ICAR-NRCE

Annual Report

2014-15

भाकृअनुप-राष्ट्रीय अश्व अनुसंधान केन्द्र

ICAR-National Research Centre on Equines

भाकृअनुप
ICAR

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Date of Publication : June 24, 2015

Editorial Board : Nitin Virmani, Taruna Anand, Bidhan C. Bera & Ajay A. Raut

The achievements and activities of the Centre from April 2014 to March 2015 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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Director's Foreword

Galloping with the winds of change, ICAR-National Research Centre on Equines has embarked onto a journey in the new era with marked confidence attained from it's glorious past, wherein the institute got recognition in national and international arena through its technological interventions in the field of diagnostics, vaccines and concerted efforts in the direction of conservation of indigenous equines.

The technologies developed by the Centre also got recognition this year and two of them viz. Equiherpabort vaccine - for control of dreaded EHV 1 infection - the leading cause of abortion and a diagnostic ELISA for equine piroplasmosis were released in the Annual General Meeting by Hon'ble Minister of Agriculture, Government of India on February 18, 2015. The Centre through its consultancy to the industry and other ancillary activities has been able to generate resources to the tune of 107 lakhs. Further, NRCE has initiated work in the frontier areas of technologies such as reverse genetics approach for equine influenza vaccine, bacterial artificial chromosomes for the viral diseases, molecular approaches to crack the riddle of latency due to herpes viruses, preparedness for the exotic diseases through development of diagnostics using synthetic peptide technology, development of biomarkers and genetic characterization of *Trypanosoma evansi* parasite, expression of recombinant equine cytokines and use of nano-technological approaches for therapeutics and diagnostics. NRCE has also initiated the process of ISO 17025 certification for its laboratories in a phased manner, so as to achieve OIE referral status for Equine Piroplasmosis followed by equine influenza and glanders, wherein the OIE twinning projects are already in progress. The Centre was able to develop a mouse model for equine

influenza, which has shown great potential for future research on host pathogen interaction and screening of vaccine candidates.

The Centre has also ventured into the area of equine nutrition through studies on requirement of ration for various stocks and how that can be met through the locally available feed and fodder. For complete characterization of donkey breeds, NRCE has taken a major step towards phenotypic and genotypic characterization of the locally available gene pool in various geographical conditions of the country. The researchers from the Centre are getting international recognitions and have been regularly visiting abroad and developing linkages for future. NRCE extends its support to stakeholders by organizing health camps and kisan goshthis and participating in exhibitions to showcase the technologies and facilities. This year, 15 equine health camps were organised. Besides, being committed towards working for the poorest of the poor, we conducted surveys in the state of Uttarakhand, where floods had ravaged the economy in 2013. The Centre intends to benefit the stakeholders in the state of Uttarakhand through organizing more field visits and helping them through consultancy in the areas of health and management.

Veterinary Type Culture Collection, an independent activity, is taking big strides now and has initiated work in the area of repositioning of bacteriophages in addition to bacteria, viruses, recombinant DNA, clones, etc, of veterinary importance along with rumen and dairy microbes. VTCC has now extended it's domain and has started using high end technologies such as whole genome sequencing and GC-FAME analysis, which have helped in identifying and characterizing many novel bacterial isolates. The Centre till now has repositioned a total of 2546 cultures/clones including 529 accessions in year 2014-15. Further, whole genome sequence of classical swine fever virus was deciphered. The work in the area of bacteriophages brought to front as unique phages from various livestock environments, which are being explored for therapeutic potential against veterinary microbes.

The role of equines especially in difficult terrains is unquestionable, however, the changing demographic patterns of equine population, their utility, climate change and rapid globalization have posed challenges for the researchers to ponder and plan strategies to overcome the present day problems especially in dealing with the new disease incursions, changing scenario of host pathogen interaction and shrinking fodder and forage lands.

NRCE has always shown its intent and commitment towards work for the welfare of equines and will continue to learn and apply the knowledge gathered in the best possible manner. I have been associated with the Centre since inception when I joined as a Scientist and we started work from a small building. The Centre is continuously evolving and I find its pragmatic approach very attractive in drawing the attention of stakeholders and scientific community.

I would like to gratefully & sincerely acknowledge the kind guidance, support and encouragement from Dr. S.Ayyappan, Hon'ble Secretary, DARE and Director General, ICAR; Dr. K.M.L. Pathak, Deputy Director General (Animal Science); Dr. Gaya Prasad, Assistant Director General (Animal Health) and principal scientists and staff at ICAR Headquarters.

Last but not the least, I whole heartedly congratulate members of publication team for timely publication of annual report and to my devoted team of scientists at NRCE to keep their wonderful work going with their robust approach and bringing out more from their shelves in coming years.

Jai Hind,

Btripathi

(B.N. Tripathi)

Executive Summary

The ICAR-National Research Centre on Equines was established on November 26, 1985 at Hisar (Haryana) and is basically charged with the responsibility for welfare of equines in the country. The campus 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 (Rajasthan) to undertake research on equine production, genetics and breeding, reproduction, physiology and nutrition. Keeping in view the livelihood of the poorest-of-the-poor (landless, small and marginal farmers) and interest of the richest-of-the-rich (Race Clubs, Breeders, Turf authorities of India) and powerful (Police, Paramilitary forces, and Army) equine keepers, NRCE has been able to tackle the problems especially regarding health coverage, health service delivery, and import/export health certification, benefits of which have reached to the end-users in several parts of the country. NRCE was entrusted with another responsibility of collection and preservation of microbes of animal origin and veterinary importance and for this Veterinary Type Culture Collection (VTCC) was established in the year 2005 and is an integral but independent part of the Centre.

The Centre is actively involved in monitoring the emerging and re-emerging diseases of equines. The NRCE scientists carry out countrywide surveillance and disease investigation on field samples in addition to testing the samples from horses under continuous movement as well as meant for export and import. The important viral diseases of concern and requiring continuous monitoring include equine influenza, EHV 1, equine infectious anaemia, and JEV/WNV. While the country had no active cases of equine influenza after 2009, the seropositivity in few cases is under investigation. EHV 1 is endemic in the country with an overall incidence of 2.53%. All samples tested for EIA were negative for antibodies by Coggins test. Amongst the parasitic conditions piroplasmosis and trypanosomosis are most important haemo-protozoan diseases. The incidence of piroplasmosis in 2014-15 was as high as 32.67%, while that of trypanosomosis was in 3.54%.

Amongst bacterial diseases, outbreaks of glanders, though of limited scale since 2006, were reported from Uttar Pradesh, Himachal Pradesh and Jammu and Kashmir.

Abortions due to EHV 1 cause huge economic losses. In quest to develop better immunoprophylactics, glycoprotein D (~48kD) and gM (~52kD) of EHV1 were expressed in eukaryotic system by transfacting sf9 cells transfected with recombinant bacmid, and the expression of protein is being further optimized with various conditions. For construction of EHV 1 bacterial artificial chromosome (BAC), gene 71 (g71) was selected as targeted region to clone EHV 1 isolate and transfection of RK-13 cells with virus and linearized plasmid showed optimistic results.

A real-time PCR assay was standardized for detection of SNP at position 2254 of ORF30 for differentiation between neurogenic and non-neurogenic EHV 1 infection. We reported the existence of neurogenic EHV 1 in the country. Genetic diversity based on the sequence analysis of partial ORF68 gene of 7 EHV 1 isolates revealed that the Indian isolates belonged to group 4 and group 5.

To meet the challenge of incursion of exotic diseases in the country, and emergency preparedness, the Centre initiated work on developing diagnostics

against exotic viral diseases of equines viz. Vesicular Stomatitis, Venezuelan Equine Encephalitis, Rift Valley Fever, etc.

Equine piroplasmosis is a tick-borne and haemoprotozoan disease of equidae and so far no drug is available, which can completely eliminate *T. equi* infection from carrier animals. We have selected some target specific drug molecules, which were tested in MASP culture of *T. equi* in *in-vitro* system. Of nine drug molecules tested, *in-vitro* growth of *T. equi* was significantly inhibited by HHD, HDTAB, HMC, Decamethonium bromide and dodecyltrimethyl ammonium bromide molecules. Further on *In-vitro* cytotoxicity trails harmaline, decamethonium bromide and NBCN salts were most promising drug molecules in inhibiting *T. equi* growth with least cytotoxicity.

Diagnosis of Trypanosomosis is a major concern in animals including equines. Working in this direction, a highly sensitive real time PCR, with detection level of 0.15pg of genomic material of parasite was developed, which compared well with gold standard: TBR PCR. Both the techniques could detect the infection after 24 hours post infection. Another approach for diagnosis of *T. evansi* employing serological assay based on Hsp70 recombinant protein could detect antibodies against *T. evansi* at 10 dpi. Nanogold based immunochromatography assay for diagnosis of *T. evansi* infection is being developed by the NRCE. RAPD typing for estimating genetic variability amongst *T. evansi* isolates revealed heterogeneity among isolates of different livestock hosts and geographical regions. In order to develop therapeutics against *T. evansi,* quinapyramine sulfate loaded-sodium alginate nanoparticles (QS-NPs) were synthesized and their safety was determined. QS-NPs were found to be safe at effective trypanocidal doses and even at doses several times higher than the effective dose.

Four recombinant equine cytokines viz. IL-2, IL-10, IL-18 and IFN-γ were purified and biological activity of two recombinant equine cytokines (IL-18 and IFN-γ) were studied. Recombinant IL-18 was able to induce secretion of IFN-γ and IL-10. Availability of biologically active recombinant equine cytokines will help us to investigate the immunological roles of cytokines in relation to equine disease, as well as their potential efficacy as a therapeutic agent or vaccine adjuvant in horse.

Currently, the Centre has two OIE twinning projects on equine influenza and glanders with Animal Health Trust, UK and Friedrich Loeffler Institute, Germany, respectively, which aim towards capacity building of NRCE to apply for the OIE referral laboratory status for the region. During the current year Dr Keith Hamilton from OIE Head Quarters at Paris, France visited NRCE and was shown the activities and capabilities in relation to equine influenza, glanders and piroplamosis. Later during the year, Scientists from AHT, UK visited NRCE for a period of one week and the surveillance system for monitoring equine influenza and processing of samples and different tests being used in laboratory were demonstrated. Under OIE twinning project on glanders two scientists from NRCE visited FLI, Germany for capacity building and learned genotyping techniques such as variable number of tandem repeats (VNTR), multilocus sequence typing (MLST) for *Burkholderia mallei.*

Donkey germplasm of six donkey populations belonging to Spiti (H.P.), Leh (J & K), Baramati (Maharashtra), Bihar, Gujarat and Rajasthan areas along with exotic Poitu breed (an outgroup) were evaluated for assessing genetic diversity within and between them using twenty four polymorphic microsatellite markers with 299 donkey DNA samples. The estimates of Fst between each pair of breeds revealed that genetic differentiation between donkey population from Gujarat and Leh were the maximum followed by Rajasthan and Leh donkey populations while donkey populations from Rajasthan and Spiti areas were the least differentiated. On comparing all the donkey populations, donkeys from Spiti and Rajasthan were observed to be very close to each other, whereas donkeys from Leh and Gujarat areas were far apart.

With objective of increased utilization of animal energy with enhanced system efficiency, the pack load capacity and draughtability of mules and

donkeys was studied under arid conditions. Results indicated that the donkeys may be comfortably used for five hours with pack load (50% of BW) in morning hours with intermittent rest in between at brick-kilns.

Nutrient requirement of horses are governed by the animal feed factors. Thus, a trial was conducted to find out the nutrient requirement of Marwari mare at late pregnancy. The animals were given feed according to the ongoing feeding regime at EPC, Bikaner which included sewan hay and green Lucerne. The feed intake by the animals under trial was 2.33 % of their body weight which meets the recommendation of NRC, 2007. A study was also conducted to assess the growth of Marwari foals on wheat straw and sewan hay based rations.

For optimization of conception rate and fertility at farm, the follicular dynamics and associated changes were studied. The mean duration of the estrous cycle remained around 27.7 days, while estrus period was 10.07 days. Mean preovulatory follicle size was observed to be 47.34 mm and corpus luteum size was 33.40 mm. The mean gestation length recorded was 334.10 days.

The socio-economic profile, existing management systems and utilization pattern of equines on Chardham Yatra in Uttarakhand were studied. Survey revealed their daily income varied from ₹ 600-1700 during yatra season. Working equines generally travel 20-24 km distance per day carrying a load of approximately one quintal.

Veterinary Type Culture Collection (VTCC) has a mandate to act as a national repository of microorganisms of animal origin comprising veterinary, rumen and dairy microbes. So far, a total of 2546 cultures/clones have been deposited in the VTCC after authentication using conventional and molecular characterization methods such as sequence analysis of 16S rRNA & important virulence genes. Recently high end technologies such as GC-FAME and whole genome sequencing have also been used for carrying out complete characterization of microbes.

In 2014-15, approximately 450 cultures were processed while a total of 227 bacterial cultures obtained from various sources were accessioned. The 16S sequence based microbial identification was completed with identification of about 122 bacterial isolates from accessioned cultures, cultures received from network units and bacterial isolations.

Many novel bacterial isolates were identified using 16s rRNA sequencing and have been added to collection which can be used as type species. About 60 isolates were identified up to species level using automated MIDI Bacterial identification system. Using seed lot system, a total of 410 accessioned cultures were additionally preserved during 2014-15. The good quality genomic DNAs of 45 bacterial cultures were also purified and preserved as ethanol precipitate at -80°C.

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 27 virus isolates viz., ORFV (2), SPPV, CMLV, BPXV, GTPV(2), NDV (2), PPRV (3), IBDV(6), CSFV, BTV (3), RDV- F strain, RDV virulent strain, Pigeon RDV, Avian reovirus, NDV (R2B strain) were identified/authenticated. Fourteen different cell lines along with three primary cultures for their use at VTCC and distribution are being maintained. Complete genome characterization of 2 isolates of CSFV was performed. This study revealed the circulation of recombinant CSFV among Indian swine population for the first time. During the period, recombinant clone library was strengthened by addition of 140 recombinant clones of various animal and poultry viruses. Validated gateway clones of 23 ORFs of immunomodulatory / virulence genes of zoonotic buffalopox virus and equine influenza virus were generated in a flexible format and the validated clones were preserved in the repository.

Along with bacteria, bacteriophages constitute an integral part of the microbiome. Isolation and characterization of a variety of Bacillus phages obtained from environmental soil and water samples was carried out. The phages were visualized by transmission electron microscopy (TEM) and protein profiles. Bacillus phages belonged to families –

Myoviridae, Siphoviridae and Tectiviridae. During 2014-15, five bacteriophages against Salmonella Gallinarum were also isolated and characterized for biological activity, pH and temperature stability. A total of 19 bacteriophages were isolated and preserved during 2014-15.

Apart from veterinary pathogens, 30 rumen bacterial isolates and 45 dairy cultures including *Lactobacillus rhamnosus, L. plantarum, L. paracasei* were also preserved.

The Centre was able to generate a revenue of ₹ 107 lakh comprising of ₹ 57.87 lakh from consultancy through disease investigation and diagnosis. Further our agriculture section made a progress and NRCE could generate revenue through sale of crops to the tune of ₹10.04 lakh.

Two technologies developed at NRCE viz. Equineherpabort vaccine - for EHV 1 infection and a diagnostic ELISA for equine piroplasmosis were released by Hon Minister of Agriculture, Sh Radha Mohan Singh Ji on February 18, 2015 during Annual General Body Meeting of ICAR at NASC, New Delhi.

Various institutional activities were executed during the period that included Foundation Day, World Veterinary day, National Science day, Interactive meet of equine and Progressive equine owners meet and drawing competition on NRCE foundation day. The Sanitation drive launched by Hon'ble PM of India as Swachch Bharat Abhiyan was duly implemented at the Centre from October 2, 2014. Further, NRCE regularly organizes Equine Health camps and Kisan Goshthis. and participated in various exhibitions to showcase its activities.

The Scientists of the Centre published 55 articles in research journals and also participated in national and international conferences and published chapters in books and abstracts in compendium. 46 Gene sequences were also submitted to GenBank.

कार्यकारी सारांश

राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार, हरियाणा की स्थापना 26 नवम्बर, 1985 को की गई थी। भारतीय कृषि अनुसंधान परिषद द्वारा, इस संस्थान को मूल रूप से अश्वों के कल्याण के लिए स्थापित किया गया था। केन्द्र के मुख्य परिसर में अश्वों के स्वास्थ्य से संबंधित प्रयोगशालाएं विषााणु विज्ञान, जीवाणु, परजीवि विज्ञान, विकृति विज्ञान, इम्यूनोलोजी, चिकित्सा, जैव रसायन तथा जैव प्रौद्योगिकी के क्षेत्र में शोध कार्य कर रही है।

इस केन्द्र का एक उप-परिसर, अश्व उत्पादन केन्द्र, बीकानेर (राजस्थान) में 1989 में स्थापित किया गया था। भारत वर्ष के गरीब से गरीब (भूमिहीन छोटे एवं सीमांत किसानों को) और अमीर से अमीर (रेस क्लब ब्रीडर्स, टर्फ) की आजीविका ओर शक्तिशाली (पुलिस अर्धसैनिक बल और सेना) को ध्यान में रखते हुए, केन्द्र ने देश में अश्व उपयागकर्ताओं को लाभ पहुंचाने की भरपूर कोशिश की है। इसमें मुख्यत: स्वास्थ्य सेवा और आयात-निर्यात, स्वास्थ्य प्रमाण-पत्र तथा अन्य स्वास्थ्य सम्बंधी जानकारियाँ देकर अश्वपालकों को लाभान्वित किया है। राष्ट्रीय अश्व अनुसंधान केन्द्र को पशु चिकित्सा महत्व के रोगाणुओं के संरक्षण और संग्रह के लिए वेटरीनरी टाईप कल्चर कलैक्शन की स्थापना वर्ष 2005 में की गई और यह केन्द्र का एक अभिन्न अंग है।

केन्द्र सक्रिय रूप से घोड़े की उभरते और फिर से उभरते रोगों की निगरानी में लगातार कार्यरत है। इस दिशा में राष्ट्रीय अश्व अनुसंधान केन्द्र के वैज्ञानिकों की टीम निरंतर रूप से देश के कोने-कोने से घोड़ों के नमूने परीक्षण के लिए लाती हैं और उनकी जांच कर रोगों का अवलोकन करती है। साथ ही निरन्तर आवाजाही वाले घोड़ों और आयात-निर्यात के घोड़ों के नमूनों का परीक्षण किया जाता है। चिंता और सतत निगरानी की आवश्यकता के महत्वपूर्ण विषाणु सम्बंधित रोगों में अश्व-फ्लू, ई.एच.वी.-1, अश्व-संक्रामक अनीमिया और जे.ई.वी./वेस्ट नाईल शामिल हैं। देश में 2009 के बाद अश्व-फ्लू का कोई सक्रिय केस नहीं मिला है ओर कुछ नमूनों में सीरोपाजिटीविटी जांच के अधीन है। ई.एच.वी.-1 देश में स्थानिक है और सतत निगरानी के दौरान, इसकी संक्रामकता 2.53 प्रतिशत पाई गई है। अश्व संक्रामक अनीमिया के सभी नमूने कागिन्स परीक्षण से एंटीबाडी के लिए नकारात्मक पाये गए हैं।

परजीवी रोगों मे पायरोप्लास्मोसिस एवं ट्रिपेनोसोमोसिस महत्वपूर्ण परजीव हैं जिनका निदान एवं उपचार अति आवश्यक हे। अश्वों के खून के नमूनों में ट्रिपोनोसोमोसिस की एंटीबाडी 3.54 प्रतिशत पाई गई जबकि पायरोप्लासमोसिस की एंटीबाडी 32.67 प्रतिशत नमूनों में मिली। अश्वों में, जीवाणुओं से होने वाले रोगों में ग्लैण्डर्स प्रमुख है और 2006 से इसका सीमित प्रकोप भारतवर्ष में जारी है। वर्ष 2014 में इस रोग का संक्रमण उत्तरप्रदेश, हिमाचल प्रदेश एवं जम्मू-कश्मीर में पाया गया। जीवाणुओं से होने वाले अन्य प्रकार के संक्रमणों में मुख्यत: स्ट्रेप्टोकोक्स इक्वी सब स्पी. जूएपीडेमिकस, स्ट्रेप्टोकोक्स इक्वी सब स्पी. इक्वी, रोडोकोकस इक्वी, क्लैबसैला एवं ई० कोलाई पाये गए। पोस्टमार्टम परीक्षण एवं उत्तक विकृति विज्ञानी द्वारा मुख्यत: सिसोसिस, नेक्रोतिजिंग आंत्रशोध, फैटी लीवर, श्वसनी फुफ्फुसपाक अवस्थाएं दर्ज की गई। ई.एच.वी.-1 अश्वों में गर्भपात का मुख्य कारण है तथा भारी आर्थिक नुकसान करता है। इसके बेहतर निदान एवं रोकथाम के लिए केन्द्र लगातार प्रयासरत है। इस क्षेत्र में कार्य करते हुए संस्थान ने सब यूनिट टीका विकसित करने के लिए यूकेरयोटिक संयोजक प्रोटीन पर कार्य शुरू किया है। वर्ष 2014-15 में जी०एम० और जी०डी० प्रोटीन एस०एफ० 9 कोशिकाओं में संयोजक बेक्मिड द्वारा ट्रांसफैक्ट करके बनाई गई। एस०डी०एस० तकनीक द्वारा जी०डी० (48 कि०डा०) एवं जी०एम० (52 कि०डा०) की पुष्टि की गई एवं वेस्टर्न ब्लॉट तकनीक द्वारा सत्यापित की गई। ई.एच.वी.-1 बैक्टीरियल कृत्रिम गुणसूत्र (बी०ए०सी०) के निर्माण के लिए जीन 71 को प्लासमिड में क्लोन करके ट्रास्फर प्लासमिड तैयार किया गया। ई.एच.वी.-1 विषाणु के साथ ट्रांसफर पलास्मिड को आर० के० 13 कोशिकाओं में ट्रांस्फेक्ट करने पर आशावादी परिणाम प्राप्त हो रहे हैं। केन्द्र ने ई.एच.वी.-1 की तंत्रिकाजन्य एवं

गैर-तंत्रिकाजन्य रोग के आनुवांशिक लक्षण के वर्णन हेतु रीयल-टाईम पी.सी.आर. तकनीक को विकसित किया। साथ ही ई.एच.वी.-1 आईसोलेट्स के बीच मौजूद आनुवांशिक विविधताओं को समझने के लिए आंशिक ओ.आर.एफ. 68 का विश्लेषण किरने पर भारतीय आइसोलेट्स को दो समूहों (समूह 4 एवं 5) में वर्गीकृत किया गया।

अप्रतिबंधित पशुओं की आवा-जाही, तेज वैश्वीकरण तथा जलवायु परिवर्तन के कारण सीमा पार के रोगों के तेजी से फैलने की सम्भावना रहती है। अत: केन्द्र ने इनके निदान एवं निगरानी हेतु कार्य शुरू किया है। इस संदर्भ में केन्द्र ने वेसीकुलर स्टोमेटाईटिस एवं वेनेजुएला इक्वाइन इन्सेफेलाइटिस विषाणु के प्रतिजनी क्षेत्रों की पहचान शुरू की है। प्रतिजनी क्षेत्रों के आधार पर डी.एन.ए. संश्लेषित करके, प्रोकेरयोटिक प्रणाली में प्रोटीन की अभिव्यक्ति का कार्य शुरू किया गया है।

इक्वाइन पायरोपलास्मोसिस एक कीट जनित रोग है जिसके संक्रमण को रोकने के लिए पूर्ण रूप से कागर कोई दवा उपलब्ध नहीं है। संस्थान ने इस क्षेत्र में कार्य करते हुए कुछ लक्ष्य विशिष्ट दवा अणुओं का चयन करके परजीवी का एम.ए. एस. पी. कल्चर में परीक्षण किया। नौ दवा अणुओं का चयन किया गया तथा उनकी इन वीट्रो सायटोटॉक्सिसिटी के अध्ययन से हार्मलिन, डेकामिथेनियम ब्रोमाईड और एन.बी.सी.एन को सुरक्षित पाया गया।

केन्द्र घोड़ों की कुछ महत्वपूर्ण बीमारियों पर बाहय एवं विदेशी वित पोषित परियोजनाओं में कार्यरत है। ऐसी ही एक महत्वकांक्षी योजना टी. इवेन्साई के निदान के विकास पर है। इसके तहत एक रीयल-टाईम पी.सी.आर. तकनीक मानकीकृत किया गया जिसकी संवेदनशीलता 0.15 पीकोग्राम परजीवी की जीनोमिक सामग्री का भी पता लगा सकती है। इसकी संवेदनशीलता गोल्ड स्टेण्डर्ड टी.बी.आर. पी.सी.आर. के समान है। टी. इवेन्साई की आनुवांशिक परिवर्तनशीलता के आकलन हेतु आर.ए.पी.डी. टाईपिंग की गई तथा अलग पशुधन मेजबान और भौगोलिक क्षेत्रों की वियोजन के बीच विविधता का पता लगाया गया। एक अन्य परियोजना में टी. इवेन्साई के निदान के लिए एच.एस.पी.-70 पुन:संयोजक प्रोटीन के आधार पर सीरम एंटीबॉडी की परख का निदान तैयार किया गया।

टी. इवेन्साई के निदान के लिए नैनोगोल्ड आधारित इम्यूनोक्रोमेटोग्राफी परख को विकसित किया गया है। टी. इवेन्साई के उपचार के लिए क्वीनापीरामीन सल्फेट-सोडियम एलजिनेट नैनोकण संश्लेषित किया गया वं उनकी सुरक्षा वीरोकोशिकाओं, हैला कोशिकाओं एवं अश्वों की रक्त कोशिकाओं में जांची गइ।

क्यू.एस.-एन.पी. की प्रभावी ट्रिपेनोसाईडल मात्रा तथा खुराक से कई गुना अधिक मात्रा सुरक्षित पाई गई। एक अन्य महम्वपूर्ण क्षेत्र में कार्य करते हुए, संस्थान ने जैविक रूप से सक्रिय पुन:संयोजक साइटोकाइनों की उपलब्धता का कार्य प्रारम्भ किया। ये साइटोकाइन हमें चिकित्सीय प्रतिनिधि या वैक्सीन सहायक भूमिकाओं में अत्याधिक उपयोगी होंगे। इस परियोजना के तहत चार पुन:संयोजक साइटोकाइन को (आई. एल.-2, आई.एल.-10, आई.एल.-18, आई.एफ.एन.-γ) किया गया एवं आई.एल.-18 और आई.एफ.एन.-γ की जैविक सक्रियता परख द्वारा जांची गई। संयोजक आई.एल.-18 द्वारा आई.एल.-10 एवं आई.एफ.एन.γ को प्रेरित करने एवं स्रवण में सक्षम पाया गया।

केन्द्र ने अपने वैश्विक स्तर को बनाने और उसे आगे बढ़ाने के लिए विदेशी प्रयोगशालाओं के साथ अनुसंधान में सहयोग किया है। केन्द्र के पास वर्तमान में अश्व-फ्लू, एवं ग्लैण्डर्स रोगों की प्रयोशाला जुड़ाव का विश्व पशु स्वास्थ्य संगठन (ओ.आई.ई.) पैरिस के साथ कार्यक्रम चल रहा है। इसके अंतर्गत अगस्त 2014 में ओ.आई.ई. से एक विशेषज्ञ डॉ० कीथ हैमिल्टन ने केन्द्र का दौरा किया। उनको अश्व फ्लू और ग्लैण्डर्स से सम्बंधित गतिविधियों एवं क्षमताओं का विस्तृतीकरण किया गया। एनिमल हैल्थ ट्रस्ट (ब्रिटेन) से नवम्बर 2014 को दो वैज्ञानिकों ने दौरा किया। इस दौरान, अश्व फ्लू प्रयोगशाला में किए जा रहे विभिन्न परीक्षणों, अश्वों के नमूने एकत्र करना एवं अश्व फ्लू की निगरानी प्रणाली का प्रदर्शन किया गया। केन्द्र के दो वैज्ञानिक क्षमता निर्माण हेतु ओ.आई.ई. की ग्लैण्डर्स परियोजना के तहत जर्मनी गए और वहां पर बुरखोल्डेरिया मेलियाई के मल्टीलोकर अनुक्रम टाईपिंग एवं वी.एन.टी.आर. की तकनीकों को सीखा।

गर्दभों के जर्मप्लाज्म और नस्ल के लक्षण-वर्णन के विश्लेषण की आवश्यकता को देखते हुए गर्दभों की आबादी वाली 6 जगहों (स्पीति, लेह, बारामती, बिहार, गुजरात एवं राजस्थान) पर आनुवंशिक विविधता का आकलन करने के लिए, 24 पोलिमार्फिक माईक्रोसेटेलाइट मार्कर का उपयोग 299 गर्दभों

के नमूनों पर किया गया। एलील गिनती, एलील आकार, हैट्रोजायगोसिटी ओर फिस वैल्यू का आंकलन किया गया। माईक्रोसेटेलाइट लोसाई द्वारा कुल मिला कर 264 एलील पाये गए। एलीलों की प्रभावी संख्या 1.5618 से 9005 तक पाई गई और प्रति लोकस एलील 6 से 22 तक देखे गए। तीन लोसाई को छोड़कर सभी लोसाई में हेट्रोजायगोसिटी देखी गई जोकि हार्डी-वीनवर्ग इक्वीलीब्रियम से विचलन दर्शाता है। गुजरात और लेह की गर्दभों की आबादी में आनुवंशिक भेद-भाव अधिकतम पाया गया जबकि राजस्थान और स्पीति के गर्दभों में यह भेद-भाव सबसे कम था। सभी गर्दभ आबादी की तुलना वाले क्षेत्रों में स्पीति और राजस्थान के गर्दभ नजदीक पाए गए जबकि गुजरात और लेह के गर्दभ एक दूसरे से काफी दूर थे। गर्दभों और खच्चरों के उत्पादन का अनुकूलन करने के लिए पशु-ऊर्जा का दक्षता से प्रयोग करना आवश्यक है। इसके लिए संस्थान में अखिल भारतीय समन्वित अनुसंधान परियोजना के अंतर्गत शुष्क परिस्थितियों में वर्क-रेस्ट साइकल पैक भार क्षमता और ड्राफ्टेबिलिटी का अध्ययन किया जा रहा है। आकलन से पता लगा है कि थकान व असमन्वय की शुरूआत पांच घण्टे के काम द्वारा पचास प्रतिशत पैक लोड और तीन घण्टे के कार्य द्वारा 66 प्रतिशत पैक लोड से होती है। इससे संकेत मिलता है कि हम गर्दभों से 50 प्रतिशत पैक लोड पर पांच घण्टे कार्य प्रात:काल में कुछ देर के लिए रूक-रूक के आराम के साथ करवा सकते हैं। तीन खच्चरों पर ड्राफ्टेबिलिटी अध्ययन के दौरान 200 न्यूटन (गर्मी के मौसम में) 300 न्यूटन (सितम्बर-अक्टूबर), एवं 450 न्यूटन (सर्दी के मौसम में) लोडिंग कार खीचने का कार्य कराने का विश्लेषण किया गया। 200 न्यूटन के साथ तीन घण्टे, 300 न्यूटन के साथ दो घण्टे और 450 न्यूटन के साथ एक घण्टा कार्य कराने पर पसीना एवं थकान के अन्य लक्षणों को देखा गया।

खेत में गर्भाधान दर और प्रजनन क्षमता के अनुकूलन के लिए फालिकुलर डायनामिक्स और उससे संबंधित जैव रासायनिक और जीव अभिव्यक्ति में परिर्वतन जो कि मारवाड़ी फिल्ली में प्यूबर्टी, एस्ट्रस चक्र और पेरीपार्टम से संबंधित हो, उनके शोध से संबंधित एक परियोजना शुरू की गई थी। यह कार्य अल्ट्रासोनोग्राफी द्वारा प्रजनन के मौसम में ओवेरिन साईकल की निगरानी के लिए शुरू किया गया था। एस्ट्रस चक्र की अवधि 27.7 दिनों के आस-पास थी जबकि एस्ट्रस 10.07 दिन के बाद हुआ। प्रीओव्युलेटरी कूप 47.34 मि.मी. पाया गया तथा पीत पिण्ड आकार 33.40 मि.मी. था।

प्रोजेस्ट्रोन एवं एस्ट्रोजन हारमोन का विश्लेषण किया गया। गर्भ-धारण काल की लम्बाई 334.10 दिन पाई गई। उत्तराखण्ड में चार धाम यात्रा में अश्वों का उपयोग होता है। इसे ध्यान में रखते हुए केन्द्र ने अश्व-पालकों का सर्वेक्षण करके उनकी सामाजिक, आर्थिक स्थिति, प्रबंधन प्रणाली एवं उपयोग स्वरूप का अध्ययन किया। इस सर्वेक्षण के लिए रूद्रप्रयाग, चमोली एवं उत्तरकाशी जिलों से 138 अश्व-पालकों से जानकारी प्राप्त की गई। अधिकतत अश्वपालक मध्यत उम्र के अनुसूचित जाति से संबंधित एवं साक्षर पाये गए। यात्रा के दौरान इनकी दैनिक आय 600-1700 रूपये तक थी जिसके लिए अश्व आमतौर पर एक क्विंटल का भार लेकर प्रतिदिन 20-24 कि.मी. तक यात्रा करते हैं। उत्तराखण्ड में हाल ही की प्राकृतिक आपदा के बाद, अश्वपालकों की पर्यटकों के एवं तीर्थ यात्रियों के कम होने के कारण, आर्थिक स्थिति प्रभावित हो रही है।

वैटरीनरी टाईप कल्चर कलैक्शन पशु चिकित्सा, रूमन और डेयरी से संबंधित सूक्ष्मजीवों के राष्ट्रीय भंडार के रूप में कार्य करने को प्रतिबद्ध है। इसकी मुख्य गतिविधियों में पशुओं के स्वास्थ्य तथा उत्पादन से संबंधित, रोगाणुओं का पृथक्कीकरण, लक्षण, संरक्षण, रख-रखाव, वितरण आदि शामिल हैं। अब तक 2546 कल्चर्स/क्लोन 16 एस.आर.आर.एन.ए. और महत्वपूर्ण विशैलापन जीन के विश्लेषण-पारम्परिक और आण्विक लक्षण वर्णन तरीकों का उपयोग कर प्रमाणीकृत करके जमा किए गए हैं। हाल ही में, उच्च अंत प्रौद्योगिकियों जैसे कि जी.सी.फेम. तकनीक तथा पूरा जीनोम अनुक्रमण का प्रयोग सम्पूर्ण विश्लेषण के लिए किया गया है। विभिन्न प्रजातियों जैसे-गोजातीय, ओवाईन, ऊँट, सुकर और मुर्गी जाति के विषाणुओं को भी शामिल किया गया है। कुल 27 विषाणु जैसे और्फ(2), एस.पी.पी.वी.,सी.एम.एल.वी., जी.टी.पी.वी.(2), एन.डी.वी.(2(, पी.पी.आर.वी.(3), आई.बी.डी.वी.(6), सी.एस.एफ.वी., बी.टी.वी.(3), आर.डी.वी., कबूतर आर.डी.वी., एवियन रियोविषाणु, एन.डी.वी. (आर. 2 बी. स्ट्रेन) की पहचान की गई है, जिनमे से 21 आईसोलेट्स (टीका बनाने वाले स्ट्रेन) सफलतापूर्वक उपयुक्त प्राथमिक सेल लाईनों में पसाज करने के बाद प्रमाणीकृत करके, संजोए गए हैं। तीन प्राथमिक सेल लाईनों के साथ, चौदह विभिन्न सैल लाईनें जैसे-वीरो, एम.डी.बी.के., एम.डी.सी.के., बी.एच.के. 21, आर.के.13, हेला, पी.के.15, हेप.2, एम.आर.सी.-5, एन.एल.बी.के. एम.ए.-104, घोडे के फेफड़े, सुअर स्थिर, सी.ई.एफ.,सी.ई.एल., बी.आर.टी. और

एल.टी को कायम रखा जा रहा है तथा इनका उपयोग विषाणुओं के अलगाव के लिए किया जा रहा है। विभिन्न सैल लाइने/प्राथमिक सेल लाइनें जैसे कि वेरो, आर.के.13, बी.एच.के. 21, हेला, एम.डी.सी.के., पी.के.15, भेड़ अंडकोष सेल लाइन का वितरण गत वर्ष के दौरान वैज्ञानिक समुदाय में किया गया। आनुवंशिक विविधता को समझने के लिए, सी.एस.एफ.वी. का पूरा जीनोम लक्षण वर्णन किया गया। इस अध्ययन द्वारा पहली बार, भारतीय सूअर आबादी के बीच पुनः संयोजक सी.एस.एफ.वी. के संचलन का पता चला। सी.एस.एफ.वी. का पूरा जीनोम लक्षण वर्णन, क्षेत्र की स्थिति में सी.एस.एफ.बी. के बीच मौजूदा आनुवंशिक विविधता की पहचान करने में मदद देगा और रोग नियंत्रण रणनीति विकसित करके वहां रिवर्स आनुवंशिक के आधार पर भविष्य के अध्ययन के लिए मार्ग प्रशस्त करेगा।

वी.टी.सी.सी. की प्रमुख गतिविधियों में से एक प्रमुख-रोगाणुओं की आनुवंशिक सामग्री की रक्षा करना है। इस अवधि के दौरान या 41 पुनः संयोजक क्लोन जो कि बफैलोपोक्स विषाणु, स्वाईनपोक्स (12 क्लोन) और वी.पी.-2 जीन (आई.बी.डी.वी.-16 क्लोन) से संबंधित थे, उन्हें शामिल किया गया। 2014-15 में करीबन, 450 कल्चर्स को प्रोसेस किया गया। जबकि, विभिन्न स्त्रोतों से प्राप्त 227 जीवाणु कल्चर्स को एक्सेशन किया गया। 165 लक्षण वर्णन के आधार पर कुल 122 जीवाणु कल्चर्स की पहचान की गई जिनमें एक्सेशंड तथा नेटवर्क इकाईयों से प्राप्त जीवाणु भी शामिल थे। कई नोवल जीवाणु जैसे स्ट्रेप्टोकोक्स प्लूरानिमेलियम, स्ट्रेप्टोकोक्स एसिडोमिनिमस, स्ट्रेप्टोकोक्स इक्वी, स्ट्रेप्टोकोक्स रूमेनिटोरम, कामोमोनास जियांग्डुएंसिस, कामोमोनास कर्सटर्ससी, एन्टेरोकोक्स फीशियम इत्यादि 16 एस.आर.आर.एन.ए. के आधार पर की गई। मिडी जीवाणु पहचान प्रणाली उपयोग करके 60 आईसोलेट्स की पहचान प्रजाति स्तर तक की गई। घोड़ी के दूध में भी जीवाणुओं की पहचान की गई। सीड़ लॉट प्रणाली का उपयोग करके, गत वर्ष में कुल 410 जीवाणुओं की एक्सेशनिंग की गई। 45 जीवाणुओं की अच्छी गुणवत्ता के जीनोमिक डी.एन.ए. भी -80 डिग्री सेंटीग्रेड में संरक्षित किए गए। बी.पी.एक्स.वी. और अश्व-फ्लू विषाणु के प्रचण्डता से जुड़े ओ.आर.एफ. के 23 क्लोन संरक्षित किए गए। ओ.आर.एफ. रिपोजीटरी पर भविष्य में अनुसंधान के लिए अश्व-फ्लू एवं बी.पी.एक्स.वी. की पुनः संयोजक प्रोटीन व्यक्त की गई।

जीवाणुओं के साथ-साथ, जीवाणुभक्षी (बैक्टीरियोफाज) माईक्रोबायोम का एक अभिन्न अंग हैं। पर्यावरण, मिट्टी और जल के नमूनों से बैसिलस जीवाणुभक्षी प्रथक और वर्णित किये गए। इन्हें टेम तकनीक द्वारा देखा गया एवं इनकी प्रोटीन प्रोफाईल विकसित की गई। बेसिलस जीवाणुभक्षियों का टेम द्वारा आकृति आकंलन करके उन्हें-मायोविरिडी, साईफोविरिडी और टेक्टीविरिडी फैमिली में शामिल किया गया। 2014-15 के दौरान, साल्मोनेला गैलिनेरम के पांच जीवाणुभक्षी प्रथक किए गए और उनकी जैविक गतिविधि, पी.एच. तथा तापमान स्थिरता जांची गई। कुल मिला कर 19 जीवाणुभक्षी संरक्षित किए गए। इसके अलावा 30 रूमन जीवाणु और 45 डेयरी जीवाणु संरक्षित किए गए।

गत वर्ष केन्द्र ने रोग-जांच एवं निदान के माध्यम से 107 लाख रूपये का राजस्व उत्पन्न किया। कृषि खण्ड ने प्रगति की एवं फसलों की बिक्री के माध्यम से 10.04 लाख रूपये का राजस्व एकत्रित किया। संस्थान ने वर्ष 2014-15 में विभिन्न गतिविधियों का आयोजन किया जिसमें प्रमुखतः भा.कृ.अनु.परि. के स्थापना दिवस पर अश्व-पालकों के साथ परस्पर संवादात्मक समागम, रा.अ.अनु.केन्द्र के स्थापना दिवस पर कला प्रतियोगिता एवं प्रगतिशील अश्वपालकों का समागम कराया। केन्द्र ने पशु चिकित्सा दिवस एवं राष्ट्रीय विज्ञान दिवस भी उत्साहपूर्वक मनाया। भारत के माननीय प्रधानमंत्री द्वारा शुरू किया गया स्वच्छता अभियान 2 अक्तूबर 2014 से केन्द्र में लागू किया गया। इस अभियान के अंर्तगत केन्द्र हर शनिवार को दो घण्टे के लिए नियमित रूप से स्वच्छता अभियान में शामिल होता है। केन्द्र ने गत वर्ष में 15 अश्व स्वास्थ्य शिविर एवं किसान गोष्ठियों का आयोजन किया। केन्द्र ने अपनी गतिविधियों को प्रदर्शित करने के लिए विभिन्न प्रदर्शनियों में भाग लिया।

केन्द्र के वैज्ञानिकों ने गत वर्ष में 55 शोध पत्र, राष्ट्रीय एवं अंतर्राष्ट्रीय ख्याति की शोध पत्रिकाओं में प्रकाशित किए। साथ ही लोकप्रिय लेख, शोध पत्र संक्षिप्तिकरण तथा 46 जीन अनुक्रम सार्वजनिक डाटाबेस में प्रकाशित किए।

Introduction

Since the dawn of Indian civilization equines find an important place in day to day life in context of our history. There might be debates about the first domestication of horse in India but they have been mentioned in the oldest scriptures of Hinduism- "Vedas" and have been the main source of transport for men and materials in past. In the present era, mechanization has decreased the utility of animal power; yet, equines have great relevance, especially in the hilly and difficult terrains of the country, where other means of transport are inaccessible. The increase of 43.34 % in mule population (0.19 m) in the recent livestock censes is a food for thought for planners and policy makers to focus more on mule production in country. At present, equine population in India is 1.14 m, which includes horses and ponies (55%), mules (17%) and donkeys (28%). Major population of these equids provide livelihood to the landless, small and marginal farmers and other sections of unprivileged rural societies.

