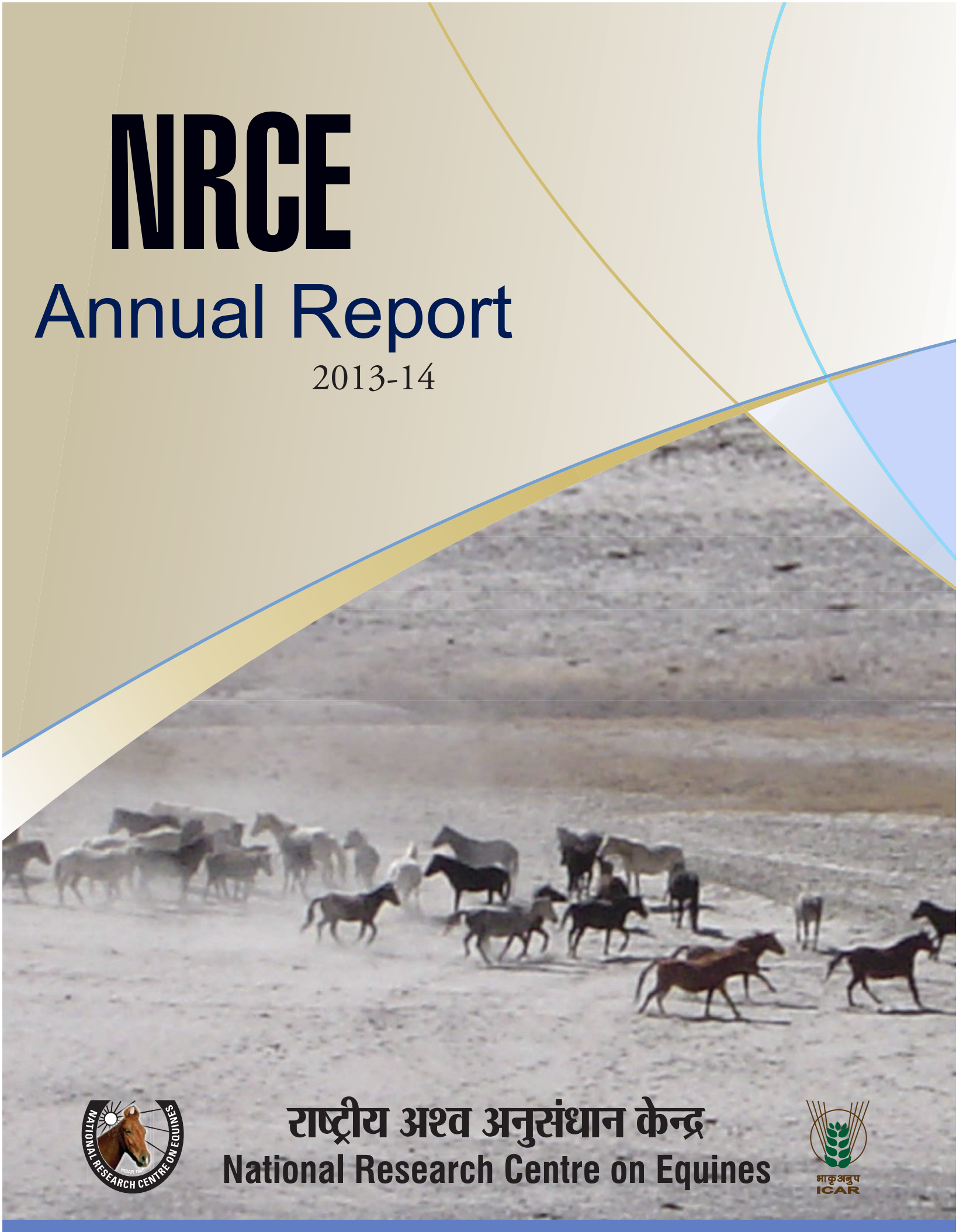
NRCE

Annual Report

2013-14



राष्ट्रीय अश्व अनुसंधान केन्द्र

National Research Centre on Equines

भाकृअनुप
ICAR

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The achievements and activities of the Centre from April 2013 to March 2014 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.

Cover: Zanskari ponies in their home tract

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Director's Foreword

I feel immense pleasure in presenting the Annual Report (2013-14) of National Research Centre on Equines, Hisar. During the journey of last 29 years, our Centre contributed immensely in the development of diagnostics and biologicals for various existing and emerging equine diseases, surveillance and monitoring of equine diseases, and providing diagnostic, advisory and consultancy services to equine owners and other stakeholders. We have also laid special emphasis on conservation and improvement of the germplasm of indigenous equine breeds like Marwari and Zanskari.

We have always believed in tackling need based field oriented problems and solving them in a time frame. In order to make a difference in life of our economically weaker equine owners; we routinely arrange equine health and disease investigation camps in field. Donkeys and mules are the lifeline source of livelihood for a class of underprivileged peasantry in rural and peri-urban communities. We have embarked on characterization of local non-descript donkeys in different geographic locations of the country. We are also working on utilization of equine energy to provide better work opportunities to use this power for economic growth of the owners as well as for maintenance of their population. A study on existing management systems and utilization of donkeys and mules for sustainable livelihood is undertaken for extension benefits. Similarly, physical, physiological and biochemical evaluation of semen is being done for their better use in artificial insemination to increase fertility rate in equines. We have also initiated work on use of smaller semen dose in AI. Selective breeding using cryopreserved semen of purebred elite Marwari stallions with desired traits through AI is on cards. We have brought Manipuri ponies from their home tract in the Eastern part of India, Manipur.

To improve the productive efficiency of the equines, Centre is working upon the various aspects of productive, reproductive and feeding management of the animals. A field oriented study was done on performance of Marwari horse on cross-country trial to study the bioavailability of vitamins and electrolytes. Extensive extension activities are conducted throughout the year for awareness of the farmers regarding various aspects of the health and management practices. NRCE team surveyed the working equids of Kolkata city and Digha beach, Kolkata and recommended measures for improving the health of equines being used for carriages. A survey was undertaken in Uttar Pradesh on management systems and utilization pattern of donkeys and mules.

Surveillance and monitoring (S&M) of the disease conditions prevalent in equines, is an important activity which drives the scientists to take up research in the diseases. Scientists regularly visit disease outbreaks in field conditions to investigate on the causes and to collect samples and epidemiological data. A major achievement of NRCE has been in preparation of dossier for data on freedom from African Horse Sickness.

The Centre is working on development of recombinant protein based diagnostics and subunit vaccine to combat against important equine pathogens like EHV1, glanders,

Trypanosomosis etc. We are carrying out studies to find out the role of diversity of Mx gene pool with Equine Influenza (EI) resistance and susceptibility in Marwari horses. We characterized TLR9 sequences of Marwari, Zanskari breeds and exotic (Poitu) and indigenous donkeys to elucidate its role in disease resistance. A study on immune-stimulatory effect of CpG-ODN against *Trypanosoma evansi* in blood cells of Marwari horses was done. Evaluation of *in vitro* growth inhibitory efficacy of some synthetic novel drug molecules against *Theileria equi* was initiated. We are doing researches on utilization of modern proteomic approaches for development of biomarkers for diagnosis of *T. evansi*.

The Centre is also endeavouring on the frontier areas of modern research such as stem cells and nano-technology. With an idea of using equine stem cells in treatment and management of ruptured or damaged tendon in race horses, equine mesenchymal stem cells derived from amniotic fluid were studied. Immunophenotypic characterization of mesenchymal stromal cells isolated from equine umbilical cord blood was also performed. Two nanoformulations of trypanocidal drug quinapyramine sulphate were synthesized, characterized and evaluated for efficacy and toxicity.

In the field of international research, NRCE has forged foreign collaboration to establish its global referral status. The Centre is dealing with three OIE laboratory twinning projects on Piroplasmosis, Glanders and Equine influenza, with path now open for OIE Reference Laboratory for Equine Piroplasmosis.

Veterinary Type Culture Collection established at NRCE, is serving as a Microbial Genetic Resource Centre for the country and has a mandate of collection of microorganisms of animal origin/significance/relevance. During 2013-14, repository has been strengthened by new additions of 342 veterinary; rumen and dairy microbes. The total present microbial accessioning of VTCC has reached a mark of 2022 accessioned microbes including clones and DNA. We have started bacteriophages isolation and collection also.

At NRCE, we have an unique futuristic strength in the sense that we have new generation of biologists who are all field scientists, laboratory scientists and also computational scientists. In our endeavour to initiate genomic characterization of our isolates, bioinformatic analysis of 2 genome sequenced bacterial isolates was completed on RAST platform. The GC-FAME facility was installed and identification of bacterial isolates was successfully initiated at VTCC. Many new bacterial isolates and viral isolates from various animal and animal microenvironments have been identified and added to and bacteriophages repository after 16S rRNA sequencing. The ORF clone library has been strengthened with addition of seven Gateway clones of ORFs.

Under our institutional activities, we conducted National workshop on Equine Health & Welfare for working equines in association with The Brooke India Ltd in March 2014. Annual review meet of Veterinary Type Culture Collection Network Project was also held in Sept., 2013. The Centre also organized trainings, interactive meet of scientists, expert lectures in various conferences and trainings. Toll-free helpline for equine owners was inaugurated at EPC Bikaner during this period. Apart from the regular activities such as Hindi week celebration, Vigilance awareness week, Health Camps, Kisan Goshthis, Exhibitions, Animal Fairs, various visitors such as farmers and students were provided with required know-how for the benefit of equines.

The researchers at the institute published about 100 research articles, popular articles, book chapters, and abstracts in conferences leading to a significant contribution to the scientific community.

I personally 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 expeditious help from Scientists and staff at ICAR Headquarters.

(A.K. Gupta)

Executive Summary

NRCE was established on November 26, 1985 at Hisar (Haryana) under the aegis of Indian Council of Agricultural Research and further a sub-campus of NRCE was established in 1989 at Bikaner (Rajasthan). NRCE has state-of-the-art laboratories and facilities for undertaking research in areas of equine virology, bacteriology, pathology, parasitology, immunology, medicine, biochemistry and biotechnology, genetics and breeding, reproduction and physiology. NRCE acts as a National Referral Centre of Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture (Govt. of India) to provide consultancy and testing for health certification and diagnostic services for various equine diseases to stake-holders.

Surveillance and monitoring of the disease conditions prevalent in equines is an important activity and drives the scientists to take up the research in the diseases which can pose threat to industry in near future. During the period under report, surveillance and monitoring (S&M) was provided for EIA, glanders, equine influenza, EHV-1, EVA, CEM, *Theileria equi, Trypanosoma evansi, Trypanosoma equiperdum, Babesia equi, Salmonella* abortus-equi, and African horse sickness. A total of 5795 serum samples from thoroughbred as well as indigenous equines were examined for EIA under S&M (1442), disease investigation (16) and contractual service (4337); 7044 serum samples were tested for glanders; 2733 for screening for equine influenza (H3N8);1782 for *T. evansi* and 1442 serum samples tested for Brucellosis and *Salmonella* Abortusequi. EHV1 could be isolated from 8 samples as well. NRCE also helped in preparation of dossier for freedom of AHS disease and tested 542 serum samples collected during 2011 to 2014 as requested by DAHDF. Various bacterial isolates were also isolated from field samples originating from Rajasthan, HP, Haryana, UP, AP, Punjab and Chhattisgarh. Clinical cases of equine glanders were reported from three states viz. Uttar Pradesh, Himachal Pradesh, and Chhattisgarh. Investigation regarding neuropathogenicities of Equine Herpes Virus-1 (EHV-1) among equines was carried out by the Centre.

The Centre is working on development of recombinant protein based diagnostics and subunit vaccine to combat against important equine pathogens. In this direction, the work was initiated for eukaryotic expression of glycoprotein D with the aim to develop an efficacious subunit vaccine against EHV-1 using indigenous isolate. Indirect ELISA using recombinant Hcp1 protein was evaluated for glanders diagnosis. It was observed that Hcp-1 ELISA was also able to diagnose human melioidosis.

Studies on C-terminal rHSP70 protein as a candidate for detection of anti-trypanosoma antibodies in donkey sera at early stage were performed in order to develop antibody ELISA for Trypanosoma diagnosis. For application in pregnancy diagnosis the gene for beta-alpha eCG was cloned, expressed and characterized by ELISA and Western Blotting. The recombinant eCG was successfully purified and analysed *in silico* and the 3D homology model of the eCG α-subunit was generated using bioinformatics tools and molecular docking studies performed to evaluate its efficacy.

The selection of disease resistant animal and study of health parameters of equines are the prime concern for the development of resistant herd through selective breeding. A study has been conducted to find out the role

of diversity of Mx gene pool with equine influenza resistance and susceptibility in Marwari horses. Comparative analysis of structural and functional motifs of horse cytokines (IL-2, IL-4, IL-10 and IL-18) with other domestic animals was performed. This information will be helpful to investigate the physiological roles of cytokine, as well as their potential efficacy as a therapeutic agent or vaccine adjuvant in horse. Further, *in vitro* immunostimulatory effect of CpG-ODN Class-A and C, against *Trypanosoma evansi* in peripheral blood mononuclear cells (PBMCs) of Marwari horses was studied. Also the immuno-stimulatory effects of CpG-ODN class C in *T. evansi* infected rabbits were determined. Evaluation of *in vitro* growth inhibitory efficacy of some synthetic novel drug molecules against *Theileria equihaemoprotozoa* was initiated. We characterized TLR9 sequences of Marwari & Zanskari breeds of horse, Poitu (exotic donkey) and indigenous donkeys to elucidate it's role in disease resistance.

Taking a leap ahead regarding proteomic approaches for development of biomarkers for diagnosis of *Trypanosoma evansi*, three cluster of immuno reactive proteins were identified as potential candidates and large scale purification of recombinant protein (hsp70) was done for further use in immunodiagnostic test. Also the deduced amino acid sequence of HSP70 protein of the Indian isolate of *T. evansi* was compared with the other HSP70 amino acid sequences of different strains of *Trypanosomes, Leshmania* and *Plasmodium* parasites circulating worldwide and it was observed that Indian *T. evansi* isolate is almost similar to *Trypanosoma brucei brucei* isolate.

The Centre is also endeavouring on the frontier areas of modern research such as stem cells and nano-technology. During this period, equine mesenchymal stem cells derived from amniotic fluid were differentiated to tenogenic lineage using BMP-12. These cells were found to express tenomodulin and decorin by RT-PCR and immunocytochemistry and hence they find a great potential in regeneration of ruptured or damaged tendon in race horses. Also immunophenotypic characterization of mesenchymal stromal cells isolated from equine umbilical cord blood was performed. Two nanoformulations of trypanocidal drug quinapyramine sulphate using different polymers were synthesized, characterized and evaluated for efficacy and toxicity.

NRCE explored the possibilities of foreign collaboration in research to establish it's global referral status. The Centre is having three OIE laboratory twinning projects on Piroplasmosis, Glanders and Equine Influenza. The Centre has successfully completed OIE sponsored Laboratory Twinning Project on Equine Piroplasmosis and consequently applied to the OIE for recommending NRCE as OIE Reference Laboratory for Equine Piroplasmosis. Under OIE Twinning Laboratory Project on Glanders, for capacity building program, scientists from NRCE visited the OIE Reference Lab. at FLI, Germany where recombinant proteins based indirect ELISA developed by NRCE was evaluated using samples from India and global collection of equine samples from the repository of OIE Reference Lab. at FLI. Also OIE Experts from parent lab (FLI, Germany) visited the candidate laboratory in December 2013. Under OIE Twinning Project on Equine Influenza, a laboratory exchange program was executed at the Animal Health Trust, UK, during which hands-on experience regarding various assays for the diagnosis of equine influenza along with molecular characterization of EI viruses was imparted to NRCE scientists.

To improve the productive efficiency of the equines, Centre is working upon various aspects of productive, reproductive and feeding management of the animals. A project has been initiated with the objective to study the follicular dynamics and associated endocrine, biochemical and gene expression changes during onset of puberty, estrous cycle, pregnancy and peripartum periods in Marwari fillies and mares. Effect of combinations of roughage feeding on digestibility and

performance of horses' young stock in arid region of Rajasthan was determined. A trial to study the bioavailability of oral supplementation of vitamin C and E in exercising horses (5 km gallop) was also conducted. Studies on oxidative stress, electrolytes requirements and therapeutic efficacy of vitamin C and Vitamin E on oral supplementation for oxidative stress in horses under galloping exercise were carried out.

We carried out the genetic characterization of Indian horse & pony breeds and found that genetic differentiation between Spiti and Thoroughbred horses was the maximum followed by Spiti and Kathiawari, while Zanskari and Manipuri were the least differentiated. Indian horse and pony breeds clustered separately while Thoroughbred formed a separate out-group. Kathiawari was the most prominent cluster as horse breed followed by Zanskari. Marwari and Bhutia appeared to have common ancestries while Kathiawari represented the oldest stock. Donkey populations from different geographic areas were phenotypically characterized during this period. This biometric data will be used as baseline information in differentiating donkey populations.

NRCE works on improvement of the working equids at the farmer's door step. Extension activities were conducted throughout the year for awareness of the farmers regarding various aspects of the health and management practices. NRCE team surveyed the working equids of Kolkata city (animal working on hard pavement) and Digha beach and recommended measures for improving the health of equines being used for Victoria carriage and joyrides. Survey study undertaken in Uttar Pradesh on management systems and utilization pattern of donkeys and mules, revealed that poor health of animals; low social status and poor management were the major constraints that had an impact on animals and livelihood of their owner's families.

The Centre is also creating the biobank of the precious biological samples. Various biological materials in the form of serum, clinical samples, microbes are being preserved and maintained. In this endeavour, Veterinary Type Culture Collection established at NRCE, is serving as a Microbial Genetic Resource Center for the country and has a mandate of collection of microorganisms of animal origin/significance/relevance. During 2013-14, repository has been strengthened by addition of 194 accessioned veterinary microbes; 53 rumen microbes and 125 dairy microbes. The accessioned veterinary microbes include 73 bacterial and 11 viral cultures; 59 recombinant clones and 38 genomic DNA of bacterial cultures. The total present microbial accessioning of VTCC has reached a mark of 2022 accessioned microbes including clones and DNA. Also as a new initiative, 13 lytic bacteriophages have been isolated and preserved in the repository. Seven Buffalopox virus (BPXV), one Swinepox virus were isolated; one Peste de Petits Ruminants (PPR) virus, one Blue Tongue virus (BTV), one Canine adenovirus, PPRV, Contagious ecthyma virus, Sheepox virus, Canine adenovirus etc. were cultured and accessioned in the repository. Bioinformatic analysis of 2 genome sequenced isolates viz. *Pasteurella multocida* and *Salmonella enterica* sub spp. enterica Serovar Gallinarum was completed using RAST software. The GC-FAME was installed and identification of bacterial isolates was successfully initiated at VTCC. This year, significant genotype based identification included isolates identified as *Moraxella ovis, Bacillus hunanensis, Corynebacterium tuscaniense, Nocardia niwae, Brevibacillus agri, Nocardia otitidiscaviarum, Streptomyces ghanaensis, Kluyvera georgiana, Rhodococcus coprophilus, Escherichia hermanii, Castellaniella denitrificans, Nocardiopsis alba, Aerococcus viridians, Pasteurella multocida, Ottowia pentelensis, Prolinoborus fasciculus, Rhodococcus aetherivorans* and others from various animal and animal micro-environments. A major achievement has been isolation of *Mannheimia varigena* from buffalo pneumonia case. The ORF clone library has been

strengthened with addition of seven Gateway clones of ORFs viz., A39R, B5R, L5R, D8L, A21L, A27L & B1R of buffalopox virus (BPXV). The entry clones were generated by cloning into Gateway vector pDONR221.

The Centre was able to generate revenue of ₹ 52.13 lacks from consultancy through disease investigation & diagnosis. Further our agriculture section made a progress and NRCE could generate revenue through sale of crops to the tune of ₹ 10.04 lakhs. Further a sum of ₹ 21.22 lakhs was received from sale of Eucalyptus trees to Haryana Forest Development Corporation.

Various institutional activities were executed during the period. National workshop on "Equine Health & Welfare of Working Equines" was organised in association with The Brooke India. Annual review meet of Veterinary Type Culture Collection Network Project was organized during the month of September, this year. The Centre also organized training on “Equine Viral Diseases” for PhD student from SKUAST, Jammu from 22-25 Sept., 2013, in which they were given the hands-on-training regarding cell culture, cultivation, isolation and identification of animal viruses such as EHV1, EVA and JEV. The Centre also convened interactive meet of scientists working on Trypanosomosis in ICAR institutes and organized a training on “*In vitro* cultivation of *Trypanosoma evansi*” for Research Fellow, IVRI. During this period, the scientists of the Centre were also invited for expert lectures in various conferences and trainings. Various dignitaries also had a visit at the Centre and interacted with scientists during this period. As an important achievement a Kisan Call Centre: Toll-free helpline for equine owners was inaugurated at EPC Bikaner on August 17, 2013. Apart from the regular activities such as Hindi Week Celebration, Vigilance Awareness Week, Health Camps, *Kisan Goshthis*, Exhibitions, Animal Fairs, various visitors such as farmers and students were provided with required know-how for the benefit of equines.

The researchers at the institute published 60 research articles, 10 popular articles, 4 book chapters, presented various abstracts in conferences and earned 67 gene accessions in public database thus leading to a significant contribution to the scientific community.

कार्यकारी सारांश

राष्ट्रीय अश्व अनुसंधान केन्द्र की स्थापना 26 नवम्बर 1985 को भारतीय कृषि परिषद् के तत्वाधान हिसार, हरियाणा में हुई थी और 1989 में एक और उपकेन्द्र की स्थापना बीकानेर, राजस्थान में की गई।

राष्ट्रीय अश्व अनुसंधान केन्द्र में अश्व प्रजाति के पशुओं पर अश्व विषाणु विज्ञान, जीवाणु विज्ञान, पैथालॉजी, परजीवी विज्ञान, रोग प्रतिरोध विज्ञान, वैद्यक-शास्त्र, जीव रसायन शास्त्र, जैव प्रौद्योगिकी आनुवांशिकी, प्रजनन, फिजीयालॉजी आदि विषयों की अत्याधुनिक प्रयोगशालाएं एवं सुविधाएं हैं। राष्ट्रीय अश्व अनुसंधान केन्द्र अश्व स्वास्थ्य एवं अश्व रोग-निदान के विषय में भारत सरकार के पशु-पालन, डेयरी एवं मात्स्यिकी विभाग, कृषि मंत्रालय को समय-समय पर रोग-निदान एवं सलाह-परामर्श देता है एवं इसके लिए एक राष्ट्रीय संप्रेषण केन्द्र के रूप में अपनी सेवाएं देता है।

भारतीय अश्व प्रजाति के पशुओं जैसे घोड़े, गर्दभ, खच्चर आदि की संक्रामक बीमारियों और प्रकोपों की रोकथाम के लिए रोग जाँच, निगरानी एवं नियंत्रण के कार्य को लेकर केन्द्र के वैज्ञानिक सतत् अनुसंधान प्रयासरत रहते हैं ताकि वर्तमान के रोगों का निदान एवं भविष्य के रोगों की रोकथाम हो सके। गत वर्ष की अवधि में विविध अश्व रोगों जैसे इक्वाइन इन्फैक्शियस एनीमिया, ग्लैण्डर्स, इक्वाइन इन्फ्लुएंजा, इक्वाइन हरपीज़ विषाणु-1, इक्वाइन वाइरल आर्टराईटिस, कन्टेजियस इक्वाइन मैट्राईटिस, थेलीरिया इक्वाई, ट्रिपैनोसोमा इवैनसाई, ट्रिपैनोसोमा इक्वीपरडम्, बैबीसिया इक्वाई, सालमोनेल्ला एबार्टस्-इक्वाई, अफ्रीकी हार्स सिकनैस रोगों पर निगरानी जाँच कार्य किया गया। थारोब्रैड एवं देसी नस्ल के अश्वों के 5795 सीरम नमूनों को इक्वाइन इन्फैक्शियस एनीमिया के लिए रोग-निगरानी (1442) रोग अन्वेषण (16) एवं संविदा संबधी कार्य (7337) के अन्तर्गत जाँचा गया। ग्लैण्डर्स के लिए 7044 सीरम नमूनों की जाँच की गई। इक्वाइन फ्लू के लिए 2733 नमूने जाँचे गए, टी० ईवैनसाई के लिए 1782 एवं साल्मोनेल्ला एवं ब्रुसेलोसिस के लिए 1442 सीरम नमूनों की जाँच की गई। अफ्रीकी हार्स सिकनैस रोग के भारत के भौगोलिक क्षेत्र से उन्मूलन के तथ्य की अधिकाधक पुष्टि हेतु फाईल तैयार करने के लिए 2011-12, 2012-13 एवं 2013-14 के 542 सीरम नमूनों की जाँच की गई और पशु-पालन डेयरी एवं मात्स्यिकी विभाग, भारत सरकार को रिपोर्ट प्रेषित की गई।

विभिन्न राज्यों जैसे राजस्थान, हिमाचल-प्रदेश, हरियाणा, उत्तर-प्रदेश, आन्ध्र-प्रदेश, पंजाब एवं छत्तीसगढ़ आदि से प्राप्त पशु-रोग नमूनों से जीवाणु पृथक्कीकरण किया गया। अश्व-ग्लैण्डर्स के रोग लाक्षणिक मामले उत्तर-प्रदेश, हिमाचल प्रदेश एवं छत्तीसगढ़ से प्रकाश में आए हैं। केन्द्र द्वारा अश्व-हरपीज विषाणु-1 के तंत्रिका व्याधिजनिक रूप पर अनुसंधान किया गया। केन्द्र विभिन्न अश्व-रोगों के नैदानिक (पुनः संयोजक प्रोटीन) एवं रोग प्रतिरोध शक्तिवर्धक उपैकिक टीकों के विकास पर कार्य कर रहा है। इस दिशा में इक्वाईन हरपीज विषाणु-1 के ग्लाईको प्रोटीन डी की यूकैरियोटिक अभिव्यक्ति पर इसके लिए उपैकिक टीका निर्माण पर कार्य शुरू किया गया है। ग्लैण्डर्स रोग के निदान के लिए पुनः संयोजक एच०सी०पी० प्रोटीन मनुष्यों में मैलिडियोसिस रोग का निदान कर सकता है। ट्रिपैनोसोमोसिस रोग निदान हेतु पुनः संयोजक एच०एस०पी० 70 प्रोटीन की पात्रता की जाँच चल रही है जिससे गदर्भों के उत्तरार्द्ध-सीरम नमूनों में ट्रिपैनोसोम रोधी प्रतिपिण्डों का पता लगे जिनका उपयोग एक प्रतिपिण्ड एलीसा निदान विधि में किया जा सके। मादा अश्वों के गर्भाधारण निदान के लिए इ०सी०जी० हारमोन के जीन की क्लोनिग, अभिव्यक्ति एवं निरूपण किया गया और उसका एलीसा एवं वैस्टर्न-ब्लाटिंग द्वारा

परीक्षण किया गया। पुन: संयोजित प्रोटीन का शुद्धिकरण एवं कम्प्यूटरीकृत अन्वेषण किया गया ताकि उसके त्रिआयामी चित्र का जैवसूचना प्रौद्योगिकी विश्लेषण किया जा सके।

रोग-प्रतिरोधी पशुओं के चयन हेतु एवं अश्वों के स्वास्थ्य सम्बंधी मापदण्डों के अध्ययन हेतु कार्य इनके रोग प्रतिरोधी रेवड बनाने और प्रजनन के लिए आवश्यक है। इसके लिए मारवाड़ी अश्वों के एम एक्सजीन की विविधता इक्वाइन फ्लू रोग के समक्ष मापी गई। अश्वों के साइटोकाईन (आईएल-2, 4, 10 एवं 18) का अन्य पशुओं के साथ उनकी बनावट और संरचना का तुलनात्मक अध्ययन किया गया। इस जानकारी का प्रयोग साइटोकाईन की फिजियोलाजिकल भूमिका पर प्रकाश डालेगा एवं उपचार हेतु अथवा टीके में एडजुवैंट के लिए प्रयोग में काम आ सकेगा।

मारवाड़ी अश्वों के परिधीय मोनोन्यूक्लियर कोशिकाओं में टी० इवैनसाई के विरुद्ध सी०पी०जी०-ओ० डी०एन० वर्ग ए एवं सी रोगप्रतिरोधी शक्ति उत्तेजन का प्रभाव देखा गया। थिलेरिया इक्वाई रक्त परजीवी के विरुद्ध इनविट्रो प्रणाली में इनके विरुद्ध कुछ कृत्रिम दवाओं के अतरोधी असर पर कार्य शुरू हुआ है। रोग प्रतिरोध में टी०एल०आर० 9 अनुक्रमों की भूमिका ज्ञात करने के लिए इन्हें मारवाड़ी एवं जांसकारी घोड़ों और पोइटू एवं देशी गदर्भों से प्राप्त कर आंका गया।

टी० इवैनसाई परजीवी के निदान हेतु इसके लिए इनमें जैवाँक चिह्न हेतु प्रोटिआयिक पद्धति का इस्तेमाल किया गया। एच०एस०पी० पुन: संयोजित प्रोटिन का बड़े पैमाने पर इम्यूनोनैदानिक कार्य के लिए शुद्धिकरण किया गया। भारतीय मूल के टी० इवैनसाई के एच०एस०पी० 70 प्रोटीन के अमीनो अम्ल के अनुक्रम की तुलना दूसरे ट्रिपैनोसोमा, लिशमेनिया एवं प्लास्मोडियम के एच०एस०पी० 70 प्रोटीन से की गई और इसे ट्रिपैनोसोमा ब्रुसियाई के समान पाया गया।

आधुनिक अनुसंधान के अग्रणी सीमांत विषयों जैसे भ्रूण जनित स्टैम कोशिकाओं एवं नैनो-तकनीक पर भी हम कार्य कर रहे हैं। हमने मादा अश्वों के भ्रूणावरण द्रव्य से मिसैनकाईमल स्टैम कोशिकाएं प्राप्त की जिन्हें कण्डरोन्मुखी बनाया जा सका है। घुड़दौड़ में अक्सर अच्छे घोड़ों को माँसपेशिकण्डरा में चोट लगने से नुकसान होता है जिसका इलाज कण्डरा के पुनर्जनन में किया जा सकता है क्योंकि इन कोशिकाओं में टीनोमोडुलिन और डेकोरिन की अभिव्यक्ति देखी गई है। घोड़ी के नाभि-रज्जु रक्त से प्राप्त मिसैनकाइमल कोशिकाओं का रोग प्रतिरक्षा लक्षणों पर अध्ययन किया गया। ट्रिपैनोसोमा परजीवी नाशी दो नैनो दवा मिश्रणों जैसे एन०एफ० एक एवं दो क्विन पाइरामिन सल्फेट को तैयार किया गया और उनकी प्रभावकारिता एवं विषाक्तता का मूल्यांकन किया गया।

राष्ट्रीय अश्व अनुसंधान केन्द्र ने अपने वैश्विक स्तर को बनाने और उसे उत्तरोत्तर आगे बढ़ाने के लिए विदेशी प्रयोगशालाओं के साथ अनुसंधान में सहयोग किया है। केन्द्र के पास वर्तमान में पाईरोपलासमोसिस, ग्लैण्डर्स एवं अश्व फ्लू रोगों की प्रयोगशाला जुड़ाव का विश्व पशु स्वास्थ्य संगठन, पैरिस के साथ कार्यक्रम चल रहा है। केन्द्र ने पाइरोप्लासमोसिस का प्रयोगशाला को पूर्ण पाइरोप्लाज़मा संदर्भ प्रयोगशाला बनाने का आवेदन भी विश्व पशु स्वास्थ्य संगठन को कर दिया गया है। ग्लैण्डर रोग प्रयोगशाला जुड़ाव कार्यक्रम के अन्तर्गत हमारे वैज्ञानिक फ्रैडरिच लोफ्लय संस्थान, जर्मनी गए जहाँ उन्होंने ग्लैण्डर्स के निदान के लिए उपयोगी पुन: संयोजित प्रोटीन पर आधारित अप्रत्यक्ष एलीसा का प्रयोग भारत से ले जाए गए सीरम नमूनों और उस प्रयोगशाला में एकत्रित नमूनों पर किया। एफ०एल०आई० प्रयोगशाला जर्मनी के वैज्ञानिक भी भारत आए और उन्होंने हमारी प्रयोगशाला देखी। विश्व पशु स्वास्थ्य संगठन के अन्तर्गत एक अन्य प्रयोगशाला जुड़ाव कार्यक्रम में हमारे वैज्ञानिकों ने एनीमल हैल्थ संबंधित नैदानिक विधियों पर एवं विषाणु आण्विक विश्लेषण पर कार्य किया।

अश्वों के उत्पादन दक्षता में निरन्तर सुधार के लिए केन्द्र अश्वों के उत्पादन प्रजनन और पोषण प्रबंधन पर लगातार कार्य कर रहा है। इस संबंध में मारवाड़ी घोड़ियों के उत्तम प्रजनन के लिए अंडाशय पुटिका और उससे संबधित अंत: स्त्रावी, जैव

