Bhardwaj, B.C.Bera, S.K. Ravi, Ramesh Dedar, Riyesh,T. and P.A. Bala participated in XII Annual Convention of Indian Society of Veterinary Immunology and Biotechnology and National Symposium on 'Immunomics and Proteogenomics in Livestock'- VIBCON 2015 organized by ICAR-National Research Centre on Equines, Sirsa Road, Hisar from December 17-19, 2015.
3. Anju Manuja attended National Conference on "Biotechnology: Emerging Trends (NCB-2016)", held at Chaudhary Devi Lal University, Sirsa, Haryana from February 11-12, 2016.
4. Anuradha Bhardwaj, Sanjay Kumar and A K Gupta participated in 6th World Congress on

Biotechnology, October 05-07, 2015 New Delhi, India.

5. Balwinder Kumar attended National Conference on "Biotechnology: Emerging Trends (NCB-2016)", held at Chaudhary Devi Lal University, Sirsa, Haryana from February 11-12, 2016.
6. B. N. Tripathi attended and delivered an invited lecture in the Technical Session on "Equine Management" in 8th National Livestock Championship & Expo-2016, Mukatsar Sahib on January 01, 2016.
7. B. N. Tripathi participated and acted as Chairman for organizing XII Annual Convention of Indian Society of Veterinary Immunology and Biotechnology and National Symposium on 'Immunomics and Proteogenomics in Livestock'-VIBCON 2015, ICAR-National Research Centre on Equines, Sirsa Road, Hisar, December 17-19, 2015.
8. B. N. Tripathi participated in one day workshop of CRP on Agrobiodiversity at NBPGR, New Delhi held under the Chairmanship of Secretary DARE & Hon'ble DG, ICAR, on October 14, 2015.
9. B. N. Tripathi participated in Workshop on "Non-Putrefying Properties of Ganga water" organized by Director, AIIMS, New Delhi on November 17, 2015.
10. B. N. Tripathi participated in XIV NAVS Convocation-cum-Conference on "Antimicrobial Resistance in Livestock Health and Production" held at IVRI, Izatnagar on November 04, 2015.
11. B. N. Tripathi participated in XXXII Annual Meeting of IAVP and National Symposium on Challenges and advances in disease diagnosis of livestock, poultry and fish oraganized by Department of Veterinary Pathology, NTR College of Veterinary Science, Sri Venkateshwara University, Gannavaram 521102, AP from December 3-5, 2015.
12. B. N. Tripathi, S. K. Khurana and Harisankar Singha participated in one day workshop on "Glanders", ICAR-National Research Centre on Equines, Sirsa Road, Hisar , October 7, 2015.
13. B. N. Tripathi delivered expert lecture in 1st Annual Conference of SRL & NAWAR and National Symposium on "Concepts in Zoonoses & Health in New Millenium" held in Nagpur from October 19-20, 2015.
14. B. R. Gulati attended XXIV National Conference of Indian Virological Society on "Transboundary Viral Diseases under One Health: Perspectives and Challenges" held at North Eastern Indira Gandhi Regional Institute of Health & Medical Sciences (NEIGRIHMS), Mawdiangdiang, Shillong, Meghalya from October 8-10, 2015.
15. B.R. Gulati and Sanjay Barua, participated and acted as Organizing Secretary and Co-organizing Secretary for XII Annual Convention of Indian Society of Veterinary Immunology and Biotechnology and National Symposium on 'Immunomics and Proteogenomics in Livestock'-VIBCON 2015, ICAR-National Research Centre on Equines, Sirsa Road, Hisar, December 17-19, 2015.
16. H. Singha participated in one day workshop on Glanders, ICAR-NRCE, Hisar, October 7, 2015.
17. Nitin Virmani participated in National Workshop on "Influenza: Risk Factors, Massive Impact and Uncertain future - 2015", Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, October 19, 2015.
18. Nitin Virmani participated in XXXII Annual Meeting of IAVP and National Symposium on "Challenges and advances in disease diagnosis of livestock, poultry and fish", Department of Veterinary Pathology, NTR College of Veterinary Science, Sri Venkateshwara University, Gannavaram, AP, December 3-5, 2015.
19. P.A. Bala attended workshop on "Disaster Management in Animals: A Renewed Approach and Future Vision", College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, February 20, 2015.
20. P.A. Bala participated in XVI Biennial Animal Nutrition Conference on "Innovative Approaches for Animal Feeding & Nutrition

