Annual Report

2010-11

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National Research Centre on Equines

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ICAR

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The achievements and activities of the Centre from April 2010 to March 2011 are presented in this Report. Mention of trademark, proprietary product or firm in the Report does not constitute an endorsement or rejection of other suitable products or firms.

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Director's Foreword

I am pleased to present the 24th Annual Report of the National Research Centre on Equines, Hisar. This report provides a succinct account of researches on various ambits of equine health, production and management as well as other activities carried out in 2010-11. The report, like previous years, attempts to provide of different laboratories and the sections of the Centre, their achievements and the general direction in which our projects and progress are moving. The report also encompasses the description and progress of NRCE entrusted Veterinary Type Culture Collection, our microbial banker.

Our pursuit towards development and refinement of equine health oriented technologies of novel and existing diagnostics, vaccine and biologicals are bringing sweet fruits and international accolades. The conservation of indigenous equine breeds is giving research dividends by increasing the baseline phenotypic and genotypic information about our precious germplasm. The relentless thrust to acquire and characterize microbial isolates is giving VTCC the critical mass to its mandated goals.

In as much as an exotic disease is a scourge on the naïve host it afflicts, it also becomes an opportunity to the research workers attempting to decipher it and learn from it to be better prepared for future. Equine influenza has been the single most important event occurring in 2008-09 and - after its successful control - we have been busy in understanding the biology of this virus. Phylogenetic analysis of M gene of the isolates led to classification of the EIV subtype circulating in India, China etc., into "Asian Clade" – a new nomenclature proposed by our scientists. Additionally, work on development of DIVA strategy, and molecular basis of diagnosis gave further impetus to research on influenza virus.

Our efforts to give finishing touches and finesse to our diagnostic and vaccine products went a notch further in EI updated vaccine, recombinant protein antigen based - ELISA kit for Equine piroplasmosis, recombinant protein antigen based – Coggin's test, indirect ELISA for EIA diagnosis and kits for Rotavirus detection and Equiherpabort vaccine validation trials.

In order to achieve a quick and accurate diagnosis, we have been using RT-PCR and PCR technologies in diagnosis and characterization of EI, Japanese encephalitis, *Rhodococcus equi* and Trypanosomosis, and research on murine monoclonal antibodies for JE and Trypanosomosis diagnosis.

The ancient disease - glanders - had again raised its ugly head in Himachal Pradesh and Uttar Pradesh (2010-2011), which were promptly attended to and control and preventive measures were taken. Seromonitoring of various equine diseases at different geographical locations along with isolation of bacterial isolates continued as part of serosurveillance and epidemiology program. A case of EIA sero-positivity was also detected in indigenous equine (mule).

Among protozoal disease of equines, Trypanosomosis diagnosis work has received a major fillip through characterization of a novel 66 kDa antigen. Work on characterization of immunological molecular markers like

equine TLRs and MHC genes is being pursued.

On the Production front, our *ex-situ* conservation strategies are bearing fruits as in the Equine sanctuary, the Zanskari nuclei placed at EPC, Bikaner is proving useful in formulation of baseline breed data. In this regard, genotypic and more phylogenetic data has been added to previous phenotypic data.

The poor man's humble beast of burden - the donkey - has not been allowed to fall into a historical neglect. On the contrary, Centre has initiated work on phenotypic characterization of indigenous donkeys. We have also attempted into developing a herd nuclei of large white indigenous donkeys. In addition, the understanding of dynamics of animal energy, especially the use of indigenous and exotic donkeys and mules in draughtability and agriculture operation work is being pursued under AICRP on "Utilization of Animal Energy". Additionally, we have focused our attention on understanding of donkey husbandry and management system and the related socio-economic parameters of their poor owners.

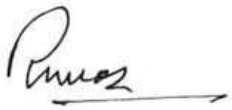
We have continued with our research efforts on semen biology, cryopreservation of semen, AI, and reproduction and have added to it by new work on semen microbiology.

Veterinary Type Culture Collection research furthered its microbial acquisition, accession and preservation work with support from network centers, with frequent meetings of the units and charting of work plan. In order to acquire newer pathogenic isolates, scientists have attended zoonotic buffalopox, sheep pox and bovine papillomatosis outbreak cases. Among bacterial microbes, a collection of pathogenic *E. coli* and *Klebsiella pneumoniae* isolates from mare abortion has been acquired. Additionally, new *Pseudomonas aeruginosa* isolates have been identified in equine semen samples.

Under developmental work at VTCC new premises the interior furnishing work is being taken up. The Agriculture Farm Section has made satisfactory progress with production of various fodder crops at Hisar and Bikaner, with Centre getting even cash proceeds from sale of oat grains worth ₹ 2.5 lakh. We continued our land reclamation, development and plantation work as well as development of lawns at NRCE premises and VTCC new premises towards contributing a bit for sustainability of the ecosystem. The sustained efforts for producing equine vermicompost have increased the production of vermin-compost to about 75 quintals. Approximately, one-fourth of equine dung generated at the farm was also composted in natural settings.

Apart from ICAR-funded research projects, we have been successful in bringing National and International externally-funded Projects on Piroplasmosis, Animal adenoviruses and glanders including ongoing OIE twinning and attempts are already on to forge links with international laboratories. Our scientists published their research findings in various international and national journals and presented their research findings in national/international fora. The research faculties have been actively deputed abroad (Singapore, USA, Japan) for training as well as across the nation for various short-term trainings, seminars and symposia. We have been very blessed in welcoming distinguished dignitaries, guests and experts in our various meetings like IRC, IMC and SRC. Among them, the visit of Dr. S. Ayyappan, DG ICAR and Secretary DARE, Dr K M L Pathak DDG (AS), Dr C.S. Prasad, ADG (ANP), and Dr. G Prasad, ADG (AH) has been very special. On this occasion, we were happy to present the launch of Kisan Call Centre service, NRCE Video film and Visitors' Room at NRCE, Hisar. We also organized Interactive meets, Scientific meets, Kisan Gosthies, training and expert lectures at Centre and village level from time to time. The Silver Jubilee year which was celebrated with the year round activities of workshops and seminars, culminated with grand function on November 26, 2011. In view of the International Biodiversity year-2010, the Interactive Meet on "Conservation of Marwari and Kathiawari Horses" at Bikaner was organized which was graced by experts and horse lovers, resulting in valuable suggestions which set forth agenda for equine biodiversity conservation. Centre celebrated the "World Environment day" with lecture on aspects of biodiversity conservation. Our routine efforts for providing consultancy and diagnostic services continued relentlessly which fetched the Centre a handsome revenue of nearly ₹ 40 lakhs plus. We have been aggressively pursuing commercialization of technologies developed at NRCE and have chalked out an ambitious plan for pushing forth commercialization of newly recognized technologies.

I firmly believe that the success and progress of NRCE has been through the goodwill and personal contributions made by its scientific, administrative, supportive and other staff and through constructive criticism of our peers and experts interacting with us in meetings and other interfaces in India and abroad. The annual report also reflects the continued advice and support we have received from the Council, to whom we are highly indebted. The light of the day seen by this Report is culmination of wonderful efforts of the Editorial Team and Centre's staff. I am extremely grateful to all of them.



R.K. Singh

Executive Summary

Ever since its inception on 26 November 1985 at Hisar, the National Research Centre on Equines (NRCE) has been striving for improvement in health and production of equines in the country. The subcampus at Bikaner was established in 1989. The Centre is contributing towards the upliftment of the landless and marginal farmers through providing diagnostic, advisory and consultancy services for major equine diseases, surveillance and monitoring along with development of diagnostics/ biologicals besides, helping in conservation and improvement of germplasm of indigenous breeds. Further, the Centre has also been entrusted with the responsibility of maintaining a National Repository of Animal Microbes along with Dairy and Rumen Microbes (Veterinary Type Culture Collection) for preservation and distribution of characterized microbes to different stake-holders of the country. During the year, the Centre has marched ahead to foray into new vistas of research.

In its quest for ensuring the availability of technologies to the equine owners, the Centre validated its equine herpes virus-1 vaccine (Equiherpabort) by conducting field trials and proving its effectiveness for control of EHV-1 infection in pregnant mares.

In its pursuit to develop state-of-the-art diagnostics, the Centre ventured into improving its existing technologies for equine influenza (EI), Japanese encephalitis (JE), equine infectious anaemia (EIA), *Rhodococcus equi* infection and Trypanosomosis. Real-time RT-PCR has been developed for rapid detection of EI. Further, in order to develop a diagnostic to differentiate infected from vaccinated animals (DIVA) for EI, recombinant NS1 proteins were immunoblotted with serum from vaccinated and infected animals. The r-protein from N-terminal is showing promising results and is under further testing. Monoclonal antibodies were also produced against equine influenza virus for antigenic characterization and development of diagnostics. Besides this, a mAb-based capture ELISA has also been standardized for diagnosis of Japanese Encephalitis. Further, a blocking ELISA was developed for detection of West Nile virus antibodies, using mAb 1H11E7. An indigenous diagnostic reagent for EIA was developed in the previous year using a recombinant protein expressed in *E. coli* from a synthetic gene of 26 kDa. It was further evaluated for use in AGID/indirect ELISA for sero-diagnosis of EIA. The initial results in the study are highly encouraging and exhibit cent percent correlation with gold standard test (Coggins test).

The usefulness of a PCR, based on amplification of cholesterol oxidase gene (*cho*E) and species-specific chromosomal region, was exploited for rapid and specific identification of pathogenic *R. equi*. PCR was 100% specific and sensitive in detecting 10 picogram of DNA. The species-specific primers can be used to identify clinical and environmental isolates of *R. equi*. Further, PCR assay was also standardized targeting different plasmid associated genes *viz. tra* (959 bp), *vap*A (286 bp), and *vap*B (477 bp).

In efforts to develop specific diagnostics for *T. evansi* infection especially to detect carrier stage, attempts are being made to identify and isolate partially purified immune-reactive proteins from mice-adapted horse (cloned) isolate of *T. evansi* whole cell lysate (WCL) antigen and its comparative evaluation is being carried out with (WCL) antigen in detection of chronic trypanosomosis using immunoblot and ELISA. Immunoblot studies revealed all infected donkeys and horses with polypeptide in the range of 55-66 kDa from second week post infection onwards suggesting that this protein is predominantly expressed in chronic stages of infection.

Infectious organisms isolated from outbreaks need authentication for establishing disease patterns. In this direction, equine influenza isolates from 2008-09 outbreaks were analyzed for matrix and nucleoprotein genes. Comparison of nucleotide sequences revealed maximum homology with Chinese and Mongolian isolates of EIV. Phylogenetic analysis revealed clustering of Indian and Chinese isolates in a separate group which was newly designated as "Asian Clade" for M gene. In contrast, the comparison of nucleotide sequence of NP gene revealed higher degree (95% to

100%) of homology with influenza A virus (H3N8) isolates of equine, canine and swine origin. In a study to determine the diversity of Mx gene and its role in resistance/susceptibility to the Influenza virus in equines, the partial nucleotide sequences of Mx genes were analyzed for polymorphism. qRT-PCR was performed to assess the changes in levels of expression of *Mx* gene. All the samples exhibited Mx gene expression.

A total of 175 donkey blood samples were analysed by PCR for MHC-DRB3 and DRB2 loci. Toll-like receptor 9 has been the focus of considerable research attention for the ability to activate innate immune responses through DNA-based immunotherapeutics. Analysis of the equine TLR 9 indicated that equine are much closer to odd-toed ungulates than to even-toed ungulates (buffalo, cattle). TLRs contain multiple repeats that are protected by special LRR-N terminal end and LRR-C-terminal end motifs. It was observed that LRR patterns towards N-terminal are conserved among all species except *Equus caballus* and *Rattus norvegicus*.

Seromonitoring of equine diseases is important to ascertain the prevalence of diseases in different geographical regions of the country. In this direction, scientists collected serum samples from 1768 equines, which were tested for various diseases. Out of these 4.87% samples for EHV-1, 21.78% samples for EI and 3.17% samples for JE were found positive. Eleven cases were found positive for glanders. Incidence of *Theileria equi* was 36.29% while 8.22% of the samples were positive for Trypanosomosis. Further, 11,909 serum samples were tested for glanders which included samples from S&M (1768), disease investigation (944) and contractual service (9197), and out of these 7 serum samples from U.P. were reported positive from U.P. and 4 serum samples from H.P. were found positive.

In May 2010, glanders was reported from Pandoh area of Mandi district (Himachal Pradesh), where four mules were found positive for glanders (1 isolation). This was followed by outbreak in December 2010 from Chandpur area of Bijnor district. Outbreaks of equine influenza were reported from several states of the country during previous years. Follow up action is continuing in affected states. 550 serum samples tested positive for equine influenza out of 3,513 serum samples, however, none of the samples tested in pair showed rise in titres. EIA was not positive in any of the 6,589 samples tested for the disease. Bacteriological analysis done on 209 samples yielded 61 isolates including *Burkholderia mallei* (4), *Rhodococcus equi* (6), *Streptococcus equi* subsp. *zooepidemicus* (33), *Streptococcus equi* subsp. *equi* (4), *Staphylococcus* sp.(1) and *E. coli* (13).

On the production front, all the six indigenous breeds of equines namely Marwari, Kathiawari, Spiti, Zanskari, Bhutia and Manipuri were previously characterized phenotypically on the basis of their biometric indices and coat colour. Genetic characterization is an important part of any breeding program, so an effort was made to study genetic diversity among different equine breeds in India, using 55 different microsats. On the basis of allele number, allelic frequencies and heterozygosity values, a high genetic diversity was observed within and between different breeds. Further, the Neighbor-Joining algorithm to study the topology and phylogenetic analysis revealed clustering of Thoroughbred horses separately while, other Indian breeds clustered into two distinctive classes. It can be inferred from the study that the geographically distant breeds are genetically also distant.

Phenotypic characterization of donkeys was initiated and 97 donkeys from Rajasthan were recorded for body length, height at withers, heart girth, foreleg length, height at knee, canon length, hind leg length, height at hock, face length, face width, ear length, ear width, pole, hoof length and width. This is an initiative to generate baseline data on donkey populations in India.

Cryopreservation of semen is an important activity for artificial insemination and conservation of breeds. In general, glycerol has toxic effect on spermatozoa, so a study was initiated with amides (methyl formamide, dimethyl formamide and dimethyl sulfoxide) as an alternative to glycerol as cryoprotectant. No statistically significant difference was observed in sperm motility and sperm livability with the use of three different cryoprotectants i.e., glycerol, dimethyl formamide and dimethyl sulfoxide in both prefreeze semen and frozen semen of Marwari stallions, Jack stallions and Zanskari stallions.

A study was also conducted on the effect of medicated (progesterone) and non-medicated intravaginal sponges to synchronize estrus in Marwari mares. Mares could hold the sponges and inoculation of 10 mg of prostaglandin to the mares holding medicated sponges could help in synchronization of estrus. Three out of four mares exhibited estrus and their insemination resulted in pregnancy in two mares.

Utilization of animal energy, an important area needs to be improved for gaining maximum efficiency. In such an attempt, NRCE has been working under AICRP on "Utilization of Animal Energy with Enhanced System Efficiency". Draughtability was studied in donkeys with work-rest cycle for equines under arid conditions with continuous work and work-rest-work cycle. Changes in physiological indices *viz.* temperature, heart rate, respiration rate and haematology were recorded. It was found that continuous work load of 6 and 8 Quintal (Q) caused fatigue in two hours while 10 Q load caused fatigue in 1 hour. Donkeys with same load but 1 hr. work and 1 hr. rest for five hours had increased physiological profile; however, there was no change in the haematology. During evaluation of exotic and indigenous donkeys engaged in agricultural work of ploughing and sowing, ploughing animals were engaged for 3 ploughing sessions of 1.5 hr. each with an hourly rest in between, revealing significant changes in the physiological parameters after 1.5 hr. of work. Average land ploughed by exotic donkeys and indigenous donkeys were 0.171 acre and 0.09 acre per hour, respectively. Exotic donkeys were able to plough 0.514 acre in two sessions at an average speed of 2.60 kmph while indigenous donkeys ploughed 0.362±0.018 acre at an average speed of 1.835 kmph. Exotic donkey could sow 0.662 acre at an average speed of 2.635 kmph in two hours. The donkey attained almost normal physiological levels after one hour of rest.

Mules and donkeys are mainly reared by resource-poor/deprived community. In order to study their livelihood sustainability, a pilot study was conducted to ascertain existing management systems and utilization of donkeys and mules in Rajasthan, Uttarakhand, Haryana and Uttar Pradesh. Majority of the respondents were illiterate middle age people from minority community, having a family size of 7-10 members with medium experience of equine husbandry and monthly income between ₹ 3,000 to 5,000. Regarding management practices, majority of the respondents were cleaning their animals twice a week. Shoeing was regularly done except in donkeys, whereas grooming and deworming was done occasionally.

The Veterinary Type Culture Centre (VTCC) – a network project with seventeen Network units located across the country for catering to the conservation of Veterinary, Dairy and Rumen microbes-strengthened its activities entailing collection of samples from different livestock species across different geographical regions, acquisition of microbial isolates from different institutes/network units along with their characterization and preservation.

The repository has accessioned 358 veterinary microbes (255 bacterial and 103 viral cultures) along with 169 recombinant clones. The important microbial isolates include, Viral isolates viz., buffalopox, camelpox, goatpox, equine influenza, Japanese encephalitis, bovine and human rotavirus, bovine herpes virus-1, equine herpes virus-1 & 4; bacterial isolates *viz. Bordetella bronchiseptica, Brucella melitensis, Rhodococcus equi, E. coli, Streptococcus* spp., *Pasteurella* spp., *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp., *Klebsiella* spp., *Aeromonas* spp., *Shigella* spp.; dairy microbes *viz., Lactobacillus* spp. *and rumen microbes* viz., Methanogenic bacteria, *Pediococcus* spp. and *Leuconostoc* species.

Outbreaks of buffalopox virus (BPXV) from Meerut, U.P. and Bovine papillomatosis cases in Hanumangarh (Rajasthan) have been investigated. To our knowledge, this is the first instance where in BPXVs have been reported to be infecting cattle, buffaloes and humans at the same time and space. A total of ten buffalopox viruses from infected animals as well as humans were isolated, characterized and preserved in the repository. A total of 23 bacterial isolates were identified as *Pseudomonas aeruginosa*, *E. coli*, *Streptomyces* spp., *Flavobacterium* spp., *E. coli*, *Pseudomonas* spp., *Pseudomonas aeruginosa*, *Acinetobacter*, *Achromobacter* spp. and *Alcaligenes* spp. in stallion semen. Besides, *E. coli* and *Klebsiella pneumoniae* have been isolated and characterized from cases of diarrhea, pneumonia, metritis and abortions from equines. Recently, isolation and culture of somatic cells of different tissues from equine, bovine and caprine has also been initiated.

कार्यकारी सारांश

राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार, 26 नवम्बर 1985 के अपने स्थापना के समय से भारतवर्ष के अश्व–नस्ल प्राणियों के उत्पादन और स्वास्थ्य में बढ़ोतरी के लिए सतत् प्रयास कर रहा है। इस केन्द्र का बीकानेर अश्व उत्पादन परिसर सन् 1989 में शुरू हुआ। केन्द्र द्वारा गरीब भूमिहीन एवं छोटे किसानों की आर्थिक उन्नति के लिए किए गए कार्य उल्लेखनीय हैं। इनमें अश्वों के मुख्य रोगों की रोकथाम के लिए तैयार की गई नैदानिक विधियां एवं रसायन, रोगों का निदान, निरन्तर निगरानी एवं अश्व–पालकों को अश्व–संबंधी जानकारी एवं परामर्श उपलब्ध कराना मुख्य कार्य हैं। इसके अलावा केन्द्र का पिछले कुछ वर्षों से भारत के अश्वों की उत्तम नस्लों के संरक्षण की दिशा में कार्य उल्लेखनीय रहा है। राष्ट्रीय अश्व अनुसंधान केन्द्र को पशु–रोगाणुओं के साथ–साथ डेयरी एवं रोमन्थी जीवाणुओं के संवर्धन के कार्य के लिए वेटरिनरी टाईप कल्चर्स कलैक्शन का महत्वपूर्ण कार्य भी सौंपा गया है। इसका ध्येय महत्वपूर्ण रोगाणुओं एवं जीवाणुओं का लम्बे समय के लिए परिसँचयन है। इन उद्देश्यों की पूर्ति हेतु केन्द्र द्वारा वर्ष 2010–11 में किए गए कार्यों का संक्षिप्त विवरण प्रस्तुत है।

अश्व–हरपीज विषाणु–1 के टीके (इक्वीहर्पाबोर्ट) का विधिमान्यकरण किया गया। इसके लिए देसी और थौरोब्रैड ग्याभिन घोड़ियों में विभिन्न स्थानों पर जाकर टीका–परीक्षण किया गया। अश्व–पालन क्षेत्र परीक्षणों में पाया गया कि इक्वीहर्पाबोर्ट टीका उच्च–क्षमता रोग–प्रतिरोधण उत्पन्न करता है और ग्याभिन घोड़ियों में अश्व हरपीज विषाणु–1 द्वारा गर्भपात की कुशल रोकथाम करता है।

अश्व–प्रजाति के रोगों जैसे अश्व–फ्लू, जापानी–बुखार, अश्व–अल्प रक्तता, रोडोकौक्कस इक्वाई एवं ट्रिपैनोसोमोसिस के निदान हेतु उच्च–तकनीकों का विकास एवं वर्तमान तकनीकों को बेहतर बनाने एवं रोगों का तुरन्त निदान हेतु कार्य की दिशा मे सतत् प्रयास किए जा रहे हैं। अश्व–फ्लू के तुरन्त–निदान के लिए मैट्रिक्स जीन पर अति–संवेदनशील आर टी–पी सी आर का मानकीकरण किया गया। क्यू आर टी–पी सी आर विधि द्वारा अश्व–फ्लू विषाणु पर परीक्षण में 100 प्रतिशत नैदानिक निश्चित्ता पाई गई। अश्व–फ्लू से ग्रसित और अश्व–फ्लू टीकाकृत अश्वों में भेद करने हेतु एन एस 1 प्रोटीन के इस्तेमाल पर प्रयोग किए गए हैं। इसके लिए एन एस 1 संयोजक प्रोटीन को ई कोलाई जीवाणुओं में रिकम्बिनैंट विधि द्वारा उसर्जित किया गया एवं प्रोटीन का परीक्षण इम्यूनोब्लाटिंग द्वारा किया गया। इस प्रोटीन के अमीनों सिरे के भाग पर किए गए परीक्षण आशादायी परिणाम दे रहे हैं और इस पर और शोध जारी है। अश्व–फ्लू रोग के नवीन नैदानिक तरीके निकालने के लिए एवं इसके विषाणु के अंतजन विश्लेषण हेतु मोनोक्लोनल एंटीबाडीज विकसित की जा रही है। वर्तमान में आठ एंटीबाडी उत्पादन करने वाले क्लोन का चयन किया गया है और उनका गुणात्मक विश्लेषण किया जा रहा है। जापानी–ज्वर के निदान हेतु एक मोनोक्लोनल एंटीबाडी पर आधारित कैप्चर एलीसा का मानकीकरण किया गया जो कि हीमएग्लुटीनेशन एवं विषाणु निशेचित्तता विधि (वी०एन०टी०) से अधिक संवेदनशील है। पश्चिमी नील विषाणु की एन्टीबाडीज की पहचान हेतु एक ब्लाकिंग एलीसा विधि का भी विकास किया गया है। जिस अश्व संक्रामक अल्परक्तता रोग के निदान हेतु गत वर्ष एक देसी नैदानिक जैविक रसायन का विकास किया गया था , उसका इस रोग के ए जी आई डी एवं एलीसा विधि द्वारा निदान के लिए विश्लेषण किया गया। अब तक के शोध से उत्साहवर्धक नतीजे मिले हैं और इस विधि और स्वर्ण मानक परीक्षण (कागिंस परीक्षण) में 100 प्रतिशत समानता पाई गई। अश्व संक्रामक अल्परक्तता रोग की निदान विधि को परखने के लिए 1,600 अश्व सीरम नमूनों का परीक्षण करने के पश्चात् आर 26 kDa प्रोटीन का ए जी आई डी परीक्षण के मुकाबले क्रमशः 100 एवं 99 प्रतिशत नैदानिक संवेदनशीलता एवं निश्चित्ता पाई गई।

रोडोकोक्कस इक्वाई रोगाणु की पहचान और अचूक निदान हेतु सी.एच.ओ.ई. के जीन पर आधारित पी सी आर विधि का इस्तेमाल किया गया, जिससे अश्व–शावकों में होने वाले इस रोग का निदान हो सके। यह विधि न्यूनतम 10 पीकोग्राम रोडोक्रोक्कस डी० एन० ए० मात्रा को 100 प्रतिशत निश्चित्ता से पहचान लेती है। इस विधि द्वारा रोगी पशुओं से और पर्यावरण से पृथक लिए गए रोडोकोक्कस रोगाणुओं की अलग–अलग पहचान संभव है। इसके अलावा इस रोगाणु के तीन विभिन्न प्लासमिड गुणसूत्र जीनों की पहचान के लिए पी सी आर विधि का प्रयोग किया गया। ट्रिपैनोसोमा इवैनसाई परजीवी के निश्चित निदान हेतु, खासतौर पर कैरियर अवस्था की पहचान हेतु नैदानिक विधि का विकास किया जा रहा है। इस सबंधं में एक अश्व से पृथक किए हुए ट्रिपैनोसोमा इवैनसाई परजीवी, जिसको चूहे में डालकर जीवित रखा गया, उसके पूर्ण कोशिका विभाजन से प्राप्त प्रोटीन सम्मिश्रण का विश्लेषण किया गया। इस अंतजन मिश्रण से प्रभावी रोग–प्रतिरोधी प्रोटीन की पहचान की जा रही है जिनका उपयोग इम्यूनोब्लाट एवं एलीसा विधि में किया जा रहा है। इम्यूनोब्लाट विधि से ज्ञात हुआ है कि सभी रोगी गर्दभों एवं अश्वों के सीरम नमूनों ने 55 से 66 kDa के प्रोटीन–बैण्डों के साथ समदकर्ण कर पहचान बनाई जिससे यह विदित होता है कि लम्बी अवधि के रोग के दौरान परजीवी द्वारा यह प्रोटीन प्रभावी रूप से स्रावित होता है।

विभिन्न प्रकार के रोग–कारक, रोगाणुओं और विषाणुओं का पृथ्क्कीकरण, उनमें विभिन्नता का विश्लेषण एवं उनका रोगों से कारक–मिलान करना आवश्यक हैं। इस दिशा में 2008–09 से प्राप्त अश्व–फ्लू विषाणुओं के मैट्रिक्स एवं न्यूक्लियोप्रोटीन जीनों का विश्लेषण किया गया। इन जीनों के अनुक्रमीकरण विश्लेषण क्रम चीन और मंगोलिया के अश्व–फ्लू से अधिकतम मेल खाते हैं। इसके अलावा हमारे विषाणु की M1एवं M2 अमीनो अम्ल एशिया के अन्य विषाणुओं से कमशः 98.41 एवं 99.41% मेल खाते हैं। एम जीन पर आधारित वँशावली विश्लेषण दिखाता है कि भारतीय एवं चीनी मूल के विषाणु एक ही समूह बनाते हैं जिसे हमारी शोध–टोली द्वारा ''एशियन समूह'' की संज्ञा दी गई है। इसके विपरीत, एन०पी० जीन के बेस अनुक्रम को जब अश्व, कुक्कर एवं सुकर फ्लू विषाणुओं के बेस अनुक्रम से मिलाया गया तो अधिक (95% से 100%) अनुरूपता पाई गई।

जापानी–ज्वर के एक विषाणु (JE/eq/India/ H225/2009) के वंशावली विश्लेषण से ज्ञात हुआ कि यह जीनो समूह–III के साथ समूह बनाता है जो कि भारत के वेल्लौर समूह के विषाणुओं के समीप है। अश्व–फ्लू के Mx जीन की विभिन्नता विश्लेषण और उसके रोग–प्रतिरोध में योगदान कारकों के अध्ययन हेतु इस जीन के एक भाग का अनुक्रमीकरण एवं उसके विभिन्न स्वरूपों का विश्लेषण किया गया। यह पाया गया कि 2080IU इन्टरफिरोन देने के पश्चात् Mx जीन एक्सप्रैशन में बढ़ोतरी होती है, जिसको क्यू आर टी–पी सी आर विधि से देखा गया।

एम०एच०सी० जीन समूह संक्रामक रोगों और वंशानुगत रोगों के विरूद्व प्रतिरोधी शक्ति के कारक हैं। एम० एच०सी०, डी०आर०बी० 3 एवं 1 लोकस का पी सी आर विश्लेषण 175 रक्त–नमूनों पर किया गया। गदर्भों में एम०एच०सी० लोकस की विभिन्नता के अध्ययन हेतु आर०एफ०एल०पी० विधि अपनाई जाएगी। अश्वों के टी०एल०आर० 9 जीन के अध्ययन से यह इंगित होता है कि यह जीन खुर–वाले पशुओं की अपेक्षा मनुष्य के जीन के अधिक समीप है। यह देखा गया कि एन–टर्मिनल की तरफ का एल आर आर गदर्भो एवं चूहों के अलावा सब में संरक्षित है।

भारत के विभिन्न भागों में अश्व–रोगों के प्रकट स्तर का पता लगाने हेतु सीरम नमूनों की जाँच, रोग–सर्वेक्षण एवं रोकथाम के लिए अत्यधिक जरूरी एवं कारगर उपाय है। इस दिशा में केन्द्र के वैज्ञानिकों ने 1,768 अश्वों के सीरम नमूने एकत्रित किए जिनको केन्द्र की प्रयोगशाला में परखा गया। सर्वेक्षण में 4.87% नमूनों में इ०एच०वी०, 21.78% नमूनों में अश्व–फ्लू एवं 3.17% नमूनों में जापानी बुखार की एन्टीबाडीज पाई गई। ग्यारह अश्व ग्लैण्डर रोग से ग्रसित पाए गए है। बेबीसिया इक्वाई के 36.29% एवं 8.22% नमूने ट्रिपैनोसोमोसिस ग्रस्त पाए गए। इसके अलावा सीरम के 11,909 नमूने, जिनमें रोग–सर्वेक्षण के 1,768, रोग–अन्वेषण के 944 और कान्ट्रैक्ट सर्विस के 9,192 नमूने शामिल हैं, को ग्लैण्डर्स रोग के लिए जाँचा गया। इनमें उत्तर प्रदेश के 7 एवं हिमाचल प्रदेश के 4 सीरम नमूने ग्लैण्डर्स एण्टीबाडीज युक्त पाए गए। मई, 2010 में हिमाचल प्रदेश के पण्डोह क्षेत्र में खच्चरों में गलैण्डर्स पाई गई। इनसे ग्लैण्डर्स रोगाणु को पृथक भी किया गया है। इसके पश्चात्

दिसम्बर 2010 में उत्तर–प्रदेश के बिजनौर क्षेत्र में रोग–प्रकोप देखा गया।

पिछले कुछ वर्षों में अश्व–फ्लू का देश–व्यापी प्रकोप देखा गया था। इन अश्व–फ्लू ग्रसित राज्यों में सीरम–नमूनों की जाँच जारी है जिसके अन्तर्गत 3515 सीरम–नमूनों की जाँच में 550 नमूनों में अश्व–फ्लू विषाणु की एन्टीबाडीज़ पाई गई, हालांकि किसी भी सीरम–नमूने जोड़ी की जाँच में सीरोपाजिटिव नमूने नहीं मिले। कुल 56 अश्व–फ्लू संक्रमण के संशय वाले अश्वों के नासिका स्त्राव नमूनों को आर टी–पी सी आर विधि द्वारा जाँचा गया जिसमें कोई नमूना अश्व–फ्लू ग्रसित नहीं पाया गया । कुल 209 नमूनों की जीवाणु जाँच में 61 जीवाणु पृथक किए गए जिसमें बरखोलडेरिया मेलियाई (4), रोडोकौक्कस इक्वाइ (6), स्ट्रैप्टोकाक्कस इक्वाइ जूएपिडैमिकस (33), एवं इक्वाई (4), स्टैफाइलोकौक्कस (1), एवं ई कोलाई (13) प्रमुख हैं।

अश्व–उत्पादन के क्षेत्र में सभी 6 देशी नस्लों जैसे मारवाड़ी, काठियावाड़ी, स्पीति, जँसकारी, भूटिया एवं मणिपुरी को शारीरिक माप–दण्ड मानकों और रंग के आधार पर विश्लेषित किया गया है। क्योंकि किसी भी नस्ल सँवर्धन कार्यक्रम में आनुवांशिक विश्लेषण एक आवश्यक अंग है इसलिए 55 विभिन्न माइक्रोसैटेलाईट मारर्कस से विभिन्न अश्व–नस्लों का विश्लेषण किया गया। इस विधि द्वारा विभिन्न नस्लों में और नस्लों के बीच में भी नैंसर्गिक भिन्नरुपता पाई गई। वंशावली अध्ययन से यह पाया गया कि थौरोब्रेड अश्व एक अलग समूह बनाता है जबकि भारतीय अश्व–नस्लें दो भागों का एक अलग समूह बनाती हैं। भारतीय समूह में एक भाग मारवाड़ी एवं काठियावाडी अश्वों का है और दूसरा समूह मणिपुरी, स्पीति, जाँसकारी एवं भूटिया टट्टुओं का बनता है। इससे यह निश्कर्ष निकलता है कि भौगोलिक रुप से विभाजित अश्व आनुवांशिक रूप से भी अलग समूह बना रहे है।

गदर्भो की कद–काठी के मापदण्डों के मूल्यांकन के अन्तर्गत राजस्थान के 97 गर्दभों के शरीर के विभिन्न अंगों को जाँचा गया। यह देश के गर्दभों के शारीरिक मापदण्डों का मानकीकरण की दिशा में एक कदम है। अश्वों के कृत्रिम–गर्भाधान एवं नस्ल के संरक्षण हेतु वीर्य का हिमीकृत सवंर्धन एक आवश्यक कार्य है। इस दिशा में मिथाईल फार्मामाइड, डाई मिथाइल फार्मामाईड और डाई मिथाईल सल्फाक्साईड का प्रयोग कर देखा गया कि यह तीनों ग्लिसरौल के मुकाबले वीर्य हिमीकृत संर्वधन में कितने सक्षम हैं। मिथाईल फार्मामाइड इस दिशा में उपयोगी सिद्ध नहीं होता क्योंकि यह शुक्राणु गतिशीलता और जीवंतता पर कोई असरदार प्रभाव नहीं दिखाता। वीर्य के 98% नमूनों में अक्षत डी०एन०ए० पाया गया।

मारवाड़ी घोड़ियों में ऋतुकाल का समय समायोजित करने हेतु हार्मोनल (प्रोजेस्टरौन) और हार्मोन–रहित स्पँाज का उपयोग देखा गया। यह पाया गया कि घोड़ियाँ स्पँाज को निकालती नहीं है और स्पँाज में 10mg की दर से प्रोस्टाग्लैंडिन इस्तेमाल करने से ऋतुकाल का समायोजन संभव है। चार में से तीन मादा अश्वों में इस विधि के प्रयोग के पश्चात् ऋतुकाल समकालीनता देखी गई और इन 3 घोड़ियों में से दो घोड़ियों मे गर्भ भी ठहर गया।

अश्व–शक्ति की उपियोगिता को देखते हुए इस क्षेत्र में अधिकतम कार्य–क्षमता प्राप्त करना महत्वपूर्ण है। इस दिशा में केन्द्र एक अखिल भारतीय समन्वयन शोध परियोजना पर कार्य कर रहा है जो कि पशु–शक्ति के उपयोग एवं अधिकतम तन्त्र कार्यकुशलता के बिन्दुओं पर केन्द्रित है। गर्दभों द्वारा शुष्क इलाकों में बोझा ढोने की कार्यकुशलता का अध्ययन कार्य– विश्राम एवं लगातार कार्य चक्र के अन्तर्गत किया गया। दैहिकी के अवयवों जैसे शारीरिक तापमान, हृदय गति आदि को नापा गया। लगातार 6 एवं 8 कुन्तल बोझा ढोने के कार्य करने पर पशु 2 घण्टे मे पस्त हो जाता है जबकि 10 कुन्तल बोझा ढोने पर गर्दभ 1 घण्टे मे थक जाता है। इतना ही बोझा ढोने वाले इन गर्दभ में जिन्हें 5 घण्टों तक एक घण्टा कार्य एक घण्टा विश्राम दिया जाता है उसके दैहिक संकेतक बढ जाते है अपितु रूधिर विज्ञान संकेतकों में कोई बदलाव नहीं होता । देसी और विदेशी दोनों प्रकार के गदर्भो में खेत की जुताई और बीजारोपण कार्य–क्षमता को भी परखा गया। जुताई में गर्दभों को 1. 5 घण्टे, तीन बार एक घण्टे के विश्राम के साथ लगाया गया। डेढ घण्टे के जुताई कार्य के बाद गदर्भो के दैहिक कारकों में महत्वपूर्ण बदलाव देखा गया। विदेशी एवं देसी गधों ने क्रमशः औसतन 0.171 एवं 0.09 एकड़ प्रति घण्टा क्षेत्रफल में जुताई करी। बड़े आकार के विदेशी गर्दभों ने देशी गर्दभों की अपेक्षा अधिक एवं द्रुत गति से जुताई करी।