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) as its main campus. It 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 like 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.

Since its inception, institute's efforts have been focused on infectious diseases confronting equines, surveillance and monitoring of equine diseases, development of diagnostics and vaccines and improvement of equine health and production which has led to its recognition at national and international levels. The vision of the Centre is to enhance the utilization of equines in agricultural and transport through development programmes in order to elevate socio-economic status of under-privileged. Veterinary Type Culture Collection (VTCC) established in the year 2005 at Hisar for collection and preservation of microbes of animal origin and veterinary importance, is an integral part of the Centre.

Mandate of NRCE

- To undertake research on health and production management in equines;
- To act as a national referral facility for diagnosis of equine diseases and
- To provide advisory and consultancy services.

Objectives

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

Major Issues

- Achieving freedom from dreaded equine diseases through development of modern diagnostics & vaccines.
- Transfer of technology for superior mule & 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 & mountainous regions.
- Income generation through market intelligence activities.

Thrust Areas

- Refinement of diagnostic tests and associative assays, kits and reagents
- Preparedness for developing newer diagnostic methods and preventive and control measures against infectious diseases of equines
- Understanding pathogen evolution through mutation or interaction of exotic genetic material, early warning against emerging/re-emerging diseases
- Emergency preparedness in terms of early diagnosis of disease, forewarning, and taking strategic control measures for the diseases with emphasis on clinical proteomics, whole genome sequencing in disease diagnosis, pathogen characterization and nanonized molecule(s) - targeted drug/vaccine delivery
- Use of bioinformatics and modern biotechnology tools in designing vaccines, drugs and stem-cell therapeutic approach for control of important equine diseases
- Development of national policy on disease control, prevention and management
- Conducting epidemiological investigations especially in widely distributed working equine populations with a statistically sound population sampling design so as to formulate disease forecast and control measures
- Establishment of equine sanctuary and *in situ* conservation of indigenous breeds of horses and donkeys by way of perfecting artificial insemination (AI) and embryo transfer technology (ETT)
- Indigenous breed conservation approaches and initiate immediate action plans with state government's/ NGO/SAUs and, agencies/ department approved by Government of India
- Initiate research work on equine welfare issues
- Generation of database and validation of ITKs in equine production and utilization
- Genetic improvement of mules, donkeys and ponies used for draught purposes
- Enhancing nutritional quality of indigenous feed/fodder for formulation of ration for equids
- Training of personnel including veterinarians and livestock assistants, educating equine breeders and farmers for adopting scientific equine practices
- Explorative research for value addition of equine products
- Equine work physiology of horses, ponies, donkeys, and mules
- Evaluating endurance potential of Marwari horses for equestrian events like Thoroughbred horses
- Evaluate donkey gut physiology and microbiome
- Assess potential of horse milk and donkey milk as of cosmetic value, sports drink for athletes, and therapeutic drink for ailing and recovering human patients for their rejuvenation
- Establishing equine sports medicine with special emphasis on creating infrastructure for studies on body scanning/mapping for kinetics of racing, athleticogenomics
- Elucidation of complete behavioural responses of equines under various physiological states

MAJOR ACHIEVEMENTS

Diagnostics for equine diseases

The Centre has been recognized as National Referral Centre for diagnosis of important equine infectious diseases by Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture (Government of India). The Centre has developed and

refined diagnostics against various equine diseases including immunodiagnostics and molecular diagnostics such as:

Equine Herpes virus 1 (EHV 1): 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. This kit tests serum samples using single dilution, thus, making it very economical. Presently the kit is under the process of commercialization.

Equine Herpes virus 4 (EHV 4): 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 stool samples. An RT-PCR using VP6 gene primers was also developed and its results were compared with the s-ELISA. The RT-PCR was found to be equally sensitive as s-ELISA.

Equine influenza virus (EIV): EIV is routinely diagnosed by haemagglutination inhibition (HI) assay. RT-PCR for equine influenza diagnosis and typing has also been developed. Furthermore, real-time RT-PCR based assay targeting M & NP genes have also been developed for diagnosis of EIV.

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.

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 surra.

Japanese encephalitis virus (JEV): Serum neutralization test (SNT) and haemagglutination inhibition (HI) assay have 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.

Equine viral arteritis: Virus neutralization is routinely used for serodiagnosis of EVA.

Small Animal models for understanding pathology and disease mechanisms

a) 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, host-pathogen interaction while simultaneously working for screening of vaccine candidates for their protective efficacy and immune response.

b) Equine Herpes Virus 1: ICAR-NRCE has standardized the BALB/c mouse model for EHV-1 for respiratory infection and abortion studies. The abortion model has been widely utilized in pathology laboratory of NRCE for protective efficacy of inactivated EHV-1 vaccine. The respiratory model has been used for immune prophylactic studies of recombinant proteins of EHV-1.

Vaccines and Immuno-biologicals developed by NRCE

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

b) Updated Equine influenza vaccine: Previously, the Centre had developed equine influenza vaccine (EI) using indigenous isolate (A/equi-2/Ludhiana/87). During 2008-09, India experienced another outbreak of equine influenza. An antigenically and genetically divergent EIV strain was isolated, which was significantly different from the previous (1987) isolate. Thus, the vaccine has been updated in 2010 incorporating epidemiologically relevant isolate {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.

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

d) Monoclonal antibodies: Monoclonal antibodies have been developed for diagnosis and characterization of equine herpes virus 1, equine rotavirus, equine influenza and Japanese encephalitis viruses.

e) Kits for disease diagnosis: HERP kit & Equiherpes B-ELISA kit (For EHV 1 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.

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. Phylogenetic analysis established that 2008 EI outbreak in India was due to eq/2 (H3N8) subtype and that Indian isolates 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 (aa) sequences, respectively. Phylogenetic analysis revealed clustering of Indian and Chinese isolates in a separate cluster designated as “Asian clade” for M gene. All the eight gene set (HA, NA, NP, NS, M, PA, PB1 & PB2) of EI isolates from 2008-09 outbreak have been cloned, sequenced and analyzed.

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): Sequence analysis of E-gene of JEV isolated from an equine indicates genotype 3 was responsible for causing the disease in equine and that the equine JEV isolate clustered with Vellore group among isolates responsible for JEV in humans in India.

In vitro* culture of *Trypanosoma evansi: 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.

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 EHV 1, EHV 4, equine rotavirus, equine influenza, Japanese encephalitis virus, West Nile virus, *Rhodococcus equi, Streptococcus equi, S. zooepidemicus, S. Equisimilis, Burkholderia mallei*, *Salmonella* Abortusequi, *Enterobacter aerogenes*, *E. 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, West Nile virus and *Trypanosoma evansi*.
- 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, EHV 1, EHV 4, EIA, JEV, EIAV, *R. equi*, *Burkholderia mallei*, *Trypanosoma evansi* and *Theileria equi.*

Indigenous breed characterization

- **Phenotypic characterization of Indigenous horse and pony breeds**

Populations of the six equine breeds registered by the Indian National Bureau of Animal Genetic Resources have drastically decreased due to indiscriminate breeding and their low utilization. These breeds namely Marwari, Kathiawari, Spiti, Zanskari, Bhutia and Manipuri, were characterized phenotypically on the basis of their biometric indices and coat colour. Significant differences among different biometric indices were observed due to breed as well as sex.

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.

In Marwari and Kathiawari breeds, both stallions and mares were found to rotate their ears at an angle of 180° making the ear tips meet in the centre, which is a typical characteristic of the two 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 out group was carried out which indicated high genetic diversity in all Indian breeds except Spiti ponies with maximum genetic differentiation between Spiti and Thoroughbred (0.1729), followed by Spiti and Kathiawari (0.1725), while Zanskari and Manipuri were the least differentiated (0.0379). Individual assignment indicated admixture in all the breeds except Thoroughbred horses.

Establishment of Nucleus Herd

- **Exotic Donkeys:** Jennies and jacks of European breed (Poitu), imported from France & UK, are being maintained at EPC, Bikaner for the improvement of indigenous donkeys and production of superior mules.
- **Marwari Horses:** In 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 (8 mares & 4 stallions) were brought from Zanskar valley, Kargil, Ladakh, Jammu & Kashmir in November, 2009. In 2014, a total of 11 Manipuri ponies (7 mares & 4 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:** Pregmare - An eCG based sandwich ELISA kit has been developed for detection of pregnancy between days 30 to 150 of gestation in mare. The kit is cost effective, horse specific and animal friendly. Also pregnancy diagnosis between days 14 and 18 post-insemination has been achieved using ultrasonography in donkey and horse mares.

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 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

The Centre was facing the problem of equine dung disposal as it cannot be utilized directly as manure in fields. It does not decompose properly due to low moisture content and poor water absorption. 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

ICAR-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.
- Assessment and transfer of technology using the latest know-how of information technology is also given due importance to extend the technologies to the end-users.
- Extension activities: To receive feedback from the equine owners, various activities like health camp, awareness and farmers meets are organized on regular basis in different areas of the country.

Veterinary Type Culture Collection

Veterinary Type Culture Collection was established at NRCE by ICAR in 2005 as a national repository of animal microbes including dairy and rumen microbes with the aims of:

- Exploration and collection of microorganisms of animal origin/significance/relevance
- Central storage of animal microbes from existing culture collection centres, institutions and universities
- Characterization, documentation and digitization of microbial database of cultures of animal microbes
- Development of a National Microbial Gene Bank for conserving the biodiversity of animal microbes
- 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.

Mandate

- To act as a national repository of micro-organisms including recombinant cultures and plasmids
- Identification, characterization and documentation of animal microbes
- Conservation, maintenance, surveillance and utilization for R & D
- Human Resource Development (HRD)

Milestone Achievements

- Several vaccine strains from livestock and poultry have been added to VTCC.
- Complete genome sequencing of two isolates of Classical swine fever virus was carried out.
- A total of 19 bacteriophage isolates against a variety of bacteria such as *Aeromonas hydrophila*, *Ecsherichia coli*, *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp. were isolated and accessioned in VTCC repository.
- Strengthening of repository with Veterinary microbes during the year:-
  - Bacteria accessioned : 227
  - Virus accessioned : 21
  - Bacteriophage : 19
  - Recombinant clones accessioned : 140
  - Genomic DNA accessioned : 47

So far, a total of 2546 cultures/clones have been deposited in VTCC 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.

VTCC repository includes bacterial isolates represented by more than 900 isolates belonging to more than 50 genera. Viral isolates *viz.* camelpox virus, buffalopox virus, goatpox virus, orf bovine herpes virus 1, equine herpes virus 1 & 4, equine influenza virus, bovine rotavirus, human rotavirus, Japanese encephalitis virus, RD virus, BTV, NDV, CSF, PPRV, Avian virus are represented in VTCC repository.

Staff position of NRCE and VTCC (as on 31.03.2015)

Name of the post	NRCE			VTCC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	1	1	0	-	-	-
Scientific	26	21	5	10	7	3
Technical	24	22	2	1	-	1
Administrative	14	11	3	-	-	-
Supporting	22	19	3	-	-	-
Total	87	74	13	11	7	4

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 Centre, 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

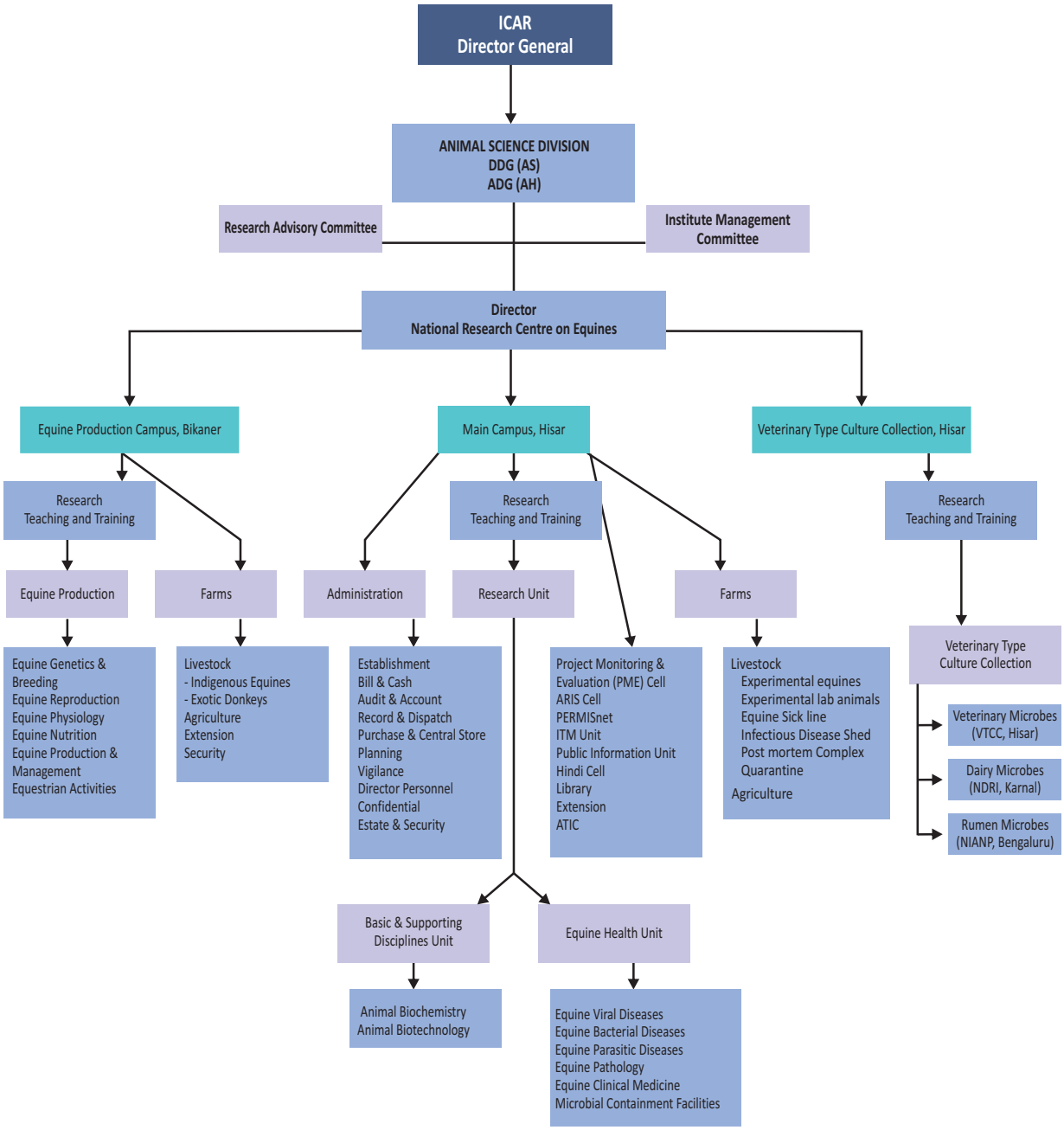
2010	Equine sanctuary for conservation of indigenous breeds of horses and indigenous donkeys initiated
2010	A new clade designated as 'Asian Clade' of Equine Influenza Virus reported
2010	Award of OIE twining project on Equine Poroplasmosis between NRCPD, Japan and NRCE, India
2010	EIA-positive mule 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	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

Summary of Expenditure & Revenue Generation

(₹ in lacs)

Summary of Expenditure		2013-14	2014-15
	Non-plan		
1.	Establishment charges including LSP/PF, wages, OTA	590.41	689.29
2.	Travelling allowances	3.99	3.98
3.	Others charges including equipments & recurring charges	322.94	326.77
4.	Works	0.00	0.00
	Total Non-Plan Expenditure	**917.34**	**1020.04**
	Plan		
1.	Establishment charges including LSP/PF, wages, OTA	0.00	0.00
2.	Traveling allowances & HRD	22.00	17.45
3.	Others charges including equipments & recurring charges	508.92	451.85
4.	Works	204.00	150.36
	Total Plan Expenditure	734.92	619.66
	Total Expenditure (Plan & Non Plan)	**1652.26**	**1639.70**
Summary of Revenue Generation		**(₹)**	**(₹)**
1.	Sale of farm produce	3127856.00	736213.00
2.	Sale of livestock	765900.00	991160.00
3.	Sale of publication and Advertisements	39201.00	292435.00
4.	License fee	110196.00	173735.00
5.	Interest on loans and advances	178750.00	328585.00
6.	Interest on short term deposits	3016067.00	2100470.00
7.	Income from internal resource generation	4541622.00	5242288.00
8.	Receipt from services	0.00	0.00
9.	Other misc. receipts	1115512.00	898309.00
	Total Revenue	**12895104.00**	**10763195.00**

Organizational Set-up



Research Achievements
Equine Health

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

The Centre is actively involved in monitoring the disease situation in equines of the country in order to combat any emerging and re-emerging diseases in this species. ICAR-NRCE carries out thorough countrywide surveillance and investigations on samples received from field and organized sectors. The Centre also conducts testing of the samples from animals under continuous movement including those being imported or exported from the country. During the year 2014-15, sero-surveillance was conducted on serum samples obtained from various States/ UTs of India including Maharashtra, Rajasthan, Delhi, Haryana, Punjab, Tamil Nadu, Uttar Pradesh, Karnataka, Andhra Pradesh, Uttarakhand, J & K, Gujarat, Pondicherry, Chandigarh, Madhya Pradesh, Manipur, Chattisgarh, Assam and West Bengal.

The emerging and re-emerging viruses cause severe health hazards in equines, which affect livelihood of poor farmers. The current situation of the important viral diseases were investigated through testing of 1626 samples for EHV-1, 1687 for JEV, 2363 for equine influenza, 105 for equine viral arteritis (EVA) and 75 samples for WNV (Table 1& 2, Fig 1). The samples (1626) tested for EHV-1 included S&M (1579), DI (44) and contractual service (3) (Fig 2). Out of 1626 samples tested, 40 serum samples under S&M and two each under DI and contractual service were

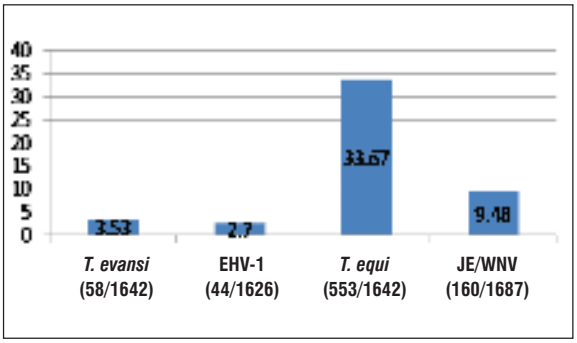


Fig. 1. Overall percent seroprevalence of various diseases under all catagories of testing (April, 2014 to March, 2015)

Table 1. Seroprevalence of important equine diseases from indigenous equines

State	EIA	EI	Glanders	*T. evansi*	EHV-1	*T. equi*	JE/WNV	*S. Abortusequi*	Brucellosis
Rajasthan	0/1058	82/1058	0/1058	17/1058	16/1058	216/1058	62/1058	0/1058	0/1058
Haryana	0/121	1/121	0/121	0/121	6/121	45/121	38/121	0/121	0/121
UP	0/374	3/374	0/374	38/374	18/374	151/374	57/374	0/374	0/374
Gujarat	0/20	0/20	0/20	1/20	0/20	4/20	2/20	0/20	0/20
Tamil Nadu	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Total	0/1579	86/1579 (5.44%)	0/1579	56/1579 (3.54%)	40/1579 (2.53)	516/1579 (32.67%)	159/1579 (10.70)	0/1579	0/1579

Table 2. Overall testing of important equine diseases under different categories

Disease	Contractual	S&M	Investigation	Total	Incidence
EIA	3560	1579	24	5163	0.00
Glanders	4521	1579	167(14)	6309(14)	0.22
EI	31(13)	1579(86)	722(62)	2332(161)	6.90
EHV-1	3(2)	1579(159)	44(2)	1626(163)	2.70
JE	75	1579(40)	33(1)	1687(41)	9.48
T. evansi	14	1579(56)	59(2)	1642(58)	3.53
T. equi	119(34)	1579(516)	17(3)	1642(553)	33.67
S. Abortusequi	-	1579	10	1589	-
Brucellosis	-	1579	5	1584	-

*Figures in parentheses indicate positive samples

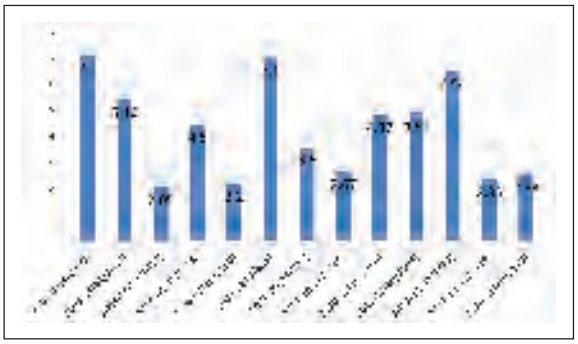


Fig. 2. Percent seroprevalence of EHV-1 under S&M (Trends)

found positive for EHV-1. The samples tested for JEV included S&M (1579), DI (33) and contractual service (75). The test results indicated 159 positive samples under S&M and one positive sample under DI, whereas no sample under contractual service was found positive for JEV antibodies. Out of nine samples tested for rotavirus by ELISA/RT-PCR, 3 samples were found to be positive. All 105 samples tested were negative for EVA by VNT under contractual service and none of the samples tested by VNT/HI was positive for WNV. In view of major outbreaks of equine influenza during 2008-09 affecting 13 states of the country, follow up surveillance for the disease in affected States continues. For this, 2363 samples under S&M (1579 samples), DI (722 samples) and contractual service (31 samples) including samples from 13 vaccinated animals from various states were tested for equine influenza (H3N8) antibodies employing haemagglutination inhibition (HI) assay, which revealed seropositivity in 161 samples (86 S&M, 62 DI and 13 contractual service samples). Twelve serum samples were screened in pair and none of them showed any rise in titres for EI. Continued existence of low antibody titres in few serum samples is under investigation. For getting disease free status for African horse sickness (AHS), necessary testing document and dossier was prepared and transmitted to OIE.

The disease status of EIA was investigated through testing of 5163 serum samples from Thoroughbred as well as indigenous equines under S&M (1579), DI (24) and contractual service (3560) (Fig. 3) employing gold standard Coggins test. All the samples tested were negative for EIA, which indicates that the disease is

State	EIA	Glanders
Delhi	715	890
Haryana	288	502
Andhra Pradesh	213	784
Maharashtra	109	231
Karnataka	937	571
Tamilnadu	239	288
West Bengal	136	62
Punjab	69	134
Rajasthan	489	541
Uttar Pradesh	207	279
Uttarakhand	26	123
Gujarat	34	43
Pondicherry	8	7
Chandigarh	26	26
Madhya Pradesh	30	15
Assam	18	9
Manipur	5	5
Chattisgarh	11	11
Total	3560	4521

* All samples tested under contractual services were negative

Fig. 3. States / UT covered for EIA and Glanders testing under contractual services

not prevalent in the country. Surveillance studies reported outbreaks of glanders, a dreaded disease of equines, from U.P., H.P. and J&K during the year. A total of 6309 serum samples were tested for glanders, which included samples from S&M (1579), DI (167) and contractual service (4521) (Fig. 3). Among these, 11 serum samples from UP were found positive under disease investigation. The causative agent *B.mallei* was isolated from a clinical sample of an infected horse from U.P. In H.P., one animal was serologically and another one was culturally positive for glanders. One serum sample was positive for glanders from J & K (Katra).

Bacteriological/mycological analysis of the samples was also carried out. A total of 1579 serum samples tested for *Brucellosis* and *Salmonella* Abortusequi (H antigen) revealed negative results. The testing of 250 samples originating from Rajasthan, Haryana, U.P and H.P including nasal swabs, rectal swabs, skin scrappings, lesion swab, tissues from PM, faecal sample, soil, stomach content from aborted foetus yielded 115 important isolates. The bacteria isolated and identified from these samples include *Rhodococcus equi* (60), *Streptococcus equi* subsp. *zooepidemicus* (3), *Streptococcus equi* subsp. *equi* (1), *Streptococcus* sp. (1), *Trichophyton* sp.(1), *Klebsiella spp.*(7), *E. coli* (32), *B. mallei* (2) etc (Table 3). Investigation of 5 samples from CMVL received for inter laboratory comparison yielded Group D *Streptococcus* (1), *Staphylococcus* species.(1), *Pseudomonas* species(1), *Klebsiella* species.(1) and *E. coli*(1). 127 swab samples from animal quarantine

Table 3. Bacteria isolated from clinical samples

Organism	No.	Site	State/Region
Rhodococcus equi	60	Nasal Swab (28), Rectal Swab (8), PM Tissue (24)	Rajasthan (60)
E. coli	32	Faecal Sample (6), Nasal Swab (1), PM Tissue (23), Stomach Content (Aborted foetus) (2)	Rajasthan (28), Haryana (4)
Streptococcus spp.	1	Nasal Swab (1)	Haryana (1)
Trichophyton spp.	1	Skin scrappings (1)	Haryana (1)
B. mallei	2	Lesion Swab (2)	UP (1), HP(1)
Klebsiella spp.	7	PM Tissues (7)	Rajasthan (7)
S. zooepidemicus	3	Nasal Swab (3)	UP (2), Haryana (1)
S. equi	1	Nasal Swab (1)	Haryana (1)
Staphylococcus spp.	5	NA Slant (5)	CMVL, UP (5)
Pseudomonas spp., Streptococcus (GDS), Klebsiella spp., E. coli	4 (1 each)		(For inter lab comparison)
Total	**115**	–	–

centres tested for CEM were negative. Antibiotic sensitivity testing of clinical isolates were done and results were conveyed to various concerned stakeholders.

Sero-surveillance on 1642 serum samples for *T. evansi* revealed positive status in 58 samples (Fig. 4). Further, 553 of 1715 serum samples tested for *Theileria equi* were found positive for antibodies by ELISA.

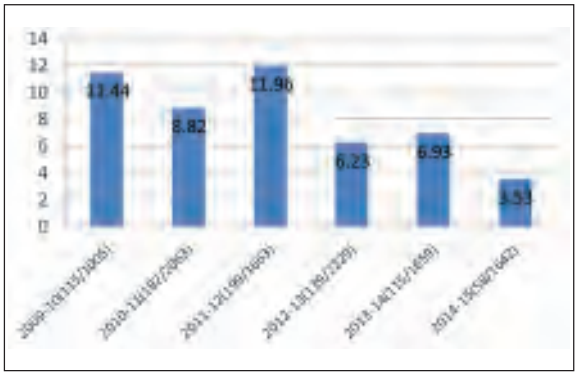


Fig. 4.Percent Seroprevalence of *T. evansi* (Trends)

Disease investigation was also carried out through post-mortem examination and histopathology on morbid material/ biopsy received from the field. Important conditions recorded (Fig. 5a to 5g) on the 21 samples included portal cirrhosis and enteritis (2),

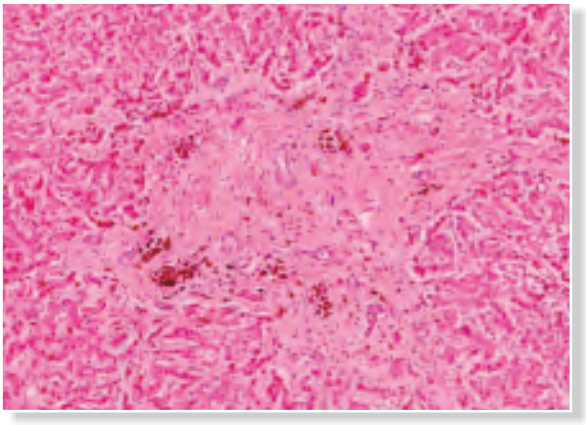


Fig. 5a. Section of liver showing massive proliferation of mature connective tissue in portal triad- Portal Cirrhosis H& E 100X

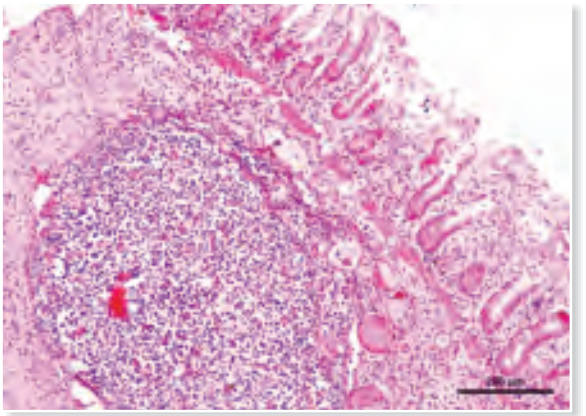


Fig. 5b. Necrotic enteritis with necrosis and desquamation of epithelium H&E100X

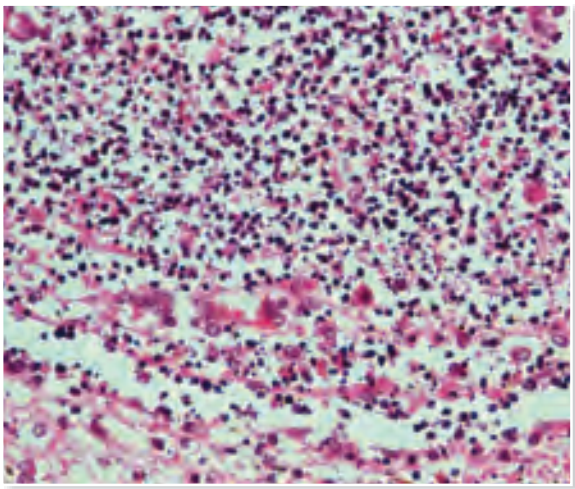


Fig. 5c. Necrosis of lymphocytes in Peyer's patches - necrotic enteritis H&E100X

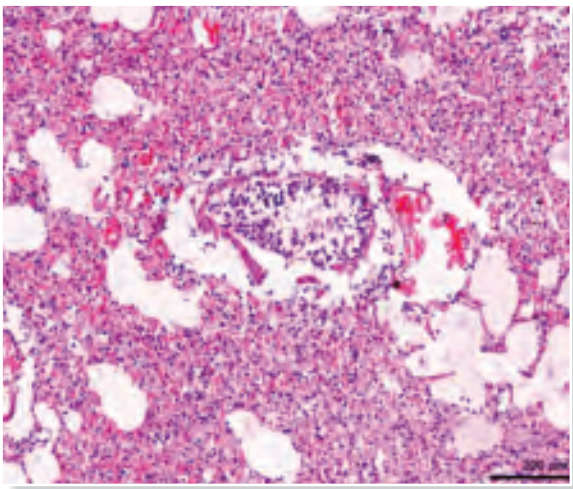


Fig. 5f. Bronchopneumonia due to *R.equi* infection in foal H&E 100X

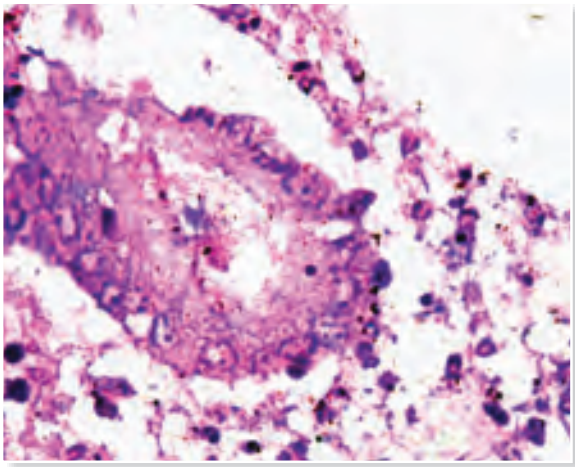


Fig. 5d. McCallum Good Pasteur stained section of intestine showing colonies of rod shaped Gram negative bacteria 100X

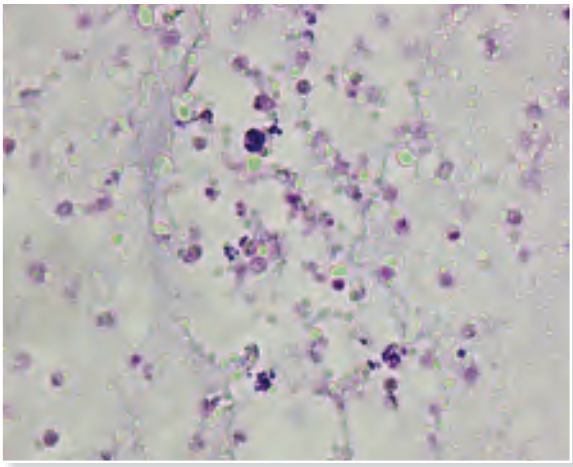


Fig. 5g. McCallum Good Pasteur stained section of lung tissue showing Gram postive coccobacilli (*R. equi*) 100X

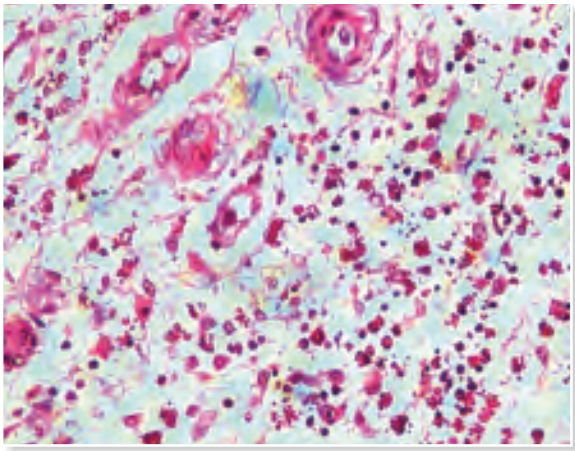


Fig. 5e. Necrotic enteritis with massive eosinophi infiltration in lamina propria and submucosa H&E100X

necrotizing enteritis (4), fatty liver along with enteritis (2), bronchopneumonia due to *R. equi* (2), interstitial pneumonia (2), enteritis (2), and abortions due to unknown infectious cause other than EHV-1(4).