रसायनिक एवं जीन-अभिव्यक्ति बदलावों का यौवन में, मादा के ऋतुकाल में, गर्भ के दौरान एवं प्रजनन काल के अंत में और पश्चात् काल में अध्ययन चल रहा है। चारे के खिलाए जाने पर उसकी पाचन क्षमता और अश्व शावकों पर उसका पोषण असर देखने की दिशा में कार्य किया गया। व्यायामक रहने वाले घोड़ों में विटामिन सी और ई की जैविक उपलब्धता का विश्लेषण किया गया। सरपट दौड़ने वाले अश्वों को चारे में विटामिन सी और ई देने के पश्चात यह देखा गया कि इन विटामिनों का आक्सीकरण तनाव, लवण उपलब्धता और ईलाज पर क्या प्रभाव पड़ता है।

राष्ट्रीय अश्व अनुसंधान केन्द्र अश्व पालक किसानों के द्वार जाकर अश्वों की कार्यक्षमता और स्वास्थ्य में सुधार का कार्य करता है। पूरे वर्ष देश के विभिन्न राज्यों में जाकर हम अश्व प्रबंधन प्रसार कार्यक्रम करते हैं। केन्द्र के ऐसे एक दल ने कलकत्ता शहर के सड़क पर चलने वाले अश्वों और दीघा समुद्र तट पर कार्य में प्रयोग होने वाले अश्वों का सर्वेक्षण किया वहाँ पर उन्होंने विक्टोरिया तांगे में इस्तेमाल किए जाने से होने वाले अश्वों के स्वास्थ्य में सुधार संबंधी सलाह दी।

उत्तर प्रदेश में भी अश्व प्रबंधन पर एक सर्वेक्षण किया गया और अश्वों और गदर्भों के प्रयोग पर सूचना एकत्रित की गई और पाया गया कि पशुओं के खराब स्वास्थ्य, निम्न सामाजिक स्थिति और खराब प्रबंधन की वजह से अश्व पालकों की आजीविका पर विपरित असर पड़ता है।

केन्द्र में अनमोल जैविक नमूनों का बैंक भी तैयार है जिनमें सीरम, नैदानिक नमूने, जीवाणु आदि का संवर्धन एवं संरक्षण किया जा रहा है। इस दिशा में वेटरीनरी टाईप कल्र्चस कलैक्शन की स्थापना एक जीवाणु आनुवांशिक संसाधन स्त्रोत के रूप में की गई। सन् 2013-14 में 194 वेटरीनरी जीवाणु संरक्षित किए गए जिनमें 54 रोमन्थी जीवाणु और 125 डेयरी जीवाणु हैं। वेटरीनरी जीवाणु में 73 बैक्टीरिया, 11 विषाणु, 59 पुन: संयोजित क्लोन एवं 38 जीनोमिक डी०एन०ए० नमूने हैं। क्लोन एवं डी०एन०ए० नमूने मिला कर अब तक कुल 2022 नमूने एकत्रित हो चुके हैं। एक नए कार्यक्रम के माध्यम से हमने 13 अपघटय जीवाणुभोजी विषाणुओं को भी पृथक कर रिपोजिटरी में संरक्षित किया है। रोगाणु पास्चुरेल्ला मल्टोसिडा एवं सालमोनेल्ला गैलीनेरम के जीनोम का जैवसूचना विश्लेषण किया गया जिसे रास्ट प्लेटफार्म पर पूरा किया गया। द्रुत गति से जीवाणुओं की पहचान के लिए जी०सी० फेम प्रणाली को प्रयोगशाला में स्थापित कर दिया गया है। इस वर्ष में महत्वपूर्ण जीवाणुओं की गुणसूत्र विश्लेषण पर आधारित पहचान की गई है जैसे- मौरेक्सेल्ला ओविस, बैसिलस हुनानैनसिस, कोराइनी बैक्टीरियम, टस्कानिएन्स, नोकार्डिया नींवे, ब्रैवीबैसिलैस एग्री, नोकार्डिया ओटाइटिडिसकावाईरम, स्ट्रैपटोमाइसेस घानाइनसिस, क्लयूवेरा जिओर्जियाना, रहोडोकाक्कस कोपरोफिलस, एस्चरिशिया हरमैनाई, कास्टैल्लानिएल्ला डिनाइट्रीफिकैन्स, नोकार्डियोपसिस अल्बा, एरोकाक्कस वीरीडन्स, पास्चुरैल्ला मल्टोसिडा, औटोविया पैनटीलैनसिस, प्रोलीनोबोरस फैसीकुलस, रहोडोकाक्कस एथिरोवोरन्स और अन्य जीवाणु एवं रोगाणु।

एक महत्वपूर्ण उपलब्धि भैंसों में निमोनिया में से मैनहीमिया वैरीजीना का पृथ्क्कीकरण है। सात भैंस चेचक विषाणु और एक सूकर चेचक विषाणु का पृथ्क्कीकरण किया गया। एक पी०पी० आर विषाणु, एक ब्लूटंग विषाणु, एक श्वान एडीनोवाइरस, कन्टेजियस एक थाईमा विषाणु, भेड़ चेचक विषाणु का प्रयोगशाला में कल्चर कर संरक्षण किया गया। औ० आर० एफ० क्लोन संग्रहण में और क्लोन एकत्रित किए गए हैं और सात और भैंस चेचक गेटवे क्लोन जैसे ए39आर, बी5आर, एल5आर, डी8एल, ए21एल, ए21एल, ए27एल और बी1आर क्लोन एकत्रित हो चुके हैं। इन प्रवेश क्लोनों को गेटवे वैक्टर पीडोनर 221 में उत्पन्न किया गया है।

गत वर्ष के दौरान संस्थान में विभिन्न प्रकार के कार्यक्रम आयोजित किए गए। ब्रूक इंडिया के साथ मिल कर केन्द्र ने एक राष्ट्रीय अश्व स्वास्थ्य और कल्याण कार्यक्रम का आयोजन किया। वेटरीनरी टाईप कल्चर्स कलैक्शन की वार्षिक समीक्षा सितम्बर माह में आयोजित की गई। केन्द्र ने शेर-ए-कश्मीर विश्वविद्यालय, जम्मू के एक पी०एच०डी० विद्यार्थी को अश्व

विषाणु रोगों पर सितम्बर 2013 में प्रशिक्षण भी दिया। इसमें अपने हाथ से कोशिका कल्चर उगाना, पृथक्कीकरण एवं पशु विषाणुओं जैसे ई०एच०वी०–1, इ०वी०ए० और जापानी बुखार विषाणु की पहचान के तरीके बताए गए। केन्द्र ने एक ट्रिपैनोसोमा परजीवी पर कार्य करने वाले वैज्ञानिक की सक्रिय गोष्ठी का आयोजन भी किया और ट्रिपैनोसोमा इवैन्साई को शरीर के बाहर उगाने की प्रक्रिया का भी प्रशिक्षण दिया। इस दौरान केन्द्र ने दूसरे संस्थानों के विशेषज्ञों को भी व्याख्यान के लिए आमंत्रित किया। केन्द्र में कई सारे गणमान्य व्यक्तियों का भी आगमन हुआ जिन्होंने वैज्ञानिकों के साथ अनुसंधान पर वैचारिक आदान–प्रदान किया। एक महत्वपूर्ण उपलब्धि बीकानेर में किसान काल सैन्टर के टॉल–मुक्त फोन नम्बर सुविधा का अनावरण रही। अब यह सुविधा हिसार एवं बीकानेर दोनों स्थानों पर उपलब्ध है।

अन्य नियमित कार्यक्रम जैसे हिन्दी पखवाड़ा, सतर्कता सप्ताह, स्वास्थ्य शिविर, किसान गोष्ठी, प्रदर्शनी, पशु–मेला, का संचालन किया गया और अन्य अतिथि जैसे किसानों और विद्यार्थियों का आगमन रहा, जिनको अश्व पालन के बारे में यथोचित जानकारी दी गई।

केन्द्र के वैज्ञानिकों द्वारा 60 शोध–पत्र, 10 लोकप्रिय लेख, 4 किताबों के अध्याय और विभिन्न शोध–पत्र सक्षिप्तिकरण लेख लिखे गए और सार्वजनिक डेटाबेस में 67 जीन अनुक्रम परिग्रहण किये गए।

Introduction

The mechanization of transport system has substantially decreased the utility of animal power but even then equines still have great relevance, especially in hilly and difficult terrains where other means of transport are inaccessible. Besides this, equines also serve as a source of livelihood to the landless, small and marginal farmers and other sections of our rural and semi-urban society.

In order to improve the health, performance and production of equines in India, the Indian Council of Agricultural Research established National Research Centre on Equines (NRCE) on November 26, 1985 at Hisar (Haryana). The main campus of NRCE is located at Hisar which has state-of-the-art laboratories and facilities for undertaking research in equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry & 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 at Hisar are supported by centralized services like animal and agriculture farms, experimental animal facility, BSLIII facility, ARIS cell, ATIC, library and Info-equine museum. The Centre has well maintained herds of Marwari, Zanskari, Manipuri horses and indigenous and exotic donkeys at Bikaner.

Since its inception, NRCE's efforts have been focused on infectious diseases confronting equines, surveillance and monitoring of equine diseases, development of diagnostics and vaccines and improvement in equine health and production which has led to its recognition at national and international level. The vision of the Centre is the enhanced utilization of equines for agricultural and transport purpose through equine development programmes in order to elevate the socio-economic status of underprivileged. 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 national referral facilities 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 under 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

- Surveillance and monitoring of important equine diseases including emerging and existing diseases with special emphasis on foal mortality and production losses.
- Development of effective, affordable and preferably

field-based diagnostics against major equine diseases threatening equine health and production in India.

- Development of effective, affordable and potent immunoprophylactics against important equine infectious diseases threatening equines in India.
- Development of effective plant-based products for management of some economically important equine diseases and to enhance performance in equines.
- To provide diagnostic and consultancy services for beneficiaries particularly equine farmers and breeders.
- Propagation of sustainable and economically viable Artificial Insemination (AI) technology for mule production using cryopreserved jack semen for use at farmers' door.
- Perfection and propagation of AI techniques in horse and pony production using frozen semen of true to breed indigenous stallions for the consortia of threatened breeds in India.
- Breed characterization and *in situ* conservation of various indigenous breed of horses.
- Effective utilization of equine draught power for economically weaker section of the society.
- Explorative research for value addition of equine products and by-products namely blood/serum, dung, urine, milk, placenta and hair.
- Extension activities through information technology and institute's development programmes for the upgradation of the indigenous breeds of equids in different parts of the country in collaboration with respective State departments.

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:

a) **Equine Herpes virus-1 (EHV-1):** A highly sensitive and specific neutralizing monoclonal antibody-based diagnostic kit Equiherpes B-ELISA was developed. This kit utilises serum samples using single dilution thus making it very economical. Presently the kit is under commercialization process.

b) **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 G has also been developed for differentiation of EHV-1 and EHV-4 and is under routine use.

c) **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. The diagnostic sensitivity (DSn) and specificity (DSp) of ELISA was 1.0 and 0.96, respectively. This assay has been validated by two external laboratories using bovine, sheep and equine rotavirus samples and detects rotavirus infection among different animals. A 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.

d) **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 gene has also been developed for diagnosis of EIV. Additionally development of monoclonal antibody based sandwich ELISA for antigenic detection is under progress.

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

ELISA kit was 0.97 and 0.96. This assay has been validated by internal and external laboratories.

f) **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.

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

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

i) **Equine viral arteritis (EVA):** Virus neutralization test is routinely used for serodiagnosis of EVA.

Vaccines and Immuno-biologicals developed

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 very encouraging results.

b) **Updated Equine influenza vaccine:** Previously, the Centre has 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 different from the 1987 isolates. 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 equines in field trials. Further, a new cell culture-based inactivated EI vaccine is being developed by the Centre.

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

d) **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

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 NRCE about the status of African horse sickness (AHS) in the country helped in declaring India free of AHS in 2006 by Office International des Epizooties (OIE).
- Outbreaks of glanders in equines have been detected since 2006-07 and control measures are being adopted for preventing its further spread. After a brief lull for two years, the disease again erupted in December 2010, in Chandpur area of Bijnor (UP). In 2012, team of scientists from NRCE investigated the cases with respiratory illness and cutaneous lesions in Bulandshahar, UP. Four mules in Ahmedpur village of Agotta Block (District Bulandshahr) and two mules in Shikarpur of the same district were found positive for glanders in clinical and serological examinations (CFT and ELISA). Cutaneous and nasal forms of glanders were observed in the affected mules. During 2012-13, twelve samples were found to be seropositive for

glanders from UP (n=7), HP (n=4) and Chattisgarh (n=1). To contain the disease, the follow up programme needs to be strengthened by the State Animal Husbandry Department, with the technical support from NRCE, in the nosoarea in view of the recurring cases of glanders from this region.

- NRCE diagnosed equine influenza (EI) in India in 2008 from Jammu region (July, 2008) that subsequently affected equines in 13 different states. The biosecurity measures were implemented in collaboration with various State Animal Husbandry Departments. No new cases of EI have been reported from India since May 2009.
- NRCE has continuously been screening equines for equine infectious anaemia from 1998. One mule has been found seropositive during 2009-10 followed by a horse detected positive in 2011-12 in Haryana state.

Molecular characterization of equine pathogens

Equine influenza virus (EIV): HA genes of EIV isolates from 2008 outbreak (A/eq/Jammu-Katra/08, A/eq/Mysore/08 and A/eq/Ahmedabad/09) were cloned and sequenced. Phylogenetic analysis established that 2008 EI outbreak in India was due to eq/2 (H3N8) subtype and 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.

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 of JE 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% 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 (14 isolates), EHV-4 (14), equine rotavirus (29), equine influenza (11), Japanese encephalitis virus (2), West Nile virus (1), *Rhodococcus equi, Streptococcus equi, S. zooepidemicus, S. equisimilis, Burkholderia mallei, Salmonella* Abortusequi, *Enterobacter aerogenes, E. coli, Staphylococcus aureus and Trypanosoma evansi* (3).
- NRCE has a number of hybridomas, secreting monoclonal antibodies against equine herpes virus-1, equine rotavirus, Japanese encephalitis virus and West Nile virus.
- NRCE has a repository of more than 15000 equine serum samples collected from different geographical locations in its Equine Serum Bank.
- NRCE has a collection of more than 100 recombinant plasmid clones with recombinant genes of pathogens including equine influenza virus, equine rotavirus, EHV-1, EHV-4, EI, JEV, EIAV, *R. equi, Burkholderia mallei, Trypanosoma evansi* and *Theileria equi*.

Indigenous breed characterization

Phenotypic characterization of Indigenous horse and pony breeds

Populations of 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. Marwari was the tallest and significantly different from other breeds in most of the biometric indices. Spiti was the shortest breed among all the six horse and pony breeds. Sex-wise differences were also observed in some of the biometric indices in different breeds.

In Marwari and Kathiawari breeds, both stallions and mares can 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. This report aims at providing reference data for identification and comparison of different breeds of equines in India with a view to raise awareness among animal geneticists and breeders for production of true to breed animals, conservation and better management of these precious genetic resources.

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, 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).

The neighbor-joining dendrogram using the allele sharing distance clearly defined clusters for most of the breeds; Indian horse and pony breeds clustered separately while Thoroughbred formed a separate out-group.

Establishment of Nucleus Herd

- **Exotic Donkeys:** Jennies and jacks of European breed (Poitu), imported from France through ODA, UK in 1990, are being maintained at EPC, Bikaner for the improvement of indigenous donkeys and production of superior mules.
- **Marwari Horses:** In 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: NRCE has initiated an *in-vivo* conservation programme in the form of developing an equine sanctuary at EPC, Bikaner. Under this, 12 Zanskari ponies (eight mares & four stallions) were brought from Zanskar valley, Kargil, Ladakh, Jammu & Kashmir in November, 2009. In 2014, a total of 11 Manipuri ponies (seven mares & four stallions) were brought from Imphal, Manipur.

Improvement in production potential of equines

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

Pregnancy diagnosis: An eCG based sandwich ELISA has been developed for detection of pregnancy between days 30 to 150 of gestation in mares. The kit is cost effective, horse specific and animal friendly.

Pregnancy diagnosis between days 14 and 18 post-insemination has been achieved using ultrasonography in donkey and horse mares.

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

Utilization of animal energy with enhanced system efficiency

- Single animal drawn matching plough, seed drill (two

furrow) and harness were designed and developed for donkeys and mules for performing various agricultural operations. Animal energy potential was utilized successfully in agricultural operations namely ploughing and sowing for different work hours without any adverse effect on the animals.

- Single exotic donkey is able to plough 0.514 acre land in three hours. Average depth and width of furrow was observed as 4.75 and 10 inches, respectively. In sowing operation, two hours continuous work was performed by exotic donkeys. Average land sowed was 0.662 acre per 2 hour by the single donkey. Average speed of operation was recorded as 2.635 kmph. The donkey attained almost normal physiological levels after one hour of rest.
- Similarly, mules can also be used efficiently in different agricultural operations as all resumed to normal physiological conditions by the next morning.
- 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 this problem, vermicompost is being prepared using equine dung in readymade vermibeds successfully and it is being applied in agricultural fields, lawns and plants.

Patents

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

Services

NRCE provides following services to the farmers and equine breeders:

- The Centre provides disease diagnostic services for various infectious and non-infectious equine diseases to equine owners, breeders, State Animal Husbandry Departments, police and army horses.
- Artificial insemination to augment the production of superior quality Marwari horses, mules and donkeys.
- Quality jacks and jennies are supplied to various states, breeding societies and farmers, for production of superior quality mules and donkeys.
- NRCE is providing health certification for movement of equines within and outside the country. This facility has helped in promotion of export of horses.
- 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. The scientific and technical staff provides clinical and diagnostic (including pregnancy diagnosis) services and consultancy to the farmers on demand in the areas of equine health and production. Farmers are imparted trainings and supplied education materials for equine management, production and health.
- 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:

a. Exploration and collection of microorganisms of animal origin/significance/relevance

b. Central storage of animal microbes from existing culture collection centres, institutions and universities

c. Characterization, Documentation and Digitization of microbial database of cultures of animal microbes

d. Development of a National Microbial Gene Bank for conserving the biodiversity of animal microbes

e. Conservation (both short-term and long-term) and utilization of microorganisms

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

Mandate

a. To act as a national repository of microorganisms including recombinant cultures and plasmids.

b. Identification, characterization and documentation of animal microbes.

c. Conservation, maintenance, surveillance and utilization for R & D.

d. Human Resource Development (HRD)

Milestone Achievements

- First isolation and characterization of *Bordetella bronchiseptica* from horse.
- First isolation and characterization of *Actionobacillus equilli* from foal.
- First isolation and characterization of *Staphylococcus hyicus* from pig.
- First isolation and characterization of *Corynebacterium pseudotuberculosis* and *Corynebacterium bovis* from horse.
- First detection of Methicillin-resistant Coagulase Negative *Staphylococcus sciuri* from goat milk.
- First detection, isolation and identification of *Mannheimia varigena* from buffalo.
- First isolation of *Providencia thailandensis* from equine oral cavity.
- First isolation and identification of *Nocardia ototidiscaviarum* from equine granulomatous pneumonia.
- First isolation and identification of *Escherichia hermanii* from equine semen.
- Isolation and identification of strain of *Enterococcus asini* from horse.
- Isolation and identification of *Nocardiopsis alba* from equine semen sample.
- Isolation and identification of *Moraxella ovis* from case of sheep conjunctivitis.
- Isolation and identification of *Rhodococcus gordoniae* from horse and *Rhodococcus coprophilus* from camel.
- Laboratory confirmed cases of Camelpox zoonosis-first report in the world.
- Isolation and characterization of camelpox virus (CMLV) from outbreaks (2009) in Delhi, Jaisalmer & Barmer.
- Isolation and characterization of zoonotic buffalopox virus (BPXV) from outbreak (2010) in Maharashtra
- Isolation and characterization of buffalopox virus (BPXV) from outbreak (2011) in cattle, buffalo and humans in Meerut, U.P.
- Strengthening of repository with Veterinary microbes during the year :
  - ❒ Bacteria accessioned : 73
  - ❒ Virus accessioned : 11
  - ❒ Bacteriophage accessioned : 13
  - ❒ Recombinant clones accessioned : 59
  - ❒ Genomic DNA accessioned : 38

VTCC repository includes bacterial isolates represented by more than 700 isolates belonging to greater than 40 Genus viz. *Rhodococcus* spp., *Corynebacterium* spp., *Trueperella* spp, *Escherichia* (*coli*, *fergusonii* & *hermanii*) *Bordetella bronchiseptica, Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Enterococcus* spp., *Pseudomonas* spp., *Brucella* spp., *Providencia* spp., *Salmonella* spp., *Proteus* spp., *Edwardsiella* spp., *Streptomyces* spp., *Nocardia* spp., *Nocardiopsis* spp., *Listeria* spp, *Campylobacter* spp., *Moraxella* spp, *Acinetobacter* spp., *Achromobacter* spp., *Flavobacterium* spp., *Branhamella* spp., *Klebsiella* spp., *Citrobacter* spp., *Serratia* spp., *Exiguobacterium* spp., *Bacillus* spp., *Lysinibacillus* spp., *Paenibacillus* spp., *Jeotga*llibacillus spp., *Brevibacillus* spp., *Aeromonas* spp., *Shigella* spp., *Actinobacillus* spp., *Pasteurella* spp., *Lactobacillus* spp., *Methanogenic bacteria, Pediococcus* spp., *Leuconostoc* spp., belonging to phylum Firmicutes, Proteobacteria and Actinobacteria. Viral isolates viz. camelpox virus, buffalopox virus, goatpox virus, bovine herpes virus-1, equine herpes virus-1 & 4, equine influenza virus, bovine rotavirus, human rotavirus, Japanese encephalitis virus.

Staff position of NRCE and VTCC (as on 31.03.2014)

Name of the post	NRCE			VTCC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	1	-	1	-	-	-
Scientific	26	21	5	10	8	2
Technical	24	22	1	1	-	1
Administrative	14	11	3	-	-	-
Supporting	22	19	3	-	-	-
Total	87	73	13	11	8	3

Major Landmarks

1985	NRCE established at Hisar with Prof. P. K. Uppal joining as Founder Director
1987	Outbreak of Equine Influenza in Northern India
1989	Sub Campus of NRCE established at Bikaner for research on production in equines
1989	Occurrence of Equine Infectious Anaemia in India
1990	Exotic donkey germplasm with Poitu blood introduced from France
1991	Artificial insemination (AI) initiated in equines using fresh extended liquid semen
1991	Early pregnancy diagnosis (15 days post insemination) using ultrasonography
1995	Ciq-ELISA developed for detection of circulating immune complexes in EIA-infected horses
1995	Development of field-oriented immune-stick ELISA kit for detection of EHV-1 latent infection in Throughbred horses
1995	Cryopreservation of Jack semen and technology of AI perfected using frozen semen with 40% conception rate
1996	Establishment of a nucleus herd of Marwari horses at Bikaner campus
1996	Crystal structure of mare milk lactoferrin deduced by crystallography
1996	New carpet fabric developed by blending of donkey and sheep hair (Assheep)
1997	Equine Influenza vaccine using indigenous isolate (A/Equi-2/Ludhiana/87) released
2001	Patent for complement fixation test based diagnostic (COFEB)
2003	An Indian patent granted to a diagnostic kit for forecasting EHV
2005	Mab-based sELISA for detection of animal rotaviruses
2005	Establishment of Veterinary Type Culture 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

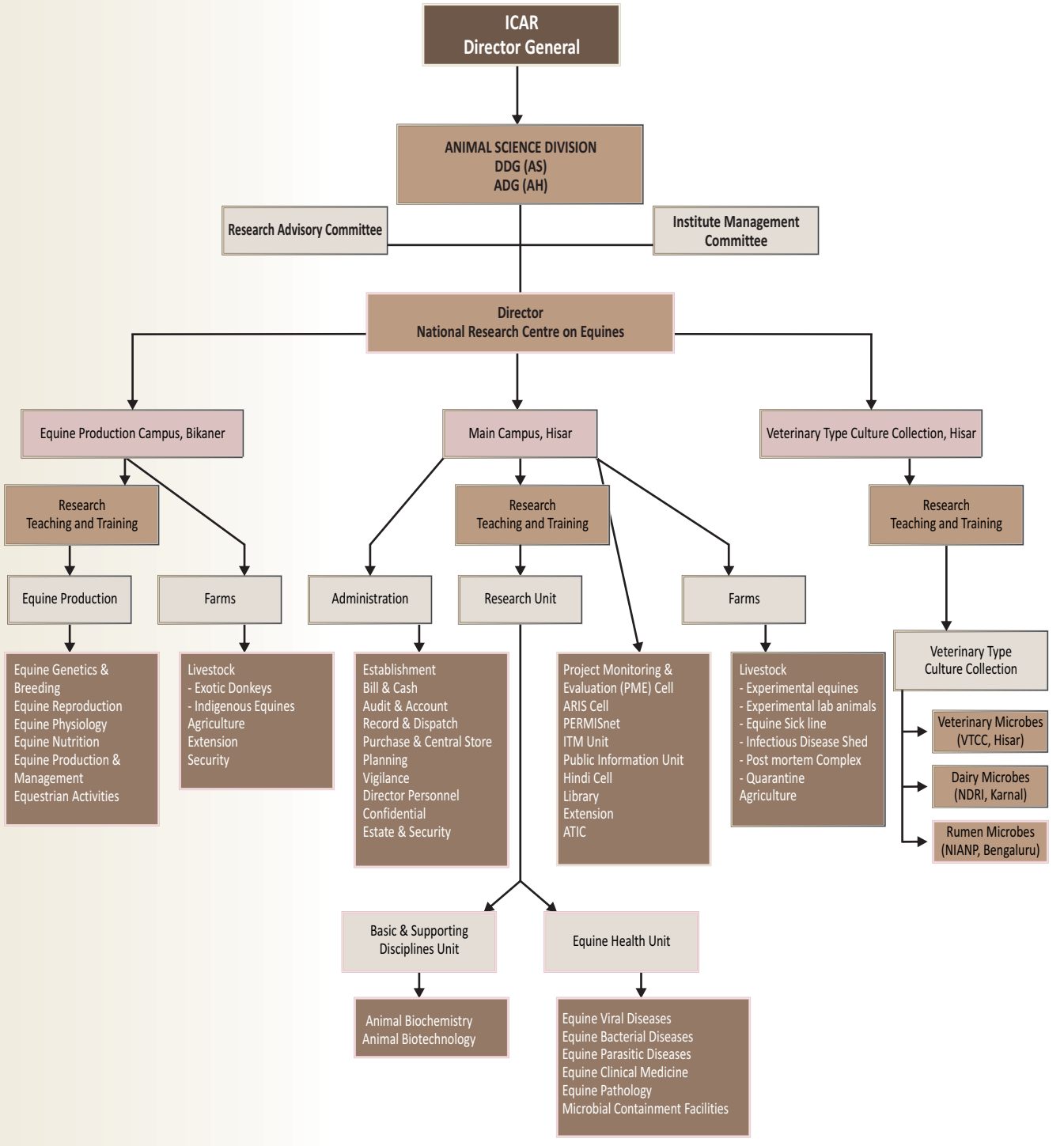
2010	Equine sanctuary for conservation of indigenous breeds of horses and indigenous donkeys initiated
2010	A new clade designated as 'Asian Clade' of Equine Influenza Virus reported
2010	Award of OIE twining project on Equine Poroplasmosis between NRCPD, Japan and NRCE, India
2010	EIA-positive mule 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 Veterinary Type Culture Collection (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
2013	Re-emergence of Glanders in Uttar Pradesh
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) has been proven as a potent diagnostic reagent for diagnosis of *Trypanosoma evansi* infection.

Summary of Expenditure & Revenue Generation

(₹ in lacs)

Summary of Expenditure		2012-13	2013-14
	Non-plan		
1.	Establishment charges including LSP/PF, wages, OTA	560.30	590.41
2.	Travelling allowances	3.99	3.99
3.	Others charges including equipments & recurring charges	406.90	322.94
4.	Works	0.00	0.00
	Total Non-Plan Expenditure	**971.19**	**917.34**
	Plan		
1.	Establishment charges including LSP/PF, wages, OTA	0.00	0.00
2.	Traveling allowances & HRD	20.16	22.00
3.	Others charges including equipments & recurring charges	668.37	508.92
4.	Works	233.06	204.00
	Total Plan Expenditure	921.59	734.92
	Total Expenditure (Plan & Non Plan)	**1892.78**	**1652.26**
Summary of Revenue Generation		**(₹)**	**(₹)**
1.	Sale of farm produce	1486307.00	3127856.00
2.	Sale of livestock	250100.00	765900.00
3.	Sale of publication and Advertisements	2020.00	39201.00
4.	License fee	58822.00	110196.00
5.	Interest on loans and advances	232490.00	178750.00
6.	Interest on short term deposits	1047019.00	3016067.00
7.	Income from internal resource generation	4672647.00	4541622.00
8.	Receipt from services	0.00	0.00
9.	Other misc. receipts	1971139.00	1115512.00
	Total Revenue	**9720544.00**	**12895104.00**

Organizational Set-Up



Research Achievements

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

To monitor the equine disease situation in the country and to combat any new incursion, team of scientists from NRCE carry our routine surveillance program in addition to investigations on the samples received from unorganized and organized sector. Apart from this, NRCE tests samples from animals under continuous movement including those being imported or exported from the country. During the year 2013-14, we conducted sero-surveillance on samples received/ collected from various states of India, viz. Maharashtra, Rajasthan, Chandigarh, Delhi, Haryana, Punjab, Tamil Nadu, Uttar Pradesh, Karnataka, Andhra Pradesh, Uttarakhand, Madhya Pradesh, Gujarat, Chhattisgarh, Himachal Pradesh, Assam and West Bengal.

Overall 1442 serum samples were collected by NRCE scientists from the above states for surveillance of various diseases (Table 1). Besides this, we received samples for disease investigation (1183 for equine influenza, 293 for EHV-1, 282 for JE/WNV, 364 for glanders) and for contractual services (4337 for EIA and 5738 for glanders).

Investigations on samples for EIA by Coggins test on a total of 5795 serum samples from Thoroughbred as well as indigenous equines revealed a negative status. Similarly haemagglutination inhibition assay on serum samples for equine influenza revealed negative status barring 32 samples with known history of vaccination. Screening of serum samples for EHV-1 revealed 81 samples to be positive for EHV-1 antibodies which included testing of 293 samples from disease investigation (Fig. 1a) where in the disease outbreaks were observed and 8 EHV-1 virus were isolated from cases of abortions. Screening of 1735 serum samples for JE/WN revealed seropositivity in 10.6%.

A total of 7044 serum samples were tested for glanders, which included samples from S&M (1442), disease investigation (364) and contractual service (5238). Twelve samples were found to be seropositive from UP (n=7), HP (n=4) and Chhattisgarh (n=1).

1782 serum samples were tested for *T. evansi*, which included S&M (1442), disease investigation (202) and contractual service (138) and 117 samples were found to be seropositive (Fig. 1b).

A total of 28 samples received from organized industry were attempted for virus isolation from samples of abortions and EHV1 could be isolated from 8 samples. In addition, 54 samples were tested and found negative for EVA under DI and 67 samples tested negative under contractual service.

NRCE helped in preparation of dossier for freedom of disease free status of African horse sickness (AHS) and tested 542 serum samples collected during 2011-12, 2012-13 and 2013-14 as per request from DAHDF. All the samples tested negative for the antibodies against AHS. In addition 30 samples were tested negative for AHS under contractual service.

1442 serum samples tested for Brucellosis and *Salmonella* Abortusequi (H antigen) revealed no positive samples.

Bacteriological analysis done on 293 samples, originating from Rajasthan, HP, Haryana, UP, AP, Punjab and Chhattisgarh including nasal swabs, vaginal swabs, uterine swab, urethral swab, tissues from PM, aborted foetus, rectal swab, faecal sample and soil sample yielded 94 isolates (Table 2) including *Burkholderia mallei* (3), *E. coli* (44), *Streptococcus equi* subsp. *zooepidemicus* (6), Group C *Streptococcus* (7), α-hemolyic *Streptococci* (4), β-hemolyic Streptococci (5), *Klebsiella* species (2), *Bacillus* species (2), *Clostridium* species (20) and unidentified Gram negative bacilli (1). A total of 201 samples from animal quarantine centres tested for CEM were negative. Antibiotic sensitivity testing was also done and results were conveyed to various concerned quarters. Cases of glanders were reported from UP, HP and Chhattisgarh.

