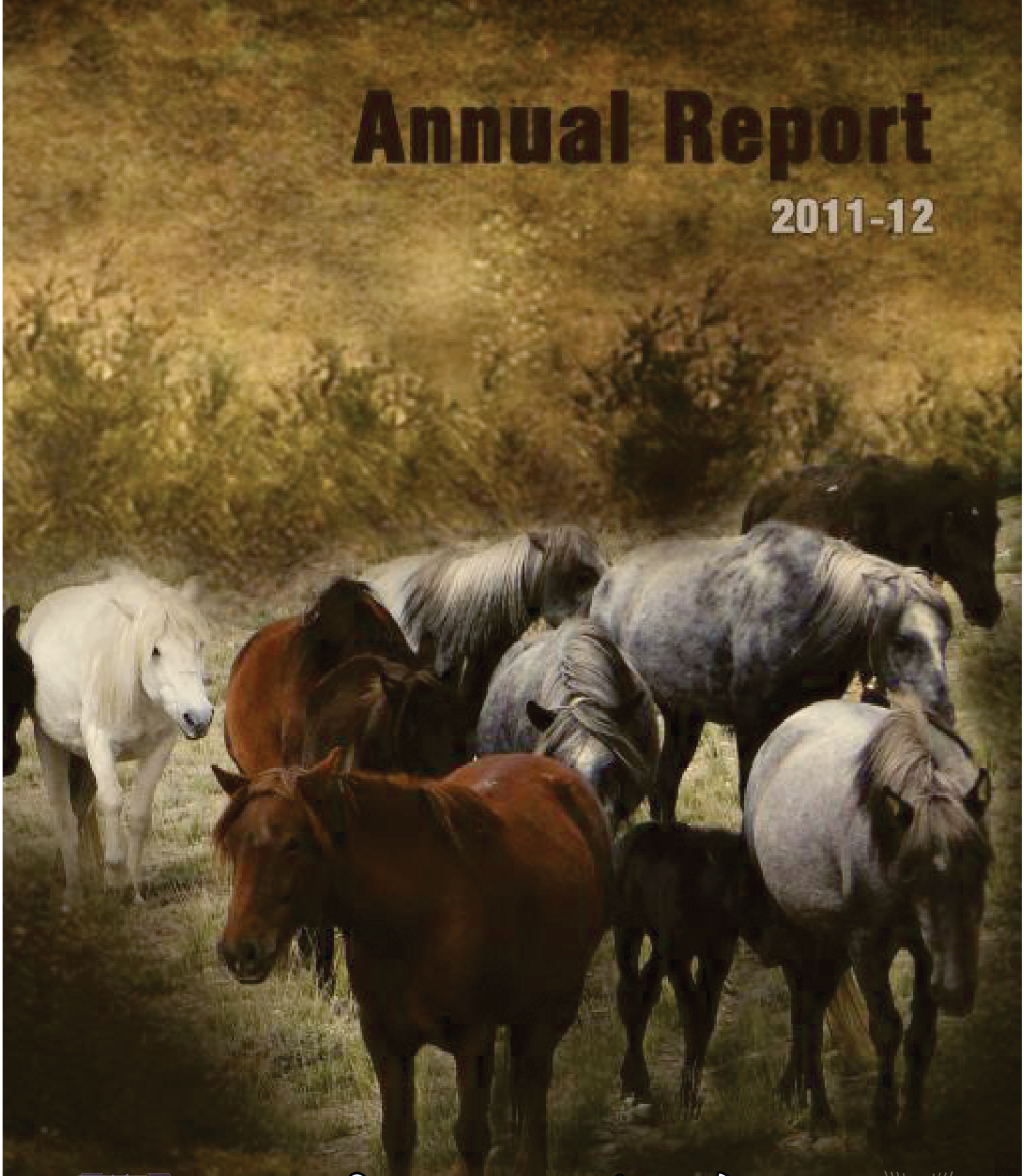
Annual Report

2011-12



राष्ट्रीय अश्व अनुसंधान केन्द्र

National Research Centre on Equines

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The achievements and activities of the Centre from April 2011 to March 2012 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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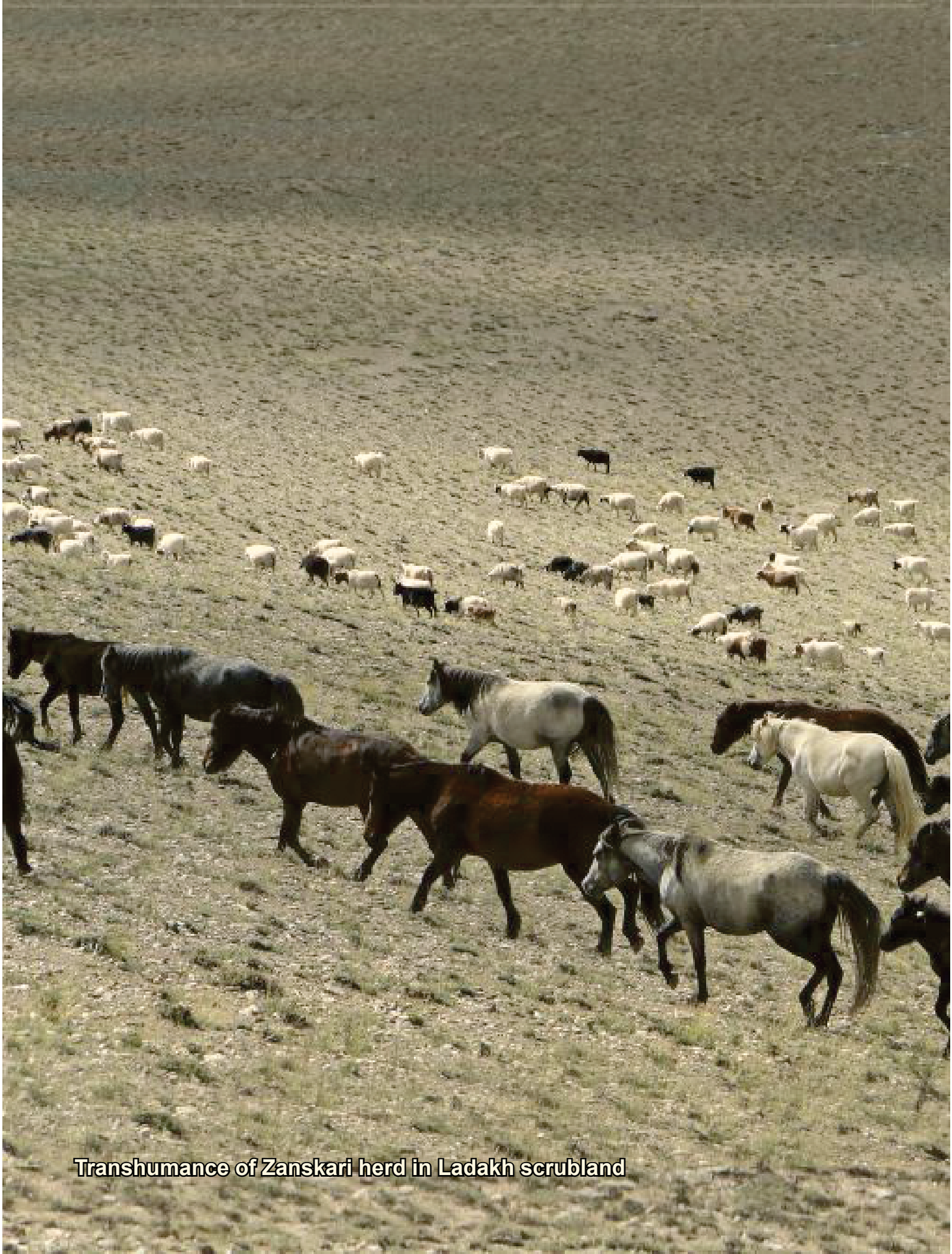


Transhumance of Zanskari herd in Ladakh scrubland

Director's Foreword

We are now well into the 2nd decade of 21st century resurgent India! A wonder of ancient world, we truly aspire to regain our humble leadership in all spheres of human endeavor. Economic development on industrial pedestal has given us the power to plan and move ahead. However, as our leaders truly believe, the wholesome development and prosperity of the nation will not be truly achieved, if our poor, socially underprivileged, landless marginal farmers and labor class do not succour the taste of economic freedom. In order to move ahead in unison, we need to empower them for a socially inclusive and sustainable growth.