(S.K. Khurana, S.C. Yadav, B.R. Gulati, Praveen Malik, Rajender Kumar, Nitin Virmani, Sanjay Kumar, Sanjay Barua, R.K. Vaid, Ramesh Dedar, H. Singha, Anju Manuja, Balvinder Manuja)

Outbreaks of glanders among indigenous equines in Uttar Pradesh, Himachal Pradesh, and Jammu & Kashmir during 2014-15

Glanders is a fatal bacterial disease of equines caused by non-motile gram-negative bacterium Burkholderia mallei. In India, glanders cases have been reported almost every year since 2006. Between April 2014 and March 2015, glanders outbreaks were reported in Uttar Pradesh, Himachal Pradesh and Jammu & Kashmir which involved 23 equines. In continuation with the past year's outbreaks in Uttar Pradesh, glanders was reported in 10 equines at Agra during September-October 2014 (Fig. 6 a). Two cases of glanders were also detected at Sarkaghat, Himachal Pradesh during February 2014. Ten equines were found to be positive for glanders at Katra in Jammu & Kashmir during March 2014 (Fig. 6b). All the infected equines were found serologically positive by CFT (titer 16-128) and in-house immuno-assays. The causative agent B. mallei was isolated from one infected horse from Uttar Pradesh and one mule from Himachal Pradesh. In glanders-endemic areas, a reasonable control, containment and eradication of disease can only be achieved by a strict implementation of 'testing and culling of positive animals' with active co-operation from State Animal Husbandry Authority in combination with provision of reasonable compensation to equine owners. NRCE is continuing all efforts for surveillance, educating equine owners, general public and field veterinarians with ultimate aim of prevention, control and eradication of glanders.

Fig. 6a. Glanders affected cases in U.P.

Fig. 6b. Glanders affected mules located at Katra, J&K

(S.K. Khurana, Harisankar Singha and Praveen Malik)

Expression and characterization of eukaryotically expressed recombinant glycoproteins of EHV1

Equine Herpes Virus 1 (EHV1) is one of the most important pathogens of equines causing abortions, respiratory disease, neurologic disorders and perinatal foal mortality. The infection due to EHV1 is endemic in India and abortion outbreaks due to EHV1 keep on occurring in India. NRCE has already developed an inactivated vaccine using indigenous EHV1 isolate. However, the control of cell associated viremia is thought to be critical for the prevention of EHV1 abortions, therefore, we need

to stimulate and strengthen the cell mediated immune responses. Looking into this, work was initiated in the area of expressing immuno-dominant glycoproteins of EHV1 in eukaryotic system and development of bacterial artificial chromosomes and to see their role in protection studies to be conducted in a mouse model.

For eukaryotic protein expression, glycoprotein B, D and M were selected and gene–specific primers were designed for amplification of extra-cytoplasmic regions with addition of RE site. Kozak sequence for efficient transcription and His-tag sequence for purification of the recombinant protein were added. The amplicons of gD & gM regions of EHV1 were directionally cloned into the donor vector followed by generation of recombinant bacmid having cloned gD and gM genes in the virus genome using site-specific transposition with Tn7 into bacmid. The optimized Sf9 cells were transfected with recombinant bacmid for gM and gD for generation of recombinant virus. Passage 1 virus was employed for protein expression studies. Glycoprotein gD (~48kD) showed expression from 40-72 hours with a peak expression at 48 hours post infection, while gM (~52kD) (Fig. 7) was expressed at 48 hours only.

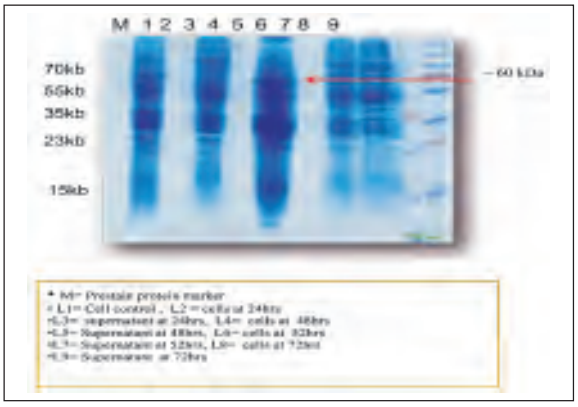


Fig. 7. Expression of rgM in sf9 cells

The protein was confirmed in Western blot analysis through staining with anti-His mAb. The expression is further being optimized with various conditions of temperature, time interval and MOI (multiplicity of infection).

For construction of EHV-1 bacterial artificial chromosome (BAC), gene 71 (g71) was selected as targeted region to clone EHV-1 isolate. The flanking regions (2.1Kb left side and 2.2Kb right side) were amplified using designed primers and a transfer vector was generated by sequential cloning of flanking regions into pUC19 vector and subsequently recombinant pUC19 construct was sub-cloned into PacI site of the mini-F plasmid. Various strategies were employed for transfection of RK13 cells with the construct and EHV-1 virus/DNA using polycationic compound at various concentration of plasmid and EHV-1 virus/DNA. Green fluorescence with cytopathic effect due to EHV-1 infection in RK-13 cells was observed 24 hours post transfection (Fig. 8a

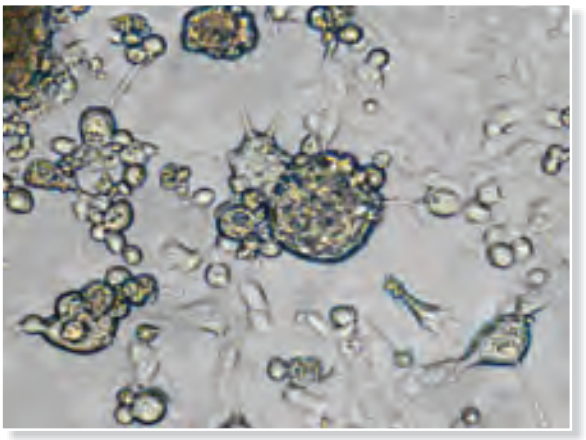


Fig. 8a. CPE in RK-13 cells transfected with EHV-1 virus and transfer plasmid at 24 hrs

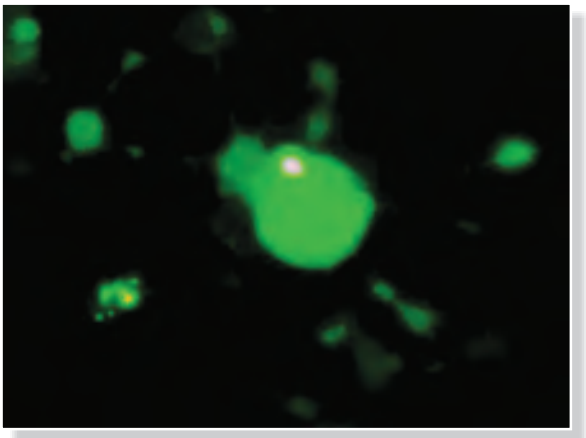


Fig. 8b. Green fluorescence due to GFP marker in transfer plasmid indicating successful homologous recombination

to 8b). Recombinant virus is being attempted for plaque purification by limited dilution methodology.

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

Investigations on neuropathogenic and non-neuropathogenic variants of equine herpes virus-1 and associated latency among equines in India

Genetic characterization of EHV-1 isolates and identification of neuropathogenic potential strains prevalent in India

Equine herpesvirus 1 (EHV1) is enzootic in horse populations worldwide and is a significant cause of economic loss to the horse industry. During past one decade, occurrence of equine herpes myeloencephalo (EHM) pony is increasing globally. EHV1 strains causing EHM show single nucleotide polymorphism (A to G) at position 2254 in the EHV1 DNA polymerase gene encoded by ORF30. We analyzed EHV1 isolates from different abortion outbreaks in India by nucleotide sequence analysis of ORF30 and ORF68 genes of EHV-1 isolates. The SNP in ORF30 was further assessed by allelic discrimination on the basis of real-time PCR assay.

ORF30 gene of 16 EHV1 isolates was PCR amplified, cloned and sequenced. Sequence analysis revealed that 14 EHV1 isolates had the nucleotide substitution 'A' at the position 2254 while two (Delhi/2008 and Tohana/2007) had nucleotide substitution 'G' at position 2254 of ORF30, indicating that they have neuropathogenic potential (Fig. 9). Overall, 87.5% of the isolates from abortion and neonatal foal mortality cases were of non-neuropathogenic genotype, while 12.5% contained the neuropathogenic marker (D752/G2254) as confirmed by sequencing.

A real-time PCR assay was standardized for detection of single nucleotide polymorphism (SNP) at position 2254 of ORF30. All the 14 isolates/ samples showing A2254 by nucleotide sequencing were detected positive by Hex probe in real-time PCR as A2254 SNP, while two G2254 by sequencing reacted with Fam probe as G2254 SNP. There was 100% agreement between results of ORF30 sequencing and real-time PCR for detection of A/G single nucleotide polymorphism. A total of 58 samples comprising 10 cell culture isolates, 18 blood samples, 22 tissue samples, 4 vaginal swabs and 4 nasal swabs were tested by both nested PCR and real-time PCR. The sensitivity and specificity of real-time PCR for detection and differentiation of EHV1 strains were 93.02% and 100%, respectively.

Genetic diversity among equine herpesvirus 1 isolates prevalent in India

EHV1 has been detected from a number of outbreaks in horses from India including abortion, still birth, neonatal foal mortality as well as paresis. However, there is not much information available about the genetic diversity existing among EHV1 isolates prevalent in India. EHV1 genome which is linear, double-stranded DNA molecule of 150,223 kbp size containing 76 open reading frames (ORFs), do not show much genetic diversity. EHV1 isolates have been

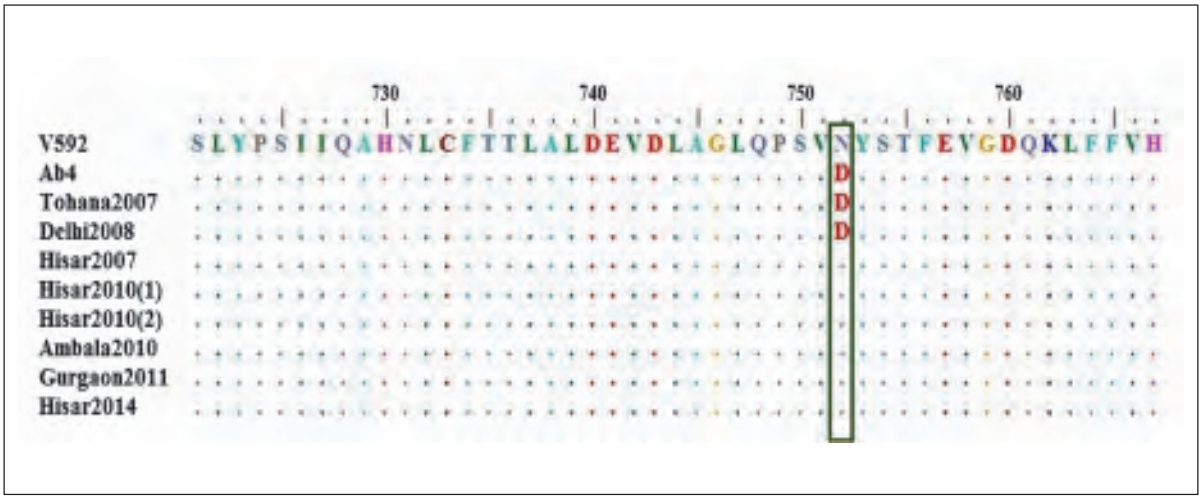


Fig. 9. Sequence analysis of ORF30 of EHV1 isolates for detection of neuropathogenicity

classified into different groups based on nucleotide polymorphism in ORF68 gene. To decipher this genetic diversity among Indian isolates, the sequence analysis of partial ORF68 gene was done for 7 EHV1 isolates. Based on SNP analysis, Indian EHV1 isolates could be classified into 2 groups (Group 4 and Group 5). Three EHV1 isolates (Jind/96, Rajasthan/98 and Delhi/07) possessed marker SNPs characteristic to Group 5, including A629, G710, A713 and 7 Gs in the homopolymeric G tract (732-739) in the partial ORF 68 region and hence were classified into Group 5. The other three Indian isolates (Delhi/98, Tohana/13 and Hisar/14) showed SNP A629 and 7G at position 732-739, indicating that they belonged to Group 4 (Fig. 10).

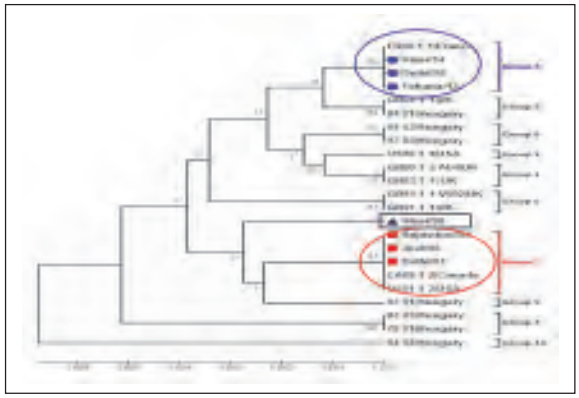


Fig. 10. Phylogenetic analysis of ORF68 genes of EHV1 used to study genetic diversity

(B.R. Gualti, Nitin Virmani and Riyesh T.)

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

The unrestricted animal movement, rapid globalization and climatic change have led to crossing of diseases through boundaries and has necessitated the preparedness for monitoring the emerging exotic diseases through development of biological reagents in terms of diagnostics and prophylaxis. Looking into this, work was initiated on developing diagnostics against exotic viral diseases of equines *viz.* Vesicular Stomatitis, Venezuelan Equine Encephalitis, Rift Valley Fever etc. For this, 5 antigenic regions from Vesicular Stomatitis Virus glycoprotein (Fig. 11a & 11b) and 12 antigenic regions from capsid and envelop proteins of Venezuelan Equine Encephalitis Virus were identified by *in silico* analysis. Constructs of 393 and 921 bases comprising multiple antigenic regions have been transferred to expression vector to express the recombinant protein in *E. coli*. For development of nucleic acid based diagnostics using synthetic gene technology for VS and VEE, the gene sequences representing serotypes of viruses were aligned to determine consensus sequences for designing primers. Serum samples were collected from different states for surveillance and monitoring

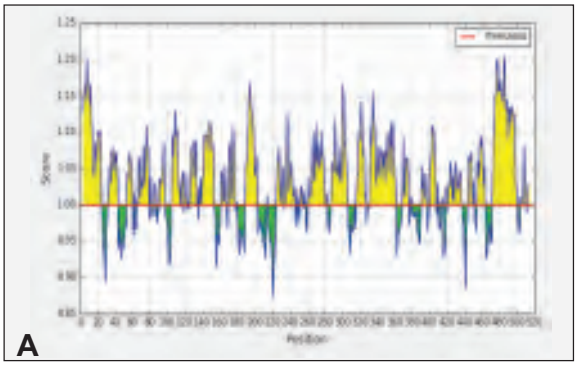


Fig. 11a. Kolaskar & Tongaonkar antigenicity prediction to predict antigenic determinants on for Vesicular Stomatitis Virus glycoprotein

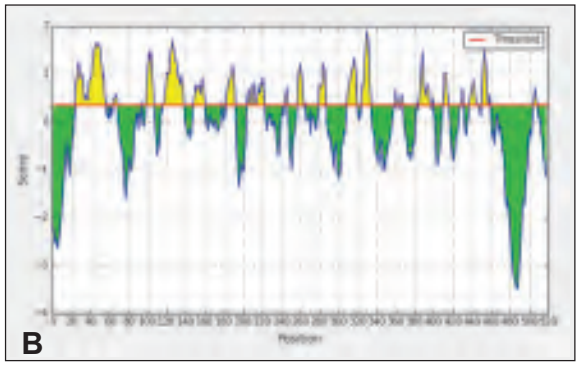


Fig. 11b. BepiPred linear epitope Prediction for Vesicular Stomatitis Virus glycoprotein

(Balvinder Kumar, Harisankar Singha, Anju Manuja and Praveen Malik)

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

Equine piroplasmosis is a tick-borne haemo-protozoan disease of equidae (horses, donkeys, mules, zebras), caused by an intra-erythrocytic protozoa *Theileria equi* and/or *Babesia caballi.* The disease caused by *T. equi* is generally more pathogenic than *B. caballi* and the parasite is more resistant to treatment as well. So far, no drug is available, which can completely eliminate *T. equi* infection from carrier animals. The development of new drugs having high chemotherapeutic efficacy against *T. equi* and low toxicity to the host are need of the day. We initiated some preliminary research work in this direction and selected target specific drug molecules and tested in MASP culture of *Theileria equi* in *in-vitro* system. Target based drug molecules were selected for this study. A total of nine drug molecules which were specific against HSP-90, choline kinase, nuclear transcription factor and flavonoid were selected. Imidocarbdipropionate was included as positive drug control so as to analyse the IC_{50} concentrations of each drug molecules.

The *in vitro* growth of *T. equi* was significantly inhibited by HHD and HDTAB drug molecule at 25, 50 and 100 µM concentration and IC_{50} values were found to be 17.42 µM and 14.0 µM, respectively. The *in vitro* growth inhibition of *T. equi* was significantly observed at 200 µM and 400 µM concentration by HMC drug molecule with IC_{50} value of 246.34 µM. The andrographolide drug did not show any significant inhibition in the growth inhibition of *T. equi* at any concentrations. The IC_{50} value of imidocarbdipropionate for growth inhibition of *T. equi* on 96h of culture was 0.139 µg/ml (Fig. 12).

In another *in vitro* trial, we planned to target phospholpid metabolism of *T. equi* by quaternary ammonium bromide salts and decamethonium bromide, dodecyltrimethyl ammonium bromide, decyltrimethyl ammonium bromide as choline kinase

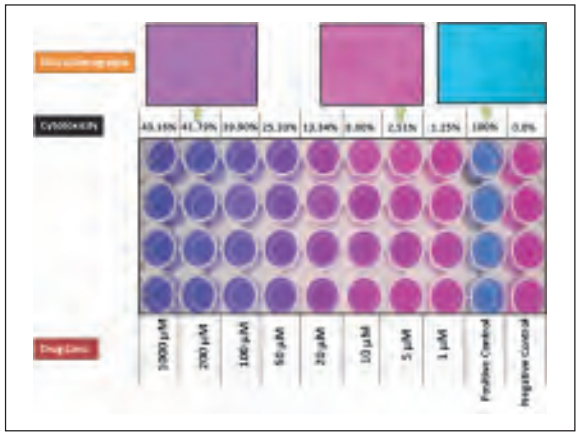


Fig. 12. Cytotoxicity assay on horse PBMC cell line using different concentrations of dodecyltrimethyl ammonium bromide drug molecule. Per cent cytotoxicity against respective drug concentration has been indicated by reduction of resazurin dye. Representative microphotographs of PBMC have also been included.

inhibitors. Decamethonium bromide showed significant inhibitory efficacy (IC_{50}) at more than 28 µm concentration, while dodecyltrimethyl ammonium bromide was able to inhibit *T. equi* growth at 14.0 µM (IC_{50}). NBCN salt and APPA are potential HSP-90 inhibitor and we tested these molecules for *T. equi* growth inhibition studies. NBCN salt showed inhibitory efficacy at 165 µM, while APPA proved to be ineffective.

Cytotoxicity of these above drugs was tested on PBMC collected form a horse and different concentrations (1 µm to 2000 µm) of these drugs were tested in analysing *in vitro* cytotoxicity. HDTAB, hesparidine and dodecyltrimethyl ammonium bromide were observed to be cytotoxic. The percent cytotoxicity of these drug molecules ranged from 31% to 58%, while harmaline, decamethonium bromide and NBCN salt are most promising drug molecules in inhibiting *T. equi* growth with least cytotoxicity. The percent cytotoxicity of these drug molecules ranged from 8% to 9.9%.

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

Pathology of equine influenza virus (H3N8) and vaccine efficacy studies in BALB/c mouse model

Equine influenza (EI) is highly contagious acute respiratory disease of equines caused by Influenza A virus (H3N8). The continuous drift in influenza viruses requires harmonization of vaccines and strain substitution. The studies in large animals for immunopathogenecity and potency testing are difficult and suitable small animal model is required for testing of the vaccine candidates prior to final testing in equines. The present investigations were undertaken to study pathology of H3N8 Influenza A virus (Sublineage Florida clade 2 virus) in mice model and to elucidate the protective efficacy of inactivated indigenous H3N8 vaccine in eliciting protective immune response in mice model. For the purpose of EIV pathogenicity and inactivated vaccine efficacy study, BALB/c mice were divided into four groups. Group A mice were mock immunized and challenged with EI virus, group B were vaccinated and challenged, group C mice were vaccinated only and group D mice were neither immunized nor challenged. Sequential studies were conducted on all the animals through humoral immune response kinetics (HAI and SRH), serum biochemistry, hematology, clinical examination, postmortem examination, histopathological examination, transmission electron microscopy, indirect immunoperoxidase assay, virus isolation and qRT-PCR. Immunization of mice resulted in protective HAI antibody titre after two boosters. Unvaccinated mice suffered severe respiratory disease and showed respiratory distress, forced expiration, ruffled coat, reduced activity and crouching at corners at 2-7 dpc with 6.34±0.21 % weight reduction at 5 dpc, whereas vaccinated mice showed minimal signs at 2-4 dpc with 3.69±0.13% weight reduction at 2 dpc. Hematology showed mild leucopenia with lymphopenia at 7 dpc and lymphocytosis at 3 dpc in group A and group B mice, respectively. Gross lesions in unvaccinated mice after challenge were congestion of nasal mucosa, grayish mucinous exudate in trachea, severe consolidation (3-4 mm × 2-3 mm) of lung parenchyma with congestion and gray discoloration at 2-5 dpc. Vaccinated mice showed only congestion of lung parenchyma without consolidation. Main histopathological changes were restricted to respiratory tract *viz*. impacted nasal turbinate and trachea with inflammatory exudates with degeneration (Fig. 13a) and sloughing of lining epithelial cells, necrosis of bronchi and bronchiolar epithelium (Fig. 13b), peribronchitis and perivascular cuffing of neutrophils and lymphocytes, diffuse interstitial pneumonia (Fig. 13c) with impacted macrophages, lymphocytes and type II pneumocytes at 5 dpc.

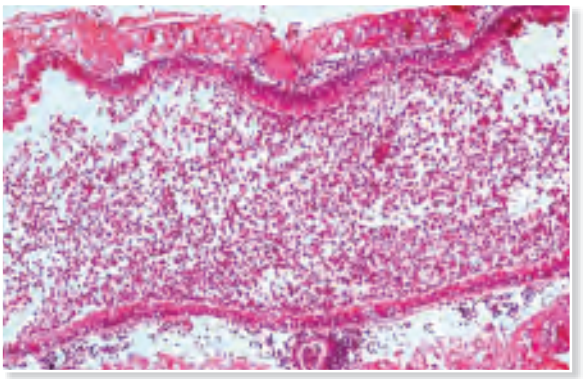


Fig. 13 a. Section of trachea from unvaccinated mice showing tracheal lumen completely impacted with denuded epithelial cells with inflammatory exudates mixed with mucus at 2

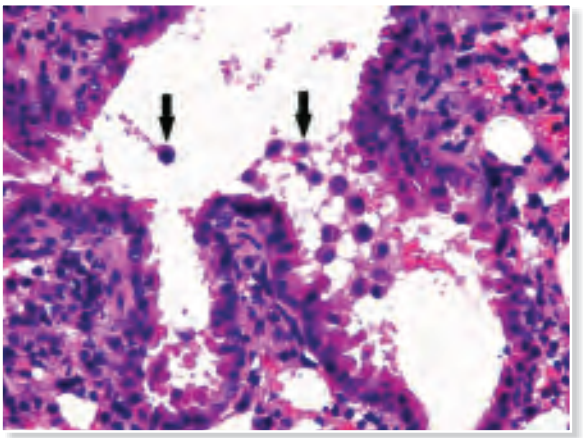


Fig. 13b. Section of lung from unvaccinated mice showing necrotic bronchiolar epithelium with macrophages in bronchial lumen (arrow) at 5 dpi H.E.X400.

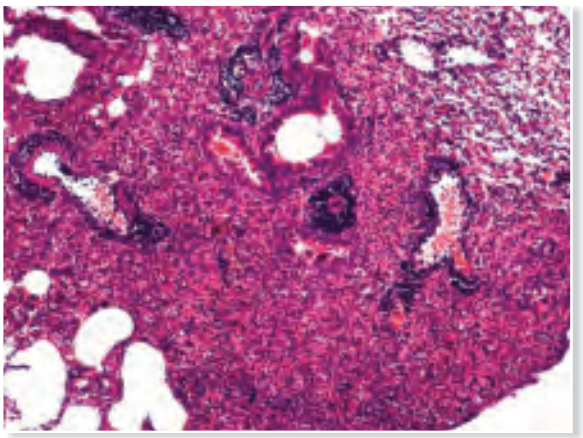


Fig. 13c. Section of lung from unvaccinated mice at 3 dpi, showing severe pulmonary consolidation H.E.X100

Gross and histopathological scoring revealed that vaccinated mice developed fewer lesions than unvaccinated mice upon challenge.

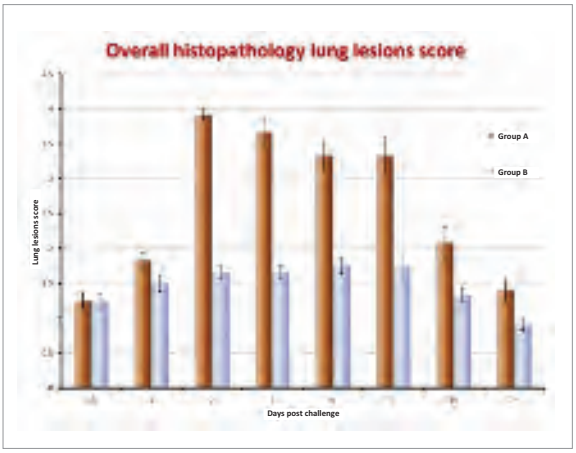


Fig. 14. Histopathological lung lesions scores for overall intensity of lung lesions at various intervals in vaccinated and unvaccinated mice after challenge with EIV

IIPT in unvaccinated mice showed EIV antigen distribution in nasal turbinate (Fig. 15a), tracheal, bronchial and bronchiolar epithelial cells (Fig. 15b), alveoli and interstitial macrophages (Fig. 15c). Vaccinated mice also showed similar EIV antigen distribution with less intensity.TEM revealed intra and inter cellular virions along with budding of virion particles from degenerating cells of trachea and lung at 3 dpc (Fig. 16) indicating establishment of infection and virus replication. Virus isolation from nasal

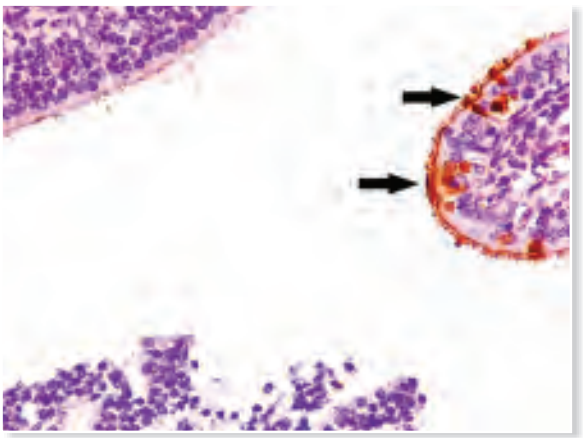


Fig. 15a. Presence of EIV antigens in cytoplasm and nucleus of degenerated and necrotic lining epithelial cells of nasal turbinate (arrow) from unvaccinated mice at 2 dpi (IIHC) X400.

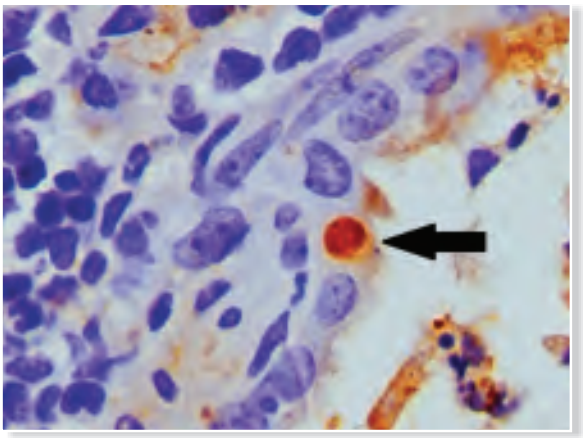


Fig. 15b. Section of lung from unvaccinated mice showing intra-cytoplasmic EIV positive antigens in bronchiolar epithelial cells (arrow) at 3dpi IIHCX1000.

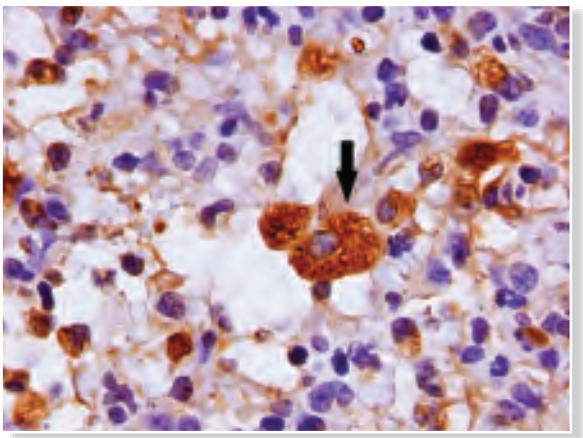


Fig. 15c. EIV positive antigens in cytoplasm of interstitial macrophages of lung at 3 dpi (arrow) from unvaccinated mice IIHCX1000.

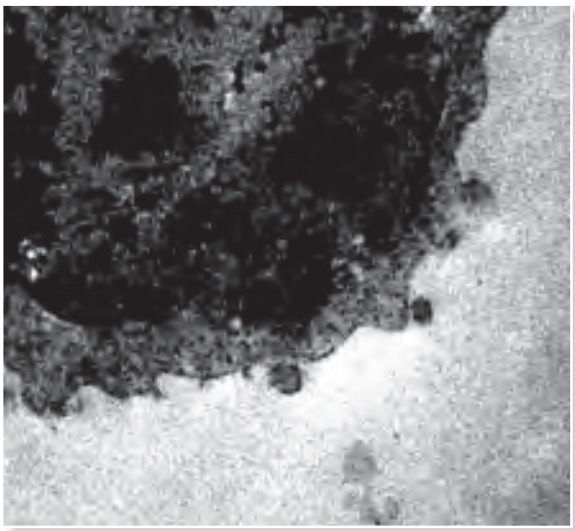


Fig. 16. Ultra thin section of lung at 3 dpi, showing budding of influenza virions from degenerating cell along with disintegration of nuclear envelope and loss of organelle architecture X 10000.

washings and lung tissues showed less virus shedding (1.25 log_{10} EID_{50}/ml) and early clearance (1 dpc) in vaccinated than unvaccinated mice (5.25 $log_{10}EID_{50}$/ml at 1 dpc and persisted up to 5 dpc). Further, qRT-PCR showed unvaccinated mice shed significantly more virus in nasal washings and lungs up to 5 dpc as compared to vaccinated mice which shed very less virus at up to 3 dpc. Thus, it has been concluded from the present investigations that EIV can infect and produce severe form of acute respiratory disease in BALB/c mice without adaptation and updated inactivated indigenous (H3N8) EIV vaccine developed at NRCE has protective efficacy in combating the disease in mice subsequent to challenge EIV. This is the first study to establish mouse model for studying equine influenza pathology and protective efficacy globally.

(Pavulraj, S. - M.V.Sc. student, Nitin Virmani and B.C. Bera)

Studies on the role of recombinant glycoprotein-B in protection against EHV-1 infection in BALB/c mice

The investigations were carried out to elucidate the protective efficacy of recombinant glycoprotein B and plasmid DNA against EHV-1 infection in BALB/c mice as compared to the conventional killed vaccine. BALB/c mice were utilized for the studies, as barring few differences related to species variation; the EHV-1 infection mimics that of natural host *viz.* equines. The activation of humoral immune response (HIR) was demonstrated by the presence of complement dependent neutralizing antibodies (NA) and by indirect ELISA post immunization with recombinant gB, plasmid as well as killed vaccine. Killed vaccine and glycoprotein vaccine showed sufficient titre of NA at 42 DPI before the challenge but DNA vaccine could not able to produce sufficient amount of viral neutralizing antibodies. Significant level of ELISA titre was observed in all the vaccinated mice before the challenge with highest level in killed vaccinated group. Maximum CMI could be observed in recombinant gB immunized mice followed by plasmid immunized group. Killed vaccine did not show any appreciable stimulation of CMI. Mice immunized with glycoprotein B showed minimum reduction in body weight followed by killed vaccine, DNA vaccine. The recovery in body weight could be seen as early as 5DPC in animals vaccinated with inactivated vaccine and rgB and followed by 7 DPC in DNA vaccinated group as compare to 14 days in control mice. On histopathology animals from all the groups exhibited characteristic lesion of EHV-1 in mice with varying severity *viz.* perivascular and peribronchiolar lymphocytic infiltration, presence of intranuclear inclusions, necrosis of nasal turbinate, trachea (Fig. 17a) and bronchiolar epithelium (Fig. 17b) with syncitia formation.

Control positive group showed maximum lesion and severity which was prominent on 3 and 5 DPC. Percentage mean score of the lesions was minimum in recombinant gB group followed by animals under killed vaccine group and maximum score was attained by non-vaccinated group in 3 or 5 DPC. The extent of lesions showed decreasing pattern in animals under immunized groups from 3 DPC onwards.

Vaccinated animals showed clearance of viral DNA from 3 DPC onward as compared to 5 DPC in control positive mice. It is concluded from the present study that recombinant glycoprotein B vaccine gave better protection in EHV-1 challenged mice as evident by

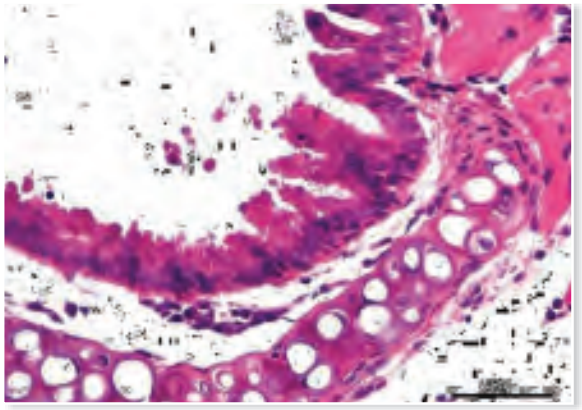


Fig. 17a. Section of trachea from EHV 1 infected mice at 3 dpc showing severe necrosis and sloughing of tracheal epithelium. H.E. X400

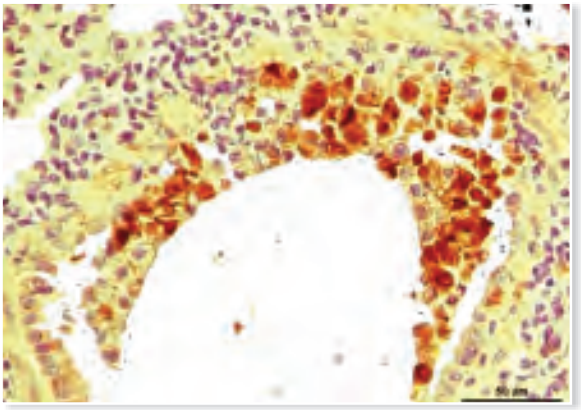


Fig. 17b. Section of lung from EHV 1 infected mice at 3 dpc showing EHV-1 positive antigens in detached lining epithelial cells and lymphocytes in bronchiole lumen I.I.H.C.X400

less reduction in body weight, early receding of clinical signs, early progress toward normal appetite and behavioral change, early attainment of pre challenge body weight, diminished histopathological lesion, least grading score at different interval, early receding of antigen from bronchiolar epithelium and vascular endothelium and rapid clearance of virus from lung.

(Alok Joshi - M.V.Sc. student, Nitin Virmani and B.C. Bera)

Expression and characterization of recombinant eCGβ protein

The recombinant eCGβ protein was expressed in *E. coli*, Sf cell lysate and Cos I cells and analysed in SDS-PAGE (Fig. 18). About 40 kDa r-eCG was produced with fusion tag- His and - Trx in *E. coli*, however, in Sf insect cell lysate about 27 kDa r-eCG was produced as no such tag was present. In Cos I mammalian cells, a band of r-eCG was observed at 65 kDa, which may be because of post-translation addition of side-chains and glycosylation as its biological counterpart. The r-eCG was purified from bacterial cells by Ni-NTA affinity chromatography and purified protein was characterized by sELISA. The biological activity was observed by ovarian hypermic reaction (OHR) in immature mice. No significant difference in weights of ovary, oviduct and uterus were observed in the control and experimental groups. The predicted amino-acid sequence of r-eCG was subjected to several bioinformatics analyses including disulphide bond prediction, hydrophilicity/hydrophobicity, antigenicity/antigenicity propensity score. DNASTAR Lasergene's Protean was used to predict and display patterns, secondary structural characteristics and locating the antigenic determinants. These analyses helped in finding different antigenic regions as well as hydrophilic and hydrophobic regions in the eCG protein through in silico approach that will be useful in future studies.

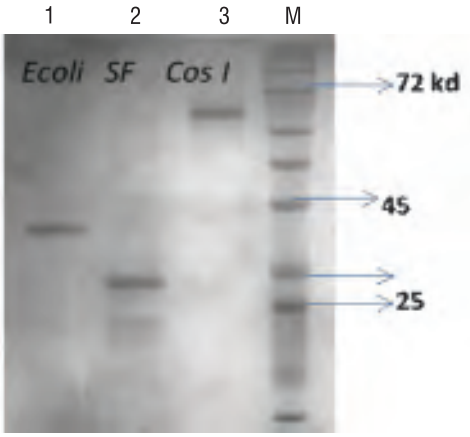


Fig. 18. Expression of recombinat eCG. M- Protein molecular weight marker, Lane 1- recombinat eCG expressed in *E. coli BL21C*, Lane 2- recombinat eCG expressed in *SF insect* lysate, Lane 3- recombinat eCG expressed in *Cos I* mammalian cells

(Anuradha Bhardwaj, A.K. Gupta and Sanjay Kumar)

All India coordinated research project on increased utilization of animal energy with enhanced system efficiency

Donkeys and mules are used for carrying bricks at brick-kilns as pack animal in northern part of the country. Indisciminate use of these animals including over loading, taking work for long durations etc., leads to injuries and metabolic disorders. To avoid cruelty to the animal and get maximum output without stress, these animals must be used judiciously under suitable work-rest cycle. The objectives of the project were to study the pack load carrying capacity as well as draughtability under arid conditions.