Table 1. Seroprevalence of important equine diseases

Sr. No.	State	EIA	EI	Glanders	Tryps	EHV-1	Piroplamosis *T. equi*	JE/WNV	*Sal.*ab.*equi*	Brucellosis
1.	Rajasthan	0/921	0/921	0/921	18/921	17/921	210/921	93/921	0/921	0/921
2.	AP	0/67	0/67	0/67	34/67	0/67	25/67	0/67	0/67	0/67
3.	Haryana	0/58	0/58	0/58	0/58	3/58	21/58	16/58	0/58	0/58
4.	West Bengal	0/93	0/93	0/93	0/93	2/93	NIL	16/93	0/93	0/93
5.	HP	0/96	0/96	0/96	0/96	4/96	12/96	0/96	0/96	0/96
6.	UP	0/147	0/147	0/147	21/147	5/147	53/147	18/147	0/147	0/147
7.	Gujarat	0/23	0/23	0/23	0/23	0/23	2/12	1/23	0/23	0/23
8.	Delhi	0/13	0/13	0/13	0/13	0/13	3/13	1/13	0/13	0/13
9.	MP	0/24	0/24	0/24	0/24	0/24	4/24	8/24	0/24	0/24
	Total	0/1442	0/1442	0/1442	73/1442 (5.06%)	31/1442 (2.14%)	330/1338 (24.66%)	153/1442 (10.61%)	0/1442	0/1442

Table 2. Bacteria isolated from clinical samples

Bacteria	No.	Sample	From
Burkholderia mallei	3	Pus (1), Nasal Swab (1), Post-mortem tissue (1)	Himachal Pradesh (2), UP (1)
E. coli	44	PM tissue (31), Nasal Swab (2), Rectal swab (3), Faecal Swab (1), Aborted foetal contents (3), Faecal sample(4)	Rajasthan (36), Haryana (8)
Streptococcus equi subsp *zooepidemicus*	6	Nasal Swab (6)	Himachal Pradesh (1), Uttar Pradesh (4), Haryana (1)
Group C streptococci	7	Nasal Swab (4), Vaginal Swab (3)	Himachal Pradesh (2), Uttar Pradesh (2), Haryana (3)
α-hemolytic streptococci	4	Nasal Swab (2), PM tissue (2)	Uttar Pradesh (2), Rajasthan (2)
β-hemolytic streptococci	5	Nasal Swab (5)	Himachal Pradesh (4), Chhattisgarh (1)
Klebsiella species	2	Nasal Swab (2)	Rajasthan (2)
Bacillus species	2	Nasal Swab (2)	Rajasthan (2)
Clostridium species	20	Faecal sample (20)	Rajasthan (20)
Gram negative bacilli	1	Aborted foetal contents (1)	Haryana (1)
Total	**94**		

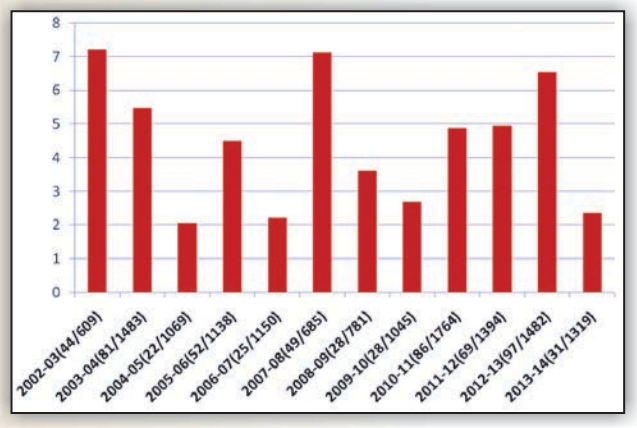


Fig. 1a. Percent seroprevalence of EHV-1 (Trends)

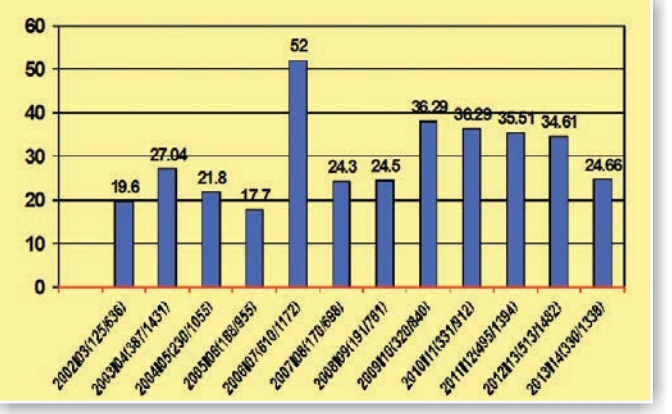


Fig. 1b. Percent seroprevalence of *T. equi* (Trends)

Disease investigation through post-mortem examination and morbid material/ biopsy received from the field was performed. Important conditions recorded on the 27 samples received for histopathology/morbid material received from the field included squamous cell carcinoma (1), necrotic haemorrhagic enteritis due to Clostridial infection (1), acute necrotic hepatitis (1), congestive heart failure (1), non-suppurative encephalitis (3), anoxia (1), toxaemia due to retained placenta (1), fatty liver and encephalopathy (2), cirrhosis and fatty degeneration of liver (2), experimental Trypanosomosis (1) and abortions due to EHV- infection (8).

(S.K. Khurana, B.K. Singh - till July, 2013, S.C. Yadav, B.R. Gulati, Praveen Malik, Rajender Kumar, Nitin Virmani, Sanjay Kumar, Sanjay Barua, R.K. Vaid, Anju Manuja, Ramesh Dedar and H. Singha)

Detection of glanders among indigenous equines in Uttar Pradesh, Himachal Pradesh, and Chhattisgarh in 2013

Burkholderia mallei, the etiologic agent of glanders, primarily infects horses, mules and donkeys. Chronically infected horses are believed to be the only reservoir of this host-adapted pathogen. The disease has gained re-emerging status due to several recent outbreaks in Central and South America, the Middle East, Africa, and Asia, and particularly in India. Detection of the clinical cases of equine glanders in three states during April, 2013 to March, 2014 is reported here. Besides complement fixation test (CFT), the 'gold standard' serological test, in-house developed indirect ELISA, western blot, and diagnostic PCR were also used to confirm the *B. mallei* infected equines. In indigenous equines, outbreaks were observed during the period in Uttar Pradesh, Himachal Pradesh and Chhattisgarh (Fig. 2, 3 & 4). In continuation with the past years outbreak in Uttar Pradesh, glanders was reported in 7 equines in Hardoi and Badaun of Uttar Pradesh during July-August, 2013. Four cases of glanders were also observed in Arki district, Himachal Pradesh in May-June, 2013 while a single case of disease was detected in Raipur, Chhattisgarh in September, 2013. All of the infected equines were tested serologically positive by CFT (titer 8-64) and in-house immune-assays. The causative agent *B. mallei* was isolated from two infected mules in Himachal Pradesh. In glanders-endemic areas, a reasonable control, containment and eradication of disease can only be achieved by a strict 'testing and culling of positive animals' policy with much sought co-operation from State Animal Husbandry Authority in combination with provision of reasonable compensation to equine owners.

Fig. 2.The typical glanders nodules are seen in a pony at Badaun district (UP).

Fig.3. Collection of biological samples from glanders infected equines at Arki, Himachal Pradesh.

Fig. 4. Nasal form of glanders (A) showing mucopurulant exudates, cutaneous glanders (B) with ulceration in hind limb was observed in mules in Dhundhan village, Arki, HP and Raipur, Chhattisgarh respectively.

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

Investigations on Neuropathogenic and Non-neuropathogenic variants of Equine Herpes Virus-1 (EHV-1) among Equines in India

Equine herpes virus type 1 (EHV-1) is responsible for respiratory diseases, abortions and neurological disorders in horses. The neurological disease due to EHV1 is called equine herpesvirus myeloencephalopathy (EHM). Although EHM is widely prevalent in various parts of the world, the problem has not been investiagated in India and cases with neurological illness hind quarter weakness and ataxia have been reported frequently from equines in India. Thus the circulation of neuropathogenic strains of EHV-1 and their role in reported neurological illnesses need to be thoroughly investigated. During 2013-14, we investigated whether strains circulating in India are having neuropathogenic traits or not.

EHV-1 viruses isolated from various outbreaks from 1990-2013 (n=7) and available in the NRCE repository were plaque purified and their ORF30 gene was amplified from nucleotide 2036 to 2689, encompassing the region of A/G single nucleotide polymorphism (SNP at position 2254). Sequence analysis showed that EHV1 isolates (Hisar-7/90, Tohana-1/96, Jind-1/96, Rajasthan-1/98, Delhi-1/98, Delhi-3 and Tohana-2) have A at position 2254 that codes for asparagine, indicating that these EHV1 isolates from abortion cases in India are non-neuropathogenic.

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

Cloning of glycoprotein D (gD) gene of EHV-1 for expression of recombinant protein in baculovirus system

Equine Herpes Virus-1 (EHV-1) is endemic in India and work has been carried out on its diagnostics, immune response, and immunoprophylaxis at NRCE. Centre has already developed an inactivated vaccine for prophylaxis and control of EHV-1. The control of cell associated viremia is thought to be critical for the prevention of EHV-1 abortions, therefore, we need to stimulate and strengthen the cell mediated immune responses. In view of the specific roles of the glycoproteins especially gB, gD and gM in infective stage of the pathogenesis of the infection and generation of the protective/neutralizing antibodies, these glycoproteins alone or in combination would be good target candidate for development of the immunoprophylaxis such as recombinant protein and/or DNA based vaccines against EHV-1. Keeping into view the above facts and with the aim to develop an efficacious subunit vaccine against EHV-1 using indigenous isolate, the work was initiated for eukaryotic expression of glycoprotein D by designing gene –specific primer for amplification of the extracytoplasmic region of the glycoprotein D gene of EHV1 virus. Various extra sequences were manually added to the primers such as restriction sites for directional cloning; kozak sequences for efficient transcription and His tag & enterokinase sequences for purification of the recombinant protein. The extra cytoplasmic region (1299 bp) of the gD gene of the EHV1 virus was successfully PCR amplified, amplicons were gel purified and used for generation of donor construct. The purified amplicons were directionally cloned into MCS sites of the donor vector pFastBac (Invitrogen) using BamHI & XhoI restriction enzymes. The donor construct pFastBac-gD was confirmed by colony PCR and RE release of the insert from the recombinant plasmids. The construct will be utilized for generation of recombinant baculovirus having cloned gD gene in the virus genome and subsequent expression of the recombinant gD protein for investigating its role in protection against EHV-1.

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

Diversity of Mx gene in horses and its association with susceptibility vis-a-vis resistance against Equine Influenza

Equine influenza (EI) is primarily an infection of the upper respiratory tract and is one of the major infectious respiratory diseases having economic significance in equines. Differences in innate immune mechanisms have

been shown to be critical in host susceptibility to many viral infections. The myxovirus resistance protein (Mx) has been reported to confer resistance to Orthomyxo virus infection by modifying cellular functions needed along the viral replication pathway. In mice, the Mx1 has been shown to protect against influenza virus infection. In the present study, the diversity of Mx gene and its association with EI resistance and susceptibility in Marwari horses was investigated. Blood samples were collected from horses declared positive for equine influenza and in contact animals with history of no clinical signs. Peripheral blood mononuclear cells were isolated and stimulated with IFN alpha/beta. Reverse transcription polymerase chain reaction was performed to amplify Mx gene using specific primers. The amplified gene products from representative samples were cloned and sequenced. Nucleotide sequences and deduced amino acid sequences were analyzed to determine association with susceptibility/ resistance to EI. Polymorphism was observed in nucleotide and deduced amino acid sequences of Mx gene in Marwari horses. Evolutionary distances based on nucleotide sequences with in equines were 0.3-2.0% and ranged between 20-24% with other species. On phylogenetic analysis all equine sequences clustered together while other species formed separate clade (Fig. 5). Out of a total 24 amino acids substitutions sorting intolerant from tolerant (SIFT) analysis predicted 13 substitutions with functional consequences. Five substitutions (V67A, W123L, E346Y, N347Y, S689N) were observed only in resistant animals.

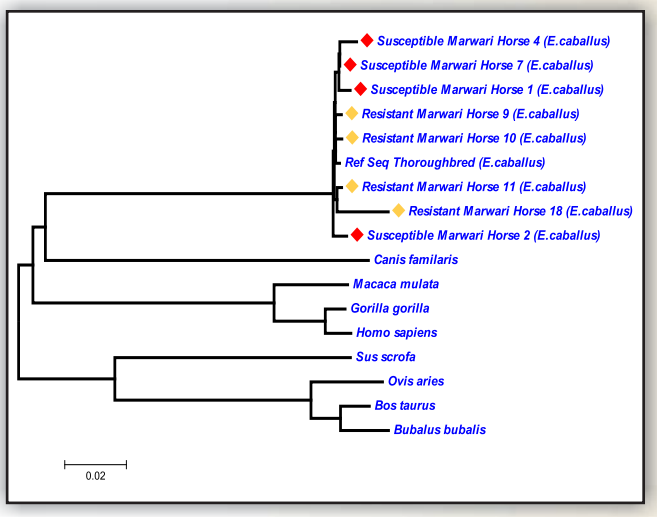


Fig. 5. Phylogenetic tree of Mx gene by neighbor-joining method using MEGA 4. Equine Influenza Resistant animals (red bullets) & susceptible animals (yellow bullets)

(Balvinder K. Manuja, Anju Manuja and R.C. Sharma)

CpG-ODN-induced effect against *Trypanosoma evansi* in peripheral blood mononuclear cells (PBMCs) of Marwari horse

Trypanosma evansi is the causative agent of surra, one of the most common equine diseases. Due to antigenic variation and the limited success in vector control, there is currently no effective preventive measure against trypanosomiosis. Synthetic oligodeoxynucleotides containing CpG-motifs (CpG-ODN) are capable of driving immunity toward a Th1 bias. No study till date was carried out on the use of CpG-ODN for enhancing immune response against *T. evansi* in equines. Considering the importance of Th1 mechanisms in resistance against parasite, *in vitro* immunostimulatory effect of CpG-ODN Class-A and C in peripheral blood mononuclear cells (PBMCs) of horses was studied. Increased Th1 cytokine production (IFN-α, TNF-α, IL12) in response to both classes of CpG-ODN was observed. The significant proliferative responses in terms of stimulation indices were observed with ODNs as well as whole cell lysate antigen of *T. evansi*. The highest stimulation index was observed when horse PBMCs were co-cultured with CpGs and whole cell lysate antigen of *T. evansi* showing synergistic effect. A number of factors produced by mononuclear cells including nitric oxide, reactive oxygen species, and tumor necrosis factor alpha, have all been shown to produce CpG-ODN-induced enhancement of host resistance to trypanosomes.

(Anju Manuja, Balvinder Kumar and Harisankar Singha)

CpG-ODN class C mediated immunostimulation in rabbit model of *Trypanosoma evansi* infection

Trypanosoma evansi produces a state of immuno-suppression, which renders the infected host more susceptible to secondary infections and results in poor immune response to bacterial and viral vaccines. Therefore, considerable interest has been generated in finding ways to stimulate the host immune system. The present work aimed to determine the immuno-stimulatory effects of CpG-ODN class C in *T. evansi* infected rabbits. Rabbits were inoculated with CpG C and challenged with *T. evansi* parasite. The reduction in parasitic load, delayed onset of clinical signs with reduced severity were observed in CpG treated and *T. evansi* challenged rabbits in comparison to that of *T. evansi* infected rabbits without CpG treatment. It also enhanced humoral immune responses in rabbits. Histopathological findings in liver and spleen revealed that CpG-ODN induced enhancement in mononuclear cell infiltration and secondary B cell follicles. These results clearly demonstrate that CpG-ODN class C, has immuno-stimulatory properties against *T. evansi* in rabbit model for trypanosomosis.

(Anju Manuja, Balvinder Kumar and Harisankar Singha)

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

Equine piroplasmosis is an economically important disease of equids and sporadic outbreaks are not uncommon. A significant segment of the equine population has carrier status to this infection, due to which the draugtability of these animals gets lowered and poor farmers suffer economically. Indian-bred horses and mules are considered to be pre-immune carriers of the *T. equi* infection. Imidocarb dipropionate, the only drug of first choice against *T. equi* infection, is also partially successful in eliminating *T. equi* from the infected horses. Recently, National Research Centre on Equines, Hisar has developed micro aerophilus stationary phase (MASP) *in vitro* culture system for *T. equi* protozoa, which is a very good biological tool for screening batteries of drug molecules for therapeutic efficacy. We selected some novel drug molecules based on targets available in *T. equi* genome. Some of the selected targets were - HSP-90; nuclear transcription factor [NF-kappaB (NF-κB)]; flavonoid, anti-oxidant; choline kinase; DOXP reductoisomearse; V-type H+ATPase; inhibitors protein synthesis the formation of β-hematin; lactate dehydrgenase. The drug molecules responsible for inhibitory effect against these targets will be tested for in vitro growth inhibitory efficacy against *Theileria equi* in MASP cultivation system and inhibitory concentration (IC50) will be determined. We have procured some of the drug molecules and *in vitro* trials are underway. Simultaneously, we are also targeting oxidative damages caused by *T. equi* parasites in RBCs. For this purpose, the protocols for estimation of oxidative stress indicators – MDA and GSH, have been optimized and standard curve were drawn as shown in figures (6 & 7).

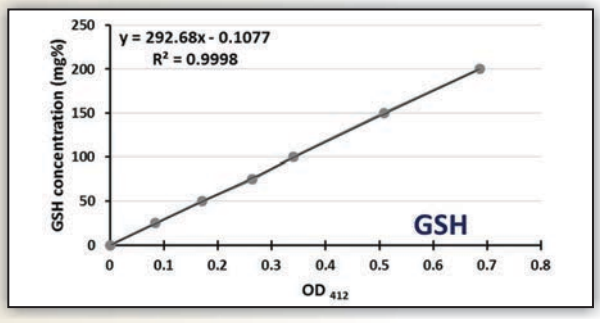


Fig. 6: Standard curve for estimation of GSH.

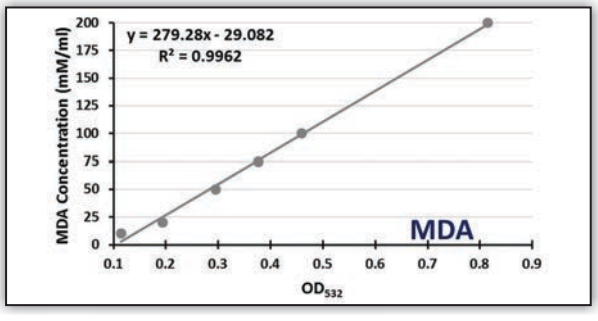


Fig. 7: Standard curve for estimation of MDA.

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

Bioavailability studies of vitamin C and E in Marwari horses

Initial studies on oral supplementation of vitamin C with normal feed and fodder revealed that supplementation has no effect on plasma ascorbic acid status as serum levels in most experiments did not increase above the endogenous pre-administration values of the vitamin. Sodium ascorbate @ 20 mg /kg body weight was given to exercising horses, 5 Km gallop in morning hours. Ascorbic acid contents were evaluated at different interval and it was observed that there was no significant difference in ascorbic acid content between supplemented and control group (Table 3).

Table 3. Plasma ascorbic acid levels in exercising horses (mg/L)

Days	Supplemented group (N=4)	Range	Control Group (N=4)	Range
1st day	3.35±0.79	1.88-5.55	2.49±0.28	2.11-3.33
15th day	2.22±0.43	1.33-3.33	2.30±0.44	1.22-3.22
30th day	2.24±0.38	1.33-3.22	2.58±0.20	2.11-3.11

exercising horses

In another study, bioavailability of orally supplementation of vitamin E @ 1mg/kg body weight in exercising horses (5 km gallop) was evaluated. It was observed that in exercising horses, oral supplementation of vitamin increased plasma vitamin E levels significantly within 10 days (Table 4).

After saturation of vitamin E in plasma, further supplementation (beyond 20 days) did not increase plasma

Table 4. Plasma vitamin E status in horses after supplementation

Days	Supplemented group (N=4) (mg/L)	Range	Unsupplemented Group(N=4)	Range
1st day	1.34±0.19	1.02-1.89	1.68±0.29	1.24-2.54
10th day	3.65±0.71	2.22-5.17	1.80±0.19	1.45-2.35
20th day	4.43±0.46	3.32-5.32	1.87±0.31	1.28-2.54
30th day	4.44±0.54	3.28-5.5	1.88±0.37	1.17-2.61

More trials are required to conclude the utility of Vitamin E supplementation through oral or intravenous route in

(R.K. Dedar, Vijay Kumar, Jitender Singh and R.A. Legha)

Electrolytes losses in exercising Marwari horses and supplement formulation

To estimate the electrolyte losses during 5 km gallop exercise, 5 horses were given exercise for 40 days in morning hours during summer season. Measurement of body mass loss is considered the gold standard for assessing rapid changes in hydration with 90% of a weight change attributed to loss or gain of body water. Sweat samples were collected three times from four horses only.

Average body weight loss was observed to be 4.35 ± 0.39 Kg after exercise per day. Sweat contained 182.83±13.51 and 46.27±4.58 mmol/ml sodium and potassium contents respectively while calcium and phosphorus contents were 25.80±2.59 and 2.47±0.54 mg/ml respectively.

After calculating sweat loss and electrolytes concentration

in sweat and digestibility of electrolytes (from previous references) in exercising horses, following electrolyte, antioxidant mix along with glucose contents was formulated to maintain the electrolyte balances in adult horses (350Kg) for 5 km gallop (12 minutes) (Table 5).

Table 5. Composition of electrolyte and antioxidant formula

	Composition
Sodium chloride	50g
Potassium chloride	20g
Calcium chloride	5.2g
Vitamin E	400 mg
Glucose	100 g

Combined effect of electrolyte and antioxidant supplementation in exercising horses

For this trial, twelve adult horses were included to evaluate the combined effect of supplementation. Electrolyte and vitamin E formulation was supplemented only to 4 horses (supplement group) that were under 5 km gallop exercise for 40 days. Rest of the horses (7) were taken as controls in two groups. First control group (CG-A) included 4 exercising horses (5 km gallop) without any supplementation while second control group (CG-B) included 3 horse which were kept in stable without any exercise and supplementation. Biomarkers of oxidative stress whole blood malondialdehyde and reduced glutathione were also estimated on three different days in all the groups (Table 6). It was found that gallop exercise increases oxidative stress in hot weather as revealed by significantly increased levels of malondialdehyde and decreased levels of vitamin C in horses under gallop exercise. Supplementation of sodium chloride, potassium chloride, calcium chloride with Vitamin E was given to replenish the electrolyte stores and combat the oxidative stress.

In supplemented group blood levels of reduced glutathione (antioxidant enzyme) were increased significantly. Higher levels of reduced glutathione in supplemented group show the increased antioxidant capacity of horses under supplementation with vitamin E.

Table 6. Average values of different biomarkers and electrolytes in exercising Marwari horses (Mean ±SE)

	Supplemented Exercise (N=4x3) Mean±SE	Unsupplemented Exercise (N=4x3) Mean±SE	Resting Control (N=3x3) Mean±SE
Malondialdehyde (nmol/ml)	346±12.30	338±9.85*	311±4.08
Reduced Glutathione (mg/dl)	19.46±1.30**	14.91±1.33	12.04±1.19
Catalase	6352±293	6293±254	5412±119
Plasma Sodium (mmol/L)	144 ±2.08	141±1.77	139±2.15
Plasma Potassium(mmol/L)	4.93±0.16	4.48±0.0.16	4.30±0.15
Plasma Calcium (mg/dl)	10.15±0.34 (n=4)	11.75±0.74 (n=4)	-

-** significantly different than unsupplemented exercise group P value < .05
-* significantly different than resting control group P value < .05

(R.K. Dedar, Vijay Kumar, Jitender Singh and R.A. Legha)

Genetic Characterization of Indian Horse & Pony Breeds

Genetic diversity analysis of Indian horse and pony breeds carried out earlier with all the six registered equine breeds along with Thoroughbred horses revealed high values of allele number, observed and expected heterozygosity, polymorphism information content for all Indian breeds except Spiti ponies in comparison to Thoroughbred horses which indicated high genetic diversity in them. Further analysis on the basis of estimates of Fst between each pair of breeds revealed that genetic differentiation between Spiti and Thoroughbred (0.1729) was the maximum followed by Spiti and Kathiawari (0.1725) while Zanskari and Manipuri were the least differentiated (0.0379). Individual

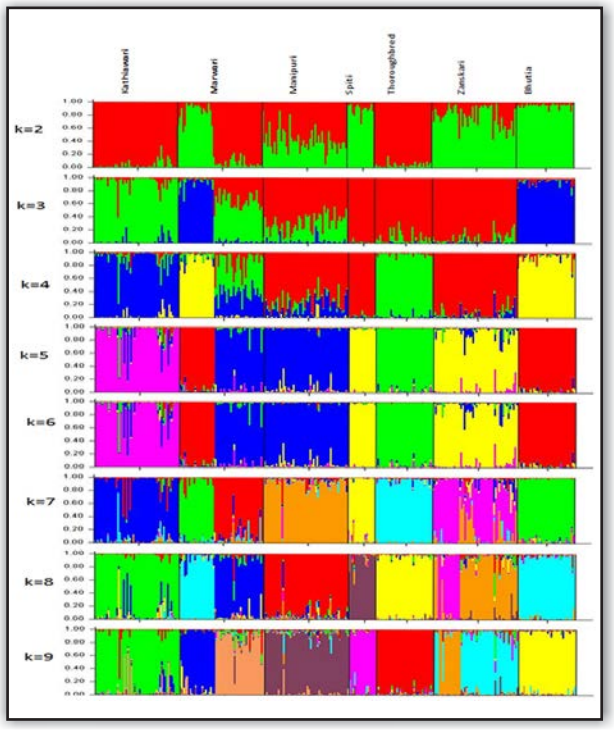


Fig. 8 : Graphical presentation of population structure analysis for a sample of 284 horses and ponies (a priory defined 7 populations including Thoroughbreds).

assignment indicated admixture in all the breeds except Thoroughbred horses. The neighbor-joining dendrogram using the allele sharing distance clearly defined clusters for most of the breeds, Indian horse and pony breeds clustered separately while Thoroughbred formed a separate out-group. Advanced Bayesian analysis using STRUCTURE revealed three distinctive clusters of Indian horse and pony breeds, Kathiawari the most prominent cluster as horse breed, second of Zanskari, Spiti and Manipuri ponies and third one having Bhutia and a sub population of Marwari horses (Fig. 8). Clustering of one sub-population of Marwari with Bhutia indicated their common ancestries which need further investigations as both these are distinct at phenotypic level and geographically isolated. Kathiawari represents the oldest stock and has contributed in other Indian breeds. Similarity of Kathiawari and Marwari horses is attributed to contiguity of their breeding tracts.

(A.K. Gupta, Anuradha Bhardwaj, S.N. Tandon, S.C. Gupta, Neelam Gupta and Yash Pal)

Phenotypic characterization of donkey populations from different geographic areas

About 50 donkeys each were selected from seven different geographic areas namely J&K (Leh), HP (Spiti), Haryana, Gujarat, Bihar, Maharashtra (Baramati) and Rajasthan for their phenotypic characterization. Fifteen biometric indices including height at wither (HW), body length (BL), heart girth (HG), face length (FL) and width(FW), ear length(EL) and width (EW), hoof length (HoL) and width (HoW), fore (FLL) and hind (HLL) leg lengths, height at knee (HK) and hock (HH), canon and gap between ears (pole) were recorded for these donkey populations along with Poitu breed of donkeys for comparative analysis.

Among indigenous donkeys, average body length of donkeys from Spiti, Gujarat, Rajasthan, Baramati and Leh areas varied from 95.76 to 103.68 cm while Poitu donkeys (138.79 cm) was significantly higher than all the seven indigenous donkey populations. Heights at wither was significantly the lowest for Leh donkeys than that for Poitou as well as all other indigenous populations included in this study. Leg lengths (fore and Hind) which contribute towards height of the donkeys were also studied. FLL, HK, canon, HLL and height at hock (HH) were significantly higher in Poitu donkeys than all Indigenous donkey populations. Among local populations, FLL, HK and HLL in Spiti and Canon and HH in Leh populations were significantly lower than other populations. Further FLL was significantly different in almost all the local Indian populations while HLL, HH and canon were almost same in donkeys from Gujarat, Rajasthan and Baramati areas..

Physical appearance of face was evaluated in terms of FL, FW, El and EW in all the indigenous donkey populations and Poitu donkeys. Poitu donkeys had maximum and significantly higher values for all these indices than all local indigenous populations. Face length of donkeys from Spiti, Gujarat and Baramati were at par with each other while donkeys from Leh had smallest face length. Face width of donkeys from Spiti (14.32 cm), Gujarat (14.23 cm) and Rajasthan (14.80 cm) was at par with each other and their values indicated lean/narrow face of these populations. Among Indigenous ones, donkey from Baramati area had significantly wider face as compared to other ones. Mean

ear length (19.95 cm) and ear width (12.97 cm) were smallest one in Spiti and Leh donkeys respectively. Ear length and width of Gujarati, Rajasthani and Baramati donkeys were almost at par with each other. Gap between ear bases (pole) was maximum and significantly higher in Baramati donkeys than Poitu, Gujarati and Rajasthani donkeys. However, the values were at par with Spiti and Leh donkey populations. This study helped in categorizing indigenous donkeys as small to medium size donkey while Poitu were categorized as large size donkeys. This biometric data will also be used as baseline information in identifying these donkey populations as separate breed(s) along with other characteristics as per norms.

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

Cloning, Expression and Characterization of eCG

A. Purification of Recombinant eCG

The gene for beta-alpha eCG was cloned and expressed in *E. coli* BL21 C cells and characterized by ELISA and Western Blotting. The recombinant eCG was successfully purified from Ni-NTA spin columns because of presence of six histidine tag at N terminal of protein. The purified r-eCG was analysed on 10% SDS-PAGE (Fig. 9). The quantitation of purified recombinant protein was done with Nanodrop

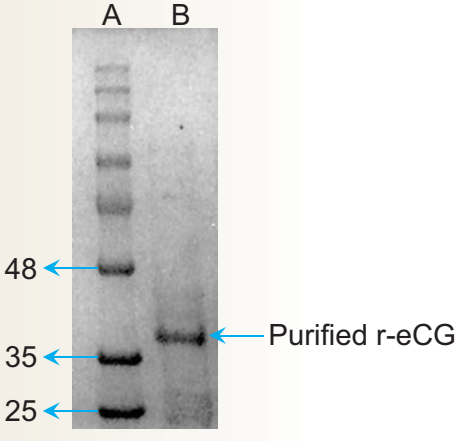


Fig. 9. Analysis of purified r-eCG on 10% SDS-PAGE. Lane A - contained protein markers while lane B contained purified r-eCG.

spectrophotometer. About 1-3 mg of pure recombinant eCG protein was obtained from 100 ml of bacterial culture.

B. *In-Silico* analysis of eCG alpha

The *in-silico* analysis of alpha eCG was done by CLC sequence viewer, DNA star and Vector NTI softwares. Geno3D (Automatic molecular modeling tool) of PBIL-IBCP Lyon-Gerland was used for construction of the homology model of eCG alpha subunit. The model generated was visualized by 3-D Molecule Viewer (a component of Vector NTI Advance 11.5.2). The 3D Homology Model (Tube diagram) of the eCG α-subunit illustrates the location of the three α- subunit loops – Loop 1, Loop 2 and Loop 3 (Fig. 10A) . This structure also illustrates the location of the region with sequences "CCFSRA" that probably may act as a binding antagonist (Fig. 10B) . The eCG alpha contains the two conserved motifs with "CKGCCFSRAYPTP" &"NHTQCYCSTCYHHK" sequences respectively. The SCRATCH Protein Predictor was used for ascertaining the antigenicity propensity score. ANTIGENpro under it is a sequence-based, alignment-free and pathogen-independant predictor of protein antigenicity. The SVM classifier summarizes the resulting predictions and predicts

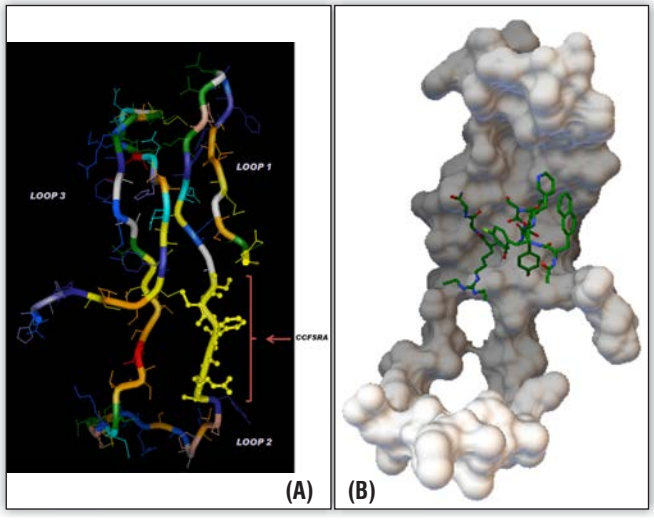


Fig. 10. (A) The 3D Homology Model (Tube diagram) of the eCG A-subunit. This figure illustrates the location of the three A-subunit loops and the location of the region with sequences "CCFSRA" that probably may act as a binding antagonist. (B) Molecular Docking studies of eCG alpha with Ganirelix. Ganirelix prevents ovulation until it is triggered by injecting human chorionic gonadotrophin (hCG). Ganirelix is known to interact with hCG.

if the protein is likely to be antigenic or not as well as the corresponding probability. We also analyzed the eCG alpha protein sequence from a diverse selection of methods for secondary structure, hydropoathy, antigenicity, amphilicity, surface probability and flexibility by using the Protean Software of the DNASTAR Lasergene software suite version 7.1 (Fig. 10B). The in-silico analysis paves the way for future studies on eCG alpha using molecular docking hunt for agonist, antagonist or drugs or inhibitors for synergism, drug discovery etc.