Research", ANSI-ICAR-NDRI, Karnal, February 6-8, 2016.

21. R. A. Legha attended one day workshop on "Climate change mitigation and adaptation in hot arid region" under NAIP project at ICAR-CAZRI, RRS, Bikaner on February 26, 2016.
22. R. A. Legha attended workshop on "Improving water productivity in IGNP- Expanding Dimensions" organized by ICAR- CAZRI, RRS, Bikaner on March 2, 2016.
23. R. A. Legha attended XXXI Annual Convention of The Indian Society for Study of Animal Reproduction (ISSAR) & National symposium on current challenges and opportunities in animal reproduction, Department of Obstetrics & Gynaecology, Veterinary College, Bengaluru, from December 3-5, 2015.
24. R. A. Legha participated and acted as convener of National Seminar on "Agriculture Resource Management for sustainability and Eco-restoration" at ICAR-CIAH, Bikaner from 11-13, March, 2016 organized by Society for Agriculture and Arid Ecology Research.
25. R. A. Legha attended 3rd Biennial National Conference of Indian Veterinary Nutrition and Animal Welfare, CSK HPKV, Palampur, HP from November 4-5, 2015.
26. R. K. Vaid attended National Conference on "Role of credible inspection and certification system in facilitating export trade held at India Habitat Centre, New Delhi on April 22, 2015.
27. R. K. Vaid participated in XIV National Convocation and Conference of National Academy of Veterinary Sciences on "Antimicrobial resistance in Livestock Health and Production" at IVRI, Izatnagar on November 4, 2015.
28. R.K. Vaid attended workshop on Next Generation Sequencing data Analysis organized by BioNivid, Chandigarh on July 14, 2015.
29. Rajender Kumar participated in XXV National Congress of Veterinary Parasitology held at Madras Veterinary College, TANUVAS, Mannuthy, Chennai from February 17-19, 2016.
30. S. K. Ravi delivered lecture in XXXI Annual Convention of The Indian Society for Study of Animal Reproduction (ISSAR) & National symposium on Current Challenges and Opportunities in Animal Reproduction held at Department of Obstetrics & Gynaecology, Veterinary College, Bengaluru from December 3-5, 2015.
31. S.K. Khurana delivered lead paper in 3rd IAVNAW Conference held at COVAS, CSKHPKV, Palampur, India from November 4-5, 2015.
32. S.K. Khurana attended one day workshop on Glanders at ICAR-NRCE, October 7, 2015.
33. Sanjay Barua delivered lecture in CRP on Agro-biodiversity (CRP-AB) Workshop held at ICAR-NBPGR, New Delhi on October 14, 2015.
34. Sanjay Barua, R.K. Vaid & Taruna Anand attended VTCC Network Unit Annual Meet at NAAS Complex, New Delhi on November 18, 2015.
35. Sanjay Kumar attended workshop of Nodal Officers of ICAR on 'Research Data Repository for Knowledge Management', from August 4 - 5, 2015.
36. Sanjay Kumar attended workshop on "Drug discovery technology: a molecular modelling approach" organized by Department of Bio And Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana from March 28 -30, 2016.
37. Sanjay Kumar attended XXV National Congress of Veterinary Parasitology and National Symposium on One Health Approach – Plausible solution for sustainable parasite control, held at Department of Veterinary Parasitology, Madras Veterinary College, Chennai, from February 17-19, 2016.