खच्चर एवं गर्दभ मूलतः कमजोर वर्ग के लोगों द्वारा प्रयोग में लाए जाने वाले पशु हैं और उनके कल्याण

कार्य की प्रायः उपेक्षा होती है। इस वर्ग की जीविका उपार्जन के बिन्दुओं की अविच्छिन्नता एवं सततता के अध्ययन हेतु एक आरम्भिक प्रयोग किया गया। यह अध्ययन राजस्थान, उत्तराखंड, हरियाणा एवं उत्तर प्रदेश राज्यों में किया गया जिससे विदित हुआ कि इनका पारिवारिक आकार 7–10 लोगों के बीच है और यह वर्ग अनपढ़ अधेड़ उम्र के व्यक्तियों का है। इनकी अश्व–प्रबंधन आदि में कम कुशलता है और इनकी मासिक आय ₹ 3,000 से 5,000 के बीच आंकी गई। अधिकतम पशुपालक अपने पशुओं की शारीरिक साफ सफाई सप्ताह में दो बार और मालिश और उदर–कृमिनाशक औषधि का प्रयोग कभी–कभार ही करते हैं।

वेटरीनरी टाईप कल्चर्स केन्द्र ने अपने आदेशपत्र के अनुसार कार्य कलापों को आगे बढ़ाया है और देश के विभिन्न भौगोलिक स्थानों से पशु–रोग प्रकोपों से नमूने एकत्रित किए है एवं विभिन्न स्थानों एवं नेटवर्क केन्द्रों से वियुक्त रोगाणु कल्चर्स एकत्रित किए है। इन रोगाणुओं का भौतिक जैवरासायनिक और आण्विक द्रष्टि से विशलेषण किया गया और उन्हें सुचारू रूप से परिरक्षित किया गया है। वर्तमान में इसमें 358 संरक्षित हैं जिनमें 255 जीवाणु एवं 163 विषाणु कल्चर्स हैं। साथ में 169 जैविक संयोजक क्लोन संरक्षित हैं। विषाणुओं में भैंस–चेचक, उष्ट्र–चेचक, बकरी–चेचक, अश्व–फ्लू, गौहरपीज़ विषाणु–1, अश्व हरपीज़ 1 एवं 4, जापानी बुखार विषाणु, गौ रोटा विषाणु, एवं मानव रोटा विषाणु शामिल हैं एवं जीवाणुओं में बोर्डिटैल्ला ब्रांकीसैप्टिका, ब्रुसैल्ला मेलीटैनसिस, रहोडोकाक्कस इक्वाई, एस्चरिशिया कोलाई, स्ट्रेप्टोकाक्कस, पास्चुरैल्ला, स्टेफाईलोकाक्कस, बैसिलस, स्यूडोमोनास, सालमोनैल्ला, क्लैबसिएल्ला, एयरोमोनास, शिगैल्ला एवं डेयरी जीवाणुओं में लैक्टोबैसिलस और रोमन्थी जीवाणुओ में मिथेनोजैनिक जीवाणु, पीडियोकाक्कस और लियूकानास्टाक शामिल हैं।

इसके अलावा वेटरीनरी टाईप कल्चर्स की तरफ से मेरठ में भैंस–चेचक एवं राजस्थान में गौ पैपिलोमैटोसिस रोग प्रकोप क्षेत्रों का भ्रमण किया गया और रोग–नमूने एकत्रित किए गए। भैंस–चेचक का प्रकोप भैंस, गौवंश एवं मनुष्यों में एक ही स्थान एवं समय पर पाया गया है। भैंस–चेचक विषाणु को पशु और मानव नमूनों में से पृथक कर लिया गया है और उनका आण्विक विश्लेषण कर उन्हें परिरक्षित कर लिया गया है। ई कोलाई और क्लैबसिएल्ला रोगाणुओं को अश्व दस्त, गर्भपात और श्वास रोग नमूनों में पाया गया एवं पृथकीकरण किया गया है। नर–अश्वों के वीर्य के नमूनों में से 23 प्रकार के जीवाणु पृथक किए गए हैं। प्रयोगशाला में पृथक कर परिरक्षित जीवाणु और विषाणुओं की क्लोंनिग कर आण्विक विशलेषण भी किया गया है। हाल में विभिन्न पशु–जातियों के उत्तकों से कोशिकाओं का पृथकीकरण एवं संवर्धन की दिशा में कार्य शुरू किया गया है जिसका उपयोग विषाणु पृथकीकरण में किया जा सकता है।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Introduction

The foundation of National Research Centre on Equines (NRCE) was laid on 26 November, 1985 at Hisar (Haryana) under the flagship of the Indian Council of Agricultural Research with a belief that equines play an important role in society and in our everyday lives. Since then NRCE has been striving hard by performing activities that have come to fruition in improvement of equine health and production in India. The institution grew stronger in 1989 when a sub-campus was established at Bikaner, thereby expanding the scope and depth of it's services. In a short span, NRCE has gained recognition as a premier research centre in the area of equine health and production. The research activities continue to bridge the gap between basic biology and clinical applications thereby providing cutting-edge translational research for improving the health and welfare of the equine population in the country. In addition to ongoing research in the areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry, biotechnology, genetics and breeding, reproduction, physiology and nutrition; the Centre is disseminating information at national and international level to equine owners through publications and news articles. The Centre has contributed significantly for upliftment of the landless and marginal farmers by helping in conservation and improvement of the indigenous equine germplasm. Recently, Veterinary Type Culture Centre has also been established in the year 2005 at NRCE for collection and storage of microbes of animal origin. NRCE looks forward to the future with great enthusiasm to extend benefits to equine population in the country.

Mandate

- To undertake research on health and production management in equines;
- To develop diagnostics / biologicals for major equine diseases;
- To act as a National Referral Facility for diagnosis, surveillance and monitoring of equine diseases;
- To provide diagnostic, 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 the development of modern diagnostics & vaccines.
- Transfer of technology for superior mule & true-to-breed indigenous horse production in their home tracts using 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 AI technology for mule production in India using cryopreserved jack semen for use at farmers' door.
- Perfection and propagation of artificial insemination techniques in horse and pony production using

frozen semen of true-to-breed indigenous stallions for the consortium of threatening breeds in India.

- Breed characterization and *in situ* conservation of various indigenous breed of horses.
- Exploiting importance 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 development programmes for the upgradation of the indigenous breeds of equids in the different parts of the country in collaboration with the 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.

Equine herpes virus-1 (EHV-1): A highly sensitive and specific neutralizing monoclonal antibody-based diagnostic kit namely **Equiherpes B-ELISA** was developed by the Centre for diagnosis of EHV-1 antibodies. This kit tests serum samples using single dilution thus making it very economical. It was formally released by Hon'ble DG, ICAR on August 20, 2008. Presently, the kit is under the process of commercialization.

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

Equine Rotavirus: A sandwich enzyme-linked immunosorbent assay (s-ELISA) was developed employing a monoclonal antibody (mAb) raised against VP6 of rotavirus, for detection of equine rotavirus (ERV) from stool samples. The diagnostic sensitivity (DSn) and specificity (DSp) of ELISA was 1.0 ($0.8076 \leq \pi \leq 1.0$) and 0.96 ($0.8541 \leq \pi \leq 0.9932$), 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.

Equine influenza virus (EIV): EIV is routinely diagnosed by haemagglutination inhibition 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 recombinant NS1 protein based immunoassay for differentiation of vaccinated and infected animals is under progress.

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

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

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

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

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

Vaccines and Immuno-biologicals developed by NRCE

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.

Equine influenza vaccine: The Centre had developed equine influenza vaccine using indigenous isolate (A/equi-2/Ludhiana/87), in view of the emergence of EI in

India. During 2008-09 an antigenically and genetically divergent EIV strain was isolated which was different from the 1987 isolates. As the vaccine developed using 1987 strain might not provide protection against the challenge with the current strain, therefore the vaccine has been updated in 2010 incorporating epidemiologically relevant isolate {A/eq/Katra-Jammu.06/08 (H3N8)} responsible for equine influenza 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 a limited experimental trial as well as in field trials. Further, a new cell culture-based inactivated equine influenza vaccine is being developed by the Centre.

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

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

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*, and kit for pregnancy diagnosis have been developed by the Centre.

Surveillance and monitoring of equine diseases in India

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 AHS in the country helped in declaring India free of African horse sickness in 2006 by Office International des Epizooties (OIE).
- Outbreaks of glanders in equine during 2006-07 and 2008-09 were detected and control measures were taken to prevent its further spread. Of late, in December 2010 the disease was once again confirmed by NRCE from Chandpur area of Bijnor district on the basis of clinical symptoms, microbiological investigations (agent isolation and identification), molecular techniques (PCR) and serological tests (CFT and ELISA). Of the total 121 samples tested from UP during 2010, seven equines were found to be affected with glanders, six from Bijnor and one from Ghaziabad district of UP. Three *Burkholderia mallei* cultures were isolated from nasal swabs. To contain the disease, the follow up monitoring and surveillance programme needs to be strengthened by the State Animal Husbandry department, with the technical support from NRCE, in the area 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 anemia from 1998. One mule has been found seropositive during 2009-10.

Molecular characterization of equine pathogens

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

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 JEV isolates responsible for JE in humans in India.

***In vitro* culture of *Trypanosoma evansi*:** The Centre succeeded in *in vitro* cultivation of bloodstream forms of *T. evansi* in artificial media by using specially formulated cell culture medium supplemented with 20% adult horse serum.

Biological resource bank

NRCE has a strong biological resource base having numerous pathogens, recombinant clones, reference

sera, equine sera, monoclonal antibody secreting hybridomas, etc.

- Pathogenic isolates (viruses, bacteria and parasites) of equine origin available with NRCE include EHV-1 (6 isolates), EHV-4 (14), equine rotavirus (29), equine influenza (11), Japanese encephalitis virus (2), West Nile virus (1), *Rhodococcus equi, Streptococcus equi, S. zooepidemicus, Burkholderia mallei*, *Salmonella* Abortusequi, *S. equisimilis*, *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 15,000 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 breeds

All the six indigenous breeds of equines namely Marwari, Kathiawari, Spiti, Zanskari, Bhutia and Manipuri, have been characterized phenotypically on the basis of their biometric indices and coat colour. True-to-breed equids (50) of each breed were selected from their home tracts in India and fifteen different biometric indices were recorded for each equid. Significant difference among different biometric indices were observed due to breed as well as sex. Some of the salient features are given below.

- Marwari and Kathiawari had wither height equivalent to 150 cm or more and as such both these breeds come under the category of horse breeds. It is well established that equids having height less than 150 cm are termed as ponies. Both mares and stallions of Kathiawari and Marwari breeds were at par as far as their height at wither is concerned. Equids of Manipuri (129.04 cm), Spiti (123.54 cm), Zanskari (126.32 cm) and Bhutia (126.94 cm) breeds had their mean wither height less than 150 cm and come under the category of Pony Breed. Spiti stallions (117.80 cm) were observed to be significantly ($P<0.05$) small among both the sexes of all the breeds.
- Equids of Marwari and Spiti breed were observed to be significantly ($P>0.05$) tall and small, respectively, among all the horse breeds. Almost similar pattern was observed in body length of all these breeds.
- Among two horse breeds (Kathiawari and Marwari), differences in heart girth were also significant ($P<0.05$). Heart girth in Spiti, Zanskari and Bhutia animals were at par with each other but significantly ($P<0.05$) higher than Manipuri equines. Canon length of Kathiawari equids was significantly ($P>0.05$) higher than Marwari, Zanskari and Bhutia breeds. Further, Bhutia ponies had longer canon than Zanskari ponies.
- Ear length and width were recorded to be maximum and significantly ($P>0.05$) higher in Spiti and Manipuri animals, respectively than all other breeds. Among Kathiawari and Marwari horses, both these indices were significantly ($P>0.05$) higher in Marwari animals. Ear width of Spiti equines was significantly ($P>0.05$) lower but with maximum ear length than rest of the breeds.
- Among all the breeds, gap between ears (pole) was significantly ($P>0.05$) low in the animals of Kathiawari breeds than other breeds. Maximum pole was observed to be in Manipuri breed.
- Among all the six breeds, face of Manipuri ponies was observed to be the broadest. Face length also varied significantly ($P>0.05$) among all the breeds with minimum in Spiti ponies and maximum value in Kathiawari animals.
- Both fore and hind leg lengths were significantly ($P<0.05$) higher in Marwari animals with lowest values in Spiti ponies. Like leg length, height at hock and knee height were maximum and significantly higher in Marwari animals than rest of the five breeds. No significant difference, due to sex, was observed in fore and hind leg lengths in mares and stallions of all the breeds.
- Hoof length was observed to be maximum and significantly higher both in Marwari and Bhutia equids, however as far as hoof width is concerned, it was maximum and significantly higher in Marwari animals than rest of the equines breeds.

Genotypic characterization of Indian equine breeds

Genetic characterization is an important part of any breeding program, so using 55 different microsats an effort was made to study genetic diversity among different equine breeds available in India. On the basis of allele number, allelic frequencies and heterozygosity

values; some of the salient features include:

- Heterozygosity analysis with different polymorphic microsats indicated the presence of high genetic diversity within and between different breeds.
- The Neighbor-Joining algorithm was used for the construction of both the topology as well as phylogenetic tree. The Thoroughbred horses expectedly clustered separately in topology as well as phylogenetic tree. Other Indian breeds clustered into two distinctive classes. One cluster grouped Kathiawari and Marwari horses while the other cluster had Manipuri, Spiti, Zanskari and Bhutia ponies. It can be inferred from the study that the geographically distant breeds are also genetically distant.
- Recent bottleneck in the population i.e. within past few dozen generations was examined by a graphical method analyzing distortion of allele frequency distribution which plots groups of alleles from a sample of many polymorphic loci into each of the ten frequency classes. All the seven breeds showed normal "L" shaped curve reflecting no bottleneck in the recent past.

Establishment of Nucleus Herds

- Exotic Donkeys: Twenty Jennies and six jacks of European breed (Poitu) were imported from France through ODA, UK in 1990, for the improvement of indigenous donkeys and production of superior mules.
- Marwari Horses: In an effort to conserve true to breed equids, the Centre has also established a nucleus herd of Marwari horse at Equine Production Centre, Bikaner.
- Zanskari Ponies: 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 bought from Zanskar valley, Kargil, Ladakh, Jammu & Kashmir, in November, 2009.
- Indigenous donkeys: The Centre has initiated the establishment of nucleus herd of small grey and large white donkeys found in India.

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, Kathiawari stallions and donkeys have been standardized. The technique of artificial insemination using frozen semen for production of superior quality Marwari horses, superior mules and donkeys has been perfected. The pure germplasm of endangered indigenous breeds of horses is being conserved using this technology.

Early pregnancy diagnosis: 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.

Kit for pregnancy diagnosis: An eCG s-ELISA kit.

Patents

- Patent has been granted by the Patent Office, Government of India entitled "A method for preparation of a diagnostic kit useful for forecasting Equine Herpes Virus-1 disease".
- A patent has been filed for "COFEB-Kit for diagnosis of *Babesia equi* infection in equines".
- A patent has been filed for "A method for preparing complement fixation test based (COFEB) kit for the diagnosis of *Babesia equi* infection in equines".
- The Centre has filed a patent for "A kit for detection of pregnancy in equines and assay thereof".

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 Centre

Veterinary Type Culture Centre 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 centers, 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 center 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 *A. 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 goats.
- Laboratory confirmed cases of Camelpox zoonosis-first laboratory confirmed camelpox zoonosis 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 at Hisar during the year–
- Bacteria accessioned : 255
- Virus accessioned : 79
- Recombinant clones accessioned : 164
- VTCC repository includes- 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; bacterial isolates *viz. Rhodococcus equi, E. coli, Bordetella bronchiseptica*, *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Brucella melitensis*, *Salmonella* spp., *Klebsiella* spp., *Bacillus* spp., *Aeromonas* spp., *Shigella* spp., *Pasteurella* spp., *Lactobacillus* spp. Methanogenic bacteria, *Pediococcus* spp., *Leuconostoc* spp., etc.

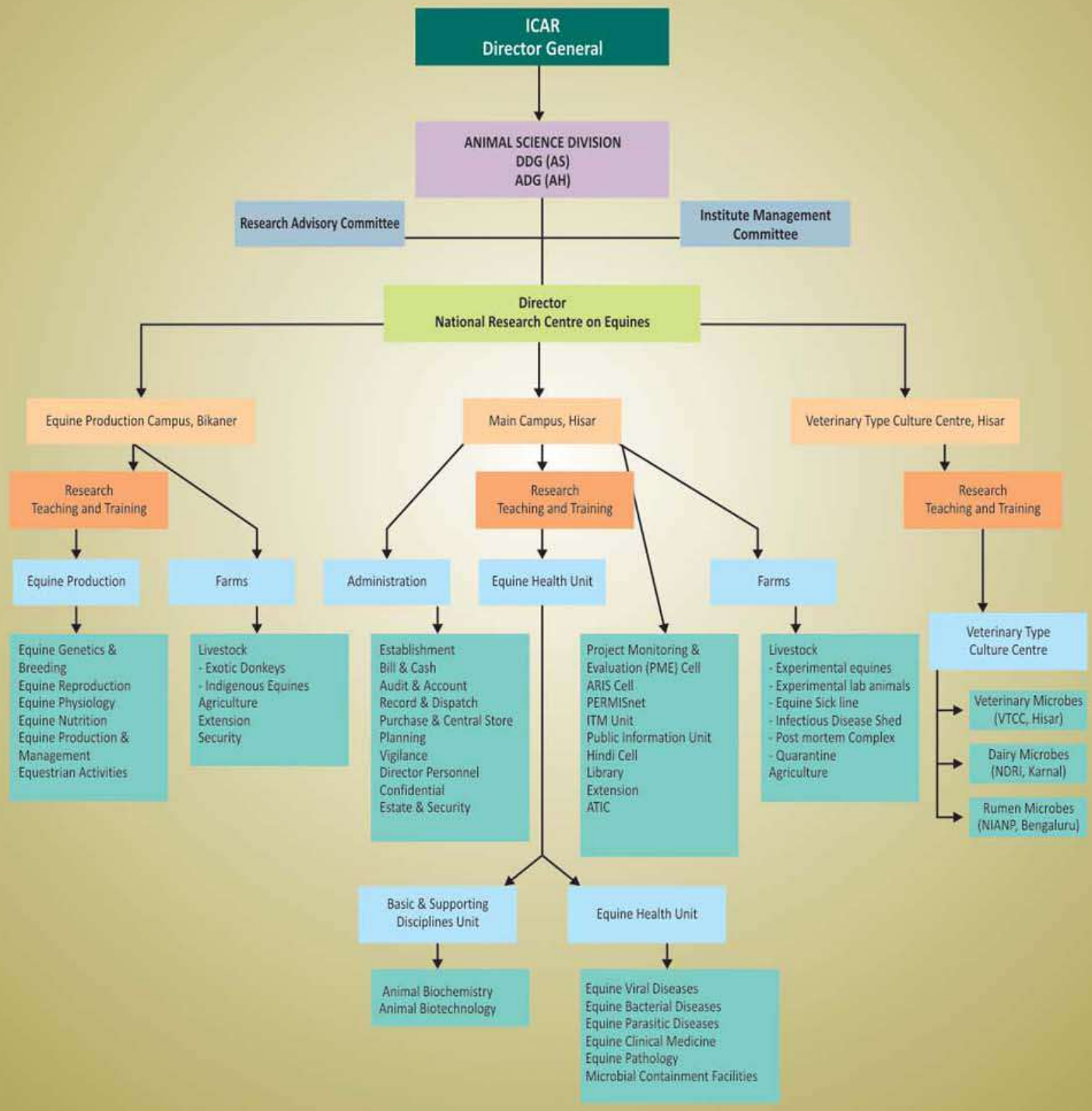
Staff position of NRCE and VTCC

Name of the post	NRCE			VTCC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	1	1	-	-	-	-
Scientific	25	23	2	10	9	1
Technical	23	22	1	1	1	0
Administrative	14	12	2	0	0	0
Supporting	22	20	2	0	0	0
Total	**85**	**78**	**7**	**11**	**10**	**1**

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 production research in equines
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
1994	An ELISA developed for differentiation of equine influenza vaccinated and infected animals (DIVA)
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)
2005	Mab-based s-ELISA 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
2008	Equine Herpes Virus-1 diagnosis kit released
2008	ELISA based pregnancy diagnosis kit for pregnancy diagnosis in mares released
2009	Development of equine herpesvirus-1 vaccine
2009	A nucleus herd of Zanskari ponies established at Bikaner
2009	First laboratory confirmed camelpox zoonosis in the world
2009	Japanese Encephalitis Virus isolated from equines in India
2009	Updation of equine influenza vaccine
2009	First isolation of *Bordetella bronchiseptica* from horse
2009	First isolation of *Actionobacillus equilli* from foal
2009	First isolation of *Staphylococcus hyicus* from pig
2009	First isolation of *Corynebacterium pseudotuberculosis* and *Corynebacterium bovis* from horse
2009	First detection of Methicillin-resistant Coagulase Negative *Staphylococcus sciuri* from goats
2010	Equine sanctuary for conservation of indigenous breeds of horses
2010	First confirmation of EIA seropositivity in indigenous equines in India
2010	A new clade designated as 'Asian Clade' of equine influenza virus identified
2010	Phenotypic characterization of all six indigenous equine breeds completed
2010	Re-emergence of glanders in HP & UP
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
2011	First laboratory confirmed report on BPXV causing disease in buffaloes, human & cows in the same time & space

Organizational Set-Up



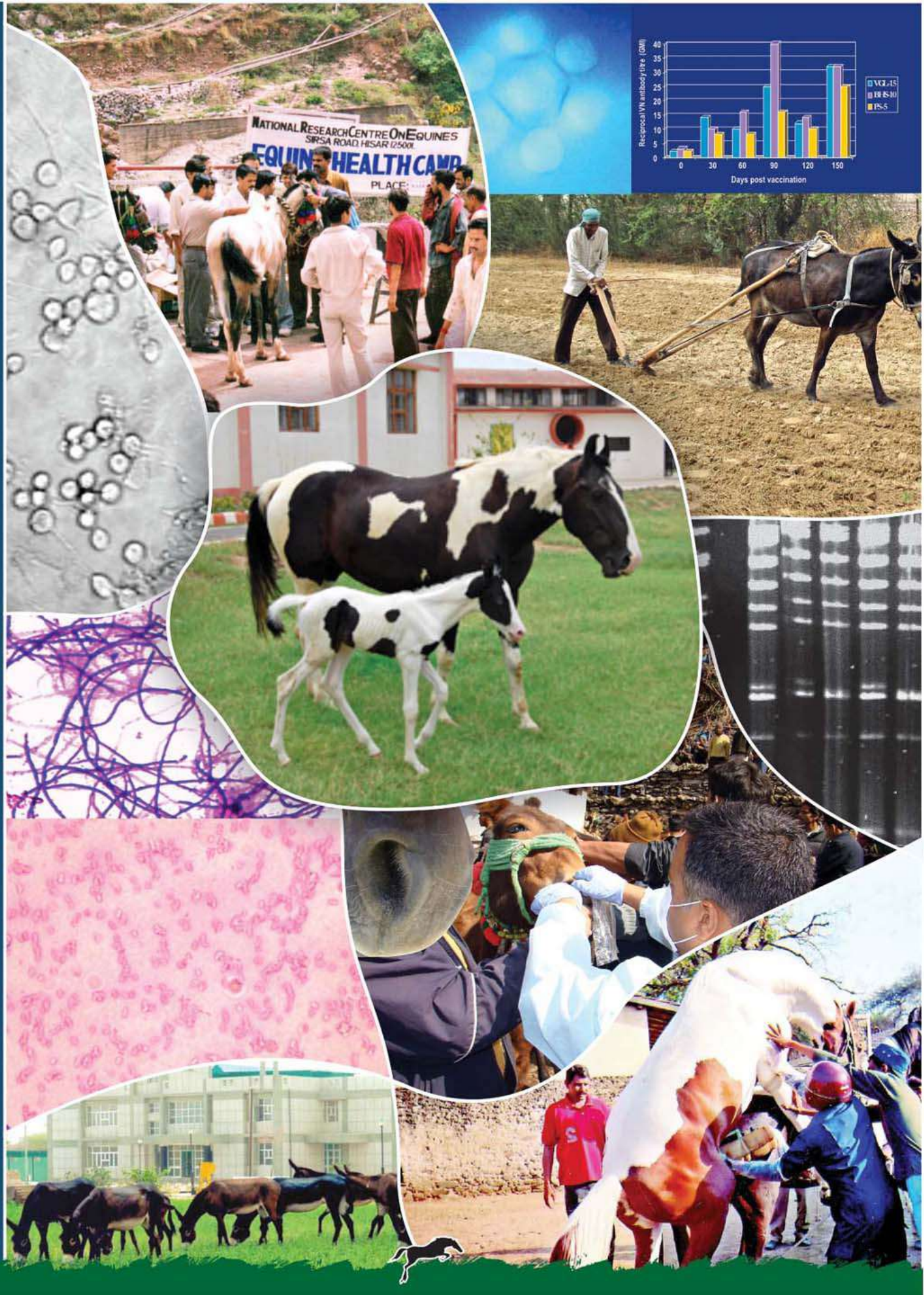
Summary of Expenditure & Revenue Generation

(₹ in lacs)

	Summary of Expenditure	2009-10	2010-11
	NON-PLAN		
1.	Establishment charges including LSP/PF, wages, OTA	484.34	498.98
2.	Traveling allowances	3.70	3.03
3.	Others charges including equipments & recurring charges	103.88	143.81
4.	Works	9.27	17.39
	Total Non-Plan Expenditure	**601.19**	**663.21**
	PLAN		
1.	Establishment charges including LSP/PF, wages, OTA	0.00	0.00
2.	Traveling allowances & HRD	6.56	8.48
3.	Others including equipments & recurring charge	236.59	426.92
4.	Works	117.69	26.63
	Total Plan Expenditure	**360.84**	**462.03**
	Total Expenditure (Plan and Non-Plan)	**962.03**	**1125.24**

(in ₹)

	Summary of Revenue Generation		
1.	Sale of farm produce	250.00	2,45,640.00
2.	Sale of livestock	0	70,000.00
3.	Sale of publication and advertisements	3540	2,160.00
4.	License fee	1,37,379.00	94,926.00
5.	Interest on loans and advances	43,599.00	99,027.00
6.	Interest on short term deposits	2,77,487.00	51,760.00
7.	Income from internal resource generation	42,88,867.00	44,49,000.00
8.	Receipt from services	0	0
9.	Other misc. receipts	17,37,752.00	14,59,826.00
	Total Revenue	**64,88,874.00**	**64,72,339.00**


NATIONAL RESEARCH CENTRE ON EQUINES
SIRSA ROAD HISAR 125001
EQUINE HEALTH CAMP
PLACE:
Reciprocal VN antibody titre (GMT)
40
35
30
25
20
15
10
5
0
0
30
60
90
120
150
Days post vaccination
VGL-15
BHS-10
PS-5

Research Achievements

Genetic and antigenic differentiation of Equine Influenza Viruses

Equine influenza is a severe respiratory disease of equine caused by influenza A virus (H3N8 and H7N7). India had an epizootic of EI in 2008-09 which involved 13 states of the country. Equine influenza virus (EIV) isolated from various regions and characterized as that belonging to clade 2 of Florida sublineage on the basis of HA gene sequence. For the purpose of genetic characterization of EIVs isolated from outbreaks of EI in different geographical regions, matrix (M) and nucleoprotein (NP) genes were cloned and sequenced. Homology search of sequences of M & NP genes was carried out using BLAST. All the related sequences were aligned and phylogenetic trees were constructed using MEGA4.0 software. Comparison of nucleotide sequence of M & NP genes of Indian isolates revealed maximum homology with Chinese and Mongolian isolates of EIV.

A new clade designated as 'Asian Clade' of equine influenza virus identified on the basis of matrix gene analysis

All isolates shared 98.41% and 99.54% homology with other Clade 2 viruses of Asian origin for M1 and M2 amino acid (aa) sequences, respectively. There were 3 and 4 unique aa residue changes, respectively in M1 and M2 proteins in all Asian isolates (Table 1). Phylogenetic analysis revealed clustering of Indian and Chinese isolates in a separate group (Fig. 1 & 2). The changes observed in the sequences of Indian and Chinese isolates indicate significant differences from EIV isolates circulating in the rest of the world and have been thus grouped in a separate cluster which has been designated here as "Asian clade" (Fig. 1 & 2). The aa residue changes *viz.* I15V, I80V and R95K in M1 protein and G21D, F48S, D85S and G89S in M2 protein were observed in all Indian isolates along with Chinese and Mongolian isolates while all other EIV isolates did not show these changes (Table 1). Intensive surveillance and structural studies of the EIVs for persistence of the changes observed in the M gene would enable to establish their significance in the host-specific adaptations.

Table 1: Amino acid changes in M1 & M2 proteins of equine influenza virus

	M1																	M2																	
STRAIN	*14*	*15*	*30*	*35*	*50*	*80*	*85*	*91*	*95*	*101*	*142*	*188*	*208*	*224*	*231*	*248*	*253*	*17*	*18*	*21*	*23*	*27*	*33*	*43*	*48*	*50*	*59*	*61*	*79*	*04*	*22*	*35*	*37*	*89*	*98*
Eq/KAT/6/08	I	I	N	K	P	I	S	N	K	R	V	A	K	S	D	M	*	C	K	G	S	V	I	L	S	F	L	R	P	E	E	N	A	S	D	S	*
Eq/MYS/12/08	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Eq/GOP/1/09	.	.	D	N	.	.	.	.	.	.	.	.	.	.	.	.	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Eq/CZECH/09	.	V	D	.	.	V	.	.	R	.	.	.	R	.	.	.	*	.	.	D	.	.	.	.	F	.	M	.	.	.	.	.	.	D	.	G	*
Eq/GAN/7/08	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	*	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Eq/XIN/1/07	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Eq/MON/8/08	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	L	*	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Eq/NEW/5/03	.	V	D	.	.	V	.	.	R	.	.	.	.	.	.	.	*	.	.	D	.	.	.	.	F	.	.	.	.	.	.	.	.	D	.	G	*
Eq/OHI/1/03	.	V	D	.	.	V	.	.	R	.	.	.	R	.	.	.	*	.	.	D	.	.	.	.	F	.	.	.	.	.	.	.	.	D	E	G	*
Eq/KEN/5/02	.	V	D	.	.	V	.	.	R	.	.	.	.	.	.	.	*	.	.	D	.	.	.	.	F	.	.	.	.	.	.	.	.	D	.	G	*
Eq/NEW/1/93	V	V	D	.	.	V	.	.	R	.	.	.	.	.	.	.	*	.	.	D	.	.	.	.	F	.	.	.	R	.	D	.	.	D	.	G	*
Eq/NEW/2/93	.	.	V	D	.	.	V	.	.	R	.	.	.	.	.	.	*	.	.	.	.	.	.	V	.	F	.	.	.	.	.	.	.	D	.	G	*

Amino acid residues are numbered from the N-terminal methionine. Residue identity to A/eq/Katra-Jammu/6/08 is shown with a dot (.) and stop codons are represented with an asterisk (*). Other reference strains are A/eq/Mysore/12/08, A/eq/Gopeshwar/1/09, A/eq/Czech-Republic/09, A/eq/Gansu/7/08, A/eq/Hubei/6/08, A/eq/Xinjiang/1/07, A/eq/Inner Mongolia/8/08, A/eq/Newmarket/5/03, A/eq/Ohio/1/03, A/eq/Kentucky/5/02, A/eq/Newmarket/1/93 and A/eq/Newmarket/2/93.

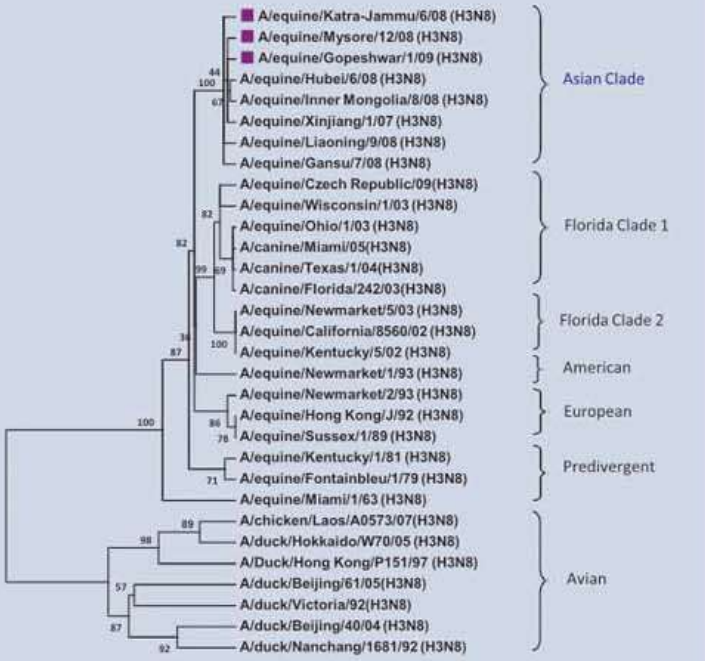


Fig. 1: Phylogenetic analysis of M1 gene

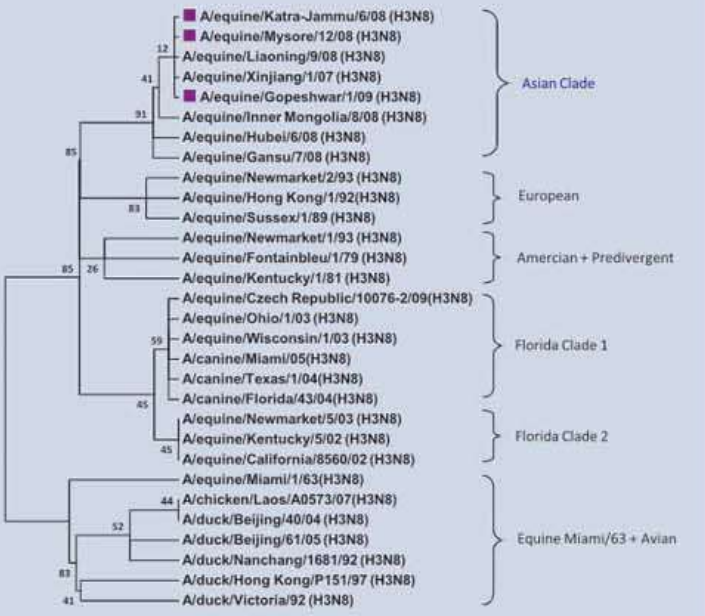


Fig. 2: Phylogenetic analysis of M2 gene

Analysis of nucleoprotein gene of equine influenza virus

Comparison of nucleotide sequence of NP gene revealed higher degree (95% to 100%) of homology with influenza A virus (H3N8) isolates of equine, canine and swine origin. The same degree of homology was also observed with other subtypes of influenza A viruses including H1N1, H2N1, H5N1, H5N2, H7N3, H3N6, H7N7, H10N6, H4N6, H11N9, H13N9, H9N2 etc. Results corroborate the previous findings that the NP gene sequence is highly conserved within the genus influenzavirus A. Phylogenetic analysis revealed close homology of Indian isolates with Clade 1 and Clade 2 viruses.

Antigenic differentiation of equine influenza viruses

Monoclonal antibodies are important immunological reagent which play crucial role in antigenic differentiation of pathogen. For antigenic differentiation of EIVs isolated from Indian outbreak of EI during 2008-2010, attempts were made to raise mAbs against the indigenous isolates which will be much more useful in assessing the antigenic profiles of EIVs. Following the standard procedures, the mice were immunized with EIV antigen, spleen collected from mouse showing highest EIV antibody titre and finally fusion experiment was done. A total of eight hybridomas showing ELISA OD values in the range of 0.474 to 2.747 were selected. First cloning of 8 hybridomas 1D12 (OD 2.224), 1G4 (OD 2.747), 4D9 (0.749), 5A7 (OD 1.645), 5 F4 (OD 1.995), 3C7 (0.973), 5F8 (0.474) and 5 G7 (0.578) has been done by limiting dilution method. They are growing well and are under process of screening for secretory activities so that they can be further processed for second cloning.