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

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

The project was initiated with the objective to study the follicular dynamics and associated endocrine, biochemical and gene expression changes during onset of puberty, estrous cycle, pregnancy and peripartum periods in Marwari fillies and mares. The ultrasonography assisted monitoring of the ovarian cyclicity during estrous cycle in breeding and non breeding seasons revealed that the cyclicity is more prevalent in the breeding season in summer than in the non breeding winter season, however majority of the mares continued to express cyclicity and estrous behavior during the major part of winter in Rajasthan. Erratic estrous behavior was noticed in about 30% of the mares during peak winter which comprised of ovarian quiescence, seasonal nymphomania and weak estrus symptoms inspite of good follicular activity. Progesterone analysis carried out in three animals during the estrus and pregnant stages revealed below 0.5 ng/ml in the estrus stage while it was higher than 1 ng/ml in the pregnant stages.

Real time conditions for the primers of Interleukin 8 and housekeeping Beta actin gene in the mare leukocytes have been set. The Tm was 58° C which gave amplification for both the genes.

(Vijay Kumar, Ramesh Kumar Dedar, Sanjay Kumar Ravi and J. Singh)

Molecular Characterization of Toll-like receptor 9 in Marwari & Zanskari breeds of horses, poitu and donkeys

Toll-like receptors (TLRs) are family of proteins that constitute a phylogenetically ancient system that are expressed in both vertebrate and invertebrate species. TLR9 has been characterized as a receptor and signaling by this receptor can trigger a pro-inflammatory cytokine response that influences both innate and adaptive immune responses. Significant structural differences in the extracellular domain of TLR9 account for species-specific recognition of CpG ODN sequences. TLR9 is extensively studied in human, mice and in some domestic animal species but there was no report of TLR9 characterization of Indian breeds of horses, donkeys and poitu. We characterized TLR9 sequences of Marwari & Zanskari breeds of horse, Poitu (exotic donkey) and indigenous donkeys. Phylogenic tree based on amino acid sequences of TLR9 protein from different species of equines in the

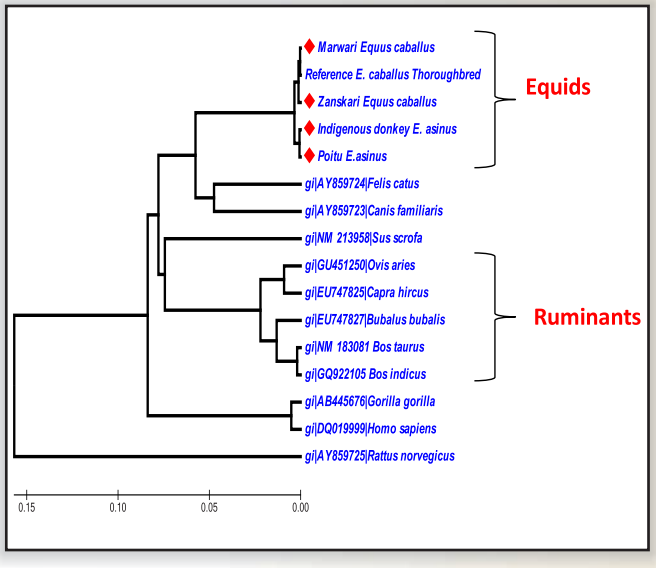


Fig. 11. Phylogenetic tree of deduced aminio acid sequences of TLR9 mRNA of eqines indicating genetic relationship with TLR9 sequences with different species retrieved from GenBank

present study and TLR9 amino acid sequences of other species retrieved from GenBank was constructed by amino acid sequence analysis using the Neighbour-Joining method (Fig 11). The TLR9 proteins from the present study clustered with *Equus caballus* protein sequences, while human, cattle, dog, sheep, mice, and buffalo formed separate clades. The analysis shows conserved sequences and close association of TLR9 proteins within species and high divergence with other species of animals. Estimates of evolutionary divergence between the sequences of TLR9 revealed *Equus caballus* Marwari breed and *Equus* asinus differ from Human by 14.7% and 14.4-14.6%, respectively; Marwari breed from Zanskari differs by 0.3%, indigenous donkey by 0.8% and Poitu by 0.3%. Blast analysis of TLR9 sequences of equines indicates that these are much closer to odd toed ungulates e.g homosapiens than to even toe ungulates (buffalo, cattle etc.). Analysis of association of variations in TLR9 gene of *Equus caballus* and *Equus asinus* was performed with reference TLR9 sequence of *Equus caballus* by sorting intolerant from tolerant (SIFT) analysis using online prediction algorithm and functional changes on the basis of amino acid substitutions were determined. Comprehensive validation of these functional changes in terms of how these structural parameters correlate with the TLR9 activation of unique host responses is further required.

(Anju Manuja, Balvinder Kumar and Harisankar Singha)

Survey report on feeding and managemental practices in Ajmer and Nagaur district

A survey was conducted in the Pushkar (Ajmer) and Tehla and Gundisar villages of Nagaur district for generating information regarding livelihood of the horsemen around the area in Rajasthan. To assess the feeding pattern and various managemental practice adopted by the equine owners, information in the form of a questionnaires was collected which included aspects of management, nutrition and housing of equines and livelihood of the farmers.

The farmers' main vocation is agriculture and horse rearing along with other animals was their side business. Majority of the farmers fed green lucerne to their horse in spring, summer, autumn and winter season. However, they preferred sorghum and moth (*Vigna aconitifolia)* in the rainy season. Farmers offered groundnut haulm, sorghum straw, wheat straw, pearl millet straw and oats straw as dry fodder year around on the basis of availability. Farmers used to feed grain mixture and green fodder once a dry, while dray fodder was offered *ad libitum* to the animals.

Feed intake and utilization studies in equines of different categories

a) Growing animals (2 years)

To estimate the energy intake and utilization by the young equine stocks, nine animals were selected in group of three. Each animals was fed with 2 kg HAFED made concentrate mixture, 5 kg green lucerne and 4 kg sewan hay. The feeding trial was conducted for 45 days. Residual matter and total feed intake was studied. During this period, a seven days digestibility trial was conducted but samples (feed sample, faeces etc) were collected only for last five days. These samples were analyzed for nutrient digestibility, total digestible crude protein and total digestible nutrients. Table (7) revealed the nutritional contents available in feed and fodders.

Feed intake varied from animal to animal and in the present findings the DMI is 2.77 %. The feed intake by the growing horses varied from 2.0 to 3.55% of the body weight (NRC, 2007). Average increase in body weight/ day after 45 days of trial was 0.13 ± 0.01 kg/young horse. Digestibility coefficient of most of nutritive components was low which may be due to inferior fodder (sewan hay) quality, and age.

b) Adult mules

Five adult mules were selected for 75 days feeding trial. Animals kept under maintenance condition, were fed with 1.88 kg concentrate, 9.4 kg green lucerne and 4.07 kg sewan hay per animal. Residual feed matter and total feed

Table 7. Nutritional contents of feed and fodder provided to equines (young horses)

Feed	Concentrate mixture	Sewan hay	Lucerne green	Dry matter intake	Digestibility coefficient (%)
Dry matter (%)	93.99±0.16	92.74±0.19	16.79±0.11	6.01±0.1	59.94±0.23
Crude protein (%)of DM	15.53±0.11	4.87±0.10	19.61±0.09	0.47±0.1	69.27±0.15
Ether extract (%) of DM	2.53±0.13	0.82±0.09	8.72±0.07	0.85±0.1	56.83±0.21
Neutral detergent fibre (%) of DM	28.17±0.08	64.70±1.2	39.01±1.01	2.23±0.1	41.80±0.22
Acid detergent fibre (%) of DM	21.52±0.09	28.83±0.91	31.86±0.92	1.24±0.1	39.35±0.24
Non fibrous carbohydrate (%) of DM	35.09±0.28	17.22±0.21	0.96±0.01	6.01±0.1	59.95±0.22
Ash (%)of DM	3.38±0.11	6.16±0.16	10.70±0.24	0.31±0.1	33.8±0.29

intake was studied and a seven days digestibility trial was conducted, with 2 days preliminary and 5 days of collection period. Then the feed sample and faeces were analyzed for nutrient digestibility, total digestible crude protein and total digestible nutrients.

Average increase in body weight after completion of trial was only 1.00 Kg/mule while feed intake was 2.25 % of body weight, which is in higher side compared to the NRC recommendations (1.8-2.0% of bd.wt). In present study, digestibility coefficients for dry matter, fat, NDF and ADF were also lower, which may be due to higher DM intake (2.25).

c) Non pregnant mares at maintenance

To estimate the energy intake and utilization by the adult mare (non pregnant), six non pregnant mares were selected and kept under maintenance condition for 45 days. Animals were fed with 4.0 kg concentrate and 8.0 kg sewan hay each. Residual feed matter and total feed intake was recorded and samples were collected for five days only. The same were analyzed for nutrient digestibility, total digestible crude protein and total digestible nutrients.

Initial and final body weight measurements revealed a net gain of 14.14±4.18 kg/animal while feed intake was observed to be about 2.16 kg per 100 kg body weight. Like other trials, digestibility coefficient of different feed nutrients was also low in this study may be due to higher DM intake (2.16%), and lower fodder quality.

d) Adult mule (under light working condition)

Four adult mules selected were subjected to light work (cart pulling for 1.15 hr with 18.0 kg force). During 5 months feeding trial, these working mules were fed 4.5 kg green oats and 7.0 kg sewan hay per animal. Residual feed matter and total feed intake was studied and samples were collected during last 5 days of digestibility trial. The feed intake was only 2.07% of body weight.

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

Foot and leg ailments in working equids

To assess the prevalence of foot and leg ailments in working equines on different surface conditions (hard pavement, sandy or kacha road), NRCE team surveyed the working equids of Kolkata city and Digha beach (200 km from Kolkata city) where animals work on hard pavement and sandy beach, respectively Besides this, the horses and mules used in carting on hard pavement for transport of agriculture produce, building material and other domestic commodities etc. (Fig. 12) in Julana (Haryana) were also assessed to recommend whether working of horses on hard pavement is good or not.

Fig. 12. Different sized horses being used with improperly-fitting poor harness

Culled horses purchased at cheap rates are being used in Victoria carriages at Kolkata and at Digha beach. There was lack basic amenities viz. proper housing (Fig. 13), access to feed and potable drinking water, saddler, shoeing and farriery where as animals at Julana are well kept and taken care off. This has led to incidence of lameness in horses at Kolkata while those at Julana are healthy and incidence of lameness was is quiet low.

Incidence of other conditions (cow hock, rope injury, frog infection, damaged hoof wall, soft non-painful swelling on fetlock joints, harness injury, contracted heal, splint bone, facial injury) was 31.90% in animals working on hard pavement in Kolkata but these afflictions were not observed in animals working on soft pavement at Digha beach. It was concluded from the findings of the study that hard pavement is not good for the horses being used currently for plying Victoria carriages in Kolkata, as use of these horses- majority of which are already having lameness and other foot problems- on hard pavement, is leading to further deterioration of their foot health. Totally sound and healthy horses with proper shoeing, defined hours of work schedule, and vigilant veterinary supervision can be used on hard pavement with least foot damage.

Fig. 13. Poor housing condition of horses kept in crowded space

(Yash Pal, Praveen Malik, Ramesh Dedar, A.A. Raut, Sanjay Kumar, Rajender Kumar and R.K. Singh)

Survey on management systems and utilization pattern of donkeys and mules

The donkey and mules are considered better than other draught animals because of inherent tolerance for dehydration and ability to work in varying climatic condition in difficult terrains. Donkeys and mules provide a means of transportion for products like farm produce, construction materials, market products, water, clay, fodder, manure and fuel more rapidly in greater amounts (Fig. 14), but at cheaper rate and in difficult terrains than motorized transportation. A survey was undertaken in Uttar Pradesh about the use of donkeys and mules regarding livelihood. Data was collected from 123 equine owners in Allahabad, Varanasi district in eastern Uttar Pradesh and Lucknow and Barabanki district in central Uttar Pradesh on socio-economic profile of equine owners, existing management systems and utilization of donkeys and mules in different activities. Survey findings on socio-economic status indicated that majority of respondents (52.03%) were from middle age group (26 to 50 years) and belonged to SC category (60.16%). Literacy level was low (53.66%). 65.04% respondents had medium family size (7 to 10 members) and about 56.10% had medium level experience in equine husbandry (11 to 20 years). Majority of respondents (55.28%) had a monthly income between ₹ 3000 to 5000.

Ownership and utilization pattern of donkeys and mules: Owners included underprivileged resource poor people in the society. They generally use these equines as pack animals or in carts for carrying different materials to earn their livelihood. The pack owners kept on an average 4-6 donkeys/mules/ponies while cart owners generally kept only one or two animals per household. Majority of owners (95.65%) in central Uttar Pradesh (Lucknow and Barabaki) were using their animals in cart transportation whereas in eastern Uttar Pradesh (Allahabad and Varanasi) majority equine owners (88.31%) were using their animals as pack for transportation of bricks at brick kilns (Fig .14). The owners used to carry 300-450 brick/cart/trip in cart transportation and 30-45 brick/trip as pack depending on size of animal and distance to be travelled. They were paid between ₹ 150-185 for 1000 bricks depending on distance and ₹ 15 for 30-40 kg load to market by cart. Daily earnings of equine owners varied between ₹ 350-800 per day.

Fig. 14. Utilization Pattern of Donkeys and Mules at Brick Kilns

Management practices: Owners of working equines in central Uttar Pradesh had good knowledge about management and welfare aspects due to interventions and awareness by NGOs whereas in eastern Uttar Pradesh owners lacked the necessary knowledge and skills to meet their animals' most basic management and welfare needs. The majority of the sores and wounds were caused due to

overloading, poor quality of harness material and uneven loads. Health problems in working equines were also mainly due to ill-treatment by owners, hakims and due to traditional and unconventional methods of treatment. Grooming practices were followed regularly by most of the respondents. The mules were shoed regularly but shoeing was not observed in case of donkeys. Deworming and vaccination of equines were observed in central Uttar Pradesh as a common practice as indicated in Fig. 15. Women were actively participating in the earning activity with males in family by assisting them in transportation of bricks at brick kilns. Activities like feeding of animals, cleaning of sheds and removal of dung and urine were done mostly by women (Fig.16).

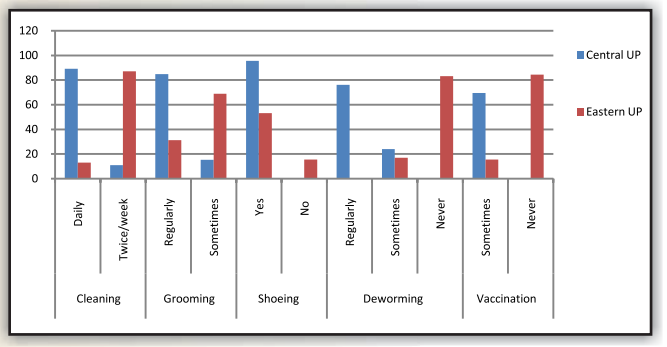


Fig. 15. Management practices followed by equine owners

Feeding and Housing Pattern: It was found that mostly equines were allowed to graze in open; however working mules and donkeys were provided locally available dry fodder and concentrates. Housing was in temporary kaccha houses made of brick and thatched roof (Fig.17). The feed provided included wheat and rice straw and wheat

Fig. 16. Women participate in Work and earn livelihood for their families.

Fig. 17. Housing, feeding and management practices among equine owners

bran. The equine owners were also providing additional supplements like Jaggery occasionally and oil in winter season to their equines.

Equine Welfare Issues and Livelihood Constraints:

The survey findings indicated that the working equines at brick kilns were mostly overloaded, overworked and undernourished. The management practices like grooming, shoeing and deworming were not regular. Wounds, sores and harness injuries were commonly observed in working animals. Equine owners generally starts working early in the monring at brick kilns (4 AM to 1 PM) in summer and from 8 AM to 4 PM in winter season. Very few equine owners were providing rest, feed and water to animals during long working hours. The availability of accessible and affordable animal health services were lacking especially in Allahabad and Varanasi districts in Uttar Pradesh. The major constraints faced by equine owners were high cost of feed and concentrates, lack of adequate employment opportunities, lack of availability of pasture land for grazing, lack of veterinary assistance and commission by contractors and middlemen at brick kilns. Poor health of animals, low social status and poor management were the major constraints that respondents claimed had an impact on their animals and livelihood of their families.

(A.A. Raut, Yash Pal, R.A. Legha, Ramesh Dedar and Jitendar Singh)

NRCE in News

दैनिक भास्कर हिसार, बुधवार, 27 नवंबर, 201

अश्व अनुसंधान केंद्र में शुरू हो सकता है घुड़सवारी प्रशिक्षण क्लब

एलएलआरयू के वीसी ने दिया सुझाव

केंद्र में पौधरोपण करते एलएलआरयू के वीसी श्रीकांत शर्मा व केंद्र के अन्य अधिकारी।

एलएलआरयू के वीसी मेजर जनरल श्रीकांत शर्मा ने कहा कि केंद्र में घुड़सवारी प्रशिक्षण क्लब शुरू करना चाहिए। इससे लोगों को घुड़सवारी सीखने की सुविधा मिलेगी। वह मंगलवार को राष्ट्रीय अश्व अनुसंधान केंद्र के 28वें स्थापना दिवस व्याख्यान के दौरान बोल रहे थे। यहां उन्होंने पौधारोपण भी किया।

उन्होंने कहा कि भारतीय संस्कृति में अश्वों का महत्वपूर्ण योगदान रहा है। नकुल स्वयं एक पशुचिकित्सक थे। रामायण, महाभारत एवं वेदों में अश्व का उल्लेख किया गया है। वर्तमान

बीकानेर अब तक

1 सितम्बर 2013, रविवार

वेविवि रोगाणु कल्चर को हिसार में संग्रहित करवाएगा

अशोक स्वामी

बीकानेर अब तक। वेटरनरी विश्वविद्यालय के कुलपति प्रो. ए.के. गहलोत ने राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार में गत दिनों वैज्ञानिक-अधिकारियों के साथ एक बैठक में विचार-विनिमय कर

गर्भाधान का कार्य यहां शुरू किया जाएगा। कुलपति प्रो. गहलोत ने बताया कि विश्वविद्यालय की इस नई पहल से अश्वपालकों को अपनी घोड़ी को उन्नत नस्ल के घोडों से प्रजनन कराने की एक वैज्ञानिक और अत्याधुनिक सुविधा प्राप्त

लॉयन एक्सप्रेस

बीकानेर | रविवार | 1 सितम्बर 2013

वेटरनरी विवि रोगाणु कल्चर को हिसार में संग्रहित करवाएगा

लॉयन न्यूज | बीकानेर| वेटरनरी विवि के कुलपति प्रो. ए.के. गहलोत ने राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार में गत दिनों वैज्ञानिक-अधिकारियों के साथ एक बैठक में विचार-विनिमय कर केन्द्र की गतिविधियों का अवलोकन किया।

अश्व अनुसंधान केन्द्र के निदेशक डॉ. आर.के. सिंह ने वेटरनरी टाइप कल्चर केन्द्र (वी.टी.सी.) की कार्यवाही की जानकारी दी। वेटरनरी विश्वविद्यालय, बीकानेर अपने यहां संग्रहित रोगाणुओं के कल्चर को चिर

कल्चर वापस लिये जा सकेंगें और देश-विदेश के शोधार्थियों के लिए अनुसंधान और बीमारियों के अध्ययन के लिए उपलब्ध रहेंगें। वेटरनरी विश्वविद्यालय, और राष्ट्रीय अश्व अनुसंधान केन्द्र, बीकानेर के बीच शिक्षा व अनुसंधान कार्यों के लिए एम.ओ.यू. के तहत घोड़ों में कृत्रिम गर्भाधान का कार्य यहां शुरू किया जाएगा। कुलपति प्रो. गहलोत ने बताया कि विश्वविद्यालय की इस नई पहल से अश्वपालकों को अपनी घोड़ी को उन्नत नस्ल के घोडों से प्रजनन कराने की

होगा एवं शोध को नए आयाम मिलेगे। घोड़ियों में कृत्रिम गर्भाधान के लिए शुरू में मारवाड़ी नस्ल के घोड़ों के सीमन का प्रयोग किया जाएगा। घोडो की मारवाड़ी नस्ल भारत वर्ष के रियासत काल में अपनी कद काठी, दम-खम और स्वामिभक्ति की एक मिसाल रही है। रेगिस्तान के मारवाड क्षेत्र की प्रसिद्ध नस्ल और प्राचीन भारत की युद्ध कला में अग्रणी भूमिका और राजपूत शासकों की शान बने मारवाडी घोडों का उद्गम 1193 ईस्वी माना गया है। इस नस्ल के घुडसवारो

राजस्थान पत्रिका

बीकानेर, रविवार, 1 सितम्बर 2013

पशु रोगाणुओं की 'कल्चर' का बनेगा रिकार्ड

बीकानेर राजस्थान पशु चिकित्सा एवं पशु विज्ञान वि.वि. बीकानेर में पशुओं होने वाले रोगाणुओं के कल्चर को वेटरनरी टाइप कल्चर केन्द्र, हिसार में संग्रहित करवाएगा। ये संग्रहित रोगाणु कल्चर शोधार्थियों के लिए अनुसंधान और बीमारियों के अध्ययन के लिए उपलब्ध रहेगें। वेटरनरी वि.वि. और राष्ट्रीय अश्व अनुसंधान केन्द्र, बीकानेर के बीच शिक्षा व अनुसंधान कार्यों हेतु हुए एम.ओ.यू. के तहत घोड़ों में कृत्रिम गर्भाधान का कार्य यहां शुरू किया जाएगा। कुलपति प्रो. गहलोत ने बताया कि इस नई पहल से

पंजाब केसरी

1.9.2013

रोगाणु कल्चर को हिसार में संग्रहित करवाएगा वैटर्नरी विश्वविद्यालय

बीकानेर, 31 अगस्त (राजेंद्र): वैटर्नरी विश्वविद्यालय के कुलपति प्रो. ए.के. गहलोत ने राष्ट्रीय अश्व अनुसंधान केन्द्र हिसार में गत दिनों वैज्ञानिकों-अधिकारियों के साथ एक बैठक में विचार-विनिमय कर केन्द्र की गतिविधियों का अवलोकन किया। अश्व अनुसंधान केन्द्र के निदेशक डा. आर.के. सिंह ने वैटर्नरी टाइप कल्चर केन्द्र (वी.टी.सी.) की कार्रवाई की जानकारी दी।

वैटर्नरी विश्वविद्यालय बीकानेर अपने यहां संग्रहित रोगाणुओं के कल्चर को चिरस्थाई संग्रहण के लिए अपने नाम से वैटर्नरी टाइप कल्चर केन्द्र हिसार में संग्रहित करवाएगा। भविष्य में आवश्यकता पड़ने पर संग्रहित रोगाणु कल्चर वापस लिए जा सकेंगे और देश-विदेश के शोधार्थियों के लिए अनुसंधान और बीमारियों के अध्ययन के लिए उपलब्ध रहेंगे। वैटर्नरी विश्वविद्यालय और राष्ट्रीय अश्व अनुसंधान केन्द्र बीकानेर के बीच शिक्षा व अनुसंधान कार्यों हेतु हुए एम.ओ.यू. के तहत घोड़ों में कृत्रिम गर्भाधान का कार्य यहां शुरू किया जाएगा।

कुलपति प्रो. गहलोत ने बताया कि विश्वविद्यालय की इस नई पहल से अश्वपालकों को अपनी घोड़ी को उन्नत नस्ल के घोड़ों से प्रजनन करवाने की एक वैज्ञानिक और अत्याधुनिक सुविधा प्राप्त हो सकेगी। वैटर्नरी विद्यार्थियों को भी इस प्रायोगिक तकनीक को समझने का अवसर प्राप्त होगा एवं शोध को नए आयाम मिलेंगे। घोड़ियों में कृत्रिम गर्भाधान के लिए शुरू में मारवाड़ी नस्ल के घोड़ों के सीमन का प्रयोग किया जाएगा। घोड़ों की मारवाड़ी नस्ल भारत वर्ष के रियासतकाल में अपनी कद, काठी, दमखम और स्वामी भक्ति की एक मिसाल हैं। रेगिस्तान के मारवाड़ क्षेत्र की प्रसिद्ध नस्ल और प्राचीन भारत की युद्धकला में अग्रणी भूमिका और राजपूत शासकों की शान बने मारवाड़ी घोड़ों का उद्गम 1193 ईसवी माना गया है। इस नस्ल के घुड़सवारों के भावनात्मक लगाव के किस्से आज भी मशहूर हैं। मेवाड़ के शासक महाराणा प्रताप का 'चेतक' भी इसी प्रजाति से था।

VTCC Accomplishment

Culture Collection: At a glance

The Veterinary Type Culture Collection (VTCC) was established at NRCE campus as a National Repository of Animal Microbes including dairy and rumen microbes. VTCC has a mandated aim of exploration and collection of microorganisms of animal origin/significance/relevance, central storage of animal microbes, and characterization, documentation and digitization of microbial database of cultures. This will serve as a Microbial Genetic Resource Center, which will provide/distribute characterized microbes to the different stake-holders of the country for further R&D in the various fields of microbiology, taxonomy, biotechnology, epidemiology and vaccinology. During 2013-14, repository has been strengthened by addition of 372 accessioned microbes including 194 veterinary microbes; 53 rumen microbes and 125 dairy microbes. The accessions include 73 bacterial and 11 viral cultures; 59 recombinant clones and 38 genomic DNA of bacterial cultures. For the first time we were also able to isolate and preserve 13 lytic bacteriophages. The total present microbial accessioning of VTCC has reached a mark of 2022 accessioned microbes including clones and DNA. Details of the cultures reposited are depicted in Table 1. The VTCC repository has been strengthened with reposition of many bacterial species (Fig 1) covering a range of host species and different pathogenic viruses (Fig 2).

Table 1. Cultures reposited during the period April 2013-March, 2014 and present strength of VTCC

Microbial Resources	Accessioned cultures (2013-14)	Accessioned cultures present strength
Veterinary Microbes		
Bacteria	73	700
Virus	11	135
Bacteriophage	13	13
Recombinant clones	59	326
Phage library	-	27
Genomic DNA	38	176
Total	194	1377
Rumen Microbes		
Anaerobic bacteria	28	101
Fungi/Yeast	17	104
Patent cultures	8	8
Total	53	213
Dairy Microbes		
Bacteria	125	432
Total	125	432
Total Strength	372	2022

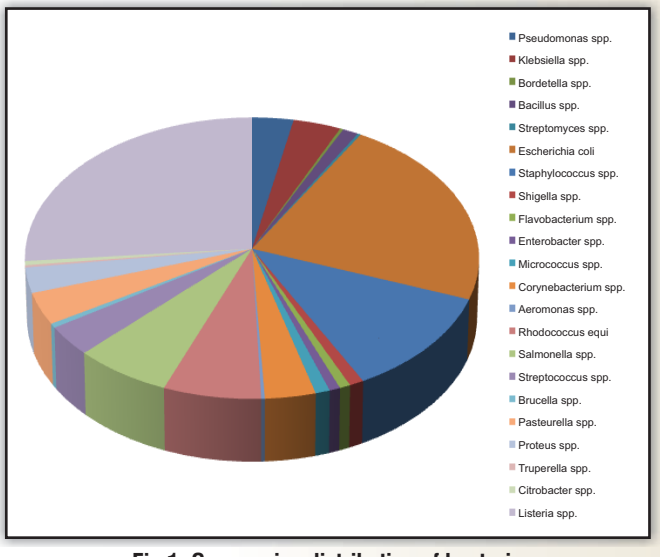


Fig 1. Genus-wise distribution of bacteria

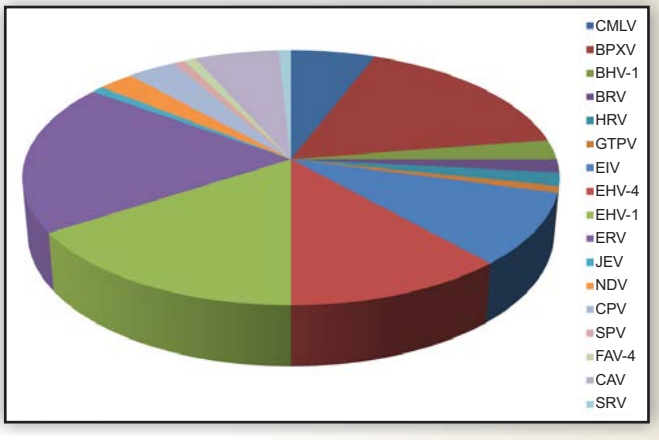


Fig 2. Distribution of viral isolates

Isolation, preservation, accessioning and reposition of virus isolates

Seven Buffalopox virus (BPXV) isolates from Buffalo (n-1) and humans (n-6) were isolated in vero cell line from an outbreak in Maharashtra (Table 2). One Swinepox virus was isolated from an outbreak in a piggery unit in Rohtak, Haryana. Besides, one Peste de Petits Ruminants (PPR) virus (CIRG Makhdoom) was authenticated in Vero cell line (Table 2). In addition, one Blue Tongue Virus (BTV) (TANUVAS) was passaged in BHK-21 cell line and one Canine adenovirus (CMVL, Meerut) was also propagated in MDCK cell line for ascertaining its viability. Furthermore, biological samples (25) collected / received from network units for isolation / reposition of PPRV, contagious ecthyma virus, sheeppox virus, canine adenovirus etc. were also passaged in Lamb testicle primary cultures, Vero, MDCK & NLBK cell lines.

Table 2. Isolation and Accessioning of Viruses in Cell lines

Virus	Host	Cells	Isolates
BPXV/Nasik/2014	Human	Vero	6
BPXV/Nasik/2014	Buffalo	Vero	1
SWPV/Rohtak/2013	Pig	PK-15	1
PPRV/CIRG/2013 (Deposit)	Goat	Vero	1
BTV/TANUVAS/2013 (Deposit)	Sheep	BHK-21	1

Table 3. Viruses/clones Accessioned in the VTCC repository

Name of Isolate / Clones	No. of Accessioned
BPXV (VTCC Deposit)	7
SWPV (VTCC Deposit)	1
BTV (TANUVAS Deposit)	1
PPRV (CIRG Deposit)	1
CAdV (CMVL Deposit)	1
Recombinant clones	36
Total	47

A total of 154 vials of characterized viral isolates, which include swinepox (14 nos), buffalo pox (98 nos), Canine adenovirus (14), PPRV (14 nos) and BTV (14 Nos) were preserved in the repository. During the period under report, 11 virus isolates were accessioned in the VTCC repository (Table 3). Recombinant clones (36 nos.) of specific genes of BPXV and SWPV have been accessioned in the repository. All the generated data of culture collection are being compiled and documented in digitized form as different registers namely Acquisition, Authentication, Accession, and Clone repository registers.

Isolation, molecular characterization and reposition of swinepox virus

Swinepox is a highly contagious viral disease of swine caused by swinepox virus (SWPV), under genus Suipoxvirus within the family Poxviridae. Swinepox is most severe in young pigs (up to 4 months of age) and adults generally develop a mild, self-limiting form of the disease. We describe herein the isolation and molecular identification of SWPV from an outbreak in a piggery unit in Rohtak, Haryana in September, 2013. This unit had a total population of 100 young pigs of which 30 animals developed generalized pustular lesions on the body (Fig. 3). The disease was characterized by dullness, depression and anorexia while the lesions exhibited in the form of papules followed by crusting. Scabs, spleen and lymphnode samples collected during post-mortem examination of an animal were used for DNA extraction. The SWPV was isolated in PK-15 cell line and preserved in the VTCC repository (Fig. 4). PCR confirmation was done by amplification of 552 bp of Orthopox virus-specific ATI gene and 522 bp product of

Fig 3. Generalized pustular lesions on the body of the pigs

Fig. 4. Isolation of swinepox virus in PK-15 cells

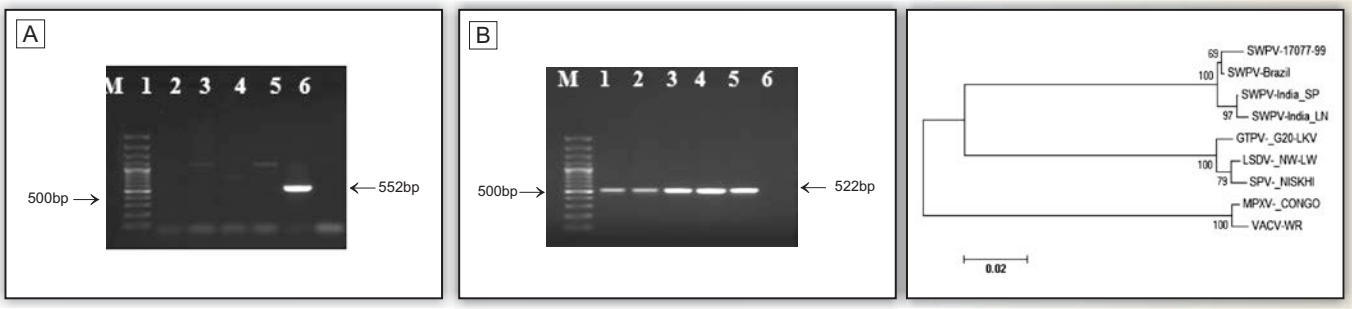


Fig 5. PCR amplification of ATI gene (A) & VLTF-3 gene (B) of SWPV isolate

Fig. 6. Phylogenetic tree of nt sequence of VLTF-3 gene of SPXV

SPXV-specific virus late transcription factor-3 gene (VLTF-3) (Fig. 5). The amplicons have been cloned, sequenced and BLAST homology and phylogeny analysis revealed that Indian isolate was closely related to Brazilian isolate (Fig. 6).