38 Sanjay Kumar participated in workshop of HRD Nodal Officers at NAARM, Hyderabad from February 10-12, 2016.

39. Taruna Anand attended workshop on 'Next Generation Sequencing data Analysis' organized by BioNivid, at Chandigarh on July 14, 2015.

40. T. R. Talluri delivered invited lecture in XXXI Annual Convention of The Indian Society for Study of Animal Reproduction (ISSAR) & National Symposium On "Current Challenges and Opportunities in Animal Reproduction" held in Bangalore from December 3-5, 2015.
41. T. R. Talluri delivered invited paper in International livestock Conference & Expo 23rd Annual Convention, ISAPM-2016 held at Hyderabad from January 28-31, 2016.
42. T. R. Talluri participated in one day workshop on " Climate change mitigation and adaptation in hot arid region" under NAIP project held at ICAR-Central Arid Zone Research Institute (CAZRI), Regional station, Bikaner on February 26, 2016.
43. Vijay Kumar attended National Conference on "New horizons of veterinary and medical forensic medicine" held at Rajasthan University of Veterinary and Animal Sciences, Bikaner from March 5-6, 2016.
44. Yash Pal attended 3rd Biennial National Conference of Indian Veterinary Nutrition and Animal Welfare at CSK HPKV, Palampur, HP. from November 4-5, 2015.
45. Yash Pal delivered lecture in training programme on 'Ultrasonography in Large Animal Reproduction for Fertility Augmentation' held at CIRB, Hisar from June 22-27, 2015.

Visit of Dignitaries

● Dr. H. Rahman, DDG (Animal Science), ICAR visited NRCE on 9th January, 2016. Dr A. K. Srivastava, Director, National Dairy Research Institute, Karnal and Dr Inderjeet Singh, Director, Central Institute for Research on Buffaloes, Hisar joined him during the visit. Dr. Rahman addressed the staff of NRCE and congratulated the whole staff for receiving the Sardar Patel Outstanding ICAR Institution Award. During his visit he was shown research and various activities at NRCE campus Hisar and VTCC. He was briefed about

the BSL-III laboratory and info-equine museum at ATIC centre. He wished the Director and staff of NRCE all the success in future endeavours.

● Dr Rameshwer Singh, Project Director, DKMA visited the Centre on 4th April 2015. Dr B.N. Tripathi, Director, NRCE accompanied him for a visit around the campus and elaborated various institutional activities. During his visit to info-equine museum at NRCE, he was briefed about evolution of equines, the breeds of horses in India, equine-related accessories and glimpses of research and extension activities at the Centre.

● Shri Dushyant Chautala, Hon'ble Member of Parliament, Govt. of India and Member of Governing body of Indian Council of Agricultural Research (ICAR), New Delhi visited Equine Production Campus (EPC), Bikaner on 4th September, 2015. Shri Chautala visited research laboratories and institute equine farm and learnt about the present practices of housing and management. He appreciated the achievements and ongoing research and emphasized on extending the research results to the farmers. He assured his full support to ICAR-NRCE towards such conducting activities for the benefit of farmers.

● Dr Arjava Sharma, Director, NBAGR visited NRCE on 23rd September 2015. He visited various laboratories, NCVTCC and info-equine museum at the Centre. Various extension activities and research activities were briefed to him. Dr Arjava Sharma was impressed about the research and services being provided by NRCE in the field of diagnostics and vaccines.

● Professor Nagendra Sharma, former Vice Chancellor, Shere-E-Kashmir University of Animal Science and Technology, Jammu and Ex-Director, NDRI Karnal visited the Centre on 30th September, 2015. Dr B.N. Tripathi, Director, NRCE briefed him about various activities of NRCE, NCVTCC and EPC campus Bikaner. He addressed the scientific community and conveyed that NRCE is well planned and well managed research centre.