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

Development of diagnostics for Equine Influenza

Diagnosis of Equine Influenza (EI) by Real-time RT-PCR (qRT-PCR)

For development of faster and quicker diagnostics for detection of EI, real-time RT-PCR test was standardized for diagnosis of Equine influenza (EI) using SYBR GREEN dye-based reaction chemistry targeting matrix (M) gene. Viral RNA isolated from purified equine influenza virus (EIV) was quantified in Nanodrop (308 ng/µl) and serial 10-fold dilution was done from 10^{-1} to 10^{-7} dilution for generation of standard curve. The RNA was isolated from 200 µl of virus having HA titre of 256. Standard curve indicated the standardization of reagents, primers and cycling condition (Fig. 3 & 4). The

estimated T_m was 80°C for the amplified product (244 bp) of the matrix gene (Fig. 5). The developed test detected as low as 1pg of viral RNA. The qRT-PCR test revealed 100% specificity in the detection of isolated EIV (Fig. 6). The developed qRT-PCR assay would be useful for rapid detection of EI.

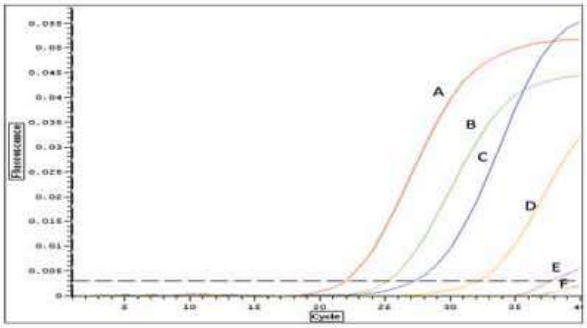


Fig. 3: Standardization of SYBR Green based real-time PCR for detection of EIV. A) 10ng RNA, B) 1ng RNA, C) 100pg RNA, D) 10pg RNA, E) 1pg RNA, F) No template control

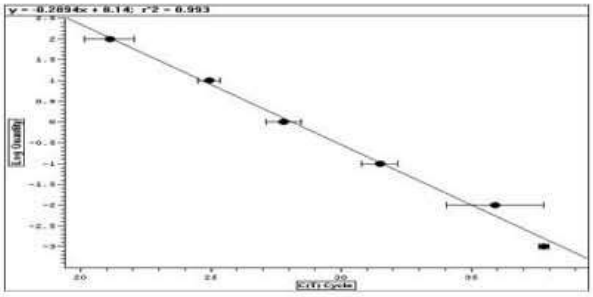


Fig. 4: Standard curve of SYBR Green based real-time PCR for detection of equine influenza virus

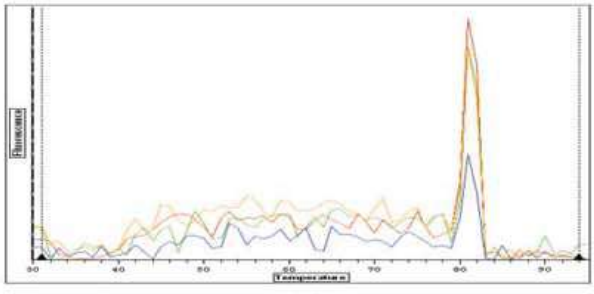


Fig. 5: Melting curve analysis: amplified product shown a specific Tm of 81.0°C in melting curve point analysis

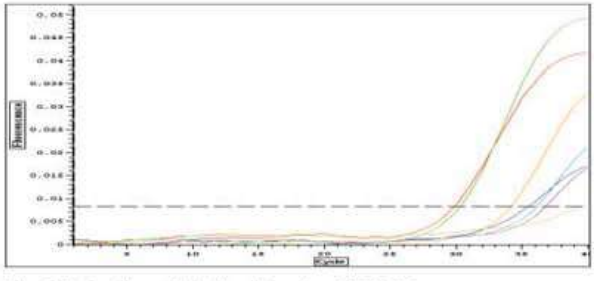


Fig. 6: Detection of viral isolates by qRT-PCR

Development of recombinant Non-structural 1 (NS1) protein-based DIVA immunoassay for differentiation of equine influenza infected and vaccinated animals

Differentiation of vaccinated and infected animals (DIVA) is an important aspect in control of infectious diseases. DIVA strategies for diagnosis of equine influenza were initiated exploiting the potential of NS1 protein to distinguish between the infected and vaccinated animals serologically. For this, recombinant NS1 proteins (full-length, N-terminal & C-terminal) were expressed in *E. coli* cells. Recombinant proteins (rProteins) were expressed as fusion protein with thioredoxin and histidine upon induction of transformed BL-21 cells with 1mM (final concentration) of IPTG for 4 hours. The expressed fusion proteins were tested by SDS-PAGE and the expected size of 43 kDa, 35 kDa & 33 kDa bands were observed for full-length, N-terminal and C-terminal of NS1 proteins, respectively (Fig. 7). Western blotting was standardized using these rProteins for detection of antibodies in serum samples (Fig. 8). Serum samples

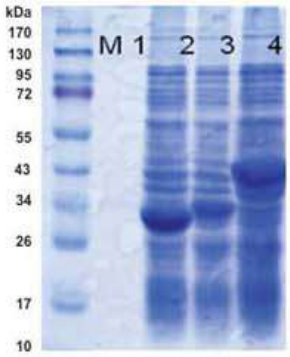


Fig. 7: SDS-PAGE bands of 43kDa (Lane 4), 35 kDa (Lane 3) & 33 kDa (Lane 2) for full length, N-terminal and C-terminal of NS1 gene respectively

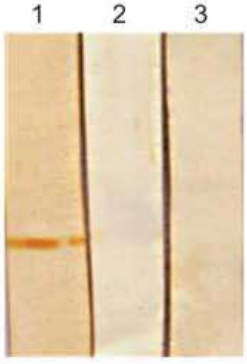


Fig. 8: Western blotting using C-Terminal r NS1 protien Serum from Infected Serum (L1), Vaccinated animal (L2) and Normal animal (L3)

from known vaccinated, infected and normal animals were utilized for standardization of the test. Serum dilution was standardized at 1:400 while anti-horse HRPO conjugate was used at the dilution of 1:700. The N-terminal as well as full-length proteins could not differentiate between the vaccinated and infected serum, however, C-terminal protein is showing promising results. Further, ELISA testing with large number of samples is under process for development of assay.

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

Development of indigenous diagnostics for Equine Infectious Anaemia (EIA)

Control of EIA is based on identification of inapparent carriers by detection of antibodies to EIA virus (EIAV) by internationally accepted agar gel immunodiffusion (AGID) test. At NRCE, AGID and cELISA tests are performed using reagents imported from approved commercial sources. There is lack of availability of the indigenous diagnostic reagents for EIA.

Recombinant protein (rP26) antigen-based ELISA

Recombinant protein was expressed in *E. coli* using synthetic gene of 26 kDa protein (Fig. 9) and evaluated rprotein for use in AGID/indirect ELISA for sero-diagnosis of EIA. The ELISA using rp26 was optimized using hyper-immune serum raised in rabbits and also using a panel of four positive and four negative samples (Fig. 10). The initial results have exhibited 100% correlation with gold standard test (Coggins test). A total of 1600 equine samples have been tested for EIAV antibodies using the optimized rp26 protein vis-à-vis standard AGID test (Fig. 11). The DSn and DSp for the test were found to be 100% and 99%, respectively. Based on the initial findings, efforts have been directed towards development of a field/lab based kit for sero-diagnosis of EIA. For this, the shelf-life and stability of reagents is being tested under various conditions and use of heavy water for thermostabilization of reagents is also being explored.

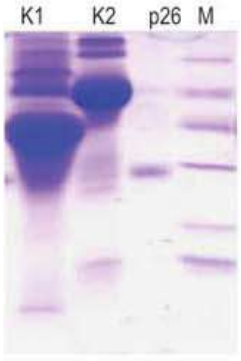


Fig. 9: Expression of rp26 protein

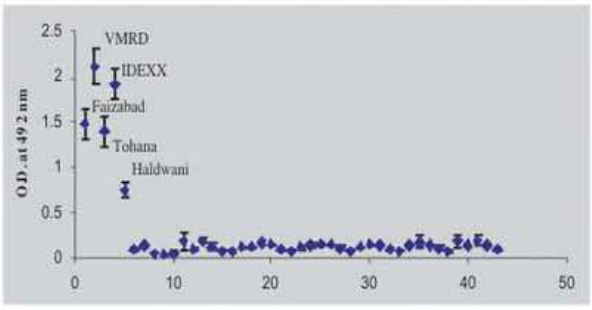


Fig. 10: Indirect ELISA using rp26 protein

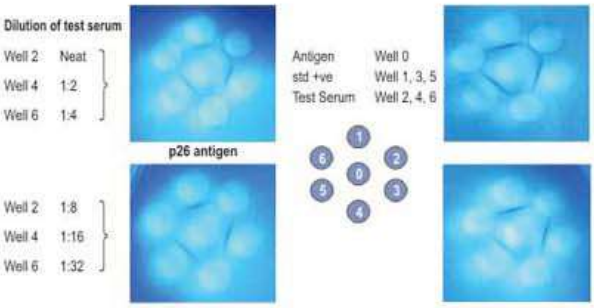


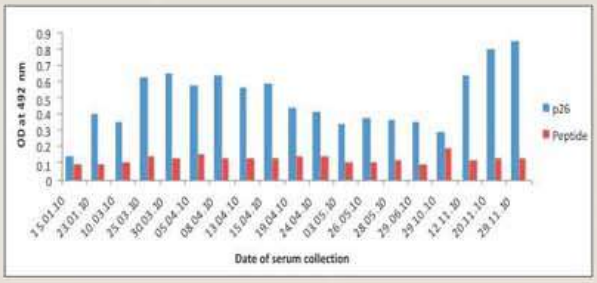
Fig. 11: Testing of samples by AGID using rp26 protein and standard kit.

EIA seropositive mule detected in Uttarakhand

Equine infectious anemia (EIA) is a chronic, debilitating retroviral disease of all equids. In Jan 2010, one mule was detected seropositive for EIA during routine active surveillance in district Nainital (Uttrakhand) after a gap of about 11 years. This is the first confirmation of EIA in indigenous equines in India. Seropositivity for EIA was indicated by immunoassays viz. Coggins and cELISA tests, however, no virus or antigen could be demonstrated by PCR, IFT or electron microscopy/ isolation.

The animal was immediately quarantined and kept in containment following all bio-safety and bio-security

Examination of visible mucous membrane



Monitoring of Immune response of EIAV naturally infected mule by ELISA

measures. The animal was regularly monitored and health status examined on daily basis (Fig.). The mule tested positive continuously by conventional Coggins test and rp26 ELISA developed by NRCE (Fig.). Several attempts to detect EIAV genomic RNA and intermediary DNA by RT-PCR and PCR, respectively revealed negative results. The animal was culled in Nov, 2011 and post-mortem conducted. None of the organs showed any significant gross or histopathological findings characteristic of EIA. Concerned authorities in Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture (Government of India) and State AH department were duly informed. Surveillance is being followed rigorously as per National Policy on EIA. It is a very important and alarming finding in view of absence of the disease in the country for quite a long period.

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

Validation of EHV-1 vaccine (Equiherpabort) in indigenous and Thoroughbred mares

Equine herpes virus-1 (EHV-1) vaccine - a killed oil emulsion mannide monooleate vaccine (OEMM) developed by the Centre for control of abortions caused by EHV-1 in equines - was subjected to validation. Field trials were conducted in 73 pregnant mares in different locations (Hanumangarh-29; Tohana-30; NRCE, Hisar-6 and NRCE, EPC, Bikaner-8). A total of 43 indigenous and 30 Thoroughbred pregnant mares from different villages / organized breeding farms were vaccinated with 2 ml vaccine dose (i/m) at 5th, 7th and 9th month of gestation. No untoward effect was seen after vaccination. Serum collected from the vaccinated animals was tested for virus neutralizing (VN) titre at monthly intervals for 6 months period. The mares were negative for VN antibody titre on the day of inoculation.

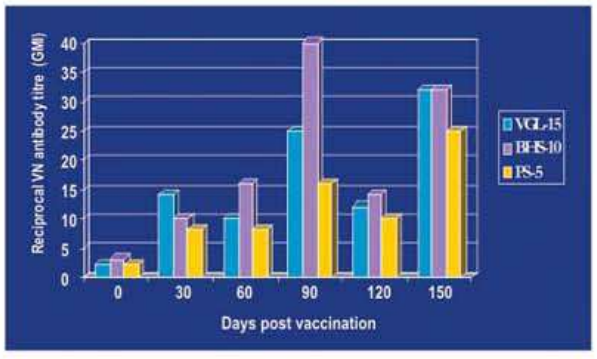


Fig. 11: Virus neutralizing antibody titre in Thoroughbred pregnant mares vaccinated with Equiherpabort vaccine

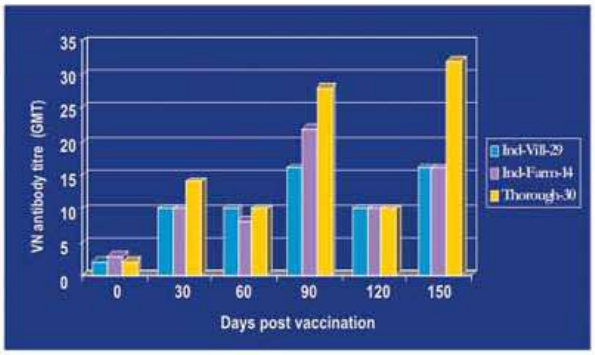


Fig. 12: VN antiibody titre in pregnant mares (indigenous vs Thoroughbred) with Equiherpabort vaccine

The reciprocal VN antibody titres against EHV-1 upon primary vaccination (30 day post vaccination) was in the range of 8-16. The booster antibody response ranged between titres of 16-64 (Fig. 11). The results were in accordance to antibody titres observed with "Pneumabort 'K'" imported vaccine as per our earlier studies. A higher antibody response was noted in Thoroughbred mares as compared to indigenous mares (Fig. 12). The vaccine was prepared in two batches and its shelf life was observed to be around 6 months. The field trials indicated that the "Equiherpabort" vaccine could be an effective alternative to the "Pneumabort 'K'" imported vaccine for large scale vaccination for control of EHV-1 in pregnant mases.

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

Development of diagnostics for Japanese Encephalitis in equines

Japanese encephalitis (JE) is an important vector-borne zoonotic disease of horses and humans in India. Efforts for development of diagnostics of JE in equines are being made through isolation and characterization of JEV isolates in India. One isolate of JEV (JE/eq/India/H225/2009) was recovered in mice and PS cell culture from a horse showing neurological signs in 2009. Phylogenetic analysis of JEV indicated that the equine JEV isolate belonged to genotype III and clustered together with Vellore group of JEV isolates from India.

Characterization and application of murine monoclonal antibodies in diagnosis of JE/WNV

During the previous years, monoclonal antibodies against (mAb) JEV (14 clones) and WNV (6 clones) were raised, cloned, amplified and cryopreserved. JEV specific mAbs EJC1, 3, 4, 6, 11 were IgM isotype while EJC7, EJC12 and EJC14 were isotyped as IgG2a. Two clones specific against E-protein of JEV i.e. EJC7 and EJC14 were selected and ascites was raised against them. mAb-based capture ELISA for JEV antibodies was standardized using anti-JEV monoclonal antibody ascites (EJC7). The assay was found to be more sensitive than HI and VNT for specific detection of JEV antibodies.

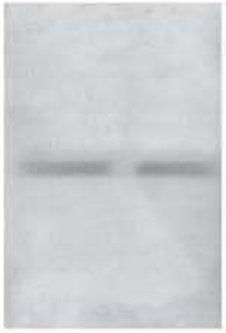


Fig. 13: Immunoblotting of WNV proteins with mAb $5A_1E_{11}$ and $1H_{11}E_7$. Both mAbs reacted with 53-kDa WNV E protein

During the year, murine mAbs were developed against WNV antigens. Two selected WNV clones i.e. 1H11E7 & 5A1E11 were isotyped as IgG2b and IgM, respectively. On western blotting both WNV specific mAbs were found to be directed against 53 kDa E-protein (Fig. 13). The mAb (5A1E11) cross-reacted with JEV E-protein, whereas mAb (1H11E7) was WNV-specific.

For WNV antibody detection, a blocking ELISA was developed using mAb 1H11E7. Using this B-ELISA, 64 equine serum samples (35 positive for WNV antibodies and 29 negative) were tested. B-ELISA detected 41 samples (34 VNT positive & 7 VNT negative) as positive and 23 samples as negative. The percent agreement between the results of VNT and B-ELISA was 87.5%. Based on these results, the sensitivity and specificity of B-ELISA in comparison to VNT was 97.14 % and 75.86 %, respectively. Further validation of these two ELISAs is in progress.

Prevalence of JEV in equine population in different geographical locations of India

A total of 1762 equine samples from 8 different states were tested for JEV antibodies and 56 (3.18%) were detected positive (Table 2). In addition, 107 pig samples from North-East states were tested for JEV antibodies and 36 (33.6%) were detected positive for JEV antibodies.

Table 2: JE antibody precedence during 2010-11 (State-wise)

State	S & M Samples	
	Nos. tested	Nos. positive (%)
Haryana	27	9 (33.33)
Rajasthan	558	22 (3.94)
Gujarat	313	6 (1.91)
Uttarakhand	299	8 (2.67)
Uttar Pradesh	135	2 (1.48)
Himachal Pradesh	76	0 (0.00)
Chattisgarh	326	9 (2.77)
Manipur	28	0 (0.00)
Total	1762	56 (3.18)

(B.R.Gulati, B.K.Singh, H.Singha and Nitin Virmani)

PCR detection of *Rhodococcus equi* targeting *cho*E and species-specific genes

The usefulness of the cholesterol oxidase gene (*choE*) and species-specific chromosomal region as targets for the rapid and specific identification of pathogenic *R. equi* was studied. *ChoE* gene encodes cholesterol oxidase, which is a membrane-damaging factor responsible for CAMP-like reaction. PCR assay was standardized for detection of *R. equi* by amplification of 959 bp *choE* and 700 bp species-specific sequence. Purified genomic

DNA of *R. equi* (isolate-98) was diluted (10- fold serial dilution) and detection limit of the *choE* and species-specific PCR were calculated. The assay was 100% specific and sensitive in detecting 10 pg of DNA (Fig. 14 & 15).

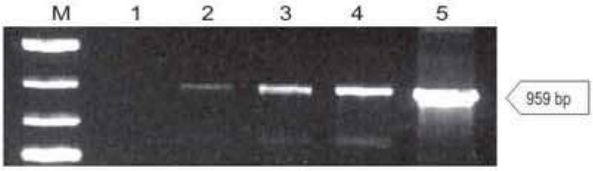


Fig. 14. Sensitivity of the *choE* PCR. Lanes: 5 to 1 (10 ng, 1 ng, 100 pg, 10 pg and 1pg, respectively). Lane: M, 1 kb DNA ladder

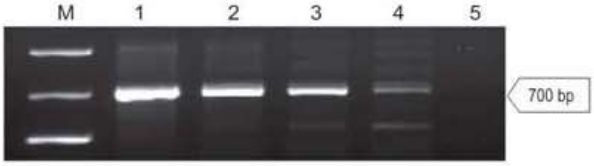


Fig. 15: Sensitivity of the species-specific PCR. Lanes: 1 to 5 (10 ng, 1 ng, 100 pg, 10 pg and 1pg, respectively). Lane M: 1 kb DNA ladder

A pair of species-specific primers for *R. equi* was used. These primers, however, are not specific for virulent organisms, because they amplify a 700-bp region of chromosomal DNA present in both virulent and avirulent *R.equi* isolates. These species-specific primers can be used in a PCR-based diagnostic test to identify clinical and environmental isolates of *R. equi.*

Characterization of plasmid category by PCR

PCR-based identification of a universal plasmid marker is essential for rapid discrimination between plasmid positive and negative strains of *R. equi.* The virulence of *R. equi* is associated with the presence of 85–90 kb plasmids in equines. These plasmids encode virulence-associated protein A (*vapA*), a 15–17-kDa surface lipoprotein antigen responsible for intra-macrophage survival, cytotoxicity, and pathogenicity. Most equine isolates are *vapA*+, as compared to humans and other animal isolates. A variant plasmid encoding VapB, a VapA-related surface antigen of larger size (18– 20 kDa), has also been identified in equines except horses. For characterization of the plasmids, PCR assay was standardized targeting different plasmid associated genes *viz., tra* (959 bp), *vapA* (286 bp) and *vapB* (477 bp) into 4 major plasmid categories *viz.*, $traA^{+}/vapA^{+}B$, $traA^{+}/vapA^{-}B^{+}$, $traA^{+}/vapAB^{-}$, and $traA^{-}/vapAB^{-}$ (plasmidless) of epidemiological significance (Fig. 16).

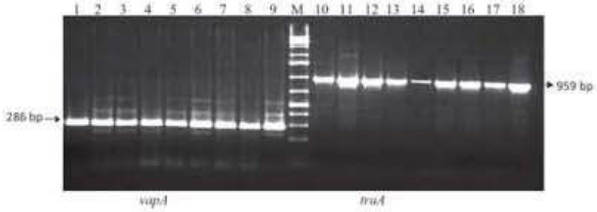


Fig. 16: PCR amplification of *vapA* and *traA* genes. L: 1 & 10, reference strain; L:2 &11, isolate 25; L:3& 12, isolate 36; L:4& 13, isolate 44; L:5 &14, isolate 48; L:6 &15, isolate 77; L: 7 & 16, isolate 79; L:8 &17, isolate 98; L:9 & 18, isolate 113; M: 1 kb DNA ladder plus

R. equi horse isolates (28) confirmed by both *cho*E and species-specific PCR revealed that all the equine isolates had plasmids not associated with a *vapB*+ marker suggesting host (horse)–driven (counter) selection of the *vapB*-type plasmid. The results clearly establish that equine isolates carry $traA^{+}/vapA^{+}B$É plasmid types.

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

Re-emergence of Glanders in Himachal Pradesh and Uttar Pradesh

Glanders is one of the fatal, contagious, notifiable, zoonotic diseases of equines, caused by *Burkholderia mallei.* It causes nodules and ulcerations in the upper respiratory tract and lungs. The skin form is known as 'farcy' where typical nodules form along the lymph vessels between affected lymph nodes. These nodules often rupture through skin to discharge yellowish pus and form deep ulcers which heal slowly. It may also cause a fatal disease in humans. The disease remerged in India after about a decade in 2006 and outbreaks of glanders were reported from many parts of the country from 2006-08. In 2010, after a gap of two years, glanders (cutaneous and nasal form) was again confirmed by NRCE in 13 horses/ponies in Raipur (Chhattisgarh).

In May 2010, glanders was reported from Pandoh area of Mandi district (Himachal Pradesh), where four mules were found positive for glanders (1 isolation). This was followed by outbreak in December 2010 from Chandpur area of Bijnor district. The disease was confirmed by NRCE based on clinical symptoms, microbiological

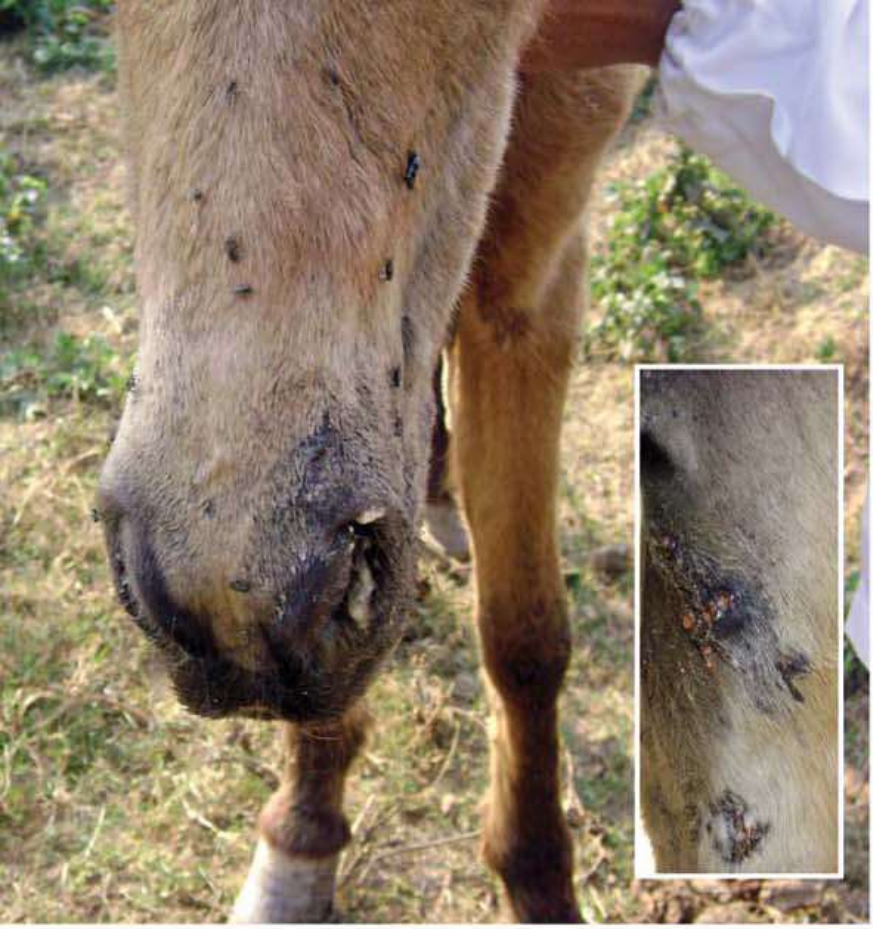


Fig. 17: Glanders affected horse showing ulcers in nasal cavity and skin nodules on hind limbs, Bijnor, U.P.

investigations (agent isolation and identification), molecular techniques (PCR) and serological tests (CFT and ELISA) and reported to the relevant authorities for further action for containment of the disease. Of the total of 121 samples tested from UP during the period, seven equines (five horses, one pony and one mule) all involved in carriage transportation in local markets were found to be affected with glanders (six from Chandpur, District Bijnor and one from Babugarh area, District Ghaziabad) (Fig. 17).

Three *B. mallei* cultures were isolated from nasal swabs. Efforts on the part of NRCE in coordination with Central and State animal husbandry authorities could control the disease effectively. To prevent its further spread, the follow up monitoring and surveillance programme needs to be strengthened by the State Animal Husbandry department, with the technical support from NRCE, in the area in view of the recurring cases of glanders from this region.

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

Purification and characterization of *T. evansi* invariable antigen and its use in diagnosis of trypanosomosis

Trypanosomosis is most severely reported in horses, at times showing nervous signs. However, disease in donkeys may occur in chronic forms which persists years together. This entails further efforts for search of immunodominant antigens, which can be universally produced using molecular biology tools and applied for seroepidemiology of trypanosomosis. During the period under report, an attempt has been made to identify and isolate partially purified immunoreactive proteins from mice-adapted horse (cloned) isolate of *T. evansi* from whole cell lysate (WCL) antigen preparation.

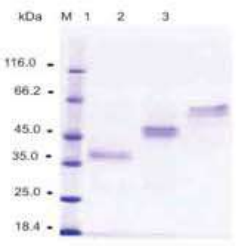


Fig. 18: Immunodominant antigens of horse isolate of *T.evansi* purified from WCL antigen

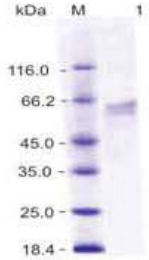


Fig. 19: Immunoblot of F1 antigen using hyperimmune serum. M: protein marker, L1: F1 (partially purified antigen)

Several preparatory SDS- PAGE were run and immuno-reactive cluster of polypeptide 62-66 kDa, 52-55 kDa and 41-43.0 kDa from horse origin antigen were identified and isolated (Fig 18). Out of three immuno-reactive proteins, 66-62kDa abbreviated as F-1 (Fig. 19), initially, was purified in large scale from gels and subsequently used in ELISA and western blot. Hyperimmune serum (HIS) was successfully raised in rabbits against purified fraction (F1) antigen and showed a high antibody titer in ELISA (Fig. 20). Further,

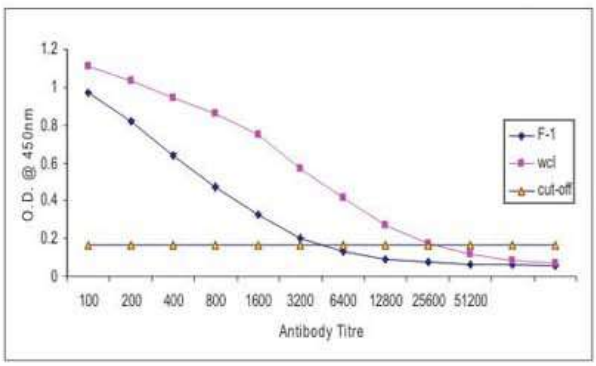


Fig. 20: ELISA titres of hyperimmune serum raised against F-1 & WCL antigen

immunoblot also showed strong reactivity with HIS raised in rabbits confirming the immunogenicity of the isolated protein (Fig. 21).

Fig. 21: Immunodominant antigens F1 (62-66 kDa) of horse isolate of *T. evansi* purified from WCL antigen. M-protein marker, L1-HIB, L2-control

The sensitivity of sonicated (WCL) and purified (F-1) antigen was comparatively evaluated using serum samples of infected equines. Both antigens detected *T. evansi* antibodies in experimentally infected donkeys from 2nd week onward and showed rising trend which reached peak by 5-7 weeks PI, there after it maintained the plateau with high antibody titre till 280 days PI (Fig. 22) Immunoblot studies revealed that all the infected donkeys strongly recognized polypeptide bands from 2nd

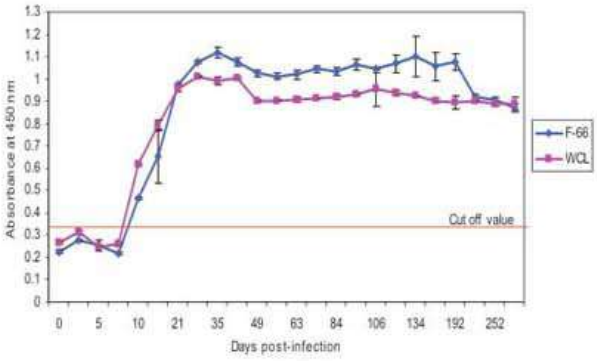


Fig. 22: Comparison of purified and crude *T. evansi* antigens in ELISA using experimentally infected donkey serum samples.

weeks PI onward in the range of 66-55 kDa. Likewise, all horse infected with *T. evansi* also recognized antibodies using this semi-purified protein. This suggests that the purified F-1 protein, which is predominantly expressed in chronic stages of infection, is equally sensitive and specific as WCL antigen in detection of chronic *T. evansi* infection.

(S.C. Yadav, Rajender Kumar and Sanjay Kumar)

Development of monoclonal antibody based assays for detection of *Trypanosoma evansi* infection in equines

The present study was undertaken to develop monoclonal antibodies and recombinant antigen-based assays for diagnosis of *Trypanosoma evansi* infection in equines. Donkey and horse isolates of *T. evansi*-cryopreserved and being maintained in laboratory- were propagated in mice/rats for preparation of antigen. At the peak of peripheral parasitaemia, trypanosomes were purified using DEAE-cellulose column. The whole cell lysate (WCL) antigen was prepared from purified trypanosomes after sonication. SDS-PAGE profile of sonicated antigen revealed major polypeptides in the molecular weights ranging from 26-81 kDa along with several other minor polypeptides. Western blot studies with serum samples from experimentally infected donkeys, naturally infected horse with *T. evansi* and hyperimmune serum raised in rabbits against WCL antigen revealed common immounogenic proteins. These proteins are being purified in large scale for development of screening assay and immunization of donor spleen mice (BALB/c).

(Rajender Kumar, S.C. Yadav, Sanjay Kumar and B.R. Gulati)

Molecular characterization of toll-like receptor 9 in Marwari horse

Toll-like receptors (TLRs) are typical type I transmembrane glycoproteins. Toll-like receptor 9 has been the focus of considerable research attention for the ability to activate innate immune responses, through DNA-based immunotherapeutics. To get mRNA complete codons of equine TLR9, different sets of primers were designed to obtain overlapping fragments of equine TLR9. Amplicons of three different overlapping TLR9 fragments were obtained from Marwari horse. These were cloned into pGEMT vector and sequenced.

BLAST analysis of TLR9 sequences of equines indicates that these are much closer to odd-toed ungulates e.g., *homo sapiens* than to even-toed ungulates (buffalo, cattle). TLRs contain multiple repeats that are protected by special LRR-N terminal end and LRR-C-terminal end motifs. Program SMART was used for comparative prediction of protein domain architectures. It was observed that LRR patterns towards N-terminal are conserved among all species except *Equus caballus* and *Rattus norvegicus*. Comparative prediction of TLR9 protein domain architectures for *Mus musculus, Homo sapiens, Gorilla gorilla, Capra hircus, Ovis aries, Bos indicus, Bos taurus, Bubalus bubalis, Canis familiaris, and Felis catus* confidently predicted three leucine rich repeats (LRRs) in partial TLR9 sequence indicating substantial conservation of TLR9 among animal species except *Equus caballus* and *Rattus norvegicus* (Fig. 23.).

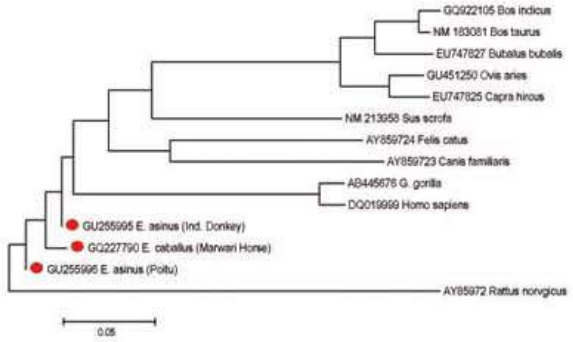


Fig. 23: Phylogenetic analysis of TLR9 of *Equus* spp.

(Anju Manuja, Balvinder Kumar, Hari Singha, Sanjay Kumar and R.K.Singh)

Diversity of *Mx* gene and association of polymorphic markers with susceptibility vis`-a-vis` resistance against Equine Influenza

In order to determine the diversity of Mx gene and its role in resistance / susceptibility to the *Orthomyxovirus* in equines, the partial nucleotide sequences of Mx genes were analyzed for polymorphism. For this purpose, blood samples collected from equine influenza positive and healthy in contact animals were stimulated with different doses of interferon α/β. RT-PCR was performed to amplify partial and full length Mx gene from different animals. A total of 27 partial overlapping fragments of Mx gene from 9 animals were amplified (Fig. 24 & 25). qRT-PCR was performed to assess the changes in levels of expression of *Mx* gene. All the samples exhibited Mx gene expression. Samples induced with 2080 IU of IFN exhibited marked increase in Mx expression (Fig. 26).

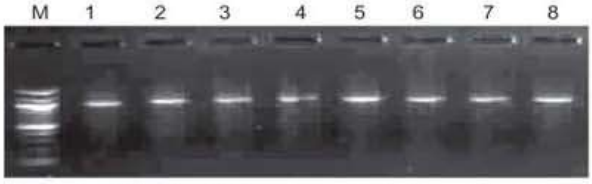


Fig. 24: Amplification of *Mx* gene upon induction with interferon. M: 100 bp ladder, L 1-8: Amplified 983bp products

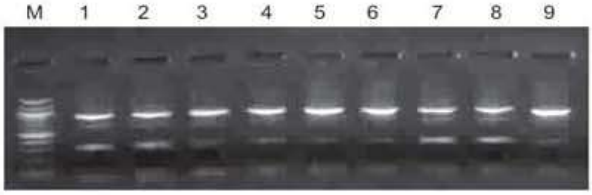


Fig. 25: Amplification of *Mx* gene upon induction with interferon. M: 100 bp ladder, L 1-9: Amplified 861bp products

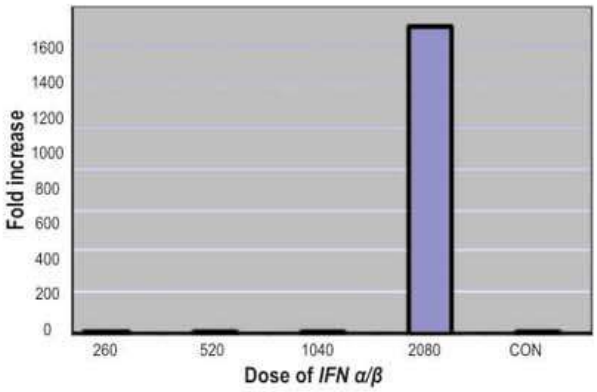


Fig. 26: Quantitative expression of *Mx* gene detected by qRT-PCR upon stimulation with IFN α/β

Sequence and evolutionary analysis of *Mx* gene of Marwari horse

Blast analysis revealed 99% nucleotide sequence homology with *Equus caballus* (ECU55216), 81% with *Homo sapiens* (AB527675) and *Pan troglodytes* (XM531569) and 80% with *Canis lupus familiaris* myxovirus (influenza virus) resistance genes. Amino

acid sequence analysis revealed 99% sequence homology with Thoroughbred horses. Phylogenetic analyses were conducted in MEGA4. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. On phylogenetic analysis the sequence formed separate clade with *Equus caballus* (Thoroughbred) Mx protein homolog (Fig 27).