Isolation and reposition of Buffalopox virus (BPXV) isolates from outbreaks in buffaloes, battle & humans at Nasik, Maharashtra

Buffalopox viruses were isolated from the infected buffaloes and animal handlers from an outbreak in a small herd consisting of 15 buffaloes and two cattle at Nasik, Maharashtra in 2013. Two buffaloes and one cattle exhibited lesions of BPXV. Six in - contact humans were also severely affected. The lesions consisted of vesicles, scab and ulcers on udder and teats of buffaloes (Fig. 7). Vesicle and scab formation were also observed on fingers, palm, fore arm, hands and head of milkers and in contact animal owners (Fig. 7). Clinical samples were collected from the affected animals and in contact humans in the form of scabs, swabs and blood. Buffalopox virus infection was confirmed by employing the BPXV specific PCR targeting C18L and orthopoxvirus specific ATI genes which resulted in amplicon sizes of 368 bp and 552 bp, respectively (Fig. 8). Although infection could be confirmed in all collected samples, virus could be isolated in vero cell line from six humans and one buffalo sample only. Besides the amplicons of the confirmed isolates have been cloned in

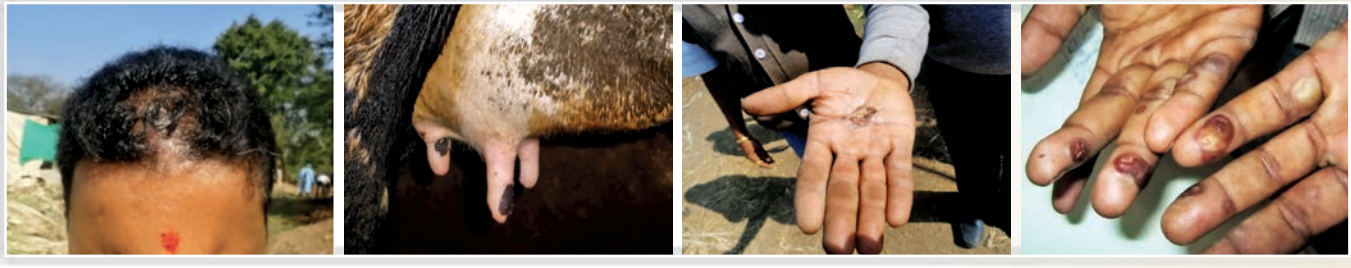


Fig. 7. Pock-like lesions in buffaloes and humans infected with BPXV in Nashik, Maharashtra

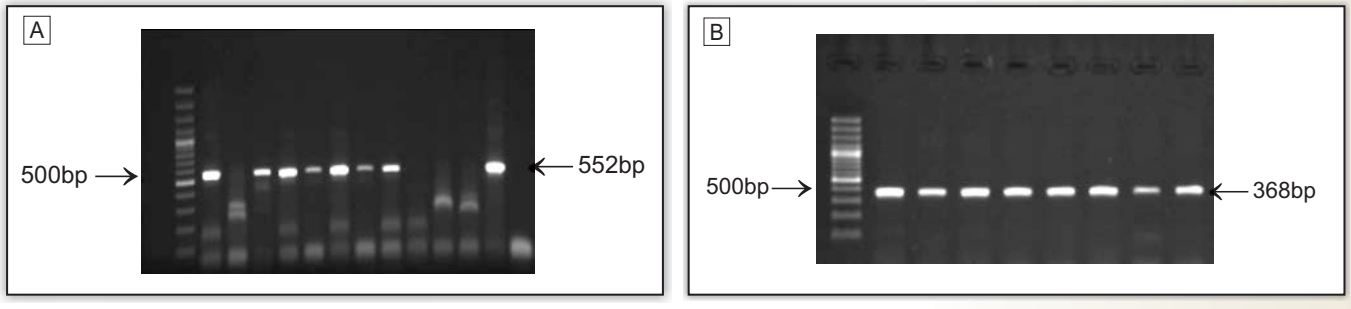


Fig. 8. PCR amplification of ATI gene (A) & C18L gene (B) of BPXV isolates

Genetic analysis of four host-range genes of human isolates of buffalopox virus

Molecular characterization of 4 host-range genes viz., E3L, K3L, C7L & B5R genes of six human isolates of BPXVs isolated from outbreaks (2013) in Nashik, Maharashtra has been carried out to elucidate the host-specific mutations as well as to add passport data of the isolates in the repository. All human and buffalo isolates showed 99.5 to 100% similarity at both nt and aa levels. Three significant point mutations (I11K; N12K & S36F) were observed only in C7L genes of Nashik isolates along with buffalo isolates from 2010 outbreak in Jalgaon in comparison to other VACV isolates and BPXV reference strain (BP4). However, mutation (D64N) observed in B5R gene in earlier human and buffalo isolates of BPXV isolated from 2010 outbreak, was not found in current isolates. 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 based on concatenated gene sequences revealed that BPXVs are to reference strain (BPXV-BP4) and other vaccinia and vaccinia-like viruses such as Passatempo and Aracatuba viruses.

Recombinant clone library of specific genes of various viruses in the repository

A total of 10 host range genes of Buffalopox virus and Swinepox virus isolate have been cloned. The genes targeted were: 4 host range genes (E3L, C7L, B5R & K3L) of BPXV along with six genes (Ankyrin, Kelch Like protein, G protein coupled receptor, MHC, Extra cellular envelope protein, A52R) of Swine poxvirus isolate. Homology analysis is underway with the available sequences in the public domain of the respective viral isolates. 72 copies of recombinant clones of specific genes of buffalopox virus (60 copies) and swinepox virus (12 copies) isolates have been have been generated by cloning into different vectors. The generated clones have been preserved and are being maintained in the repository.

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

Bioinformatic analysis of whole genome sequences of *Pasteurella multocida* and *Salmonella enterica* sub spp. *enterica* serovar Gallinarum

Bioinformatic analysis of 2 sequenced isolates, i.e., *Pasteurella multocida* Strain Bu1, a B:2 serotype strain, Acc No VTCCBAA264 (NCBI Accession No. ALYC00000000), and *Salmonella enterica* sub spp. *enterica* Serovar Gallinarum (Strain Sal40) was completed on RAST platform. This preliminary bioinformatic analysis of *P. multocida* isolate revealed a genome of 2,23,6312 bp size, mapped to 59 Contigs after Contiguator analysis with ref. Genomes (Table 4, Fig. 9), and it gave 2183 predicted ORFs (Pegs), and 52 rRNA genes. The *Salmonella* Gallinarum

Table 4. Contiguator analysis of *Pasteurella multocida* & *Salmonella* Gallinarum with reference genomes

Category	Reference replicon (*Pasteurella multocida*) gi.378773696.ref.NC.016808.1 (Strain 36950)		Reference replicon (*Salmonella* Gallinarum) gi/15601865/ref/NC_002663.1/(Pm70)	
	#	bp	#	bp
Input contigs	97	2283802	97	2283802
Mapped Contigs	59	2236312	61	2238989
UnMapped (UM) contigs	38	47490	36	44813
UM Short	33	20917	23	6345
UM poor coverage	4	23180	9	30200
UM duplicated hits	1	3393	4	8268

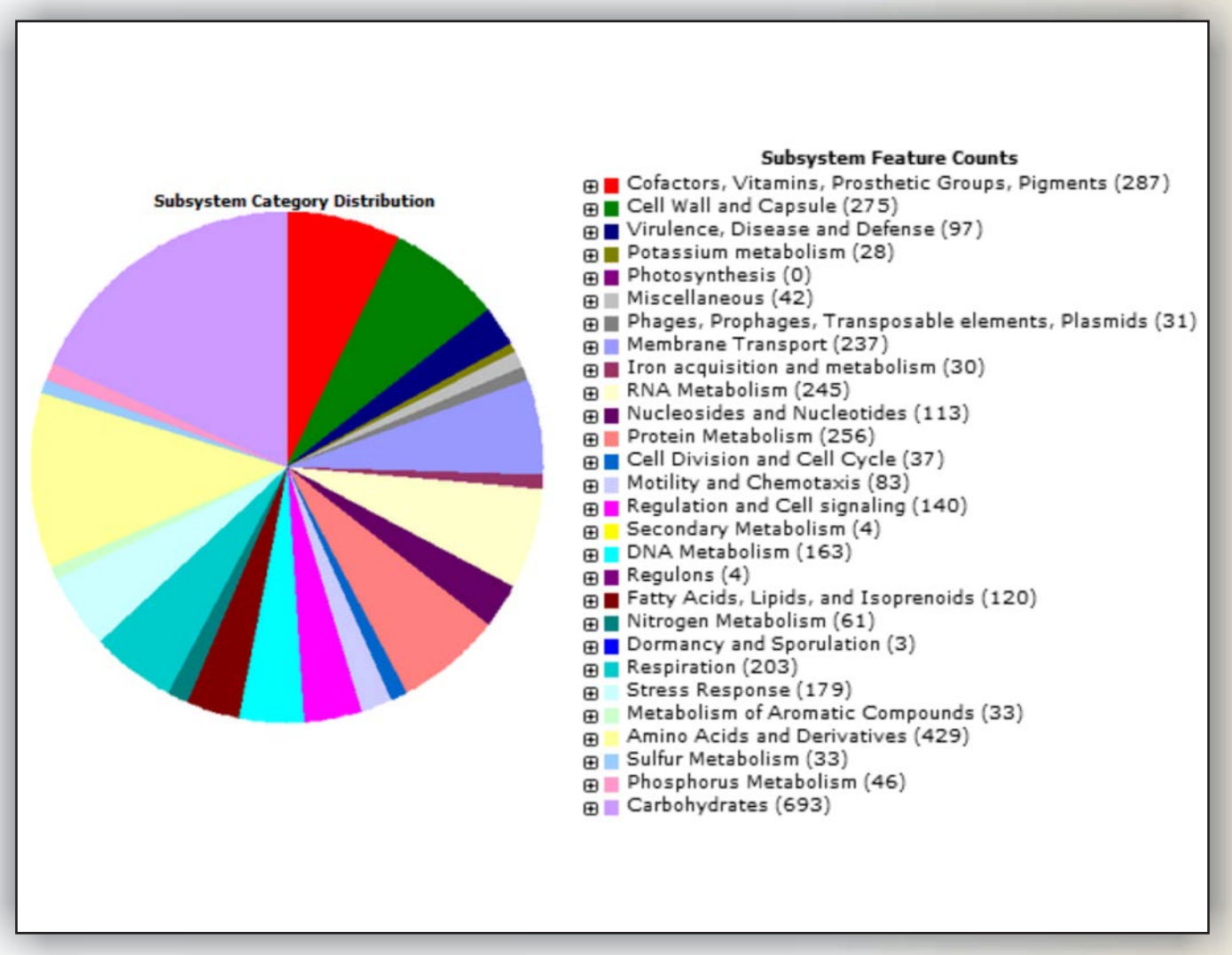


Fig. 9. Subsystem genetic information for *Salmonella* Gallinarum

genome of 4,688,112bp size was mapped to 92 Contigs after Contiguator analysis with ref. genomes, and it gave 4707 predicted ORFs (Pegs), and 72 rRNA genes. Antibiotic resistance genes were found in both the pathogenic isolates. Total 3872 features including virulence, disease & defence; Phages, prophages, transposons & plasmids; Iron acquisition & metabolism were revealed in RAST analysis (Fig. 9). In *P. multocida* genome, antimicrobial resistance genes including, Penicillin-binding protein 2 (PBP-2); bicyclomycin resistance protein; acriflavin resistance protein; Macrolide-specific efflux protein MacA & Multiple antibiotic resistance protein MarC are detected. Further analysis is underway.

GC-FAME analysis of culture isolates

The Microbial Identification System analyzes & identifies microorganism isolated in pure culture on artificial media. After the sample preparation procedure and GC step, it yields qualitatively and quantitatively reproducible fatty acid composition profiles, which are compared to given library for identification. The GC-FAME analysis and identification of bacterial isolates was successfully started, and 12 isolates (Table 5.) were identified up to species level using

Table 5. Identification of isolates with high similarity index up to species level by FAME analysis.

Isolate	Sim Index	Identification
Bu34B	0.554	*Morganella morganii*
Bu34C	0.539	*Salmonella enterica* Typhimurium
Bu34B1	0.607	*Serratia plymuthica*
Bu34D	0.623	*Bacillus pumilus* GC Subgrp B
Eq153	0.751	*Kluyvera cryocrescens*
Bu17	0.835	*Salmonella bongori* enterica
Eq50A	0.520	*Klebsiella pneumoniae* sub spp. ozaenae
Eq131	0.720	*Bacillus pumilus* GC Subgrp B
Bu34G	-	*Salmonella enterica* sub spp. *enterica*
C2	-	*Bacillus* spp.

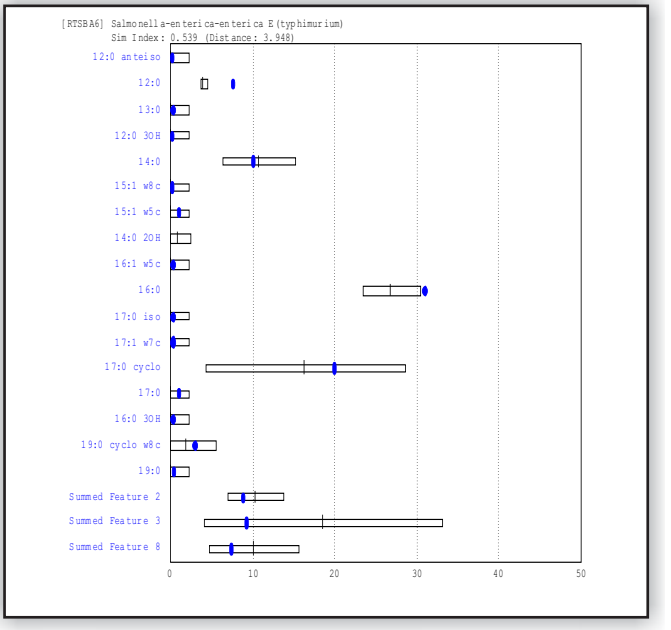


Fig. 10. Identification of *Salmonella* isolate from buffalo by FAME analysis

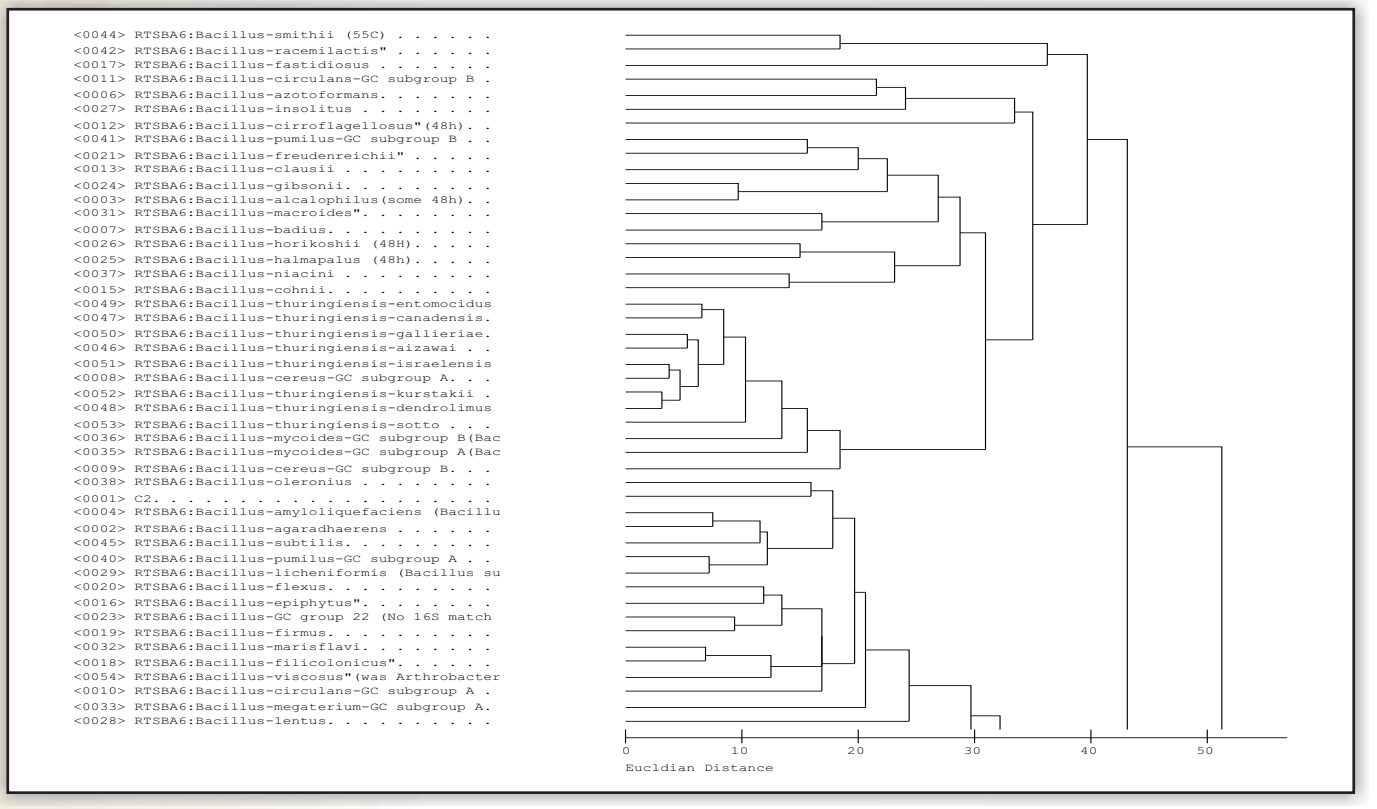


Fig. 11. *Bacillus* spp. isolate (C2) phylogenetically analysed as a putative novel species

automation. One isolate of *Salmonella enterica* was identified as Typhimurium serovar by FAME analysis (Fig 10.).One isolate of *Bacillus* spp. was revealed to be putatively a novel species, which needs further polyphasic analysis (Fig 11).

Isolation of four species of Rhodococci from animal sources

Rhodococcus is a genus of non-motile, non-sporulating, aerobic gram-positive filamentous rods of the phylum Actinobacteria. We have in our culture collection 4 representative species of *Rhodococcus* genus, including *Rhodococcus equi* from horse, *R. gordoniae* from donkey, *R. coprophilus* from camel and *R. trifolii* from horse. *Rhodococcus equi* is a well known pathogen which causes suppurative pneumonia and enteritis associated with lymphadenitis in 1 to 6 month old foals. However, in search of *R. equi* isolates from susceptible species, we have been able to isolate other species of rhodococci. *Rhodococcus gordoniae* has originally been described as a blood-borne clinical and soil isolate; *R. aetherivorans* from activated sludge; *Rhodococcus coprophilus* has been suggested as a specific indicator organism of farm animal fecal contamination; and *R. trifolii* has been previously isolated from leafy surface. We have isolated all 4 isolates from respective animal's dung and identified by sequence analysis and phylogenetic position is confirmed by analysis.

Significant isolations, identifications and accessioning of new bacterial isolates

At VTCC, the bacterial cultures are purified, phenotypically characterized, and sequencing of 16S rRNA gene is done for the identification of biochemically atypical bacteria, significant isolates, for speciation purpose or rarely encountered bacteria.

This year, significant genotype based identification included isolates identified as *Moraxella ovis, Bacillus hunanensis, Corynebacterium tuscaniense, Nocardia niwae* (Fig. 12), *Brevibacillus agri, Nocardia otitidiscaviarum, Streptomyces ghanaensis, Kluyvera georgiana, Rhodococcus coprophilus, Escherichia hermanii, Castellaniella denitrificans, Nocardiopsis alba, Aerococcus viridians, Pasteurella multocida, Ottowia pentelensis, Prolinoborus fasciculus, Rhodococcus aetherivorans* and others from various animal and animal microenvironments.

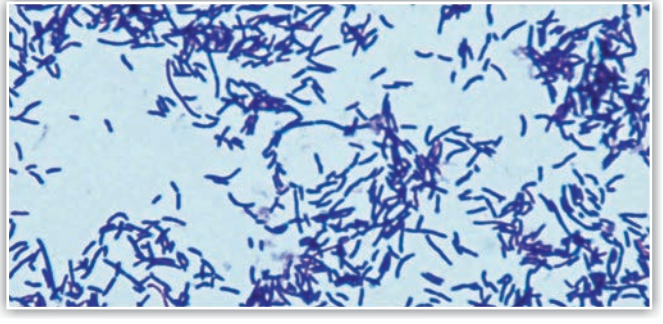


Fig. 12. Filamentous cells of *Nocardia niwae* (Grams stain 1000x)

A major achievement has been isolation of *Mannheimia varigena* from buffalo pneumonia case. This is probably the first report of this species from India. The isolate has been confirmed by 16S sequence and phylogenetic analysis.

In 2013-14, 73 cultures were authenticated and accessioned in VTCC. Bacterial isolates like *Pseudomonas* spp., *Klebsiella pneumoniae*, *Escherichia coli* of various Serogroups, *Salmonella Typhimurium*, Staphylococcus aureus, *Pasteurella multocida*, *Corynebacterium* spp, *Nocardia niwae*, *Bacillus* spp, *Mannheimia varigena* and *Streptococcus equi* sub spp. equi are preserved by cryopreservation and accessioned. Apart from these cultures, we have additionally preserved/repositioned 211 bacterial cultures which are in various stages of identification/ authentication.

Clone repository of 16S rRNA genes and DNA library of various bacterial isolates

Multiple copies of sixteen (16) recombinant clones of 16S rRNA genes of various bacterial isolates have been generated, sequence validated, preserved and accessioned and are being maintained in the VTC repository. VTCC repository has been strengthened with development of DNA repository. The good quality genomic DNAs of 38 bacterial cultures have been purified and preserved as ethanol precipitate at -80°C. At present, the DNA repository has been strengthened with preserved DNA of total 176 bacteria of different genera.

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

Isolation and characterization of T4-like bacteriophages from different bacterial isolates

The T4-like bacteriophages were isolated from pathogenic *Escherichia coli* (O12 serogroup), *Enterobacter aerogenes*, *Aeromonas* spp. and *Bacillus* spp. from soil and sewage samples collected from animal farm. Phages were isolated using agar overlay technique. The bacteriophage culture enrichment from sewage/soil samples was achieved by incubating the sample aliquot with the host bacteria followed by centrifugation and filtration through 0.45 μm membrane filter, and finally plating in molten agar with host bacterial culture. The plates were incubated overnight at 37°C and examined for the presence of plaques. Plaques were purified three times using the same procedure. The initial phage titre was estimated to be ~1.02×10^{10} for various phages. The phage preparations were stored at 4°C in SM buffer and later used for large scale preparation of phage stocks. Pancreatic DNaseI and RNase were used to degrade any host DNA and bacteriophage particles were precipitated using PEG8000. The DNA content of phage was visualized in 0.7% agarose gel. One of the phages was also visualized by transmission electron microscopy and observed to have icosahedral head morphology. The morphological appearance of the isolated bacteriophages as visualized by TEM indicated their similarity to coliphages of the myoviridae family. Out of 15 phage isolates, analysed by PCR for amplification of gp23 gene (major capsid protein) - 7 isolates showed amplification of the desired gene (Fig. 13). After phylogenetic analysis phages were found belonging to family Myoviridae, genus -T4 like virus (Fig. 14).

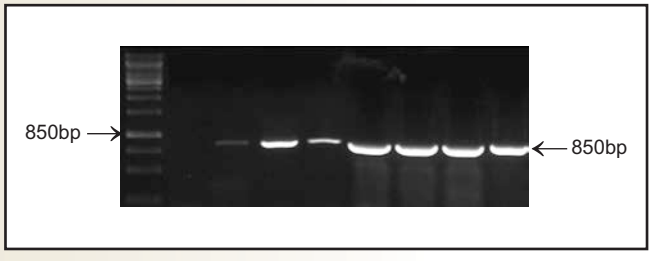


Fig. 13. PCR using T4 - specific primers for amplification of gp23 gene of phage isolates

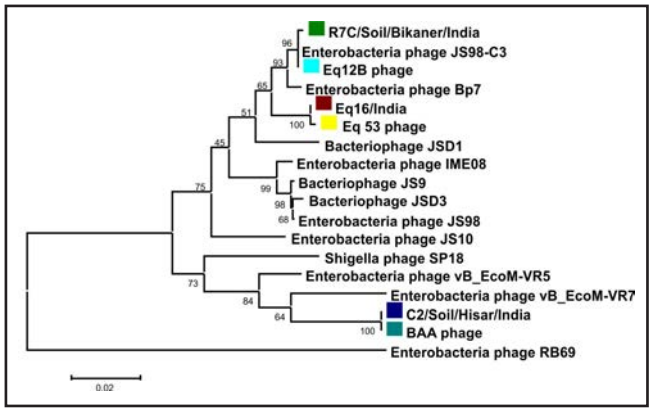


Fig. 14. Neighbour-joining tree based on the alignment of the gp23 gene from various phage isolates.

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

Generated repository of ORF clone library of virulence associated genes of zoonotic buffalopox virus (BPXV)

The ORF clone library has been strengthened with addition of seven Gateway clones of ORFs viz., A39R, B5R, L5R, D8L, A21L, A27L & B1R of buffalopox virus (BPXV). The entry clones were generated by cloning into Gateway vector pDONR221, Invitrogen Bioscience by homologous recombination based cloning strategy. The ORFs - specific primers of BPXV were designed using ORF-specific primer designing software by omitting stop codons of each ORFs. The phage attachment site (att) was manually added at the end of the each primer to facilitate homologous recombination based cloning. Two stage PCRs were employed for each ORFs to incorporate complete sequence of att site into the amplicons. First PCR was carried out using the ORF-specific primers and second round PCR was carried out using the universal left over att primers. The recombinant clones were generated on the basis of suicidal

action of the non-recombinant clones due to the presence of suicide gene (ccd) in the vector at recombinational site. The developed clones were validated by colony PCR, sequencing of recombinant plasmids and BLAST homology analysis of the sequence data. A total of 7 entry clones of virulence associated genes viz., A39R, B5R, L5R, D8L, A21L, A27L & B1R of BPXV/buffalo/Meerut/2011 isolate were generated. Five clones of each gene were cryopreserved and accessioned. The developed ORF library consists of 19 Gateway clones of BPXV virus and one Gateway clone of equine influenza virus genes.

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

Rumen Microbes - VTCC Network Component

Isolation and characterization of rumen bacteria and their reposition

During the year, a total of 28 rumen bacteria have been accessioned in the VTCC repository. Rumen bacteria were isolated from the faeces of cattle, goat, sheep and buffalo and from wild ruminants - nilgai (*Boselaphus tragocamelus*) and mithun (*Bos frontalis*). The faecal sample was enriched and tested for gas production before a serial dilution of the sample. The inoculum was added to the Hungate roll tubes containing suitable medium, and incubated at 39°C for a week. The discrete single colonies were taken for purification and for the molecular characterization. The bacteria were identified based on the 16S rRNA homology with existing bacteria in the GenBank. The cellulolytic and tannin degrading properties of the isolated bacteria were analyzed. Seven tannin degrading rumen bacteria (*Streptococcus gallolyticus*) were isolated from goat and all cultures were characterized by cloning and sequencing of sodA & 16S rRNA genes. The *Escherichia coli* isolated from buffalo was found with the property of Nitrate reduction and methane inhibition. The fibre degrading *Ruminococcus flavefaciens* (4 nos.) were isolated from cross bred cattle characterized by 16S rRNA gene sequence analysis. The following gut microbes were isolated from domestic and wild ruminants and reposited in the culture collection- *Streptococcus infantarius, Streptococcus lutetiensis, Streptococcus bovis, Streptococcus equinus, Clostridium* spp., *Clostridium bifermentans, Escherichia coli, Clostridium botulinum, Escherichia fergusonii, Bacteriodes uniformis, Streptococcus macedonicus, Shigella flexinerii, Streptococcus luteciae, Streptococcus parteurianus, Shigella boydii, Paprabacteriodes distasonis, Pseudobutyrivibrio ruminis, Pseudobutyrivibrio xylanivorans, Roseburia hominis, Streptococcus salivarius, Selenomonas ruminantium, Selenomonas bovis, Mitsuokella jalaludinni, Prevotella albensis, Prevotella melaninogenica, Prevotella salivae, Salmonella enterica* and *Succinivibrio detrinosolvens.*

Isolation and characterization of rumen fungi & and their reposition

Rumen fungi have been described as an efficient fiber degrader and thus after screening these for fibrolytic activities, an isolate can be used as direct-fed microbial, whereas bacterial cultures have also been vastly reported for their different roles in rumen microbial ecosystem. The VTCC culture collection has been strengthened with reposition of seventeen (17) rumen fungi. The important anaerobic rumen fungi available in the repository includes- *Neocallimastix* spp. from African elephant, Black buck, buffalo, cattle, goat, hippopotamus, Indian elephant, Mithun, Nilgai and Spotted deer; *Piromyces* spp. from African elephant, Black buck, Buffalo, cattle, goat, Hippopotamus, Indian elephant, Mithun, Nilgai and Spotted deer; *Anaeromyces* & *Orpi nomyces* spp. from buffaloes.

Dairy Microbes - VTCC Network Component

Isolation, characterization and preservation of important dairy microbes in the repository

Strains of lactic acid bacteria have been isolated from different sources such as fermented milks, vegetables, fruits, fermented foods like dahi, lassi, chhas, shrikhand, idli batter, dosa batter, jalebi batter etc from different places across the country. Also mixed strains O and LD type cultures from National Collection of Dairy Cultures were isolated. More than 600 different lactic acid bacterial cultures belong to *Lactococcus*, *Streptococcus thermophilus*, *Leuconostoc* spp. and *Lactobacillus* spp. have been isolated, identified and characterized. Approximately, 400 cultures have been preserved as freeze dried ampoules and deposited under VTCC culture collection, fifty cultures are in process of accession and more than 200 cultures isolates are in the process of characterization. Seven *Leuconostoc* isolates (D6, D14, D54, D50, D51, D52, D55) are flavour and EPS positive. *L. lactis* isolates C12 and C16 are fast acidifier. Combination of C12 (*L. lactis* ssp *lactis*) and D54 (*Leuconostoc mesenteroides* ssp. *mesenteroides*) is very suitable for dahi and lassi preparation. Two technological valuable strains (fast acid production & proteolytic acivity) A9 and E13 are for the preparation of dahi/curd. Two new species - *S. macedonicus* (SRC) and *S. infantarius* (HRL) have been identified. Five different species of *Propionibacterium* spp. have been procured form DSMZ culture collection, Germany and deposited in culture bank. Six *Lactobacillus* spp. having phytase degrading potential and strong antifungal activity have been isolated form milk-cereal fermented products (Rabadi samples). Several other potential cultures have been identified such as: amylytic strain of *Pediococcus acidolactici* (1), EPS producing strains of Leuconostoc spp. (15), aast acidifying *Lactococcus lactis* (30), and galactose positive *Streptococcus thermophilus* (10). Dextran producing strains of *Leuconostoc* spp. have also been isolated and characterized.

Impact assessment and practical utility of isolated dairy cultures

EPS positive *Lactobacillus* spp. have been isolated, identified and characterized for preparation of low fat lassi, cultured Buttermilk and sweetened Dahi. Fast acidifying *Streptococcus thermophilus* cultures were screened for sugar tolerance and 8 potential strains have been selected for preparation of Misti Doi. Combination of *L. lactis* ssp *lactis* C12 and *Leuconostoc mesenteroides* ssp. *mesenteroides* D54 is very suitable for dahi and lassi preparation. The fast, medium and slow acidifier strains of *Lactococcus lactis* ssp. *lactis* and proteolytic strains of *Lactococcus lactis* can be used for preparation cultured buttermilk, dahi, lassi etc. Galactose fermenting strains of *Streptococcus thermophilus* can be used for preparation of low galactose fermented milks including Yoghurt and low browning Mozzarella cheese. Mannitol producing *Leuconostoc* spp. can be used for preparation of reduced caloric lassi. Phytase degrading *Lactobacillus* spp. can be used in defined strain starter culture for cereal-milk fermented products.

Externally Funded Projects

Inter–Institutional and Externally Funded Projects

Charaterization of *Trypanosoma evansi* isolates of different livestock hosts and agro-ecological zones of India by microsatellite genotyping

Genetic characterization of *Trypanosoma evansi* isolates from various animal species and from different geographical areas is important for evaluating the molecular epidemiology of the disease, clinical and evolutionary research and vaccine and drug design. The present study deals with microsatellite based genotyping of *T. evansi* isolates from different geographical locations in north and north-western parts of India (Table 1). In this study, DNA of six isolates of *T. evansi* from horse, camel, donkey and cattle were analysed. Fifteen microsatellite primer pairs (published reports) targeting the flanking regions of selected microsatellites were chosen on the basis of perfect repeating motifs from different chromosomes to minimise the chance of linkage, using data of *T. brucei* genome project. Twelve out of 15 microsatellite loci were found useful after evaluation on different *T. evansi* isolates. Using these microsatellite markers, the genotype studies were carried out. The reverse primers for 12 microsatellite loci were labelled with a fluorescent dye at their 5' end. Six of these (TB11/13, TB2/19, TB10/19, TB10/1, TB1/8 and TB3/3) were labelled with FAM (Applied Biosystems), five reverse primers (TB11/1, TB8/11, TB11/29, TB7/12 and TB4/2) were labelled with HEX (Applied Biosystems) and one reverse primer (TB8/1) was labelled with ROX (Applied Biosystems) at their 5' termini. All fluorescently labelled PCR amplicons were analysed on an ABI 3730xl genetic analyzer (Applied Biosystems) in conjunction with a GeneScan 500 LIZ size standard (Applied Biosystems). Allele identification was performed using the ABI program GeneMapper V4.0 (Applied Biosystems).