Studies on the load carrying capacity of Indian donkeys as pack animal under work rest cycle

Adult donkeys (n=4) carrying a pack load equivalent to 50 and 66% of their body weight were tested during summer and winter seasons respectively, till the onset of fatigue symptoms. Work conditions were simulated to those prevailing at brick kilns (work-rest-work cycle). Donkeys carried a pack load for 500 m followed by unloading and repeating the process. The animals walked at an average speed of approximately 0.5 m/s (1.9 km/h). The Onset of fatigue symptoms such as profuse sweating, in-coordination of legs, improper and forced forward movement, unwillingness to work, etc., were recorded along with physiological and biochemical parameters.

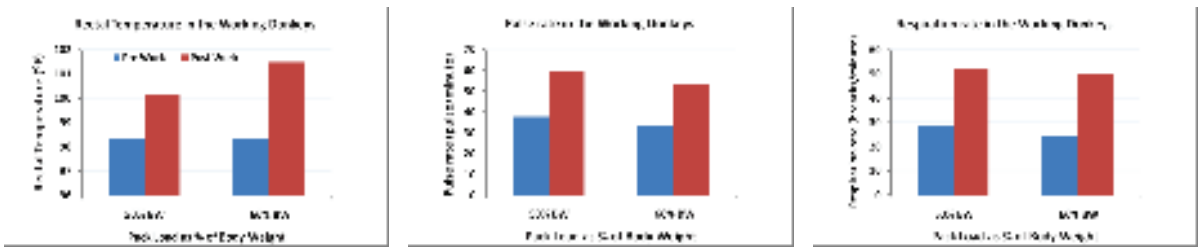
Fatigue symptoms such as unwillingness to continue operation, in-coordination between hind and forelegs were seen at 5 h of work in the case of 50% pack load and 3 h of work with 66% pack load, respectively. Among physiological indices, rectal temperature, pulse rate and respiration rate increased significantly after work (Fig. 19a-c) under both the pack loads but came to normal levels by the next morning.

Similarly, hemoglobin, packed cell volume, red blood cells and white blood cells increased significantly after work. Activity of serum LDH and lactate content didn't differ significantly as compared to control barring lactate levels at 50% load, which increased significantly post work. This study indicated that the donkeys can be comfortably used for five hours with pack load (50% of BW) and for short duration with 66% load in morning hours at brick-kilns under work rest work cycle prevailing there.

Draughtability studies with equines under arid conditions

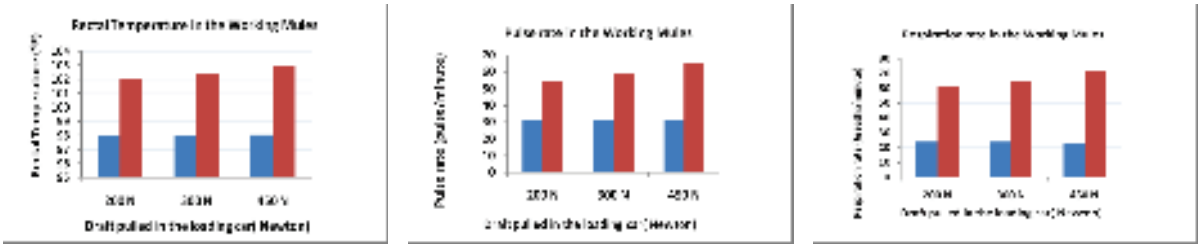
Three apparently healthy mules of 8-10 years of age and weighing between 350-400 kg were used in loading car for pulling draft of 200 Newton (N) during summer season, 300 N during September and October and 450 N during winter season till the onset of fatigue symptom. The mules walked at normal speed during the experiment. Physiological responses mainly rectal temperature (RT), pulse rate (PR) and respiration rate (RR) were recorded before start and just after completion of the work. Vigorous sweating and other fatigue symptoms such as unwillingness to continue operation were observed after 3h of contiuous work at 200 N draft load, 2 h with 300N and 1h only with 450N draft. All physiological indices increased significantly after work in all the groups (Fig. 20a-c). However, these indices attained normal levels by the next morning.

Hematology and biochemical parameters estimated in the mules with 300N draft load,indicating



(A) (B) (C)

Fig. 19a-c. Rectal temperature, pulse rate and respiration rate variation observed in the working donkeys in relation to the pack load



(A) (B) (C)

Fig. 20a -c. Rectal temperature, pulse rate and respiration rate variation observed in the working mules in relation to pulled draft in loading car

significant increase in LDH activity post work. Similarly hemoglobin, packed cell volume, red blood cells and white blood cells also increased significantly after work. This study indicated that the mules may be used for 3h continuously for pulling 200 N draft without any adverse effects on the animal health.

(R. A. Legha, Yash Pal and Vijay Kumar)

Effect of combinations of roughage on digestibility in horses

Evaluation of nutrient intake and digestibility in advanced pregnant mares

In advanced pregnant mares (~ 411 Kg), maintained on 4 Kg concentrate along with feeding of dry and green fodder (1:3 ratio), feed intake was about 2.33 % of their body weights which meets the recommendation of NRC, 2007. During the last trimester, average body weight gain (35.44 Kg) was very rapid which may be due to their preparation for lactation and growth of fetus. Digestibility coefficients were 57.96% for dry matter, 69.85% for crude protein, 47.57% for crude fibre and 80.48% for ether extract. All the animals had a body condition score between 6 and 7 on a 0-9 scale which is considered to be quite good.

Nutrient digestibility and growth performance of young foals on different ration

Nutrition plays a pivotal role in achieving optimum growth rates and size in horses. Regular weighing and measuring horses for at least the first 18 months of life and adjusting nutrition as and when needed is necessary to maintain a steady growth rate in young foals. A study to measure the growth of Marwari foals on wheat straw and sewan hay based rations was carried out.

Young foals (divided in 3 groups) between 18 to 24 months were fed 50% grain mixture and 50% sewan hay (group 1), 50% grain mixture and 50% wheat straw (group 2) and 50% grain mixture (25% sewan hay and 25% wheat straw – group 3) on dry matter basis. The ration for the animals was formulated to provide feed @ 3.5% of their body weight. No significant difference in growth rate was observed in all the 3 groups due to variations in ration which indicated that foals can be maintained in any of the combinations available. Digestibility evaluation of nutrient indicated both sewan hay and wheat straw were of lower quality and thus supplement of grains mixture is required for foals.

Nutrient digestibility and growth performance of young foals fed on groundnut haulm and sewan hay

Young growing horses groomed for competition or work require diet that will result in growth and development as well as is necessary to satisfy maximum expression of the genetic vigour. In the Marwar region (Rajasthan), farmers generally prefer sewan hay (along with grain mixture) for horses, while donkeys are fed groundnut haulm, wheat straw or grasses without grain supplementation as single feed. A study was conducted to evaluate the effect of groundnut haulm and sewan hay on the growing animals.

Nine young foals (~167 kg each) divided into three groups of three animals each, were fed on 50% grain mixture and 50% sewan hay (group 1), 50% grain mixture and 50% groundnut haulm (group 2) and 50% grain mixture, 25% sewan hay and 25% groundnut haulm (group 3) on dry matter basis.

Ration was prepared to provide dry matter of more than 3.5% of the body weight along with extra 5 Kg green Lucerne.

In the present study, the growth rate in Group-1 (333 g/day) and Group-2 (294 g/day) foals was little lower than the recommended growth rate. For young foals (6 months to 1 year), expected growth rate is 360 g per day (NRC, 2007). This lower growth rate may be due to the fact that these animals were recently weaned. However, the growth rate of Group-3 was 379 g/day.

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

Evaluation of total mixed rations for maintenance horses

Information pertaining to the dietary requirement for maintenance of Marwari horses is meager. A survey of the farmers in Marwar region revealed an urgent need for formulating a ration and scientific intervention specifically for maintenance of horses.

To prepare concentrate mixture and TMR best suited for this region with locally available feed ingredients, 24 feed samples were collected from Bikaner and EPC campus. Proximate principles and Van soest's fibre analysis were done for all the 24 feed samples (Table 4).

Local grasses are high in fibre contents, thus along with concentrate mixture it would make a good ration for the horses. As the horses are grazers, a ration with good quality grass (viz. doob) can be the maintenance feed for horses and donkeys. However, for working equines extra supplementation of grain mixture will be compulsory.

Table 4. Nutrients composition of feeds

	Parameters (range in %DM basis)						
Type of feed	Dry matter	Crude protein	Crude fibre	NDF	ADF	Ether extract	Ash
Energy supplements (n=5)	86.2-89.7	8.8-12.6	2.6-13.9	13.6-35.5	3.6-16.2	1.7-5.4	1.6-3.0
Protein supplements (n=3)	89.4-95.0	34.9-49.0	7.0-12.7	14.0-28.4	9.4-15.6	5.3-10.1	5.8-8.3
Local grasses (n=10)	85.9-94.9	3.8-10.2	28.8-45.0	62.8-79.6	33.7-51.9	0.3-2.7	6.7-13.0
Roughages & fillers (n=6)	87.0-92.8	4.2-18.2	10.4-41.5	34.4-77.5	13.5-50.0	1.4-14.4	6.2-16.1

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

Genetic studies in donkey population belonging to different geographic areas

Genetic diversity within and between different donkey population

Six donkey population belonging to Spiti (H.P.), Leh (J&K), Baramati (Maharashtra), Bihar, Gujarat and Rajasthan areas along with an exotic Poitu breed (an out group) (Fig. 21a–g) were evaluated for assessing genetic diversity within and between them using twenty four polymorphic microsatellite markers with 299 donkey DNA samples. Allele count, allele size, heterozygosity and Fis values across different loci were evaluated. In total, 264 alleles were obtained with all the microsatellite loci across all the donkey populations studied. The observed number of alleles (na) per locus ranged from 6 (LEX 68; LEX 73) to 22 (AHT05) with a mean value of 11.083±0.1599 and effective number of alleles ranged from 1.5618 (COR022) to 9,005 (COR069) with a mean value of 3.8685 0.0795. For all the loci, value of effective number of alleles was less than that of observed alleles. All the studied microsatellites loci were polymorphic, which indicated that the microsatellites used were highly suitable for genetic diversity analysis. The observed value of heterozygosity was lowest (0.1161) at locus ABS17 and highest (0.8396) at AHT05 with an average value of 0.5374 ± 0.010. The expected value of heterozygosity (Nei) ranged from 0.3597 (COR022) to 0.8890 (COR069) with a mean of 0.682 ± 0.010. Except three loci (LEX33, LEX034 AND HTG 07), the expected heterozygosity at all other loci was higher than observed heterozygosity indicating

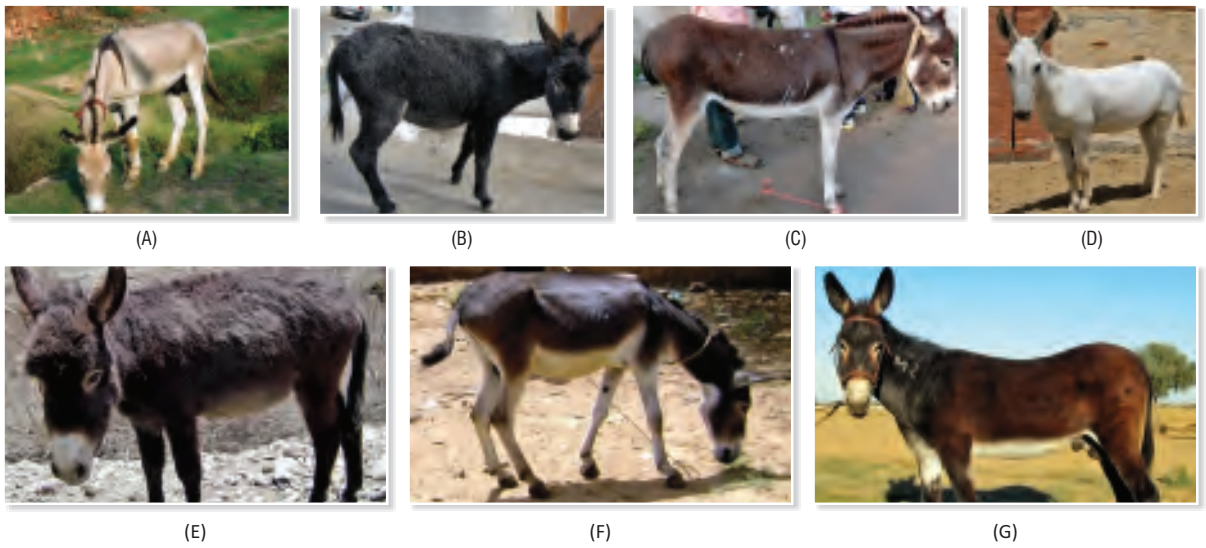


Fig. 21a-g. Donkeys from various regions a. Bihar; b. Leh; c. Maharashtra; d. Gujarat; e. Spiti; f. Rajasthan; g. Poitou

that most of the loci showed deviation from Hardy-Weinberg equilibrium. Inbreeding coefficient values (Fis) ranged from -0.0169 (HTG 07) to 0.7774 (ASB 17) with a mean value of 0.2365±0.011. Three loci (LEX33, LEX 34 and HTG 07) had negative values indicating heterozygosity deficiency while values for nine loci were less than 0.2 indicating low inbreeding among the indigenous populations.

The gene flow analysis revealed a large effective number of migrants among donkey populations between Rajasthan & Spiti and Bihar & Leh donkeys. The estimates of Fst between each pair of breeds revealed that genetic differentiation between donkey population from Gujarat & Leh were the maximum (0.5259) followed by Rajasthan & Leh donkey populations, while donkey populations from Rajasthan and Spiti areas were the least differentiated (0.0759). Among different individual donkey populations, mean number of alleles ranged from 5.2000±0.2751 (Spiti, HP) to 7.4400±0.2825 (Haryana). Both average observed and expected heterozygosity values were highest (0.5400, 0.6951) for donkey population from Haryana, respectively, while values were lowest for donkeys from Spiti area (0.4226, 0.5153) respectively. Significant deviations from Hardy-Weinberg equilibrium were also detected in all the populations for most of the loci. Data were analyzed for testing for Hardy Weinberg equilibrium using Genepop v 4.0. Except locus ASB02, none of the other microsatellite showed variation in all the donkey populations, while the rest deviated from 2 to 3 populations only. The results point towards non random mating of gametes leading to deviation of loci from HW equilibrium and presence of population structure due to inbreeding.

Population relationship and structure analysis

Topology of various donkey population was prepared on the basis of genetic distances estimated on allele sharing basis. On comparing all the donkey population, donkeys from Spiti and Rajasthan were found to be very close to each other as genetic distance among them was the least (0.0759), whereas donkeys from Leh and Gujarat areas were far apart with a genetic distance of 0.5259. The neighbor-joining algorithm was used for the construction of phylogenetic tree (Fig. 22).

Fig. 22. Phylogenetic tree depicting genetic closeness and distances among different donkey populations from various geographic areas.

Phylogenetic tree indicated that Rajasthan donkey population was very close to Spiti population, while donkey population from Bihar was close to Leh donkey populations. Donkey populations can be divided in 2 groups : one consisting of Bihar and Leh donkeys while other group consisting of donkeys from Gujarat, Maharashtra Rajasthan, Spiti and Haryana.

Bottleneck studies in donkey population from seven geographic areas

Recent bottlenecks in various donkey populations i.e. within past few dozen generations were examined by a graphical method analyzing distortion of allele frequency distribution which plots groups of alleles from a sample of many polymorphic loci into each of ten frequency classes. All the seven donkey population belonging to different geographic locations showed normal "L" shaped curve (Fig. 23)

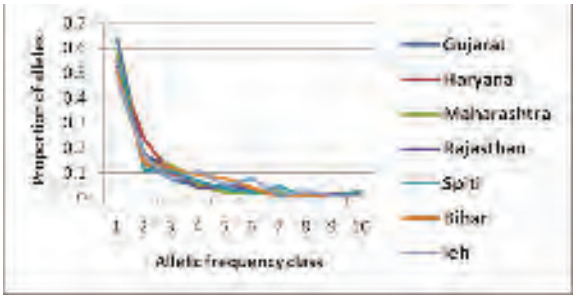


Fig. 23. Graphic distribution of proportion of alleles and their distribution in different donkey population.

reflecting no bottleneck has occurred in the recent past.

However, bottleneck needs to be further evaluated based on three models of mutation equilibrium for final clarification of the fact.

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

Network Project on Animal Genetic Resources on Characterization of donkeys of Rajasthan

Twenty six biometric indices of 50 donkeys (including seven foals of less than one year of age) from different areas of Bikaner district were recorded for characterization of donkeys. The donkeys were light grey, dark grey and brown in colour (Fig. 24). Tail switch was non-distinct and pole was prominent. Nasal bone was concave, whereas fore-head was convex in shape. Zebra marking on legs of few donkeys, shoulder strip, dark outline markings, light coloured under parts were recorded. Ears were erect & horizontal. White markings of muzzle, eyes, legs and belly were common. Hairs were of medium length, dull in appearance and straight. The foals had long hairs with glossy appearance. Colour of skin was grey/black in donkeys. One to three donkeys were

Fig. 24. Donkeys in Rajasthan

being maintained by the sheep herders to carry the luggage. In most of the donkeys tail was either above or up to hock, but in few donkeys it was beyond hock. The work is in progress.

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

Follicular dynamics, hormonal profile and pregnancy diagnosis in Marwari mares

Ultrasonography assisted monitoring of ovarian cyclicity during estrous cycle in Marwari mares was carried out to generate basic information for optimium production. In Marwari mares, estrous cyclicity was more prevalent in the breeding season (summer) than in the non breeding season (winter). The mean duration of the estrous cycle remained around 27.7 days while estrus period was 10.07 days. Mean pre-ovulatory follicle size was observed to be 47.34 mm and corpus luteum size was 33.40 mm. It was observed that the onset of estrus symptoms in the adult mares initiated when the growing follicle size reached 26.51 mm. Mares showing erratic estrous behavior during peak winter (long period of ovarian quiescence, seasonal nymphomania, weak estrus expression) expressed normal estrus behavior

during the long day photoperiod in summers. Progesterone remained lower than 0.5 ng/ml plasma during the estrus period until ovulation. Post ovulation the level increased in the luteal phase (1.512 ng/ml) when functional corpus luteum was observed in the animal. The levels of the estrogen hormone remained lower during the luteal phase (4.19 ng/ml) and at the onset of estrus (3.57 ng/ml), and increased to peak levels when the pre-ovulatory follicle was present (5.6 ng/ml).

In the pregnant mares, the levels of progesterone increased and remained 2.07 ng/ml in 3 month pregnant mares, 10.17 ng/ml in 6 month pregnant mares and 3.2 ng/ml in nine month pregnant mares. This trend shows that the progesterone increased during pregnancy till mid pregnancy and then in late pregnancy, its role was overtaken by other progestogens. Use of ultrasonography for pregnancy diagnosis was successful for detection of the embryonic vesicle at about one week of conception (Fig. 25). However, by two weeks, the vesicle became prominent and after a month of conception, it attained >30 mm size. The embryonic growth curve indicated a continuous increase in vesicle size. The mean gestation length was 334.10 days while average foal weight at birth was 32.5 kg.

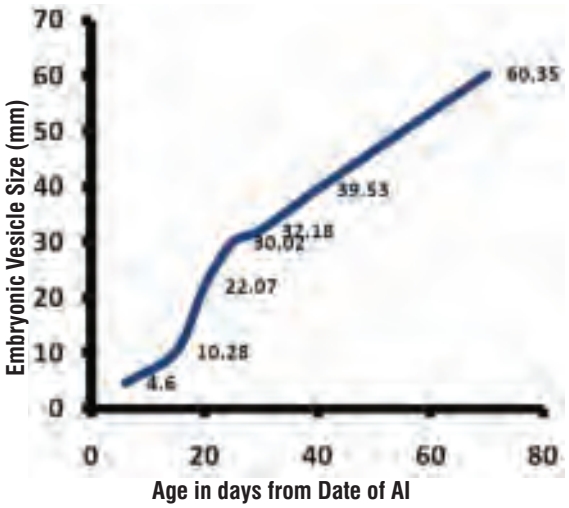


Fig. 25. Embryonic vesicle size during early gestation period in Marwari mare

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

Survey of working equines on Chardham Yatra route in Uttarakhand

Equines were the reliable and quickest means for transportation for humans before the invention of motorized vehicles. Working equines are used on Chardham Yatra in Uttarakhand for carrying pilgrims to the places of worship and religious importance. Keeping this in mind a survey was undertaken on working equines in Rudraprayag, Chamoli and Uttarkashi districts on Chardham Yatra route in Uttarakhand. Survey was conducted and information was collected from 138 equine keepers on socio-economic profile, existing management systems and utilization pattern.

Socio-economic profile of equine owners

In Uttarakhand, as indicated in Fig. 26 majority of equine owners (72.46%) were from middle age group between 26 to 43 years, mostly belonging to scheduled caste (62.32%). Literacy level was found to be 82.61%. Majority of equine owners (76.09%) had medium level of experience 9 to 25 years in equine husbandry, having medium family size (88.41%) i.e. of 4 to 7 members per family and (81.88%) have daily income between ` 600-1700 during yatra season.

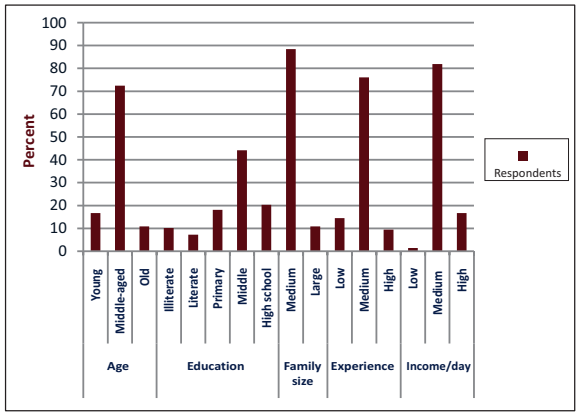


Fig. 26. Socio-economic profile of respondents

The socio-economic profile of equine owners indicate that most of the equine owners were educated and had reasonable experience on management of equines, which could be useful in developing any training programme on equine husbandry and management.

Management practices followed by equine owners

Constraints related to management and livelihood opportunities were identified during interaction with

equine owners. As depicted in Table 5, deworming was done regularly by most of the equine owners (46.15%) in Rudraprayag, whereas in Chamoli (55.56%) and Uttarkashi (35.71%), respondents were occasionally deworming their equines. Others were providing digestive herbal mixture to their equines. Vaccination in equines was not a regular practice among equine owners. 6.52% respondents vaccinated their equines for tetanus only. Data on cleaning, shoeing and grooming are presented in the Table 8. Various types of wounds, injuries, rope marks and saddle sores (Fig. 27) were observed in about half of the equines surveyed, but these problems were mostly managemental.

Fig. 27 Saddle sores on back of mules

Table 5: Management practices adopted by the respondents

S.No.	Parameter	Category	Percentage of respondents			
			Rudraprayag (n1=78)	Chamoli (n2=18)	Uttarkashi (n3=42)	Total (N=138)
1.	Deworming	Never	12.82	44.44	64.29	32.61
		Occasionally	41.03	55.56	35.71	41.30
		Regular	46.15	0.00	0.00	26.09
2.	Vaccination	Never	88.46	100.00	0.00	63.04
		Sometimes	11.54	0.00	0.00	6.52
3.	Cleaning	Never	0.00	0.00	0.00	0.00
		Sometimes	70.51	66.67	73.81	71.01
		Regular	29.49	33.33	26.19	28.99
4.	Shoeing	Yes	100.00	100.00	100.00	100.00
		No	0.00	0.00	0.00	0.00
5.	Grooming	Sometimes	62.82	27.78	66.67	59.42
		Regular	37.18	72.22	33.33	40.58

The saddles and harness material used by working equine owners are made locally using old clothes, rug and other raw material enclosed by polythene sheets. The absence of proper pack saddles and lack of rest for working equines with back sores compounded the problem.

Housing pattern of equines

Mostly temporary sheds made of polythene and iron sheets, bamboo, plastic bags and bricks were observed for keeping equines at base camps on the yatra route. The floor of these temporary stables was uneven and mostly made of stones which was not congenial to the equids (Fig. 28). At base camps some of the equine owners rented space and shed form local people for ₹ 1000-3000 per month for staying and keeping their animals during the yatra season.

Fig. 28 Temporary shed at Govindghat

In villages, permanent sheds made of stone wall and bricks with thatched roof or covered with tins were observed. At most of the places equine owners were keeping the animals in the ground floor of their houses.

During winter season equine owners keep their animals in sheds which lacked proper height and ventilation (Fig. 29). Sheds were not cleaned regularly by most of the equine owners. At most of the places stone or cemented floor was observed and there was no provision of drainage in sheds. Disinfectants were rarely applied.

Fig. 29. Village sheds used for keeping equines

Feeding pattern of equines

The equine owners were feeding 5-6 kg dry fodder like wheat straw which is locally available. They also provided 0.5-1 kg gram, 0.5 kg wheat flour and 2-3 kg/animal/day as concentrate mixture, which is mostly purchased. In villages, equine were left for grazing during off hours. However, during yatra season the owners fed green grass 8-10 kg/animal/day which were procured at high rates of about ₹ 8-10 per kg. The feed is provided in equally distributed ration twice a day generally in morning before work and in evening or night after working hours (Fig. 30). Additional supplements like Jaggery 250g/animal/day and 100-150 gm/animal oil were fed to their animals occasionally. Besides some of the equine owners were providing homemade or purchased herbal digestive mixture to their animals regularly.

Fig. 30. Feeding of equines

Ownership and utilization pattern of equines

In hilly areas of Uttarakhand, mules are biggest source of employment and transportation. Mules mostly work in group of two, therefore, most of the equine owners owned a pair of mules. A mule is a big asset for a family and costs between ₹ 60,000 to 1 lakh. Majority of equine owners (88.31%) were using equines as pack for transportation of tourists and pilgrims on chardham yatra at Kedarnath, Yamunotri and Hemkund Sahib and for transportation of agricultural produce from farms and construction material and goods in hills during non-yatra season. They carry one person on their back and usually carry load upto one quintal as pack. The working equines generally travel 20-24 km distance per day. The equine owners earn on an average ₹ 40000- 70000/ animal/season from April to October. Although the rates for carrying tourist and pilgrims are fixed by the district administration and yatra committee, in the event of less number of pilgrims, the people bargaining with equine owners fetch them less than the prescribed fees. During the non yatra season, the equine owners return to their native villages and they use equines in transportation of farm produce to village and for transporting construction materials and other goods in villages.

Factors affecting livelihood of equine owners at pilgrimage places on Chardham Yatra

In Garwal region of Uttarakhand equines are mostly engaged for yatra route, transporting food, construction material and various other commodities. Earning activity is limited to mostly for 4-6 months during yatra season which sustain them throughout the year. Most of the equine owners come from nearby areas or states and stay in make-shift tents away from family during yatra season. Religious tourism is a major source of employment for majority of the people living on Chardham route. After the recent natural disaster in Uttarakhand, low congregation of tourists has adversely affected the livelihood of the equine owners.

(A.A. Raut, Yashpal, R.A. Legha and Ramesh Dedar)

Approaches for derivation of induced pluripotent stem cells from cattle

A non-viral transposon method for reprogramming of bovine somatic cells to iPS cells. I compared two transposon systems namely, piggyBac (PB) and Sleeping Beauty (SB) employing different combinations of reprogramming factors. Initially the PB and SB transposon systems have been tested for the derivation of iPS cells from cells of inbred (BL6) and outbred (NMRI) mice, respectively. The murine fibroblasts derived from an inbred BL/6 mouse line carrying a pluripotency reporter, Oct4-EGFP, allowed to follow reprogramming. The reprogramming PB transposon encoded the cDNAs of reprogramming factors OCT4, SOX2, KLF4, c-MYC, LIN28 and NANOG, driven by the chimeric CAGGS promoter, whereas SB transposon encoded for the same transcription factors excluding LIN28 and NANOG. Both transposon systems resulted in the successful isolation of murine iPS cell lines. For targeted differentiation a transgenic mouse model with expression of a vital fluorophore reporter, tdTomato, IPS cells from the transgenic mice were generated by SB reprogramming and these iPS cells were differentiated into lentoid bodies *in-vitro*.

Finally, the optimized conditions for transposon reprogramming were used to derive bovine iPS (biPS) cells from fetal fibroblasts electroporated with SB and PB systems, respectively. By using bFGF (8 ng/ml) and hLIF (1000 U/ml) supplementation a stable bovine iPS culture, biPS-1, could be established by PB reprogramming. The derived biPS line expressed typical endogenous markers of embryonic stem cells, proliferated rapidly, showed long term proliferation and readily formed teratomas. This study is the first demonstration that biPS cells can be generated by a non-viral transposon system, it suggests that ectopic NANOG and LIN 28 are necessary for reprogramming of bovine cells. These results are a major step towards the routine derivation of biPS cells and will facilitate the genetic modifications of the bovine genome.

(T. Rao Talluri)

Effect of dietary entry of n-3 PUFA on ovarian function, embryonic development and semen quality in horses

An investigation was carried out to assess the effect of dietary fish oil, a rich source of n-3 PUFA on development of follicle , corpus luteum (CL) and conceptus as well as changes in plasma estradiol, progesterone, metabolites and conception rate in mares. In mares fish oil was supplemented in diet @0.25ml/kg BW daily for 70 days. In stallions, influence of n-3 PUFA was examined on various seminal parameters by feeding fish oil supplementation @ 0.25ml/kg BW daily for 14 weeks.

There was no significant (P>0.05) effect of dietary fish oil supplementation on total number of follicles in different class size (small, medium, large) or irrespective of class size counted on D0, whereas diameter of follicle was significantly greater on D0 (P<0.05) and day 2, 4, 5 of estrus (P<0.01); and one day before ovulation (D_{ov-1}; P<0.01). There was significant (P<0.01) increase in CL diameter both on 7 and 15 day PO with fish oil supplementation. Plasma estradiol was significantly higher on D0 (P<0.05), day 3 of estrus (P<0.01) and tended to rise on D_{ov} (P=0.05). Plasma estradiol concentration on D_{ov} had significant (P<0.001) positive correlation with ovulatory follicle diameter on D_{ov-1} in mares of Gr. B, whereas the positive correlation between two reached to significance (P=0.05) in group A. Progesterone concentration was significantly (P<0.01) higher on 15 day PO and had significant (P<0.05) positive correlation with concurrent CL size in mares of Gr. B. There was significant increase in diameter of embryonic vesicle (P<0.01) on 15 day PO as well as length and width of embryo proper (P<0.01) on 28 day PO. Conception rate was significantly (P<0.01) higher with less number of AI per conception in mares of Gr. B. The concentration of cholesterol did not alter significantly (P>0.05), whereas significant (P<0.05) decrease in triglyceride and NEFA was inconsistent with respect to day of sampling in mares of Gr. B. Fish oil supplementation to stallions.

(S.K. Ravi)

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
Anagha G M.V.Sc. Student	Dr. B.R. Gulati Principal Scietist	IVRI, Izatnagar	Characterization of Equine herpesvirus 1 isolates for neuropathogenic potential	Completed
Himanshu Sharma Ph.D. Student	Dr. B.R. Gulati Principal Scietist	LUVAS, Hisar	Studies on Latency in Equine Herpesvirus-1 Infection among Equines in India	Continuing
Ameya Gupte M.V.Sc. Student	Dr. B.R. Gulati Principal Scietist	LUVAS, Hisar	Development of peptide ELISA for serodiagnosis of Equine Herpesvirus1	Continuing
Shashi Kant Kankar M.V.Sc. Student	Dr. Rajender Kumar National Fellow	IVRI, Izatnagar	Development and application of a quantitative real time PCR for diagnosis of surra in equines	Completed
Pavulraj S M.V.Sc. Student	Dr. Nitin Virmani Principal Scientist	IVRI, Izatnagar	Pathology of equine influenza virus (H3N8) and vaccine efficacy studies in BALB/c mouse model	Completed
Alok Joshi M.V.Sc. Student	Dr. Nitin Virmani Principal Scientist	LUVAS, Hisar	Studies on the role of recombinant glycoprotien-B in protection against EHV-1 infection in BALB/c mice	Completed
Ramesh Kumar Ph.D. Student	Dr. Nitin Virmani Principal Scientist	LUVAS Hisar	Pathological investigation and protective immunity of recombinant vaccine candidates of equine influenza virus in BALB/c mice	Continuing
Gopalakrishnan A M.V.Sc. Student	Dr. Sanjay Kumar Principal Scientist	IVRI, Izatnagar	Evaluation of oxidative damage and anti piroplasmic activity of some novel drug molecules against *in vitro* cultured *T.equi*	Completed
Rajesh Kumar Ph.D. Student	Dr. Sanjay Kumar Principal Scientist	Chaudhary Devi LalUniversity, Sirsa	Studies on the genomic diversity of *Theileria equi* among different geographic isolates	Continuing
Suthar Abhinav Navinchandra M.V.Sc. Student	Dr. Sanjay Kumar Principal Scientist	IVRI, Izatnagar	In vitro evaluation of cytotoxic damage and anti-piroplasmic activity of some novel drug molecules targeting phospholipid metabolism and heat shock protein of *Theileria equi*	Continuing
Sheetal Saini Ph.D. Student	Dr. H.S. Singha Scientist	Chaudhary Devi LalUniversity, Sirsa	Expression of recominant equine cytokines and analysis of their biological activities	Continuing

VTCC Accomplishments

Culture Collection: At a glance

Veterinary Type Culture Collection (VTCC) 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. So far, a total of 2546 cultures/clones have been deposited in VTCC after authentication, and conventional and molecular characterization including GC-FAME and sequence analysis of various genes. These microbes are being contributed from 19 source including veterinary (7), rumen (8), and dairy (4) network units, and other ICAR institutes and State Agricultural and Veterinary Universities. These cultures/clones are veterinary pathogens including viruses, bacteria, bacteriophages, rumen microbes comprising anaerobic bacteria and fungi, dairy microbes and clones. The Centre is also maintaining various cell lines. More than 70 genera of bacteria are represented in VTCC repository, including some novel taxa, 13 families of viral pathogens and bacteriophages of various families. The dairy cultures were categorised as exopolysaccharide (EPS) producing and bio-protective cultures (*Lactobacillus rhamnosus, L. plantarum, L. paracasei*) based on their functional characteristics. A summary of the deposits in VTCC repository is given in Table 1.

Table 1. Present Status of the Microbial Repository

Microbial Resources	(2014-15)	Present strength
Veterinary Microbes		
Bacteria	227	927
Virus	21	156
Fungus	-	-
Recombinant clones	140	466
Phage library	-	27
Bacteriophage	19	32
Genomic DNA	47	223
Total	454	1831
Rumen Microbes		
Anaerobic bacteria	30	176
Fungi/Yeast	-	86
Methanogenic Archeae	-	8
Total	30	270
Dairy Microbes		
Bacteria	45	445
Total	529	2546

Authentication, preservation and reposition of virus isolates

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 27 virus isolates viz., ORFV (2), SPPV, CMLV, BPXV, GTPV(2), NDV (2), PPRV (3), IBDV(6), CSFV, BTV (3), RDV- F strain, RDV virulent strain, Pigeon RDV, Avian reovirus, NDV (R2B strain) were identified/authenticated by PCR amplification of virus-specific regions. 21 of these, isolates were successfully passaged in appropriate cell lines/primary cultures for subsequent reposition in the repository (Table 2 & Fig 1-2). A total of 325 vials of twenty one characterized viral isolates have been cryopreserved in the repository.

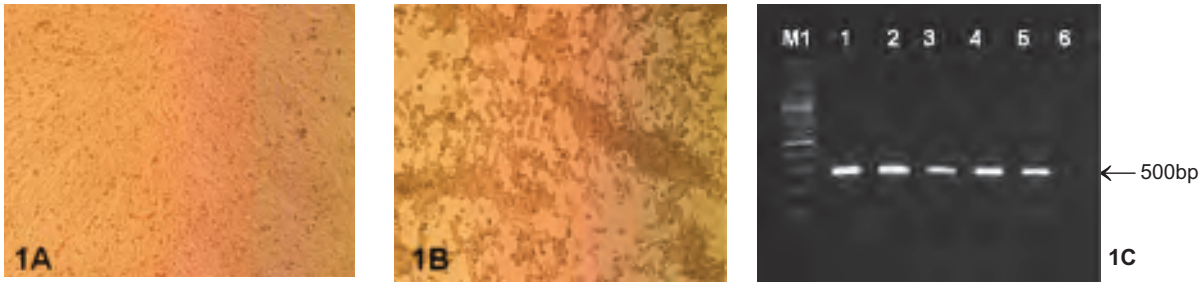


Fig. 1. Characterisation of Bluetongue virus; 1 A-B: Propagation of Bluetongue virus in BHK-21 cells: 1A : Control BHK-21 cells; 1 B: CPE in BHK-21 cells after 48 hpi; 1 C : PCR confirmation of Bluetongue virus : Lane M1: 100bp DNA ladder; Lane 1: Positive control; Lane 2: BTV 2 TN-P1, Lane 3: BTV-18 (IVRI), Lane 4: BTV-19 (IVRI); Lane 5: BTV-20 (IVRI); Lane 6: Negative

Table 2. Virus isolates successfully passaged in cell culture

Sr. No.	Virus	Propagated	Isolates
1	GTPV/CIRG//2014	LambTesticle	1
2	ORFV/CIRG/2014	LambTesticle	1
3	ORFV/IVRI/ Mukteswar/2014	LambTesticle	1
4	SPPV/IVRI/Srinagar/2014	VERO	1
5	GTPV/IVRI/ Uttarkashi/2014	VERO	1
6	BPXV/Vij96/IVRI/2014	VERO	1
7	CMLV1/97/IVRI/2014	VERO	1
8	PPRV/IVRI/ Sungri96/2014	VERO	1
9	PPRV/IVRI/ Revati06/2014	VERO	1
10	PPRV/IVRI/ Jhansi03/2014	VERO	1
11	CSF cell culture vaccine (IVRI)	PK 15	1
12	NDV/TANUVAS/2013	CEF	2
13	BTV/IVRI/ MKD18/08/IND/2014	BHK 21	1
14	BTV/IVRI/ MKD19/08/IND/2014	BHK 21	1
15	BTV /IVRI/MKD20/08/IND/2014	BHK 21	1
16	RD-F Strain	CEF	1
17	RD Virulent virus velogenic	CEF	1
18	Pigeon RD virus lentogenic	CEF	1
19	Avian Reovirus (viral arthritis)	CEF	1
20	NDV R2B Mukteswar Strain	CEF	1
	Total		**21**

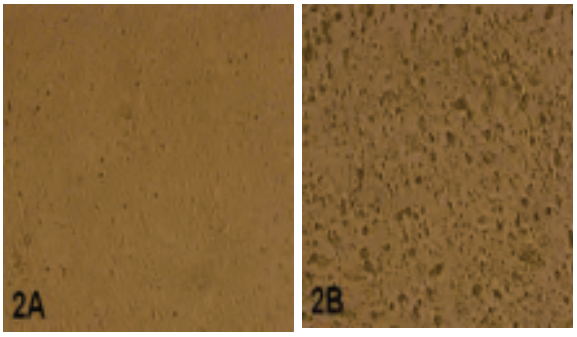


Fig. 2 A-B: Propagation of New castle disease virus in CFF cells : 2A : Control CEF cells; 2B: CPE in CEF cells after 48 hpi

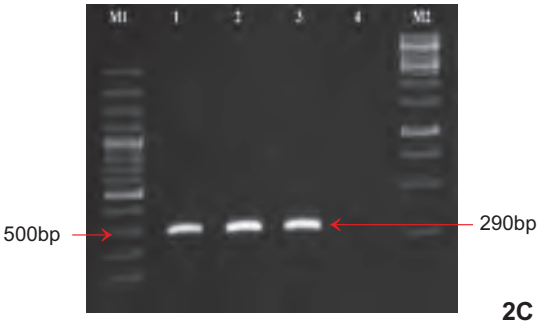


Fig 2 C: PCR confirmation of Newcastle disease virus : Lane M1: 100 bp DNA ladder; Lane 1: Positive control; Lane 2: NDV-2K3, Lane 3: NDV-2K35, Lane 4: Negative control

Preservation, maintenance and distribution of cell lines

Fourteen different cell lines/three primary cultures *viz*., Vero, MDBK, MDCK, BHK21, RK13, HELA, PK15, HEP2, MRC5, NLBK, MA104, Equine lung, Porcine Stable, CEF, CEL, BRT and LT are being maintained for isolation of different viruses in the repository. Various cell lines /primary cultures viz., Vero, RK13, BHK21, HELA, MDCK, PK15and Lamb testicle, were distributed to the scientific community from TANUVAS Chennai, COVAS Palampur, CMVL Meerut, LUVAS Hisar and NRCE, Hisar.