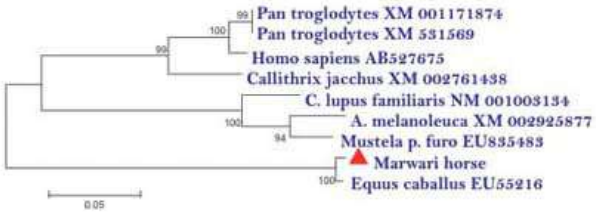


Fig. 27: Phylogenetic tree of Mx gene of Marwari horse

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

Analysis of class I and II genes of Major Histocompatibility Complex in donkeys

Major histocompatibility complex (MHC) provides major genetic component for resistance or susceptibility to infectious/autoimmune diseases and regulates the basic immune response in the animals. For carrying out studies on MHC class I and II genes in donkeys, a total of 175 blood samples (comprising of 147 indigenous donkeys belonging to different parts of Rajasthan and Gujarat states and 28 Poitu donkeys of EPC farm) were collected. The DNA was extracted from the blood samples using the standard method. The PCR conditions for amplification of MHC-DRB3 and DRB2 loci were optimized and respective fragments of 309 bp and 276 bp were successfully amplified (Fig 28 & 29) using published primers. These amplicons will be used for RFLP to ascertain polymorphism at MHC loci in donkeys.

Fig. 28: MHC-DRB3 gene in indigenous donkeys

Fig. 29: MHC-DRB2 gene in indigenous donkeys

(R. C. Sharma, Balvinder Kumar and A. K. Gupta)

Seromonitoring of existing and emerging diseases of equines

Seromonitoring of equine diseases is important to ascertain the prevalence of diseases in different geographical regions of the country with the aim of prevention, control and eradication of equine diseases. During the year, sero-survey was conducted in various states / UTs of India, *viz*., Maharashtra, Rajasthan, Chandigarh, Delhi, Haryana, Punjab, Tamil Nadu, Uttar Pradesh, Karnataka, Andhra Pradesh, Uttarakhand, Madhya Pradesh, Gujarat, Chhattisgarh, Manipur, Himachal Pradesh, and West Bengal. A total of 1768 serum samples from equines were screened for various diseases. Serum samples (1768) tested for EIA, Brucellosis and *Salmonella* Abortusequi (H antigen) revealed no positive result while11 out of 1768 serum samples tested for glanders were found positive. Further, 356 out of 1634 serum samples (21.78%) tested for equine influenza were found positive. 86 out of 1764 serum samples (4.87%) tested for equine herpes virus-1 (EHV-1) were found positive. 331 out of 912 serum samples (36.29%) tested for *Theileria equi* were found positive, 56 out of 1762 serum samples (3.17%) tested for JE were found positive and 145 out of 1762 serum samples (8.22%) tested for *T. evansi* were found positive (Table 3).

For EIA, 6589 serum samples from Thoroughbred as well as indigenous equines were examined by Coggin's test under S&M, diseases investigation and contractual service. A total of 11909 serum samples were tested for glanders in 2010-11 which included S&M (1768), disease investigation (944) and contractual service (9197), out of which 7 serum samples from U.P. and 4 serum samples from H.P. were positive for glanders. The

major achievement was control of glanders outbreak in states of H.P. and U.P. by relentless follow up action for its control. Outbreaks of equine influenza were reported from several states of the country during previous years. Follow up action continued in affected states. 550 serum samples tested for equine influenza out of 3513 serum samples were found positive, however, none of the samples tested in pair showed rise in titres. Nasal swabs from 56 animals suspected for EI were also tested employing RT-PCR but none of the samples were found positive. Testing of 154 samples under disease investigation revealed 22 samples to be positive for *T. evansi* infection.

Similarly, 50 samples out of 291 tested for EHV-1 under DI/ contractual service were found positive but none of 89 samples tested for EVA were found positive. Bacteriological analysis done on 209 samples, originating from Rajasthan, Haryana, UP, HP, Punjab, Gujarat, Karnataka and Tamil Nadu including nasal swabs, vaginal/cervical/uterine swabs, ocular swab, whole blood, pus, tissues from PM, lesion swab, faecal sample, aborted foetii and contents and soil sample including two cultures from CMVL,Meerut for confirmation yielded 61 isolates including *Burkholderia mallei* (4), *Rhodococcus equi* (6), *Streptococcus equi* subsp. *zooepidemicus* (33), *Streptococcus equi* subsp. *equi* (4), *Staphylococcus* sp.(1), *E. coli* (13) (Table 4). 21 nasal swab samples of camel origin were also tested where no bacteria of pathogenic significance was isolated. 526 samples from Delhi and Chennai animal

Table 3: Seroprevalence of various diseases under S & M among indigenous equines

Sl. No.	State	EIA	Brucellosis	*S.* Abortusequi	Glanders	EI	EHV-1	*T. equi*	JE	*T. evansi*
1.	Chhatisgarh	0/330	0/330	0/330	0/330	105/248	20/326	14/82	9/326	30/330
2.	Manipur	0/28	0/28	0/28	0/28	0/13	0/28	-	0/28	2/28
3.	Uttar Pradesh	0/135	0/135	0/135	7/135	36/98	4/135	20/85	2/135	3/135
4.	Rajasthan	0/560	0/560	0/560	0/560	128/560	30/560	67/213	22/558	33/554
5.	Gujarat	0/313	0/313	0/313	0/313	35/313	0/313	188/313	6/313	4/313
6.	Haryana	0/27	0/27	0/27	0/27	13/27	0/27	12/27	9/27	2/27
7.	H.P.	0/76	0/76	0/76	4/76	0/76	4/76	-	0/76	14/76
8.	U.K.	0/299	0/299	0/299	0/299	39/299	28/299	30/188	8/299	57/299
	Total	**0/1768**	**0/1768**	**0/1768**	**11/1768**	**356/1634 (21.78%)**	**86/1764 (4.87%)**	**331/912 (36.29%)**	**56/1762 (3.17%)**	**145/1762 (8.22%)**

Table 4: Details of isolates recovered

Isolate	No. of isolates	Originated from (Sample)	Originated from (State)
Streptococcus equi subsp *equi*	4	Nasal Swab (2), Culture for confirmation (2)	Gujarat (1), Rajasthan (1), Uttar Pradesh (2)
Streptococcus equi subsp *zooepidemicus*	33	P M Tissue (4), Nasal Swab (22), Pus (2), Reproductive tract Swab (1), Aborted foetus (1), Lesion Swab (3)	Gujarat (12), Uttar Pradesh (7), Himachal Pradesh (7), Haryana (3), Rajasthan (4)
Escherichia coli	13	P M Tissue (1), Reproductive tract Swab (5), Aborted foetus (6), Faecal sample (1)	Haryana (11), Rajasthan (2)
Burkholderia mallei	4	Nasal Swab (4)	Himachal Pradesh (1), Uttar Pradesh (3)
Rhodococcus equi	6	Nasal Swab (6)	Haryana (6)
Staphylococcus species	1	Nasal Swab (1)	Punjab (1)
Total	61*		

*Including 2 *S equi* isolates received from CMVL, Meerut (UP) confirmed by the laboratory

quarantine centres including 365 vaginal swabs and 161 preputial swabs tested for CEM were negative. Antibiotic sensitivity testing of clinical samples was also done and results were conveyed to various concerned quarters. Seroprevalence of EHV-1 during the years 2002-03 to 2010-11 revealed that the prevalence rates hovered in between 2.05 to 7.2% (Fig. 30). Seroprevalence of *Theileria equi* from 2002-03 to 2010-11 revealed that the prevalence rates hovered around 20% (Fig. 31).

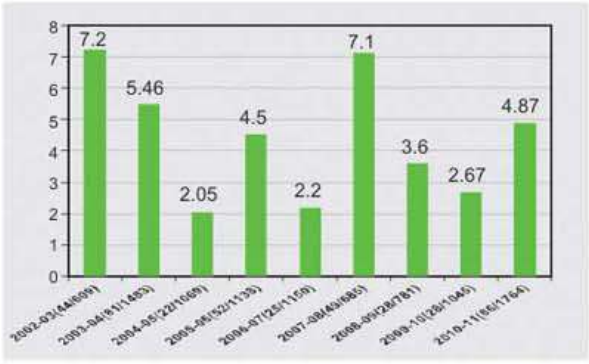


Fig. 30: Seroprevalence of EHV-1 among indigenous equines

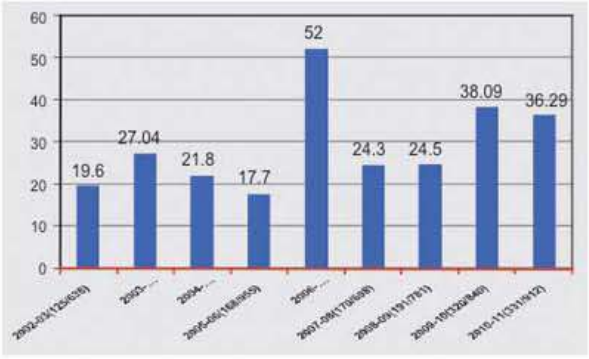


Fig. 31: Seroprevalence of *Theileria equi* among indigenous equines

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

Genotypic characterization of Indian equine breeds

Population of true-to-breed indigenous equines have decreased drastically during the last few decades mainly due to indiscriminate breeding and their decreased use in routine activities. Some of the breeds *viz.*, Spiti, Bhutia, Zanskari and Manipuri have come under the category of endangered species as their population is less than 10,000 animals per breed. It is important to conserve these breeds by adopting proper breeding and managemental tools. Genetic characterization is an important part of any breeding program and therefore, an effort was made to study genetic diversity among different equine breeds available in India. These studies were carried out using 55 pairs of polymorphic microsats (approved by FAO) with more than 280 DNA samples of true-to-breed animals of six indigenous equine breeds along with Thoroughbred horses. Samples were collected from different locations in home-tract of each breed. Nine different multiplex PCR, were carried out using fluorescent labeled 55 microsat primers. Multiplexing was done in such a way that PCR products of same size had different labels so that there was no difficulty in assessing their size. The electropherograms drawn through GeneScan program were used to extract DNA fragment sizing details using Gene Mapper software (version 3.0) (Applied Biosystems). The size, number, and frequency of alleles were used for further analysis. Heterozygosity analysis with 48 different polymorphic microsats indicated the presence of genetic diversity within and between different breeds. Topology of Indian horses was prepared on the basis of genetic distances estimated on allele sharing basis (Fig. 32). The Neighbor joining algorithm was used for the construction of both the topology as well as phylogenetic tree.

The Thoroughbred horses expectedly clustered separately in topology as well as phylogenetic tree (Fig 33). Other Indian breeds clustered into two distinctive

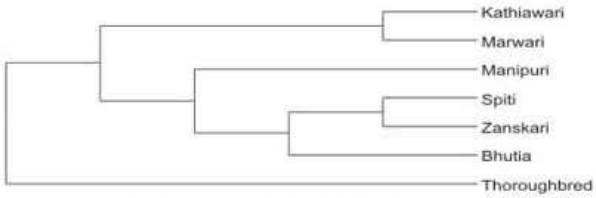


Fig. 32: Topology of Indian breeds

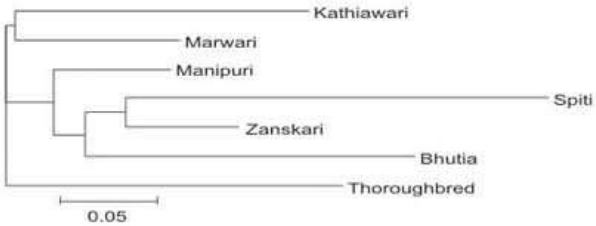


Fig. 33: Phylogenetic tree of equine breeds

classes. One cluster grouped Kathiawari and Marwari horses while the other cluster had Manipuri, Spiti, Zanskari and Bhutia ponies. It can be inferred from the study that the geographically distant breeds are also genetically distant.

The correspondence analysis (multivariate analysis) was carried out wherein each spot represented one individual and different populations were given different colors (Fig. 34). The contribution of the Axis 1, 2 and 3 to the total variation was 25.07%, 22.08% and 16.34%, to the total population, respectively which helped in differentiating between Bhutia (Extreme left) and Thoroughbreds (pink colored). Rest of the population was as per the genetic distance measures.

Recent bottleneck in the population *i.e.* within past few dozen generations was examined by a graphical method analyzing distortion of allele frequency distribution which

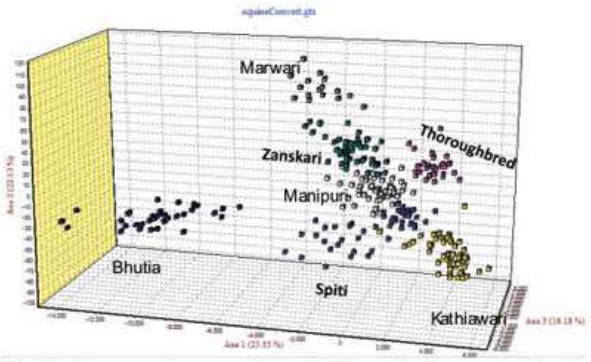


Fig. 34: Correspondence analysis of different Indian equine breeds

plots groups of alleles from a sample of many polymorphic loci into each of the ten frequency classes. All the seven breeds show normal "L" shaped curve reflecting no bottleneck has occurred in the recent past.

(A.K. Gupta and A. Bhardwaj)

Phenotypic characterization of donkey population from Rajasthan state

Biometric indices of 97 adult, healthy, male donkeys were recorded for phenotypic characterization of local donkeys from Rajasthan state. Average age of donkeys was 5.76±2.12 years. It was observed that 70% of the randomly selected donkeys had grey colour and remaining animals were of white colour. Body length, height at withers, heart girth, fore leg length, knee height, canon length, hind leg length, height at hock, face length, face width, ear length, ear width, pole, hoof length and width were 97.30±5.05, 97.62±4.93, 101.54±5.69, 66.83±3.66, 31.54±2.36, 17.99±1.55, 71.85±4.84, 40.10±2.41, 47.16±3.35, 14.67±1.39, 21.82±1.63, 18.29±1.12, 10.23±0.81, 6.25±0.70 and 6.51± 0.80 cm, respectively. This is an initiative to generate baseline data on different donkey populations in India.

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

Cryopreservation of equid semen using amides

Glycerol is the primary cryoprotectant used for freezing semen, however, it has toxic effects on spermatozoa along with contraceptive effects in the mare. As spermatozoa cannot survive freezing without a cryoprotectant, there is a need to evaluate other cryoprotectants that might be less toxic than glycerol to stallion spermatozoa. With this aim, we evaluated amides as an alternative to glycerol for use as cryoprotectant.

The basic semen characteristics were studied in three Zanskari stallions (37 ejaculates), four Exotic Jacks (23 ejaculates) and in five Marwari Stallions (68 ejaculates). The semen was collected using artificial vagina upon filtrated through gauge filter to remove gel fractions and centrifuged with primary extender (Citrate EDTA). The semen was re-suspended in secondary (Lactose 11%) extender containing cryoprotectants *viz.* Glycerol, Methyl Formamide (MF), Dimethyl Formamide (DMF) and Dimethyl Sulfoxide (DMSO) @ 2% to the total volume. Thereafter the diluted semen was kept in the semen cooling cabinet for equilibration cum cooling period of 2 hrs followed by cryopreservation and storage in LN_2 (-196° C).

The cryoprotectant Methyl Formamide did not work as an effective cryoprotectant as pre-freeze and post-thaw motility were very low (below 10%). The other two amide based cryoprotectants *viz.*, Dimethyl Formamide and Dimethyl sulfoxide yielded good post thaw motility ($\geqslant$ 35%) and viability ($\geqslant$ 50%) in frozen semen (Table 5). No statistically significant difference was observed in

Table 5: Sperm motility and livability in pre-freeze and frozen semen of three equine breeds with different cryoprotectants.

BREEDS	PRE-FREEZE MOTILITY (%)			POST- THAW MOTILITY (%)			PRE-FREEZE LIVABILITY (%)			POST-THAW LIVABILITY (%)		
	GLY	DMSO	DMF	GLY	DMSO	DMF	GLY	DMSO	DMF	GLY	DMSO	DMF
Jacks(n=4, N=23)	71.03± 1.95	71.48± 1.83	74.43± 1.62	35.87± 0.77	31.45± 1.39	38.43± 1.26	72.74± 1.31	70.64± 1.55	73.67± 1.69	52.89± 2.69	51.18± 3.00	56.14± 2.53
Zanskari(n=3, N=37)	69.64± 1.52	64.53± 2.53	60.11± 4.51	36.04± 2.12	25.73± 2.69	37.61± 1.35	65.01± 2.77	61.65± 3.49	64.39± 3.80	49.14± 2.55	33.10± 3.14	43.94± 2.84
Marwari(n=5, N= 68)	66.84± 1.29	66.25± 1.43	67.56± 1.28	35.28± 6.30	35.28± 5.91	35.42± 6.76	68.43± 1.22	67.17± 1.53	71.02± 1.55	46.55± 1.52	45.38± 2.31	48.08± 2.02

(n= Number of animals used; N= Total number of collections) (GLY=Glycerol, DMSO=Dimethyl Sulfoxide, DMF= Dimethyl Formamide)

sperm motility and sperm livability with the use of three different cryoprotectants i.e Glycerol, Dimethyl Formamide and Dimethyl sulfoxide in both prefreeze semen and frozen semen of Marwari stallions, Jack stallions and Zanskari stallions semen. DNA intactness was studied using Acridine orange stain and it was found that 98% samples had intact DNA in all groups.

(A. Arangasamy, Thirumala Rao Talluri and Sanjay Kumar Ravi)

Cryopreservation of embryos for conservation of Marwari horses

Embryo transfer is a valuable tool to improve reproductive efficiency and genetic upliftment. We have initiated embryo transfer technology in Marwari mares with an aim to preserve and conserve the true-to-breed Marwari germplasm by cryopreserving the embryos.

In present study, non surgical collection of embryos was carried out from 17 mares subsequent to synchronization of estrus which was done by inculcation of 2 ml (10 mg) of Lutalyse ($PGF_2\alpha$) I/m. Out of 17 animals, 14 responded to the treatment and came to estrus. For embryo transfer programme, seven mares were kept as donor mares and six mares were kept as recipient. Flushing of the donor mares was carried at 6th day of AI. One embryo was recovered out of total seven flushings (day 6th post ovulation) (Fig.35). The embryo was immediately transferred to the synchronized recipient mare. The recipient mare was given Progesterone (250 mg) after 24 and 48 hours of transfer. The pregnancy verification was conducted on 20th day of post ovulation, however, it was not successful. Further studies are in progress to standardize embryo transfer technology.

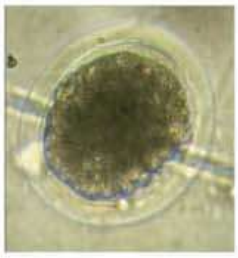


Fig. 35: Embryo recovered on day 6th post-ovulation

(A. Arangasamy, Thirumala Rao Talluri, Yash Pal and S.K. Khurana)

Development of intravaginal device for estrus control in mares

The estrous cycle of the mare is characterized by a long and variable follicular phase and time of ovulation. Synchronization of ovulation may be useful for batch insemination of mares or for preparation of potential recipient mares in an embryo transfer regime. The aim of this study was to evaluate cost-effective intravaginal device containing slow release progesterone as an estrus synchronizer in Marwari mares.

Progesterone impregnated (Medicated) and non-medicated (sponges without progesterone) intravaginal sponges along with sponge applicator were prepared at CSWRI, Avikanagar and sent to EPC/NRCE, Bikaner for their use in the mares. In the first trial, all the six mares (100%) retained the non-medicated intravaginal sponges for a period of six days successfully. In the second trial, out of six mares inserted with non-

medicated intravaginal sponges, four (66.66%) could retain it successfully till the day of their removal (day 12). Similar trial was conducted with medicated (progesterone impregnated) sponges in six mares for a period of 13 days. Out of six mares, four (66.66%) mares could retain sponges successfully till the day of removal (day13) while two mares failed to retain it. In four mares that could retain the sponges, 2 ml (10 mg) of prostaglandin was administered intramuscularly on day 13th (day of sponge removal) of insertion. Three (75%) out of four mares exhibited estrus on 3rd day after removal of medicated intravaginal sponges. All three mares were inseminated on 5th and 6th day of estrus. Upon rectal examination after 20 days of insemination, pregnancy was found to be established in two out of three (66.66%) mares.

(S.M.K. Naqui, S. Sejian, Thirumala Rao Talluri and S.K. Ravi)

Work stress studies on equines using rotary mode systems

Donkeys were trained to work in "Rotary Mode" unit. Initially, problems related to harness was faced and we used GI pipe for pulling iron rod but it was creating problem in moving of the animal. Then cotton rope was used in place of GI pipe, but it was also giving some rubbing effect. Cotton rope wrapped with cotton strip gave encouraging results. In the trials, single donkey was able to pull the shaft of the Rotary Mode (Fig. 36), when the battery (two batteries of 12 volt each attached parallel) was placed at a distance from the alternator (24 volt), however, animal faced difficulty in pulling it, when batteries are placed closer to alternator. The current was flowing from alternator, but batteries were not getting charged. The observations were discussed in XII workshop of "AICRP on increased utilization of animal energy with enhanced system efficiency", at University of Agricultural Sciences, Raichur during February 4-6, 2011. It was decided to use the rotary mode unit for other operations *viz*. chaff cutting, wheat flour grinding and winnowing, among others.

Fig. 36: Donkeys working in rotary mode unit

Draughtability studies with work-rest cycle for equines under arid conditions

Continuous work

Three exotic female donkeys were used in carting with 6, 8, and 10 quintals of load under continuous work for three hours. The average body weight of female donkeys was 345 kg. They were offered 2 kg concentrate mixture (Oat: Gram: Wheat bran) daily, *ad lib* dry fodder and 10 kg green fodder. Changes in physiological indices during carting load of 6 Q and 8 Q are presented (Table 6 & 7). Physical changes (frothing, profuse sweating, watery discharge from nostrils and eyes, pause in between work after 2 hour work) were also observed during 6 and 8 Q load. No specific change in haematological values (Hb, PCV, TEC, TLC) after work were observed during 6 and 8 Q load. However, under 10 Q load, the animals showed total fatigue in 1 hour of work with accompanying physical changes (frothing, in-coordination of legs, profuse sweating, watery discharge from nostrils and eyes, pause in between work) appearing in a very short time of 1 hour.

Table 6: Changes in physiological responses during carting load of 6 Q

Parameters/time	Control	1 h Work	2 h Work	3 h Work	1 h Rest
PR (per min)	37.00±3.03	71.67±7.72	83.33±6.61	88.50±5.39	42.00±3.50
RR (per min)	34.50±2.49	57.67±5.33	64.67±4.91	67.67±3.23	40.±2.35
RT (°C)	99.80±0.25	101.8±0.47	102.6±0.38	102.76±0.51	100.4±0.30

Table 7: Changes in physiological responses during carting load of 8 Q

Parameters/time	Control	1 h Work	2 h Work	3 h Work	1 h Rest
PR (per min)	39.60±0.96	72.00±2.91	78.60±4.23	84.80±4.77	43.00±2.80
RR (per min)	36.0±0.86	56.80±3.77	63.6-±4.35	67.20±3.30	38.00±3.52
RT (°C)	100.20±0.27	101.88±0.56	102.2±0.52	102.36±0.64	100.6±0.35

Work-rest-work cycle

Two exotic female donkeys were used in carting with 6, 8 and 10 quintals of load under work-rest cycle (1hW-1hR-1hW-1hR-1hW) (Fig. 37). Physical changes (frothing, profuse sweating, watery discharge from nostrils and pause in between work) were more prominent during 10Q load rather than 6 & 8 Q load (Fig. 38). Physiological indices increased with all the three loads but no changes in haematological indices were recorded.

Fig. 37: Donkey during carting experiment

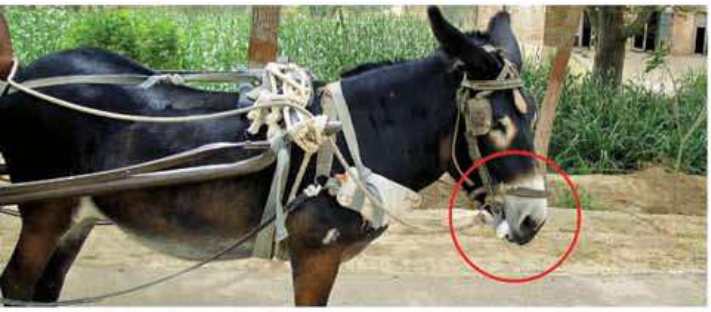


Fig. 38: Frothing during carting in donkey

Survey of draught equine population and their utilization in agricultural operations and transport

Under the project, a survey to study indigenous technical know-how and socio-economic status of equine owners and feeding, housing, health care and general management of equines Nainital (Uttarakhand) and UP was done.

Survey of horse owners in Nainital

About 150 horse owners in Nainital (Uttarakhand) provide horses to tourists for sightseeing. Most of the horse owners belong to minority community and every owner had a castrated horse. The horse owners, owing to the uncertainty of livelihood in this trade, were not optimistic of their profession, handed down to them as a legacy. Migrants from UP about 30-40 years back, they are progressive in educating their children. Horses were fed with rice polish (5 kg), wheat bran (3 kg), gram (1 kg) and locally available green fodder (3-4 kg) per day to each horse. Each horse generally had to perform 2-3 rounds of 10 Km each per day. The working hours are from morning to evening or until the tourists are available there. Government has constructed *pucca* sheds for animals to provide accommodation for horse owners. They reported that they are not getting any veterinary facility. The condition of the horses varied between fair to good without any injury, lameness or gall formation. The saddle was of good quality. Regular shoeing was being done @Rs 100 per shoeing at weekly interval. One farrier was there to perform 8-10 shoeing daily. Deworming was done 2 to 3 times a year. A few of the horse owners were providing digestive *masala* to their horses. The horses are purchased from animal fairs held in UP. Castration, to make animal docile and suitable for tourist operations, was performed in plains after which a rest of 15-20 days was given to animal.

Horses/ponies used for ferrying passengers and school going children

In plains of Uttarakhand and parts of UP, pony/horse

Fig. 39: Horse cart carrying people

Fig. 40: Horse cart carrying school-going children

carts are in use to carry passengers from village to cities or nearest bus stops and vice-versa. The animals carry 10-15 passengers in a pneumatic tyre cart (Fig. 39) and these carts are also used for carrying children to schools (Fig. 40). Horse cover about 30-40 km per day. The owners start in the morning at 7-8 AM and continue till evening, earning nearly ₹ 400–500/ day with an expenditure of ₹ 100-150/animal/day. For feed, horses are offered about 2 kg horse gram, 2 kg *choker* and *ad lib* wheat *bhusa* per day, and left for grazing after work. Ordinary salt (20-30 gm) is given daily to the horse. As the cart is to be pulled both on *pucca* and *kuchcha* road, horses are shoed regularly.

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

Development and evaluation of donkey drawn agricultural implements suitable to arid region of Rajasthan

Use of exotic donkeys in ploughing

In India, various animal species *viz.*, bullocks, he-buffaloes, camels, yaks, donkeys and mules are used for draught purpose. Donkey is mainly used as a pack animal and for carting. Donkeys are capable of exerting a draught equivalent to 24-32% of their body weight, which is much higher than that of cattle, buffalo, and camel. Four male donkeys reared on standard feeding and

Fig. 41: Matching plough for single donkey

Fig. 42: Donkey being used in ploughing operation

management were trained and used in ploughing operation. They were offered 10 kg sorghum as green fodder, *ad lib* sewan as dry roughage, and 2 kg concentrate mixture (Oat: Gram: Wheat bran) daily. The average body weight of donkeys was 298 kg. A single animal drawn matching plough was designed (Fig. 41) and male and female adult donkeys were trained for ploughing operation (Fig. 42). The whole operation was planned as 3 ploughing sessions of 1.5 h. each with an hourly rest in between. Changes in pulse rate (PR), respiration rate (RR) and rectal temperature (RT) were recorded (Table 8). Average land ploughed was 0.171 acre per hour by the donkey. Donkeys were able to plough 0.514 acre in two sessions at an average speed of 2.60 kmph.

Table 8. Changes in physiological responses

Parameters/time	Control	1.5 h Work	1 h Rest	1.5 h Work
PR (per min)	42.16±1.97	84.5±2.66	45.5±0.93	86.33±4.38
RR (per min)	36.33±1.22	62.0±1.44	42.66±2.36	62.67±2.39
RT (°C)	99.76±0.31	101.76±0.22	100.2±0.10	101.9±0.15

Use of male exotic donkeys in sowing

The exotic donkeys were employed in bajra (pearl milet) sowing operation to evaluate their efficiency in this aspect of farm operations. Three donkeys of 300 kgs average weight, reared on standard feeding and management were trained and used in sowing operation. Single animal drawn matching plough with two furrows was designed (Fig. 43) and male adult donkeys were trained for 2 hours continuous work regimen (Fig. 44). Changes in pulse rate (PR), respiration rate (RR) and rectal temperature (RT) were recorded (Table 9). In 2 hours, 1 donkey sowed 0.662 acre at an average speed of 2.635 kmph. The donkey attained almost normal physiological levels after one hour of rest. Slight increase in Hb, PCV, TEC and TLC values was observed during sowing.

Fig. 43: Two furrow seed drill developed for donkeys

Fig. 44: Sowing operation using exotic donkey

Table 9. Physiological responses during sowing operation

Parameters/time	Control	1 h Work	2 h Work	1 h Rest
PR (per min)	35.33±2.86	77.0±6.28	86.0±8.6	40.33±3.34
RR (per min)	34.33±2.86	59.67±2.67	71.0±5.52	38.33±3.34
RT (°C)	99.86±0.31	102.67±0.22	102.1±0.10	100.3±0.15

Use of indigenous donkey in ploughing operation

Indigenous donkey is small-sized and is mainly used as pack animal or for carting. They are capable of exerting a draft equivalent to 24-32% of their body weight. Keeping in view, single animal drawn matching plough was designed and male adult donkeys were trained for ploughing operation. Four donkeys reared on standard feeding and management were trained and used in ploughing (Fig. 45 & 46). Average body weight of local donkeys was 112 kg. Donkeys were employed in 4 sessions of 2 hours each with a two hourly rest in

Fig. 45: Ploughing by local donkey

Fig. 46: Use of Mule in plouging

between each session. Changes in pulse rate (PR), respiration rate (RR) and rectal temperature (RT) are depicted (Table 10). Donkeys were able to plough an average of 0.09 acre/hour and total land ploughed was 0.362±0.018 acre at an average speed of 1.835 kmph.

Table 10: Changes in physiological responses

Parameters/time	Control	2 h Work	2 h Rest	2 h Work
PR (per min)	32.14±5.28	57.28±2.31	48.14±2.20	60.71±2.52
RR (per min)	20.28±3.17	40.43±3.84	32.57±2.23	43.71±2.46
RT (°C)	97.8±0.33	100.74±0.25	100.17±0.45	100.9±0.21

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

Management systems and utilization of donkeys and mules for sustainable livelihood

Horses, ponies, mules and donkeys are reared in almost all parts of India, especially, in hilly and difficult terrains. During the last fifty years, the equine population declined at alarming rate except mule which has increased in spite of mechanization. Further, some traits of donkeys like low-pricing, low-cost maintenance, easy to train, resistance to some diseases and undemanding feeding habits make them animal of choice. Donkeys seem to be less affected by some of the common disease that debilitate or kill other working animals, notably trypanosomosis and African horse sickness. Since mules and donkeys are mainly reared by resource poor/deprived community, their welfare is often neglected. The new knowledge on equine husbandry is not reaching to resource-poor farmers especially on Complex, Diverse and Risk-prone areas (CDR). As such, pilot studies had been conducted regarding existing management systems and utilization of donkeys and mules.

The equine populated states Rajasthan, Haryana, Uttar Pradesh and Uttrakhand were selected for the present study. The survey was conducted and data was collected from 38 respondents from Sardarshar and Churu in Rajasthan, 33 respondents in Haldwani, Uttrakhand, 18 respondents from Rewari and Mundal in Haryana and 34 respondents from Jamapur, Nagina, Chandpur in Bijnor from Uttar Pradesh. Majority of respondents were from middle age group (i.e.36-50 years), from minority community, mostly illiterate having medium family size (7-10 members) with medium experience of equine husbandry and monthly income between ₹ 3000 to 5000 (Fig. 47). Regarding management practices, majority of the respondents were cleaning their animals twice a week, shoeing was regularly done except in donkeys, whereas grooming and deworming was done occasionally (Fig. 48).

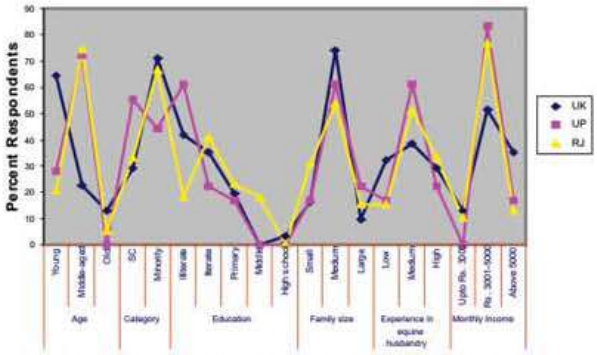


Fig. 47: Comparative socio-economic profile

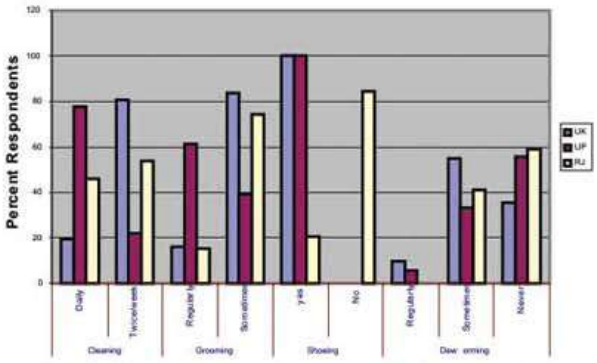


Fig. 48: Management practices followed by respondents

In Haldwani, the Mules were used by respondents in carts (80.65%) and also as pack animals (19.35%) as a source of livelihood. The animals were mainly used for transportation of sand and gravel from Gola river. In Haldwani, the small animals were used as pack whereas sturdy animals were used in cart transportation by equine owners. In Churu and Sardarshahar in Rajasthan, the ownership of donkeys was found high

Table 11. Utilization, feeding and housing pattern of equines

Parameter Utilization of equines	Uttarakhand	Uttar Pradesh	Rajasthan
Use of Cart	Transport of gravel, cement and sand	Transporti of bricks, goods and people	Transport of grains, vegetables, milk and goods from market
Load	4-6 quintal/cart/trip	250-350 bricks/cart/trip	2-3 quintal/cart/trip
Work timings	7-12 AM	8AM-1PM	8 AM-6 PM (2 hrs break at noon)
Earnings	₹ 100-150/trip depending on distance	₹ 150 for 1000 bricks	₹ 100-150/trip or ₹ 15-25 /quintal depending on goods and distance
No of trips	4-6 trips/day	5-7 trips/day	3-8 trips/day depending on distance
Earnings/day/cart	₹ 350-700	₹ 250-600	₹ 200-350
Feeding and housing pattern of equines			
Dry Fodder	Rice/wheat straw, kutti (5-7kg)	Rice/wheat straw (5-6kg)	wheat bhusa, gram bhusa (4-5kg)
Green	Grass/berseem (2-4kg)	Grazing	Groundnut leaves (2-3 kg)
Concentrate	Chokar (1Kg)/Gram (1 kg)	Chokar (1Kg)	Crushed Bajra/barley/gram (1-1.5 kg)
Supplements	Jaggery occasionally and oil in winter	Churna, masala, Jaggery occasionally and oil in winter	Jaggery and oil in winter
Housing	Kaccha with tin roof or bricks walls with tin roof	Kaccha with thatched roof	Kaccha with thatched roof

Fig. 49: Use of Equines in transportation

(84.62%) as compared to mules (20.51%). All the respondents were utilizing their equines for carts and were used in transporting goods and other materials (Fig. 49). In Bijnor and Nagina in Uttar Pradesh, the Mules were used by respondents in carts for transportation of bricks at brick kilns as a main source of livelihood. Data on various aspects are depicted in Table 11.