Ten microsattelite loci could amplify correctly with microsatellite primers, while 2 were rejected due to poor amplification and statistical error noted in their analysis. Ten microsatellite markers were successfully amplified in 6 isolates of *T. evansi* and six of them were found polymorphic. At four microsatellite loci (TB11/13, TB2/19, TB11/1 and TB8/1) observed (na=2) as well as effective (ne=2) number of allele were same. These loci were considered as non-polymorphic and thus not found useful for the further genetic diversity studies among *T. evansi* isolates. At six microsatellite loci (TB8/11, TB11/29, TB10/1, TB1/8, TB3/3 and TB4/2) observed number of alleles per locus ranged from 3 (TB8/11) to 6 (TB4/2) with a mean value of 4.8333±1.7529 and effective number of

Table 1. Details of *Trypanosoma evansi* isolates collected from different agro-climatic zones of India

T. evansi isolates	Lab ID	Agro-climatic zones*
T.ev-India NRCE-Horse1 /Hisar/Haryana	PH-1	Trans Gangetic Plains Region
T.ev-India-NRCE-Camel 1/Bikaner/ Rajasthan	CB-2	Western Dry region
T.ev-India-NRCE-Donkey 2/Junagarh/Gujarat	DJ-3	Gujarat Plains and Hill Regions
T.ev-India-NRCE-Donkey1/Hardoi/Uttar Pradesh	DH-4	Upper Gangetic Plains Region
T.ev-India- NRCE-Cattle1/ Karnal/Haryana	CK-5	Trans Gangetic Plains Region
T.ev-India-NRCE-Horse 2 /Karnal/Haryana	PK-6	Trans Gangetic Plains Region

alleles ranged from 2.1176 (TB8/1) to 5 (TB4/2) with a mean value of 3.3227±1.165. All the loci had lower value of effective number of alleles in comparison to observed number of alleles. Shannon's information index values ranged from minimum value in TB8/1 (0.6365) and maximum in TB4/2 (1.6957) with the mean value of 1.0661±0.4928. The observed heterozygosity varied from 0.167(TB8/11) to 1(TB11/13, B2/19, TB10/1, TB1/8 and TB4/2) with a mean value of 0.7666±0.3713. The expected value of heterozygosity (Levene's) varied from 0.485 (TB8/1) to 0.889 (TB4/2) with a mean value of 0.5932±0.2369. PIC value ranged from 0.346 (TB8/1) to 0.772 (TB4/2), indicating that some microsatellites (TB11/29, TB10/1, TB1/8, TB3/3 and TB4/2) were highly informative (PIC>0.5). Allelic variation at 6 microsatellite loci indicated significant genetic diversity among different isolates of *T. evansi* from different agro-climatic zones in India. At all loci we have found significant observed (Ho) and expected heterozygosity (He).

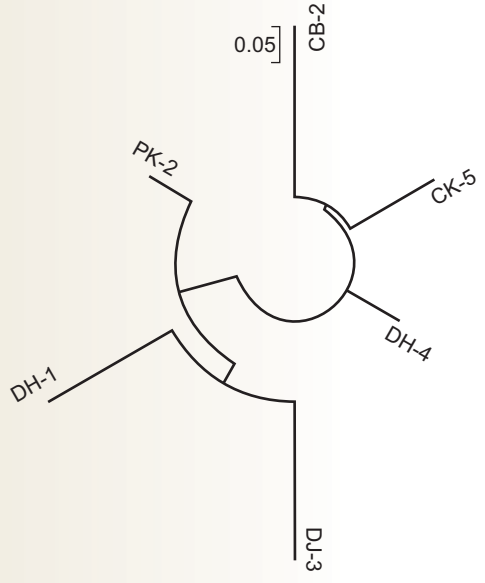


Fig. 1. N-J tree based on share allele (DAS) genetic distances showing relationship between different isolates of *Trypanosoma evansi* (circle tree). Scale indicates a genetic distance of 0.05.

Genetic distance study indicated different clustering of isolates based on allelic sharing. Neighbour-Joining tree analysis based on allelic sharing analysis indicated that DJ-3 isolate is more closer PH-1 isolate whereas, CB-2 isolate is closer to CK-5 isolate. The DH-4 and PK-6 isolates of *T. evansi* were found different from each other as well as from other isolates. (Fig. 1). Six sets of microsatellite primers have been found useful for genetic diversity study.

However, for evaluating appropriate genetic diversity mapping and molecular epidemiology of the disease, more number of isolates is required from different endemic agro-climatic zones and to search more number of informative microsatellite makers genome sequencing data of *T. evansi* is needed.

(Rajender Kumar)

Development of biomarkers for diagnosis of *Trypanosoma evansi* infection in animals using proteomic approach

Three clusters of immuno reactive proteins of *T. evansi* were identified using immunoblot and further purified by SDS-PAGE preparatory gel method (Fig. 2). 62-66k Da cluster was subjected to mass spectroscopy, which revealed five proteins. The gene encoding heat shock protein (hsp70) out of these proteins has been amplified and cloned in cloning and expression vector. The kinetics of expression studies were standardized and large scale purification of recombinant protein were made for further use in immunodiagnostic test.

The deduced amino acid sequence of hsp70 protein of the Indian isolate of *T. evansi* was compared with the other HSP70 amino acid sequences of different strains of Trypanosomes, *Leshmania* and *Plasmodium* parasites circulating worldwide. The Indian isolate of *T. evansi* showed similarity of 85% to 99% with other isolates. It has been observed that Indian *T. evansi* isolate is almost similar to *Trypanosoma brucei brucei* isolate differing at three amino acid substitutions: two changes (Q139R & L202R) at N-terminal region and one point mutation (V636E) at C-terminal region. Comparative analysis also revealed that N-terminal region of the HSP70 protein is highly conserved in

Trypanosomes. The major changes in HSP70 protein are present in C- terminal region. A stretch of 662-690 and 654-678 amino acids are present in *T. evansi* and *T. cruzi* respectively, however they are absent in other trypanosomes.

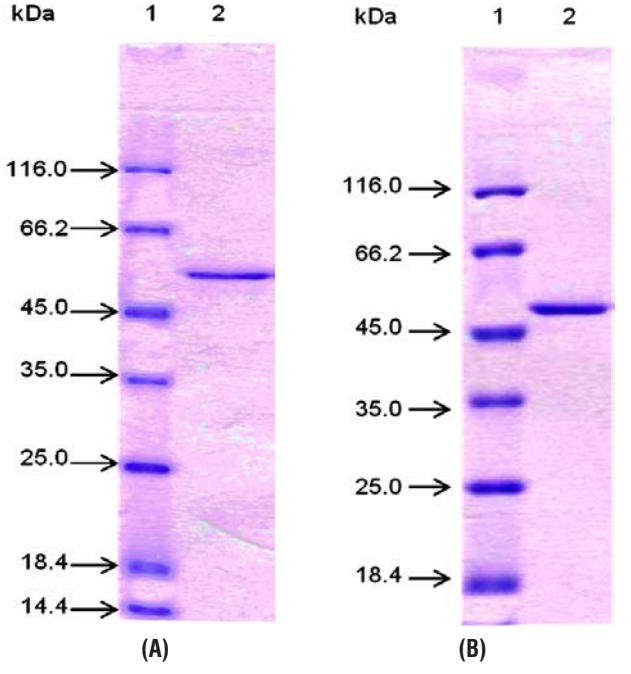


Fig. 2. Purification of rHSP70 (N-terminal) 55 kDa (A) and rHSP70 (C-terminal) 50 kDa (B) by Ni-NTA agarose beads. Lane 1- Marker unstained; Lane 2- Recombinant protein (N –terminal) & (B) C- terminal r protein.

Phylogenetic analysis of HSP70

The homology search of the nucleotide sequences of hsp70 gene was carried out using BLAST, NCBI. Phylogenetic analysis based on the deduced amino acid sequences of HSP70 protein of *T. evansi* and other isolates showed that Indian isolate of *T. evansi* clustered within a trypanomsomes clade and was most closely grouped with *T. brucei brucei* and *T. brucei* (Fig. 3). The mitochondrial HSP70 and cytosol HSP70 of *T. congolense* was laid separately in different clade. Similar topologies to the consensus maximum likelihood tree were obtained with other phylogenetic analyses (neighbour joining, Maximum Parsimony), with the exception that *T. congolense* mitochondrial hsp70 clustered with *Plasmodium berghei* in Maximum Parsimony analyses.

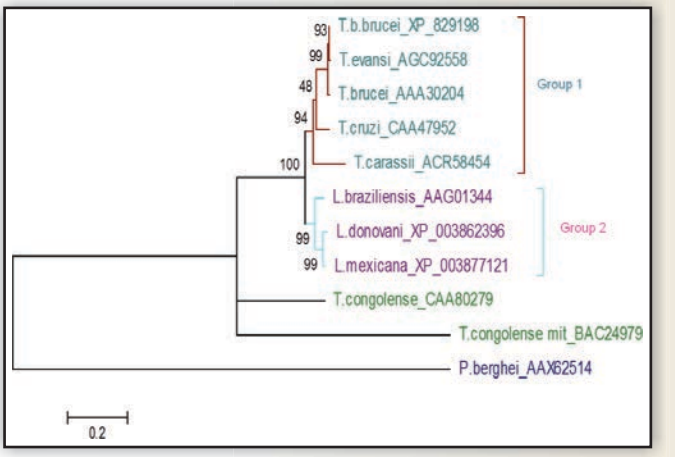


Fig. 3. Phylogenetic tree of HSP 70 protein constructed by maximum likelihood method

Antibody ELISA using rHSP antigen

To evaluate the diagnostic potential of these recombinant proteins, a series of checker board titration were conducted and optimum concentration of antigen, conjugate were evaluated. The cut off value for the antibody ELISA was established as the mean ± 2 SD of the OD value of uninfected donkeys serum samples. The mean OD value of negative sera was 0.235 with an SD (0.057) giving a cut off value (0.350) for N-terminal rHSP70 (Fig. 4), whereas for C-terminal rHSP70 mean OD of negative sera was 0.197 with an SD (0.062) giving a cut off value (0.323) (Fig. 5). It is evident that C terminal rHSP70 protein is capable of detection of antibodies at early stage i.e. 14 DPI, showing peak at 42 DPI in pooled serum samples. Thereafter, gradual fall in titre was observed but remained above cut-off value throughout the experiment i.e.191 DPI in

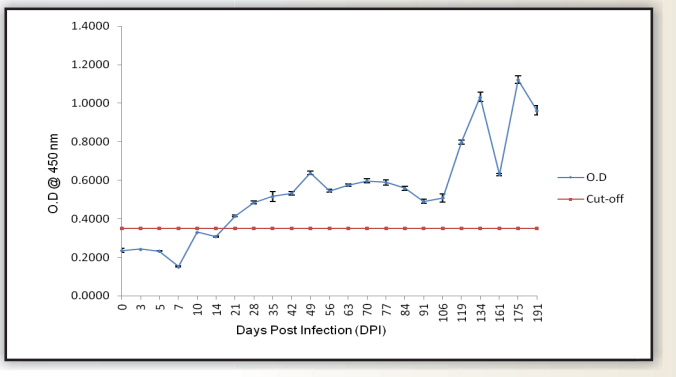


Fig. 4. Antibody response of recombinant HSP70 (N-terminal protein) in donkeys (pooled sera) experientially infected with *T. evansi*

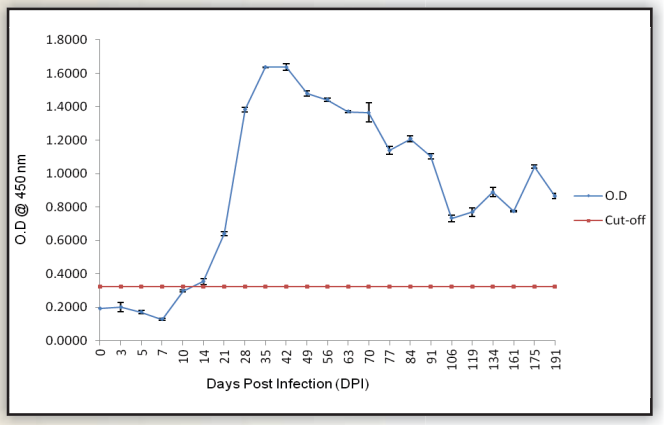


Fig 5. Antibody response of recombinant HSP70 (C-terminal protein) in donkeys (pooled sera) experientially infected with *T. evansi*

experimentally infected serum samples. However, N terminal rHSP70 recombinant protein detected the infection a 28 DPI initially (with low antibody titre) and peak observed at later stage of infection in contrast to observations for C terminal. Similar evaluation of truncated N & C terminal rHSP 70 antigen using experimentally infected ponies and field serum samples are in progress.

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

Studies on *Burkholderia mallei* for rapid diagnosis of glanders in equines using molecular tools

Optimization and validation of recombinant Hcp1 protein based sero-diagnostics of glanders

The development of accurate diagnostic tests for detection of glanders, specifically the carrier horses, is utmost necessity for quarantine and elimination of infected animals. Because of the inherent complexity and drawback of complement fixation test (CFT)- the OIE recommended serological test – the development of a modern diagnostics with defined immuno-dominant protein is the need of the time. In the previous year, two immunogenic proteins namely TssB and Hcp-1 of type six secretion system clusters of *B. mallei* were expressed and diagnostic efficacy of recombinant TssB protein was assessed. In continuation with the previous attempt, indirect ELISA using recombinant Hcp1 protein was evaluated for glanders diagnosis.

Optimum concentration of ELISA reagents were determined by checkerboard titration analysis using known glanders positive (n=56) and negative (n=30) equine serum samples. The optimum cut-off value of ELISA was determined by receiver operative curves (ROC curves) analysis using normalized OD_{492} values (PP%) (Fig.6). After optimization of reagents, 3235 equine serum samples were assayed in duplicate by indirect ELISA. Relative sensitivity and specificity of the ELISA were 100% in comparison to CFT. Cross-reactivity of the ELISA was tested using human melioidosis positive (n=6) and negative (n=10) serum sample as well as equine serum obtained from unrelated bacterial infection (n=15). It was observed that Hcp-1 ELISA was also able to diagnose human melioidosis. The aetiological agent of melioidosis is *Burkholderia pseudomallei*, an evolutionary ancestor of *Burkholderia mallei* - the causative pathogen of glanders. Cross-

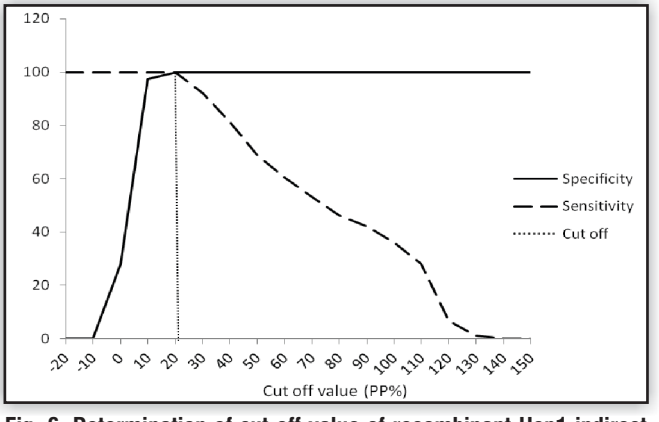


Fig. 6. Determination of cut-off value of recombinant Hcp1 indirect enzyme-linked immunosorbent assay using known positive (n=56) and known negative (n=30) according to sensitivity and specificity values.

reactivities of ELISA with *B. mallei* and *B. pseudomallei* antibodies suggests that the assay can be used for both glanders and melioidosis diagnosis.

(Praveen Malik, H. Singha, S.K. Khurana and R.K. Singh)

Differentiation of Equine Mesenchymal Stem Cells derived from Amniotic Fluid to Tenogenic Lineage using BMP-12

Tendon injuries are common in race horses and mesenchymal stem cells (MSCs) isolated from adult and fetal tissues have been used for tendon regeneration. In the present study, we evaluated equine amniotic fluid (AF) as a source of MSCs and standardized methodology and markers for their *in vitro* tenogenic differentiation. Plastic-adherent colonies were isolated from 12 of 20 AF samples by day 6 post-seeding and 70-80% cell confluency was reached by day 17. These cells expressed mesenchymal surface markers (CD73, CD90 and CD105) as detected by RT-PCR and immunocytochemistry, but did not express haematopoietic markers (CD34, CD45 and CD14). In flow cytometry, the expression of CD29, CD44, CD73 and CD90 was observed in 68.83±1.27%, 93.66±1.80%, 96.96±0.44% and 93.7±1.89% of AF-MSCs, respectively. Osteogenic, chondrogenic and adipogenic differentiation of MSCs was confirmed by Von Kossa, Alizarin Red S, Alcian Blue and Oil Red O staining, respectively. Upon supplementation of MSC growth media with 50 ng/ml bone morphogenic protein-12 (BMP-12), AF-MSCs differentiated to tenocytes within 14 days. The differentiated cells were more slender, elongated, spindle shaped with thinner and longer cytoplasmic processes and showed expression of tenomodulin and decorin by RT-PCR and immunocytochemistry. In flow cytometry, 96.7±1.90% and 80.9±6.4% of differentiated cells expressed tenomodulin and decorin in comparison to 1.6% and 3.1% in undifferentiated control cells, respectively. Our results suggest that AF is an easily accessible and effective source of MSCs. On BMP-12 supplementation, AF-MSCs can be differentiated to tenocytes, which could be exploited for regeneration of ruptured or damaged tendon in race horses.

Immunophenotypic characterization of mesenchymal stromal cells isolated from equine umbilical cord blood

Mesenchymal stem cells (MSCs) isolated from equine fetal adnexa are not fully characterized due to lack of species-specific markers. We isolated and characterized MSCs from equine umbilical cord blood (UCB) using cross-reactive markers. The conditions for adipogenic differentiation of equine MSCs were optimized. The plastic adherent cells isolated from 13 out of 20 UCB samples proliferated till passage 20 with average cell doubling time of 46.40±2.86 h. These cells expressed mesenchymal surface markers (CD29, CD44, CD73, CD90 and CD105) but did not express haematopoietic/ leucocytic markers (CD34, CD45 and CD14) by RT-PCR and immunocytochemistry. The expression of CD29, CD44, CD73 and CD90 was shown by 96.36% (±1.28), 93.40% (±0.70), 73.23% (±1.29) and 46.75% (±3.95) cells, respectively in flow cytometry. Cytochemical staining and RT-PCR confirmed that these cells were capable of undergoing directed differentiation into osteocytes and chondrocytes. Adipogenic differentiation could be done by culturing cells in media supplemented with 15% rabbit serum and 20 ng/ml of recombinant human insulin for 10 days. This was confirmed by demonstration of oil red O stained neutral triglycerides droplets and expression of PPAR-ã and adiponectin by RT-PCR. MSCs in equines can be characterized unequivocally using the protocol and phenotypic markers identified in this study.

(B.R. Gulati, Pawan Kumar, Prem Singh Yadav, Taruna Anand and B.K. Singh)

Synthesis, characterization and evaluation of drug loaded nano-formulation against *Trypanosoma evansi* in animal model

Two nanoformulations (NFI and NFII) of trypanocidal drug Quinapyramine sulphate using different polymers were synthesized, characterized and evaluated for efficacy and toxicity. Nanoparticles were well formed and regular in shape. The TEM micrographs show encapsulated quinapyramine sulphate-loaded nanoparticles of NFI and NFII in aggregates. The release of drugs showed an initial burst effect followed by slow release of the drug. Both the formulated drug-loaded nanoformulations showed trypanocidal effect in mice and were able to clear the *T. evansi* parasite at much lower concentrations as compared to the conventional drug. The drug in form of nanoformulation had remarkably less cytotoxicity as compared to conventional quinapyramine sulphate drug. Formulated drug-loaded nanoformulations are nontoxic, biocompatible, biodegradable, and stable and are highly effective against parasite *T. evansi* at highly reduced dose.

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

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

Comparative ananlysis of structural and functional motifs of horse cytokines and other domestic animals

Structural and functional motifs in deduced amino acid sequences of horse IL-2, IL-4, IL-10 and IL-18 were analyzed and compared with other domestic animal species. The position of cysteine residues, glycosylation, myrisoylation, and phosphorylation motifs in deduced amino acids of respective cytokines were studied using bioinformatic tools. Horse IL-2 has 33-38 amino acid substitutions and five amino acid deletions at position 91-95. It contains four cystein residues (at position 9, 78, 121, and 141), one potential N-linked glycosylation motif (at position 106-108), seven phosphorylation motif (four Ser-, three Thr- and one Tyr- phosphosites), and two myristoylation motifs (at position 47 and 111) (Fig. 7a). Conservation of cysteine residues in IL-2 among livestock animals and horse suggests evolutionary conservation of secondary structure of this molecule across the species. Position of N-linked glycosylation site in horse was homologous to pig and camel IL-2. Location and number of phosphorylation and myristoylation motifs in IL-2 sequences were distinct among the animal species.

The horse IL-4 has 25-33 amino acid substitutions. Among the seven cysteine residues in mature IL-4, only four cysteine residues showed homologous conservation which indicates horse IL-4 may differ from disulfide bonds position identified in other animals. Divergence in number and position of possible N-linked glycosylation sites were prominent in IL-4. Interestingly structural divergence was also observed in equine IL-4 receptor. Together the structural differences in IL-4 cytokine and its receptor may explain the species-specific activity of this cytokine. A single conserved myristoylation site was present in cattle, buffalo, sheep, goat, pig and camel at position 92, while horse IL-4 contains three myristoylation sites (positions 95, 102, and 106). Phosphorylation sites in IL-4 sequences were not conserved between livestock animals and horse (Fig. 7b).

A very high degree of sequence and structural homology was observed between horse IL-10 and companion animals. Deduced amino acid sequences of IL-10 of Marwari horse contains six Cys residues (positions 8, 9, 30, 80, 126, and 132), two N-linked glycosylation sites (positions 67-69 and 134-136), two myristoylation sites

Fig. 7a

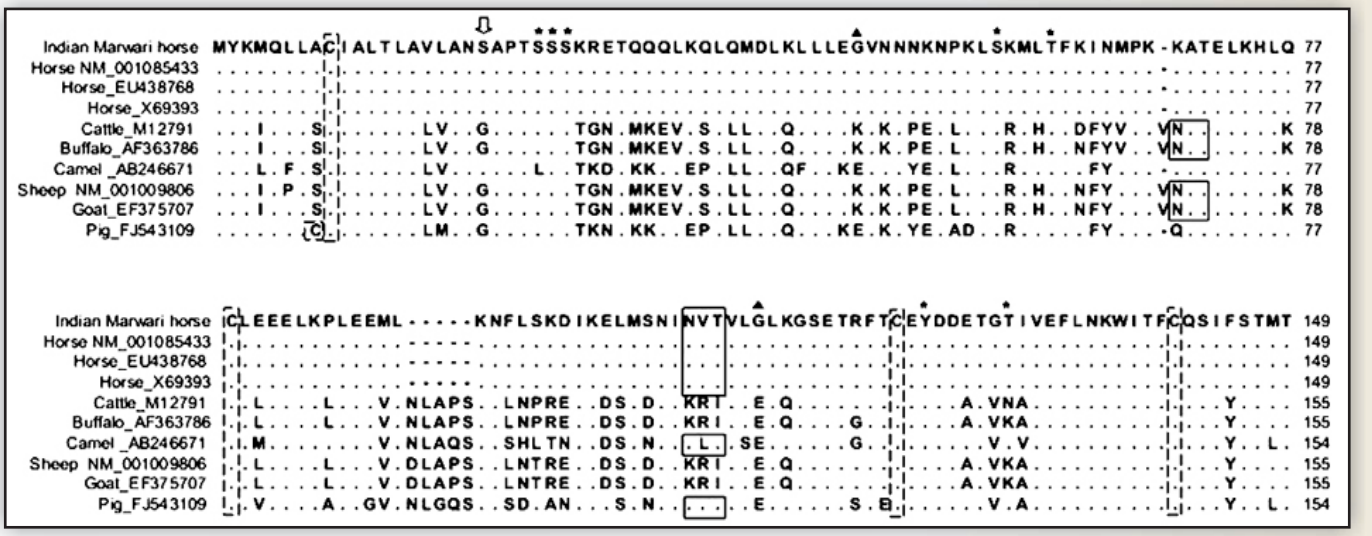


Fig. 7b

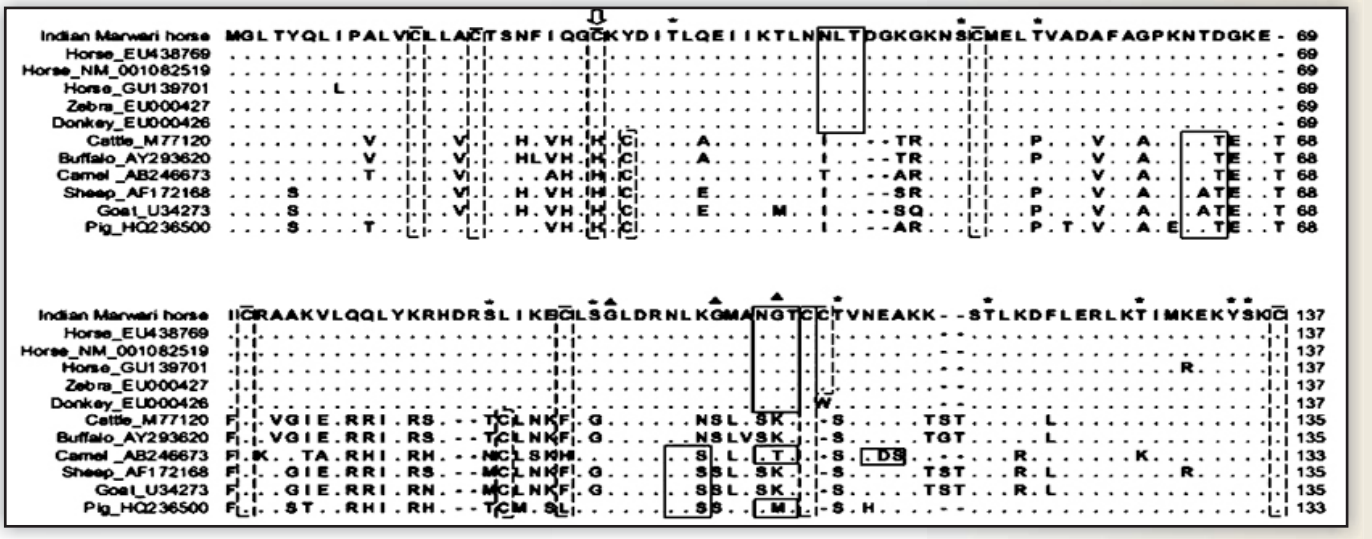


Fig. 7c

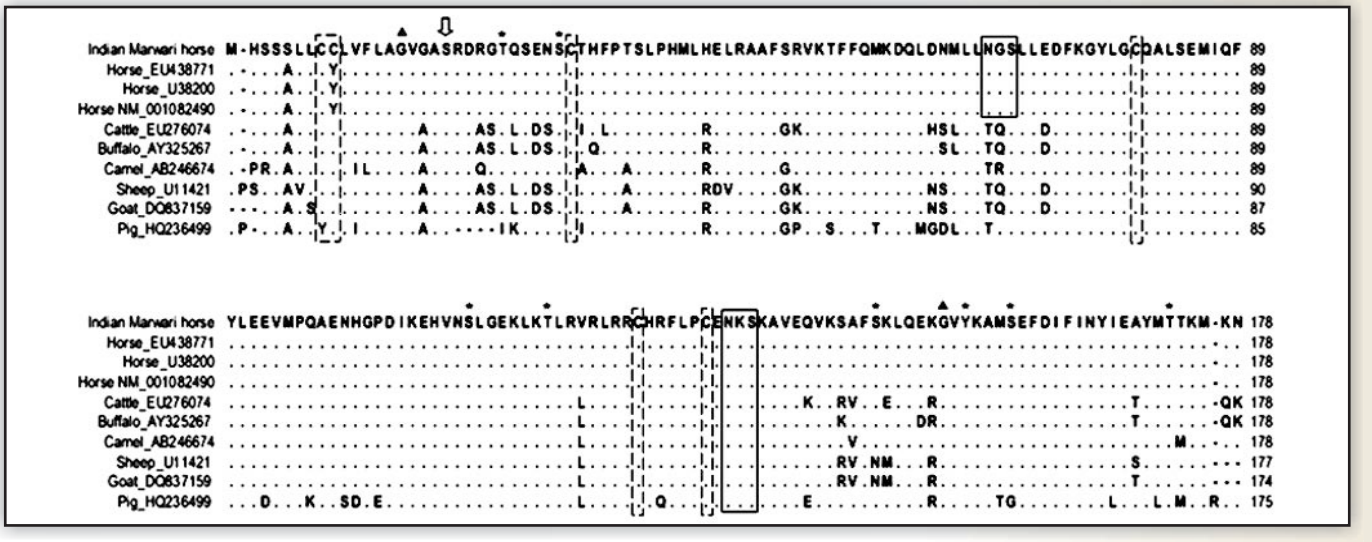


Fig. 7d

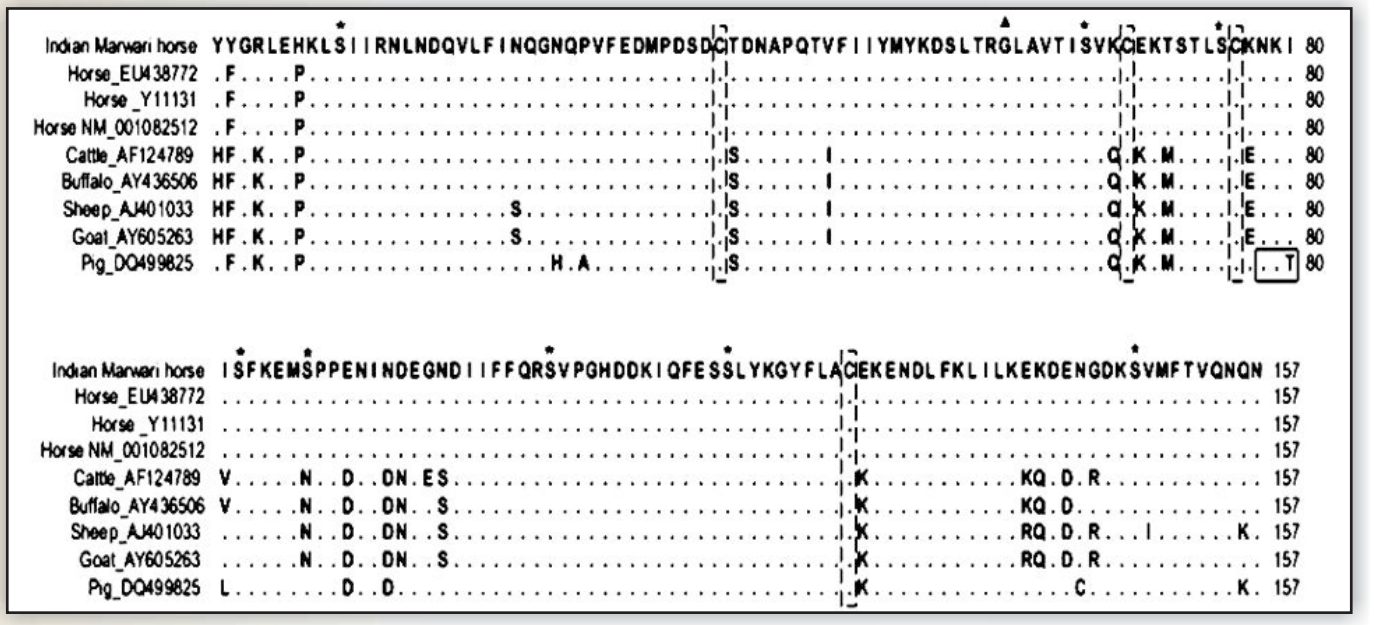


Fig. 7. Alignment of the deduced amino acid sequence of Indian Marwari horse cytokine cDNAs with those of other mammalian species. (A) IL-2, (B) IL-4, (C) IL-10, (D) IL-18. The identical amino acid residues are indicated by dots (.). A dash (-) denotes the absence of amino acids. White arrowheads ( ) indicate the amino acid terminus of the mature proteins. The conserved Cys residues or potential N-linked glycosylation sites are delineated by dashed boxes and solid boxes, respectively. Potential phosphorylation sites are indicated by a star (⋆), while myristoylation sites are indicated by black triangle (▲).