- Dr R K Singh, Director cum Vice Chancellor, IVRI, Izzatnagar, Bareilly visited the Centre on 18th December, 2015 during XXII Annual Convention (VIBCON-2015) of ISVIB. Being former Director of NRCE, Dr Singh was very keen to know about the

progress of the Centre. He said that his memories were refreshed during this visit and found the maintenance of the Centre and museum excellent.

- Padma Bhushan Dr R.S. Paroda, Ex-Director General ICAR and Secretary, DARE, visited NRCE on 1st February, 2016. During his visit, he was briefed about the research activities of NRCE-Hisar, EPC-Bikaner and NCVTCC. He visited various facilities viz. BSL-III

laboratory, info-equine museum, NCVTCC and different laboratories of the Centre. He congratulated the Director and whole staff of NRCE for receiving the Sardar Patel Outstanding ICAR Institution Award.

Personnel
Milestones

Awards and Recognition

- ICAR-NRCE was conferred Sardar Patel Outstanding ICAR Institution Award for the year 2014 for research conducted in the last five years. The award was conferred by Hon'ble Prime Minister of India, Shri Narendra Modi Ji on '87th Foundation Day' of ICAR organized in Patna, Bihar on July 25, 2015.

- ISO 9001:2008 certification of the Centre. The Centre was awarded the ISO 9001:2008 certificate by URS Certification Pvt. Ltd., NOIDA, U.P.

- Dr B. N. Tripathi received Fellowship of Indian Society for Veterinary Immunology and Biotechnology during XII Annual Convention of Indian Society of Veterinary Immunology and Biotechnology organized by ICAR-National Research Centre on Equines, Sirsa Road, Hisar, December 17-19, 2015.

- Dr B. R. Gulati was awarded the Fellowship of National Academy of Veterinary Sciences on October 28, 2015 at Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P.

- Dr Pavulraj, S. M.V.Sc student guided by Dr Nitin Virmani received Best M.V.Sc Thesis Award conferred by Indian association of Veterinary Pathologists for work on 'Development of mouse model for equine influenza' during XXXII Annual Meeting of IAVP organized by Department of Veterinary Pathology, NTR College of Veterinary Science, Sri Venkateshwara University, Gannavaram, Andhra Pradesh, December 3-5, 2015.

- Dr Pavulraj, S. M.V.Sc student of Dr Nitin Virmani and their team including B.C. Bera, Alok Joshi, Taruna Anand, M. Virmani, B.N. Tripathi received 'Young Scientist Award' for the paper entitled "Pathology of equine influenza vaccine (H3N8) and vaccine efficacy studies in novel small animal model" by Indian association of Veterinary

Pathologists during XXXII Annual Meeting of IAVP organized by Department of Veterinary Pathology, NTR College of Veterinary Science, Sri Venkateshwara University, Gannavaram, Andhra Pradesh, December 3-5, 2015.

- Dr R.A. Legha and team received 2nd best poster presentation award for the poster presented on "Nutrients Intake and Their Digestibility in Adult indigenous Donkeys in Arid region of Rajasthan" in 3rd Biennial National Conference of Indian Academy of Veterinary Nutrition and Animal Welfare, COVAS, CSK HPKV in collaboration with IGFRI and IVRI Station, Palampur, HP., November 4-5, 2015.
- Dr Anju Manuja edited special issue "Therapeutic Interventions against Trypanosomiasis" in Journal "Current Topics in Medical Chemistry.
- Dr Ramesh Dedar was awarded Gold Medal for PhD research work entitled "Biomarkers of oxidative stress and therapeutic efficacy of antioxidants in donkeys in varying ambient temperatures" by RAJUVAS, Bikaner, September 16, 2015.
- Dr S. K. Ravi and team received 'Best Presentation Award' in Young Scientist Category for the paper entitled "Supplementation of omega-3 fatty acids in diet improved ovarian function, conceptus development and conception in mare" by The Indian Society for Study of Animal Reproduction (ISSAR), Dept. of Obstetrics & Gynaecology, Veterinary College, Bengaluru, December 3-5, 2015.
- Dr Thirumala R. Talluri received first prize for poster presentation on the topic "Characterization of Sleeping Beauty Transposon Transgenic Founder Mice to Establish Homozygous Transgenic Lines" at FLI Young Scientist meeting in Greifswald, Germany.
- Dr Vijay Kumar was awarded with '3rd Best Oral Presentation' for the topic entitled "Work performance of indigenous donkeys at pack load of 50% of live body weight in the subtropical climate" in National Conference on "New Horizons of Veterinary and Medical Forensic Medicine" held at RAJUVAS, Bikaner, March 5-6, 2016.