(Rajender Kumar, A. A. Raut, Yash Pal and R. A. Legha)

New Initiatives

Comparative study on seminal plasma protein profiles in three different breeds of equines

Eight semen ejaculates were obtained from Marwari stallions (2), Zanskari (2) and two Exotic Jacks (*Martina franca*). Total protein from the seminal plasma was estimated by Lowry Method and the molecular weight was analysed by SDS-PAGE. The total protein concentration was found to be 0.71±0.02, 2.24±0.22 and 2.21±0.05 gm/dl in Marwari, Zanskari and Exotic Jack seminal plasma, respectively. The differences in protein concentration were found to be significantly higher ($P<0.01$) in Zanskari and jack stallions as compared to Marwari stallion. There was no significant difference in total protein values between Zanskari and jack stallions. A total of twelve proteins bands were observed in the seminal plasma of both Marwari and Zanskari stallions where as, fifteen proteins were observed in the seminal plasma of Exotic jacks (Fig-50).

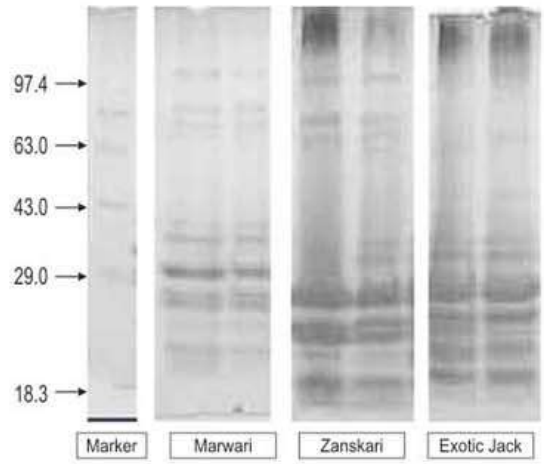


Fig.50: Comparative Protein Profile of Marwari, Zanskari stallions and Exotic Jack Stallions

The seminal plasma proteins in Marwari and Zanskari stallions ranged from 11.45 kDa to 130.23 kDa and that of Exotic Jack ranged between 11.45 to 162.83 kDa. There were three unique proteins in Jack stallion with molecular weight of 36.52, 39.25 and 1602.83 kDa. It can be concluded that there are at least 12 proteins common between all the three breeds of equines and that seminal plasma protein profile of jack stallion is different from Marwari and Zanskari stallions.

(Thirumala Rao Talluri, Gorakh Mal and Sanjay Kumar Ravi)

Evaluation of fresh and frozen semen from different breeds of equines

A study was conducted to establish the correlation between the supravital staining, progressive motility and functional integrity of spermatozoa in fresh and frozen-thawed semen samples of Marwari, Zanskari and exotic Jack stallions. In the fresh semen samples of Marwari stallions (0.16) and exotic Jacks (0.18), positive correlation was observed between progressive motility and HOS-reacted sperms. A positive correlation was also noted between the post-thaw motility and HOS-reacted sperms in frozen-thawed semen of all the three stallion breeds. Further a significant correlation was observed between sperm liveability and HOS positive sperms in both fresh and frozen thawed semen of Marwari (0.53 & 0.37), Zanskari (0.36 & 0.48) and exotic Jacks (0.35 & 0.30) (Fig. 51a & b).

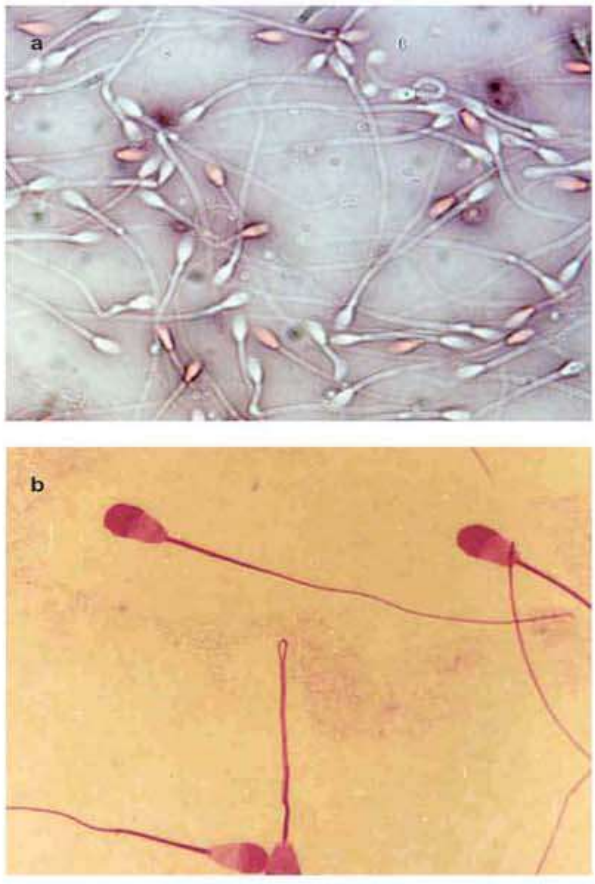


Fig. 51: (A) Live and Dead Sperms of Zanskari Stallion.
(B) Sperm Acrosomal Integrity of Zanskari stallion

(Thirumala Rao Talluri and Sanjay Kumar Ravi)

Isolation of stem cells from equines

Mesenchymal stromal cells (MSCs) are promising subpopulation of adult stem cells for cell-based regenerative therapies in veterinary medicine, especially for the treatment of equine orthopedic diseases, with particular attention to ligament and tendon injuries. There is an increasing interest in application of MSCs isolated from various extra-embryonic neonatal tissues, since their collection is safe, ethical and easy. Extra-embryonic tissues like umbilical cord tissue, umbilical cord blood, amniotic membranes are known to be good source of MSCs in human beings. We initiated work on isolation and characterization of MSCs from equines. The techniques for isolation of MSCs from equine umbilical cord matrix, umbilical cord blood, amniotic fluid and amniotic membranes were perfected (Fig. 52 & 53). In addition, the histomorphy and histology of the equine umbilical cord was studied in Thoroughbred mares (n=6). Histo-architecture of umbilical artery and vein varied at different sites in relation to thickness and constituents of tunics (intima, media and adventitia). Wharton's jelly constituted by different cell types presented structural variations studied at the levels of perivascular, intervascular and subamnion regions. MSCs were isolated from the foal end of the UC tissues (n=4), with classical morphology of adherent fibroblastoid spindle shaped cells. The present findings may help in identifying the most suitable site of the UC for isolation of mesenchymal stem cells in equines.

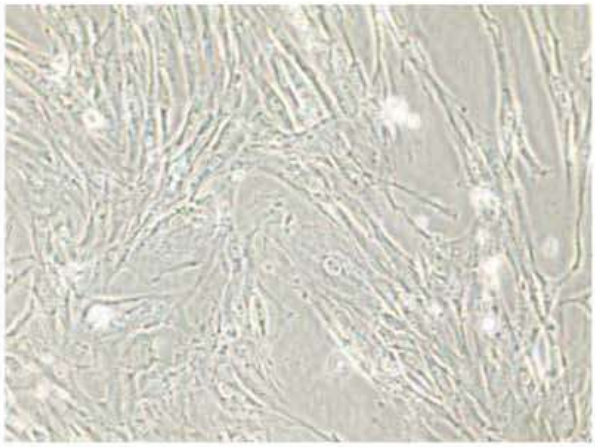


Fig. 52: MSC isolated from umbilical cord matrix at passage 9 level

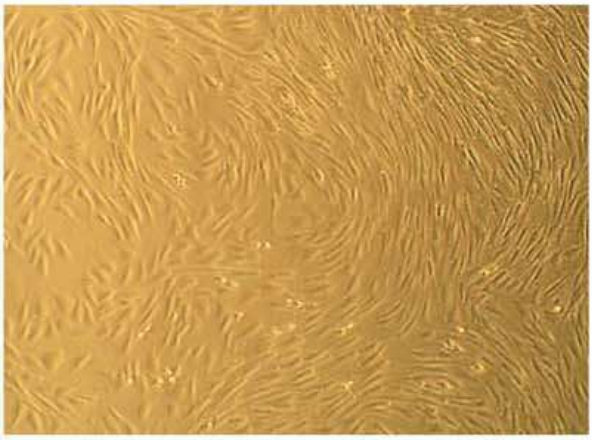


Fig. 53: MSC isolated from umbilical cord blood at passage 3 level

(B.R. Gulati and T. Anand)

VTCC Research Achievements

Investigation of outbreak of Buffalopox Virus (BPXV)

A buffalopox outbreak involving approx. 25 buffaloes, 20 cattle and 15 humans was attended in Baatnor village, Meerut, U.P. in February-March, 2010. The pox lesions in affected buffaloes and cattle were primarily confined to the udder and teats (Fig. 54 & 55). Characteristic

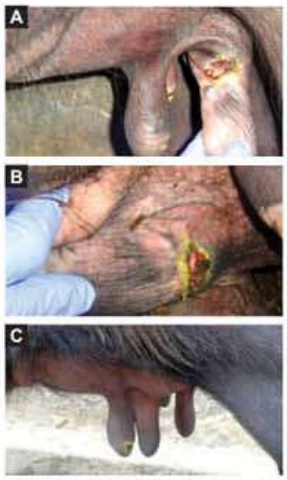


Fig. 54: Pock-like lesions in teats of infected buffaloes

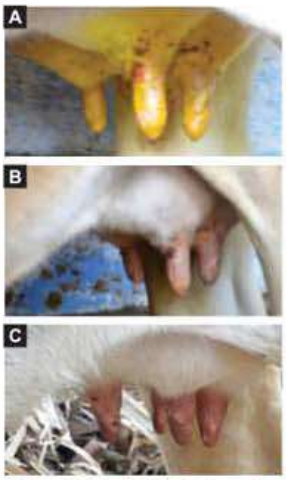


Fig. 55: Pock-like lesions in teats of infected cattle

circumscribed ulcerated lesions with raised edges were observed, which were painful on palpation. The pock-like lesions in animal handlers were present on fingers, forearms, eye, leg and back side of the head (Fig. 56). The lesions on eye were very severe with swelling and ulceration of the eye lids. Biological samples (blood, scabs & swabs) were collected from infected animals (buffaloes & cattle) and humans for identification and characterization of the etiological agent. All the samples were collected under aseptic conditions in viral transport medium and were transported chilled. The disease was further confirmed on the basis of isolation of the virus in Vero cell culture as well as by PCR amplification of the genomic DNA extracted from the scab and swab targeting the buffalopox virus–specific region of *C18L* & *ATI* genes using published primers. Buffalo, cattle and human samples were PCR positive and resulted in amplification of the expected product size of 552bp for *ATI* and 368bp for *C18L* genes (Fig. 57 A & B). BPXV isolates recovered in Vero cell culture from this outbreak

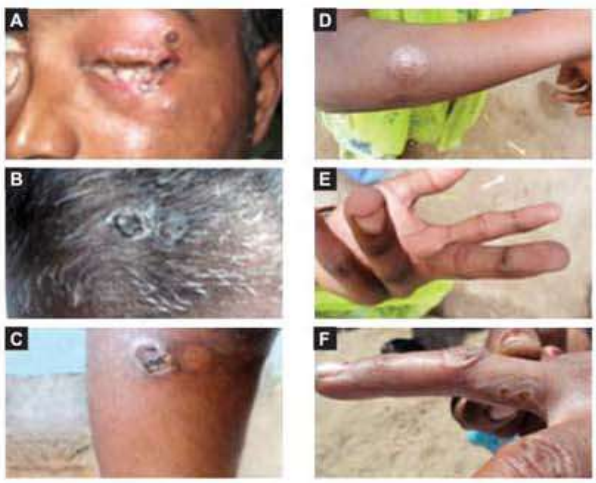


Fig 56. Clinical lesions in animal handlers on (A) eye, (B) backside of the head, (C) leg, (D) hand, and (E, F) fingers.

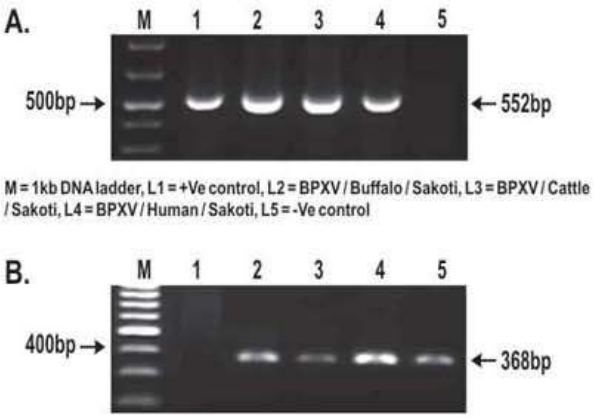


Fig. 57: PCR amplification of (A) *ATI* gene of BPXV and (B) *C18L* gene of BPXV isolates.

included 4 from buffaloes; 3 from cattle & 3 from human samples. Circulation of BPXV in buffalo, human and cows is a serious concern in view of huge population which is naive to smallpox infection.

(Sanjay Barua, B.C. Bera, Riyesh T., Shanmugasundaram K., P. Malik & R.K. Singh)

Accidental laboratory infection of a researcher with Buffalopox Virus (BPXV)

A laboratory infection of buffalopox virus occurred through accidental insertion of broken piece of viral ampoule in the palm of a researcher during freeze-drying process in December 2010 at Veterinary Type Culture Centre, NRCE, Hisar. Although, he was wearing disposable nitrile gloves, the broken glass piece cut through the gloves pierced the palm skin. Four days after injury, erythematus lesion around the wound was noted and on sixth day formation of a small vesicle over the wound was observed. Severe fever with oedema on the thumb region (Fig. 58) and swelling of axillary lymph node were noticed on eleven days after accident. The hand lesions were surgically excised to remove the necrotic tissue (Fig. 59). The pustular material and blood samples were collected for identification of the virus. The condition of patient improved slowly and the lesions healed in approximately nine weeks. The case was confirmed by isolation and identification of BPXV in Vero cell culture; detection of nucleic acids in tissue samples and isolated virus by amplification of BPXV-specific 368bp amplicons of *C18L* gene (Fig. 60) and detection of antibodies against BPXV in serum by plaque reduction neutralization test (PRNT) and immunoperoxidase test. This accidental case proved the severity of the local infection caused by BPXV which warrants the need for vaccination of vaccinia virus (VACV) lab workers and implementation of strict biosafety and biosecurity measures during handling of BPXV to prevent such accidental cases.

Fig. 58: Visible edema on the thumb region

Fig. 59: Wound after surgical excision

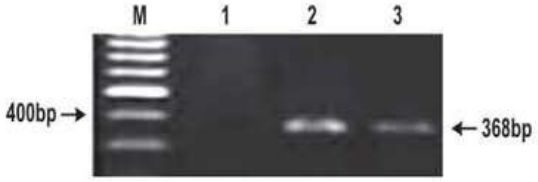


Fig. 60: PCR amplification of *C18L* gene of buffalopox virus. M = 1kb DNA ladder, L1 = -Ve control, L2 = +Ve control, L3 = BPXV / Buffalo / Lab isolate

(Riyesh T., Shanmugasundaram K., B.C. Bera, Sarita Yadav, R.K. Vaid, P. Malik and R.K. Singh)

Investigation of Bovine Papillomavirus (BPV) outbreak in Rajasthan: Mixed infection with BPV-1 and BPV-2

The suspected cases of papillomatosis were investigated in cows in a *gaushala* in Gandibadi village (Bhadra) of Hanumangarh Distt, Rajasthan in March 2011. Six to seven cases were observed with varying degrees of infection. The primary lesions were in the form of warts of variable size distributed all over the body but they were specifically confined to head and neck region (Fig. 61). Warts were collected upon excision for identification of the causative agent. The etiological agent was confirmed by PCR amplification of bovine papilloma virus (BPV) specific amplicons of 301bp and 164bp for BPV-1 and BPV-2 subtypes, respectively using published primers (Fig. 62).

Fig. 61: Papilloma warts on head and neck region of cattle

Fig. 62: PCR amplification of *L1* gene of BPV-1 & BPV-2. M = 1kb DNA ladder, L1 to L3 = +Ve amplification for BPV-1, L4 = -Ve control, L5 to L7 = +Ve amplification for BPV-2

(Shanmugasundaram K., B.C. Bera, Sanjay Barua, Nitin Virmani, Riyesh T., Taruna Anand, Praveen Malik and R.K. Singh)

Mixed infection of Sheeppox and Orf in sheep

Five sheeppox virus suspected samples were sent to VTCC laboratory from Central Sheep Breeding Farm (CSBF). The samples included scab materials, lung and spleen tissues collected after postmortem. Sheeppox virus infection was confirmed by PCR amplification of *T3A* peptide gene. PCR resulted in an amplicon size of 289 bp in three out of five samples tested (Fig. 63). PCR was also employed for Orf virus by targeting the *F1L* gene because of the suspicion of a mixed infection. The

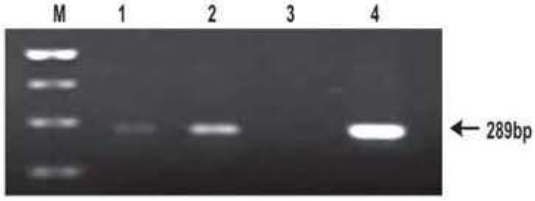


Fig. 63: PCR amplification of *T3A* gene of Sheeppox virus. M = 1kb DNA ladder, L1 = Scab, L2 = Lung, L3 = -Ve control, L4 = spleen

Orf virus-specific PCR resulted in an amplicon size of 708 bp in two out of five samples tested (Fig. 64). These

Fig. 64: PCR amplification of *F1L* gene of Orf virus. M = 1kb DNA ladder, L1 = Scab1, L2 = Scab2, L3 = -Ve control

results confirmed the presence of a mixed viral infection consisting of Sheeppox and Orf virus in CSBF. The virus isolation was tried in Vero cell line, however, the virus could not be isolated even after five blind passages.

(Riyesh T., Sanjay Barua, Nitin Virmani, B.C. Bera, Shanmugasundaram K. and Naresh Jindal)

Isolation and characterization of Enteropathogenic and Enteroaggregative *Escherichia coli* and *Klebsiella pneumoniae* from equine abortions, diahhorea and other conditions

Escherichia coli and *Klebsiella pneumoniae* have been considered to be major opportunistic pathogens of equines and they have been associated with cases of diahorrea, pneumonia in equines, metritis and abortions in mares and infections of foals. We report isolation and identification of these pathogens in majority from sporadic abortions and equine clinical cases related to intestinal discomfort like colic or respiratory discomfort. Clinical and pathological samples of heart blood, spleen, stomach content of aborted fetuses, vaginal and cervical swab of aborted mares, nasal swab, PM lung samples and PM intestinal content of colic horse were brought to laboratory and subjected to bacteriological analysis by plating on rich Sheep Blood Agar and McConkeys Lactose Agar. Out of 15 clinical cases of various presentations, 12 isolates of *E. coli* and 3 isolates of *K. pnemoniae* were identified after morphological and biochemical identification. In order to further characterize the virulent traits of *E. coli* isolates, these were subjected to PCR amplification targeting different virulence-associated genes *viz.*, enterohaemorrhagic, heat-labile toxin, heat stable toxin, entero-aggregative and bundle-forming pilus genes. All *E. coli* isolates were confirmed to species level by species-specific PCR (Fig. 65). Five *E. coli* isolates were positive for bundle-forming pilus gene which indicates the isolates to be Enteropathogenic. Two isolates were confirmed as Enteroaggregative *E. coli* (Fig. 66). None of the *E. coli* indicated carriage of toxin or enterohemolysine genes.

Fig. 65: PCR amplification of species-specific region of *E. coli*. M = 1kb DNA ladder, L1 to L12 = +Ve amplification for species-specific region of *E. coli* isolates and L13 = -Ve control

Fig. 66: PCR amplification of entero-aggregative gene of *E. coli*. M = 1kb DNA ladder, L1 to L2 = +Ve for *eae* gene

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

Enumeration and identification of microbes from stallion semen

Artificial insemination (AI) is a practical method for horse breed improvement and equine conservation. Sixteen semen samples (8 frozen semen straw, 8 fresh semen samples) were subjected to microbiological examination by enumeration of semen by employing SPC on Nutrient Agar and enumeration on SBA for TVC, MLA for selective isolation and SDA for fungal counts. The quantitative enumeration of aerobic bacteria showed an overall bacterial count range of $1x10^2$ to $6.1x10^3$ cfu/ml in frozen semen and $1x10^2$ to $2.3x10^5$ cfu/ml in fresh semen. However, in general, the bacterial counts were lower in frozen samples as compared to fresh semen samples. Of the 16 samples examined, none was found to be sterile, while the rest yielded a predominantly mixed flora comprising 1 to 4 bacterial genera. Out of 16 equine semen samples, 23 isolates were picked up, purified and subjected to biochemical identification. Out of 23 isolates, 18 were Gram-negative and 5 were Gram-positive. Potential venereal pathogens isolated from samples were identified as *Pseudomonas aeruginosa* (3 samples) and *Escherichia coli* (1 sample). Out of 23 isolates, majority (15) cultures were classified into Gram-negative Glucose Non-fermentor group, and were subjected to further tests. On the basis of growth and colony characteristics, these were identified as *Streptomyces* spp. (2), *Flavobacterium* spp. (1), *Escherichia coli* (1), *Pseudomonas* spp. (4), *Pseudomonas aeruginosa* (4), *Acinetobacter* (2), *Achromobacter* spp. (1) and *Alcaligenes* spp. (3) (Fig. 67) and 4 cultures were unidentified. The identity of microbes indicate environment as the source of contamination.

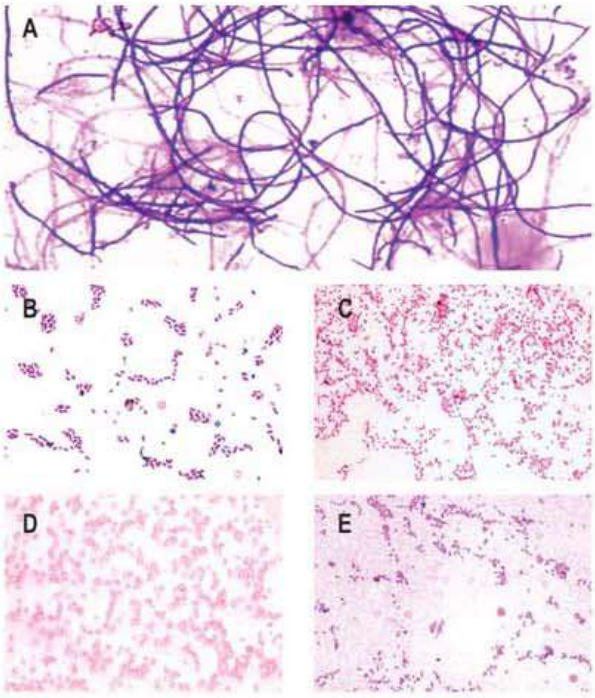


Fig. 67: Bacterial isolates : A) *Streptomyces* spp., B) Pleomorphic *E. coli, C) Pseudomonas* spp., D) *Acinetobacter* spp. & E) Pleomorphic thin rods of *Achromobacter* spp.

(R.K. Vaid, Shanamugasundaram K., A. Arangasamy and Thirumala Rao Talluri)

Molecular characterization of *Rhodococcus equi*

Rhodococcus equi is an opportunistic pathogen capable of infecting a wide range of hosts including equine, porcine, camel and humans. Foals below 6 months of age are more susceptible causing >3% deaths worldwide, thereby leading to huge economic losses to the equine industry. The virulence of *R. equi* is associated with plasmids encoding genes *viz. vap*A, -B, -C, -D, -E, -F, -G, -H, and –I. Identification of the virulence plasmids and antigens is important in characterization of pathogenic epidemic strains of *R. equi*. The present study was undertaken to characterize the *vap* gene family viz., *vap*A, -B, -C, -D, -E, -F, -G and -H of *R. equi* isolates by multiplex-PCR. The DNA was purified by CTAB method from *R. equi* isolates (59) from horses and donkeys and subjected to species-specific PCR. Positive isolates were further characterized by amplification of plasmid associated gene (*traA*). Thereafter, *traA*$^+$ isolates were screened for the presence of *vap* genes by multiplex-PCR. A total of 9 isolates were found to be positive for *vap*A, -C, -D, -E, -F, -G and –H genes yielding 550, 700, 400, 600, 350, 450, 500bp amplicons, respectively (Fig. 68). None of the isolates were positive for *vap*B gene. The *vap* positive isolates belonged to cases of respiratory infections in equines. Isolates obtained from healthy animals were negative for *traA* and *vap* genes. The study revealed the

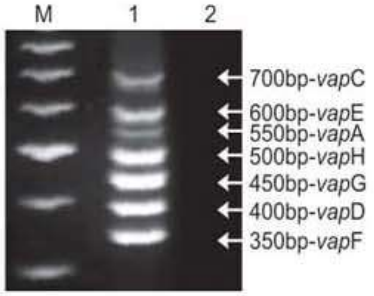


Fig. 68: Multiplex PCR of *vap* genes of *Rhodococcus equi*. M = 100bp DNA ladder, L1 = amplification products and L2 = -ve control

molecular profile of pathogenic isolates to be *vap*A,-H except *vap*B in Northern India. The presence of *vap*A gene in *R. equi* clinical isolates indicates the presence of virulent epidemic strains of *R. equi* in farm environment. The multiplex PCR assay for the *vap* gene family allowed molecular profiling of virulent isolates in a simple one-step reaction which would be a useful tool for epidemiological investigations as well as for prompt diagnosis of the infection.

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

New Initiative

Development of fibroblast cell cultures of animal origin

The Veterinary Type Culture Centre has been established with a mandate of reposition of animal microbes including viruses of animal origin. This envisages the isolation of large number of viruses from the clinical samples which requires appropriate species-specific cell lines. This warrants the generation of a battery of cell lines including somatic cell lines for isolation as well as production and testing of biologicals. Keeping this in view, we collected ear tissue samples (Mule-1, Buffalo-2, Goat-1, Sheep-1) which were seeded individually in tissue culture flasks containing DMEM supplemented with 10% fetal bovine serum. The cultures (Fig. 69 & 70) were established within 10-12 days by tissue explant method. For subsequent passaging, primary fibroblast cells were harvested by trypsinization upon 80–90% confluence and divided in 1:3 split ratios. For cryopreservation, the harvested cells (P5) were resuspended in freezing medium (10% DMSO with 20% fetal bovine serum) dispensed into sterile plastic cryogenic vials, sealed and kept in LN_2. The developed cultures could be expected to accelerate the pace of isolation of viruses from clinical samples.

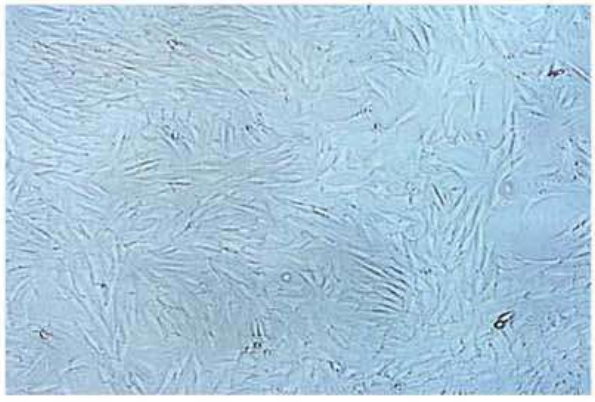


Fig. 69: Culture of fibroblast cells at passage 5 derived from mule ear pinna

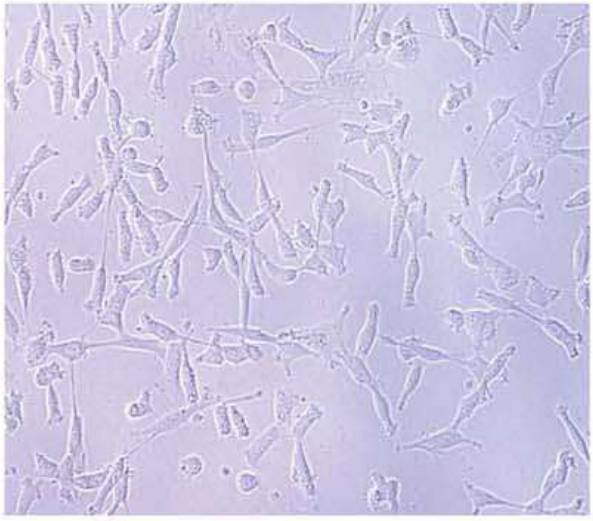


Fig. 70: Primary culture of fibroblast cells derived from buffalo ear pinna

(Taruna Anand, B.C. Bera, Sanjay Barua, Shanmugasundaram K., Sarita Yadav and P. Malik)

Externally-funded Research Projects

OIE Twinning Project on Equine Piroplasmosis between NRCPD, Japan and NRCE, India

MASP *in-vitro* culture: We initiated the *in-vitro* MASP culture by using Anaero Pouch®. Culture stocks of LN_2 preserved *Theileria equi* and *Babesia caballi* were cultured in 24-well culture plate. Medium was replenished every other day and fresh RBC's were added after every 4th day. The parasites were observed on 14th day of the culture [both *T. equi* (1-1.5%) and *B. caballi* (0.5-1%)]. The cultures were subcultured and parasitaemia of 3-4% was observed in both the protozoan cultures.

qPCR: The pre-designed primers (Kim et al 2008) were used for this study. For standardizing, calculation for copy number of parasite/plasmid, we cloned full-length 18s RNA gene (Accession no. Z15105; 1600bp) of *T. equi* in TOPO TA cloning vector. DNA concentration and copy no. of plasmid per µl was worked out.

Real-time PCR was performed using this cloned plasmid as standard (30 to $30x10^7$ molecules per reaction) alongwith some unknown DNA samples (Fig. 71). Initial results were encouraging, but more data need to be generated.

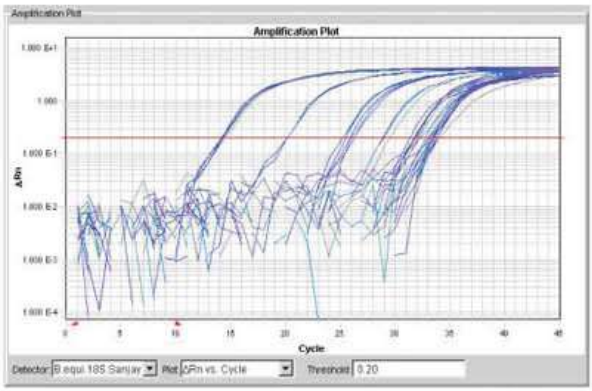


Fig. 71: qRT-PCR for detection of *T. equi*

Expression of EMA-1 and EMA-2 protein from *T. equi*

The primers for specific amplification and expression of truncated EMA-1 and EMA-2 proteins were designed so as to include maximum hydrophilic region of the expressed protein. The PCR products were cloned and expressed in pGEX4T-1 vector. R-protein product of 48 kDa and 50 kDa from EMA-1 and EMA-2, respectively were expressed. Immunogenicity of these proteins was confirmed on western blot analysis using reference *T. equi* positive serum. We are now in process of optimizing the ELISA using these proteins and will analyze its suitability in sero-surveillance studies.

Immuno-chromatographic Test (ICT): As a pen-side ICT strips were prepared using r-EMA-2 antigen. This test is very simple to use in the field. A drop of serum is required and result can be read in 10 minutes time. Using the r-protein, about 400 ICT strips were prepared and its suitability will be assessed on the serum samples collected from field (from Indian equid population).

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

DBT Project: Isolation and Characterization of Animal Adenoviruses for Development of a Novel Viral Vector for Vaccine Delivery

Adenoviruses have several properties that make them attractive candidates for developing vaccine delivery vectors. Vectors based on non-human adenoviruses have great potential as they are safe and induce desired immune response in experimental animals. Adenoviruses from buffaloes and horses can be used as vectors for delivery of protective antigens to develop recombinant vaccines for humans and animals. During

the year, we attempted to identify and characterize non-pathogenic adenoviruses from buffaloes in a DBT-funded research project.

Nasal and stool samples (156 each) collected from two organized buffalo farms in Hisar were screened for adenovirus by multiplex and nested PCR. Multiplex PCR detected 7 stool and 30 nasal samples positive while nested PCR detected 1 stools and 16 nasal samples positive for bovine adenovirus. Virus could be isolated by adapting to grow in BU cells (two samples) and SJMRF cells (6 samples) (Fig. 72). The PCR product (430 bp) from nPCR was cloned and sequenced. The isolated virus F-74 was typed as bovine adenovirus serotype 8 by sequence analysis. Further characterization of bovine adenoviruses is in progress for their use as candidate vectors.

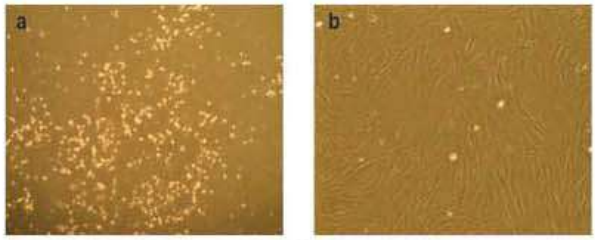


Fig. 72: Bovine adenovirus NS138 at passage 3 in SJMRF cells showing specific CPE (a); control cells show no CPE (b)

(Baldev R. Gulati)

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

PCR based detection of *Burkholderia mallei*

Primers targeting different gene fragment have been designed for specific detection of the agent. Initially *fliP* gene based primer has been used for identification of *B. mallei* which amplified a 700 bp product (Fig. 73). The cycling condition was; initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min. Fifty (50) field samples collected from outbreaks have been tested by PCR and the results corroborated with the isolation of bacteria and serological tests.

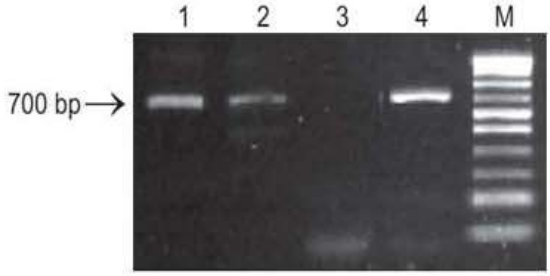


Fig. 73: PCR amplification of *flip* gene of *B. mallei* for diagnosis of glanders Lane 1: Sample 2645, Lane2: Sample 2651, Lane 3: Negative control, Lane 4: Positive control, Lane M: 100 bp DNA ladder

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

Technology Development & Assessment

Updated Equine Influenza Vaccine

An updated equine influenza vaccine (inactivated) was developed by NRCE, utilizing recent EI epizootic isolates. For this purpose, EIVs isolated from Katra (Jammu), Mysore (Karnataka), Ahmedabad (Gujarat) and Gopeshwar (Uttarakhand) were characterized by sequence analysis of HA gene for selecting the virus for updation of the vaccine. The sequence analysis classified the viruses to the clade 2 of Florida sublineage of American lineage. The EIV isolate-A/eq/Katra (Jammu)/06/08 (H3N8) was selected for updating the vaccine. The inactivated virus was checked for sterility and inactivated prior to vaccine production. The HA content of the virus was quantified and adjusted to 20 µg/dose. The vaccine was tested for potency in the Guinea pigs followed by safety and potency testing in equines. None of the horses showed any untoward reaction. The animals were given booster dose of the vaccine after four weeks of first vaccination. Isotype-ELISA was used to measure post vaccine isotype kinematics. The updated vaccine generated primarily IgGa, followed by IgGb and IgT response two weeks post-vaccination which persisted for more than 8 weeks post-vaccination period. The dosage of the vaccine is 1ml (I/M) for animals above six months of age with booster at 4 weeks after the first dose. Vaccination should be repeated annually.

Updated Equine Influenza Vaccine (inactivated virus)

Recombinant protein antigen-based ELISA (r-ELISA) for detection of Theileria equi antibodies

Equine piroplasmosis - caused by *Theileria equi* or *Babesia caballi* - is a tick-borne haemoprotozoan disease of equids (horses, donkeys, mules and zebras). Recombinant equine merozoite surface antigen-2 (rEMA-2) - a 52 kDa recombinant protein based ELISA (r-ELISA) was developed for detection of specific antibodies for diagnosis of *T. equi* infection in equine serum. Different components of r-ELISA were stabilized and the assay was transformed in the form of a diagnostic kit. This kit was validated vis-à-vis OIE approved CI ELISA on 60 serum samples of known disease status (33 known positive and 27 known negative). Diagnostic specificity and sensitivity of these two assays was compared and a very high correlation was observed. The accuracy of the results obtained by r-ELISA was also compared with Western blot analysis on selected number of serum samples which confirmed the results.

r-Ag based ELISA KIT

During 2007 to 2009, 2571 serum samples were collected/received from different geographical areas of India. A total 971 serum samples were found positive for *T. equi* antibodies, indicating prevalence of specific antibodies in 37.76% Indian equine population.