National Research Centre on Equines at Hisar made a humble beginning in last century. We proudly completed 25 glorious years in 2009-10, when we again took stock of our achievements and charted out Vision 2020. The development of diagnostics/biological for major equine diseases, surveillance and monitoring of equine diseases, and providing diagnostic, advisory and consultancy services to stakeholders besides helping in conservation and improvement of the germplasm of indigenous equines breeds has been our forte. Our cutting-edge research efforts for the development of diagnostics and biologicals for major equine diseases are continuing in right earnest. NRCE has contributed a great deal towards diagnosis/management/ elimination of diseases like equine influenza outbreaks in India during 1987-1989 and 2008-2009; equine infectious anaemia 1991-1998; glanders outbreaks during 2006-2007, 2010, and of late in 2011-12. Sustained surveillance and monitoring of equine diseases has led to: (1) the control of many diseases like equine infectious anaemia, equine influenza, etc.; (ii) kept a check on diseases which are re-emerging frequently like glanders, and (iii) ensured that new equine diseases such as contagious equine metritis do not enter the country with animals imported from outside.

With the explosion of sequence and structural information available to researchers, the field of bioinformatics is playing an increasingly larger role in the study of fundamental biomedical problems. We at NRCE have the unique futuristic strength in the sense that we are new generation of biologists who are both laboratory scientists and computational scientists. The genetic nucleotide sequence of a pathogen encompasses in it, all the information about the pathogen. We have sequenced the whole genome of Japanese Encephalitis virus isolated from equines in India. Its comparison with other JEV genomes and phylogenetic analysis helps us to understand the type of JEV lurking in this region. Similarly, under VTCC mandate, we collaborated with researchers to sequence a pathogenic strain of *Pasteurella multocida* isolated from a septicaemic buffalo calf.

Equine Influenza (EI) was single most important scourge to hit our equine population in 2008-09. We attended to the outbreaks and detected and confirmed the disease agent promptly, which helped in timely control of disease. We scouted for the infectious agent and netted six isolates of EIV from different locations. In order to be ready for any future eventuality, we updated the EI vaccine and stocked a seed armory. However, for the sake of novel improved future diagnostics, we have set our sights on researches in exploiting the isolated agents. We have analyzed all the 6 isolates by amplifying and sequencing 3 genes of each isolate and done phylogenetic analysis of EIV. The accurate differentiation of EI infected and vaccinated by use of a recombinant NS1 protein based immunoassay is underway. We are also exploring monoclonal antibody (MAbs)-based sandwich ELISA (S-ELISA) for detection of equine influenza viruses (EIVs). To exploit the innate resistance of indigenous breeds of horses to EIV, we are also studying Mx

gene expression in different cohorts.

Apart from EI, Glanders, Japanese encephalitis (JE) and Trypanosomosis are some of the pressing infectious problems in equines today which are being investigated. Glanders has raised its ugly head again in UP, where we confirmed cases with respiratory illness in mules by clinical and serological examinations. We are constantly monitoring the situation in collaboration with state authorities. Trypanosomosis is responsible for considerable morbidity and economic loss; especially to small and medium equine farmers in unorganized sector. For a major head start in field of trypanosome diagnostics, the *in vitro* cultivation studies of parasites have met with success. For immunodiagnostics against Trypanosomosis, three immuno-dominant antigens have been applied successfully in detection of antibodies in experimentally infected animals using immunoblot and ELISA.

A constant vigil to ascertain the prevalence of various equine diseases in different parts of the country is important for rapid control and prevention of an outbreak. For this, our scientists constantly visit various states to collect serum and other samples for testing. Incursion of EI in Mongolia led us- as part of emergency preparedness on expedition to middle Himalayas in order to detect any unforeseen foci of EI. We planned a survey of various areas of Jammu and Kashmir right from Srinagar in the west to Leh in the east. None of the samples were, however, found positive for EI, even though positivity for trypanosomosis, piroplasmosis and EHV-1 was a finding. Testing of serum samples from 18 states revealed negative status for EI, EIA, EAV, brucellosis, CEM and *Salmonella* Abortusequi. Bacteriological analysis and antibiotic sensitivity testing of clinical isolates helped the stake-holders to moderate the treatment accordingly. Under our nanotechnology platform, we successfully tested chitosan nanoparticles-mediated sustained targeted release of hydrophilic drug taking Trypanosomosis as a model.