Appointments and Transfers

- Dr Praveen Malik, Pr. Scientist & Incharge NCVTCC, deputed as Director, NIAH, Baghpat, U.P. on April 9, 2015.
- Dr Naveen Kumar, Sr. Scientist (Animal Biotechnology) joint NCVTCC after transfer from ICAR-CIRG Makhdoom to ICAR-NRCE, Hisar on 27-04-2015.
- Shri A.G. Barapatre, Administrative Officer joined at ICAR-NRCE, Hisar on 16-10-2015.
- Shri R.A. Pachori, Technical Officer was transferred from ICAR-CIRB, Hisar to ICAR-NRCE, EPC, Bikaner on 15-04-2015.
- Dr Ajaykumar Ashok Rao Raut, Scientist (Extension) has been transferred from ICAR-NRCE to ICAR- Zonal Project Directorate, Zone VII, JNKVV Campus, Adhartal, Jabalpur (M.P.) on 30-12-2015.

Promotions

- Shri Subhash Chander, Shri Ram Singh, Shri Ishwar Singh, Shri Mahabir Prasad Meena and Shri Raju Ram, Skilled Supporting Staff have been financially upgraded under MACP Scheme in 2015.
- Assessment of Shri Om Prakash to Technical Assistant, Shri Raghubir Singh to Senior Technician and Shri Gopal Nath to Technician on 22.03.2016.
- Shri Subhash Chander, Assistant; Shri Pratap Singh, Assistant; Shri Sunil, Assistant and Smt Soma, Skilled Supporting Staff cleared their probation in 2015.

NRCE in News

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घोड़ों को इन्फ्लुएंजा बीमारी से मिलेगा निदान

पशुओं के बांझपन निवारण पर काम करें विज्ञानी : डॉ. श्रीकांत

नभ-छोर हिसार

8

अश्व अनुसंधान केंद्र में स्थापना दिवस पर करवाई गई चित्रकला प्रतियोगिता

हिसार. राष्ट्रीय अश्व अनुसंधान केंद्र हिसार में कल 31 वां स्थापना दिवस मनाया जाएगा जिसके अंतर्गत विभिन्न कार्यक्रमों का आयोजन किया जाएगा। राष्ट्रीय अश्व अनुसंधान केंद्र के स्थापना दिवस समारोह में मुख्य अतिथि के रूप में डॉ. पीके उप्पल, सलाहकार पंजाब सरकार तथा अश्व प्रजनन फार्म, हिसार के कमांडेंट ब्रिगेडियर बीएस कश्यप विशिष्ट अतिथि के रूप में उपस्थित होंगे। केंद्र के निदेशक डॉ. बीएन त्रिपाठी ने बताया कि स्थापना दिवस के उपलक्ष्य में राष्ट्रीय अश्व अनुसंधान केंद्र हिसार में 'अश्व- एक सहयोगी पशु' विषय पर स्कूल के छात्रों द्वारा चित्रकला प्रतियोगिता का आयोजन किया गया। इस चित्रकला प्रतियोगिता में हिसार के विभिन्न स्कूलों से 42 छात्र एवं छात्राएं सहभागी हुए। प्रतियोगिता के विभिन्न वर्गों में क्रमश: प्रथम, द्वितीय एवं तृतीय स्थान प्राप्त करने वाले छात्रों को कल आयोजित स्थापना दिवस समारोह में पुरस्कृत किया जाएगा। स्थापना दिवस के अवसर पर कल अश्व-पालक किसान गोष्ठी आयोजन किया जाएगा जिसमें अश्व-पालक वैज्ञानिकों से अश्वों में होने वाली विभिन्न बीमारियों एवं समस्याओं के बारे में वार्तालाप कर लाभान्वित हो सकते हैं।