Recombinant protein antigen-based AGID/ indirect ELISA tests for diagnosis of EIA

The Centre has developed AGID/indirect ELISA tests based on recombinant p26 protein for diagnosis of EIA. For this purpose, recombinant protein was expressed in *E. coli* from a synthetic gene of 26 kDa and it was evaluated for use in AGID/indirect ELISA for sero-diagnosis of EIA. The ELISA was optimized using hyper-immune serum raised in rabbits and with the equine samples using a panel of four positive and four negative samples. The results showed 100% correlation with gold standard test. A total of 1600 equine samples have been tested for EIAV antibodies using the optimized p26 protein antigen-based *vis-a-vis* standard AGID. The diagnostic specificity and sensitivity for the test was found to be 100% and 99%, respectively. Based on the initial findings, efforts have been directed towards development of a field/lab based kit for sero-diagnosis of EIA. Further, the shelf-life and stability of reagents is being tested under various conditions and use of heavy water is also being explored.

Equi Rotavirus test for diagnosis of rotavirus in animal and human stool samples

EQUI ROTAVIRUS TEST is a sensitive and specific sandwich enzyme-linked immunosorbent assay (sELISA) developed employing a mAb raised against group-specific protein VP6 of rotavirus, for detection of mammalian group A rotaviruses from stool samples. The test employs a polyclonal anti-rotavirus serum as coating antibody to capture the rotavirus antigen and a monoclonal antibody for detection of group-specific antigen present on the captured rotaviruses. Stool suspension or control sample is added in the ELISA modules coated with polyclonal antibody to capture rotavirus antigens. To the captured rotavirus in microwell, murine anti-rotavirus mAb is added, which is further detected by incubation with anti-murine horseradish peroxidase. A chromogen substrate is added in the wells to show the presence of bound conjugate. The color intensity in comparison with the negative controls indicates the presence of rotavirus in the samples.

Validation of EHV-1 vaccine (Equiherpabort) in indigenous and Thoroughbred mares

Equine herpes virus-1 (EHV-1) vaccine - a killed oil emulsion mannide monooleate vaccine (OEMM) - developed by the Centre for control of abortions caused by EHV-1 in equines was subjected to validation. Field trials were conducted in 73 pregnant mares in different locations (Hanumangarh-29; Tohana-30; NRCE, Hisar-6 and NRCE, EPC, Bikaner-8). A total of 43 indigenous and 30 Thoroughbred pregnant mares from different villages / organized breeding farms were vaccinated with 2 ml dose (I/M) at 5th, 7th and 9th months of gestation. No untowards effect were seen after vaccination. Serum collected from the vaccinated animals was tested for virus neutralizing (VN) titres at monthly intervals for a 6 months period. The mares were negative for VN antibody titres on the day of inoculation. The booster antibody response ranged between titres of 16-64. The results were in accordance to antibody titres observed with Pneumabort 'K' imported vaccine as per our earlier studies. A higher antibody response was noted in Thoroughbred mares as compared to indigenous mares. The vaccine was prepared in two batches and its shelf-life was found to be around 6 months. The field trials indicated that the "Equiherpabort" vaccine could be an effective alternative to the "Pneumabort 'K' imported vaccine for large scale vaccination for control of EHV-1 in pregnant mares.

Consultancy & Commercialization of Technology

Consultancy

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

During the period under report, diagnostic services were provided to various stakeholders for EIA, glanders, equine influenza, EHV-1, EVA, CEM, *Theileria equi*, *Trypanosoma evansi*, *Trypanosoma equiperdum*, *Babesia equi*, *Salmonella* Abortusequi, and African horse sickness. A total of 4558 serum samples for EIA and 9197 serum samples for glanders were tested. Out of these 11 samples (7 from U.P. and 4 from H.P) were found positive for glanders, however, none of the samples were found positive for EIA. The major achievement was control of glanders in H.P. and U.P. by followup action. Out of 3513 serum samples tested for equine influenza, 550 samples gave positive reaction, however, the rise of titre in pairwise sera was not detected. Out of 291 samples tested for EHV-1, 50 were found positive. None of 89 samples tested for EVA was found positive. The agent for Contagious Equine Metritis was tested in 498 vaginal swabs and 28 preputial swabs obtained from Delhi and Chennai quarantine centres, in which no CEM agent was detected. Samples tested for *T. evansi* under disease investigation showed 22 samples positive out of 154. The Centre generated a revenue of ₹ 40,48,400 through testing of biological samples.

Commercialization of Technology

Two technologies namely Equi Herpes B-ELISA Kit for diagnosis of EHV-1 infection in equines and Pregmare kit for pregnancy diagnosis in mares, developed by the centre are ready for commercialization. These technologies have been discussed in public-private interface meeting held at IVRI, Izatnagar on March 3, 2011. Following discussions at IVRI, a private company- M/s Ubio Biotechnology System Pvt. Ltd., Cochin, Kerala has shown interest to purchase these technologies for commercialization. The mode of transfer of technologies and other clarifications are being discussed with the representative of the firm.

Technologies Developed by NRCE

- Updated Equine Influenza Vaccine
- Equi Herpes B-ELISA Kit
- Equine herpes virus-1 vaccine (Equiherpabort)
- mAb- ELISA kit for diagnosis of rota virus infection in equines
- Recombinant protein antigen-based ELISA kit for *Theileria equi* diagnosis
- Pregmare kit for pregnancy diagnosis in mares
- Cryopreservation of equine semen
- Recombinant protein antigen-based ELISA for diagnosis of EIA

Education & Training

An Interactive Meet on "Conservation of Marwari and Kathiawari Horses"

A one-day interactive meet on "Conservation of Marwari and Kathiawari Horses" was organized at Equine Production Campus, NRCE, Bikaner on September 7, 2010. Dr B.K. Joshi, Director, NBAGR, Karnal graced the occasion as the Chief-Guest. Besides, Sh. Narayan Singh Manaklaw (Ex MP) and Dr Gaya Prasad, ADG, Animal Health were the guests of honor. Director, NRCE briefed on conservation of Marwari and Kathiawari horses and emphasized on the importance of extension services to equine breeders. Dr B.K. Joshi emphasized the need of equine production and conservation. During the technical session, Director, NRCE further stressed upon the efforts the Centre is making for equine breeders. He also enumerated on the techniques for conservation of equines. The problems faced by the equine breeders were also discussed in detail. Valuable suggestions on the efforts to conserve indigenous breeds were put forth by the officials of different equine societies. The session concluded with the finalization of recommendations which are later submitted to DAHDF, GOI. The issues debated upon during the meet will be very needful to the Marwari and Kathiawari horse breeders in time to come.

Dignitaries releasing the compendium on Interactive Meet at Bikaner

Annual Scientific meet of VTCC

The first Annual Scientific Meet of Veterinary Type Cultures Network Project was held at NRCE, Hisar on September 21, 2010. The meeting was chaired by Prof. K.M.L. Pathak, Deputy Director General (Animal Sciences). Prof. Gaya Prasad, Assistant Director General (Animal Health) also attended the meet. Director, National Research Centre on Equines coordinated the meet. Nodal Officers of 15 network units from 11 states including veterinary, dairy and rumen microbe components participated in the meeting.

1st Annual Scientific Meet of VTCC

The annual progress reports of various components of VTCC were presented and discussed. Addressing the meeting, the DDG (Animal Sciences) commended the work done so far and congratulated the VTCC team for their hard work in terms of repository establishment and its strengthening in a short period of time. ADG also commended the progress made by VTC Network and underlined the need to increase the funding of the centres excelling in culture collection and deposition.

Training programme on "Diagnosis and Control of Japanese Encephalitis"

A training programme was conducted for Microbiologists from North Estern Region, Disease Diagnostic

NERDDL officials at NRCE Laboratory

Laboratory (NERDDL), Guwahati. Dr Amitav Chakravarti and Dr (Mrs) Shiney George were imparted training on "Diagnosis and Control of Japanese Encephalitis" during July 12-17, 2010. The NERDDL officials were given hands-on practical training on JEV detection and diagnostic techniques.

Preparation of educational video on NRCE activities

In order to provide a glimpse of various research and extension activities of NRCE and also to educate visitors, stakeholders and farmers on the utility of equines in different activities, an educational video film "Illustrious Equines" was prepared. The video film is available for visitors to get acquainted to the activities and research programmes at NRCE.

Besides, display material depicting mission, vision, mandate and other milestones and research accomplishments were also prepared in the form of posters for display to provide a glimpse of Centre's activities. Extension material on different breeds of equines were also prepared in Hindi and English languages in ATIC for benefit of visitors and farmers.

Dr S. Ayyappan, Secretary DARE and Director General, ICAR watching the video documentary "Illustrious Equines" in Visitor's room at NRCE

Trainings/Workshops Seminars organized

- A training for Microbiologists from NERDDL, Guwahati on "Diagnosis and Control of Japanese Encephalitis" during July 12-17, 2010.
- Interactive meet of VTCC Network units of Veterinary Microbes on August 28, 2010.
- Interactive Meet on "*Conservation of Marwari and Kathiawari Horses*" held at Equine Production Campus, NRCE, Bikaner (Rajasthan) on September 7, 2010.
- *Kisan Goshthi* at the Centre on the occasion of Foundation Day on November 26, 2010.

Expert Lectures at NRCE

- Mr. Manoj Kumar, Senior Technical Officer, Rajbhasha Vibhag, ICAR visited NRCE to monitor the progress of Hindi activities at NRCE on August 28, 2010. He delivered a lecture on "Rajbhasha Niyamo ka Palan"
- Dr Utpal Tatu, Professor, Department of Biochemistry, Indian Institute of Science, Bangalore visited NRCE on August 31, 2010 and delivered a lecture on "Proteomics of Infectious Diseases: Applications in Biomarker Identification, Diagnostics and Drug Development"
- Dr Sanjay Dhar, Director (Research) Aesthetic and Plastic Surgery Institute, University of California, Irvine visited NRCE on September 18, 2010 and delivered lecture on "Nerve Tissue Engineering-Bench to Bedside"
- Dr R. K. Singh, Director NRCE delivered a lecture on "Stem Cell Therapy in Equines- Present Status and Future Prospects" on January 22, 2011.
- Dr A. K. Gupta, Principal Scientist NRCE delivered a lecture on "Mitochondrial DNA and its Significance in Molecular Genetics" on March 28, 2011.
- Dr B. K. Singh, Principal Scientist NRCE delivered a lecture on "Understanding Equine Neurological Disorders" on March 28, 2011.

Expert Lectures Outside

- Virmani Nitin. 2010. An overview of viral diseases in equines and trends in diagnosis. ICAR Sponsored Winter School On Recent Concepts In Veterinary Laboratory Diagnostics, Department of Veterinary Pathology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana October 12 to November 1, 2010.
- Singh, R.K. and Virmani, N. 2010. Interspecies Transmission of Influenza A viruses and Zoonosis with a Focus on Equine Influenza (H3N8) Virus, NAVS- National Convention on "Zoonotic Diseases: Present Status and Future Road Map" on October 20, 2010, NDRI, Karnal, India October 30, 2010.
- Singh, R.K., Director, 2010. Delivered a lecture on importance of Equine Influenza at NDRI, Karnal on October 30, 2010.
- Singh, R.K., Director, 2010. Delivered invited lecture on "Stem cell therapy in equines : Potential & future prospects" on November 12, 2010 during the

building in livestock under changing climate scenario" and annual meeting of SAPI held at IVRI, Izatnagar.

- Vaid, R. K. 2010. Lead paper "Microbial Resource Centers: Source of genome biological information that can be used" in V Convention of Society for Immunology and Immunopathology and National Symposium on Immunobiotechnology organised at Institute of Biotechnology, G B Pant University of Agriculture and Technology, Patwadangar, Nainital (Uttarakhand) from December 17-19, 2010.

Students Guided

S.No.	Name of Scientist/ Guide/Co-Guide	Name of Student	Course	Year	Research Work/ Thesis title	University/ Institute
1.	Dr A.K. Gupta	Shalini Goyat	M.Sc. (Biotechnology)	2010	Preliminary Studies on Microsaellite based Genetic Diversity in Bhutia Ponies	Kurukshetra University, Kurukshetra (Haryana)
		Priyanka Saini	M.Sc. (Biotechnology)	2010	Molecular characterization of non-descript donkeys by using microsatellite markers	Lovely Professional University, Phagwara (Punjab)
		Lalita	M.Sc. (Biochemistry)	2010	Microsatellite marker based genetic diversity studies in Bhutia breed of equines	Kurukshetra University, Kurukshetra (Haryana)
		Ajender Singh	M.Sc. (Biochemistry)	2010	Microsatellite marker based genetic diversity studies in Spiti breed of equines	Kurukshetra University, Kurukshetra (Haryana)
2.	Dr B.K.Singh	Ms Pushplata	M.Sc. (Biochemistry)	2010	Production of hybridomas secreting mAb against equine influenza virus (H3N8)	Lovely Professional University, Phagwara (Punjab)
3.	Dr S. C. Yadav	Hariom Choudhary	M.Sc. (Biotechnology)	2010	Identification of infection specific proteinoses of *T. evansi.*	Lords International College Alwar (Rajasthan)
4.	Dr B. R. Gulati	Ms Vinti Kumari	M.Sc. (Biotechnology)	2009	Purificationof immunologial characterization of recombinat partial envolop protein of Japanese Encephalitis virus.	MDU Rohtak (Haryana)
5.	Dr Sandeep Khurana	Ms Monika Sihag	M.Sc. (Biotechnology)	2009	Molecular characterization of *Rhodococcus equi* isolates using polymerase chain reaction for *cho*E gene	Lovely Professional University, Phagwara (Punjab)
6.	Dr Nitin Virmani	B.N. Shukla	M.Sc. (Biotechnology)	2010	Genetic analysis of Matrix gene of Equine Influenza virus from epizootic in India 2008-09 and serological investigation for equine influenza employing HI assay	Jaipur National University, Jaipur (Rajasthan)
		Sakshi Narang	M.Sc. (Biotechnology)	2010	Characterization of EHV-4 isolate and validation of r-protein based ELISA for differentiation of EHV-1 & EHV-4 infections.	Maharishi Dayanand University, Rohtak (Hry.)
7.	Dr Sanjay Barua	Ms Zeenat Wadhwa	M.Sc. (Biotechnology)	2009	Molecular Characterization of Ankyrin gene of Camelpox virus	GJUS&T, Hisar (Haryana)
8.	Dr R.K.Vaid	Muktakshi Malhotra	M.Sc. (Biotechnology)	2009	Equine bacterial isolates of Enterob-acteriacae family: Biochemical and molecular characterization	Amity University, NOIDA, (U.P.)
9.	Dr Anju Manuja	Jyoti Kaushik	M.Sc. (Biotechnology)	2009	Transcriptional expressionof TLR9 in Zanskari and Kathiawari breeds of horses	Lovely Professional University, Phagwara (Punjab)
10.	Dr H.Singha	Ms Tarunesh	M.Sc. (Biochemistry)	2010	Expression and purification of envelop protein of Japanese encephalitis virus	Kurukshetra University, Kurukshetra (Haryana)
11.	Dr B.C.Bera	Anu Gupta	M.Sc. (Biotechnology)	2010	Molecular characterization of Schafen gene of Camelpox virus	GJUS&T, Hisar (Haryana)

QRT, RAC, IRC & IMC Meetings

32nd Institute Management Committee Meeting

The 32nd meeting of IMC was held on June 17, 2010 at ICAR, Krishi Bhawan, New Delhi. Among the nominated members Dr Lal Krishna, ADG (AH); Dr Vinay Mohan, Asstt. Director, (AH), Punjab; Col Umaid Singh, Jodhpur; Dr S.B.S. Yadav, Dean CoVSc, Bikaner; Dr S.N. Singh, Technical Manager, Biovet, Bangalore; Dr Dharmendra Singh, Dy. Dir (AH), Haryana and Sh K.K. Sharma, AFAO, IASRI, New Delhi were present. The committee was appraised about the discard of vehicle, replacement of items against those approved equipments in XI Plan, purchase of animals of indigenous breed for EPC, Bikaner, etc. The committee agreed to all the agenda items including purchase of vehicle and need-based equipments. The IMC adopted and confirmed the proceedings of 31st IMC meeting.

XIII Research Advisory Committee Meeting

The XIII, RAC meeting was held under the chairmanship of Dr A.T. Sherikar, former Vice-Chancellor, Maharashtra Animal and Fishery Sciences University, Maharashtra on May 31, 2010 to discuss ongoing research activities. The RAC members who participated in the meeting included Col (Dr) B. Raut, Dr R.C. Katoch, Dr S.N. Maurya, Dr D.V. Rangnekar, Col (Dr) Umaid Singh Rathore, Dr Lal Krishna and Dr R.K. Singh, Director, NRCE. The RAC reviewed the ongoing research projects in the area of equine production, health, extension and Veterinary Type Cultures and also approved six new research projects. The Chairman RAC congratulated the Director and applauded the efforts of NRCE scientists for their sincere contribution in the ongoing research programmes and other developmental activities of the Centre.

Research Advisory Committee Members

- Dr A.T. Sherikar, Ex-VC, MAFSU, Mumbai
- Col (Dr) B. Raut, Consultant DRDO, FRL, Chandigarh
- Dr R.C. Katoch, Ex-Dean, COVS, CSKHPKV, Palampur
- Dr S.N. Maurya, Ex-OSD & VC, (UPDDUPC VVVAS), Mathura
- Dr D.V. Rangnekar, Ex-Vice President, BAIF, Pune
- Dr Lal Krishna, ADG (AH), ICAR, New Delhi
- Dr R.K. Singh, Director, NRCE, Hisar

New Research Projects Approved by RAC

- Characterization of indigenous, non-descript, geographically distinct donkeys and their evolutionary links.
- Development of monoclonal antibodies and recombinant antigens based assays for detection of *Trypanosoma evansi* infection in equines.
- Cloning, expression and characterization of equine chorionic gonadotropin (eCG)
- Characterization of Toll-like receptor 9 and its role in CpG immuno-modulation in equines
- Development of recombinant protein/peptide based diagnostic(s) for equine infectious anemia.
- Development of targeted drug release therapeutics using nano-particles in equine medicine.

Institute Research Committee Meeting

The annual meeting of Institute Research Committee was held under the chairmanship of Dr R.K. Singh, Director, NRCE, Hisar on August 7, 2010 to review the research achievements of the ongoing projects for the year 2009-10 and to consider new research project proposals. In the IRC, Dr Gaya Prasad, Prof. P.K. Uppal, Prof. O.P. Dhanda, Dr R.S. Khatri and Dr M.B. Chhabra were invited as external experts. The IRC reviewed the progress of ongoing research projects in the area of equine production, health, extension and Veterinary Type Culture Centre. New research projects to be taken up by the Centre were also discussed and approved during the meeting.

Workshop, Seminar & Institutional Activities

"Kisan Call Centre" for equine owners at NRCE Hisar inaugurated by Dr. S. Ayyappan, Hon'ble DG (ICAR) & Secretary (DARE)

The Kisan Call centre - a helpline for equine owners - was inaugurated by Hon'ble DG. ICAR and Secretary DARE, Dr S. Ayyappan on December 9, 2010 by answering a phone call from an equine owner from Julana in Jind District of Haryana. Prof. K.M.L. Pathak,

Hon'ble Director General and Secretary DARE Dr S. Ayyappan inaugurating Kisan Call centre

DDG (AS), Dr C.S. Prasad, ADG (AN), Dr R. K. Sethi, Director, CIRB and Dr R. K. Singh, Director, NRCE were also present. This toll-free helpline will provide advisory services to equine owners from ATIC at NRCE on the lines of "Kisan Call Centre". The NRCE toll-free helpline number 1800-180-1233 can be accessed by equine owners from all over the country. The queries of the farmers are addressed by the experts from NRCE and relevant advice is provided to equine owners on health and management aspects of equine husbandry. Currently, the Kisan Call Centre of NRCE is also addressing the issues related to buffalo production in consultation with Central Institute for Research on Buffaloes, Hisar.

Dr S. Ayyappan, Secretary (DARE) and Director General (ICAR) inaugurated Visitors' Room at NRCE, Hisar

Honorable Director General, ICAR and Secretary DARE, Dr S. Ayyappan visited NRCE on December 9, 2010. He was accompanied by Prof. K.M.L. Pathak, DDG (AS) and Dr C.S. Prasad, ADG (AN). On this occasion, Dr S. Ayyappan also inaugurated the newly renovated Visitors' Room at NRCE gate. Plantation was also done by Hon'ble Director General in the premises of NRCE. Dr S. Ayyappan visited different laboratories of NRCE, Animal shed, BSL-III facility, ATIC, VTCC new building and Agriculture Farm.

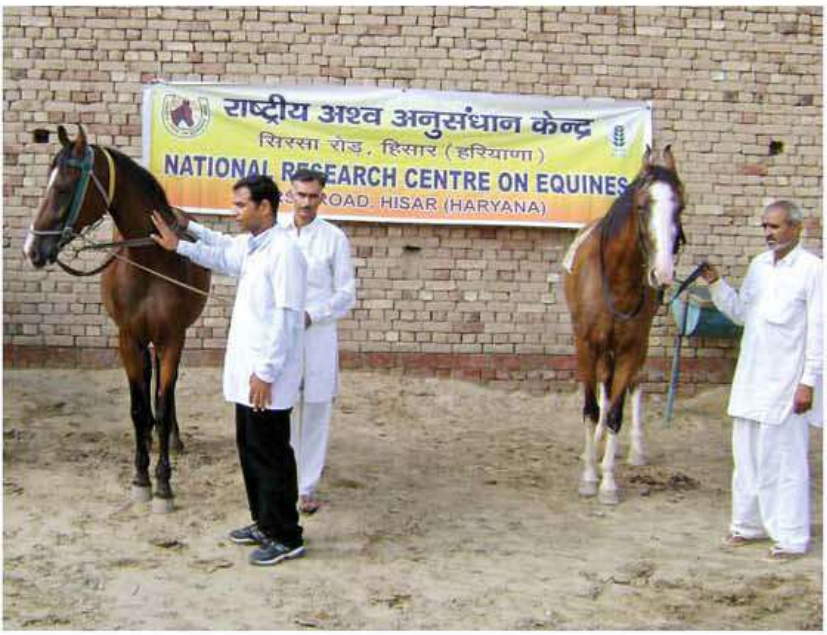
Dr R.K. Singh, Director, NRCE, explained Hon'ble Director General about various ongoing research activities and salient achievements of the Centre. During interaction with scientists, Dr S. Ayyappan emphasized that scientist should put dedicated efforts to get excellent research outcome for the benefit of the farmers and the equine owners. On this occasion, he discussed with scientists about the future programmes for XIIth Five year plan, He also addressed the joint interactive meeting of scientists and staff of NRCE and CIRB.

Dr S. Ayyappan, Director General (ICAR) and Secretary (DARE) inaugurating Visitors' Room at NRCE

Equine health camp at Sardarshahar

Equine health camps

NRCE regularly organizes equine health camps to provide timely help and support to the under-privileged equine owners. During the year 2010-11, twelve health camps were organized and animals treated for various ailments like colic, lameness, respiratory problems, body wounds, parasitic infestation etc. Medicines, deworming tablets and mineral mixture were provided free of cost to the equine owners. The interaction between scientists and farmers helped to understand the problems faced by equine owners and farmers were adviced on various health and management problems in equines.



Equine health camp at Hanumangarh

Equine health camp at Rajli

Equine health camps organized during 2010-11

Place of Organization	Date	Activities
Churu Distt., Rajasthan	April 28, 2010	The animals were examined for various ailments by the experts from NRCE. Free medicines and treatment was provided to diseased animals at the camp. Pregnancy diagnosis was done during the camps and deworming tablets and mineral mixture were provided to the equine owners free of cost.
Sardarshahar, Churu Distt., Rajasthan	April 29, 2010	
Jodhpur Distt., Rajasthan	April 7, 2010	
EPC, Bikaner, Rajasthan	July 8, 2010	
Jodhpur Distt., Rajasthan	July 15, 2010	
Hanumangarh Distt., Rajasthan	August 16, 2010	
Shergarh, Jodhpur Distt., Rajasthan	September 18, 2010	
Pali, Sadri Distt., Rajasthan	November 11, 2010	
Vautha, Ahmedabad Distt., Gujarat	November 15, 2010	
Ganganagar Distt., Rajasthan	November 22, 2010	
Rajli, Hisar Distt., Haryana	October 28, 2010	
Hanumangarh Distt., Rajasthan	February 18-19, 2011	

Kisan Goshthies and equine welfare activities

During 2010-11, nine Kisan Goshthis were organized in Haryana, Rajasthan, Uttar Pradesh and Uttarakhand. During these Kisan Goshthies, scientists from NRCE interacted with equine owners on equine welfare issues to understand their problems and constraints. Interactive equine owners' meets were also organized in which the equine owners were benefited through interaction with scientists about the various aspects of equine husbandry and management. They were advised regarding equine health, feeding and diseases in equines. Also, literature in hindi on various aspects of equine husbandry and management was distributed to the equine owners.

Participation of NRCE in exhibitions and animal fairs

During 2010-11, NRCE participated in eight exhibitions and animal fairs at national level with NRCE exhibition stall displaying different technologies developed at NRCE. NRCE participated at AgriExpo 2010, Bawal, Rewari Dist. Haryana (October 4-6, 2010); CIPHET, Ludhiana, Punjab (October 20, 2010); IVRI Kisan Mela – 2010 (November 1-3, 2010); National Livestock Championship, Muktsar, Punjab (January 8-12, 2011); Buffalo Mela, CIRB, Hisar, Haryana (February 1, 2011); Bhatner Ashav Mela, Hanumangarth, Rajasthan (February 17-19, 2011); Nagore Animal Fair, Rajasthan (February 9-11, 2011) and Animal fair at Karauli, Rajasthan (February 19-21, 2011). During these exhibitions and animal fairs, information was provided to equine owners and visitors about activities and services

Dignitaries at NRCE stall during CIRB Mela

Scientists interacting with equine owners

provided by NRCE. Extension literature on various aspects of equine husbandry and management to equine owners were also distributed to the equine owners.

Punjab Deputy CM Sh. Sukhbir Singh Badal at NRCE Stall during National Livestock Championship, Muktsar

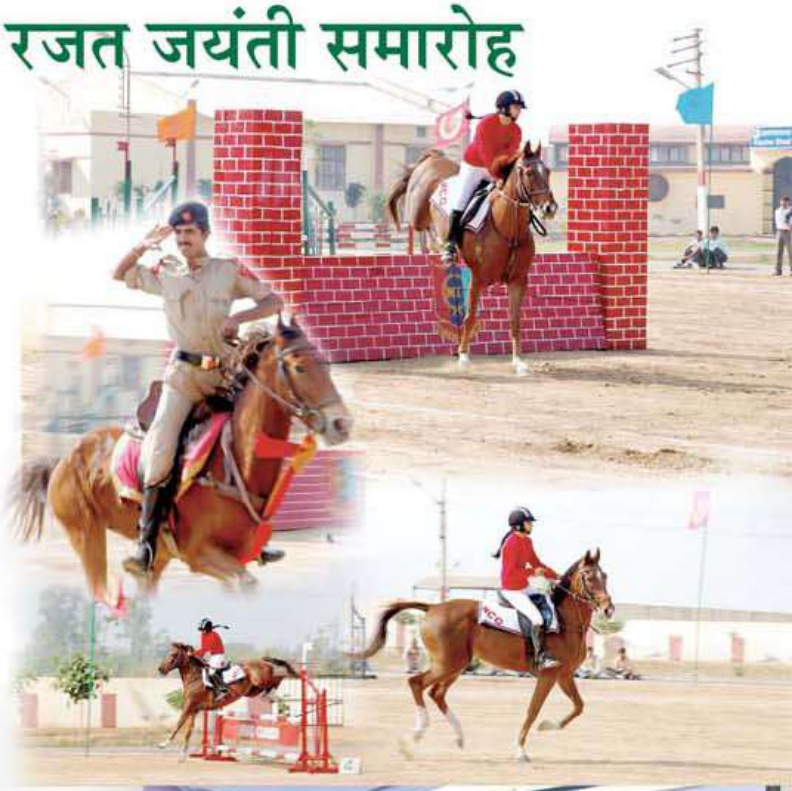
Students Educational Tours and Exposure Visit of Farmers

During 2010-11, visitors from different places, including farmers, students from SAUs and other visitors visited NRCE as part of educational tours and exposure visit. During visits, visitors were briefed about the research activities and different extension and field activities of NRCE for benefit of equine owners. The visitors visited Equine Museum at ATIC and animal shed at NRCE and were made aware of various on-going programmes of NRCE.

स्थापना दिवस एवं रजत जयंती समारोह

दिनांक 26 नवम्बर, 2010 को राष्ट्रीय अश्व अनुसंधान केंद्र में स्थापना दिवस एवं रजत जयंती समापन समारोह बड़ी धूमधाम से मनाया गया। इस अवसर पर कार्यक्रम के मुख्य अतिथि, प्रो. कृष्ण मुरारी लाल पाठक, उपमहानिदेशक (पशु–विज्ञान), भारतीय कृषि अनुसंधान परिषद्, ने आयोजित अश्व प्रदर्शनी का उद्घाटन किया।

इस मौके पर कार्यक्रम की अध्यक्षता हेतु डॉ. कृष्ण सिंह खोखर, कुलपति, चौधरी चरण सिंह हरियाणा कृषि विश्वविद्यालय, हिसार, ने पधारकर कार्यक्रम की शोभा बढाई। केंद्र के स्थापन निदेशक डॉ. प्रेम कुमार उप्पल, पूर्व निदेशक डॉ. महेन्द्र पाल यादव तथा डॉ. शैलेन्द्र कुमार द्विवेदी विशिष्ट अतिथि के रूप में उपस्थित थे। कार्यक्रम का शुभारम्भ करते हुए अपने स्वागत भाषण में केंद्र के निदेशक डॉ. राज कुमार सिंह ने आधुनिक युग में मशीनीकरण के बावजूद अश्वों की उपयोगिता की तरफ सबका ध्यान केन्द्रित किया तथा केंद्र की पिछले 25 वर्षों की



उपलब्धियों का विवरण भी दिया। इस अवसर पर मुख्य अतिथि ने अन्य गणमान्य अतिथियों के साथ संस्थान परिसर में वृक्षारोपण भी किया।

मुख्य अतिथि प्रो. कृष्ण मुरारी लाल पाठक ने केंद्र के अनुसंधान कार्यों को सराहा और केंद्र द्वारा किये जा रहे रोग निदान, रोग निवारण एवं नस्ल संरक्षण के क्षेत्र में किये जा रहे प्रयासों की प्रशंसा की। कार्यक्रम के अध्यक्ष डॉ. खोखर ने भी संस्थान के उत्कृष्ट कार्यों की सराहना की।रजत जयंती समारोह पर आयोजित अश्व क्रीड़ा, घुड़सवारी प्रदर्शन व अन्य प्रतियोगिताओं में राष्ट्रीय कैडेट कोर हिसार के पशुचिकित्सा विज्ञान महाविद्यालय, मधुबन पुलिस तथा हरियाणा, राजस्थान एवं पंजाब राज्य से आए हुए घुड़सवारों ने अपने–अपने करतब दिखाकर सबको मन्त्रमुग्ध कर दिया। इस अवसर पर आयोजित अश्व–पालक किसान गोष्ठी में दूर–दूर से आये हुए करीब 200 अश्व पालकों ने भाग लिया। किसान गोष्ठी में अश्व–पालकों ने अश्वों में होने वाली विभिन्न बीमारियों एवं समस्याओं के बारे में वैज्ञानिकों से वार्तालाप कर अपने संशय दूर किए और लाभान्वित हुए। वैज्ञानिकों ने अश्व–पालकों को अश्वों के रोग निदान के बारे में महत्वपूर्ण जानकारी भी दी।

हिन्दी सप्ताह का आयोजन

राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार में सितम्बर 8–15, 2010 तक हिन्दी सप्ताह का आयोजन किया गया। दिनांक सितम्बर 8, 2010 को केन्द्र के निदेशक, डॉ. राज कुमार सिंह एवं कार्यक्रम के मुख्य अतिथि डॉ. रमेश कुमार सेठी, निदेशक, केन्द्रीय भैंस अनुसंधान संस्थान, हिसार, के द्वारा द्वीप प्रज्जवलन कर कार्यक्रम का शुभारंभ किया गया। इस अवसर पर केन्द्र में विभिन्न प्रतियोगिताएं आयोजित की गई। हिसार स्थित केन्द्रीय कर्मचारियों एवं रा०अ०अनु० केन्द्र के कर्मचारियों ने प्रतियोगिताओं में बढ़–चढ़ कर भाग लिया।

सितम्बर 14, 2010 को राज्यकवि श्री उदयभानु हंस जी की अध्यक्षता में सम्पन्न हुई काव्य गोष्ठी में हिसार के गणमान्य कवियों ने शिरकत की व अपनी रचनाएं प्रस्तुत की। निदेशक व कार्यक्रम के मुख्य अतिथि डॉ. रणधीर सिंह दलाल, सदस्य सचिव, हरियाणा किसान आयोग एवं विशिष्ट अतिथि श्री विजय राजदान, निदेशक दूरदर्शन केंद्र, हिसार, द्वारा द्वीप प्रज्जवलन कर हिंदी काव्य गोष्ठी का शुभारंभ किया गया। काव्य गोष्ठी में श्री राधेश्याम शुक्ल, श्री रघुवीर अनाम, श्री महेन्द्र जैन, श्री नरेश शर्मा, श्री ओमप्रकाश दिलबर, डॉ. चंद्रशेखर, प्रद्युमन भल्ला एवं डॉ. इंद्रजीत सिंह जैसे गणमान्य कवि मौजूद थे।

मुख्य अतिथि द्वारा द्वीप प्रज्जवलन कर हिन्दी सप्ताह का शुभारंभ

सितम्बर 8, 2010 को आयोजित भाषण प्रतियोगिता के अंतर्गत श्रीमती शम्मी त्यागी को प्रथम पुरस्कार तथा कविता प्रतियोगिता में श्री अनुरंजन कपूर जी को प्रथम पुरस्कार प्राप्त हुआ। सितम्बर 9, 2010 को आयोजित अंग्रेजी शब्दावली का हिंदी में अनुवाद प्रतियोगिता में श्री राम अवतार पराशर को प्रथम पुरस्कार मिला। प्रमुख अतिथि एवं विशिष्ट अतिथि ने इस बात पर जोर दिया कि देश को आगे बढ़ाना है तो यह हिंदी को साथ लेकर ही संभव है। निदेशक ने संस्थान में हिंदी के प्रयोग को दिन–प्रतिदिन ज्यादा से ज्यादा प्रयोग में लाने पर जोर दिया।

अश्व उत्पादन परिसर, बीकानेर में हिन्दी पखवाड़ा समारोह का आयोजन

अश्व उत्पादन परिसर, रा०अ०अनु० केन्द्र, बीकानेर में सितम्बर 14–28, 2010 तक 'हिन्दी पखवाड़ा' समारोह का आयोजन किया गया। प्रभारी महोदय (डॉ. सूर्य नारायण टण्डन) ने अपने अध्यक्षीय भाषण में हिन्दी भाषा की महत्ता उजागर करते हुए हिन्दी भाषा से संबंधित कार्यो को सुचारू रूप से एवं सहज ढंग से अपनाने पर जोर दिया।

इस क्रम में सितम्बर 24, 2010 को हिन्दी पखवाड़ा समारोह के दौरान निबन्ध, लेखन व सुलेख प्रतियोगिता का आयोजन किया गया। निबन्ध प्रतियोगिता में प्रथम पुरस्कार श्री कमल सिंह एवं द्वितीय पुरस्कार डा. जितेन्द्र सिंह व सुलेख प्रतियोगिता में प्रथम पुरस्कार डा. बलविन्दर कुमार, एवं द्वितीय श्री बृजलाल को प्रभारी महोदय द्वारा दिया गया। समारोह के दौरान आयोजित प्रतियोगिताओं में सभी वर्ग के कर्मचारियों एवं अधिकारियों ने बढ़–चढ़कर भाग लिया।

अश्व अत्पादन परिसर, बीकानेर में आयोजित हिन्दी पखवाड़ा

स्वतन्त्रता दिवस समारोह का आयोजन

राष्ट्रीय अश्व अनुसंधान केन्द्र में अगस्त 15, 2010 को स्वतंत्रता दिवस बड़ी धूमधाम से मनाया गया। इस अवसर पर प्रो. गया प्रसाद, सहायक महानिदेशक (पशु स्वास्थ्य) मुख्य अतिथि के रूप में उपस्थित थे। केन्द्र के निदेशक एवं मुख्य अतिथि द्वारा सम्मिलित ध्वजारोहण कर कार्यक्रम का शुभारंभ किया गया। प्रो. गया प्रसाद एवं डॉ. सिंह, दोनों ने इस अवसर पर केन्द्र के कर्मचारियों को सम्बोधित करते हुए राष्ट्र की अपेक्षाओं पर खरा उतरने हेतु स्वयं का आकलन करने की आवश्यकता पर बल दिया। केन्द्र के कर्मचारियों के परिवार के सदस्य भी इस अवसर पर उपस्थित थे।