In order for fruits of research to reach needy clients, we have set forth our agenda on technology development and assessment also. The validation of recombinant protein (rp26) based AGID/ELISA for equine infectious anaemia & Immuno-chromatographic Test (ICT) for detection of *Thelieria equi* antibodies has progressed well. We have filed new patent applications in the field of trypanosome drug delivery and nano-delivery of drug. In order to ensure that the developed technologies are transferred, we are in active consultations with National Research Development Corporation for transfer of six technologies. We actively provide the diagnostic, advisory and consultancy services to various stake-holders for disease investigation and testing for health certification in the country. During the current year, Centre generated handsome revenue of ` 54, 28,150 through testing of samples. The newly build BSL-3 facility has been functioning and was finally validated on April 28, 2012 and will now be in regular use paving the way for surveillance and R&D work on dangerous and/or exotic pathogens. This facility will be useful for whole of this region and shall be a good resource facility for training of scientists and students.

Strong belief in the adage of the founding fathers of the nation, which exhorts us to think on the ways in which we can make a difference in life of our economically less empowered sections; took ourselves to work harder for making a difference in the lives of the landless and marginal farmers also. Donkeys and mules have often been associated with poverty but these animals of drudgery are lifeline source of livelihood for a class of underprivileged peasantry in rural and peri-urban communities. The donkeys and mules enable cheap mobility, and put cash in the kitty of poor households. These humble animals are mainly used for transporting goods by cart or as pack animals, thus benefitting these communities by providing a source of income. The utility of such animals in cheap transportation through narrow lanes, tricky tracts, arduous mountains, *kutcha* roads and smouldering sands make them irreplaceable in work and war. Therefore, we have embarked on characterization of local non-descript donkeys in different geographic locations of the country. In All India Co-ordinated research project on Increased Utilization of Animal Energy, the studies were performed for estimating optimum usage of mules and donkeys in different seasons. We are also spearheading a study on existing management systems and utilization of donkeys and mules for sustainable livelihood, which has given us interesting information. Similarly, physical and biochemical evaluation of semen of indigenous Jacks was done for their further use in artificial insemination (AI), so that the population of large white donkeys can be sustained. We are practicing conservation and improvement of germplasm of indigenous breeds of not only Marwari horses and Zanskari ponies but also large white and small grey donkeys, which are becoming harder to find.

For horse breed improvement and equine conservation activities, as well as mule production; research on equine semen characteristics, preservation and utilization has been already going on in elite Marwari horses. Now it has been extended to include Zanskari stallions and indigenous jacks also. Similarly, physical and biochemical evaluation of semen of indigenous Jacks was done to assess their individual potential by studying their seminal characteristics

for their further use in AI. In order to develop future expertise in certification of semen free from pathogenic agents, studies on bacteriological quality of fresh and frozen Zanskari semen were also performed, the results of which warranted implementation of quality management program in semen collection and cryopreservation.

VTCC (Veterinary Type Culture Collection) has a mandated aim of long-term preservation and distribution of veterinary pathogens along with Dairy and Rumen microbes. This distribution of characterized microbes to the different stake-holders of the country for further research and development promises to give impetus to development of the livestock sector. During the year 2011-12, VTCC labs were shifted into its newly constructed premises, and marched further with its mandate to foray into the new vistas of its repository endeavors. The availability of a fully sequenced bacterial culture and JE virus in the VTCC culture collection not only enhances the value of collection, but also opens up new vistas of research on a pathogen which are an important cause of economic losses in ruminants and equines, respectively. The repository increased its collection from previous 358 (2010-11) numbers to present 546 (2011-12) of accessioned veterinary microbes including 440 bacterial and 106 viral cultures along with 180 accessioned recombinant clones. Some notable new microbial inclusions in the repository include those of *Trueperella pyogenes,* from buffalo, *Rhodococcus equi* from double-humped camel from Ladakh, *Enterococcus asini* from horse, *Exiguobacterium* spp. from pig, *Lysinibacillus fusiformis* from donkey, and more such isolates. The culture collection has recently acquired a rich repository of various *Listeria* isolates. We continued to characterize our isolates at molecular level. Molecular and phylogenetic analysis of host-range genes of buffalopox virus have been completed and preserved in the repository. We are also reporting the first isolation and identification of NDV from donkey & sheep and suggest that this virus be included in the screening of viruses from non-avian hosts.