दुष्यंत चौटाला
सांसद लोकसभा (लोक सभा)
Dushyant Chautala
Member of Parliament
(Lok Sabha)

डॉ. बी. एन. त्रिपाठी जी,

नमस्कार।

राष्ट्रीय अश्व अनुसंधान केन्द्र बीकानेर की विजिट करने का मुझे मौका मिला। संस्थान की कार्यप्रणाली को देखने का मौका मिला। संस्थान में चहुमुखी एवं अच्छे तरीके से जो काम-संस्थान एवं स्टाफ दिया उसे कहीं भी भुला नहीं पाऊंगा। आपके कुशल संचालन एवं कर्मचारियों की मेहनत से संस्थान दिन दुगुनी रात चौगुनी तरक्की कर रहा है।

इस व्यावसायिक विजिट के लिए मैं आपका व समस्त स्टाफ का आभार व्यक्त करता हूँ और उज्ज्वल भविष्य की कामना करता हूँ।

धन्यवाद सहित।

दुष्यंत चौटाला

NRCE in News

घोड़ों में एक्वाइन फ्लू बीमारी का टीका तैयार

अश्व पीढ़ी की पहचान को हिसार में बनेगी देश की पहली लैब

राष्ट्रीय अश्व अनुसंधान केंद्र को मिला सरदार पटेल उत्कृष्ट आईसीएआर संस्थान पुरस्कार

NRCE vaccine to prevent equine abortions boon for horse owners

HISAR: An indigenous vaccine developed by the National Research Centre on Equines (NRCE) here to prevent abortion in equines (horses, mules and donkeys) has proved to be a boon for horse owners.

Working under the aegis of Indian Council of Agricultural Research (ICAR), the NRCE released vaccine 'Equilvac pabor' early this year after years of testing and research.

The equine owners who have tried this vaccine on their horses

• A museum on the campus of the National Research Centre on Equines in Hisar.

Rajasthan was also all praise for

tions and to save the owners from

DainikBhaskar.com

घोड़ों में एक्वाइन फ्लू बीमारी का टीका तैयार

Staff at NRCE

Director: Dr B. N. Tripathi

Scientists at NRCE, Hisar Campus

1. Dr. A.K. Gupta, Principal Scientist
2. Dr. S.C. Yadav, Principal Scientist
3. Dr. Yash Pal, Principal Scientist
4. Dr. B.R. Gulati, Principal Scientist
5. Dr. Rajender Kumar, National Fellow
6. Dr. S.K. Khurana, Principal Scientist
7. Dr. Nitin Virmani, Principal Scientist
8. Dr. Sanjay Kumar, Principal Scientist
9. Dr. Mamta Chauhan, Sr. Scientist
10. Dr. Anju Manuja, Sr. Scientist
11. Dr. Balvinder Kumar, Sr. Scientist
12. Dr. Anuradha Bhardwaj, Scientist
13. Dr. Harishankar Singha, Scientist
14. Dr. Ajaykumar Ashok Rao Raut, Scientist (Transferred to ZPD, JNKW)

Scientists at VTCC, NRCE, Hisar

1. Dr Praveen Malik, Principal Scientist, Veterinary Microbiology (On Deputation from 09.04.2015)
2. Dr Sanjay Barua, Principal Scientist, Veterinary Microbiology
3. Dr R.K. Vaid, Principal Scientist, Veterinary Public Health
4. Dr Naveen Kumar, Senior Scientist (from 27.04.2015)
5. Dr Taruna Anand, Scientist, Animal Biotechnology
6. Dr B.C. Bera, Scientist, Animal Biotechnology
7. Dr K. Shanamugasundaram, Scientist, Veterinary Pathology
8. Dr Riyesh T., Scientist, Veterinary Microbiology