Complete genome sequencing of Classical swine fever virus

To understand the genetic heterogeneity of existing classical swine fever viruses (CSFV) in-field conditions, the complete genome characterization of two virulent classical swine fever viruses (CSFV) was done by amplifiing sixteen overlapping fragments by RT-PCR. The amplified complete genome sequences of the viruses CSFV/Challenge Virus/IVRI/Std/India (IVRI strain) and CSFV/VTCC/ Haryana/India (VTCC strain) were 12294 and 12295 nucleotides (nt) in length, respectively. The 5' UTR was 373 nt in both the viruses, while, the amplified length of 3′ UTR was 224 nt in IVRI strain and 225 nt in VTCC strain.

The 3¢ UTR of IVRI strain showed a notable T rich insertion (CTTTTCATTTTTCTTTTTTATATATTATTTATATCTTTT) of about 39 nt in length at position 12130-12167. Complete genome of IVRI and VTCC strains shared a nt identity of 82.8%-94.1% and 83.4-95.5% with other reference CSFV strains, while the coding region shared aa identity of 88.8%-97.6% and 90.5%-95.8% with other reported CSFV strains. Phylogenetic analysis based on full length genome sequence as well as the individual regions Erns, E2 and NS5B revealed that IVRI isolate belonged to subgenotype 1.1 of genotype 1 and closely related to the highly virulent strain CSFV/Shimen/HVRI/China, while the phylogenetic analysis of VTCC isolate revealed that it belonged to subgentoype 2.2 of genotype 2 and was closely related to CSFV/IND/UK/LAL/290|India (Fig. 3).

Both viruses exhibited less nt and aa similarity with vaccine viruses. Recombination analysis detected the presence of intragenotypic recombination in VTCC strain. The study revealed the circulation of recombinant CSFV among Indian swine population for the first time. Complete genome characterization of CSFV will help in identifying the genetic heterogeneity existing among CSFV in field conditions and also pave the way for future studies based on reverse genetics and there by developing disease control strategies.

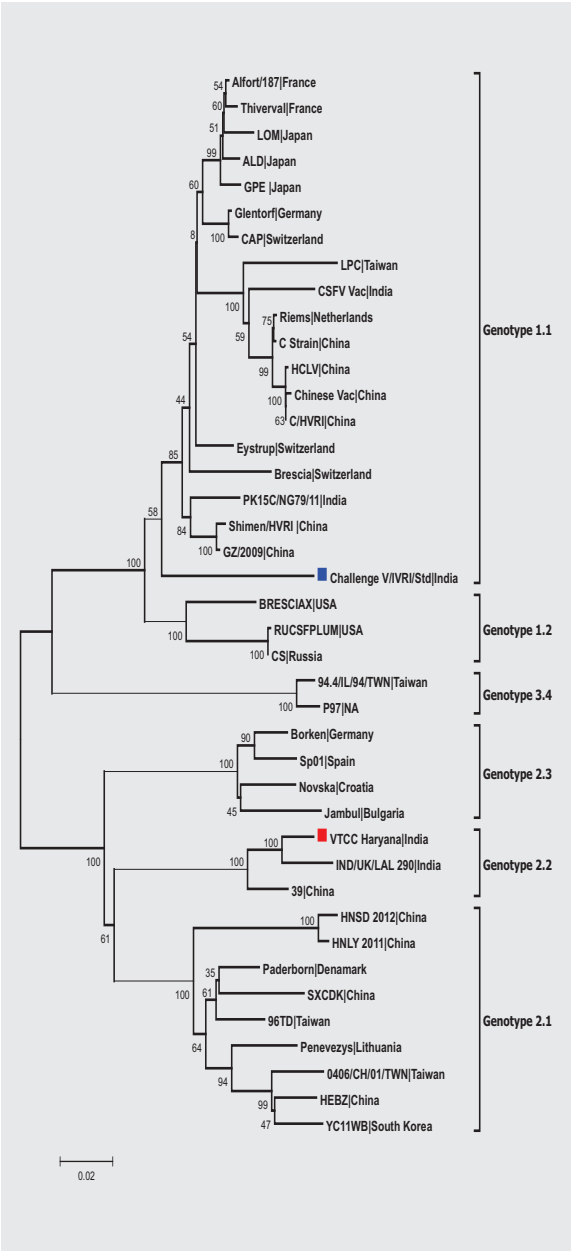


Fig 3: Phylogenetic tree of E2 region of CSFV

Development of recombinant clones of virulence genes

One of the major activities of the VTCC repository is to preserve the genetic materials of the microbes. In this direction, recombinant clone repository is being developed by cloning, sequencing, preserving and maintaining the clones of various genes of microbes. During the period, recombinant clone library was strengthened by addition of 41 recombinant clones of various virulence-genes of buffalopox virus isolates (13), swinepox virus : host-range genes (12) [Ankyrin-repeat protein (ANK), Kelch-like protein (KLP), extracellular enveloped virus protein (EEV), G protein-coupled receptor (GCR) and A52-Like protein (A52L)] and 16 clones of VP2 genes of IBDV in the VTCC repository. All generated clones have been preserved and are being maintained in the repository. All the generated data of the repository is being compiled, documented and maintained in digitized form.

Molecular characterization of host-range genes of buffalopox virus isolates from buffalo, cattle & humans

To elucidate the host-specific mutations as well as to add passport data of the isolates in the repository, sequencing of 6 host-range genes viz., E3L, K1L, K3L, C2L, C7L & B5R genes of BPXV isolates (n=6) from outbreaks in Nashik, Maharashtra was carried out. The BLAST-NCBI homology analysis revealed 99.5 to 100% similarity of all genes at both nt & aa levels among buffalo, cattle & human isolates. More significant point mutations at positions 11 (I to K); 12 (N to K) & 36 (S to F) were observed in C7L gene in most of the isolates in comparison to other VACV isolates and BPXV reference strain (BP4). However, mutation (D64N) observed in B5R gene in the earlier human and buffalo isolates of BPXV isolated from 2010 outbreak, was not found in isolates from 2014 outbreak. The mutations in C7L could play an important role in adaptation of BPXV in human and cattle which needs further functional studies. The phylogeny constructed on the basis of concatenated gene sequences revealed that BPXVs were closest to reference strain (BPXV-BP4) and other vaccinia and vaccinia-like viruses such as Passatempo and Aracatuba viruses.

Molecular characterization of host-range genes of swinepox virus from India

Various host-range genes *viz.*, Ankyrin-repeat protein (ANK), Kelch-like protein (KLP), extracellular enveloped virus protein (EEV), G protein-coupled receptor (GCR) and A52-Like protein (A52L) of swinepox virus (SWPV) were sequenced. Sequence analysis revealed that the SWPV from India was very closely related to the SWPV isolate 17077-99 (>96% identity at nt level). Sequence analysis of extracellular enveloped virus protein (ORF120) revealed an insertion of 3 nt 408CAA410 which codes for an asparagine (N138). Among the four ORFs (ORF141, ORF142, ORF143 and ORF144) encoding ankyrin protein of SWPV, analysis of ORF143 and ORF144 of SWPV/VTCC/AVA121 showed close similarity with SWPV isolate 17077-99 and did not reveal any deletion or insertion. Analysis of A52-like protein encoded by ORF 133 revealed only two aa changes in SWPV from India. The ORF 146 encoding for G protein-coupled receptors depicted single aa deletion at position 21 (D21) and an insertion at position 205 (N205) when compared to SWPV isolate 17077-99. Phylogenetic tree based on extra cellular enveloped protein gene 120 (SPV 120) showed the close clustering of SWPV/VTCC/AVA121 with SWPV isolate 17077-99 (Fig. 4).

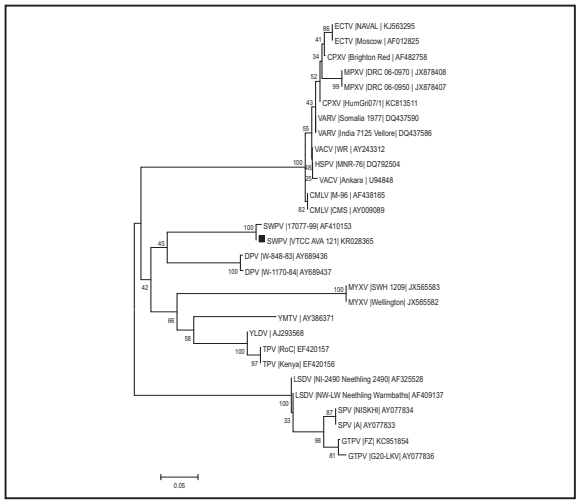


Fig. 4. Phylogenetic tree of extra cellular enveloped gene (SPV120)

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

Accessioning of Bacteria

In 2014-15, approximately 450 cultures were processed, while a total of 227 cultures obtained from various sources *viz.* Tamil Nadu University of Veterinary & Animal Sciences, Chennai; IVRI, Izatnagar; Central Institute for Research on Goats, Makhdoom; College of Veterinary Sciences, AAU, Khanpara; CSKHPKV Palampur HP; SKUAST, Jammu and collected by isolation at VTCC, NRCE, Hisar were accessioned. Apart from Network Units, isolates were also obtained from Sardar Krushinagar Vishvidhyalaya, Gujarat; Deptt of VPH, Mathura Veterinary College; NIVEDI Bangalore; Deptt of VPH Nagpur and National Institute of Biotic Stress Management, Raipur. A total of 114 bacterial isolates were obtained from samples collected or received at VTCC laboratory are preserved till identification.

Bacterial isolates like *Pasteurella multocida* sub sp. multocida, *Aeromonas hydrophila* sub sp. dhakensis, 25 serovars of *Salmonella enterica*, 5 isolates of Listeria from Goa, *Brucella melitensis* from Himachal Pradesh, 5 *Escherichia coli* from Goat and cattle; 8 isolates of S*taphylococcus* from yak, *Staphylococcus* from buffalo, Gujarat; 31 isolates of *Streptococcus* from VTCC, Hisar were received in the laboratory/ isolated, purified, checked for authentication by phenotypic/genotypic methods and preserved by cryopreservation and accessioned.

Authentication of bacteria by 16S rRNA gene sequencing

The 16S rRNA sequence based microbial identification was completed for 122 bacterial isolates. The isolates include curved gram-negative bacterium from buffalo like Comamonas kerstersii and Comamonas jiangduensis; a number of species of *Streptococcus* have been identified such as *Streptococcus infantarius* sub spp. coli (4 isolates), *Streptococcus dysgalactiae* sub spp. dysgalactiae, *Streptococcus porcinus*, *Streptococcus equi* sub spp. equi (8 isolates), *Streptococcus dysgalactiae* sub spp. equisimilis, *Streptococcus pluranimalium, Streptococcus acidominimus, Streptococcus equi* sub sp. ruminatorum.

Equine isolates of *Escherichia coli, Escherichia fergusonii* and *Escherichia hermanii* were identified at molecular level. *Enterococcus* is an important genus with probiotic potential. We have identified isolates in the genus as *Enterococcus faecium* (mice), *Enterococcus devriesei* (2 isolates from pig), *Enterococcus gilvus, Enterococcus raffinosus* and *Enterococcus hirae* (2 mice isolates). A buffalo isolate of *Acinetobacter indicus* with 96.5% similarity with *Acinetobacter indicus* Type Strain CIP 110367 is a good candidate for investigation as a novel species. The Centre has a good collection of *Rhodococcus* isolates including *Rhodococcus equi* (19) and *Rhodococcus rhodochrous* (9). *Salmonella* Gallinarum serovars have also been identified by 16S sequence to be *Salmonella enterica* (2 isolates). An oxidase positive Staphylococcus isolate from Sardar Krushinagar Veterinary College, Gujarat was identified as *Staphylococcus sciuri* sub sp. sciuri. Two isolates of Pasteurella multocida have been authenticated by 16S sequence also.

The sheep isolates of *Moraxella ovis* (2 isolates) confirmed by biochemical and 16S sequencing were accessioned. Other isolates include *Delftia lacustris*, *Acinetobacter soli* and *Achromobacter pulmonis*. Four isolates from buffalo have been identified as *Escherichia coli, Shigella sonneii, Serratia marcescens* subsp. sakuensis and *Shigella flexneri*. Strains of *Streptomyces albidiflavus* and *Achromobacter rhulandii, Corynebacterium lipophiloflavum, Corynebacterium glutamicum, Corynebactrium stationis, Citrobacter sedlakii*, and *Providencia thailandensis*, isolated from equines have been identified. Other significant isolates include *Moraxella ovis, Bacillus hunanensis, Corynebacterium tuscaniense, Nocardia niwae, Brevibacillus agri, Streptomyces ghanaensis, Kluyvera georgiana, Rhodococcus coprophilus, Escherichia hermanii, Castellaniella denitrificans, Nocardiopsis alba, Aerococcus viridians, Pasteurella multocida, Ottowia pentelensis, Prolinoborus fasciculus, Rhodococcus aetherivorans* from various animals and animal microenvironments. Many of the 16S sequences have been analysed by phylogeny methods.

Isolation and identification of aerobic Gram-positive cocci

Most common aerobic Gram-positive cocci of economic significance in animals with considerable public health significance include those belonging to Staphylococus, Streptococcus and Enterococcus genera. Present day literature indicates that these biodiverse groups of versatile prokaryotes comprise of 110 species of Streptococci, 54 species of Enterococci and 51 Staphylococcal species. In order to capture the biodiversity of these genera, we have undertaken targeted identification of such isolates preserved in VTCC. A number of species of streptococci isolated from water buffalo, equines and porcines have been identified which includes *Streptococcus infantarius* sub spp. coli (4 isolates), *Streptococcus dysgalactiae* sub spp. dysgalactiae, *Streptococcus porcinus, Streptococcus equi* sub spp. equi (8 isolates) (Fig. 5a-b), *Streptococcus dysgalactiae* sub spp. *equisimilis, Streptococcus pluranimalium, Streptococcus acidominimus* and *Streptococcus equi* sub sp. ruminatorum. Also characterized *S.aureus* isolate (Fig. 6).

Enterococcus is an important genus with probiotic potential. We have identified isolates in the genus as *Enterococcus faecium* (mice), *Enterococcus devriesei* (2 isolates from pig), *Enterococcus gilvus, Enterococcus raffinosus*, and *Enterococcus hirae* (2 mice isolates). We have used GC-FAME analysis for identification of staphylococcal isolates (Fig 7). The culture growth conditions, culture processing conditions were standardized and 60 isolates were identified up to species level. Some of the species identified are *Staphylococcus cohnii, S. vitulinus, S. equorum, S. lutrae, S. xylosus, S. simulans, S. lentus, S. schleiferi, S felis, S. aureus, S. cohnii, S. arlettae, S. gallinarum, S. chromogenes* and *S. saprophyticus*.

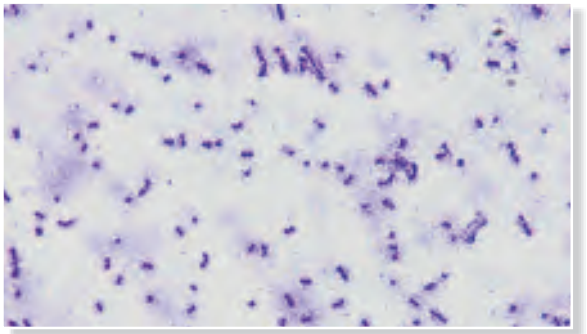


Fig 5a. Short chains of gram-positive cocci of *Streptococcus equi* with capsule can be seen

Fig. 5b. *Streptococcus equi* sub spp. *equi*: mucoid coalescing colonies with wide zone of haemolysis .

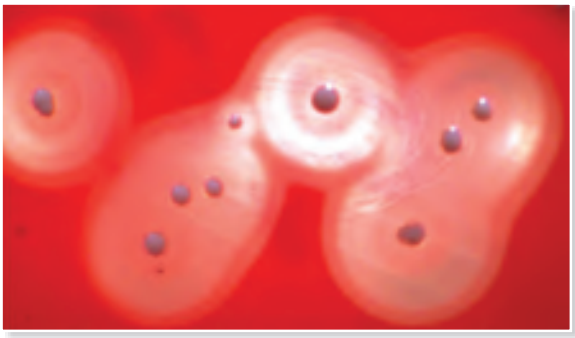


Fig. 5c. Characteristic double zone haemolytic *Staphylococcus aureus* colonies

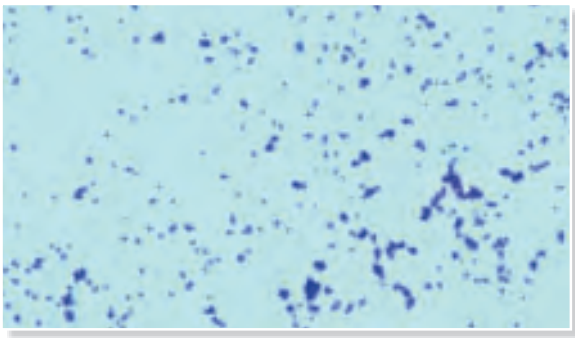


Fig. 6. Gram-positive cocci of *Enterococcus hirae* distributed in pairs or single cells

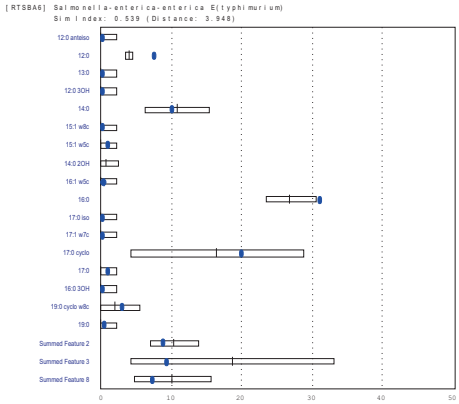


Fig. 7. Identification of *Staphylococcus simulans* by GC-FAME analysis

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

Generation of gateway ORF clone library of buffalopox and equine influenza viruses

In recent times, buffalopox virus (BPXV), a close variant of vaccinia virus (VACV) has emerged as a disease of zoonotic importance causing high morbidity in buffaloes and a health hazard to the milkers in the country. Further, epizootic of equine influenza occurred in the country in 2008-09 affecting equine population of 14 states. Like other Influenza A viruses, EI viruses undergo frequent mutational changes that render vaccines ineffective. Thus to understand the disease pathogenesis as well as to develop effective prophylaxis, there is need to study the proteome of these microbes.

Various virulence associated ORFs of BPXV & EIV were amplified, cloned and repository of validated gateway clones of immunomodulatory/ virulence genes of zoonotic buffalopox virus and equine influenza virus were generated in a flexible format. 21 ORFs of buffalopox virus isolated from outbreak associated with zoonotic infection, were amplified from purified DNA (Fig.8). The 2 ORFs viz., M & NS1 of equine influenza virus isolate (Katra-Jammu/6/2008) were also amplified by RT-PCR using genomic RNA isolated from the EIV (Fig 8).

Upon second round of PCR, complete lambda phage att site required for homologous recombination was incorporated in the amplicons. PCR products were purified and cloned into gateway vector by homologous recombination strategy. Gateway entry clones of 21 ORFs of BPXV and 2 ORFs of EIV were confirmed. The validated clones having correct ORF were preserved in the repository. The present strength of the developed ORF library consists of 39 gateway entry clones of buffalopox virus; 3 gateway entry clones of equine influenza virus and 10 gateway destination clones of BPXV ORFs and all clones were accessioned and preserved in the VTCC repository. The generated clones will serve as resource to study the proteome i.e., functional activities of the proteins encoded by genome of these viruses to identify vaccine candidate, disease mechanisms and drug discovery.

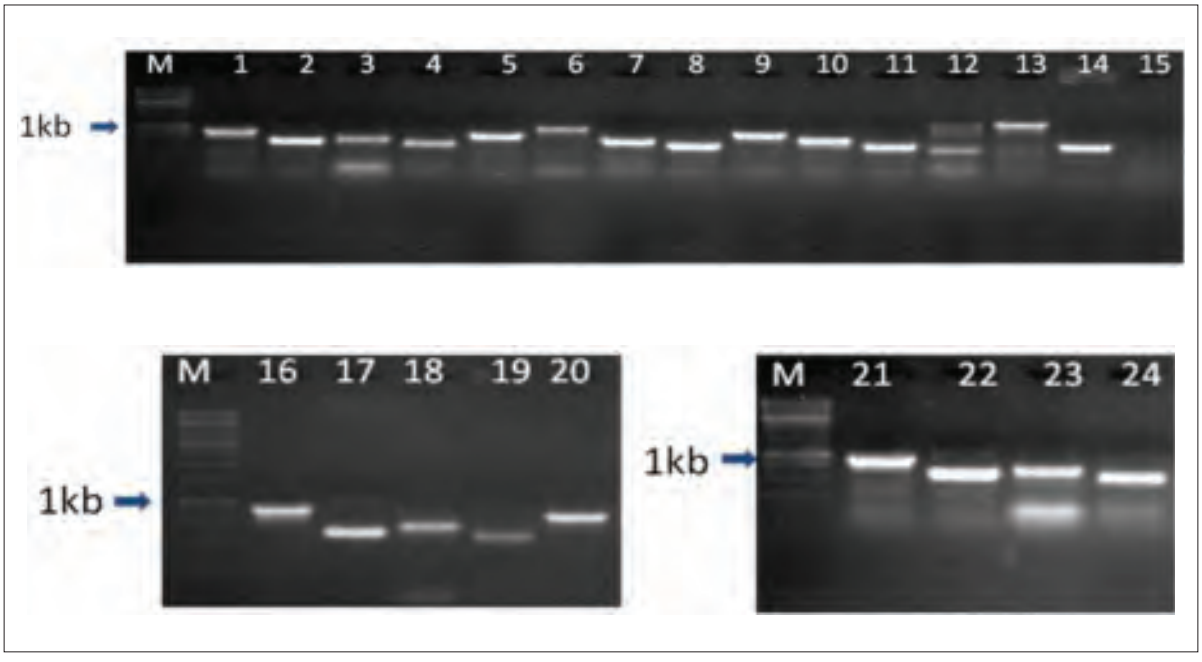


Fig. 8. PCR amplification of ORFs of BPXV & EIV. L1= DNA marker, L2-24: Amplicons of ORFs of BPXV & EIV

Validation of developed gateway clone library by expression of recombinant proteins of cloned ORFs

One of the major applications of the gateway ORF library is the expression of recombinant proteins in various formats by subcloning the validated ORFs from gateway entry clones into different destination vectors. Such studies depict the validity as well as utility of the developed gateway ORF library for future research in the areas of studies of protein-protein interactions, host-pathogen interactions and drug and vaccine development. For this purpose, 10 ORFs of BPXV were subcloned from gateway entry clones into gateway prokaryotic destination vector - pDEST15. Recombinant plasmid construct (pDEST-ORFs) were confirmed and subsequently transformed into BL-21 (DE3) cells. Recombinant proteins were expressed upon stimulation with IPTG and confirmed the expressed proteins by SDS-PAGE (Fig. 9).

This indicates that developed gateway library could be used for production of authenticated large numbers of recombinant proteins of many ORFs easily in different destination format which augment the future research in many facets of the targeted viruses.

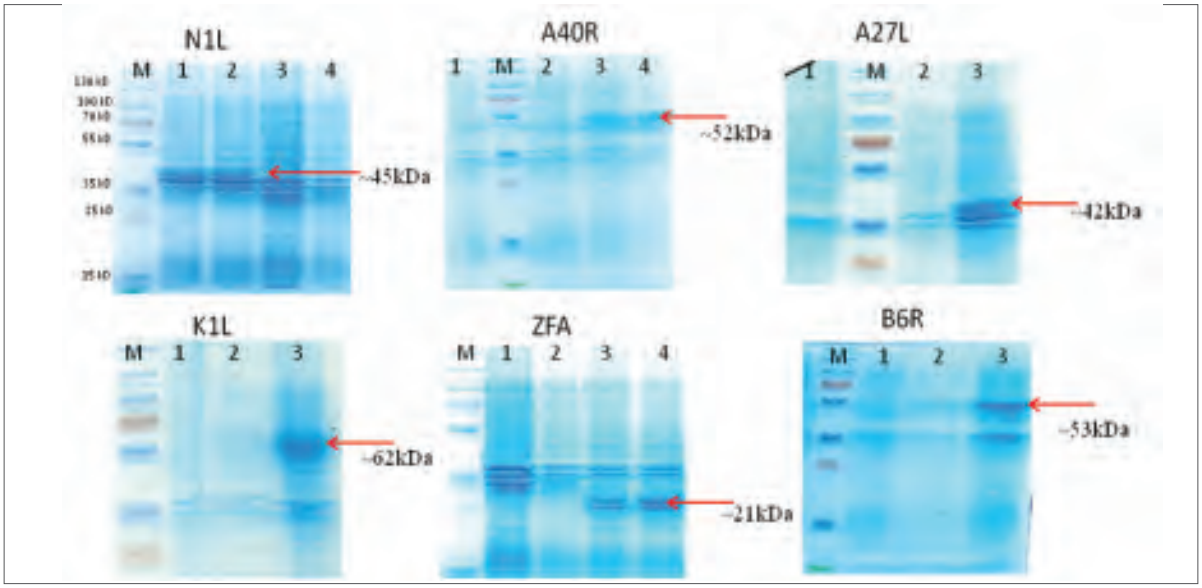


Fig. 9. Expression of recombinant proteins of representative ORFs of gateway clones of BPXV

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

Development of environmental *Bacillus* phage repository

Together with bacteria, bacteriophages constitute an integral part of the microbiome. Bacteriophages are abundant in earth's biosphere and play a major role in the evolution of host bacteria, mediate horizontal gene transfer and maintain environmental and ecological balance.

We report isolation and characterization of a variety of *Bacillus* phages obtained from environmental soil and water samples. The bacteriophage enrichment from soil/water sample was achieved with the *Bacillus* host isolated and purified from the same sample. The NA plates were examined for the presence of plaques (Fig. 10a) and plaque characteristics were recorded. Plaques were purified

three times and phage preparation was stored at 4°C in SM buffer and later used for large scale preparation of phage stocks. The phages were visualized by transmission electron microscopy (TEM) and protein profile was developed by running concentrated phage preparations in 12% SDS-PAGE gels. As indicated by TEM (Fig. 10b-c), phage morphology of Bacillus phages (VTCCBPA8, VTCCBPA12, VTCCBPA11) were found to resemble to families – *Myoviridae* (icosahedral with contractile tail), *Siphoviridae* (icosahedral with non-contractile tail), and *Tectiviridae* (a relatively rare group of tail less phages), respectively. Protein analysis by SDS-PAGE showed major protein bands of 58kDa, 63kDa and 60kDa, respectively. Two phages (VTCCBPA13 and VTCCBPA32) which were analysed by SDS-PAGE only, showed major protein bands of 50kDa (Fig. 10d) and 35kDa, respectively. Thus, we observed diversity in terms of morphology and structural protein profile for *Bacillus* phages.

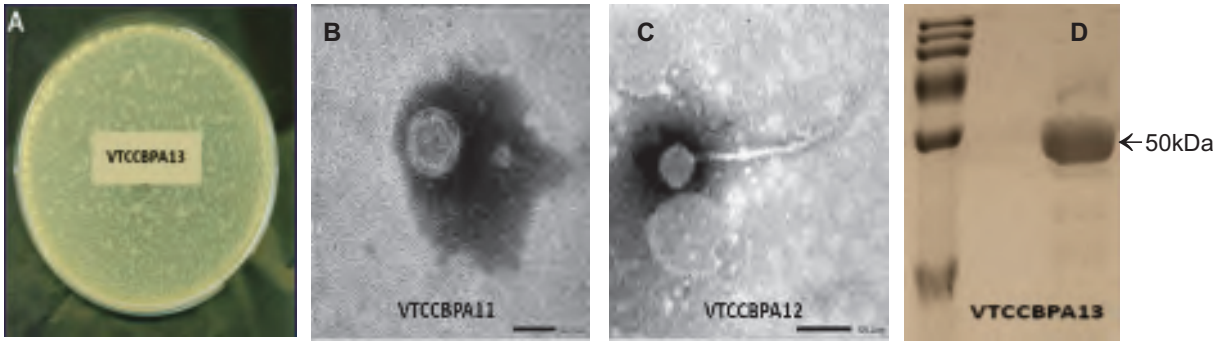


Fig. 10a-d. Characterization of Bacillus phages - A:Plaque characteristics; B & C : Electron microscopy (TEM); D : PAGE profile (VTCCBPA13)

Isolation and characterization of bacteriophages against *Salmonella* Gallinarum

The isolation and characterization of five bacteriophages (VTCCBPA25, 26, 28, 29 &30) infecting *S*. Gallinarum was carried out. Ten bacterial isolates of *S*. Gallinarum were used separately for enrichment of bacteriophages from poultry litter. The bacteriophages yielded clear plaques ranging in dia.- 1mm (VTCCBPA25) to 6mm (VTCCBPA30). The phage isolates were bulk cultured, concentrated using PEG and used for protein profiling by SDS-PAGE. Major protein band of 58 kDa was observed in all of them with another prominent protein band of 48 kDa (in VTCCBPA26) and 35 kDa (in VTCCBPA25). The phage biological activity was screened for temperature sensitivity over a range of 4°C to 80°C (Fig. 11). VTCCBPA25 & VTCCBPA30 phage isolates lost the biological activity completely at 65°C. VTCCBPA28 & VTCCBPA29 were able to survive this temperature;

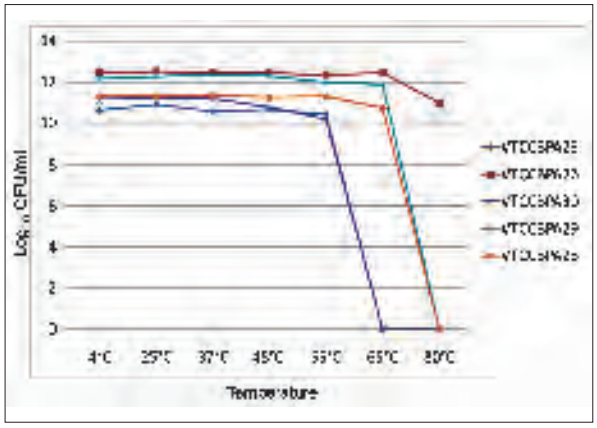


Fig 11. Temperature sensitivity test of bacteriophages

however they lost biological activity completely at 80°C. VTCCBPA26 was able to survive beyond 80°C as well. These were further tested for their biological activity (in vitro) against 81 bacterial isolates including *S*. Gallinarum and *S*. Typhimurium.

VTCCBPA26 showed a broad host range and was active against 97.8% of *S*. Gallinarum as well as 61.1% of *S.* Typhimurium isolates checked by spot test. The currently reported bacteriophages may be effective alternative to antibiotics for the control of fowl typhoid disease in chickens.

Table 3. Bacteriophages accessioned during the period

S.No.	Phage accession Nos.	Host	Source of Phage
1	VTCCBPA5	*Aeromonas hydrophila*	Pond water
2	VTCCBPA13	*Bacillus pumilus*	Soil
3	VTCCBPA14	*Pseudomonas mendocina*	Pond water
4	VTCCBPA15	*Paenibacillus spp.*	Pond water
5	VTCCBPA18	*E. coli*	Farm sewage
6	VTCCBPA22	*Pseudomonas spp.*	Farm sewage
7	VTCCBPA20	*Pseudomonas spp.*	Animal feed (Green chara)
8	VTCCBPA21	*Pseudomonas spp.*	Animal feed (Green chara)
9	VTCCBPA16	*Sphingobacterium spp.*	Farm sewage
10	VTCCBPA17	*Stenotrophomonas spp.*	Farm sewage
11	VTCCBPA23	*E. coli*	Farm soil
12	VTCCBPA24	*Acinetobacter baumanii*	Farm sewage
13	VTCCBPA25	*Salmonella* Gallinarum	Poultry litter
14	VTCCBPA26	*Salmonella* Gallinarum	Poultry litter
15	VTCCBPA27	*Salmonella* Gallinarum	Poultry litter
16	VTCCBPA28	*Salmonella* Gallinarum	Poultry litter
17	VTCCBPA29	*Salmonella* Gallinarum	Poultry litter
18	VTCCBPA30	*Salmonella* Gallinarum	Poultry litter
19	VTCCBPA31	*Staphylococcus aureus*	Submitted by CIRG

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

Inter-Institutional and externally funded projects

National Fellow Scheme - Development of sensitive and specific diagnostic assays for detection of *Trypanosoma evansi* infection in animals using modern molecular tools

Comparative evaluation of diagnostic sensitivity of q-PCR with conventional parasitological techniques in experimentally infected mice

Optimization of SYBR dye based qPCR

A real time PCR was standardized employing TBR primers on *T. evansi* isolate of pony origin propagated in Swiss albino mice. Genomic DNA was extracted from purified and counted parasites as well as from whole blood for standardization of qPCR. The conserved sequence of ITS 1 gene of *T. evansi* isolates was used for designing of primers, which amplified 151 bp product between 18S and 5.8S sub unit of rRNA in qPCR (Fig 1a & 1b).

The sensitivity of qPCR was found to be 1.5 parasitic equivalence or equal to 0.15 pg of parasite genomic DNA. By using DNA extracted from *T. evansi* infected whole blood the sensitivity was found equal to 0.82 parasites/µl of blood, Primer specificity of assay was confirmed by using DNA samples extracted from related blood protozoan *Theileria equi*.

Experimental infection of mice with *T. evansi* and evaluation of diagnostic sensitivity of different assays

Blood was collected from experimentally infected mice (n=6) after a regular interval of 24 hpi and subjected to analysis by different diagnostic techniques. Comparative evaluation of diagnostic sensitivity in experimentally infected mice indicated that sensitivity of real-time PCR was near to gold standard TBR-PCR. Both the techniques were able to detect the infection after 24 hpi in 33% (2/6) of mice, whereas parasitological technique was unable to detect the infection after 24 hpi. On 2nd dpi Wet blood film (WBF), thin blood smear (TBS), microhematocrit centrifugation test (MHCT), PCR and

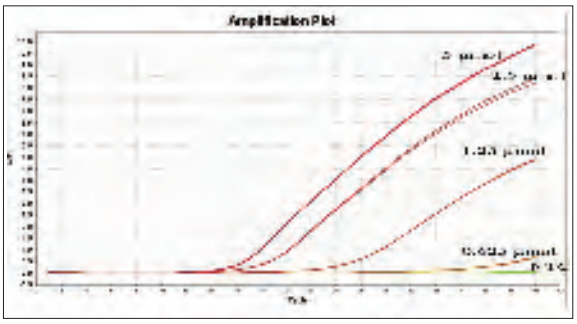


Fig 1a. Real time amplification of ITS-1 with different concentrations of TBR primers

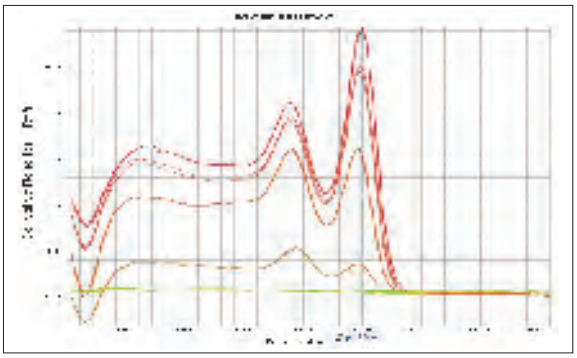


Fig 1b: Post amplification acquisition of melting curve with different concentrations of primers leading to formation of two melting peaks (approximate at 73°C and 79°C).

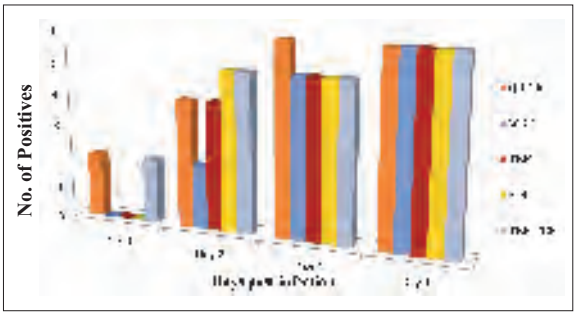


Fig 2: Comparative diagnostic sensitivity of qPCR with conventional parasitological techniques

real-time PCR detected 33% (2/6), 50% (3/6), 67% (4/6), 83% (5/6) and 67% (4/6) positivity, respectively. After 3rd dpi all the conventional parasitological techniques including PCR detected infection in 83% (5/6) of mice, while 100% mice (6/6) were positive by real-time PCR. After 4th dpi 100% (6/6) mice were positive by all the techniques with parasitaemia ranging from 1.6x105 to 2.1x108. (Fig 2). The study indicated that qPCR could be applied for quantitative estimation of parasitaemia in equines and could help to understand disease stage, risk of transmission of *T. evansi* and drug efficacy.