स्वतंत्रता दिवस के अवसर पर ध्वजारोहण करते हुए मुख्य अतिथि एवं निदेशक महोदय

गणतंत्र दिवस पर रंगारंग कार्यक्रम

राष्ट्रीय अश्व अनुसंधान केन्द्र में जनवरी 26, 2011 को गणतंत्र दिवस धूमधाम से मनाया गया। संस्थान के निदेशक डा. राजकुमार सिंह ने तिरंगा फहराकर गणतंत्र दिवस समारोह का शुभारम्भ किया। इस अवसर पर उन्होंने वैज्ञानिकों एवं कर्मचारियों को गणतन्त्र दिवस की शुभकामनाएं देते हुए अपने सम्बोधन में केन्द्र की उपलब्धियों का विवरण दिया एवं सभी कर्मचारियों से केन्द्र के विकास के लिए पूरी तरह समर्पित होने का आह्वान किया। इस अवसर पर केन्द्र के सभागार में बच्चों द्वारा देशभक्ति से परिपूर्ण रंगारंग कार्यक्रम का आयोजन भी किया गया।

गणतंत्र दिवस पर ध्वजारोहण कार्यक्रम

बच्चों द्वारा देश भक्ति कार्यक्रम

सतर्कता जागरूकता सप्ताह का आयोजन

राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार में दिनांक अक्टुबर 25 से नवम्बर 1, 2010 तक सतर्कता जागरूकता सप्ताह मनाया गया। इस अवसर पर केन्द्र के निदेशक ने केन्द्र के सभी कर्मचारियों एवं अधिकारियों को कार्यालयी सतर्कता की ओर सजग रहने की शपथ दिलाई। कार्यक्रम का उद्घाटन श्री बलबीर सिंह मलिक जी, आयुक्त हिसार मण्डल, हिसार, के कर कमलों द्वारा द्वीप प्रज्ज्वलित करके किया गया। डा0 रमेश कुमार सेठी निदेशक, केन्द्रीय भैंस अनुसंधान संस्थान, हिसार, तथा श्री संत सिंह रेवड़ी, समाज सेवी, विशिष्ट अतिथि के रूप में उपस्थित थे। इस अवसर पर मुख्य अतिथि द्वारा संस्थान के प्राँगण में पौधारोपण भी किया गया। मुख्य अतिथि ने अपने अभिभाषण में जोर देते हुए कहा कि सतर्कता के लिये हमें अपना जीवन समर्पित करना चाहिए तथा समाज में भ्रष्टाचार के खिलाफ संगठित प्रयास करना चाहिए। उन्होंने जोर देकर कहा कि भ्रष्टाचार को समाप्त करने के लिए सामाजिक उत्थान, अच्छे संस्कार व व्यक्ति विशेष की संवेदनशीलता अति आवश्यक है, तभी इस सामाजिक कुरीति पर काबू पाया जा सकता है। इस कार्यक्रम में शहर के प्रबुद्ध विद्वान व वक्ताओं क्रमशः प्रो. राधेश्याम शुक्ल जी, प्रो. रघुबीर अनाम जी, डा. इन्द्रजीत नाहल जी आदि ने भ्रष्टाचार के खिलाफ जागरूकता पर अपने विचार व्यक्त किये। इस कार्यक्रम के दौरान एक भाषण प्रतियोगिता का आयोजन किया गया जिसमें शहर के विभिन्न स्कूलों के विद्यार्थियो ने "भ्रष्टाचार के विरूद्व जागरूकता एवं प्रचार प्रसार" विषय पर अपने विचार व्यक्त किये। सतर्कता सप्ताह कार्यक्रम का समापन कार्यक्रम नवम्बर 1, 2010 को हुआ जिसमें श्री नवीन जैन, लेखानियन्ता, चौ0 चरण सिंह हरियाणा कृषि विश्वविधालय, हिसार, कार्यक्रम के मुख्य अतिथि थे। केन्द्र के निदेशक डा. राजकुमार सिंह ने अपने सम्बोधन में भ्रष्टाचार को समाप्त करने के लिए विभिन्न कार्य बिन्दुओं एवं सुझावों से अवगत कराया तथा इस सामाजिक कुरीति को सगंठित होकर उखाड़ फैकने का आह्वान किया। मुख्य अतिथि ने अपने सम्बोधन में भ्रष्टाचार के उन्मुलन के लिये पहला कदम अपने घर से ही उठाने का आह्वान किया।

सतर्कता जागरूकता सप्ताह कार्यक्रम

सतर्कता जागरूकता सप्ताह के अवसर पर शपथ लेते हुए केन्द्र के कर्मचारी एवं अधिकारी

कौमी एकता सप्ताह का आयोजन

राष्ट्रीय अश्व अनुसंधान केन्द्र में नवम्बर 19–25, 2010 तक कौमी एकता सप्ताह (राष्ट्रीय एकता सप्ताह) मनाया गया। आर्य समाज मंदिर के श्री आनंद मुनि जी ने राष्ट्रीय एकता दिवस के उपलक्ष्य पर अपने विचारों से सभा को सम्बोधित किया। नवम्बर 22, 2010 को भाषाई सद्भावना दिवस के उपलक्ष्य पर विभिन्न भाषाओं में कविता पाठ का आयोजन किया गया। जिसमें वैज्ञानिक डा. शणमुगसुंदरम्, डा. बिधान चंद्र बेरा, डा. तरुणा आनन्द, डा. राजेश कुमार वैद एवं टी रियेश ने क्रमशः तमिल, बांगला, मलयालम एवं हिन्दी भाषाओं में कविता पाठ कर अनेकता मे एकता का परिचय दिया। नवम्बर 25, 2010 को पर्यावरण संरक्षण दिवस के उपलक्ष्य पर केन्द्र के निदेशक महोदय ने अपने विचार प्रकट किये। इस अवसर पर वृक्षमित्र समिति, हिसार के सदस्य भी उपस्थित थे।

सद्भावना दिवस के उपलक्ष्य पर कविता पाठ का आयोजन

NRCE in News

हिसार में पकड़ी गई वायरस की चालाकी

पैराप्लाजमोसिस ट्यूनिंग सेंटर खुला

बीकानेर में बनेगा अश्व अभ्यारण्य

ईएचवी से लड़ने के लिए वेक्सीन तैयार

हिम्मत ही मेरा ...

इंसार : राष्ट्रीय अश्व अनुसंधान केंद्र की स्थापना के रजत जयंती समारोह में करतब दिखाता जीद का अश्व पालक महेश लर्मा। उसने अपने जबड़े में कड़े अभ्यास से वह कर दिखाया कि लोग दांतों तले उंगली दबाने पर मजबूर हाथ में तिरंगा थामे दो घोड़ों की सवारी करता पालक। गुलशन

बीमार घोड़ों से इंसानों को बचाना होगा आसान

राष्ट्रीय अश्व अनुसंधान केंद्र के वैज्ञानिक ग्लैंडर्स का मॉलीक्यूलर टेस्ट तैयार करने के करीब

हॉर्स फ्लू का टीका ईजाद

अब खच्चर व गधे चलाएंगे खेतों में हल

ग्लैंडर्स के कारण स्थगित हुआ पुलिस हार्स शो

12 से 19 फरवरी तक चूरे के मुरादाबाद में होना था आयोजन

Visit of Dignitaries

- Prof Utpal Tatu, Prof. of Biochemistry Department of Biochemistry, Indian Institute of Science, Banglore visited NRCE on August 31, 2010. He delivered lecture on "Proteomics of Infectious Diseases: Applications in Biomarker Identification, Diagnostics and Drug Development" under collaborative program on Trypanosomosis.
- Dr Sanjay Dhar, Director Research, Aesthetic and Plastic Surgery Institute, University of California, USA visited NRCE on September 18, 2010 and delivered a lecture on "Nerve Tissue Engineering: Bench-to-Bedside"

Dr Sanjay Dhar delivering lecture at NRCE

- Prof K. M. L. Pathak DDG (AS) and Dr Gaya Prasad ADG (AH) visited NRCE on September 21, 2010 on the occasion of 1st Annual Scientific Meet of Veterinary Type Cultures Network Project. Prof Pathak appreciated the progress of work at VTCC and exhorted the VTCC team to still work hard towards enhancing the output in terms of repository establishment and its strengthening on war footing. Dr Gaya Prasad desired that the microbial repository infrastructure of VTCC Network Units should be strengthened in the XII plan.
- Dr Peter Mertens, Vector-Borne Diseases Programme Institute for Animal Health, Pirbright, UK. He visited various laboratories and equine shed complex of NRCE on November 29, 2010. He also

Dr Peter Mertens at NRCE Hisar

interacted with scientists of the Centre.

- Dr S. Ayyappan, Hon'ble Director General (ICAR) and Secretary (DARE) visited NRCE on 9th December, 2010. He was accompanied by Prof. K.M.L. Pathak, DDG (AS) and Dr C.S. Prasad, ADG (AN). On this occasion, plantation was done by Dr S. Ayyappan, Prof K.M.L. Pathak, Dr C.S. Prasad in the VTCC premises. Hon'ble DG and Secretary DARE visited Animal Shed Complex, farm section, BSLIII, ATIC and different laboratories of the Centre. On this occasion Dr S. Ayyappan inaugurated Visitors' Room and Kisan Call Centre at NRCE, Hisar. He also interacted with the various scientists about ongoing research activities and commended the technologies developed by the Centre. Hon'ble DG and Secretary DARE also visited the VTCC new building and

Hon'ble DG ICAR at Animal Shed

Hon'ble Director General at VTCC new building at NRCE Hisar

addressed the Scientists from NRCE and CIRB in a joint session at NRCE.

- Prof Sagar M Goyal, Prof. University of Minnesota, USA visited NRCE on December 20, 2010 and interacted with scientists and delivered a lecture on "Role of Biotechnology in the Detection and Control of Animal Pathogens".

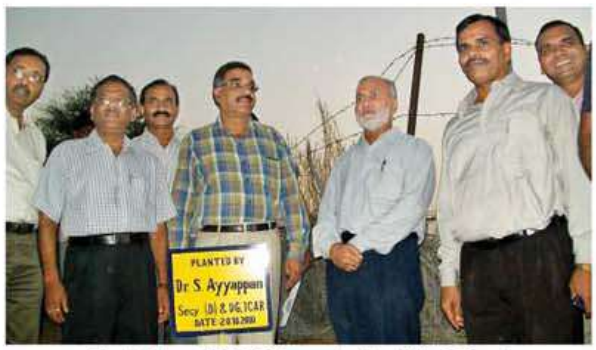
Dr Sagar M Goyal delivering lecture at NRCE

At EPC/NRCE, Bikaner

- Deputy Director Generals from ICAR [Dr Swapan K Datta, DDG(AS); Dr H P Singh, DDG (Hort); Dr K D Kokate, DDG (Ag. Extn); Dr A K Singh, DDG (NRM); Dr M M Pandey, DDG (Engg); Dr Arvind Kumar, DDG (Edn)], Dr Bangali Baboo, National Director (NAIP); Dr T.P. Trivedi, Project Director (DKMA); Dr JS Samra, Chief Executive Officer, National Rainfed Area Authority, New Delhi and Vice Chancellors from SAUs (Dr A M Sheikh, VC, AAU, Anand, Dr N C Patel, VC, JAU, Junagadh) visited Equine Production Campus, Bikaner on October 22-23, 2010, respectively. Dr R.K. Singh, Director, NRCE accompanied the dignitaries and explained in detail the status of livestock and ongoing research programmes at this Centre.

- Dr S. Ayyappan, visited Bikaner sub-campus of NRCE on October 20, 2010. On this occasion, horse/donkey/mule - driven electricity production unit was inaugurated. He was apprised about the ongoing research activities at the Centre and the research vision for the XIIth Five Year Plan. Prof K.M.L. Pathak, DDG (AS), Dr C.S. Prasad, ADG (ANP) and senior officers from ICAR also graced the occasion. Dr R.K. Singh, Director, NRCE briefed on the achievements and the future plan of this Centre to Hon'ble DG, Dr S. Ayyappan and the dignitaries. On this occasion, Dr S. Ayyappan also interacted with scientists.

Hon'ble Director General interacting with scientists at EPC Bikaner



Plantation at EPC Bikaner by Hon'ble DG and Secretary DARE, Dr S. Ayyappan

Director NRCE briefing dignitaries

Infrastructure & Developmental Activities

Infrastructure Development at VTCC

The developmental works at Veterinary Type Culture Centre's new premises have gained momentum. The first phase building of the VTCC costing ₹ 1.49 crores has been completed and taken over from CPWD. However, to make it functional and initiate the working in the new building, interior furnishing of the building is also being taken up and is likely to be completed shortly. The boundary wall, parking, internal approach road and security post have also been developed at the cost of ₹ 73.00 Lakhs and has been taken over. The agreement with Hospital Services Consultancy Corporation (under Ministry of Health & Family Welfare, GOI) for development of BSL-III laboratories for VTCC with a provision of about Rs 8.80 crores is under processing and the draft agreement is being considered by the Council. The process for development of second phase of VTCC building has also been initiated. The expression of interest for the developmental works has been called for and the draft agreement for the same is due for vetting at the Council. The agricultural land reclamation process was initiated with admirable success. New equipments worth ₹ 32.00 Lakhs have also been added in VTCC inventory, thereby, strengthening the working of VTCC. In the meantime, activities of VTCC have been keeping pace in the existing laboratories, despite constraint of space. The microbes of animal origin originated from various sources have been isolated, characterized and preserved following authentication. Accession numbers have been assigned to 358 Veterinary microbes including 103 viruses and 255 bacteria along with 169 recombinant clones and 334 microbes including 79 viruses, 255 bacteria and 164 clones have been added to the repository during the year. The team investigated various outbreaks including that of zoonotic buffalopox in cows, buffaloes and humans and bovine papillomatosis in cattle. Besides, the Centre acquired 158 microbes from other network units working with VTCC.

Construction of BSL-3 Laboratory completed

The work on the construction of biosafety level 3 laboratory at Hisar campus has been completed. The internal validation of the laboratory has been done and the process of external validation of the facility is under progress. The laboratory has been constructed at a cost of ` 4.0 crores including the biological liquid effluent decontamination plant. The laboratory after validation is likely to be handed over to the Centre very soon. The BSL-3 laboratory will cater to the need of working on highly infectious animal diseases that can spread rapidly from animals to animals and also on diseases that can be transmitted from animals to humans.

Dr Ayyappan, DG ICAR during his visit to the laboratory on December 9, 2010 appreciated the state-of-the-art facilities in the laboratory and highlighted that the facility will pave the way for research on emerging and exotic animal pathogens.

Agriculture Farm Production

Production of crops

During the period, a total of 1926 quintal of green fodder, 130 Qt. dry fodder and 300 Qt. of grains of different crops were produced (Table). In spite of high water table and salinity in most of the farm area, vigorous efforts were made to produce maximum feed & fodder. The efforts put in this activity not only resulted in self-sufficiency of the Centre in terms of fodder requirement but surplus yield was also utilized for revenue generation. A sum of ₹ 2, 45, 640.00 was generated through the sale of 127 Qt. of oat grain produced in previous year.

At our Equine Production Campus, Bikaner; 1446.91 Qt. of green fodder, 340 Qt. of dry chaffed bajra, 60 Qt. dry (sewan) fodder along with 4 Qt. Barley grain and 13 Qt. of barley bhusa was produced.

Crop production at Agriculture Farm, NRCE, Hisar (2010-11)

Name of Crop	Cultivated Area (Acre)	Quantity (Qt)
Oat	2	116
Berseem	2.5	88
Lucern	1.5	154
Maize	5	158
SSG + Cowpea	19	891
Bajra	1	93
Oat + Berseem	4	426
Total Green fodder	**35**	**1926**
Other Produce		
Oat grain	26	150 (approx)
Oat straw		130
Mustard	30	150 (approx)

Crop production at Agriculture Farm, EPC/NRCE, Bikaner (2010-11)

Name of Crop	Quantity (Qt)
Barley grain	4
Total dry fodder	413
Total green fodder	1446.91

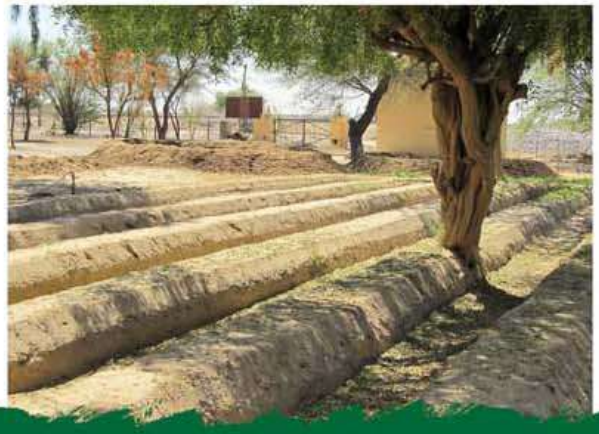
Reclamation, Development and Plantation work

About fifty acres of land behind the animal shed was developed for cultivation of crops. Different species of plants were planted in this area to develop agri-silvi-pasture system of agro-forestry. Mustard crop was cultivated in thirty acres of land in this area for field reclamation which led to revenue generation as well.

Lawns were developed within the Centre's premises adjacent to the generator room, animal shed, ATIC, VTCC buildings for improving the ecosystem. Air layering and cutting methods were used to propagate and plant different species of plants at NRCE Campus Hisar and EPC, Bikaner. In addition, different species of flowering, ornamental and shady plants were also planted in the campus premises.

Vermi-composting

Animal wastes are mainly used for making compost to apply in crop fields for fertilization, supplement organic matter, and improve soil conditions for better yield. Animal wastes are also responsible for environmental pollution if not handled/disposed off properly. Horse manure contains plenty of fibre, after decomposition it become an excellent soil conditioner. Composting is the process whereby naturally occurring microbes break down organic matter. At Equine Production Campus,



Bikaner, we are maintaining about 100 horses and donkeys and since many years the dung was not utilized for any purpose and dumped at nearby areas of stables. Equine dung-due to low moisture content and poor absorption of moisture-does not decompose properly and it cannot be utilized directly as manure in crops. Keeping in view the sufficient availability of equine dung for composting and problem of disposal of equines dung from farm, we initiated the trial of simple composting in pit and vermicomposting in trenches to explore the possibility of composting and vermicomposting at our farm. During the period under report, two batches of vermicompost was prepared and a total of 150 bags (50 Kg. each) were prepared. About 300-400 quintals of simple compost in six trenches was also prepared for use in plants, lawns and fodder crop fields.

Livestock

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

Herd Strength at Equine Production Campus, Bikaner (2010-11)

Category	Sex	Horses		Ponies		Donkeys		Mules	Total
		Marwari	Kathiawari	Zanskari	Indigenous	Exotic	Indigenous		
Stock as on 01.4.2010	M	16	01	04	-	13	-	03	
	F	36	-	07	03	16	-	02	101
Births during the year	M	05	-	03	-	-	-	-	
	F	08	-	-	-	01	-	-	17
Purchased during the year	M	-	-	-	-	-	14	-	
	F	-	-	-	-	-	07	-	21
Deaths during the year	M	-	-	-	-	-	-	-	
	F	-	-	-	-	-	-	-	00
Sold during the year	M	-	-	-	-	01	-	-	
	F	-	-	-	-	-	-	-	01
Balance as on 31.3.2011		**65**	**01**	**14**	**03**	**31**	**21**	**05**	**140**

Herd strength at Main Campus, Hisar (2010-11)

Category	Horses		Non-descript ponies	Mules	Poitou Donkeys	Total
	Marwari	Others				
Adult Male	0	1	0	2	2	5
Adult Female	13	0	2	0	3	18
0-3 years	07	0	2	0	1	10
Total	20	1	4	2	6	33

Personnel Milestones

New Joining

- Dr Sanjay Kumar Ravi, Scientist (Animal Reproduction & Gynaecology) joined the Centre on 23.4.2010.
- Dr Riyesh T., Scientist (Vety. Microbiology) joined VTCC, NRCE, Hisar on 30.4.2010.
- Dr Praveen Malik, Pr Scientist (Vety. Microbiology) joined VTCC, NRCE, Hisar on 20.9.2010.
- Sh A.K. Maithani, joined as A.O. on 12.11.2010.

Promotions

- Sh Pratap Singh, LDC has been notionally promoted to the post of UDC w.e.f. 23.3.2004.
- Sh D.D. Sharma, LDC has been promoted to the post of UDC w.e.f. 26.6.2010.
- Sh Deepak Kumar, SSS has been appointed to the post of LDC w.e.f. 17.7.2010.
- Sh Rampal, Asstt has been promoted to the post of AAO w.e.f. 23.9.2010.
- Sh Ashok Arora, Steno Gr.-III has been promoted to the post of PA w.e.f. 23.9.2010.
- Sh Pratap Singh, UDC has been promoted to the post of Asstt. w.e.f. 18.12.2010.
- Sh Om Prakash, LDC has been promoted to the post of UDC. w.e.f. 18.12.2010.

Selection/appointment

- Dr Jitender Singh has been appointed to the post of T-6 (Cat.-III), Farm Manager (Livestock) w.e.f. 22.1.2011 at EPC, Bikaner.

Transfers

- Dr Niranjan Lal, Scientist (Vety. Extension) was relieved on 17.5.2010 to enable him to join Central Avian Research Institute, Izatnagar.
- Sh R.A. Parashar, AF&AO was relieved on 31.3.2011. He has been promoted to the post of Finance & Accounts Officer at NIASM, Baramati.
- Sh A.K. Maithani, A.O. was relieved on 03.02.2011 to enable him to join at Project Directorate on Horticulture, New Delhi.

Study Leave

- Sh Manoj Kumar, T-3 (Lab Tech.) has been granted study leave to undergo Ph.D. for a period of three years w.e.f. 13.1.2011 to 12.1.2014.

Superannuation

- Dr S.N. Tandon, Pr. Scientist retired from Council's service upon superannuation on 31.10.2010.

Awards and Recognitions

- Dr Anju Manuja, Senior Scientist, has been awarded Bharat Jyoti award and Certificate of Excellence by IIFS, New Delhi for "Meritorious achievements and outstanding performance" at seminar on "Global Participation in India's Economic Development" held at New Delhi, on May 24, 2010.
- Dr Balvinder Kumar Manuja, Senior Scientist, has been bestowed with Dr. V.D. Kashyap and Ms. Manju Utreja Gold medals for Ph D research by CCS Haryana Agricultural University, Hisar in convocation held in December, 2010.
- Dr B.R. Gulati, Principal Scientist has been sanctioned a research project by DBT with a grant of

₹ 21.87 lakh on the Research Project "Isolation and characterization of animal adenoviruses for development of a novel viral vector for vaccine delivery".

- Dr B.R. Gulati, Principal Scientist, was bestowed with ISVIB Mid-Career Scientist Award-2010 in recognition to the research work entitled "Isolation and genetic characterization of Japanese encephalitis virus from equines in India" in: XVII National Convention of Indian Society for Veterinary Immunologists and Biotechnologists (ISVIB), RAU, Bikaner, December 29-31, 2010.
- Dr Parveen Malik, H. Singha, S.K. Khurana & R.K. Singh have been sanctioned a research project "Studies on *Burkholderia mallei* for rapid diagnosis of glanders in equines using molecular tools" by Life Sciences Research Board, DRDO, New Delhi with a grant of ₹ 25.12 Lakh.
- Dr Rajender Kumar, Senior Scientist, has been nominated as Member IMC, Central Avian Research Institute, Izatnagar for three years from September 17, 2010.
- Dr Rajender Kumar: awarded "National Fellow" by ICAR for "Development of monoclonal antibodies based assays for detection of *T. evansi* infection in equines."
- Dr R.K. Singh was conferred fellowships of Indian Society of Vety. Immunology & Biotechnology (April 8, 2010) at Veterinary College, Nammakkal (TN) and Academy of Environment (Sept. 29, 2011) DDCWFR, Bhimtal, Nainital (Uttarkhand).
- Dr R.K. Vaid, conferred upon the Award of "Fellow of Society for Immunology and Immunopathology" in recognition of outstanding contributions in the field of Immunology and Immunopathology at Vth Convention of Society for Immunology and Immunopathology and National Symposium on Immunobiotechnology organised at Institute of Biotechnology, G B Pant University of Agriculture and Technology, Patwadangar, Nainital (Uttarakhand) from December 17-19, 2010.
- Dr Sanjay Kumar, Rajender Kumar and R.K. Singh awarded OIE Twinning Laboratory project in equine Piroplasmosis between NRCPD, Obihiro, Japan and NRCE, Hisar

Staff at NRCE

Director: Dr R. K. Singh

Scientists at NRCE, Hisar

1. Dr A. K. Gupta, Principal Scientist
2. Dr B. K. Singh, Principal Scientist
3. Dr S. C. Yadav, Principal Scientist
4. Dr Baldev R. Gulati, Principal Scientist
5. Dr Rajender Kumar, National Fellow, ICAR
6. Dr S. K. Khurana, Senior Scientist
7. Dr Nitin Virmani, Senior Scientist
8. Dr Sanjay Kumar, Senior Scientist
9. Dr Mamta Chauhan, Senior Scientist
10. Dr Anju Manuja, Senior Scientist
11. Dr H. Singha, Scientist
12. Dr Anuradha Bhardwaj, Scientist
13. Dr A.A. Raut, Scientist

Scientists at EPC, Bikaner

1. Dr Yash Pal. Senior Scientist
2. Dr R. C. Sharma, Senior Scientist
3. Dr R. A. Legha, Senior Scientist
4. Dr Balvinder Kumar, Senior Scientist
5. Dr A. Arangasamy, Scientist
6. Dr Ramesh Dedar, Scientist
7. Dr P.A. Bala, Scientist
8. Dr T. Rao Talluri, Scientist
9. Dr Sanjay Kumar Ravi, Scientist

Scientists at VTCC, Hisar

1. Dr Praveen Malik, Principal Scientist
2. Dr Sanjay Barua, Senior Scientist
3. Dr R. K. Vaid, Senior Scientist
4. Dr Mamta Tigga, Scientist
5. Dr B.C. Bera, Scientist
6. Dr Shanmugasundaram K., Scientist
7. Dr Sarita Yadav, Scientist
8. Dr Taruna Anand, Scientist
9. Dr Riyesh T., Scientist

Technical Staff at EPC, Bikaner

1. Dr Jitender Singh, T-6 Veterinary Officer
2. Sh K.K. Singh, T-5 Technical Officer
3. Sh Brij Lal, T-5 Livestock Assistant
4. Sh N.K. Chauhan, T-4 Farm Technician
5. Sh Om Prakash, T-3 Tractor Driver
6. Sh S.N. Paswan, T-2 Livestock Assistant
7. Sh Rajendra Singh, T-2 Lab. Assistant
8. Sh Gopal Nath, T-1 Vehicle Driver

Technical Staff at VTCC, Hisar

1. Sh Manoj Kumar, T-3 Lab. Technician

Technical Staff at NRCE, Hisar

1. Sh R.K. Chaturvedi, T-6 Technical Officer
2. Sh K.S. Meena, T-5 Farm Manager
3. Sh P.P. Chaudhary, T-5 Lab. Technician
4. Sh Ajmer Singh, T-5 Livestock Assistant
5. Sh D.D. Pandey, T-5 Lab. Technician
6. Sh Sita Ram, T-5 Lab. Technician
7. Sh S.K. Chhabra, T-5 Lab. Technician
8. Sh Joginder Singh, T-3 Lab. Technician
9. Sh Mukesh Chand, T-3 Lab. Technician
10. Sh Raj Kumar Dayal, T-3 Lab. Technician
11. Sh Sajjan Kumar, T-3 Driver
12. Sh Suresh Kumar, T-3 Driver
13. Sh Arun Chand, T-2 Tractor Driver
14. Sh Raghubir Singh T-1, Driver

Administrative Staff at NRCE, Hisar

1. Sh A.K. Maithani, AO (12.11.10-03.02.11)
2. Sh R.A. Parashar, AFAO
3. Sh Hawa Singh, AAO
4. Sh Ram Pal, AAO
5. Sh S.P. Kaushik, Assistant
6. Sh Subhash Chander, Assistant
7. Sh Ashok Arora, PA
8. Sh Pratap Singh, UDC
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 Satbir Singh
11. Sh Hanuman Singh
12. Sh Subhash Chander
13. Sh Ishwar Singh
14. Sh Ram Singh
15. Smt Ram Kali
16. Smt Santra
17. Sh Sant Ram
18. Smt Soma Devi

Supporting Staff at EPC, Bikaner

1. Sh Raju Ram
2. Sh Mahabir Prasad

List of Ongoing Research Projects

Equine Health (2010-11)

S.No.	Title	Team	Date of Start	Date of Completion
1.	Development of vaccine(s) against equine herpes virus-I infection.	B.K. Singh*, B.R. Gulati & N. Virmani	June, 2003	March, 2010; Extended up to March, 2011
2.	Development of sensitive and specific diagnostics for Japanese encephalitis in Equines	Baldev R. Gulati*, B.K. Singh, N. Virmani & H. Singha	Oct., 2006	March, 2010; Extended up to March, 2011
3.	Surveillance, Monitoring and Control of Emerging and Existing Diseases of Equines	R.K. Singh*, B.K. Singh, S.K. Khurana, S.C. Yadav, Baldev R. Gulati, Rajender Kumar, P. Malik, Sanjay Kumar, Nitin Virmani, Sanjay Barua, Rajesh Kumar Vaid, A. Arangasamy & Ramesh Dedar	April, 1995	Continuous Service Project
4.	Cysteine proteinase, a defined antigen of *T. evansi* for control of trypanosomosis	S.C. Yadav*, Rajender Kumar, Sanjay Kumar & A.K. Gupta	Sept., 2008	Aug., 2010; Extended up to Aug., 2011
5.	Genetic and antigenic differentiation of equine influenza viruses	B. K. Singh*, Nitin Virmani, B. C. Bera, B. R. Gulati, K Shanmugasundaram. & R. K. Singh	Sept., 2009	Aug., 2012
6.	Development of diagnostics for equine influenza	Nitin Virmani*, Baldev. R.Gulati, Bidhan C. Bera, B.K.Singh & R.K.Singh	Sept., 2009	Aug., 2012
7.	Evaluation and Updation of the inactivated equine influenza virus vaccine	R.K. Singh*, Baldev R. Gulati, Nitin Virmani, A.K. Gupta & B.K. Singh	Oct., 2009	Sept., 2012
8.	Diversity of Mx gene and association of polymorphic markers with susceptibility vis-à-vis resistance against Equine Influenza	Balvinder Kumar*, R.C. Sharma, Anju Manuja & R.K. Singh	Sept., 2009	Aug., 2012
9.	Development of monoclonal antibodies and recombinant antigens based assays for detection of *Trypanosoma evansi* infection in equines	Rajender Kumar*, S.C. Yadav, Sanjay Kumar & Baldev R. Gulati	Sept., 2010	Sept., 2012
10.	Characterization of Toll-like receptor 9 and its role in CpG immuno-modulation in equines	Anju Manuja*, Balvinder Kumar, Sanjay Kumar, H. Singha & R.K. Singh	Oct., 2010	Oct., 2013
11.	Development of recombinant protein-based immune-diagnostic kit for equine infectious anemia (EIA)	R.K. Singh*, Praveen Malik, H. Singha & Sameer Srivastava	Sept., 2010	Aug., 2012
12.	Development of targeted drug release therapeutics using nanoparticles in equine medicine	Anju Manuja*, Neeraj Dilbaghi, Sandeep Kumar, Rajender Kumar, Balvinder Kumar & S.C. Yadav	Oct., 2010	Oct., 2013
13.	Studies on donkey lactoferrin and its therapeutics application	Sanjay Gupta*, A.K. Gupta, T.P. Singh, R. Kumar, Rajesh Vaid, Anju Manuja & R.K. Singh	Sept., 2009	Aug., 2010

Equine Production (2010-11)

S.No.	Title	Team	Date of Start	Date of Completion
1.	Molecular characterization of indigenous breeds of horse for genetic diversity within and between different breeds.	A.K. Gupta* & Anuradha Bhardwaj	Oct., 2006	March, 2011
2.	Characterization of indigenous non-descript and geographically distinct donkeys	A.K. Gupta*, Yash Pal, R.C. Sharma, Anuradha Bhardwaj & Sanjay Kumar	Aug., 2010	July, 2014
3.	Enhancing reproductive efficiency in horses through semen cryopreservation and embryo transfer technologies	A. Arangasamy*, Thirumala Rao Talluri, Sanjay Kumar Ravi, Yash Pal & Jitendar Singh	Sep., 2009	Aug., 2011
4.	Studies on class I and II genes of Major Histocompatibility Complex in donkeys	R. C. Sharma*, Balvinder Kumar & A. K. Gupta	April, 2010	March, 2013
5.	Draughtability studies and utilization of equine (mule and donkey) energy in agricultural operations including transport (AICRP)	Yash Pal*, R.A. Legha, P. A. Bala, N. Lal & A.K. Gupta	April, 2009	March, 2011
6.	Cloning, Expression and characterization of equine chorionic gonadotropin (eCG)	Anuradha Bhardwaj*, A.K. Gupta, Sanjay Kumar & Varij Nayan	Dec., 2010	Nov., 2013

Extension (2010-11)

S.No.	Title	Team	Date of Start	Date of Completion
1.	A study on existing management systems and utilization of donkeys and mules for sustainable livelihood.	Rajender Kumar*, A.A. Raut, Yash Pal & R.A. Legha	Sept., 2009	Aug., 2011

VTCC (2010-11)

S.No.	Title	Team	Date of Start	Date of Completion
1.	Isolation, maintenance and characterization of bacterial pathogens and their molecular identification	R. K. Vaid*, Sanjay Barua, Mamta Tigga, Shanmugasundaram, K., B.C. Bera & Taruna Anand	June, 2007	May, 2010; Extended up to May, 2011
2.	Isolation, molecular characterization and reposition of viruses of animal origin	R.K. Singh*, Sanjay Barua, B.C. Bera, R. K. Vaid, Shanmugasundaran, K., B.R. Gulati, T. Riyesh & Sarita Yadav	Sep., 2009	Aug., 2012

*Principal Investigator

Participation in Trainings, Conferences & Symposia

Participation in Trainings

1. R.C. Sharma, Senior Scientist, participated in Workshop on "Inventorization and Documentation of Local Specific Problems Requiring S&T Intervention" organized by Department of Science & Technology, Govt. of Rajasthan on April 23, 2010 at CVAS, Bikaner.
2. A.K. Gupta, Principal Scientist, **a**ttended FAO regional training workshop on "*In vivo* Conservation of Animal Genetic Resources" at NASC complex organized by NBAGR, Karnal from October 28-30, 2010.
3. B.R. Gulati, Principal Scientist, participated in sensitization training on "Soft Computing techniques in Animal Bioinformatics" at NBAGR, Karnal from November 8-12, 2010.
4. T. Rao Talluri, Scientist, attended 21 days "Hands on Training on Techniques for Assessing Ovarian Follicular function in Cattle and Buffalo" at National Institute of Animal Nutrition and Physiology, Bangalore, from November 16 to December 6, 2010.
5. B.R. Gulati, Principal Scientist, participated in Partners' Meet of NAIP-NABG Project at NBAGR, Karnal on November 19, 2010.
6. R.A. Legha, Senior Scientist, attended workshop on "Present status and future strategies to improve the utility of donkeys and mules" held at CVAS, Bikaner on December 13, 2010.
7. R.C. Sharma, Senior Scientist, participated in Workshop on "Present Status and Future Strategies to Improve Utility of Donkeys and Mules" held at RUVAS, Bikaner on December 13, 2010.
8. Yash Pal, Senior Scientist attended a Workshop on "Present status and future strategies to improve the utility of donkeys and mules" held at CVAS, Bikaner on December 13, 2010.
9. Sanjay Kumar Ravi, Scientist, attended workshop on "Minitube Sperm Vision™ CASA", organized by Chemtron Instruments Pvt. Limited in collaboration with Minitub GmbH, Germany at Hotel Relax Inn, New Delhi on December 16, 2010.
10. Praveen Malik, Principal Scientist, attended 3 day training programme on "Biosafety-III protocols" at Microbial Containment Complex (MCC), National Institute of Virology, Pune from January 10-14, 2011.
11. B.R. Gulati, Principal Scientist, participated in "Preliminary Training for working in BSL-III laboratory" at Microbial Containment Complex, National Institute of Virology (ICMR), Pune, January 11-13, 2011.
12. B.C. Bera, Scientist, attended training on "Metagenomics: Methods and Applications in Microbiology" at National Bureau of Agriculturally Important Microorganisms, Kusmaur, Mau Nath Bhanjan, UP from January 11-20, 2011.
13. K.S. Meena, T-5 participated in the training course on Research Station management at ICRISAT, Patancheru (Andhra Pradesh) on January 17-22, 2011.
14. Balvinder Kumar Manuja, Senior Scientist, attended training on "Bioinformatics Resources and Tools for Agricultural Research" during January 24-29, 2011 under National Agricultural Bioinformatics Grid (NABG-NAIP) at IASRI, New Delhi.
15. Yash Pal, Senior Scientist, attended XIIth Workshop of "AICRP on increased utilization of animal energy with enhanced system efficiency" held at University of Agricultural Sciences, Raichur during February 4-6, 2011.
16. R.A. Legha, Senior Scientist attended XII workshop of AICRP on increased utilization of animal energy with enhanced system efficiency held at University of Agricultural Sciences, Raichur during February 4-6, 2011.
17. Anju Manuja, Senior Scientist attended Brain

Storming workshop on "Prospects of Nano-technology in agricultural chain" held at NAARM Hyderabad on February 22, 2011.