Technical Staff at NRCE, Hisar

1. Sh. K.K. Gupta, Chief Tech. Officer
2. Sh. K.S.Meena, Farm Manager
3. Sh. P.P.Chaudhary, Tech. Officer
4. Sh. D.D.Pandey, Tech. Officer
5. Sh. Sita Ram, Tech. Officer
6. Sh. Ajmer Singh, Tech. Officer
7. Sh. Sanjeev Kumar, Tech. Officer
8. Sh. Joginder Singh, Sr. Tech. Officer
9. Sh. Mukesh Chand, Sr. Tech. Officer
10. Sh. Sajjan Kumar, Sr. Tech. Officer
11. Sh. Suresh Kumar, Sr. Tech. Officer
12. Sh. Raj Kumar Dayal, Tech. Asstt.
13. Sh. Arun Chand, Sr. Technician
14. Sh. Raghbir Singh, Technician

Administrative Staff at NRCE, Hisar

1. Sh. A.G. Barapatre, Administrative Officer
2. Smt. Shammi Tyagi, Assistant Finance & Accounts Officer
3. Sh.Ram Pal, Assistant Administrative Officer
4. Sh.S.P.Kaushik, Assistant Administrative Officer
5. Sh.Ashok Kumar, Personal Assistant
6. Sh.Subhash Chander, Assistant
7. Sh.Pratap Singh, Assistant
8. Sh. Sunil, Assistant
9. Sh.Dinesh Datt Sharma, Upper Division Clerk
10. Sh.Omparkash, Upper Division Clerk
11. Sh. Deepak Kumar, Lower Division Clerk

Supporting Staff at NRCE, Hisar

1. Sh. Ishwar Singh
2. Sh. Guru Datt
3. Sh. Jai Singh
4. Sh. Mahabir Prasad
5. Sh. Ramesh Chander
6. Sh. Mardan
7. Sh. Desh Raj
8. Sh. Ishwar Chander
9. Sh. Om Parkash
10. Sh. Hanuman Singh
11. Sh. Subhash Chander
12. Sh. Ishwar Singh
13. Sh. Ram Singh
14. Smt. Santra
15. Sh. Sant Ram
16. Sh. Soma Devi
17. Sh. Lilu Ram

Scientists at EPC (NRCE), Bikaner Campus

1. Dr. R.A. Legha, Principal Scientist
2. Dr. Vijay Kumar, Scientist (Sr.Scale)
3. Dr. Ramesh Kumar Dedar, Scientist
4. Dr. Prokasananda Bala, Scientist
5. Dr. T.R. Tilluri, Scientist
6. Dr. Sanjay Kumar Ravi, Scientist

Technical Staff at EPC, Bikaner

1. Dr. Jitender Singh, Sr. Technical Officer
2. Sh. K.K.Singh , Sr. Technical Officer
3. Sh. Brij Lal , Technical Officer
4. Sh. Narender Chauhan, Technical Officer
5. Sh. R.A. Pachori, Technical Officer
6. Sh. S.N.Paswan , Technical Assistant
7. Sh. Rajender Singh, Technical Assistant
8. Sh. Om Parkash , Sr. Technical Assistant
9. Sh. Gopal Nath, Sr. Technician

Administrative Staff at EPC, Bikaner

1. Sh Mahender Singh, LDC

Supporting Staff at EPC, Bikaner

1. Sh M.P. Meena, SSS
2. Sh Raju Ram, SSS

Improving equine health & productivity is the priority of NRCE

ICAR-NRCE conferred with
Sardar Patel Outstanding ICAR Institution Award
by Hon'ble Prime Minister of India Shri Narendra Modi Ji
on 87th Foundation Day of ICAR
organized at Patna, Bihar on July 25, 2015

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