(positions 15 and 153) and nine phosphorylation (five Ser-, three Thr-, and one Tyr- phosphorylation) sites (Fig. 7c). Interestingly, number and position of Cys residues, N-linked glycosylation sites, and myristoylation sites were well conserved in IL-10 of all the animal species including horse which suggests conservation of tertiary structure and functional integrity of this molecule in horse and other animal species as well. Overall the sequence and structural identity of horse IL-10 was nearly identical to camel IL-10 protein.

Similar to IL-10, IL-18 had high degree of sequence and structural conservation across mammalian species which includes number of amino acids, number and position of cystein residues, serine phosphorylation sites, and myristoylation sites. Sequence and structural identity of horse IL-18 was very similar to pig IL-18 protein. Deduced amino acid sequences of mature IL-18 of horse and other mammals contain four Cys residues at position 38, 68, 76 and 127. Conservation of functional motifs in IL-18 suggests the evolutionary necessity of maintaining the important function of this molecule across the mammalian species. N-linked glycosylation motif was not present in mature IL-18 protein of horse, cattle, buffalo, sheep, and goat (Fig. 7d).

The present work contributes to the understanding of the IL-2, IL-4, IL-10, and IL-18 sequence of Marwari horse in respect of conservation of structural and functional motifs among domestic animals. Interestingly, number of amino acid sequences, structure, and functional motifs of horse IL-10 and IL-18 were highly conserved however, IL-4 and IL-2 showed marked variation in sequences and other vital structure. The availability of biologically active recombinant equine cytokines will be helpful to investigate the physiological roles of this cytokine, as well as its potential efficacy as a therapeutic agent or vaccine adjuvant in horse.

(H. Singha and Praveen Malik)

OIE Laboratory Twinning Project on Equine Piroplasmosis

NRCE successfully completed OIE sponsored Laboratory Twinning Project on Equine Piroplasmosis and consequently NRCE applied to the OIE, (through Indian OIE delegate) for recommending NRCE as OIE Reference Laboratory for Equine Piroplasmosis. This request was considered in the OIE Biological Standards Commission meeting in February, 2014. In the communication received from the OIE, the OIE Biological Commission agreed that Centre is active in the field of equine piroplasmosis research and diagnostics, and that application revealed its high level of scientific and technical expertise. The Commission pointed out that the laboratory does not have appropriate quality management system equivalent of ISO 17025, which is essential requirement for OIE Reference Laboratory. NRCE is pursuing towards processing the documents for ISO 17025 certification of Equine Piroplasmosis laboratory.

(Sanjay Kumar, Rajender Kumar and R.K. Singh)

OIE Twinning Laboratory Project on Glanders with Institute of Bacterial Infections and Zoonoses, FLI, Jena, Germany

Under OIE twinning Glanders a capacity building program for the Scientists of NRCE, India was organized at FLI Laboratory, Germany. The long-term objective of NRCE is to become Referral Centre for SAARC region besides imparting training to the competent researchers/scientists from these countries so that a systematic surveillance for the disease condition can be carried out. Under this project, two scientists Praveen Malik and Harisankar Singha from the candidate lab, NRCE, Hisar, were trained in the parent laboratory at Germany in May 2013 as part of the capacity building programme. (Fig. 8) Considering the intrinsic problems with CFT for diagnosis of glanders a better assay is mandated. The two recombinant proteins developed by NRCE were evaluated indirect ELISA, using samples from India and global collection of equine samples from the repository of OIE Reference Lab. The results were compared with FLI –LPS containing antigen based western blot and complement fixation test. These tests have the potential to become the alternative test for glanders. Two *B. mallei* isolates recovered from clinical specimens (nasal swabs = 2) obtained from glanders infected equines from Himachal Pradesh were identified by cultural and biochemical characteristics, fliP PCR and sequencing of PCR amplicons.

Fig. 8. OIE experts from the parent lab, Prof Heinrich Neubauer and Dr Mandy Elschner along with Dr Praveen Malik are observing the results of ELISA and CFT in the candidate lab, NRCE, Hisar.

OIE Experts from parent lab, Prof. H. Neubauer and Dr. Mandy Elschner visited the candidate laboratory in December 2013. The scientists observed the functioning and capacity of the NRCE in the areas of surveillance of glanders in the field and diagnosis in the laboratory. They have expressed their satisfaction on the working and progress of NRCE.

(Praveen Malik, H. Singha and R.K. Singh)

OIE Twinning Project on Equine Influenza between NRCE, India and Animal Health Trust, UK

A Laboratory Exchange Program was executed at the Animal Health Trust, UK (AHT) from 8th August, 2013 till 15th September, 2013, as described in the technical program of the OIE twinning project on equine influenza. The primary purpose was to build capacity at the candidate laboratory (National Research Centre on Equines, India, NRCE) in the area of advanced molecular epidemiology, diagnostics and surveillance of equine influenza. The visiting team comprised Dr R K Singh (Director, NRCE), Dr Nitin Virmani (Principal Scientist, NRCE Hisar), Dr Rajesh Kumar Vaid (Senior Scientist, VTCC/NRCE, Hisar) and Dr B. C. Bera (Scientist VTCC/NRCE, Hisar) (Fig. 9). Scientists from NRCE had extensive discussions and hands-on experience performing various assays for the diagnosis and surveillance of equine influenza along with molecular characterization of EI viruses. Training was provided in a number of techniques used regularly at the AHT including quantitative RT PCR and sandwich ELISA for diagnostic detection of virus, haemagglutination-inhibition assay for antigenic characterisation of virus isolates, single radial haemolysis (SRH) assay for measurement of antibody levels and single radial diffusion (SRD) assay for quantitation of haemagglutinin content of virus preparations. Comparison of methods used in the host laboratory and first-hand experience of the techniques by NRCE scientists highlighted differences and potential modifications that can be applied to optimise the methods used at NRCE. The long term aim is to fully validate the methods used at NRCE to OIE standards. In addition, an in depth series of experiments was carried out by both NRCE and AHT scientists to assess an NRCE assay for the detection of equine influenza, with the aim of using this as a straightforward and relatively low cost diagnostic assay in Indian laboratories. A detailed investigation was carried out for the antigen capture ELISA developed at the NRCE for the detection of equine influenza virus in equine nasal swabs. The NRCE assay differed from that used at the AHT primarily in that the monoclonal antibody used for detection of bound virus antigen reacted against the viral haemagglutinin (HA) rather than the nucleoprotein (NP). Extensive work was carried out to determine the sensitivity of the assay, using egg-grown stocks of viruses belonging to Florida clades 1 and 2 and the European and Kentucky sublineages. These studies showed that the assay could detect antigen equivalent to a virus stock containing 1,000 genome copies, determined by qRT-PCR. The NRCE assay requires further experiments to investigate the possibility that the anti-HA monoclonal antibody recognises a specific glycosylated form of the virus, present in egg grown virus, or another conformationally-dependent epitope as the

Fig. 9. NRCE Scientists visited AHT, UK

results with equine samples are variable. Alternative strategies will also be sought, including the generation of NP monoclonal antibodies at NRCE. During the exchange visit, genome sequencing strategies were discussed and demonstrated at the AHT, including the use of both a low cost Sanger sequencing method developed at the AHT and next generation sequencing approaches. The aim is to apply next generation sequencing to isolates from the recent Indian outbreak during the next visit by NRCE scientists. In addition NRCE and AHT had joint laboratory meetings held using Skype for discussions on the areas of respective research and understanding the picture of equine influenza in respective countries.

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

Consultancy and Commercialization of Technologies

Consultancy

NRCE acts as a National Referral Centre to provide 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 and expert technical advice is also provided to the stakeholders. 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 for EIA, glanders, equine influenza, EHV-1, EVA, CEM, *Theileria equi, Trypanosoma evansi, Trypanosoma equiperdum, Babesia equi, Salmonella* Abortus-equi, and African horse sickness were provided to various stakeholders. A total of 5795 serum samples from Thoroughbred as well as indigenous equines were examined by Coggins test for EIA under S&M (1442), disease investigation (16) and contractual service (4337), none was positive. Similarly a total of 7044 serum samples were tested for glanders, which included samples received under S&M (1442), disease investigation (364) and contractual service (5238). Seven samples from U.P., four from H.P. and one from Chattisgarh, respectively were found to be seropositive. Testing of 2733 samples under S&M and DI and 98 samples (57 vaccinated and 41 unvaccinated) under contractual service from various states for screening for equine influenza (H3N8) antibodies, employing Haemagglutination inhibition assay revealed none to be positive for active equine influenza infection. NRCE helped in preparation of dossier for disease free status of AHS and tested 542 serum samples collected during year 2011 to 14 which were negative for antibodies. In addition 30 samples were tested negative for AHS under contractual service. The Centre generated a revenue of ₹ 52.13 lakhs through testing of samples.

Following is the list of technologies under process of commercialization through National Research Development Corporation, New Delhi

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

NRCE has submitted Patent applications to Indian Patent Office, New Delhi for grant of following Patents : -

List of technologies applied for patents

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

Technologies Developed and Assessed

Assessment of *Burkholderia mallei* type six secretory proteins TssB and Hcp1 for serological diagnosis of glanders

For diagnosis of glanders, development of a modern diagnostic with defined immuno-dominant protein is the need of the time because of the inherent complexity and drawback of complement fixation test (CFT) which is OIE recommended serological test. Two immunogenic proteins namely TssB and Hcp1 of type six secretion system clusters of *Burkholderia mallei* were expressed and purified. The diagnostic efficacy of recombinant TssB protein was assessed by western blot and indirect ELISA using a panel of equine glanders negative (n=30) and positive (n=50) serum, human melioidosis positive (n=10) and negative (n=15), and more than 3000 equine serum samples (Fig. 1). Relative sensitivity and specificity of the ELISAs were 100% in comparison to CFT. The result demonstrates that TssB (Fig. 2) was not reacting with melioidosis human serum. In contrast, the Hcp1 could be used for diagnosis of both glanders and melioidosis, caused by *B. pseudomallei* a close relative of *B. mallei,* the causative agent of glanders.

The indirect ELISA method using recombinant TssB and Hcp1 proteins offered safer, rapid and efficient means of serodiagnosis of glanders in equines. Further, validation of the assay with equine serum from glanders endemic and glanders free countries will be useful for wider acceptance of the recombinant protein based ELISA.

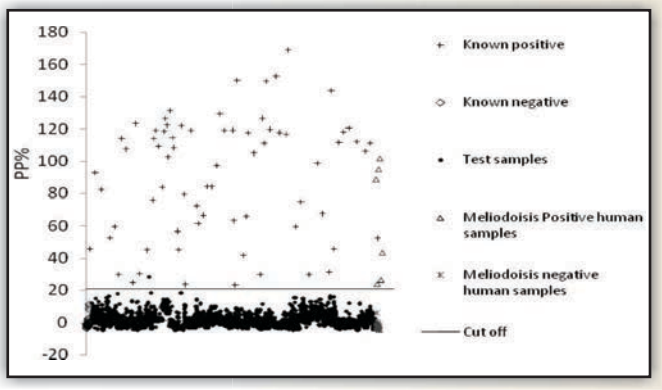


Fig. 1. Distribution of PP% values of equine serum samples (n=3235) as determined by iELISA. The PP% value was calculated by the formula: Percent positivity (PP%)= [(OD_{492} sample serum- OD_{492} negative control)/ [(OD_{492} positive control - OD_{492} negative control)]x 100%. The PP % value of 22 was calculated as diagnostic cut-off.

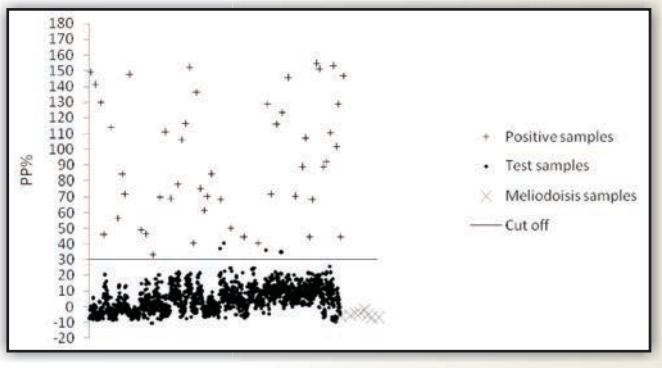


Fig. 2. TssB ELISA: distribution of PP% value of positive and negative serum samples. The horizontal line represents the cut-off value [percent positivity (PP%= 30)] Glanders positive serum are indicated by + sign, dot represent the test serum samples, melioidosis positive human serum are indicated by x symbol.

(Praveen Malik and Harisankar Singha)

Education and Training

Annual Review Meet of Veterinary Type Culture Collection Network Project

The fourth Annual Review Meet of Veterinary Type Culture Collection Network Project was held at NRCE, Hisar on September 13, 2013 under the chairmanship of Dr Gaya Prasad, ADG (AH). In his opening remarks, he requested the PIs of all network units to work according to their mandate to contribute maximum for strengthening of the VTCC repository. He wished that in the coming years, VTCC would be able to provide microbial cultures to the industry for commercialization. The Chairman further opined that VTCC should distribute microbial cultures and cell lines for teaching and research to the stakeholders. He also emphasized that VTCC should characterize the microbes available in the repository so as to create a bank of reference microorganisms. Director, NRCE remarked that the publications made under the VTCC project by the network units need to be acknowledged and copies of publications should be included with the VTCC Annual Report. Finally, the Chairman in his concluding remarks, besides appreciating the achievements for the financial year 2012-13, stated that the current policy of accessioning/ repositioning of microbial cultures should be more stringent and focused on acquisition of unique and important microbes. It was also emphasized that the idea of including so many network units in the project was to tap the vast microbial diversity in the culture collection; in this endeavor all the network units should contribute in the development of the repository. He further urged the scientists to explore the presence of bacteriophage(s) in the environment.

VTCC Annual Review meeting under progress

Trainings/Workshops/Seminars Organized

- Dr S. C. Yadav, Pr. Scientist, organized and convened an interactive meet of scientists working on Trypanosomosis in ICAR institutes on 26th November, 2013 at NRCE Hisar, as per the recommendations of RAC (2013). A need was felt to develop an effective control strategies through development of Network programme on Trypanosomosis.
- Two days workshop on "Health & welfare of Working Equines" was organized jointly by NRCE and Brooke Hospital for Animals (India) from 21-22 March, 2014 at NASC Complex, New Delhi.

Expert Lectures at NRCE

- Dr Jayaseelan Murugaiyan, from Freie Universitat Berlin, Germany, delivered lecture on "Application of MALDI-TOF in microbial identification" on 20 Nov., 2013.

Expert Lectures Outside

- Dr Anju Manuja delivered lectures in trainings organized at LUVAS, Hisar on nano based drug and vaccine delivery on 17 March, 2014.
- Dr Balvinder Kumar delivered a lecture in short course on 'Sequence Analysis" at Department of Nanotechnology, GJUS&T, Hisar on 8 March, 2014.

- Dr Balvinder Kumar delivered lectures in trainings organized at Department of Animal Biotechnology, LUVAS, Hisar on "Application of Nanotechnology in Animal Sciences" on 14 March, 2014
- Dr B.C. Bera delivered an expert lecture on "Generation and Application of Bacterial Artificial Chromosome of Virus Genome" in training course on "Nucleic acid based diagnostics and cell culture"" organized by Department of Animal Biotechnology, LUVAS, Hisar on March 20, 2014.
- Dr B.R. Gulati delivered a lecture on "Infectious Diseases of Equine Respiratory Tract" in Training on "Essentials of Equine Practice" organized by Teaching Veterinary Clinical Complex, LUVAS, Hisar on June 9, 2013.
- Dr B.R. Gulati delivered a lecture on 'Trends in Diagnosis and Control of Japanese Encephalitis in Animals' in 26th CAFT Training on Diagnosis and Control of Infectious Diseases of Small Ruminants, organized by Department of Veterinary Microbiology, LUVAS, Hisar on October 1, 2013.
- Dr B.R. Gulati conducted practical on 'Serodiagnosis of Japanese Encephalitis in Animals' in 26th CAFT Training on Diagnosis and Control of Infectious Diseases of Small Ruminants, organized by Department of Veterinary Microbiology, LLRUVAS, Hisar on 1 October, 2013.
- Dr B.R. Gulati delivered an expert lecture on "Viral Diversity" in training course on "Techniques in Genetic Engineering & Bioinformatics" organized by Department of Animal Biotechnology, LUVAS, Hisar, October 22, 2013.
- Dr B.R. Gulati delivered an expert lecture on "Biosafety and Risk Assessment in Research Laboratories", in Department of Nanotechnology, GJUS&T, Hisar, March 25, 2014.
- Dr B.R. Gulati delivered an expert lecture on "Risk Assessment, Biosafety and Biosecurity Measures in Research Laboratories", in DBT sponsored Short Course on Reproductive Biotechnology– An integrated Approach to Improve Reproductive Efficiency at Central Institute for Research on Buffaloes, Hisar, March 11-26, 2014.

RAC, IRC & IMC Meetings

XVI Research Advisory Committee (RAC) Meeting

The XVI Research Advisory Committee meeting of the Centre was held under the Chairmanship of Dr S.K. Dwivedi, Ex-Director, NRCE, Hisar on June 18, 2013 at NRCE, Hisar for appraisal of the research achievements of the ongoing projects and to consider new research project proposals. The RAC members who participated in the meeting included Dr Arun Varma, Ex-ADG (AN&P); Dr Gaya Prasad, ADH (AH) ICAR, New Delhi; Dr (Professor) Arvind Kumar, College of Veterinary Science, LUVAS, Hisar; ProCol (Dr) Devender Kumar, Dr R.K. Singh, Director, NRCE Hisar and Dr Yash Pal, Pr. Scientist, I/c PME & Member Secretary. Dr R.K.Singh presented various research activities being carried out at NRCE. The Chairman RAC applauded the scientists for their sincere contribution in the ongoing research programs and other developmental activities of the Centre. He opined that NRCE should have an equine clinic, and advised the scientists to empower themselves for providing efficient referral clinical services. RAC members were of opinion that NRCE should have the best indigenous animals, good breed of horses (true-to-breed horses and donkeys). RAC members emphasized that bio-security of microbial cultures is very important to prevent misuse of these agents (bio-warfare) and VTCC needs to emphasize on security of the preserved cultures & Centre should focus on the enhancement of indigenous equines through development of performance enhancer including nutraceuticals. The Chairman also emphasized on the training of technicians in farriery. Use of stem cells in disease management and therapy was recommended. It was suggested that research projects should be based on need of farmers and facilities and vision needs to be created for equine emergency management for example: scarcity of fodder, earthquake, flood, etc. Chairman emphasized that the Institute should strongly put efforts on utilization of equine energy in agricultural operations to enhance the value of equine power in agriculture sector.

Institute Research Committee (IRC) Meeting

The annual meeting of Institute Research Committee (IRC) was held under the chairmanship of Dr R. K. Singh, Director, NRCE, Hisar on July 23-24, 2013 at NRCE, Hisar for appraisal of the research achievements of the ongoing research projects and to consider new research project proposals for the year 2013-14. The IRC reviewed the progress of ongoing research projects in the area of Equine Production, Health, Extension, Veterinary Type Culture Collection and Externally Funded Projects. Thorough discussion was done on each project regarding outcomes, shortcomings and future course of work. Chairman, IRC emphasized that more progress has to be done in terms of the patents, technologies and publications in high impact journals.

IRC meeting in progress

XVII Research Advisory Committee (RAC) Meeting

The XVII Research Advisory Committee meeting of the Centre was held on March 10, 2014 under the Chairmanship of Dr S.K. Dwivedi, Ex-Director, NRCE, Hisar.

The RAC members who participated in the meeting included Dr Arun Varma Ex-ADG (AN&P), Dr Gaya Prasad, ADH (AH), Dr Arvind Kumar, Dr A.K. Gupta, Acting Director (NRCE), Sh G.P.S. Posana and Dr Yash Pal, I/c PME & Member Secretary. Dr. A. K. Gupta, Director, NRCE gave a presentation of the salient achievements of different research projects going on at the Centre. The Chairman RAC appreciated the work done by the scientists in the mandated area of the Centre and emphasized for generation of demand-driven technologies for the benefit of stakeholders as well as need based research. The Chairman also expressed concern over the dwindling equine populations and need to speed up the research in this endeavor by NRCE. He suggested that being the only Centre on equines, we should have top quality equine clinicians to meet the demand of stakeholders in equine sector. NRCE should have package of practices on feed and fodder for different regions and environment including disaster management. The RAC again strongly recommended that the Centre should be upgraded to National Institute on Equine Research. The Chairman and other members emphasized that NRCE should focus on development of semen bank of elite stallions. For this semen may be collected from field from superior quality stallions. The committee further stressed upon the need of ISO certification of NRCE Laboratories for international accreditation.

RAC meeting in progress

35th Institute Management Committee Meeting (IMC)

The 35th meeting of Institute Management Committee (IMC) was held on April 21, 2013 at NRCE, Hisar under the Chairmanship of Dr R.K. Singh, Director, NRCE, Hisar. The esteemed members of IMC such as Dr R.L. Sandal, (Asstt. Director, Animal Husbandry, Himachal Pradesh-Representative of the Director, Animal Husbandry, HP); Dr S.C. Mehta (Principal Scientist, NRCC, Bikaner); Dr R.K. Sawal, (Principal Scientist, Regional Station CSWRI, Bikaner); Dr Yash Pal (Pr. Scientist); Col (Dr) Umaid Singh Rathore; Sh A.P. Sharma (F&AO, IASRI, New Delhi); and Sh R.B. Saxena (Admn. Officer, NRCE, Hisar) attended the meeting. Prof (Dr) R.N. Shreenivasa Gowda, Chairman, QRT and Dr A.K. Gupta, Member Secretary, QRT were special invitees for IMC meeting. The IMC adopted and confirmed the proceedings of 34th IMC meeting and discussed about the various issues of the Centre. The committee approved the agenda items like use of Type-V Quarter for the students, additional sanction for interior furnishing of VTCC building at NRCE, Hisar; additional sanction for construction of Stables (6 Nos.) at EPC, Bikaner; creation of sports cell with indoor sports facility, sharing of resource generated through EIA/Glanders/CEM testing under contractual service, etc. The IMC emphasized upon purchase of equipments, stores and execution of some works in the financial year 2013-14. Prof (Dr) R.N. Shreenivasa Gowda, Chairman, QRT of NRCE apprised the IMC of the salient features of QRT report. The IMC agreed with the recommendations made by QRT.

Workshop, Seminar and Institutional Activities

National Workshop on Welfare of Working Equines

The Brooke India and National Research Centre on Equines, Hisar jointly organized National workshop on Equine Health and Welfare during March 21-22, 2014 at National Agriculture Science Center, New Delhi. The workshop was inaugurated by Dr S. Ayyappan, Hon'ble Secretary DARE and DG, ICAR. On this occasion, Dr Suresh S Honnappagol, Commissioner, Animal Husbandry Department Govt. of India; Gen. N.S. Kanwar, DG RVS, AHQ, New Delhi; Maj. Gen. (Retd.) Dr. R.M. Kharb AVSM Chairman, AWBI; Dr Gaya Prasad, ADG (AH), ICAR were present as Guest of Honor. Rear Admiral (Retd.) V. K. Malhotra AVSM, VSM, Director Brooke India, Ltd. welcomed the participants to the National Workshop on Welfare of Working Equines. Dr A.K. Gupta, Director, NRCE, Hisar presented the brief outline of scenario of equine husbandry in India and presented "Current challenges in equine sector in India- Role of NRCE". The workshop was attended by 75 scientists, veterinary officers, officials from Department of Animal Husbandry, Dairying & Fisheries, Govt. of India, Director or their representative from State Animal Husbandry Departments, NGOs, Brooke (India) & NRCE Scientists.

Inaugural speech by Dr. S. Ayyappan, Secretary DARE and DG, ICAR

Interactive Technical Sessions on Equine Health & Welfare

During the two day workshop Interactive Technical Sessions on Equine Health & Welfare: Status & Challenges, Equine Health and Welfare in Equine Fairs & Pilgrimage Sites, Current Status of Equine Diseases in India- A way Forward, Equine Production and Management in working equines were organized along with a poster session on display of new technologies in Equine Health and Production. Scientists from NRCE, officials from Brooke India and participants in the seminar deliberated on various aspects of Equine Health & Welfare during the workshop.

हिन्दी सप्ताह का आयोजन

केन्द्र में 1-7 अक्टूबर, 2013 को हिन्दी सप्ताह का आयोजन किया गया। इस अवसर पर केन्द्र में हिन्दी के अधिकाधिक प्रयोग हेतु हिन्दी भाषा से सम्बन्धित विभिन्न स्पर्धाओं का आयोजन किया गया जिसमें केन्द्र के अधिकारियों, कर्मचारियों व केन्द्र सरकार से सम्बंधित अन्य संस्थाओं के कर्मचारियों ने बढ़-चढ़ कर हिस्सा लिया। 7 अक्टूबर, 2013 को आयोजित हिन्दी सप्ताह समापन एवं पुरस्कार वितरण समारोह आयोजित किया गया। इस कार्यक्रम में डॉ. पी.एस. लाम्बा, पूर्व उप-कुलपति मुख्य अतिथि

के रूप में उपस्थित थे। मुख्य अतिथि ने अपने भाषण में दैनिक कामकाज में हिन्दी के अधिकाधिक प्रयोग पर जोर दिया। कार्यक्रम में मुख्य अतिथि द्वारा विभिन्न प्रतियोगिताओं में पुरस्कार वितरण किया गया। हिन्दी भाषान्तर प्रतियोगिता प्रथम पुरस्कार श्री के.एस. मीणा, द्वितीय पुरस्कार श्री डी.डी. शर्मा व तृतीय पुरस्कार श्रीमती शम्मी त्यागी ने प्राप्त किया।

निबन्ध प्रतियोगिता में प्रथम पुरस्कार श्रीमती आशमा, द्वितीय पुरस्कार श्रीमती शम्मी त्यागी व तृतीय पुरस्कार श्रीमती पार्वती शर्मा ने प्राप्त किया। केन्द्रीय कर्मचारियों के काव्य पाठ प्रतियोगिता में प्रथम पुरस्कार श्री नरेश शर्मा, द्वितीय पुरस्कार श्री नरेश दत्त व तृतीय पुरस्कार श्रीमती कोमल सरदाना ने प्राप्त किया।

हिन्दी सप्ताह का आयोजन

Kisan Call Centre: Toll-free helpline for equine owners inaugurated at EPC, Bikaner

Toll-free helpline providing advisory services to equine owners from EPC, Bikaner on the lines of "Kisan Call Centre" was inaugurated by Dr K. M. L. Pathak, Hon'ble DDG (AS) on August 17, 2013. The EPC, Bikaner toll-free helpline number 1800-180-6225 can be accessed by equine owners from all over the country. The queries of the farmers are addressed by the experts at EPC, Bikaner and relevant advice is provided to equine owners on health and management aspects of equine husbandry.

Hon'ble DDG (AS) Dr K.M.L. Pathak inaugurating Kisan Call Centre at EPC, Bikaner

Vigilance Awareness Week at the Centre

Vigilance Awareness Week was observed from 28th October to 2nd November 2013. As part of Vigilance Awareness Week celebration Dr R.K. Singh, Director, NRCE administered pledge to the scientists and staff of NRCE on October 28, 2013 for honesty and transparency in public life. He also stressed on coordinated efforts for eliminating the menace of corruption from the society. As part of Vigilance Awareness Week celebration on October 30,

2013 a Lecture by Sh. Patram ji Hon'ble SP Vigilance, Hisar on "Promoting Good Governance - Positive Contribution of Vigilance" was organized at NRCE.

Vigilance Awareness Week at the Centre

Foundation Day Celebration at NRCE

National Research Centre on Equines celebrated its 28th foundation day on November 26, 2013. A foundation day lecture was delivered by Maj. Gen. Shri Kant Sharma Vice-Chancellor LUVAS, Hisar. Brigadier Desh Raj, Equine Breeding Stud, Hisar and Dr R. K. Sethi, Former Director CIRB were present as guest of honour on the occasion. In his foundation day lecture, Maj. Gen. Shri Kant emphasized on role of equines in Indian culture and history and utility of equines in modern times. On this occasion a plantation drive was organized where in chief guest and other dignitaries planted trees near animal shed complex at NRCE.

Foundation Day Celebration at NRCE

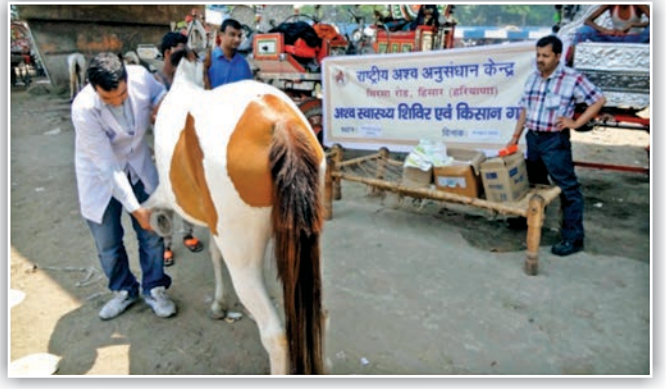
Equine Health Camps and Kisan Goshthis Organized

A total of 16 equine health camps, awareness camps, interactive meet of equine owners and scientists and Kisan Goshthis were organized at Jodhpur (April 03-04, 13 & August 22, 2013), Gujron ka Mohalla; Bikaner (May 25, 2013), Ridmalsar, Bikaner (June 28, 2013), Bundi (July 04, 2013), Suratgarh (July 28, 2013), Tehla (October 17, 2013), Degana (October 18, 2013), Thanwala (December 30, 2013), Jassusar Gate; Bikaner (March 31, 2014), in Rajasthan, Julana (April 05, 2013), Rajli (April 27, 2013) in Haryana, Kolkata and Digha (April 10-11, 2013) in West Bengal, Allahabad and Varanasi (February 23-25, 2014) & Barabaki and Lucknow (March 1-2, 2014) in Uttar Pradesh. During equine health 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 during the camps. The interaction between scientists and equine owners, Kisan Goshthis and Interaction Meet with equine owners helped to

understand the problems of equine owners and information on health, production and management aspects of equine husbandry was given to the equine owners.

Equine Health Camp at Suratgarh

Pregnancy diagnosis at Equine Health Camp in Jodhpur



Equine Health Camp at Kolkata

Interactive meet of NRCE Scientists & Brooke staff with equine owners at Lucknow

Participation in Exhibitions /Animal Fairs/ Kisan Mela

NRCE participated in various exhibitions, animal fairs and kisan melas at state and national level displaying different technologies developed at 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 screened 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.

Participation in Exhibitions, Animal Fairs and Kisan Mela

S. No	Place	Date
1.	Marwari horse show, Jodhpur, Rajasthan	April 03-04, 13
2.	Kisan Mela at Dera Sacha Sauda, Sirsa, Haryana	September 26-27, 2013
3.	IIHTC Durgapura Jaipur organized by RAJUVAS, Bikaner, Rajasthan	September 28-29, 2013
4.	National Livestock Championship Muktsar, Punjab	January 8-12, 2014
5.	Kisan Sammelan, Jhajjar, Haryana	January 19, 2014
6.	CIRB Buffalo Mela, Hisar, Haryana	February 01, 2014
7.	IVRI Kisan Mela, Izatnagar, Uttar Pradesh	February 28, 2014

Exposure Visit of Farmers/Educational Tours of Students/Study Tours & Visits

During 2013-14, visitors from different places viz. students from SAU's and schools, farmers and other visitors visiting NRCE were briefed about the research activities and different extension and field activities of NRCE for benefit of equine owners and various on-going programmes of NRCE.

Sr. No.	Details of Visitors	Date	No. of Visitors
1.	Study tour of trainees from CCSHAU, Hisar	April 25, 2013	22
2.	Study tour of BVSc. students form LUVAS, Hisar	June 06, 2013	24
3.	Study tour of BVSc. students form CVAS Bikaner	October 11, 2013	28
4.	Exposure visit of farmers from Ellnabad, Sirsa, Haryana	September 13, 2013	26
5.	Exposure visit of farmers from Suratgarh, Rajasthan	November 27, 2013	18
6.	Exposure visit of HCS officers, Haryana	December 04, 2013	20

National Livestock Championship, Muktsar

CIRB Buffalo Mela, Hisar

IVRI Kisan Mela, Izatnagar

Kisan Sammelan, Jhajjar

Infrastructure Development and Developmental Activities

Veterinary Type Culture Collection

The first phase of construction of VTCC building has been completed and the laboratory building has become fully functional. The internal furnishing of the individual laboratories, including the Microbial repository and other support facilities has also been completed. Besides the entrance lobby of the VTCC building has been renovated to include a Reception cum visitors room at the ground floor and Seminar/Conference room on the first floor. Furthermore, the work of converting the space for Seminar /Conference room including furnishing has been initiated by the executing agency. The second phase construction of the VTCC building is also in full swing and is likely to be completed shortly which would help in augmenting the current research activities. This building will have the provision of walk in incubator/freezer as well as a teaching laboratory for Bioinformatics. Besides, the work for the construction of Laboratory Animal House Phase-II has also been awarded to NPCC, New Delhi. A dedicated hotline connection has been provided by DHBVN. For electricity backup, a 500 KVA DG set has also been procured. Installation and commissioning is underway.