Genetic differentiation among *T. evansi* isolates of different livestock hosts by RAPD typing

To know the genetic variability among six isolates of *T. evansi* obtained from different livestock species (horse, donkey, camel and cattle) and geographical regions, genomic DNA was amplified by polymerase chain reaction using 17 decamer arbitrary primers. Of the 17 primers, 13 were able to amplify polymorphic DNA fragments from the genome of *T. evansi* and produced 179 scorable bands, 38 of which were polymorphic. The pattern of RAPD disclosed high polymorphism among different isolates of *T evansi.* Depending upon the *T. evansi* isolate primer combination, DNA fingerprints of 300 to 4000 bp were amplified, suggesting minor and major differences in the RAPD profiles. In all the assays performed, a few fragments were amplified more efficiently, giving more intense bands than other reproducible bands in the same reaction. The RAPD-PCR revealed strong reaction products of 300 bp – 4000 bp range. The primer PR2 showed different polymorphic DNA fragments from different isolates of *T. evansi* which were unique for particular isolate and all these band were scorable as either distinct or likely distinct. The RAPD analysis revealed heterogeneity among *T. evansi* isolates of different livestock hosts and geographical regions (Fig. 3).

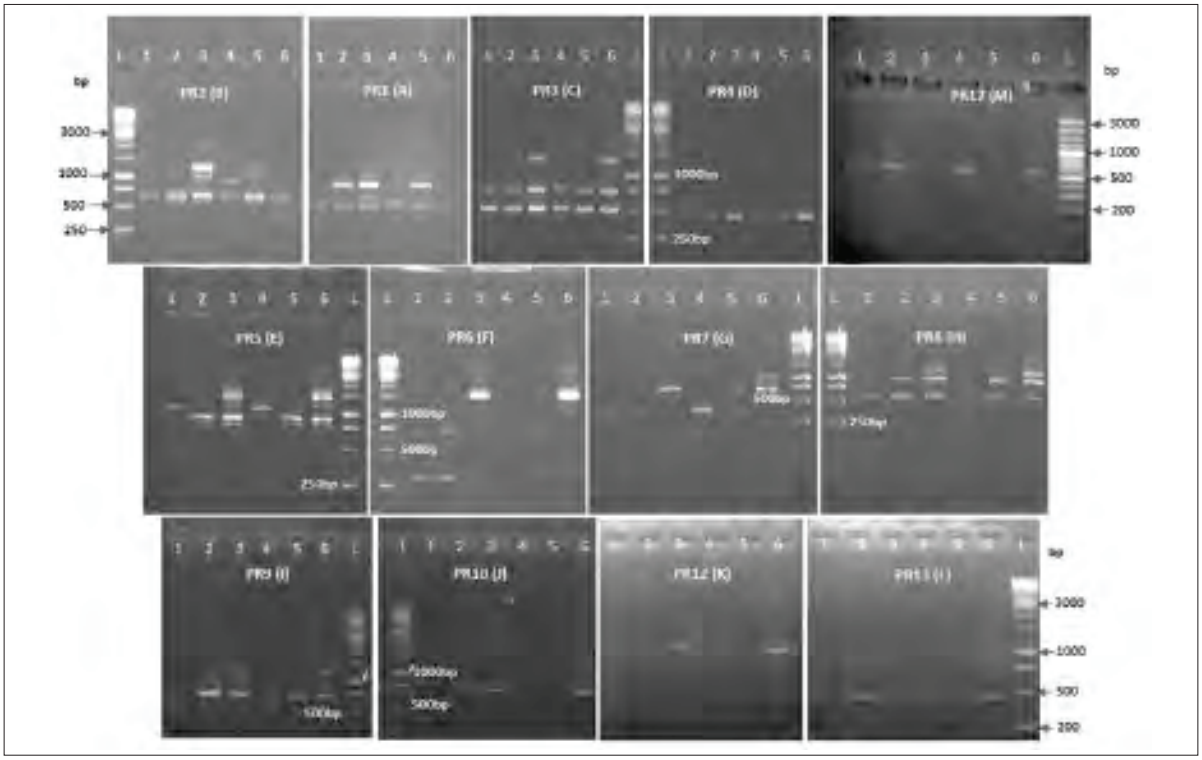


Fig. 3. (A-M) RAPD amplicons obtained using different primers with six isolates of *Trypanosoma evansi*. Lane 1: Pony (Karnal, Haryana); Lane 2: Cattle (Karnal. Haryana): Lane : Donkey (Hardoi, Uttar Pradesh): Lane 4: Pony (Hisar, Haryana); Lane 5: Donkey (Junagarh, Gujarat) ; Lane 6: Camel (Bikaner, Rajasthan); Lane 1: Ladder. Different types of primers shows different pattern of polymorphism.

(Rajender Kumar)

Development of Biomarker(s) for diagnosis of *Trypanosoma evansi* infection in animals using proteomic approach (DBT Project)

Expression of recombinant immuno reactive C- terminal fragments of heat shock protein 70 (hsp70)

The different fragments of immuno reactive C-terminal region of heat shock protein 70 (hsp70) were identified and amplified separately using specific primers as per the strategies depicted in diagram (Fig 4). Apart from 50 kDa truncated C- terminal HSP 70 recombinant protein, two more fragments encoding for C- terminal (440 -690 and 514-690 amino acids) were also cloned and expressed during the year, resulting in 27 kDa and 20 kDa recombinant proteins (Fig 5). The kinetics of expression studies of each fragment were standardized and large scale purification of recombinant proteins in complete purity were achieved using Ni NTA column for use in the immunodiagnostic test. Thereafter, all the four fragments designated as rHSP1, rHSP2, rHSP3, & rHSP4, respectively, were comparatively evaluated using reference serum raised in ponies experimentally infected with *T. evansi.*

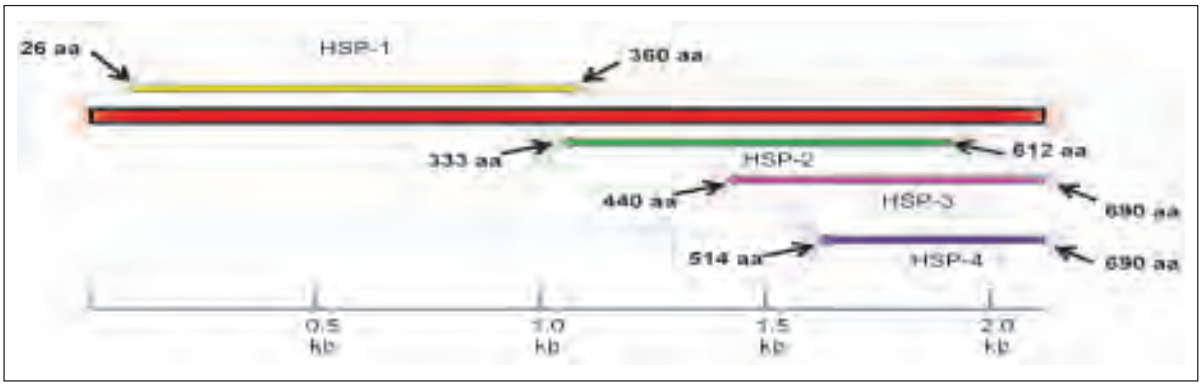


Fig. 4. Strategy of cloning N and C terminal fragments derived from full length c DNA of hsp70 of *T. evansi*. HSP-1 :- 26-360 aa residues; HSP-2 :- 333-612 aa residues; HSP-3:- 440-690 aa residues & HSP-4:- 514-690 aa residues.

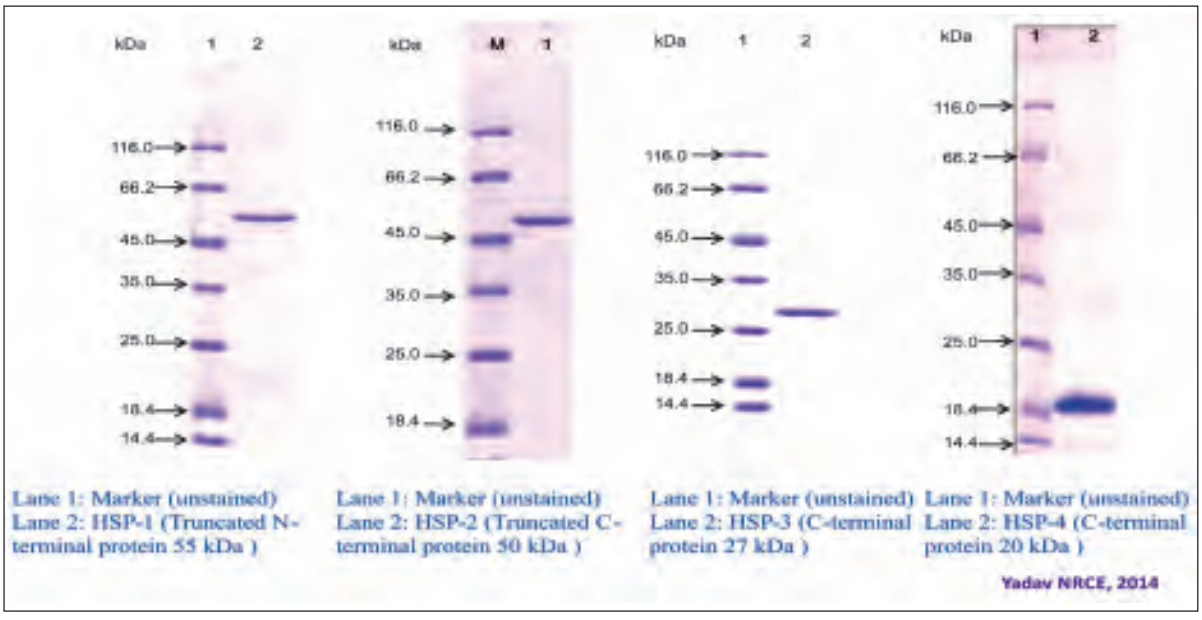


Fig. 5. Recombinant HSP 70 (N- & C- terminal) proteins purified by Ni-NTA agarose column

Evaluation of the diagnostic potential of all expressed recombinant HSP70 proteins

To evaluate the diagnostic potential of all expressed recombinant proteins in experimentally infected ponies, cut off value for the antibody ELISA were established as the mean plus four standard deviation (SD) of the OD value of uninfected ponies serum samples. It is evident that C terminal rHSP70 proteins (50kDa, 27 kDa and 20kDa) are capable of detection of antibodies at early stage i.e. 10 dpi, showing peak at 42 dpi in pooled serum samples, however, N terminal recombinant antigen showed poor response and detected antibodies at 14 dpi with comparatively low antibody titre (Fig. 6). Thereafter, gradual decline in antibody titre were observed at 42 dpi in all recombinant antigens, while WCL antigen maintained increasing trend till termination of experiment. However, antibody titre remained above cut-off value, throughout the experiment in all antigen groups.

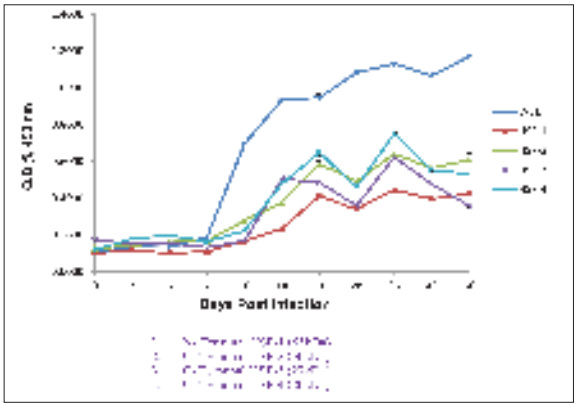


Fig. 6. Antibody response in ponies (pooled serum samples) using recombinant HSP70 antigen (s) along with WCL antigen.

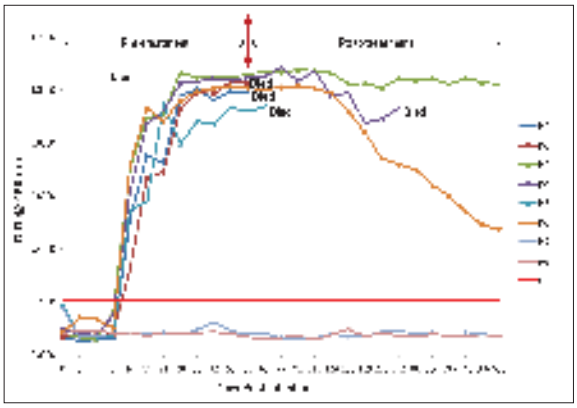


Fig. 7a. Pre and Post treatment antibody titre in ponies using Whole Cell Lysate (WCL) antigen

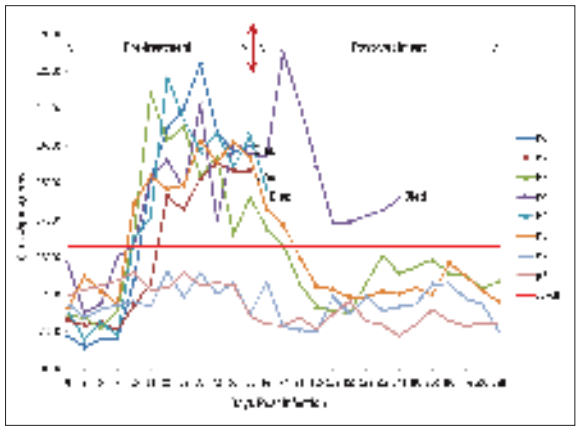


Fig. 7b. Pre and Post treatment antibody titre in ponies using recombinant Heat Shock Protein 70 (C-terminal 27 kDa) antigen

It was interesting to observe that post-treated PCR negative mice had antibody titre to recombinant HSP3 (27 kDa) below the cut off value after 30 days (Fig 7a & 7b). However, WCL antigen using same set of samples continued to show high antibody titre, which persisted for long period (530 days) even after treatment. Shelf life of rHSP70 antigen (27 kDa) on ELISA plates was found to be upto 7 months.

(S.C. Yadav, Rajender Kumar and B.C. Bera)

Development of nanogold based immunochromatography / immuno dot blot synthesis and characterization of gold nanoparticles

The aim of the project is to develop nanoparticle based effective diagnostics for *T. evansi*. The colloidal gold nanoparticles were prepared by the citrate reduction method. The UV-Vis measurement of the synthesized gold nanoparticles showed the characteristic absorbance maximum at lmax ~ 518 (Fig. 8a). The synthesized gold nanoparticles were stable (zeta potential -41.1 mV, Fig. 8a), spherical and in the size range of 12-15 nm as characterized by TEM (Fig. 8a-8b).

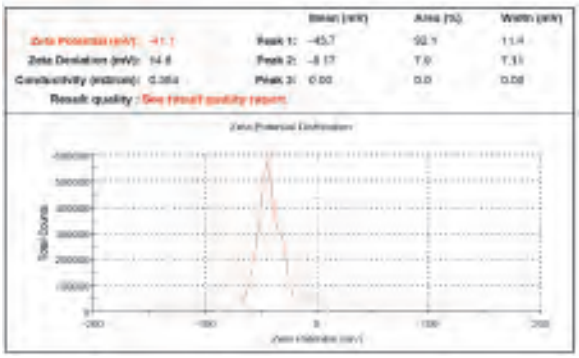


Fig. 8 a: Zeta potential of Gold Nanoparticles

Zeta potential measured by Zeta sizer showed the synthesis of stable gold nano particles

Further, in order to prepare anti-horse IgG conjugate using above characterized gold nanoparticles, hyperimmune serum (HIS) was raised against purified horse IgG in rabbits and thereafter anti-horse IgG was purified for the conjugation process. The HIS raised against IgG was titrated by ELISA. Further, the purification of anti-horse immunoglobulins from hyper immunized rabbits is in progress.

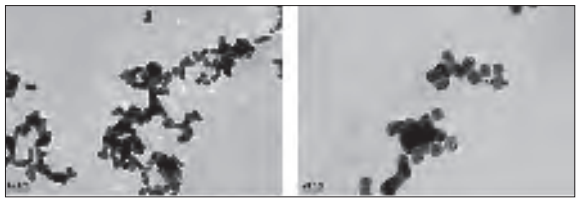


Fig. 8b : TEM images of Gold Nanoparticles

TEM images showed the uniform distribution of spherical shaped gold nanoparticles with size range of 12-15 nm

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

Eukaryotic expression of important equine cytokines and analysis of their biological activities

Five cytokine genes (IL-2, 4, 10, 18 and IFN-γ) were mobilized from gateway entry vector to N-term baculovirus vector for the purpose of expression of recombinant cytokines in insect cells. Insect cells (sf9) were transfected with recombinant baculovirus and transfectants were selectively enriched by ganciclovir selection.

After transfection, insect cells were observed under inverted microscope at 24h interval. After 48 hour, reduced cell growth was observed as compared to control cells. Enlargement of cell nuclei and cell diameter, polyhedron formation inside cells were also seen (Fig. 9a & 9b). Cell culture supernatants (P1 viral stock) were harvested after 72 hour post-transfection period, which was used for subsequent propagation of P2 and P3 viral stock. Expression of proteins in insect cells was evaluated by SDS-PAGE (Fig. 10). Four recombinant equine cytokines like IL-2, IL-10, IL-18 and IFN-γ were purified by Nickel-NTA column chromatography (Fig. 11). Biological activity of two recombinant equine cytokines (IL-18 and IFN-γ) in terms of their ability of inducing cell proliferation by XTT assay and stimulation of in vitro cytokine production by cytokine ELISAs was assessed in equine peripheral blood mononuclear cells (PBMC). Lymphocyte proliferation assay and cytokine ELISAs were optimized using different concentration of purified recombinant cytokines ranging from 500 ng-1500 ng/ml. Recombinant IL-18 was able to induce secretion of IFN-γ and IL-10 as determined by cytokine ELISAs using PBMCs culture supernatant. The availability of biologically active recombinant

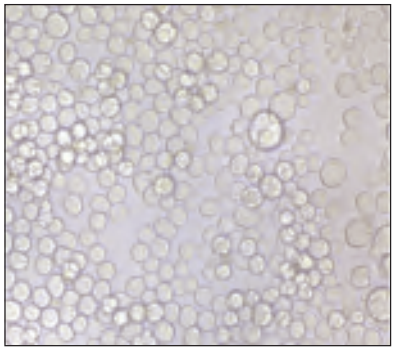


Fig. 9a. Control sf9 insect cells Recombinant baculovirus transfected sf9 insect cells

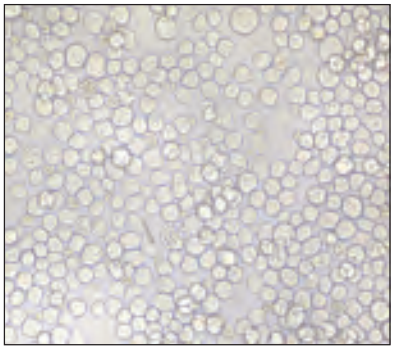


Fig. 9b. Morphological changes of cells like enlargement of cells polyhedron formation are evident in transfected cells.

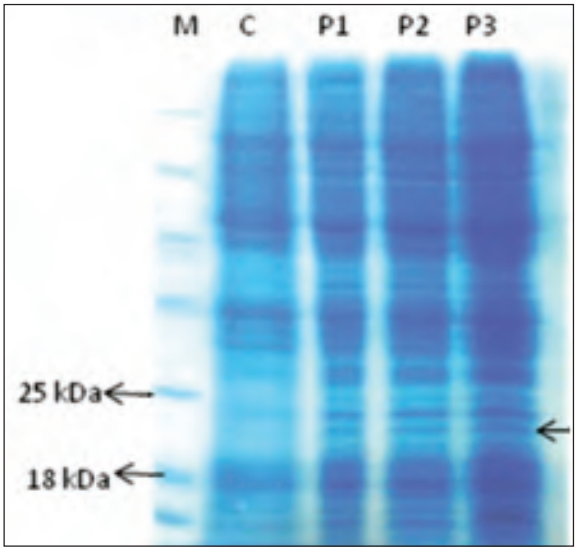


Fig. 10. SDS-PAGE analysis of recombinant IL-2 produced in insect cells. Lanes M: Protein molecular weight marker, C: control sf9 cells, P1: P1 baculovirus transfected cells P2: P2 virus infected cells, P3: P3 virus infected cells

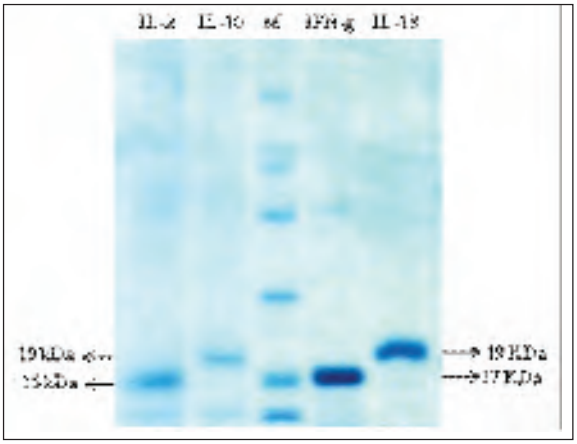


Fig. 11. Purified recombinant horse cytokines.
M: protein molecular weight marker.

equine cytokines will be helpful to investigate the immunological roles of this cytokine in relation to equine disease, as well as its potential efficacy as a therapeutic agent or vaccine adjuvant in horse.

(H.S. Singha and Praveen Malik)

Toxicological evaluation of quinapyramine sulphate-loaded nanoparticles

With the aim of development of the potential nanoparticle based therapeutics against economically important disease caused by *T. evansi*, quinapyramine sulfate loaded-sodium alginate nanoparticles (QS-NPs) were synthesized. In the present investigation, the safety of the formulated nanoparticles and biocompatibility of synthesized QS-NPs was determined using Vero & Hela cell lines and horse erythrocytes in a dose-dependent manner. The experiments unveiled a concentration-dependent safety/cytotoxicity (metabolic activity), genotoxicity (DNA damage, chromosomal aberrations), production of reactive oxygen species and hemolysis in QS-NPs treated cells. Annexin-V propidium iodide (PI) staining did not show massive apoptosis or necrosis. Compatibility testing of the QS-NPs performed by comparison with QS revealed decreased hemolytic rate as well as decreased oxidative stress. The levels of MDA/hydroperoxides were significantly higher in all the concentrations of

QS compared to QS-NPs treated cells and untreated controls. The nitric oxide production by QS-NP was significantly lower ($p < 0.05$) than QS at 1000 µg/ml concentration, whereas the lower concentrations did not show any significant differences ($p > 0.05$) amongst each other. QS-NPs were safe at effective trypanocidal doses and even at doses several times higher than the effective dose. The current approach highlights the potential for simplified therapy against economically important surra caused by *T. evansi* and merits evaluation in a higher animal model.

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

OIE Twinning Laboratory Project on Equine Influenza

An OIE twinning program on equine influenza was initiated with basic aim of capacity building at ICAR-NRCE, quality assurance of diagnostics and compliance with OIE standards for diagnosis of equine influenza and to finally submit a proposal to the OIE to designate ICAR-NRCE, India laboratory as 'OIE reference laboratory for equine influenza.' During the current year, Dr Keith Hamilton from OIE headquarters visited NRCE on August 24, 2014 and was shown scientific and laboratory activities being carried out on equine influenza. Later during the year, scientists from OIE referral laboratory, Animal Health Trust, United Kingdom- Dr Debra Elton, Head, Division of Virology and Dr Elizabeth Medcalf, Head, Diagnostics, visited NRCE, Hisar from November 14-21, 2014 (Fig. 12a-12c). During the visit, NRCE demonstrated the surveillance system for monitoring equine influenza besides processing of samples and different tests (HI, SRH, qRT-PCR & virus isolation) being employed at NRCE for testing. Also a field visit was conducted and an equine health camp was organized at Hanumangarh to demonstrate various aspects of sample collection, surveillance and liason being developed by NRCE with farmers for better dissemination of information from both ends.

Fig. 12b. Interacting with farmers in Equine Health Camp and Kisan Goshthi at Hanumangarh

Different aspects of biosecurity issues were also discussed for the control of the disease during outbreaks. A lecture was delivered by Dr Debra Elton on biosecurity and biosafety for equine influenza along with monitoring system in UK. All scientists of ICAR-NRCE participated in the talk. The AHT has recent experience of ISO 17025 certification and offered technical support and guidance through this process. On March 6, 2015, NRCE participated in the Expert Surveillance Panel meeting through SKYPE with other members at OIE Head-quarter's in France on equine influenza for strategizing the vaccine strain selection.

Fig 12a. Scientists from Animal Health Trust, UK. with Scientist and Staff of Equine Influenza lab at NRCE

Fig. 12c. Scientist from AHT interacting with EI lab personnel

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

OIE Twinning Laboratory Project on Glanders

Glanders is an important disease in context to India and we are facing continuous outbreaks since 2006. NRCE has developed advanced diagnostic facilities for glanders and foresees itself to cater to the region. With this thought in mind, an OIE twinning project on Glanders was initiated in July 2012 with Friedrich Loeffler Institute, Jena, Germany. The basic aim of the project is capacity building of the Centre and to become OIE Reference Laboratory for SAARC region besides imparting training to the competent researchers/scientists from these countries so that a systematic surveillance for the disease condition could be carried out. For capacity building, two scientists - Dr Praveen Malik and Dr Harisankar Singha from the candidate lab, ICAR-NRCE, Hisar visited FLI, Germany (Fig. 13) for two weeks from June 15-28, 2014. During the visit, scientists were provided hands on training on genotyping techniques of *Burkholderia mallei* such as variable number of tandem repeats (VNTR), multilocus sequence typing (MLST), etc. Although the planned activity of the project was to genetically characterize the field isolate of *B. mallei* available at ICAR-NRCE, *Burkholderia mallei* reference strain from FLI was used for the experiment. VNTR and MLST typing were performed by targeting 23 and 7 different loci, respectively. The DNA sequence was analysed using bioinformatics tools for number of tandem repeat analysis and assigning allele number to MLST sequences. Besides genotyping, the diagnostic potential of ELISA using recombinant TssA protein was also evaluated using panel of glanders positive and negative equine serum from FLI repository. The result was encouraging and

Fig. 13. Scientists from candidate lab ICAR-NRCE in parent lab at FLI Germany

comparable with CFT as it was seen with other two recombinant proteins (Hcp1 and TssB) during first visit. During the last phase of visit, a training module for 10 days workshop was developed and discussion about the third year program of the project was carried out.

Accordingly, work was initiated on genotyping of *B. mallei* isolates in India using published primers. MLST typing of three *B. mallei* isolates revealed that they belong to sequence type 734 along with reference *B. mallei* strains like ATCC23344, Bogor, Zagreb and Mukteswar. For the issues regarding planned laboratory workshop on glanders and future candidate of OIE-reference laboratory, an improvement of infrastructural and equipment facilities of the candidate lab and sharing of biologicals between laboratories need to be strengthened.

(Praveen Malik, H.S. Singha and B.N. Tripathi)

Consultancy and commercialization of technologies

Consultancy

NRCE, being a nodal agency and National Referral Centre of Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Govt. of India provides consultancy and testing for health certification and diagnostic services for various equine diseases to 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 various diseases/ infections such as EIA, glanders, equine influenza, EHV 1, EVA, CEM, *Theileria equi, Trypanosoma evansi, Trypanosoma equiperdum, Babesia equi, Salmonella abortus-equi*, and African horse sickness. All of 5163 serum samples of Thoroughbred as well as indigenous equines examined by Coggins test for EIA under various schemes were found to be negative. Testing of 6309 serum samples for glanders revealed positive cases from U.P. (n=11), H.P. (n=2) and J&K (n=3). *B. mallei* was isolated from clinical sample of an infected horse from U.P. NRCE helped in preparation of dossier for freedom of disease free status of AHS. Overall the Centre generated a revenue of ₹ 57.87 lakhs through testing of samples.

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

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, and
8. Recombinant protein based ELISA for differentiation of EHV 1 and EHV 4 infections.

Name of Patent (Technology) filed by the Centre

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

Technologies Developed and Assessed

Release of two technologies developed at NRCE by Hon'ble Minister of Agriculture, Sh. Radha Mohan Singh ji on 18 February 2015 during Annual General Meeting of ICAR at NASC, New Delhi

(i) Equiherpabort vaccine

Equine herpes virus 1 (EHV1), a widely prevalent equine viral pathogen causes a range of clinical signs including respiratory disease in young horses, abortion storms or sporadic abortions in pregnant mares, early neonatal death and neurological illness resulting in paresis/paralysis in foals. The greatest clinical threat posed by EHV1 infection is the late term abortions in pregnant mares and birth of weak foals causing early neonatal mortality. In a national assessment of EHV1 infection among equines in India, overall seropositivity to EHV1 has been reported to be 13.5%. In view of carrier status and latency, vaccination remains an appropriate option for preventing infection. Thus, vaccine is of immense value in control of EHV 1 abortion when used in conjunction with adequate management practices. Although live and inactivated vaccines are commercially available in the international market, there is no indigenously manufactured EHV1 vaccine available for control of abortion storms in India.

We developed inactivated vaccine using Indian strain of equine herpesvirus 1 (Strain Hisar-90-7) grown in VERO cell culture. Being a inactivated vaccine, it does not have the risk of reversion to virulent virus, which might be the case with the live viral vaccines. This vaccine provides good protective immunity against abortions due to EHV1 in pregnant mares. Vaccine is safe, potent and without any adverse reaction. The vaccine is stable at room temperature and has a shelf life of six months at 4°C.

(Developed by B.K. Singh, Nitin Virmani and B.R. Gulati)

(ii) *Theileria equi* antibody detection ELISA kit

Equine piroplasmosis - is an acute, sub-acute or chronic tick-borne disease of equids, caused by an intra-erythrocytic protozoa *Theileria equi* or *Babesia caballi*, which adversely affects the performance of the working equids (sero-prevalence up to 35%). Equine piroplasmosis caused by *T. equi* – is more widespread and pathogenic than that by *B. caballi*. Sero-diagnosis of the disease is carried out by employing an imported kit (OIE recommended CI ELISA Kit), which is expensive.

Recently, a team of scientists from NRCE developed recombinant merozoite antigen-2 (EMA-2) protein antigen-based ELISA for detection of antibodies against *T. equi* parasite in equine serum. The kit provides ready-to-use ELISA plate which makes it simpler to use in two steps, viz., serum sample application and conjugate addition – and the results can be

obtained in just 90 min after application of serum samples. The ELISA has a diagnostic sensitivity (DSn) of 94% and diagnostic specificity (DSp) of 96% in comparison to OIE recommended CI ELISA Kit. Serum samples shipped to the laboratory at ambient temperature can be utilized for testing by kit and with same practice now the new kit is being used at NRCE routinely.

(Developed by Sanjay Kumar, Rajender Kumar, A.K. Gupta and S.C. Yadav)

Internal validation of recombinant Hcp1 and TssA protein based ELISAs for serological diagnosis of glanders

Two indirect ELISAs using recombinant Hcp1 and TssA protein of Burkholderia mallei was developed for detection of B. mallei specific antibodies in equines. Analytical and diagnostic performance characteristics of the assay were determined using a panel of glanders positive serum (n=75), negative serum (n=100) and other equine disease control serum. The assays were evaluated with large number of equine serum samples. The diagnostic specificity and sensitivity of the assay were 99.8% and 100%, respectively. Repeatability (within laboratory) and reproducibility (between laboratories) of the assays were determined on 44 samples (positive= 20, negative=24) along with a reference positive and negative serum. Reproducibility was tested in 3 laboratories of the institute. Randomly ordered samples, coated ELISA plate, and related reagents were provided to the participating laboratories. The ELISAs were performed according to the optimized protocol. The results were shown to be highly repeatable and reproducible. Further, reproducibility study of these ELISAs in 3 diagnostic laboratories of different institutes has been initiated. However, these assays need to be validated according to the principles and methods of OIE validation pathway to support the routine use of this test in laboratories providing diagnostic service to equine glanders.

(H.S. Singha and Praveen Malik)

Training and Capacity Building

Field Experience Training of FOCARS trainees at ICAR-NRCE

The Centre hosted the Field Experience Training (FET) for a group of six FOCARS trainee scientists from ICAR-NAARM, Hyderabad from 19 February to 11 March, 2015. Dr A.A. Raut (Scientist), NRCE was entrusted with responsibility of Local Coordinator for the FET. Field experience training comprised of study in the village Rajli in Hisar district using PRA tools, exposure visits to agri-based industries, educational and research institutions in Hisar. The FET group presented village seminar in Rajli village on 2 March, 2015 and discussed various problems related to agriculture and animal husbandry with farmers and provided suggestions for improvement of the associated problems. The findings of work done during FET were presented in the institute seminar at NRCE by FOCARS trainees in presence of scientists from the Centre including Director NRCE and Dr. J. R. Rao, the monitoring faculty for Field Experience Training (FET) from NAARM, Hyderabad.

Village seminar at Rajli by FET trainees from NAARM

Institute Seminar at NRCE by FET trainees from NAARM

Annual Review Meet of Veterinary Type Culture Collection Network Project

The fifth Annual meet of Network Units of Veterinary Type Culture Collection Network Project was held at the College of Veterinary Sciences, Assam Agricultural University, Khanapara, Guwahati on October 13, 2014 under the chairmanship of Prof Gaya Prasad, ADG (AH), ICAR. Dr Apurba Chakraborty, Director Research (Veterinary), AAU, Khanapara welcomed the participants for VTCC annual review meet. Dr B. N. Tripathi, Director, ICAR-NRCE & VTCC, Hisar addressed the members of the Network Project and highlighted the significance of microbial repositions and their potential use in future. Prof Gaya Prasad emphasized upon conservation of microbial diversity, its preservation and its distribution to stakeholders. The technical session started with the presentation by Dr B. N. Tripathi. He briefed about status of the cultures submitted and accessioned in the network project and discussed on various issues for smooth

VTCC Annual Review meet at Guwahati

management of the project. Different modalities related to culture submission and accessioning were thoroughly discussed among all nodal officers. Subsequently, the progress made during the year was presented by nodal officers from network units - Veterinary Microbes Component, Rumen Microbes Component and Dairy Microbes Component. Dr R. N.

Goswami, Dean, College of Veterinary Sciences, Khanapara in his address mentioned that VTCC has made its impact in the country by focusing on three areas i.e. Veterinary, Dairy and Rumen microbes, however, emphasis needs to be given to North-Eastern states of country to monitor incursion of exotic and transboundary animal diseases from this region of the country.

Expert Lectures at NRCE

- Dr Keith Hamilton from OIE Headquarters Paris, France delivered a lecture on "Activities and role of OIE in control of diseases of livestock" on August 25, 2014.

Dr Keith Hamilton delivered a lecture in NRCE

- Dr Gaya Prasad, ADG (AH), ICAR delivered lecture on "Scientific Revolution" on occasion of ICAR-NRCE Foundation Day Celebration. The dignitaries, equine owners, scientists and staff from ICAR-NRCE and ICAR-CIRB attended the lecture.
- Dr Debra Elton, OIE expert on Equine Influenza from Animal Health Trust, UK delivered lecture on "Biosecurity and biosafety for equine influenza along with monitoring systems in UK" on November 20, 2014. Scientists from NRCE participated in talk and discussed various aspects of surveillance & dissemination of information. Different aspects of biosecurity and biosafety issues were also discussed for the control of the disease during outbreaks.
- Dr B. R. Gulati delivered lecture on "Risk Assessment and Biosafety Practices in Research Laboratories" during Science Day Celebration at ICAR-NRCE, Hisar on February 28, 2015.
- Dr Rajender Kumar, National Fellow, ICAR-NRCE delivered lecture on "Open Access to Agricultural Knowledge for Inclusive Growth and Development" during Science day Celebration at the Centre on February 28, 2015.

Expert Lectures Outside

- Dr B.N. Tripathi, Director, NRCE delivered a talk on "Role of NRCE in Diagnosis & Control of Diseases in Equines" during the National Seminar on "Veterinary Service Provision in India: An Equine Perspective" organized by Brooke Hospital for Animals (India) and National Academy of Veterinary Sciences (NAVS) at Kisan Bhawan, DUVASU, Mathura on February 25, 2015.
- Dr Rajender Kumar delivered an expert lecture on "Introduction to Veterinary Parasitology" during Raksha Knowledge Summit-2014 organized by Indian Immunologicals Ltd., at Hyderabad on August 23, 2014.
- Dr B.R. Gulati delivered lecture on "Risk Assessment, Biosafety Principles and Practices in Research Laboratories" in ICAR Short Course on "Frontier Approaches in Diagnosis and Control of Animal Viral Diseases with Quality Assurance and Quality Control for Veterinary Biologicals", IVRI, Izatnagar from November 10-19, 2014.
- Dr B.R. Gulati delivered lecture on "Risk Assessment and Biosafety Practices in Research Laboratories" in Department of Animal Biotechnology, LUVAS, Hisar on February 13, 2015.
- Dr T. Rao delivered lecture on"Derivation of bovine induced pluripotent stem cells by piggyBac mediated reprogramming" at 41st Annual Conference of the International Embryo Transfer Society (IETS), Versailles, France, January 10-13, 2015.

IRC & IMC Meetings

Institute Research Committee (IRC) Meeting

The Annual Institute Research Committee meeting for the year 2013-14 was held under the chairmanship of Dr A.K. Gupta, Acting Director on April 17, 2014 at NRCE, Hisar. The IRC assessed the achievements of the research projects executed during last year in the area of equine health, production, extension, Veterinary Type Culture Collection and externally funded projects. The Chairman, IRC raised concern over the development of equine specific formulation of feed using locally available feed and fodders. Chairman also emphasized on development of more practical

IRC meeting in progress

oriented technologies for utilization and conservation of equines and for combating the emerging and re-emerging diseases.