18. Sanjay Kumar Ravi, Scientist, attended 21 days training on "Advances in Diagnostic techniques in Veterinary Theriogenology" organized at GADVASU, Ludhiana from February 16 to March, 8 2011.
19. Balvinder Kumar Manuja, Senior Scientist, attended training on "Bioinformatics for Animal Genomics and Proteomics" from February 24 to March 9, 2011 under National Agricultural Bioinformatics Grid (NAIP) at National Bureau of Animal Genetic Resources, Karnal.
20. R.A. Legha, Senior Scientist attended one-day workshop on Patent awareness organized by Department of Science & Technology, Govt of Rajasthan, Regional Office, Bikaner and RAJUVAS, Bikaner on February 25, 2011.
21. R.C. Sharma, Senior Scientist, participated in Workshop on "Patent Awareness" organized by Department of Science & Technology, Govt. of Rajasthan on February 25, 2011 at RUVAS, Bikaner.
22. Yash Pal, Senior Scientist, attended one-day workshop on "Patent awareness" organized by Department of Science & Technology, Government of Rajasthan, Regional Office, Bikaner and RAJUVAS, Bikaner on February 25, 2011.
23. A.A. Raut, Scientist, attended training on Data Analysis using SAS under NAIP Consortium Program "Strengthening Statistical Computing for NARS", organized on March 7-12, 2011 by National Dairy Research Institute, Karnal at Central Institute for Research on Buffaloes, Hisar.
24. A. Bharadwaj, Scientist, participated in 21 days National Training Program on "Databases and Softwares for Analysis of Animal Genetics and Breeding Data", from March 10-30, 2011 at Centre of Advanced Faculty Training (Animal Genetics and Breeding), Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal.
25. Anju Manuja, Senior Scientist, participated in training on "Nanotechnology: Opportunities and Applications in Veterinary Sciences" at Punjab Agricultural University, Ludhiana, from March 7-12, 2011.
26. Sanjay Barua, Senior Scientist, participated in a workshop on Results-Framework-Document at Vigyan Bhawan, New Delhi on March 22, 2011 organized by the Performance Management Division, Cabinet Secretariat, New Delhi.
27. K.S. Meena, T-5 participated in the National training workshop-cum-travelling seminar on resource conservation technologies in different cropping system during March 24-27, 2011 at DHRM, CCSHAU, Hisar.

Participation in Symposia and Conferences

1. R.K. Singh, Director, presented a lead paper in the XVI Annual Convention of ISVIB and National Symposium on "Biotechnology and Immunology in Mitigation of Climate Change Impact" and acted as panelist in technical sessions, held at TANUVAS, Namakkal on April 08, 2010.
2. R.K. Singh, Director, panelist in Technical Session-II "Management of genetic resources: emerging issues" during National Consultation on Agro-biodiversity meeting held at the NASC, New Delhi on May 27, 2010.
3. R.C. Sharma, Senior Scientist, participated in Interactive Meet on "Conservation of Marwari and Kathiawari Horses" held at Equine Production Campus, NRCE, Bikaner on September 7, 2010.
4. R.K. Singh, Director, participated in Workshop on "National strategy for conservation of indigenous breeds of livestock" at Yojana Bhavan, New Delhi on September 28, 2010.
5. R.K. Singh, Director, delivered a lecture on September 29, 2010 in the National Consultation on "Biodiversity of High Altitude Aquatic Resources, Conservation & Utilization" held at Directorate of Coldwater Fisheries Research, Bhimtal from September 29-30, 2010.
6. R.K. Singh, Director, participated in National Symposium on "Climate Change and Livestock Productivity in India" from October 7-8, 2010 at NDRI, Karnal and presented a paper in the symposium. He also chaired a technical session on Zoonosis.
7. Anju Manuja, Senior Scientist, attended International Conference on Nanosensors & Technology at Central Scientific Instruments

Organization, Chandigarh from October 28-31, 2010.

8. Rajender Kumar, Senior Scientist, participated in XXII National Congress of Parasitology jointly organised by Indian Society for Parasitology and Department of Zoology, University of Kalyani, Kalyani from October 30 to November 1, 2010.
9. Sanjay Kumar Ravi, Scientist, attended and presented paper (oral) in International Symposium on "Biotechnologies for optimization of Reproductive Efficiency of Farm & Companion Animals to Improve Global Food Security & Human Health" and XXVI Annual Convention of ISSAR" at Pantnagar, U.S. Nagar, Uttarakhand, during November 10-12, 2010.
10. R.K. Singh, Director, participated in a Brain Storming Session on "Research Priorities of HSADL for the next Decade" as expert group member at HSADL, Bhopal on December 1, 2010.
11. R.K. Singh, Director, addressed the workshop on "Present status and future strategies to improve utility of donkey and mules" on December13, 2010 at Dept. of Animal Breeding and Genetics in College of Veterinary & Animal Sciences, Bikaner.
12. R.K. Vaid, Senior Scientist, attended Vth Convention of Society for Immunology and Immunopathology and National Symposium on Immunobiotechnology, at Institute of Biotechnology, G.B. Pant University of Agriculture and Technology, Patwadangar, Nainital (Uttarakhand) from December 17-19, 2010.
13. B.C. Bera, Scientist, participated in International Symposium on "Role of Biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" organized by ISVIB, from December 29-31, 2010 at Bikaner, India.
14. R.K. Dedar, Scientist, attended International Symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" & XVII Annual Convention of Indian Society of Veterinary Immunology & Biotechnology, held at Rajasthan University of Veterinary & Animal Sciences, Bikaner during 29-31 December, 2010.
15. B.R. Gulati, Principal Scientist, participated and presented a paper entitled 'Isolation and genetic characterization of Japanese encephalitis virus from equines in India' in XVII National Convention of Indian Society for Veterinary Immunologists and Biotechnologists, RAU, Bikaner, December 29-31, 2010.
16. Balvinder Kumar Manuja, Senior Scientist, participated in International Symposium on "Role of Biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" organized by ISVIB, from December 29-31, 2010 at Bikaner, India.
17. Anju Manuja, Senior Scientist, attended International Symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" & XVII Annual Convention of Indian Society of Veterinary Immunology & Biotechnology, held at Rajasthan University of Veterinary & Animal Sciences, Bikaner during December 29-31, 2010.
18. Sanjay Kumar Ravi, Scientist, attended and presented paper in International Symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" & XVII Annual Convention of Indian Society of Veterinary Immunologists & Biotechnologists, at College of Veterinary & Animal Science, RUVAS, Bikaner from December 29-31, 2010.
19. R.C. Sharma, Senior Scientist, participated in International Symposium on "Role of Biotechnology in Conserving Bio-diversity and Livestock Development for Food Security and Poverty Alleviation" held at RUVAS, Bikaner, during December 29-31, 2010.
20. R.K. Singh, Director, presented a lead paper on "Technology – led roadmap for livestock" during the ISVIB Conference held at RAJUVAS from December 29-31, 2010.
21. Rajender Kumar, Senior Scientist, participated in XXI National Congress of Veterinary Parasitology jointly organised by Indian Association for the Advancement of Veterinary Parasitology and Department of Veterinary Parasitology, Bombay Veterinary College, Mumbai, from January 5-7, 2011.
22. R.K. Singh, Director, participated in National Consultation on "Animal Disease Monitoring & Surveillance" on January 25, 2011 under the Chairmanship of Secretary, DARE & DG (ICAR) at New Delhi

23. Taruna Anand, Scientist, attended International Conference on Frontiers in Reproductive Biotechnology & 21st Annual Meeting of Indian Society for the Study of Reproduction and Fertility (ISSRF) from February 9-11, 2011 at NDRI, Karnal.
24. K.S. Meena, T-5 participated in Zonal Seminar on "Plant Stress Physiology in perspectives of Agri-Horticulture and Climate change" and presented a poster entitled "Weed control in soybean in rainfed conditions" at SKN College of Agriculture, Jobner, SKRAU, Bikaner, organized by Department of Plant Physiology and ISPP, New Delhi on February 25, 2011.

Trainings and Visits abroad

1. Sanjay Kumar, Senior Scientist, attended three months NAIP sponsored training in Bioinformatics, at Bio-informatics Institute, Singapore, from June 1 to August, 31 2010.
2. Sanjay Barua, Senior Scientist, attended a three months International training in Biosecurity (Animal Science) sponsored by National Agricultural Innovation Project, ICAR, N. Delhi at Veterinary Diagnostic Laboratory, Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, Minneapolis USA from October 27, 2010 to January 24, 2011.
3. R.K. Vaid, Senior Scientist, attended 3 month International training sponsored by National Agricultural Innovation Project, ICAR, N. Delhi at Department of Bioinformatics and Computational Biology, George Mason University, Manassas, North Virginia, USA in Bioinformatics (Animal Science) from January 17 to April 16, 2011.
4. R.K. Singh, Director, participated in training programme under "OIE twinning programme for Equine Piroplasmosis" between National Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan and National Research Centre on Equines, Hisar, India at Obihiro, Japan from February 19 to 24, 2011.
5. Rajender Kumar, Senior Scientist, participated in training programme under "OIE twinning programme for Equine Piroplasmosis" between National Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan and National Research Centre on Equines, Hisar, India at Obihiro, Japan from February 19 to March 20, 2011.
6. Sanjay Kumar, Senior Scientist visited NRCPD, Obihiro Japan under the capacity building program of "OIE twinning programme for Equine Piroplasmosis" as an expert from February, 19 to March 20, 2011.
7. Sanjay Kumar, Senior Scientist, attended a Feedback workshop at OIE, Paris, France from March 30-31, 2011 under OIE Laboratory Twinning Project on Equine Piroplasmosis between National Research Centre for Protozoan Diseases (NRCPD), Obihiro, Japan and National Research Centre on Equines, Hisar, India.

List of Publications

List of Published Papers

1. Arangasamy, A., Singh, J., Sharma, R.C., Singh, R.K., Bansal, R.S. and Tandon, S.N. 2010. Fetal resorption in a Poitu jenny with persistent corpus luteum. *Indian Veterinary Journal,* **87**:812-813.
2. Bansal, R.S., Yash Pal and Pareek, P.K. 2009. Ultrasonographic imaging for early pregnancy diagnosis in mares. *Indian Journal of Animal Reproduction,* **30**:52-53.
3. Bera, B.C., Shanmugasundaram, K., Barua, S., Venkatesan, G., Riyesh, T., Bhanuprakash, V., Gulati, B.R., Vaid, R.K., Virmani, N., Kakker, N.K., Malik, P., Bansal, M., Gadvi, S., Singh, R.V., Yadav, V., Sardarilal, Nagarajan, G., Balamurugan, V., Hosamani, M., Pathak, K.M.L. and Singh, R.K. 2010. Zoonotic cases of Camelpox infection in India. *Veterinary Microbiology,* doi: 10.1016 ld.vetmic. 2011.04.010.
4. Chauhan, M., Gupta, A.K. and Dhillon, S. 2010. Genetic diversity and population structure of three Indian horse breeds. *Molecular Biology and Reproduction,* doi: 10.1007/s11033-010-0461-z.
5. Gulati B.R., Singha H., Singh, B.K., Virmani, N., Khurana, S. K. and Singh, R.K. 2011. Serosurveillance for Japanese encephalitis virus infection among equines in India. *Journal of Veterinary Sciences* (Accepted).
6. Malik, P., Khurana, S.K. and Dwivedi, S.K. 2010. Re-emergence of Glanders in India-Report of Maharashtra state. *Indian Journal of Microbiology,* **50**:345-348.
7. Pallavi, R., Roy, N., Nageshan, R.K., Talukdar, P., Pavithra, S.R., Reddy, R., Venketesh, S., Kumar, R., Gupta, A.K., Singh, R.K., Yadav, S.C. and Tatu, U. 2010. Heat shock protein 90 as a drug target against protozoan infections: biochemical characterization of HSP90 from *Plasmodium falciparum* and *Trypanosoma evansi* and evaluation of its inhibitor as a candidate drug. *Journal of Biological Chemistry*, **285**:37964-37975.
8. Rao, T. Thirumala, Arangasamy, A., Singh, J., Singh, R.K. and Tandon S.N. 2010. Scrotal Biometry in Marwari Stallions. *Indian Veterinary Journal,* **87**:1059-1060.
9. Rao, T. Thirumala, Singh, J. and Ravi, S.K. 2010. Detection of Postpartum Endometritis in a Marwari mare and its treatment. *The Indian Veterinary Journal* (Accepted).
10. Roy, N., Nageshan, R.K., Rani, Pallavi, Chakravarthy, H., Chandran, S., Rajender Kumar, Gupta, A.K., Singh, R.K., Yadav, S.C. and Tatu, U. 2010. Proteomics of *Trypanosoma evansi* infection in Rodents. *PLoS ONE,* **5**:1-10.
11. Singh, B.R., Chauhan, M., Sindhu, R.K., Gulati, B.R., Khurana, S.K., Singh, B., Singh, H.S. and Yadav, R.P. 2010. Diseases prevalent in equids in India: a survey of veterinary practitioners. *Asian Journal of Animal and Veterinary Advances,* **5**:1-10.
12. Singh, B.R., Gulati, B.R., Virmani, N. and Chauhan, M. 2010. Outbreak of abortions and infertility in thoroughbred mares associated with waterborne *Aeromonas hydrophila*. *Indian Journal of Microbiology,* **51**:212-216.
13. Vaid, R.K. 2010. Prokaryotic Wealth. Correspondence, In *Current Science*, **98**:8.
14. Virmani, N., Bera, B.C., Gulati, B.R., Shanmugasundaram, K, Singh, B.K., Vaid, R.K., Kumar, S., Kumar, R., Malik, P., Khurana, S.K., Singh, J., Manuja, A., Dedar, R., Gupta, A.K., Yadav, S.C., Chugh, P.K., Narwal, P.S., Thakur, V.L.N., Kaul, R., Kanani, A., Rautmare, S.S. and Singh, R.K. 2010. Descriptive epidemiology of equine influenza in India (2008-2009): temporal and spatial trends. *Veterinaria Italiana*, **46:**449-458.
15. Virmani, N., Bera, B.C., Shanumugasundaram, K., Singh, B.K., Gulati, B.R. and Singh, R.K. 2011. Genetic analysis of the matrix and non-structural genes of equine influenza virus (H3N8) from epizootic of 2008-09 in India. *Veterinary*

Microbiology, doi: 10.1016 Id.vetmic.2011.04.011

16. Yadav, S.C., Kumar, R., Kumar, S., Tatu, U., Singh, R.K. and Gupta, A.K. 2011. Identification and characterization of cysteine proteinases of *Trypanosoma evansi*. *Parasitology Research*. doi : 10.1007/s00436-011-2284-9.
17. Yash Pal, Arangasamy A., Legha, R.A., Singh J., Bansal, R.S., Khurana, S.K. and Tandon, S.N. 2011. Freezability and Fertility of Marwari Stallion Semen. *Indian Journal of Animal Sciences* (Accepted).
18. Yash Pal, Legha, R.A. and Khurana, S.K. 2010. An easy technique for cryopreservation of stallion semen under field conditions. *Indian Veterinary Journal* (Accepted).

List of publications in affiliation/ collaboration with other institutes/ organizations

19. Balamurugan, V., Sen, A., Venkatesan, G., Yadav, V., Bhanuprakash, V. and Singh, R.K. 2010. Isolation and identification of virulent *peste des petits ruminants* viruses from PPR outbreaks in India. *Tropical Animal Health and Production,* **42**:1043–1046.
20. Balamurugan, V., Sen, A., Venkatesan, G., Yadav, V., Bhanot, V., Riyesh, T., Bhanuprakash, V., and Singh, R.K. 2010. Sequence and phylogenetic analyses of the structural genes of virulent Isolates and vaccine strains of *peste des petits ruminants* virus from India. *Transboundary and Emerging Diseases,* **57**:352-364. (DOI: doi:10.1111/j.1865-1682.2010.01156.x).
21. Balamurugan, V., Sen, A., Venkatesan, G., Yadav, V., Bhanot, V., Bhanuprakash, V. and Singh, R.K. 2010. Application of Semi-quantitative M Gene-Based Hydrolysis Probe (TaqMan) Real-Time RT-PCR Assay for the Detection of *Peste des petitis ruminants* virus in the clinical samples for investigation into clinical prevalence of disease. *Transboundary and Emerging Diseases,* **57**:383-395. (DOI: doi:10.1111/j.1865-1682.2010.01160.x).
22. Bhanuprakash, V., Venkatesan, G., Balamurugan, V., Hosamani, M., Yogisharadhya, R., Chauhan, R.S., Pande, A., Mondal, B. and Singh, R.K. 2010. Pox outbreaks in sheep and goats at Makhdoom (Uttar Pradesh), India: evidence of sheeppox virus infection in goats. *Transboundary and Emerging Diseases,* **57**:375-82.
23. Bhanuprakash, V., Balamurugan, V., Hosamani, M., Venkatesan, G., Chauhan, Bina, Srinivasan, V.A., Chauhan, R.S., Pathak, K.M.L. and Singh, R.K. 2010. Isolation and characterization of Indian isolates of camel pox virus. *Tropical Animal Health and Production,* **42**:1271–1275.
24. Bhanuprakash, V., Venkatesan, G., Balamurugan, V., Hosamani, M., Yogisharadhya, R., Gandhale, P., Reddy, K.V., Damle, A.S., Kher, H.N., Chandel, B.S., Chauhan, H.C. and Singh, R.K. 2010. Zoonotic Infections of Buffalopox in India. *Zoonoses and Public Health,* **57**:e149-e155 (DOI: 10.1111/j.1863-2378.2009.01314.x).
25. Chandranaik, B.M., Singh, R.K., Hosamani, M., Krishnappa, G., Harish, B.R., Chethana, C.S. and Renukaprasad, C. 2010. Comparative sequence analysis of B5R gene of zoonotic buffalo pox virus isolates with other orthopoxviruses. *Tropical Animal Health and Production,* **43**:287-290.
26. Chaturvedi, U., Kalim, Shahina., Kumar, R., Sawant, P., Tiwari, S., Khurana, S. K., Sahoo, A. P., Palia, S. and Tiwari, A. K. 2010. Cloning and expression of chicken granulocyte-macrophage colony stimulating factor (GMCSF) gene. *Indian Journal of Experimental Biology,* **48**:1175-1180.
27. Chugh, M., Gulati, B.R. and Gakhar, S.K. 2010. Monoclonal antibodies AC-43 and AC-29 disrupt the *Plasmodium vivax* development in Indian malaria vector *Anopheles culicifacies* (Diptera culicidae). *Journal of Biosciences,* **35**:87-94.
28. De, S., Singh, R.K. and Brahma, B. 2011. Allelic Diversity of Major Histocompatibility Complex (MHC) Class II DRB gene in Indian cattle and buffalo. *Molecular Biology International.* (doi: 10.4061/2011/120176).
29. Manuja, A., Manchanda Sonia, Kumar, B., Khanna, S. and Sethi, R.K. 2010. Evaluation of different DNA extraction methods from semen of buffalo (*Bubalus bubalis)* bull. *Buffalo Bulletin,* **29**:109-115.
30. Manuja, B.K., Prasad, M., Manuja, A., Gulati, B.R. and Prasad, G. 2010. Comparative efficacy of immunological, molecular and culture assays for detection of group A rotavirus from faecal samples of buffalo calves. *Tropical Animal Health and Production*, **42**:1817-20.
31. Mukherjee, A., Polley, S., Roy, B., Anand, T. and Kumar, D. 2009. Assisted reproductive technologies: tools to augment animal production.

Indian Buffalo Journal, **7**: 33-40.

32. Rana, N., Khanna, S., Raut, A., Bhardwaj, S.R, Manuja, A., Manuja, B., Saini, A., Kakkar, S., Khurana, K.L. and Sethi, R. K. 2010. Retrospective epidemiological analysis of mortality trends in neonatal and growing Murrah buffalo calves at an organized herd. *Indian Journal of Animal Sciences*, **80**:976–979.
33. Rana, N., Manuja, A., Raut, A., Khanna, S. and Mehrara, K.L. 2010. Leptospiral abortions in Nili-Ravi buffaloes in Punjab state of India. *Indian Journal of Animal Sciences*, **81**:143-145.
34. Rao, T. Thirumala., Rao, M.M., Rao, K.B. and Naidu, K.V. 2010. Studies on ejaculate characteristics and fertility in Ongole Bulls. *The Indian Veterinary Journal,* **88**:36-37.
35. Ravi S.K., Shiv Prasad, Beerendra Singh, Prasad, J.K. and Singhal Sumit. 2011. Hormonal profile in superovulated buffaloes following ablation of dominant follicle. *Indian Veterinary Journal,* **88**:25-27.
36. Ravi, S.K., Shiv Prasad, Beerendra Singh, Prasad, J.K. and Sumit Singhal. 2011. Superovulatory response following ablation of dominant follicle in buffaloes. *Indian Veterinary Journal,* **88**:79-80.
37. Saugandhika, S., Kumar, D., Singh, M.K., Shah, R., Anand, T., Chauhan, M.S., Manik, R.S., Singla, S.K. and Palta, P. 2010. Effect of nitric oxide on in vitro development of buffalo (*Bubalus bubalis*) embryos. *Reproduction in Domestic Animals,* **45**:931-933.
38. Singh, S.R., Sangwan, A.K, Manuja, A., Kadian, S.K. and Nichani, A.K. 2011. Heterogeneity of antigenic proteins in Indian isolates of *Theileria annulata*. *Indian Journal of Parasitology* (Accepted).
39. Tupperwar, N., Tiwari, A.K., Kataria, R.S., Kumar, S., Khurana, S.K. and Rai, A. 2010. Expression of IBD virus VP2 gene in eukaryotic expression system for use as DNA vaccine. *Journal of Immunology and Immunopathology*, **12**:52-58.
40. Venkatesan, G., Balamurugan, V., Prabhu, M., Yogisharadhya, R., Bora, D.P., Gandhale, P.N., Siva Sankar, M.S., Kulkarni, A.M., Singh, R.K. and Bhanuprakash, V. 2010. Emerging and re-emerging zoonotic buffalopox infection: a severe outbreak in Kolhapur (Maharashtra), India. *Veterinaria Italiana,* **46**: 439-448.
41. Venkatesan, G., Balamurugan, V., Singh, R.K. and Bhanuprakash, V. 2010. Goatpox virus isolated from an outbreak at Akola, Maharashtra (India) phylogenetically related to Chinese strain. *Tropical Animal Health and Production,* **42**:1053-1056.
42. Yadav, S., Hosamani, M., Balamurugan, V., Bhanuprakash, V. and Singh, R.K. 2010. Partial genetic characterization of viruses isolated from pox-like infection in cattle and buffaloes: evidence of buffalo pox virus circulation in Indian cows. *Archives of Virology,* **155**:255-61.

Research Papers Presented in Conferences

1. Anand, T., Kumar, D., Singh, M.K., Shah, R.A., Chauhan, M.S., Manik, R.S., Singla, S.K. and Palta, P. 2010. Buffalo (*Bubalus bubalis*) embryonic stem cell-like cells and preimplantation embryos exhibit comparable expression of pluripotency-related antigens. National Conference on Medical Biotechnology-Vision 2020, Advanced Centre for Biotechnology, Maharshi Dayanand University, Rohtak, April 16-18, 2010, pp 70.
2. Bera, B.C., Shanmugasundaram, K., Barua, S., Gupta, A., Zeenat, Riyesh, T., Bansal, M., Gulati, B.R., Vaid, R.K. and Singh, R.K. 2010. Sequence analysis of Schlafen gene of Camelpox virus (CMLV) isolated from recent outbreak (2009) in Rajasthan. Fifth convention of "Society for Immunology and Immunopathology and National Symposium on Immunobiotechnology" at Institute of Biotechnology, G. B. Pant University of Agriculture and Technology, Patwadanagar, Nainital, Uttarakhand from December 17-19, 2010 *Indian J. Immuno. Immunopathol.* 12(2), PP-171-172.
3. Dedar, R.K., Yash Pal, Kumar, S., Ghorui, S.K., Legha, R.A. and Singh, R.K. 2010. Therapeutic evaluation of ivermectin against endoparasites of donkey. In International Symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" & XVII Annual convention of Indian Society of Veterinary Immunology & Biotechnology, held at Rajasthan University of Veterinary & Animal Sciences, Bikaner during December 29-31, 2010 pp 60.

4. Kaur, H., Dilbaghi, N., Kumar, S., Bhanjana, G. and Manuja, A. 2010. Evaluation of Alginic acid nanoparticles for nanobased therapeutics, In Proceedings of International Conference on Nanosensores & Technology, Chandigarh, October 28-31, 2010.

5. Khurana, S.K., Garg, D.N. and Singh, Y. 2010. Molecular characterization of *Mycoplasma equigenitalium* using restriction fragment length polymorphism. IX National Conference of IOM, Government Medical College Hospital, Chandigarh, September 17-18, 2010.

6. Khurana, S.K., Kanupriya, Namita Singh, Singha, H. and Sarika Punia. 2010. Molecular characterization of *Rhodococcus equi* on the basis of protein profile. National Conference on Multidisciplinary approach in frontier areas of Environmental Science and Engineering. GJU, Hisar, March 4-5, 2011.

7. Kumar, D., Singh, K.P., Anand, T., Singh, M.K., Shah, R.A., Chauhan, M.S., Palta, P., Singla, S.K. and Manik, R.S. 2011. Generation of buffalo embryonic stem cells from *in vitro* produced day 8 hatched and day 9 expanded blastocysts. International Conference on Frontiers in Reproductive Biotechnology & 21st Annual Meeting of ISSRF, NDRI Karnal, February 9-11, 2011, pp 131.

8. Kumar, R., Kumar, S. and Yadav, S.C. 2011. Cultivation of bloodstream forms of *Trypanosoma evansi* in an axenic culture system. In: Proceedings, XXI National Congress of Veterinary Parasitology organized by Department of Veterinary Parasitology, Bombay Veterinary College, Mumbai, January 5-7, 2011, pp. 115-116.

9. Kumar, R,, Yadav, S.C., Kumar, S. and Goyal, L. 2010. Transplacental transmission of *Trypanosoma evansi* in a donkey neonatal foal. In: Proceedings of 22nd National Congress of Parasitology organized by Department of Zoology, University of Kalyani, Kalyani October 30 to November 1, 2010. pp. 129.

10. Manuja, A., Manuja, B.K., Singha, H., Kumar, S. and Singh, R.K. 2010. Molecular characterization of toll-like receptor 9 in Marwari horse, Poitu and indigenous donkey. In: Proceedings of International Symposium on Role of Biotechnology in Conserving Bio-diversity and Livestock Development for Food Security and Poverty Alleviation" held at RUVAS, Bikaner during December 29-31, 2010.

11. Manuja, B.K., Sharma, R.C., Manuja, A. and Singh, R.K. 2010. Transcription expression and phylogenetic analysis of *Mx* gene sequence in Marwari horse (*Equus caballus*). In: Proceedings of International Symposium on Role of Biotechnology in Conserving Bio-diversity and Livestock Development for Food Security and Poverty Alleviation" held at RUVAS, Bikaner during December 29-31, 2010. pp 91.

12. Muzaffar, M., Anand, T., Lalaji, N.S., Zandi, M., Kala, S., Saini, M., Singh, M.K., Chauhan, M.S., Palta, P., Singla, S.K. and Manik, R.S. 2011. Effect of ascorbic acid supplementation on *in vitro* culture of buffalo embryos. International Conference on Frontiers in Reproductive Biotechnology & 21st Annual Meeting of ISSRF, NDRI Karnal, February 9-11, 2011, pp 223.

13. Nagarajan, G., Swami, S.K., Ghorui, S.K., Kumar, Sanjay, Narnaware, S.D., Pathak, K.M.L. and Singh, R.K. 2010. Amplification and cloning of the virus interferon resistant (VIR) gene of Indian camel contagious echthyma virus isolates. In: Souvenir of National symposium on "Novel Biotechnological and immunological interventions in mitigation of climate change on production and protection of livestock and poultry, organized by Indian Society of Veterinary Immunology and Biotechnology (ISVIB), Namakkal, Tamil Nadu, April 8-10, 2010, pp 40.

14. Rao, T. Thirumala, Ravi, S.K., Singh, J., Singh, R.K. and Tandon, S.N. 2010. Effect of foal birth weight on gestation length in marwari mares. *In*: XXVI Annual Convention of the Indian society for study of animal reproduction & International Symposium on "Biotechnologies for optimization of reproductive efficiency of farm and companion animals to improve global food security & human health" November 10-12, 2010 held Pantnagar, Uttrakhand.

15. Rao, T. Thirumala, Gorakh Mal, Ravi, S.K., Singh, R.K. and Patil, N.V. 2010. Comparative study on seminal plasma protein profile in three different breeds of equines. In: International Symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" & XVII Annual Convention of Indian Society of Veterinary Immunology & Biotechnology, December 29-31, 2010 at Bikaner, Rajasthan.

16. Raut, A.A. and Goel, R.K. 2010. Livestock and

Climate Change: Vulnerability and Mitigation Mechanism. International Conference on Cooling the Earth, Tactics for Restoring Climate Order and Saving the Living Planet, at Department of Environmental Sciences, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar. November 15-17, 2010.

17. Ravi, S.K., Rao, T. Thirumala, Singh J., Singh, R.K. and Tandon, S.N. 2010. Effect of gestation length and foal sex on foal birth weight in Marwari mares. In: XXVI Annual Convention of the Indian society for study of animal reproduction & International Symposium on "Biotechnologies for optimization of reproductive efficiency of farm and companion animals to improve global food security & human health" November 10th-12th held Pantnagar, Uttrakhand.
18. Scagliarini, A., McInnes, C., Mercer, A., Buttner, M., Trindade, G., Singh, R K., Reynolds, M. and Damon, I. 2010. Zoonotic poxvirus gap analysis: prioritisation of research needs to improve control tools. Disease Control Tools (*http://www.discontools.eu/home/index*), European Union.
19. Shanmugasundaram, K., Bera, B.C., Barua, S., Gulati, B.R., Vaid, R.K., Riyesh, T., Khurana, S.K., Rautmare, S.S., Kulkarni, A.M. and Singh, R.K. 2010. Sequence analysis of host range genes of zoonotic buffalopox virus isolated from outbreak (2010) in Jalgaon District of Maharashtra" In: Proceedings of International Symposium on Role of Biotechnology in Conserving Bio-diversity and Livestock Development for Food Security and Poverty Alleviation" held at RUVAS, Bikaner during December 29-31, 2010.
20. Sharma, R.C., Mehta, S.C., Manuja, B.K. and Singh, R.K. 2010. Polymorphism analysis of MHC class II genes in Marwari horses. In: Proceedings of International Symposium on "Role of Biotechnology in Conserving Biodiversity and Livestock Development for Food security and Poverty Alleviation" held at RUVAS, Bikaner during December 29-31, 2010. pp 91.
21. Singh, R.K. and Kumar, S. 2010. Climate change and emerging diseases of animals. Lead paper Abstract in Souvenir of National symposium on "Novel Biotechnological and immunological interventions in mitigation of climate change on production and protection of livestock and poultry, organized by Indian Society of Veterinary Immunology and Biotechnology (ISVIB), Namakkal, Tamil Nadu (April 8-10, 2010), p 16 (Lead paper Abstract L3).
22. Singh, M.K., Singh, K.P., Kumar, D., Shah, R.A., Anand, T., Chauhan, M.S., Manik, R.S., Singla, S.K. and Palta, P. 2011. Induced differentiation of buffalo embryonic stem cell into mouse-like cells. International Conference on Frontiers in Reproductive Biotechnology & 21st Annual Meeting of ISSRF, NDRI Karnal, February 9-11, 2011, pp 137.
23. Vaid, R.K., Bera, B. C., Anand, T., Tigga, M., Shanmugasundaram, K., Saini, S., Kumar, A., Singha, H., Virmani, N., Barua, S. and Singh, R.K. 2010. Atypical *Rhodococcus equi*: Biochemical, Epidemiological and Molecular Studies. In: Proceedings of International Symposium on Role of Biotechnology in Conserving Bio-diversity and Livestock Development for Food Security and Poverty Alleviation" held at RUVAS, Bikaner during December 29-31, 2010, pp 45.
24. Yadav, S.C, Kumar, R., Kumar, S. and Gupta, A.K. 2011. Comparative biochemical/immunological profile of camel and equine isolate antigens of *Trypanosoma evansi*" Proceedings, XXI National Congress of Veterinary Parasitology organized by Department of Veterinary Parasitology, Bombay Veterinary College, Mumbai, January 5-7, 2011, pp. 112.
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26. Yash Pal. 2010. Utilization of donkey and mules in India. In workshop on "Present status and future strategies to improve the utility of donkeys and mules" held at CVAS, Bikaner (Rajasthan) on December 13, 2010.
27. Yash Pal, Legha, R.A. and Singh, R.K. 2010. Hemato-biochemical indices of Zanskari ponies at Bikaner. In International Symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" & XVII Annual convention of Indian

Society of Veterinary Immunology & Biotechnology, held at Rajasthan University of Veterinary & Animal Sciences, Bikaner during December 29-31, 2010 pp 87.

28. Yash Pal, Legha, R.A. and Singh, R.K. 2010. Hemato-biochemical indices of Zanskari ponies at Bikaner. In International Symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" & XVII Annual convention of Indian Society of Veterinary Immunology & Biotechnology, held at Rajasthan University of Veterinary & Animal Sciences, Bikaner during December 29-31, 2010. pp 87.

Chapter in Technical Bulletins/Book/Compendium

1. Anand, T. and Kumar, D. 2009. Deriving germ cells from stem cells- A step towards immortality. *Indian Buffalo Journal*, 7: 99-101.
2. Gandhale, P.N., Bhanuprakash, V., Pandey, A.B., Venkatesan, G. and Singh, R.K. 2010. Synthetic Attnuated Virus Engineering (SAVE): An approach for designing novel vaccines. *In:* Applied Aspects of Biotechnology (Eds: Srivastava, S; Sonal; Kumar, S), Publishers: Indian Veterinary Research Institute, Izatnagar-243 122, India; pp 245-250 .
3. Gupta, A.K. 2010. Kathiawari horse– present perspective. In Compendium "Conservation of Marwari and Kathiawari Horses" organized by Equine Production Campus, National Research Centre on Equines, Bikaner on September, 7, 2010. pp19-22.
4. Rajak, K.K., Sen, A., Sudhakar, S.B., Patel, A., Ahuja, A., Bisht, S., Bhanuprakash, V., Pandey, A.B. and Singh, R.K. 2010. Recent developments in viral vaccine therapy. *In:* Applied Aspects of Biotechnology (Eds: Srivastava, S,; Sonal; Kumar, S), Publishers : Indian Veterinary Research Institute, Izatnagar-243 122, India; pp 267-278.
5. Sharma, R.C. 2010. Historical perspectives of Marwari and Kathiawari horses. In: Souvenir of Interactive Meet on *Conservation of Marwari and Kathiawari Horses* held at Equine Production Campus, NRCE, Bikaner (Rajasthan) on September 7, 2010. pp 20-22.
6. Singh, R.K., Malik, P. and Kumar, R. 2011. Conservation of Marwari horses: Challenges & solutions. All India Marwari Horse Society Souvenir-2011: 41-46.
7. Singh, R.K., Yash Pal and Gupta, A.K. 2010. Equine biodiversity in India. In Souvenir of Interactive meet on "Conservation of Marwari and Kathiawari horses" held at NRCE Equine Production Campus, Bikaner, during September 7, 2010. pp 1-15.
8. Singh, R.K., Yash Pal and Gupta, A.K.. 2010. Strategies for conservation of equine biodiversity in India. In Souvenir of Interactive meet on "Conservation of Marwari and Kathiawari horses" held at NRCE Equine Production Campus, Bikaner, during September, 7, 2010. pp 23-31.
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