Agriculture Farm Production at NRCE, Hisar

Production of crops

During the period under report, about ninety nine acres of land was used under crop rotation scheme for growing both leguminous and non-leguminous 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 1348 Qt. green and 260 Qt. dry fodder was supplied to Centre's animal farm which proved to be step towards self sufficiency in terms of fodder requirement is concerned (Table 1). Besides this, surplus was being used for revenue generation.

Table 1. Production of Crops

Sr. No.	Crop	Area (Acre)	Production (Qt.)
1.	Oat + Berseem	2.5	251
2.	Berseem		52
3.	Sorghum sudan grass + Cowpea	12.5	449
4.	Sorghum sudan grass		240
5.	Lucerne	1.0	191
6.	Maize + Cowpea + Bajra	7.5	165
7.	Oat	35.5	-
8.	Mustard	40.0	-

Reclamation and development of field

About thirty acres of land, near pond, was weeded out and developed for future planning of crop cultivation.

1) **Revenue generation:** A sum of ₹ 10, 04, 990/- (₹ Ten lac four thousand nine hundred ninety only) was generated through sale of 62.50 Qt. oat straw, 273.66 Qt. oat grain & 142.40 Qt. mustard grain. A sum of ₹ 21,22,866/- was received from sale of eucalyptus to Haryana Forest Development Corporation, Hisar. Total revenue of Rs. 31,27,856/- (Rs. Thirty one lac twenty seven thousand eight hundred fifty six only) was received during the year.

2) **Landscape and plantation work:** Different species of flowering, ornamental and shady plants were planted to improve environmental condition of the campus. The plantation around animal sheds & farm area will provide shelter to the farm people and animals.

Agriculture Farm Production at EPC Bikaner

During the period under report a total of 2403.22 quintals of green fodder (Lucerne 578.00 Qtls + Oat 691.30 Qtls+Millet 681.45Qtls + sorghum 452.47 Quintals) and 110.00 quintals dry fodder of Oats was produced at the agriculture farm of EPC Bikaner for the animals. Furthermore, the expenditure incurred every year on the purchase of chaffed sewan grass was saved by the production of maintenance fodder. The farm has also been able to produce 51.05 quintals of Oats grains in 30 acres and Lucerne in 5.05 acres of land. EPC has also been maintaining a lawn in 4 acres & with 1000 new plantations. Besides 8-10 acres land has also been reclaimed at the sub campus Bikaner.

Vermicomposting

Vermicomposting from equine dung is a continuing activity at EPC, Bikaner. During the period under report, about 160 quintals (Four batches of four vermin bags) of vermicompost was produced. The vermicompost was utilized for maintaining the lawns and plantations at Equine Production Campus, Bikaner.

Oat crop at EPC, Bikaner farm

Lucerne crop at EPC, Bikaner farm

Livestock strength at NRCE, Hisar & EPC, Bikaner

Herd Strength at Equine Production Campus, Bikaner (2013-14)

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 01.4.2013	27	52	09	07	00	00	00	03	10	18	18	07	03	02	156
Purchased during the year	00	00	00	00	04	07	00	00	00	00	00	00	00	00	11
Births during the year	02	01	00	00	00	02	00	00	04	03	01	00	00	00	13
Deaths during the year	00	07	00	01	00	00	00	00	01	00	01	00	00	00	10
Auction/sold during the year	11	10	03	00	00	00	00	01	02	03	08	00	00	00	38
Balance as on 31.03.2014	18	36	06	06	04	09	00	02	11	18	10	07	03	02	132

Herd Strength at NRCE Main Campus, Hisar

The present herd strength of NRCE main campus is 27 animals which include Marwari Horses (20 Nos.), Pony (2 Nos.), Exotic Donkey (4 Nos.) and Mule (1 No.)

Cryopreserved Semen Bank at EPC Bikaner

During the period of April, 2013 to March, 2014, a total of 331 semen doses from Marwari stallions of NRCE were cryopreserved at Farm. Besides, twenty five frozen semen doses (200 number of 0.5 ml straw) were cryopreserved from an elite stallion “Prince” in field at Tehla, Nagaur (Rajasthan).

On-Going Research Projects (2013-14)

Equine Health

Sr. No.	Title	Team	From	To	PIMS Code
1.	Surveillance, Monitoring and Control of Emerging and Existing Diseases of Equines	S.K. Khurana*, B.K. Singh (upto July 31, 2013), S.C. Yadav, B.R. Gulati, Rajender Kumar, P. Malik, Sanjay Kumar, Nitin Virmani, Sanjay Barua, R.K. Vaid, Anju Manuja, H.S. Singha and Ramesh Dedar	April, 1995	Continuous Service Project	IXX00257
2.	Evaluation of *in vitro* growth inhibitory efficacy of some novel synthetic drug molecules against *Theileria equi* haemoprotozoa	Sanjay Kumar*, Rajender Kumar and A.K. Gupta	Nov., 2013	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. Gualti*, 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

Equine Production

Sr. No.	Title	Team	From	To	PIMS Code
1.	Characterization of indigenous non-descript and geographically distinct donkeys	A.K. Gupta*, Yash Pal, Anuradha Bhardwaj and Sanjay Kumar	Aug., 2010	Mar., 2014	IXX00274
2.	Cloning, expression and characterization of equine chorionic gonadotropin (eCG)	Anuradha Bhardwaj*, A.K. Gupta, Sanjay Kumar and Varij Nayan	Dec., 2010	Mar., 2014	IXX02769
3.	Characterization of donkey milk with emphasis on important milk proteins	Yash Pal*, Sanjay Kumar, R.A. Legha and Anuradha Bhardwaj	Oct., 2012	Sept., 2015	IXX07761
4.	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	Mar., 2015	IXX07762

Sr. No.	Title	Team	From	To	PIMS Code
5.	Therapeutic and performance enhancing capacity of antioxidants in equines	R.K. Dedar*, Vijay Kumar, Jitender Singh (upto March 9, 2014) and A.P. Singh	July 2012	Mar., 2015	IXX09641
6.	Endocrine, biochemical and gene expression profiling of reproductive states in Marwari Mares	Vijay Kumar*, Sanjay Kr. Ravi, R.K. Dedar, Raghvendra Singh and J. Singh (upto March 9, 2014)	Oct., 2012	Mar., 2015	IXX09663

Extension

Sr. No.	Title	Team	From	To	PIMS Code
1.	Studies on existing management systems and utilization of donkeys and mules for sustainable livelihood	A.A. Raut*, Yash Pal and R.A. Legha, R.K. Dedar and J. Singh (upto March 9, 2014)	Sept., 2009	Sept., 2014	IXX00268

VTCC

Sr. No.	Title	Team	From	To	PIMS Code
1.	Isolation, maintenance and characterization of bacterial pathogens and their molecular identification	R.K. Vaid*, Sanjay Barua, B.C. Bera, Taruna Anand (from July, 2010), T. Riyesh (from January, 2012)	June, 2007	Mar., 2014	IXX00269
2.	Isolation, molecular characterization and reposition of viruses of animal origin	Sanjay Barua*, B.C. Bera, R.K. Vaid, B.R. Gulati, T. Riyesh, and Taruna Anand (from Dec. 3, 2011)	Sept., 2009	Mar., 2014	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

Externally Funded Research Projects

Sr. 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*	Apr., 2011	Apr., 2016	OXX01431
2.	All India coordinated research project on utilization of animal energy with enhanced system efficiency	R.A. Legha* and Yash Pal	July, 2009	Mar., 2015	OXX00486
3.	Isolation characterization of animal adenoviruses development of a novel viral vector for vaccine delivery	Sudhanshu Vrati*, B.R. Gulati, Minakshi, K. Kumanan, M. Parthiban, Amarjit Singh and Ramnek	June, 2010	May, 2013	OXX00393

Sr. No.	Title	Team	From	To	PIMS Code
4.	Studies on *B. mallei* for rapid diagnosis of glanders in equines using molecular tools.	Praveen Malik*, S.K. Khurana, H.S. Singha and R.K. Singh (upto Feb, 4, 2014)	Aug., 2010	July 2013	OXX00394
5.	Development of biomarker(s) for diagnosis of *Trypanosoma evansi* infection animals using proteomic approach	Utpal Tatu*, S.C. Yadav, Rajender Kumar and B.C. Bera	June, 2011	May, 2014	OXX01616
6.	Synthesis, characterization and evaluation of drug loaded nano-formulation against *Trypanosoma evansi* in animal model	Anju Manuja*, Neeraj Dilbahgi, Sandeep Kumar, Rajender Kumar, Balvinder Kumar and S.C. Yadav	Mar., 2012	Mar., 2015	OXX01526
7.	Isolation and characterization of equine Mesenchymal stem cells from amniotic fluid	B.R. Gulati*, Pawan Kumar, Prem Singh Yadav, Taruna Anand and B.K. Singh (upto July 31, 2013)	Apr., 2012 (for 18th Months)	Sept., 2013	OXX02186
8.	Eukaryotic expression of important equine cytokines and analysis of their biological activities	H.S. Singha*	Jan., 2013	Dec., 2015	OXX02228
9.	OIE Twinning program for Glanders	Praveen Malik*, H.S. Singha and R.K. Singh (upto Feb 4, 2014)	July, 2012	June, 2015	OXX02428
10	OIE Twinning program on *Equine Influenza*	Nitin Virmani*,B.C. Bera, R.K. Viad and R.K. Singh (upto Feb 4, 2014)	Oct., 2012	Sept., 2015	OXX02429
11.	Thermo-stabilization of recombinant protein antigens in diagnostic assay/kits using heavy water	R.K. Singh*	Mar., 2013	Mar., 2016	OXX02435

* Principal Investigator

Research

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12. Gulati, B.R., Kumar, R., Mohanty, N., Kumar, P., Somasundaram, R.K. and Yadav, P.S. 2014. Bone morphogenetic protein-12 induces tenogenic differentiation of mesenchymal stem cells derived from equine amniotic fluid. *Cells Tissue Org,* DOI 10.1159/000358231.
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Participation in Training, Workshop Conferences and Symposia

International Trainings and Visits Abroad

1. Dr Praveen Malik and Dr Hari Singha attended a capacity building programme on serodiagnosis of glanders at OIE referral lab on glanders (FLI, IBIZ, Jena, Germany) under OIE Twinning laboratories project from April 26, 2013- May 31, 2013.
2. Dr S.C. Yadav attended annual meeting of Group of Non-Tsetse transmitted Animal Trypanosomosis (NTTAT) meeting held on May 26, 2013 Paris, France, sponsored by DBT New Delhi. He presented regarding an outbreak of Trypanosomosis in Thoroughbred horses from Indian Race courses. In the meeting he also explored the possibility of collaboration between NRCE and OIE referral laboratory at NRCPD, Japan and the same in principle was agreed by Head of respective laboratory.
3. Dr R. K. Singh attended "Leadership Decision Making" program at Harvard Kennedy School, USA, from 23- 28 Jun 2013. This program was sponsored under NAIP scheme of the ICAR, New Delhi. This program helped in learning decision making process in executive leadership.
4. Dr Taruna Anand attended NAIP, ICAR funded International training in the area of "Stem Cell Research" at Federal Research Institute for Animal health (FLI), Institute of Farm Animal Genetics, Neustadt, Germany from July 12, 2013 to October 9, 2013. During the training she learned various techniques for derivation of the induced pluripotent stem (iPS) cells from transgenic (crytom) mice somatic cells by transfection of reprogramming cassette carrying transposon and sleeping beauty transposase genes. She subsequently examined their potential for differentiation to eye lens lineage.
5. Dr Nitin Virmani, Dr Rajesh Kumar Vaid and Dr B.C. Bera attended Laboratory Exchange Program, for capacity building of NRCE Scientists in the area of advanced molecular epidemiology and diagnosis and surveillance of equine influenza under OIE Twinning Laboratory program on Equine Influenza between Animal Health Trust, Lanwades Park, Kentford, New Market, UK and National Research Centre on Equines, Hisar, Haryana, India from 8th August, 2013 till 15th September, 2013.
6. Dr R.K. Singh visited Animal Health Trust, Lanwades Park, Kentford, New Market, UK from 8th August, 2013 till 12th August, 2013 under OIE Twinning Laboratory program on Equine Influenza for finalization of collaborative work to be taken up under the project.
7. Dr. Anuradha Bhardwaj attended NAIP-ICAR International training for three months in the area of "Marker Assisted Selection" in the lab of Dr MF Rothschild, Department of Animal Sciences, Iowa State University, Ames, Iowa, USA from 12th September to 10th December 2013. The training was provided for utilization of molecular markers such as SNPs and Microsatellite markers for studying genetic diversity and population structure in animals. Training on computer languages eg UNIX, Linux and PERL, bioinformatics software such as 'R', PLINK and STRUCTURE and tutorials on NGS and RNASeq analysis.
8. Dr Hari Singha attended NAIP international training on the subject area of 'Allele Mining' from 19th Sept-11th December, 2013 at Gluck Equine Research Center, University of Kentucky, USA. He worked in the area of Dissection of naturally occurring variation at candidate genes/loci' using novel genomic tools referred as allele mining.
9. Dr R.K. Singh attended five days meeting on "Infectious Diseases of the Working Horses and Donkeys" from Nov. 18-22, 2013 at Addis Ababa, Ethiopia at ILRI-Ethiopia Campus. This meeting was sponsored by Havemeyer Foundation of New York, with additional support from SPANA, the Brooke and

the Donkey Sanctuary. Dr Singh also presented a paper on infectious diseases of working equinds in India and discussed various aspects of new research initiatives so as to improve the living of working equids.

10. Dr Praveen Malik attended, as Expert, the meeting of 'the OIE ad hoc group on Glanders' in Paris on November 26-28, 2013 to review the current chapter on glanders in the OIE Terrestrial Animal Health Code, and to discuss the status of Glanders to be included as 'official disease status'.

11. Dr Nitin Virmani, attended NAIP, ICAR funded international training in the area of "Molecular diagnostics" on detection and differentiation of neurogenic and non-neurogenic forms of EHV-1 in the laboratory of Klaus Osterrieder, Institut Fur Virologie, Freie Universitat Berlin, Germany from 08th Oct., 2013 to 30 Dec., 2013. During the training several molecular approaches viz. real-time PCR based differentiation neurogenic and non-neurogenic strains of EHV-1, generation of bacterial artificial chromosome (BAC) of EHV-1 genome, restriction analysis of amplicons of ORF30, sequencing of ORF68 & molecular grouping of EHV-1 and expression & functional analysis of baculovirus expressed recombinant gE protein of EHV-1 were learnt.

12. Dr B.C. Bera attended NAIP, ICAR funded International training in the area of "Biomolecules" at Institut Fur Virologie, Freie Universitat Berlin, Germany from 08th Oct., 2013 to 30 Dec., 2013. During the program he got familiarized with techniques associated with the generation of bacterial artificial chromosome (BAC) clone of EHV-1 genome, maintenance of BACs in bacteria, regeneration of virus from BAC clone and mutagenesis of virus genome in bacteria. The experience and scientific skills gathered through training in BAC technology, will be of immense use in generation of BAC library of Indian isolates of various viruses viz., herpesvirus, buffalopox virus, goatpox virus, swinepox virus etc. available in the VTCC repository. The viral BAC clones will significantly contribute to decipher the molecular pathogenesis and development of marker vaccines to combat against diseases.

13. Dr Nitin Virmani attended Expert Surveillance Panel meeting on Equine Influenza for vaccine strain selection organized by OIE at their head quarters in Paris, France on March 4, 2014. Equine Surveillance Panel meeting is conducted every year at the HQ of OIE in Paris to review the equine influenza activity and for deliberations on viruses isolated and vaccine performance. Dr. Nitin Virmani gave a presentation on equine influenza scenario in India apart from active discussions for strategizing the vaccine strain selection for the year 2014-15.

Participation in Trainings

- Dr A.K. Gupta, Dr S.C. Yadav, B.R. Gulati and Balvinder Kumar attended Internal Auditors training programme on "Quality Management Systems as per the ISO 9001; 2008" on Oct. 30. 2013 held at NRC on Equines.
- Dr Balvinder Kumar attended International Course in Laboratory Animal Science Based on FELASA Category 'C' Certification, Jointly organized by CSIR-IGIB and CPCSEA, from November 18-29, 2013.
- Dr Nitin Virmani attended workshop on IPv6 (Internet Protocol Version 6) in DARE/ICAR held on February 27, 2014 at NASC Complex, PUSA, New Delhi. The objective of the workshop was to sensitize and create awareness about IPv6 among ICAR institutes and implement same.
- Dr Sanjay Kumar attended four days training workshop on "Quantitative Proteomics Technologies" at Institute of Bioinformatics, Bangalore from 28-31 August, 2013.

Participation in Conferences, Workshops and Symposia

1. A.A. Raut attended workshop cum installation training programme on SAS at Statistical Computing Hub-II (NDRI, Karnal). NAIP Consortium Strengthening Statistical Computing for NARS during September 30, 2013 to October 1, 2013.

2. A.K. Gupta, S.C. Yadav, B.R. Gulati, Yash Pal, Rajender Kumar, P. Malik, S.K. Khurana, R.A. Legha, Sanjay Kumar, Balvinder Kumar, R.K. Dedar, A.A. Raut and S.K. Ravi attended National Workshop on Health and Welfare of Working Equines Organized

jointly by Brooke Hospitals for Animals and National Research Centre on Equines at NASC complex New Delhi from March 21-22, 2014.

3. Anju Manuja attended National conference on "Nanoscience and Biotechnology: Present and future prospective" on April 20, 2013 at Integral University, Lucknow.
4. Anju Manuja attended National Symposium on "Emerging Challenges & Opportunities in Veterinary Immunology & Biotechnology for Improved Animal Health and Productivity" organized by ISVIB, from Nov., 11-13, 2013 at Department of Veterinary Microbiology, College of Veterinary & Animal Sciences, CSKHPKV, Palampur,
5. B. R. Gulati participated and presented a paper entitled 'Characterization of Equine Adenovirus Isolates for use as a Vector for Vaccine Delivery' in Asia Pacific Congress of Virology (Virocon-2013), Amity University, Noida, Uttar Pradesh, December 17-20, 2013.
6. B. R. Gulati participated and presented an invited paper entitled 'Diagnosis and Control of Japanese Encephalitis among Animals in India: Progress and Perspective' in Asia Pacific Congress of Virology (Virocon-2013), Amity University, Noida, Uttar Pradesh, December 17-20, 2013.
7. B. R. Gulati participated and presented a paper entitled 'Immunophenotyping and gene expression profiling of mesenchymal stem cells derived from equine umbilical cord blood' in International Conference on Reproductive Health: Issues and Strategies under Changing Climate Scenario and 24th Annual Meeting of Indian Society for the Study of Reproduction and Fertility, Indian Veterinary Research Institute, Izatnagar (India), February 6-8, 2014.
8. B. R. Gulati participated and presented an Invited Paper entitled 'Effect of bone morphogenic protein-12 on in vitro tenogenic differentiation of equine amniotic fluid derived mesenchymal stem cells' in International Conference on Reproductive Health: Issues and Strategies under Changing Climate Scenario and 24th Annual Meeting of Indian Society for the Study of Reproduction and Fertility, Indian Veterinary Research Institute, Izatnagar (India), February 6-8, 2014.
9. B. R. Gulati presented an invited paper entitled 'An Overview of Equine Viral Diseases' in National Workshop on Health and Welfare of Working Equines, organized by Brooke Hospital for Animals (India) and NRCE Hisar, NASC Complex, New Delhi, March 21-22, 2014.
10. Balvinder Kumar attended International conference on "Nanotechnology: Lessons from nature and emerging technologies" from July, 25-26, 2013 at Ansal University, Gurgaon.
11. Balvinder Kumar attended National conference on "Nanoscience and Biotechnology: Present and future prospective" on April 20, 2013 at Integral University, Lucknow.
12. Balvinder Kumar attended National Symposium on "Emerging Challenges & Opportunities in Veterinary Immunology & Biotechnology for Improved Animal Health and Productivity" organized by ISVIB, from Nov. 11-13, 2013 at Department of Veterinary Microbiology, College of Veterinary & Animal Sciences, CSKHPKV, Palampur, India.
13. Balvinder Kumar attended interaction on "Maintenance and functioning of BSL III facility" on March 20, 2014, at NIV Pune.
14. Praveen Malik participated in National symposium and XXVII annual convention of IAVMI, December 13-15, 2013 at Deparment of Animal Husbandry, Lucknow, and presented paper on "Detection of glanders among indigenous equines in Uttar Pradesh, Himachal Pradesh and Chhattisgarh. U.P".
15. Praveen Malik attended the Asia-pacific congress of Virology (Virocon-2013) Organized by Amity institute of Virology & Immunology, Noida (New Delhi NCR) and Indian Virological Society from December 17-20, 2013 at NOIDA and co-chaired a session on vector-borne and zoonotic viral infections.
16. R. A. Legha attended one day Workshop on "Wildlife Management and Health" & Hands on Training on "Immobilization of wild animals for transportation and treatment" on August 13, 2013 held at Centre for Studies on Wildlife Management & Health, RAJUVAS, Bikaner, Rajasthan.

17. R. A. Legha participated and presented the progress report of the project in XIII Biennial Workshop of the AICRP on UAE held at GBPUAT, Pantnagar during 13-15 June, 2013.
18. R. A. Legha Participated and presented the technical programme of the project in XIII Coordination Committee Meeting of the AICRP on UAE held at IGKVV, Raipur during 21-22 October, 2013.
19. Rajender Kumar participated in 7th SIR Conference and Meditation retreat on "Role of Researchers in Shaping the Society "organised by Spiritual Application Research Centre, Mount Abu from Aug 30-Sept. 03, 2013.
20. Rajender Kumar participated in XXIV National Congress of Veterinary Parasitology organized by College of Veterinary &Animal Sciences, Mannuthy, Thrissur, Kerala from Feb.5-7, 2014.
21. R. K. Singh attended the ISVIB conference at IVRI Izatnagar on April 08-09, 2013.
22. R. K. Singh attended the XX Annual Convention of Indian Society for Veterinary Immunology & Biotechnology and National Symposium on "Emerging Challenges & Opportunity in Veterinary Immunology & Biotechnology for Improved Animal Health & Productivity" from 10-12 Nov., 2013 as President of ISVIB and also chaired one of the technical sessions at Palampur from Nov. 09-13, 2013.
23. R. K. Singh participated in Asia-Pacific Congress of Virology (VIROCON-2013) organized by Indian Virological Society from December 17-20, 2013 at Amity University, Sector-125, Noida (New Delhi NCR), India.
24. R. K. Singh participated in the Roundtable on "Preparedness to Combat Wildlife Diseases in India" held at NASC Complex, New Delhi on January 11, 2014.
25. R. K. Singh, Praveen Malik, N. Virmani, R. K. Vaid, Hari Singha, B. C. Bera and Taruna Anand attended the NAVS National convention on 'Role of veterinarians in quality assurance of livestock products and international trade' & XII Convocation of National Academy of Veterinary Sciences (I) held from January 28-29, 2014 at LUVAS, Hisar.
26. R. K. Vaid participated in "Group meeting on Culture Collection of Importance in Agriculture & Allied Sectors" organized on December 13, 2013 at National Bureau of Agriculturally Important Microorganisms, Maunath Bhajan Kusumaur, UP.
27. S. K. Khurana delivered a lecture on Diseases of working equids: Bacterial, fungal and Rickettsial. National workshop on health and welfare of working equines. 21-12 March 2014, BHA (India) and NRCE, New Delhi.
28. S.C. Yadav attended 5th National conference & Proteomic society meeting and presented the investigation report on Trypanosomosis in round table discussion organized by the proteomic society of India held on Nov. 27, 2013 at IISc Banglore.
29. S.C. Yadav attended and presented lead paper in XXIV National Congress of Veterinary Parasitology and National Symposium held at COVAS, Mannuthy, Kerala from February 5-7, 2014.
30. 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 Aug. 26, 2013 at DBT New Delhi.
31. S.C. Yadav was invited to present the lead paper on the topic on "Proteomic approach in diagnosis of T. evansi infection in animals" during XXIV National Congress of Veterinary Parasitology & National Symposium on 'Towards Food Security Through Sustainable Animal Production and Integrated Parasite Management' organized by the Department of Veterinary Parasitology, COVAS, Mannuthy, Kerala Veterinary and Animal Science University, Pookode, Wayanad from February 5-7, 2014.
32. Taruna Anand and Riyesh T. participated in Asia-Pacific Congress of Virology (VIROCON-2013) organized by Indian Virological Society from December 17-20, 2013 at Amity University, Sector-125, Noida (New Delhi NCR), India.

Visit of Dignitaries

- Sh. D.S. Nehra, DG Audit, Chandigarh, visited NRCE on April 12, 2013. He visited ATIC, BSL III laboratory, animal shed complex, VTCC and different labs at the Centre.
- Prof Dr R.N.S. Gowda, Ex. VC, KVAFSU, Bidar, Karnataka and Chairman QRT team NRCE visited NRCE on April 21, 2013. He visited different labs at the Centre and interacted with scientists at NRCE.
- Dr Ram Lal Sandal, Asst Director, Animal Health/Breeding Spiti at Kaza, Dist. Lahul Spiti visited NRCE on April 21, 2013.
- Dr Deepak Choudhari, Project Coordinator (Utilization of Animal Energy), CIAE, Bhopal visited NRCE on September 3, 2013 to participate in meeting on AICRP on Utilization of Animal Energy at NRCE to assess the progress made under the UAE project.
- Sh. Arvind Kaushal, Secretary, ICAR & Addl. Secretary DARE visited NRCE on September 13, 2013. During his visit, he interacted with scientists of different labs, Director NRCE briefed him about the various ongoing research and extension activities at NRCE. During the visit at NRCE Addl. Secretary DARE & Secretary, ICAR also addressed the staff of NRCE and CIRB.

Sh. Arvind Kaushal, Secretary, ICAR & Addl. Secretary DARE at VTCC

Plantation by Sh. Arvind Kaushal, Secretary, ICAR & Addl. Secretary DARE

- Dr B. Meenakumari, DDG (Fisheries) visited NRCE on October 19, 2013 and interacted with the scientists of different NRCE labs and VTCC.

Dr B. Meenakumari, DDG (Fisheries) at VTCC

- Sh Patram ji, SP Vigilance, Hisar visited NRCE on October 30, 2013 on the occasion of Vigilance Awareness Week at NRCE and delivered a lecture as well.
- Prof Heinrich Neubauer and Dr Mandy Elschner, OIE experts from FLI, Germany visited the NRCE, Hisar during December 10-15, 2013. During visit at NRCE they visited different labs and also had field vist for interaction with equine owners.

- Dr Umesh K. Mishra, VC, Chhattisgarh Kamdhenu Vishwavidyalaya, Durg, Chhattisgarh visited NRCE on

Lt. Gen. N.S. Kanwar, DG RVS at Info-equine Museum, NRCE

At EPC, Bikaner

- Dr K. M. L. Pathak, Hon'ble DDG (AS) visited EPC, Bikaner on August 17, 2013. During visit, he inaugurated Kisan Call Centre (Toll free helpline for equine owners No. 1800-180-6225) at EPC Bikaner.
- Dr S. K. Garg, Former VC, DUVASU, Mathura visited EPC, Bikaner on November 28, 2013 during visit he visited he interacted with scientists at EPC, Bikaner and visited different laboratories and animal shed complex.

Dr S. K. Garg at EPC Bikaner

- Prof. Satya Parida, Head of Vaccine Department, Pirbright Laboratory, Institute of Animal Health, UK visited EPC, Bikaner on January 1, 2014.
- Dr A.K. Srivastava, Director NDRI Karnal visited EPC Bikaner on January 3, 2014. Director NRCE briefed Director NDRI about the research and infrastructure facilities and ongoing research activates at EPC, Bikaner.

Director NDRI at EPC Bikaner

- Mr. S. Vellaiangiri, Senior Deputy Accountant General visited EPC, Bikaner on January 10, 2014.
- Shri. K. P. S. Gill, Ex-DGP, Punjab Police, Padamshree awardee visited EPC Bikaner on January 17, 2014 along with Mrs. C. D. Hellner and Mrs. Tricin Everest from USA. During visit he and interacted with scientists at EPC, Bikaner and visited different laboratories and animal shed complex.

Hon'ble Shri. K. P. S. Gill with delegates from USA at EPC, Bikaner

Personnel

Milestones

Awards & Recognitions

- Dr R.K. Vaid, Principal Scientist was adjudged runner up in chess competition at ICAR North Zone Inter-institutional games & sports tournament at IIPR Kanpur on March 20, 2014.

New Joining

- Shri K.K. Gupta, joined the Centre as Chief Technical Officer on transfer from HSADL, Bhopal on 18.07.2013.
- Shri Lilu Ram, Casual Labour (Temp. Status) joined Centre as Skilled Supporting Staff on 03.12.2013.

Appointment and Transfer

- Dr R.K. Singh, Director, NRCE, Hisar joined as Director, IVRI, Izatnagar on 04.02.2014.
- Dr A.K. Gupta, nominated as Acting Director, NRCE, Hisar w.e.f. 06.02.2014.
- Dr R.C. Sharma, Sr. Scientist was relieved from Centre on 28.05.2013 to join as Principal Scientist at CSWRI, Avikanagar.

Promotion

- Dr S.K. Khurana, Sr. Scientist has been promoted to post of Principal Scientist w.e.f. 01.01.2011.
- Dr Sanjay Kumar, Sr. Scientist has been promoted to post of Principal Scientist w.e.f. 30.10.2012.
- Dr R.K. Vaid, Sr.Scientist has been promoted to post of Principal Scientist w.e.f. 06.12.2012.
- Shri S.P. Kaushik, Assistant has been promoted to post of Assistant Administrative Officer w.e.f. 19.08.2013.
- Dr R.A. Legha, Sr. Scientist has been promoted to post of Principal Scientist w.e.f. 05.08.2013.

Superannuation

- Shri R.B. Saxena, Administrative Officer retired from NRCE upon Superannuation on 31.05.2013.
- Dr B.K. Singh, Principal Scientist retired from NRCE upon Superannuation on 31.07.2013.

Farewell of Dr B.K. Singh upon Superannuation

Staff at NRCE

Acting Director: Dr A K Gupta

Scientists at NRCE, Hisar Campus

1. Dr A.K. Gupta, Principal Scientist, Biochemistry
2. Dr S.C. Yadav, Principal Scientist, Veterinary Parasitology
3. Dr B.R. Gulati, Principal Scientist, Veterinary Microbiology
4. Dr Yash Pal, Principal Scientist, Animal Physiology
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 P.A. Bala, Scientist, Animal Nutrition
3. Dr T. Rao Talluri, Scientist, Veterinary Reproduction & Gynaecology
4. Dr Ramesh Dedar, Scientist, Veterinary Medicine
5. Dr Sanjay Kr. Ravi, Scientist, Animal Reproduction
6. Dr Vijay Kumar, Scientist Sr. Scale, Animal Physiology

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 Mamta Tigga, Scientist, Veterinary Pathology
5. Dr K. Shanamugasundaram, Scientist, Veterinary Pathology
6. Dr Taruna Anand, Scientist, Animal Biotechnology
7. Dr B.C. Bera, Scientist, Animal Biotechnology
8. 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
3. Sh P.P. Chaudhary, Technical Officer
4. Sh Ajmer Singh, Technical Officer
5. Sh D.D. Pandey, Technical Officer
6. Sh Sita Ram, Technical Officer
7. Sh S.K. Chhabra, Technical Officer
8. Sh Joginder Singh, Sr.Technical Asstt.
9. Sh Sajjan Kumar, Sr.Technical Asstt.
10. Sh Suresh Kumar, Sr.Technical Asstt.
11. Sh Mukesh Chand, Sr.Technical Asstt.
12. Sh Raj Kumar Dayal, Technical Asstt.
13. Sh Arun Chand, Sr. Technician
14. 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 (Additional 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

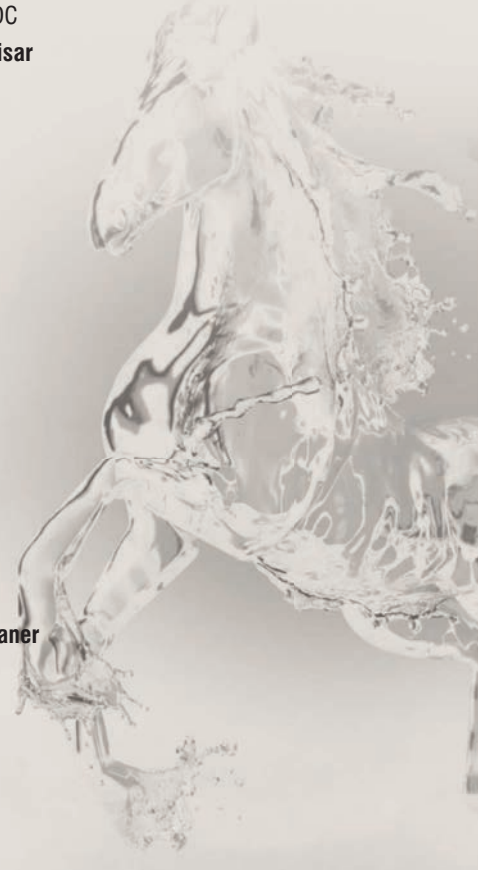
1. Sh Mahender Singh, LDC

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



Equines at NRCE, Hisar

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