Half yearly meeting of Institute Research Committee (IRC)

The half yearly meeting of Institute Research Committee (IRC) was conducted under the Chairmanship of Director, NRCE, Hisar on November 10-11, 2014 for the evaluation of research projects in the area of equine health, production, VTCC and extension. The In-charge PME welcomed the chairman and scientists, and the meeting started with the opening remarks by Dr B.N. Tripathi, Director & Chairman, IRC. All the In-charges of the units of NRCE, VTCC & EPC presented overall progress of research work executed and various issues of the respective units, followed by project presentations. The Chairman, IRC exhorted the scientists to be proactive on various issues like initiation of the transfer of technologies for vaccine and diagnostics production; formulation of new externally funded research

IRC meeting in progress

projects involving basic research in areas like donkey immunology; harnessing novel technologies such as reverse genetics, RNAi and biosensors for improving equine health; exploration of the newer areas like dope testing; research on combined vaccines and on athletogenomics.

36th Institute Management Committee Meeting (IMC)

The 36th Institute Management Committee meeting (IMC) was held on April 16, 2014 at NRCE, Hisar under the chairmanship of Director, NRCE. The meeting was attended by Dr. Gurdial Singh, Dean College of Veterinary Science, LUVAS, Hisar; Dr. M. S. Chauhan, Pr. Scientist, Animal Biotechnology Centre, NDRI, Karnal; Dr P.S. Yadav, Pr. Scientist, CIRB, Hisar; Dr Praveen Malik, Pr. Scientist, VTCC, Hisar; Shri Gajendra Pal Singh, AIMHS, Jodhpur; Shri Ved Prakash, Finance & Accounts Officer, CSSRI, Karnal and Shri Chetan Issar, Administrative Officer, NRCE, as Member Secretary. The meeting started with the welcome address by Dr A.K. Gupta, Chairman and discussions were held about the issues of previous meetings and current issues of the Centre. The IMC confirmed and adopted the proceedings of the 35th Meeting of the Centre. Various agenda items viz. construction of stables at EPC, Bikaner; construction of Animal House phase II for VTCC, NRCE; External furnishing of VTCC laboratory; ISO-17025 certification of the laboratories as per OIE recommendation etc., were thoroughly discussed among the members and consent was given. The IMC emphasized on the strengthening of the Centre with modern equipments and pro-activeness in resolving the construction works of the Centre.

Workshop, Seminar and Institutional Activities

Organisation of interactive meet of equine owners on ICAR Foundation Day

ICAR-NRCE organized an interactive meet of equine owners and scientists on July 16, 2014 on the occasion of ICAR foundation day. Equine owners from Hisar, Rohtak, Bhiwani, Jind, Kurukshetra, Fatehabad and Ambala districts attended the meet. During the meet equine owners and scientists interacted on various aspects of health and management of equines and issues affecting equine welfare. The stakeholders raised the questions related to health which were addressed by the scientists. The information about artificial insemination, pregnancy diagnosis, diagnostic and consultancy services provided by NRCE was given to the equine owners. Information on preventive measures in disease control, deworming, hoof care and feeding was given to equine owners by the scientists of the Centre.

Interactive meet of equine owners on ICAR foundation day

Organization of drawing competition on "Horse as a companion animal" at ICAR-NRCE

On the eve of ICAR-NRCE foundation day, drawing competition for school children was organized at NRCE Hisar on the theme "Horse as a companion animal" on November 25, 2014. Students from 11 different schools in Hisar participated in the event. Ms Amisha Periwal, Class XI student from O.P. Jindal Modern School, Hisar received 1st prize. Lavany Mittal, Class X student from The Aryan School, Hisar and Rakshit, Class XII student from Thakur Dass Bhargava Sr. Sec. Model School received 2nd and 3rd prize, respectively. Shri Sachdev Mann, Shri Bhimsingh Beriwal and Shri Brijendra Singh acted as members of judging committee for the drawing competition. Dr B.N. Tripathi, Director NRCE distributed certificates and drawing kit to all the participating students. The students also visited the Info-equine museum at NRCE. Chief Guest, Gen (Dr) Sri Kant Sharma, Vice Chancellor, LUVAS, Hisar presented prize to the winners of drawing competition on the occasion of Foundation day celebration on November 26, 2015.

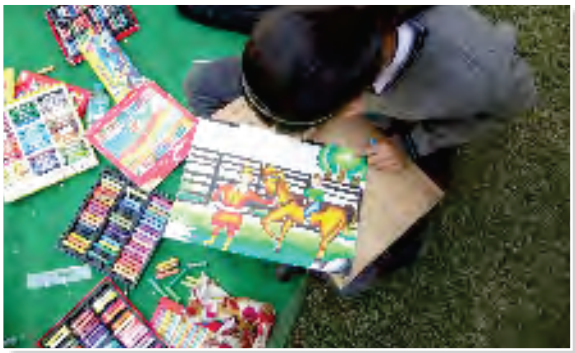
1st prize - Amisha Periwal

2nd prize- Lavany Mittal

3rd prize- Rakshit

Participants of drawing competition with NRCE staff

Students during drawing competition



Young mind working on canvas

Distribution of certificates to participants

ICAR-NRCE Foundation Day Celebration

30th Foundation Day of ICAR-National Research Centre on Equines was celebrated on November 26, 2014. Different activities like drawing competition for school children, Foundation day lecture, progressive equine owners meet and plantation of trees in the NRCE campus were organized to commemorate Foundation Day celebration. Maj Gen (Dr) Sri Kant Sharma, SM VSM, Vice Chancellor, LUVAS, Hisar graced the occasion as the Chief Guest during Foundation Day celebration programme at NRCE. Prof. (Dr) Gaya Prasad, ADG (AH), ICAR; Dr Dev Raj, Commandant, Equine Breeding Stud, Hisar and Dr Indarjeet Singh, Director, ICAR-CIRB, Hisar were present as Guests of Honour during the Foundation Day celebration. Dr B.N. Tripathi, Director, ICAR-NRCE welcomed the chief guest, guests of honour, equine owners, dignitaries and briefed about the research and extension activities of NRCE and the achievements made during the past 30 years. Chief Guest Maj Gen (Dr) Sri Kant Sharma highlighted the importance of equines in remote and hilly terrain and emphasized upon their utility in different activities like racing, equestrian events, transportation, pleasure riding, endurance racing, etc.

Director ICAR-NRCE addressing the gathering on Foundation Day

On this occasion, Chief Guest also released technical bulletin entitled "Exotic Equine Diseases". Progressive equine owners were also felicitated by the Chief Guest on this occasion. Prof. (Dr) Gaya Prasad, ADG (AH), ICAR delivered Foundation day lecture on "Scientific Revolution" on the occasion. The plantation was done at NRCE campus by Chief Guest, Guests of Honour and other dignitaries to commemorate ICAR-NRCE Foundation Day.

Distribution of prize to winner of drawing competition by Chief Guest

Release of technical bulletin on "Exotic Equine Diseases"

Plantation at NRCE campus by the Chief Guest

Foundation Day lecture by Prof. Gaya Prasad

Progressive equine owners meet organized on occasion of ICAR-NRCE Foundation Day

Progressive equine owners meet was organized on November 26, 2014 on the occasion of ICAR-NRCE foundation day in which progressive equine owners from Haryana, Rajasthan and Uttar Pradesh participated. During the progressive equine owners meet, scientists and equine owners interacted on different aspects of equine husbandry and management practices. The problems faced by the equine owners in the equine husbandry were discussed and scientists provided valuable information on feeding, hoof care, artificial insemination in equines, and preventive measures for different diseases to the equine owners. The progressive equine owners were also felicitated by the Chief Guest Maj Gen (Dr) Sri Kant Sharma, Vice Chancellor, LUVAS, Hisar.

Felicitation of progressive equine owners

हिन्दी सप्ताह का आयोजन

केन्द्र में 22-27 सितंबर 2014 तक हिन्दी सप्ताह का आयोजन किया गया। इस अवसर पर केन्द्र में हिन्दी के अधिकाधिक प्रयोग हेतु हिन्दी भाषा से संबधिंत विभिन्न प्रतियोगिताओं का आयोजन किया गया जिसमें केन्द्र के अधिकारियों, कर्मचारियों व केन्द्र सरकार की अन्य संस्थाओं के कर्मचारियों ने बढ़-चढ़ कर हिस्सा लिया। हिन्दी सप्ताह के दौरान निबंध लेखन, काव्य पाठ, हिन्दी शब्द अनुवाद, हिन्दी टिप्पणी एवं प्रारूप लेखन, हिन्दी प्रश्नोत्तरी तथा सुलेख प्रतियोगिता का आयोजन किया गया। दिनांक 22 सितंबर, 2014 को "इन्टरनेट के फायदे एवं नुकसान" विषय पर निबंध प्रतियोगिता आयोजित की गई। इस प्रतियोगिता में श्रीमती पार्वती शर्मा, अनुसंधान सहयोगी को प्रथम पुरस्कार प्राप्त हुआ। अनीता सपरा ने द्वितीय एवं गगनदीप सिंह ने तृतीय पुरस्कार प्राप्त किया। 23 सितंबर, 2014 को आयोजित हिन्दी शब्दानुवाद प्रतियोगिता में श्रीमती सविता सिंह को प्रथम पुरस्कार प्राप्त हुआ तथा कुमारी गीत व अनीता सपरा को क्रमशः द्वितीय एवं तृतीय पुरस्कार प्राप्त हुआ। 24 सितंबर, 2014 को आयोजित हिन्दी सुलेख प्रतियोगिता में डॉ० तरूणा आनन्द को प्रथम पुरस्कार प्राप्त हुआ तथा पार्वती शर्मा एवं श्री दीपक कुमार को क्रमशः द्वितीय एवं तृतीय पुरस्कार प्राप्त हुआ। 27 सितंबर, 2014 को आयोजित काव्य पाठ प्रतियोगिता में श्री नरेश शर्मा को प्रथम पुरस्कार प्राप्त हुआ तथा श्रीमती शम्मी त्यागी व श्री गुरजीत सिंह को क्रमशः द्वितीय एवं तृतीय पुरस्कार प्राप्त हुआ। 27 सितंबर 2014 को हिन्दी सप्ताह समापन एवं पुरस्कार वितरण समारोह आयोजित किया गया। इस कार्यक्रम में श्री हिम्मत सिंह, निदेशक, उत्तरी क्षेत्र कृषि मशीनरी प्रशिक्षण एवं परीक्षण संस्थान, हिसार मुख्य अतिथि के रूप में एवं श्री एम. पी. कुलश्रेष्ठ, निदेशक, एन.आई.सी., हिसार विशेष अतिथि के रूप में उपस्थित थे। मुख्य अतिथि ने अपने सम्बोधन में दैनिक कामकाज में हिन्दी के अधिकाधिक प्रयोग पर ज़ोर दिया। मुख्य अतिथि द्वारा विभिन्न प्रतियोगिताओं में विजयी प्रतिभागियों को पुरस्कार वितरण किया गया।

काव्य पाठ प्रतियोगिता का आयोजन

हिन्दी सप्ताह पुरस्कार वितरण समारोह

Vigilance Awareness Week at the Centre

Vigilance Awareness Week was observed at the Centre from October 27, 2014 to November 1, 2014. As part of Vigilance Awareness Week celebration, Director, NRCE administered pledge to the scientists and staff of NRCE on October 27, 2014 for honesty and transparency in public life. He also stressed on coordinated efforts for eliminating the menace of corruption from the society.

A Progressive Equine Owner bags Innovative Farmer Award

ICAR-NRCE nominated a progressive equine owner Sh. Sanjeev Beniwal, who received "ICAR-IARI Innovative Farmer Award 2015". Sh. Sanjeev Beniwal is a renowned progressive farmer and equine breeder from Peer Kamaria village in the Hanumangarh district of Rajasthan. He is actively involved in farming and animal husbandry since last thirty years. Sh. Sanjeev Beniwal is working for conservation of Marwari breed of horses. He started equine husbandry with three horses, which has now increased 22 horses due to his consistent effort in the last 8 years. He is associated with NRCE for consultancy and advisory services on equine health and management and is founder member and former secretary of 'Ashwa Palak Samiti, Hanumangarh' which has been organizing equine fair at Hanumagarh, Rajasthan, regularly since last six years for promotion and upliftment of equine husbandry in the region. He participates regularly in Kisan Mela and Animal Fairs to get acquaintance with latest technologies. His horses won prize money amounting 10 lakh rupees in different State and National livestock championships and equine fairs. Sh. Beniwal received the award from Hon'ble Minister of State for Agriculture, Government of India Dr Sanjeev Balyan, during "Pusa Krishi Vigyan Mela" at New Delhi on 12 March, 2015.

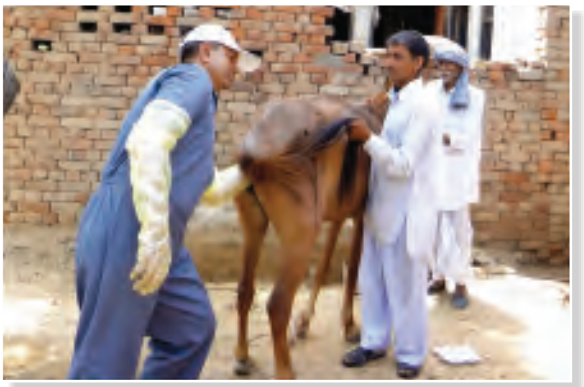
Sh. Sanjeev Beniwal receiving IARI Innovative Farmers Award

Equine Health Camp organized on World Veterinary Day

An Equine Health Camp and interactive meet of equine owners was organized by NRCE on April 26, 2014 on the occasion of World Veterinary Day at Rajli and Gurana village in Hisar. During the health camp equines were examined for various ailments by a multidisciplinary team of scientists and technical officers from NRCE in Rajli and Gurana villages. Deworming tablets and mineral mixture were provided to equine owners free of cost and treatment was given to sick and wounded equines. Pregnancy diagnosis was also done at farmer's door during the camp.

Equine owners interacted with NRCE scientists on various aspects of equine husbandry and management practices. During the interaction meeting, the equine owners were also made aware about the equine welfare, importance of rest during work periods, proper and timely shoeing and grooming of animals and adoption of improved equine husbandry and management practices.

Equine health camp at Rajli



Pregnancy diagnosis at farmers door at Gurana

National Science Day Celebration

National Science Day was celebrated at the Centre on February 28, 2014 and lectures were given on this occasion. Dr B.R. Gulati presented an overview on "Risk assessment and biosafety practices in research laboratories". Dr Rajender Kumar, National Fellow, ICAR-NRCE while delivering a lecture on "Open Access to Agricultural Knowledge for Inclusive Growth and Development" summarized about the availability of different Open Access resources to Agricultural Knowledge in different disciplines. The National Science Day programme was attended by NRCE Scientists, technical staff, FOCARS probationers at NRCE, research associates, SRF and young professionals from different laboratories in NRCE.

Dr Rajender Kumar delivering Lecture on Open Access Resources to Agricultural Knowledge

Swachh Bharat Abhiyan at ICAR-NRCE

Swachh Bharat Mission was initiated at the Centre on October 2, 2015 and all the Scientists/officers/staff members joined the campaign and cleaned their respective laboratory/office/working place. This activity was followed by Cleanliness Oath (Swachhata Shapath) by Dr B. N. Tripathi, Director, ICAR-NRCE and National Sanitation Campaign was inaugurated formally. All the Scientists/ officers/staff members took various activities and involved themselves in cleaning lawns, main entry gate area, approach road and building premises. Since then this activity is being carried out on regular basis. In the month of October, 2014, this campaign was taken up eight times and various areas of NRCE complex were cleaned. A human chain was also made on the occasion of January 26, 2015, so as to mark the commitment of the NRCE staff towards this activity. This campaign has been adopted as a routine and regular activity on each Saturday of the week for two hours (3:00 to 5:00 PM). All the NRCE-staff members participate in this activity and a roster of planned activities has been worked out and circulated among the staff members. There is a group leader for individual weekly activity, who plans and executes the campaign for that day. As such 13 weekly activities have been completed and different areas/laboratories/premises were cleaned.

Swachh Bharat Abhiyan at ICAR-NRCE

Swachh Bharat Abhiyan at ICAR-NRCE

Swachh Bharat Abhiyan at ICAR-NRCE

During equine health camps and kisan goshthis, equine owners are sensitised about "Swach Bharat Abhiyan" and importance of cleanliness in animal shed and day to day life. After the equine health camps ICAR-NRCE team, staff at veterinary hospital and equine owners collectively cleaned the veterinary hospital campus by collecting waste material from the area.

Organization of Equine Health Camps and Kisan Goshthis

A total of 15 equine health camps, awareness camps, interactive meet with equine owners and Kisan Goshthis were organized at different places. During camps, the animals were observed and treated for various ailments like colic, lameness, parasitic infections, body wounds and injuries by providing on the spot treatment. Deworming tablets and mineral mixture were provided free of cost to the equine owners. Pregnancy diagnosis was also done in mares. The interaction between scientists and equine owners during Kisan Goshthis and Interaction meet helped to understand the problems of equine owners. Information on health, production and management aspects of equine husbandry was given to the equine owners.

Health camps, Kisan Goshtis and Interactive meet of equine owners organized

S.No.	Place	Date
1.	Rajli, Haryana	April 27, 2014
2.	Degana, Nagore, Rajasthan	July 23, 2014
3.	Julana, Haryana	September 19, 2014
4.	Lwara and Sonprayag, Uttarakhand	September 25, 2014
5.	Jankichatti, Uttarkashi, Uttarakhand	September 28, 2014
6.	Ridmalsar, Bikaner, Rajasthan	September 28, 2014
7.	Maham, Haryana	November 07,2014
8.	Hanumangarh, Rajasthan	November 18, 2014
9.	Umra, Hisar, Haryana	January 20, 2015
10.	Nagore, Rajasthan	January 25-26, 2015
11.	Dhingsari, Bikaner	February 2, 2015
12.	Mukam, Nagore, Rajasthan	February 13, 2015
13.	Indri, Karnal, Haryana	March 25,2015
14.	Shamsukh, Hisar, Haryana	March 27,2015
15.	Tilwada, Badmer, Rajasthan	March 16-18, 2015

Equines Health Camp at Tilwada

Equines Health Camp at Sonprayag

Interactive meet with equine owners at Hanumangarh

Distribution of medicines to equine owners at Indri, Karnal

Participation in Exhibitions /Animal Fairs/ Kisan Mela

During April 2014 to March 2015, the Centre participated in various exhibitions, animal fairs and kisan mela at state and national levels displaying different technologies developed by the NRCE. During these exhibitions and animal fairs, information was provided to equine owners and visitors about activities and services provided by NRCE. Video film documentary of NRCE "Ashwa Gatha" was played during the exhibitions. Equine owners interacted with scientists on various aspects of equine husbandry. Extension literature on various aspects of equine husbandry and management were distributed to the equine owners.

S.No.	Activity	Date
1.	Kisan Goshthi on ICAR foundation day at NRCE, Hisar	July 23, 2014
2.	Exhibition at Loonkaransar, Bikaner	October 3, 2014
3.	ICAR Institute-SAU-Development and Stakeholder Interface at ICAR-NDRI, Karnal	October 18, 2014
4.	Livestock Expo, Mukatsar	January 8-11, 2015
5.	AgriExpo ICAR-NDRI, Karnal	February 3-6, 2015
6.	CIRB Buffalo Mela, Hisar	March 18, 2015
7.	Northern Zone Agricultural Fair at ICAR-IVRI, Izatnagar	March 17-20, 2015

Dignitaries at ICAR-NRCE exhibition stall at ICAR-CIRB

ICAR-NRCE exhibition stall at Mukatsar

Dignitaries at NRCE exhibition stall at ICAR-IVRI

Dignitaries at NRCE exhibition stall at ICAR-NDRI

Exposure Visit of Farmers/ Educational Tours of Students

During 2014-15, visitors from different places including farmers, students from SAU's, schools, visited ICAR-NRCE and Info-equine Museum at ATIC. Visitors were briefed about the research activities and different extension and field activities of NRCE for the benefit of equine owners and various on-going programmes of NRCE.

S.No.	Details of Visitors	Date	No. of Visitors
1.	Study tour of BVSc & AH, 5th Yr students from College of Veterinary Science & AH, Sardar Krishinagar, Gujarat	May 07, 2014	83 students & 2 faculty
2.	Exposure visit of farmers from Dhule, Maharashtra	September 16, 2014	4 farmers
3.	Study tour of BVSc & AH, 3rd Yr students from College of Veterinary Science & AH, GBPUA&T, Pantnagar	September 24, 2014	53 students & 2 faculty
4.	Study tour of BVSc & AH, 4th Yr students from College of Veterinary Science & AH, Parbhani, , Maharashtra	October 07, 2014	29 students & 2 faculty
5.	Exposure visit of farmers from Andhra Pradesh	November 02, 2014	15 farmers & 2 officials
6.	Study tour of students from Har Bilas Goyal Inter College Ujhani, Badaun, Uttar Pradesh	November 18, 2014	24 students & 2 faculty
7.	Exposure visit of school students from Hisar, Haryana	November 25, 2014	37 students & 11 faculty
8.	Exposure visit of Sheep breeders from Karnataka	December 04, 2014	25 farmers & 2 officials

NRCE in News

सांसद दुष्यंत चौटाला ने अश्व अनुसंधान केंद्र का किया दौरा

केंद्र का दौरा करने वाले पहले सांसद बने

अश्व अनुसंधान केंद्र का स्थापना दिवस मनाया

मेला परवान पर, सांस्कृतिक कार्यक्रम

तिलवाड़ा पशु मेले में अब तक हुई 6 हजार पशुधन की आवक, आज से शुरू होगी प्रतियोगिताएं

गांव की पशुधन का उपचार

मेले में विभिन्न रोगों से ग्रसित पशुओं का अस्पताल व चुक ट्रेडिया दिल्ली व राष्ट्रीय अश्व अनुसंधान केंद्र बीकानेर की ओर से पशुधन का उपचार किया जा रहा है। मेले में 35 घोड़ा, 65 उष्ट्रवंश तथा 400 घोड़ों का उपचार किया गया। वहीं विभाग के पशु चिकित्साधिकारी

अनुसंधान केंद्र बीकानेर द्वारा 55 मादा अश्वों की सोनोग्राफी के जरिए गर्भ परीक्षण किया गया। केंद्र के डॉ. रमेश ने बताया कि कोई भी अश्व पालक अखिल भारतीय टोल फ्री नंबर 1800 180 6225 पर जानकारी प्राप्त कर सकता है। चिकित्सक दल में डॉ. हेमंतकुमार,

गांव पीरकामड़िया में ब्रिटेन के वैज्ञानिकों ने जानी मारवाड़ी घोड़ों की खूबियां

ब्रिटेन के माइक्रो बॉयलोजी व राष्ट्रीय अश्व अनुसंधान केंद्र हिसार की टीम कर रही है रिसर्च

आज होगी अश्वों के विभिन्न रोगों की जांच

चित्रकला प्रतियोगिता में अमीशा ने बाजी मारी

हिसार के राष्ट्रीय अश्व अनुसंधान केंद्र ने चित्रकला प्रतियोगिता में आठ लेरो बच्चे।

भास्कर न्यूज | हिसार

राष्ट्रीय अश्व अनुसंधान केंद्र में मंगलवार को अश्व एक सहयोगी पशु विषय पर चित्रकला प्रतियोगिता करवाई गई। इसमें विभिन्न स्कूलों के बच्चों ने चित्रकला के माध्यम से अपनी प्रतिभा को दर्शाया। अनुसंधान केंद्र के स्थापना दिवस पर प्रतियोगिता में शहर के 11 स्कूलों के छात्र-छात्राओं ने हिस्सा लिया। इसमें छात्र-छात्राओं ने अश्व एक सहयोगी पशु की थीम पर चित्र तैयार किए। निर्णायक मंडल में ओपी जिंदल

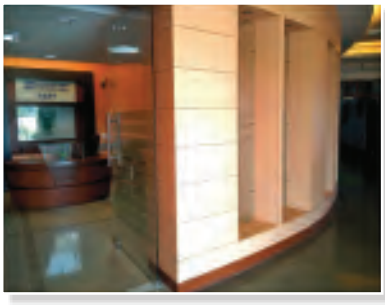
पेरीगल ग्रुप्स, दी आर्यन स्कूल के छात्र स्वाक्ष्य मित्तल को द्वितीय तथा लक्ष्मीपत भार्गव सीनियर सेकेंडरी मॉडल स्कूल के छात्र रक्षित को तीसरे स्थान पर चुना। चित्रकला प्रतियोगिता में सचदेव मान, भीम सिंह बेरवाल और बिजेंद्र सिंह ने निर्णायक मंडल के रूप में पुरस्कार प्राप्त छात्र-छात्राओं का चयन किया। केंद्र के निदेशक डॉ. वीएन त्रिपाठी सभी प्रतिभागियों को प्रमाण पत्र वितरित किए। आयोजन समिति के सचिव डॉ. एससी यादव ने प्रतिभागियों को उनकी सुंदर

देर रात तक गाव-विनोद दर्शक

मेले में सोमवार रात बालोतरा ट्रीटमेंट प्लांट ट्रस्ट और लोकसंस्कृति लोककला संस्थान के तत्वावधान में हुई सांस्कृतिक संध्या में कलाकारों ने मनमोहक प्रस्तुतियां दी। देर रात लोग कार्यक्रम में जमे रहे। मेला मैदान में बुधवार से पशु प्रतियोगिताएं शुरू होगी, जो शुक्रवार तक चलेंगी। शुक्रवार को विजेता पशुओं व पशुपालकों को पशुपालन विभाग की ओर से पुरस्कृत किया जाएगा। मेले में पशुपालन विभाग की ओर से अब तक 35 घोड़ों, 25 ऊंट व 400 घोड़ों का उपचार किया गया। राष्ट्रीय अश्व अनुसंधान केंद्र बीकानेर की ओर से 55 मादा अश्वों की सोनोग्राफी की गई। जांच कर उपचार किया गया।

Infrastructure and Developmental Activities

Infrastructure Development at VTCC



Reception area on ground floor at VTCC

Conference Hall of the VTCC building

Second phase of the VTCC building

The developmental works at Veterinary Type Culture Collection are underway. The construction of second phase of the building has been completed; however, to make it functional and initiate working in the new building, interior furnishing of the building is to be taken up shortly. The second phase of the building will further strengthen the laboratory facilities of VTCC. Furthermore, the lobby in the first phase building has been renovated as a reception area, while area on the first floor has been developed as a conference facility. The small animal house facility is also nearing completion which would further facilitate the mandated activities of the repository. The front lawn and adjoining areas in the premises are also being maintained

Agriculture Farm Production at ICAR-NRCE, Hisar

Production of crops

During the period under report, about 105 acre land was used under crop rotation scheme for cultivation of different types of crops. In spite of high water table and salinity in most of the farm area, vigorous efforts were made to produce maximum feed & fodder. A total of 1095 Qt. of green and 148 Qt. of dry fodder was supplied to animals at main campus during the year. 195.29 Qt. oat grain was produced in agriculture farm and 175.55 Qt. grain was supplied for feeding to animals at EPC, Bikaner and 19.74 Qt. grain used as seed for sowing of crop in agriculture farm at Hisar.

Table 1. Production of Green Fodder

Sr. No.	Crop	Area (Acre)	Production (Qt.)
1.	Oat+ Berseem	2	219.00
2.	Berseem		74.00
3.	Sorghum sudan grass + Cowpea	8.5	159.00
4.	Sorghum sudan grass		457.50
5.	Lucern	1.5	93.50
6.	Cowpea	3.5	48.00
7.	Oat	1.5	44.00

Reclamation and development of field: About thirty five acre land near pond was weeded out and developed for future planning of crop cultivation.

Revenue generation: A sum of ₹ 6,38,513/- (Six lakh thirty eight thousand five hundred thirteen only) was generated through sale of 203.26 Qt. of mustard.

Agriculture Farm Production at EPC Bikaner

During the period under report a total of 2282.95 Qt. of green fodder (Lucerne 554.10 Qt. + oat 816.21 Qt. + Millet 301.60 Qt. + sorghum sudan grass 617.04 Qt.) and 130.00 Qt. dry fodder and 38 Qt. grain of oats and barley was produced at the agriculture farm of EPC Bikaner for the animals. EPC has also been maintaining a lawn in 4 acres and 1000 plantations. Besides, 5 acres land has also been reclaimed.

Agricultural farm at ICAR-NRCE, Hisar

Agricultural farm at EPC, Bikaner

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

The Centre has a nucleus herd of Marwari horses along with Zanskari and Manipuri ponies and exotic donkeys at Hisar and Bikaner campuses. The stallions at Bikaner campus are primarily used for collection and cryopreservation of semen for artificial insemination. Besides, frozen semen is used for propagation of indigenous germplasm and superior mule production.

Livestock strength at NRCE, Hisar & EPC, Bikaner

Equine Herd Strength at Equine Production Campus, Bikaner (2014-15)

Category	Marwari Horse		Pony						Donkey				Mule		Total
			Zanskari		Manipuri		Indigenous		Poitou		Indigenous				
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Stock as on 01.04.2014	18	36	06	06	04	09	--	02	11	18	10	07	03	02	132
Purchased during the year	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Births during the year	06	08	02	01	03	--	--	--	03	01	01	02	--	--	27
Deaths during the year	02	01	01	--	01	02	--	--	01	01	--	--	--	--	09
Auction/sold during the year	04	13	01	--	--	--	--	02	03	07	03	01	01	01	36
Balance as on 31.03.2015	18	30	06	07	06	07	--	--	10	11	08	08	02	01	114

Herd Strength at NRCE Main Campus, Hisar

The present herd strength of NRCE main campus is 27 animals, which include 20 Marwari horses, 2 ponies, 4 Poitu exotic donkey and 1 mule.

Cryopreserved Semen Bank at EPC Bikaner

On farm semen cryopreservation at EPC

During the period 2014 - 2015, a total of 315 frozen semen doses of equines including Marwari, Manipuri, Zanskari horses and indigenous donkeys were cryopreserved at NRCE, Bikaner. Semen from Manipuri horses were cryopreserved at the Centre for the first time.

Semen cryopreservation in field

During the period of 2014 -2015, a total of forty five (45) semen doses were cryopreserved from five elite stallions in field at Peerkamria, Hanumangarh (Rajasthan).

Temporary laboratory set up under field conditions at Peerkamria (Hanumangarh)

Semen collection from an elite stallion at Peerkamria (Hanumangarh)

On-Going
Research Projects (2014-15)

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, Praveen Malik, Sanjay Kumar, Nitin Virmani, Sanjay Barua, R.K. Vaid, Anju Manuja, H. Singha, Balvinder Kumar and Ramesh Dedar	April, 1985	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	Oct., 2016	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	Aug., 2016	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	Sept., 2016	IXX10287
5.	Development of diagnostics for emergency preparedness and monitoring of emerging equine viral diseases	Baldev Kumar*, H.S. Singha, Anju Manuja and Praveen Malik	April, 2014	March, 2017	IXX10853

*Principal Investigator

VTCC

S.No.	Title	Team	From	To	PIMS Code
1.	Isolation, maintenance and characterization of bacterial pathogens and their molecular identification	Rajesh Kumar Vaid*, Sanjay Barua, B.C. Bera, Taruna Anand and Riyesh T.	June, 2007	March, 2015	IXX00269
2.	Isolation, molecular characterization and reposition of viruses of animal origin	Sanjay Barua*, B.C. Bera, R.K. Vaid, B.R. Gulati, RiyeshT. and Taruna Anad	Sept., 2009	March, 2015	IXX00270
3.	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	Dec., 2015	IXX07760
4.	Development of bacteriophage repository	Taruna Anand*, R.K. Vaid, Sanjay Barua and B.C. Bera	Oct., 2013	Nov., 2016	IXX10698

Extension

S.No.	Title	Team	From	To	PIMS Code
1.	A study on existing management systems and utilization of donkeys and mules for sustainable livelihood	A.A. Raut*, Yash Pal, R.A. Legha and R.K. Dedar	Sept., 2009	March, 2015	IXX00268

*Principal Investigator

Equine Production

S.No.	Title	Team	From	To	PIMS Code
1.	Cloning, expression and characterization of equine chorionic gonadotropin (eCG)	Anuradha Bhardwaj*, A.K. Gupta and Sanjay Kumar	Dec., 2010	March, 2015	IXX02769
2.	Characterization of donkey milk with emphasis on important milk proteins	Yash Pal*, Sanjay Kumar, R.A. Legha, A. Bhardwaj and A.K. Mohanty	Oct., 2012	Sept., 2015	IXX07761
3.	Effect of feeding various combinations of dry roughages available in arid region of Rajasthan on growth and nutrient utilization in growing horses	R.A. Legha*, P.A. Bala and N.V. Patil	June, 2012	March, 2015	IXX07762
4.	Therapeutic and performance enhancing capacity of antioxidants in equines	R.K. Dedar*, Vijay Kumar and A.P. Singh	July, 2012	March, 2015	IXX09641
5.	Endocrine, biochemical and gene expression profiling of reproductive states in Marwari Mares	Vijay Kumar*, Sanjay K. Ravi, R.K. Dedar and Raghvendra Singh	Oct., 2012	March, 2015	IXX09663
6.	Evaluation of total mixed rations for maintenance horses	P.A. Bala*, R.K. Dedar and N.V. Patil	March, 2014	Feb., 2017	IXX11646
7.	Development of rapid diagnostic test for pregnancy diagnosis in horse mares	A.K. Gupta*, Yash Pal, Sanjay Kumar and Sanjay K. Ravi	Jan., 2015	June, 2017	IXX11645

*Principal Investigator

Externally funded projects

S.No.	Title	Team	From	To	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*	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 (upto Jan. 4, 2015) and Vijay Kumar (from Jan. 5, 2015)	July, 2012	March, 2017	IXX00486

S.No.	Title	Team	From	To	PIMS Code
3.	Development of biomarker(s) for diagnosis of *Trypanosoma evansi* infection animals using proteomic approach	Prof. Utpal Tatu*, S.C. Yadav, Rajender Kumar and B.C. Bera	June, 2011	Dec., 2014	OXX01616
4.	Synthesis, characterization and evaluation of drug loaded nano-formulation against *Trypanosoma evansi* in animal model(DST)	Anju Manuja*, Neeraja Dilbahgi, Sandeep Kumar, Rajender Kumar, Balvinder Kumar and S.C. Yadav	March, 2012	Sept., 2015	OXX01526
5.	Eukaryotic expression of important equine cytokines and analysis of their biological activities	H. S. Singha*	Jan., 2013	Dec., 2015	OXX02228
6.	Characterization of donkeys of Rajasthan Network Project from NBAGR, Karnal	Yash Pal*, A.K. Gupta and R.K. Dedar	April, 2014	Oct., 2015	OXX02851
7.	DBT-NER Centre for Advanced Animal Diagnostics and Services on Animal Health and Diseases (ADSAHD)	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	OXX02933
8.	OIE Twining program for Glander	Praveen Malik*, H.S. Singha and B.N. Tripathi	July, 2012	June, 2015	OXX02428
9.	OIE Twining program for Equine Influenza	Nitin Virmani*, R.K. Vaid, B.C. Bera and B.N. Tripathi	Oct., 2012	Sept., 2015	OXX02429
10.	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	March, 2014	March, 2016	OXX02873
11.	All India Network Programme on Neonatal Mortality in Farm Animal	Sanjay Kumar*, Ramesh Dedar, B.R. Gulati, Nitin Virmani and S.K. Khurana	Jan., 2015	March, 2017	OXX03934

Research

Publications

Research Articles

1. Anand, T. Bera, B.C, Vaid, R.K., Shanmugasundaram, K., Sharma, G., Virmani, N., Shukla, B.N., Barua, S., Malik, P. and Singh, R.K. 2014. Molecular characterization of virulence-associated protein (Vap) family genes of pathogenic *Rhodococcus equi* isolates from clinical cases of Indian equines. *Indian Journal of Biotechnology*. 13:195-202.
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3. Arangasamy, A., Talluri, T.R., Ravi, S.K., Singh, R.K. and Pal, Y. 2013. Freezing of indigenous stallion and poitou jack semen with amide as cryoprotectants. *Veterinary Practitioner*, 14(2) Supple. 1:469-470.
4. Arangasamy, A., Talluri, T.R., Singh, R.K., Pal, Y. and Tandon, S.N. 2015. Unilateral cryptorchidism in a marwari stallion. *Veterinary Practioner* (Accepted).
5. Bala, P.A., Kundu, S.S., Dutt, C., Rani, R.R., and Prusty, S. 2014. Comparative digestibility of forages by cattle and horses. *Veterinary Practitioner*, 15:2.
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12. Khurana, S.K., Singha, H., Malik, P., Gulati, B.R. and Singh, R.K. 2015. Molecular characterization of clinical isolates of *Rhodococcus equi* with PCR assay based on virulence plasmid marker. *Indian Journal of Animal Science,* (Accepted).
13. Kumar, J., Chaudhury, A., Bera, B.C., Kumar, R., Kumar R. and Tatu, U. and Yadav, S.C. 2015. Production and preliminary evaluation of *Trypanosoma evansi* HSP70 for antibody detection in equids. *Acta Parasitologica,* (Accepted).
14. Kumar, P., Kumar, R., Kumar, B., Singha, H., Sharma, A., Virmani, N., Yadav, S. C. and Manuja, A. 2014. CpG-ODN class C mediated immunostimulation in rabbits experimentally infected with horse isolate of *Trypanosoma evansi.* PLoS one. (Accepted).
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2. Barua, S. and Riyesh, T. Preservation and characterization of animal viruses. in ICAR Short Course "Frontier approaches in diagnosis and control of viral diseases with quality assurance and quality control of veterinary biologicals" at IVRI, Izatnagar w.e.f. 10-19 November 2014.

Abstracts

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11. Gulati, B.R., Sharma, H., Kapoor, S., Riyesh, T. and Virmani, N. Trends and diagnosis and control of equine herpesvirus infections. XXIII National Conference on "Recent Trends in Virology Research in the Omics Era"- VIROCON 2014 organized by Tamil Nadu Agricultural University, Coimbatore from December 18-20, 2014. Pp 199.
12. Kumar, B., Riyesh, T. and Manuja, A. Cell based toxicity investigations of nanoformulations intended for animal use. Proc. International Conference on Nano Science & Engineering Applications. JNTU, Hyderabad. Pp 64.
13. Kumar, D., Talluri, T.R. and Kues, W.A. Non-viral reprogramming of mCherry expressing porcine fibroblasts into induced pluripotent stem cells by piggyBac transposons. 41st Annual Conference of the International Embryo Transfer Society (IETS), Versailles, France from January 10-13, 2015.
14. Kumar, V, Dedar, R.K., Bala, P.A., Singh, J. and Legha, R.A. Exercise Performances of Marwari mares in a ten kilometer trail ride during peak winter in subtropical desert climate. The 2nd International Conference on Bio-resource and Stress Management held at PJTSAU, Rajendranagar, Hyderabad, India from January 7-10, 2015. Pp 157.
15. Manuja, A., Kumar, B., Chopra, M., Kumar, S. and Dilbaghi, N. Biodistribution of trypanocidal drug-loaded sodium alginate nanoformulation in various organs of mice. Proceeding of International Conference on Nano Science & Engineering Applications. JNTU, Hyderabad. Pp54.
16. Manuja, A., Kumar, B., Singha, H. and Singh, S. Molecular Characterization of Toll like receptor 9 in Marwari & Zanskari breeds of horses, Poitu and indigenous donkeys. Proc. International Symposium on Livestock Diseases Affecting Livelihood Options and Global Trade-Strategies and Solutions. Pp 92-93.
17. Manuja, B., Manuja, A., Singh, S., Dahiya, R., Sharma, R.C. and Gahlot, S.K. Diversity of Mx gene in horses and its association with susceptibility *vis-a-vis* resistance against Equine Influenza. Proc. International Symposium on Livestock Diseases Affecting Livelihood Options and Global Trade-Strategies and Solutions. Pp 92.
18. Riyesh, T., Dhar, P., Barua, S., Jindal, N., Bera, B.C., Upmanyu, V., Anand, T., Vaid, R.K., Yadav, M., Anagha, G., Tiwari, A.K., Malik, P., and

Pandey, A.B. Evidence of natural recombination in the non-structural protein of classical swine fever virus from India. XXIII National Conference on "Recent Trends in Virology Research in the Omics Era"- VIROCON 2014 organized by Tamil Nadu Agricultural University, Coimbatore from December 18-20, 2014. Pp 243.

19. Saini, S., Singha, H. and Malik, P. Cloning and expression of Marwari horse Interferon gamma. National Symposium and XXVIII Annual Convention of IAVMI on 'Challenges and opportunities in Animal health at the face of globalization and climate change' held from 30th October- 1st November, 2014 at DUVASU, Mathura. Pp 181.
20. Singha, H., Malik, P., Goyal, S.K., Elschner, M. and Neubauer, H. Evaluation of recombinant proteins for surveillance of equine glanders. National Congress on Veterinary Public Health & National Symposium on 'Food security and Public Health: Present status and future road map' Organized by Association of Public Health Veterinarians (APHV) at NASC, New Delhi from 24-25th November, 2014. Pp232.
21. Talluri, T.R., Kumar, D., Glage, S., Garrels, W., Debowski, K., Behr, R., Niemann, H. and Kues, W.A. Derivation of bovine induced pluripotent stem cells by a transposon approach. World Congress of Reproductive Biology, Edinburgh, September 2-4, 2014.
22. Talluri, T.R., Kumar, D., Glage, S., Garrels, W., Niemann, H., Debowski, K., Behr, R. and Kues, W.A. Derivation of bovine induced pluripotent stem cells by piggyBac mediated reprogramming. 41st Annual Conference of the International Embryo Transfer Society (IETS), Versailles, France from January 10-13, 2015.
23. Talluri, T.R., Kumar, D., Niemann, H., Glage, S., Garrels, W., Debowski, K., Behr, R. and Kues, W.A. PiggyBac Transposon-mediated derivation of bovine iPS cells. 5th International Congress on stem cells and tissue formation at Dresden, Germany, from 8-11 July, 2014.

GenBank publications

1. Anand, T., Bera, B.C., Vaid, R.K., Barua, S., Riyesh, T., Kundu, S. And Malik, P. Aeromonas phage VTCCBP2 partial g23 gene for major capsid gp23 protein. KM111298.
2. Anand, T., Bera, B.C., Vaid, R.K., Barua, S., Riyesh, T., Kundu, S. And Malik, P. Bacillus phage VTCCBP3 partial g23 gene for major capsid gp23 protein. KM111299.
3. Anand,T., Bera,B.C., Vaid,R.K., Barua,S., Riyesh, T., Kundu,S. And Malik,P. Enterobacteria phage VTCCBP1 partial g23 gene for major capsid gp23 protein. KM111297.
4. Bera, B.C., Barua, S., Shanmugasundaram, K., Anand, T., Riyesh, T.,Vaid, R.K., Virmani, N., Bansal, M., Kundu, S., Shukla, B.N., Malik, P. and Singh, R.K. Camelpox virus isolate CMLV-DELHI09 M-T4-like virulence protein (M-T4) gene, complete cds. KM234479.
5. Bera, B.C., Barua, S., Shanmugasundaram, K., Anand, T., Riyesh, T., Vaid, R.K., Virmani, N., Bansal, M., Kundu, S., Shukla, B.N., Malik, P. and Singh, R.K. Camelpox virus isolate CMLV-BARMER09 M-T4-like virulence protein (M-T4) gene, complete cds. KM234480.
6. Bera, B.C., Barua, S., Shanmugasundaram, K., Anand, T., Riyesh, T., Vaid, R.K., Virmani, N., Bansal, M., Kundu, S., Shukla, B.N., Malik, P. and Singh, R.K. Camelpox virus isolate CMLV-BIKANER09 M-T4-like virulence protein (M-T4) gene, complete cds. KM234481.
7. Bera, B.C., Barua, S., Shanmugasundaram, K., Anand, T., Riyesh, T., Vaid, R.K., Virmani, N., Bansal, M., Kundu, S., Shukla, B.N., Malik, P. and Singh, R.K. Camelpox virus isolate CMLV-JAISALMER09 M-T4-like virulence protein (M-T4) gene, complete cds. KM234482.
8. Bera, B.C., Barua, S., Shanmugasundaram, K., Riyesh, T., Anand, T., Vaid, R.K., Virmani, N., Bansal, M., Shukla, B.N., Malik, P. and Singh, R.K. Camelpox virus isolate CMLV-DELHI09 Schlafen-like protein (v-slfn) gene, complete cds. KM234483.
9. Bera, B.C., Barua, S., Shanmugasundaram, K., Riyesh, T., Anand, T., Vaid, R.K., Virmani, N., Bansal, M., Shukla, B.N., Malik, P. and Singh, R.K. Camelpox virus isolate CMLV-BARMER09 Schlafen-like protein (v-slfn) gene, complete

cds. KM234484.

10. Bera, B.C., Barua, S., Shanmugasundaram, K., Riyesh, T., Anand, T., Vaid, R.K., Virmani, N., Bansal, M., Shukla, B.N., Malik, P. and Singh, R.K. Camelpox virus isolate CMLV-BIKANER09 Schlafen-like protein(v-slfn) gene, complete cds. KM234485.
11. Bera, B.C., Barua, S., Shanmugasundaram, K., Riyesh, T., Anand, T., Vaid, R.K., Virmani, N., Bansal, M., Shukla, B.N., Malik, P. and Singh, R.K. Camelpox virus isolate CMLV-JAISALMER09 Schlafen-like protein(v-slfn) gene, complete cds. KM234486
12. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Hisar_7_90 membrane protein gene, partial cds, 829 bp linear DNA KM285392.1.
13. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Hisar_2_14 membrane protein gene, partial cds, 829 bp linear DNA KM285391.1.
14. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Tohana_2_13 membrane protein gene, partial cds, 829 bp linear DNA, KM285390.1.
15. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Delhi_07 membrane protein gene, partial cds, 829 bp linear DNA KM285389.1.
16. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Delhi_98 membrane protein gene, partial cds, 829 bp linear DNA KM285388.1.
17. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Rajasthan_98 membrane protein gene, partial cds, 829 bp linear DNA, KM285387.1.
18. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Jind_96 membrane protein gene, partial cds, 829 bp linear DNA, KM285386.1.
19. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Gurgaon_11 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA, KM285385.1.
20. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Hisar_14 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA, KM285384.1.
21. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Ambala_10 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA, KM285383.1.
22. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Delhi_08 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA. KM285380.1.
23. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Hisar_10-1 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA, KM285381.1.
24. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Hisar_10-2 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA,KM285382.1.
25. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Hisar_2_14 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA, KM285377.1.
26. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Hisar_07 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA, KM285379.1.
27. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Tohana_07 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA, KM285378.1.
28. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Tohana_2_13 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA, KM285376.1.
29. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Rajasthan_98 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA, KM285374.1.
30. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Delhi_07 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA. KM285375.1.
31. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Delhi_98 DNA

polymerase catalytic subunit gene, partial cds, 654 bp linear DNA. KM285373.1.

32. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Jind_96 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA .KM285372.1.
33. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Tohana_1_96 DNA polymerase catalytic subunit gene, partial cds, 654 bp linear DNA. KM285371.1.
34. Gulati, B.R., Anagha, G., Riyesh, T. and Virmani, N. Equid herpesvirus 1 isolate Hisar_7_90 DNA polymerase catalytic subunit gene, partial cds. 654 bp linear DNA. KM285370.1.
35. Jaideep, Bera, B.C., Chaudhary, A., Kumar, R., Rochani, A., Tatu, U and Yadav, S.C. Sequence analysis of variant surface glycoprotein (VSG) gene of *Trypanosoma evansi*. KJ13572.
36. Riyesh, T., Barua, S., Bera, B.C., Jindal, N., Arora, D., Mahajan, N.K., Anand, T., Yadav, M., Poonia, P., Rinwa, S., Vaid, R.K., Malik, P. and Singh, R.K. Swinepox virus strain VTCC/AVA/121 putative extracellular enveloped glycoprotein (SPV119) gene, complete cds 558 bp linear DNA. KJ725379.1
37. Riyesh, T., Barua, S., Bera, B.C., Jindal, N., Arora, D., Mahajan, N.K., Anand, T., Yadav, M., Vaid, R.K., Malik, P. and Singh, R.K. Swinepox virus strain VTCC/AVA/121 putative extracellular enveloped glycoprotein (SPV120) gene, complete cds 513bp linear DNA. KR028365.
38. Riyesh, T., Barua, S., Bera, B.C., Jindal, N., Arora, D., Mahajan, N.K., Anand, T., Yadav, M., Vaid, R.K., Malik, P. and Singh, R.K. Swinepox virus strain VTCC/AVA/121 ankyrin repeat protein (SPV144) gene, complete cds 1482bp linear DNA. KR028367.
39. Riyesh, T., Barua, S., Bera, B.C., Jindal, N., Arora, D., Mahajan, N.K., Anand, T., Yadav, M., Vaid, R.K., Malik, P. and Singh, R.K. Swinepox virus strain VTCC/AVA/121 A52R family-like protien (SPV133) gene, complete cds 540bp linear DNA. KR028369.
40. Riyesh, T., Barua, S., Bera, B.C., Jindal, N., Arora, D., Mahajan, N.K., Anand, T., Yadav, M., Vaid, R.K., Malik, P. and Singh, R.K. Swinepox virus strain VTCC/AVA/121 Kelch-like protein (SPV006) gene, complete cds 1590bp linear DNA. KR028370.
41. Riyesh, T., Barua, S., Bera, B.C., Jindal, N., Arora, D., Mahajan, N.K., Anand, T., Yadav, M., Anagha, G., Chandel, S.S, Vaid, R.K., Malik, P. and Singh, R.K. Classical swine fever virus, CSFV-03_2013. NS5B gene, partial cds 449bp linear DNA. KR028371.
42. Riyesh, T., Barua, S., Bera, B.C., Jindal, N., Arora, D., Mahajan, N.K., Anand, T., Yadav, M., Anagha, G., Chandel, S.S, Vaid, R.K., Malik, P. and Singh, R.K. Classical swine fever virus, CSFV-06_2013. NS5B gene, partial cds 449bp linear DNA. KR028372.
43. Riyesh, T., Barua, S., Bera, B.C., Jindal, N., Arora, D., Mahajan, N.K., Anand, T., Yadav, M., Anagha, G., Chandel S.S, Vaid,R.K., Malik,P. and Singh,R.K. Classical swine fever virus, CSFV-08_2013. NS5B gene, partial cds 449bp linear DNA. KR028373
44. Riyesh, T., Barua, S., Bera, B.C., Jindal, N., Arora, D., Mahajan, N.K., Anand, T., Yadav, M., Anagha, G., Chandel, S.S, Vaid, R.K., Malik, P. and Singh, R.K. Classical swine fever virus, CSFV-34_2013. NS5B gene, partial cds 449bp linear DNA. KR028374.
45. Riyesh,T., Barua,S., Bera,B.C., Jindal,N., Arora,D., Mahajan,N.K., Anand,T., Yadav,M., Poonia,P., Rinwa,S., Vaid,R.K., Malik,P. and Singh,R.K. Swinepox virus strain VTCC/AVA/121 ankyrin repeat protein (SPV143) gene, complete cds 1,293 bp linear DNA KJ725378.1 GI:657709838
46. Riyesh,T., Barua,S., Bera,B.C., Jindal,N., Arora,D., Mahajan,N.K., Anand,T., Yadav,M., Vaid,R.K., Malik,P. and Singh,R.K. Swinepox virus strain VTCC/AVA/121 Kelch-like protein (SPV136) gene, complete cds 1725bp linear DNA. KR028366.

Participation in Training, Workshop, Conferences and Symposia

International trainings and visits abroad:

1. Dr. H.S. Singha attended capacity building training program at OIE referral lab on glanders, Germany under OIE Twinning laboratories project from June16 - 28, 2014.
2. Dr. Thirumala Rao Talluri attended 5th International Congress on Stem Cells and Tissue Formation, from July 8-11, 2014 at Dresden, Germany.
3. Dr. Thirumala Rao Talluri attended World Congress of Reproductive Biology, from September 2-4, 2014 at Edinburgh, United Kingdom.
4. Dr. Thirumala Rao Talluri attended the 41st Annual Conference of the International Embryo Transfer Society (IETS) at Versailles, France, from January 10-13, 2015.

Participation in Trainings:

1. Dr. B.R. Gulati completed "Management Development Programme on Leadership Development (a pre-RMP Programme)" at National Academy of Agricultural Research Management, Hyderabad, from July 15 – 26, 2014.
2. Dr. Ajay Raut participated in ICAR sponsored winter school on "Livestock based Integrated Farming Systems for Enhancing Resource use Efficiency and Improving Livelihood of Small and Marginal Farmers" organized at ICAR- Indian Grassland & Fodder Research Institute, Jhansi (UP) from November 20–December 10, 2014.
3. Dr. Taruna Anand attended 21 days training in Capacity building for safe exchange of bovine germplasm conforming to SPS standards organised by Department of Veterinary Microbiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, from February 3 - 23, 2015.

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

1. Dr. A. K. Gupta attended interactive conference of Vice Chancellors and Directors of ICAR institutes at NASC, New Delhi on April 28, 2014.
2. Dr. A. K. Gupta attended meeting of NABMGR under the chairmanship of Dr. R.S. Paroda at NBPGR, New Delhi on June 16, 2014.
3. Dr. A.K. Gupta attended Vice-Chancellor's and Director's conference at NASC complex, New Delhi on July 29-30, 2014.
4. Dr. A.K. Gupta attended XXVI meeting of the regional Committee No. VI at CSSRI, Karnal under the Chairmanship of DDG (AS) on April 23, 2014.
5. Dr. B.C. Bera attended 2nd International conference on "Animal and Dairy Sciences" held at Hyderabad organized by OMICS group, from September 15-17, 2014.
6. Dr. B.C. Bera participated in workshop on "Culture of Responsibility, Pathogen Inventory Management and Safety is the Rule: Fundamentals of working with Biosafety Cabinets" held at ICAR- National Institute of High Security Animal Diseases (NIHSAD), Bhopal during March 13-14, 2015.
7. Dr. Balvinder Manuja participated in International Conference on Nano Science & Engineering Applications, held at JNTU, Hyderabad during June 26-28, 2014.
8. Dr. Balvinder Manuja participated in International Symposium on "Livestock Diseases Affecting Livelihood Options and Global Trade-Strategies and Solutions" organized by ISVIB at TANUVAS, Chennai, from July 17-19, 2014.
9. Dr. Balvinder Manuja participated in Review meeting of DST project on "Synthesis, characterization and evaluation of drug-loaded

nano-formulations against *Trypanosoma evansi* in animal model" at Thanjavur from January 31-February 2, 2015.

10. Dr. B.R. Gulati participated and presented an invited paper entitled "Diagnosis, Control and prevention of zoonotics of Japanese Encephalitis' 4th Annual Conference on Scientific Awareness on Zoonosis Disease Control organised by Heart Care Foundation of India, New Delhi, on July 6, 2014.
11. Dr. B.R. Gulati participated in XXIII Annual Conference of Society of Animal Physiologists of India (SAPI) & National Symposium on Physiological Determinants of Climate Resilient and Sustainable Animal Production, organized by ICAR-Central Institute for Research on Buffaloes, on November 27-28, 2014.
12. Dr. B.R. Gulati participated in XXIII National Conference on "Recent Trends in Virology Research in the Omics Era", organized by Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, from December 18-20, 2014.
13. Dr. H.S. Singha attended National congress on veterinary public health & National symposium on 'Food security and Public Health: Present status and future road map' Organized by Association of Public Health Veterinarians (APHV) at NASC, New Delhi from November 24-25, 2014.
14. Dr. H. S. Singha attended XXVIII Annual convention of Indian association of veterinary microbiologist immunologists and specialists in infectious diseases (IAVMI) & International conference on 'Challenges and opportunities in Animal health at the face of globalization and climate change' held from October, 30 - November 1, 2014 at DUVASU, Mathura.
15. Dr. H.S. Singha and Dr. B.C. Bera participated in exhibition in 12th Agricultural Science Congress & Expo at ICAR-NDRI, Karnal and from February 3-6, 2015.
16. Dr. Nitin Virmani attended an International workshop addressing Production Animal Health and Welfare Research organized jointly by Indian Council of Agricultural Research in association with The University of Edinburgh's Royal (Dick) School of Veterinary Studies and Roslin Institute at NAS complex, ICAR, New Delhi on February 16-17, 2015.
17. Dr. Nitin Virmani attended XXIII National Conference on "Recent Trends in Virology Research in the Omics Era"- VIROCON 2014 organized by Tamil Nadu Agricultural University, Coimbatore on December 18-20, 2014.
18. Dr. R. A. Legha attended XXIII Annual National Conference of Society of Animal Physiologists of India and National Symposium on "Physiological Determinants of Climate Resilient and Sustainable Animal Production" held at ICAR-CIRB, Hisar from November 27-28, 2014.
19. Dr. R. A. Legha participated in Farm innovators day organized at Ridmalsar, Bikaner on Sept. 9, 2014.
20. Dr. R. K. Vaid attended 2nd International conference on "Animal and Dairy Sciences" held at Hyderabad organized by OMICS group, from September 15-17, 2014.
21. Dr. R.A. Legha attended 14th Biennial Workshop of AICRP on UAE Held at MPUA&T, Udaipur (Rajasthan) from February, 18-20, 2015.
22. Dr. R.K. Dedar & Dr. P.A. Bala attended Workshop on Disaster Management in Animals - entitled "Role of Equines in Disaster Management of Animals" on February 20, 2015.
23. Dr. Rajender Kumar participated in training programme on "Laboratory Quality System & Internal Audit" as per IS/ISO/IEC/17025 organised by National Institute of Training for Standardization, Noida (UP) from August 11-14, 2014.
24. Dr. Rajender Kumar participated in Workshop on "Open Access to Agricultural Knowledge for Inclusive Growth and Development" organised by NAARM, Hyderabad, from October 29-30, 2014.
25. Dr. S. C. Yadav attended annual task force meeting for discussion on inter institutional

project entitled "Development of biomarker(s) for diagnosis of *Trypanosoma evansi* infection in animals using proteomic approach" on July 30, 2014 at DBT New Delhi.

26. Dr. S. C. Yadav attended annual task force meeting for discussion on inter institutional project entitled "Development of Nano gold based immunochromatography/ immunodot blot assay for detection of *Trypanosoma evansi* infection in animals" at Punjab University Chandigarh.
27. Dr. S. C. Yadav attended meeting of IAEC on March 27, 2015 at LUVAS Hisar, Haryana.
28. Dr. S. C. Yadav attended meeting of IAEC on March 30, 2015 at MDU Rohtak, Haryana.
29. Dr. S. C. Yadav was invited as resource person and delivered lecture on topic "Common helminths of Yaks and its control" on June 18, 2014 at NRC on Yak Dirang, Arunachal Pradesh.
30. Dr. S. K. Ravi participated in Farm innovators day organized at Ridmalsar, Bikaner on Sept. 9, 2014.
31. Dr. S.K. Ravi attended one day workshop on "Disaster Management in Animals–A Renewed Approach and Future Vision" at Centre for Disaster Management Technology for Animals Rajasthan University of Veterinary & Animal Sciences, Bikaner on February 20, 2015.
32. Dr. S.K. Ravi participated in XXX Annual Convention of The Indian Society for Study of Animal Reproduction (ISSAR) & National symposium on Research and Innovations to Improve Animal Fertility and Fecundity held at Department of Obstetrics & Gynaecology, College of Veterinary Science and Animal Husbandry, UP Pt. Deen Dyal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura-281001, India from November 20-22, 2014.
33. Dr. Sanjay Barua attended the Annual Review Meet of the VTCC Network project held at College of Veterinary Science, AAU, Khanapara, Guwahati on October 13, 2014.
34. Dr. Sanjay Barua attended the DBT NER project partners meeting cum workshop held at AAU, Khanapara, Guwahati from February 25 -27, 2015.
35. Dr. Sanjay Barua attended the ICAR Short Course "Frontier approaches in diagnosis and control of Animal viral diseases with quality assurance and quality control of veterinary biologicals" held at Division of Biological Standardization, IVRI Izatnagar during November 10-19, 2014.
36. Dr. Sanjay Kumar attended one day workshop of HRD nodal officers of ICAR at NAARM, Hyderabad on February 26, 2015.
37. Dr. Taruna Anand attended 2nd International conference on "Animal and Dairy Sciences" held at Hyderabad organized by OMICS group, from September 15-17, 2014.
38. Dr. Taruna Anand attended XXIII Annual National Conference of Society of Animal Physiologists of India and National Symposium on "Physiological Determinants of Climate Resilient and Sustainable Animal Production" held at ICAR-CIRB, Hisar from November 27-28, 2014.
39. Dr. Thirumala Rao Talluri attended one day workshop on "Disaster Management in Animals – A Renewed Approach and Future Vision" at Centre for Disaster Management Technology for Animals Rajasthan University of Veterinary & Animal Sciences, Bikaner on February 20, 2015.
40. Dr. Vijay Kumar attended the 14th Biennial meeting of the AICRP on UAE held at Udaipur from February 18-20, 2015.
41. Dr. Vijay Kumar attended XXIII Annual National Conference of Society of Animal Physiologists of India and National Symposium on "Physiological Determinants of Climate Resilient and Sustainable Animal Production" held at the Central Institute for Research on Buffaloes, Hisar from November 27-28, 2014.
42. Dr. Yashpal attended XXIII Annual National Conference of Society of Animal Physiologists of India and National Symposium on "Physiological Determinants of Climate Resilient and Sustainable Animal Production" held at the Central Institute for Research on Buffaloes, Hisar from November 27-28, 2014.

Annual Performance Evaluation Report (April 1, 2013 to March 31, 2014) in respect of RFD 2013-2014

Name of the Division : Animal Science
Name of the Institution : National Research Centre on Equines, Hisar
RFD Nodal Officer : Dr. Sanjay Barua, Pr. Scientist

S. No.	Objective(s)	Weight (%)	Action(s)	Success Indicator(s)	Unit	Weight	Target/Criteria Value					Achievements	Performance		Percent achievements against target values of 90% Col.	Reasons for shortfalls or excessive achievements, if applicable
							Excellent 100%	Very Good 90%	Good 80%	Fair 70%	Poor 60%		Raw Score	Weighted Score		
1.	To develop diagnostics/biologicals for major equine diseases	20	Development of diagnostic kits for detection of antibodies against Glanders & EIA	Diagnostic kits developed	Number	20	2	1	0	0	0	**2**	100	20	200.0	Targeted two Diagnostic assays developed
2.	Diagnosis, surveillance and monitoring or equine diseases	20	Surveillance, monitoring and control of common equine diseases	Testing of biological samples for equine diseases	Number	20	1300	1200	1100	1000	900	**1442**	100	20	120.2	During surveillance visits higher nos. or animals were available for sampling
3.	Maintenance of National repository of microorganisms of animal origin	17	Identification, characterization and reposition of animal microbes	Reposition of reference microbial strains/isolates, plasmids, genomic DNA/RNA, Clones.	Number	17	300	250	200	150	100	**309**	100	17	123.6	Receipt of a large no. of microbial isolates from VTCC network units and other depositors
4.	Conservation & Enhancing productivity of equines	17	Cryopreservation of equid semen	Number of semen doses cryopreserved	Number	9	350	300	250	200	150	**356**	100	9	118.7	Availability of additional animals from the field
			Artificial inseminations in mares & jennies	Number of artificial inseminations	Number	8	40	30	20	10	5	**43**	100	8	143.3	Availability of additional animals in field for AI

5. Providing diagnostic, advisory and consultancy services	15	Testing of biological samples of equines for various diseases	Testing of serum samples for EIA and Glanders	Number	7.5	7000	6500	6000	5500	5000	**9597**	100	7.5	147.6	Receipt of large number of serum samples from stakeholders
		Organisation of Equine Health Camps	Health Camps organised	Number	7.5	12	10	8	4	2	**12**	100	7.5	120.0	Targeted Health camps organised
6. Efficient Functioning of the RFD System	3	Timely submission of Draft RFD (2013-14) for approval	On-time submission	Date	2	May 15 2013	May 16 2013	May 17 2013	May 20 2013	May 21 2013	**May 15 2013**	100	2	—	—
		Timely submission of Results for RFD (2012-13)	On-time submission	Date	1	May 1 2013	May 2 2013	May 5 2013	May 6 2013	May 7 2013	**April 16 2013**	100	1	—	—
7. Administrative Reforms	4	Implement ISO 9001 as per the approved action plan.	% Implement-ation	Percent	2	100	95	90	85	80	**0**	0	0	—	ISO 9001 action plan is under implem-entation stage
		Prepare an action plan for Innovation	On-time submission	Date	2	July 7 2013	Aug. 10 2013	Aug. 20 2013	Aug. 30 2013	Sept.10 2013	**July 27 2013**	100	2	—	—
8. Improving Internal Efficiency/ responsiveness/ service delivery of Ministry/ department	17	Implementation of Sevottam	Independent Audit of Implementation of Citizen's Charter	Percent	2	100	95	90	85	80	**100**	100	2	—	—
			Independent Audit of Imple-mentation of public griev-ance redressal system	Percent	2	100	95	90	85	80	**100**	100	2	—	—

Total Composite Score : 98.0

Rating : Excellent

Visit of Dignitaries

- **Dr Debra Elton**, Head, Department of Virology, and **Elizabeth Medcalf,** Head, Diagnostics, Animal Health Trust, United Kindom visited the Centre during November 14-21, 2015 under OIE twinning project on Equine Influenza. During the visit, Equine influenza laboratory of the Centre demonstrated the surveillance system for monitoring equine influenza besides processing of samples and discussion about different diagnostic assays. Further, visitors were shown various facilities such as ATIC, BSL-III laboratory, animal shed complex, VTCC and different laboratories at the main campus and EPC, Bikaner. They also attended an equine health camp and interactive meet with progressive equine owners at the village Peer Kamaria in Hanumangarh District, Rajasthan on November 18, 2014.

Dr Elizabeth Medcalf & Dr Debra Elton examining horse at Hanumangarh

Dr Debra Elton and Elizabeth Medcalf in discussion with Director, ICAR-NRCE

- **Dr M. L. Madan**, Former DDG (AS) & Vice-Chancellor Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola and U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura visited the Centre along with Dr B. S. Prakash ADG (AN&P), ICAR, New Delhi on November 28, 2014. They visited various facilities viz. Info-equine Museum, BSL-III laboratory, Animal Shed Complex, VTCC and different laboratories at the Centre.

Dr M. L. Madan interacting with scientists at NRCE

- **Dr Keith Hamilton** from OIE headquarters visited ICAR-NRCE on August 25, 2014. He delivered a lecture on "Activities of OIE", had discussion with scientists and visited laboratories of the Centre. During visit, he was shown laboratories and had interaction with scientists.

Dr Keith Hamilton interacting with scientists

- **Maj Gen (Dr) Sri Kant Sharma, SM VSM,** Vice Chancellor, LUVAS, Hisar visited ICAR-NRCE as the Chief Guest during Foundation Day celebration programme at ICAR-NRCE on November 26, 2014.
- **Dr Indu Gupta,** Additional Director, LASTEC, DRDO, Ministry of Defence, New Delhi, visited ICAR-NRCE on December 27, 2014.
- **Brigadier S. S. Kashyap**, Commandant, Equine Breeding Stud, Hisar visited ICAR-NRCE on January 19, 2015. He visited different laboratories and Info-equine museum at the Centre. Director, ICAR-NRCE briefed him about the various ongoing research and extension activities at the Centre.

Brigadier S. S. Kashyap at Info-equine Museum

- **Dr J. R. Rao,** Scientist Emeritus, ICAR-NAARM, Hyderabad visited the Centre as a monitoring faculty for Field Experience Training (FET) of six FOCARS trainees from ICAR-NAARM, Hyderabad on March 04, 2015.
- **Sh. Dushyant Chautala,** Hon'ble Member of Parliament, Hisar visited ICAR-NRCE on February 11, 2015. Director, NRCE briefed him about the research facilities and various ongoing research and extension activities at NRCE. On this occasion, plantation was done in the campus by Sh. Daushyant Chautala. He visited various laboratories and museum at the Centre and was mightily impressed by the state-of-the–art facilities available at NRCE.

Sh. Dushyant Chautala at VTCC/NRCE

- **Lt. Gen. Jagvinder Singh, VSM**, Director General, RVC, AHQ, New Delhi visited ICAR-NRCE on March 13, 2015. He visited Info-equine Museum, and different laboratories at the Centre. In-length discussion was carried out between scientists at NRCE and Lt. Gen. Jagvinder Singh regarding collaborations and identification of areas of common interest for mutual co-operation.

Lt. Gen. Jagvinder Singh at Info-equine Museum

Personnel

Milestones

Awards and Recognition

Dr Thirumala Rao Talluri, Scientist, ICAR-NRCE was awarded PhD degree in the field of Animal Reproduction and Biotechnology from the University of Veterinary Medicine, Hannover, Germany under ICAR International Fellowship. He worked under the project entitled "Approaches for derivation of induced Pluripotent stem cells from Cattle". Dr Thirumala Rao Talluri joined NRCE on 11.02.2015 after completing his PhD.

Appointment and Transfer

- Dr B.N. Tripathi joined as Director, ICAR-NRCE, Hisar on 20.08.2014.
- Dr Mamta Tigga, Scientist was relieved from Centre on 14.11.2014 subsequent to her transfer to ICAR-National Institute for Biotic Stress Management Raipur, Chhattisgarh.

Promotion

- Dr Anuradha. Bhardwaj, Scientist has been promoted to Scientist (Sr. Scale) w.e.f. 07.01.2012.
- Dr Harishankar Singha, Scientist has been promoted to Scientist (Sr. Scale) w.e.f. 07.01.2012.
- Dr Taruna Anand, Scientist has been promoted to Scientist (Sr. Scale) w.e.f. 07.01.2012.
- Dr B.C. Bera , Scientist has been promoted to Scientist (Sr. Scale) w.e.f. 07.01.2012.

Staff at NRCE

Director: Dr B. N. Tripathi

Scientists at NRCE, Hisar Campus

1. Dr A.K. Gupta, Principal Scientist, Biochemistry
2. Dr S.C. Yadav, Principal Scientist, Veterinary Parasitology
3. Dr Yash Pal, Principal Scientist, Animal Physiology
4. Dr B.R. Gulati, Principal Scientist, Veterinary Microbiology
5. Dr S.K. Khurana, Principal Scientist, Veterinary Public Health
6. Dr Nitin Virmani, Principal Scientist, Veterinary Pathology
7. Dr Sanjay Kumar, Principal Scientist, Veterinary Medicine
8. Dr Anju Manuja, Senior Scientist, Veterinary Medicine
9. Dr Balvinder Kumar, Senior Scientist, Biotechnology
10. Dr A. Bhardwaj, Scientist, Animal Biotechnology
11. Dr H.S. Singha, Scientist, Animal Biotechnology
12. Dr A.A. Raut, Scientist, Extension

National Fellow (ICAR), NRCE, Hisar

1. Dr Rajender Kumar, National Fellow, Veterinary Parasitology

Scientists at EPC (NRCE), Bikaner Campus

1. Dr R. A. Legha, Principal Scientist, LPM
2. Dr Vijay Kumar, Scientist, Animal Physiology
3. Dr P.A. Bala, Scientist, Animal Nutrition
4. Dr T. Rao Talluri, Scientist, Veterinary Reproduction & Gynecology
5. Dr Ramesh Dedar, Scientist, Veterinary Medicine
6. Dr Sanjay Kr. Ravi, Scientist, Animal Reproduction

Scientists at VTCC, NRCE, Hisar

1. Dr Praveen Malik, Principal Scientist, Veterinary Microbiology
2. Dr Sanjay Barua, Principal Scientist, Veterinary Microbiology
3. Dr R.K. Vaid, Principal Scientist, Veterinary Public Health
4. Dr Taruna Anand, Scientist, Animal Biotechnology
5. Dr B.C. Bera, Scientist, Animal Biotechnology
6. Dr K. Shanamugasundaram, Scientist, Veterinary Pathology
7. Dr Riyesh T., Scientist, Veterinary Microbiology

Technical Staff at NRCE, Hisar

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

Technical Staff at EPC, Bikaner

1. Dr Jitender Singh, Sr. Technical Officer
2. Sh K.K. Singh, Technical Officer
3. Sh Brij Lal, Technical Officer
4. Sh N.K. Chauhan, Technical Officer
5. Sh Om Prakash, Technical Asstt.
6. Sh S.N. Paswan, Technical Asstt.
7. Sh Rajendra Singh, Technical Asstt.
8. Sh Gopal Nath, Technician

Administrative Staff at NRCE, Hisar

1. Sh Chetan Issar, AO, CIRB (Addl Charge of NRCE)
2. Smt Shammi Tyagi, AF&AO
3. Sh Ram Pal, AAO
4. Sh S.P. Kaushik, AAO
5. Sh Subhash Chander, Assistant
6. Sh Pratap Singh, Assistant
7. Sh Sunil, Assistant
8. Sh Ashok Arora, Personal Assistant
9. Sh D.D. Sharma, UDC
10. Sh Om Prakash, UDC
11. Sh Deepak Kumar, LDC

Administrative Staff at EPC, Bikaner

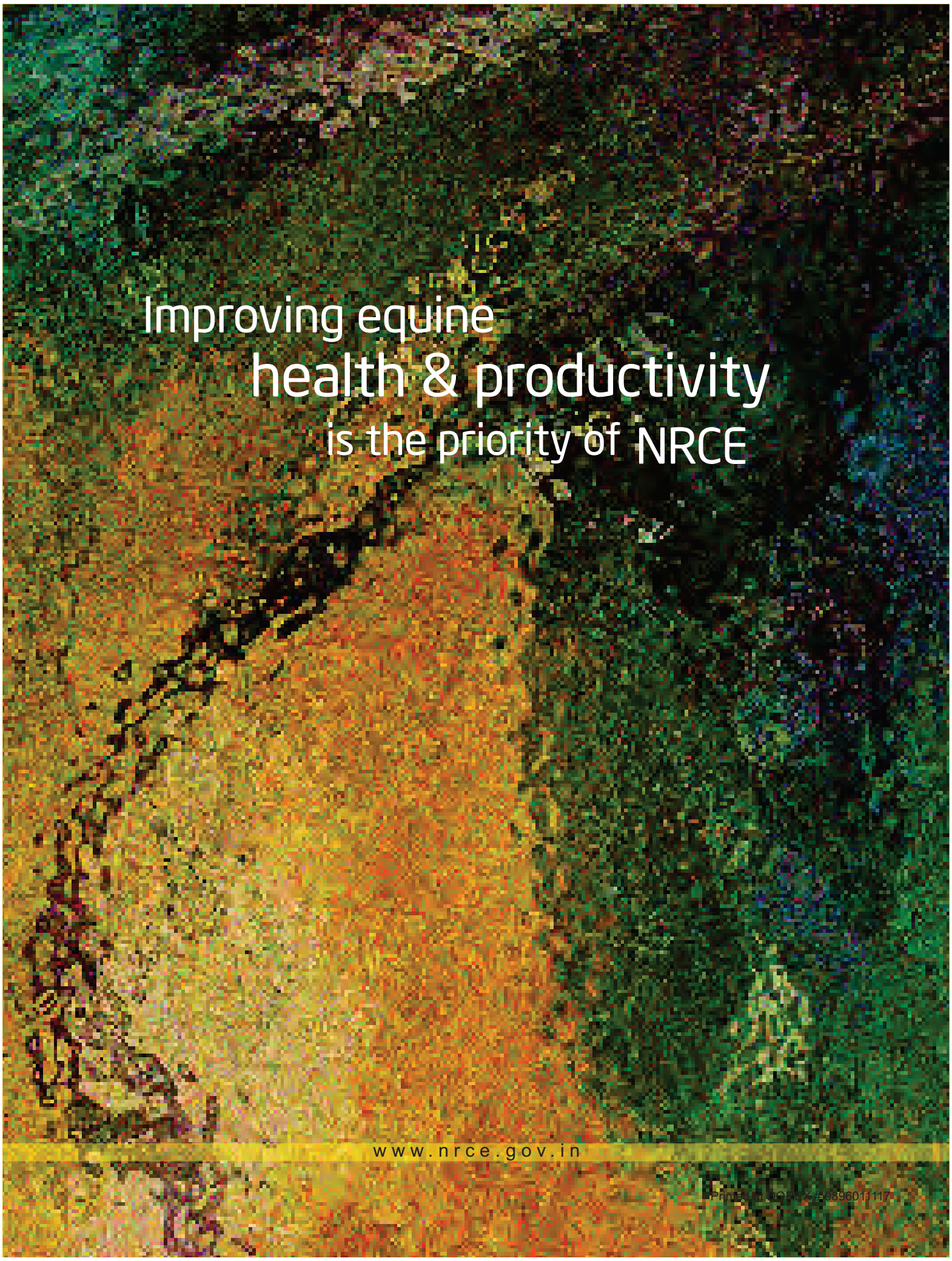
1. Sh Mahender Singh, LDC

Supporting Staff at NRCE, Hisar

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

Supporting Staff at EPC, Bikaner

1. Sh Raju Ram
2. Sh Mahabir Prasad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