The Agriculture Section also made a great progress and continued high production of fodder crops at its farmland in Bikaner and Hisar. We have been able to sell excess grain yield and generated cash proceeds to the tune of ` 8,27,974/- in last year. This year the crop production has been to the tune of approximately 200Q of oats and 175Q of mustard besides green fodder and dry fodder at both the campuses. We continued our work of reclamation and usage of land with afforestation activities with plantation of tree saplings at Bikaner and Hisar premises. We have been able to utilize the equine dung produced at the farm premises for the generation of vermicompost which is again utilized to increase the land productivity and soil health. We have strived for water conservation by application of sprinkler system, wherever possible.

Apart from ICAR funded institutional research projects, we were successful to bag many national and international research grants. Our Twinning program on Piroplasmosis is progressing well, and we had the honour of hosting our foreign research component PI in our laboratory. Further, two new twinning proposals for Glanders with Germany and EI with UK were agreed by OIE. The twinning for Glanders is to commence from July, 2012. We have new projects on nanotechnology drug formulation, recombinant protein based ELISA for *Theileria equi*, characterization of adenoviruses; and we expect new international projects accepted in future. We continue our efforts to forge new international links to foster collaborative research. Our scientists also continued to publish their research in international and national journals and attended International and National conferences and symposia to present their findings. The research faculties were deputed for research, conferences and trainings abroad (Japan, Australia) as well as in India. We have been honoured by blessings and guidance by various distinguished dignitaries, guests and experts in various fields in our IRC, IMC and RAC meetings. We also organised interactive meets, scientific conference, *Kisan gosthis*, trainings and expert-lectures in NRCE. Farmers meet such as Stakeholder Meet and *Bhagidar Sammelan*- An Interactive meet of Equine Owners was organised at NRCE. We successfully staged XX National Conference of Indian Virological Society (VIROCON-2011) at NRCE, Hisar. Other institutional activities included celebrations of International Donkey Week, World Veterinary Day, Hindi *Pakhwara*, Communal Harmony Week, and Vigilance Awareness Week at the Centre. In the developmental activities, we got an MoU signed for Construction of Microbial Containment BSL III Laboratory at VTCC. An academic MoU was also reached between NRCE and GJUS&T, Hisar and RAJUVAS.

While commending our research and organizational advancements, we need to have a vision and a focus on the challenges so that a sure foot can be kept ahead. XII-Five year plan has initiated this year and we have in our quest to excellence planned to focus on expanding our activities in relation to development of new generation diagnostics and vaccines, strengthen clinical diagnosis and work on whole genome sequence of indigenous breeds of equines.

R.K. Singh

Executive Summary

National Research Centre on Equines (NRCE) began its journey with establishment of its main campus at Hisar (Haryana) on 26th November 1985 and its production sub-campus at Bikaner (Rajasthan) in 1989. After a humble beginning in the last century, NRCE has not looked back in bringing in improvements in health and productivity of equines in India. The Centre is striving at its best in making a difference in the lives of the landless and marginal farmers by providing diagnostic, advisory, and consultancy services for augmenting equine productivity for overall development of equine sector. The scientists of NRCE have been concentrating on development of diagnostics and biologicals for major equine diseases along with surveillance and monitoring of equine diseases. Apart from this, we have been instrumental in bringing about action-oriented production, utilization, and conservation of the germplasm of indigenous equines breeds. Further, the strengthening of Veterinary Type Culture Collection- a Microbial Genetic Resource Centre of microbes of animal origin -is one of our recent challenging responsibilities.

During the year 2011-12, NRCE forged ahead into the future with its mandate to blaze new paths in its research endeavors. Towards creating a better understanding of the pathogens, deciphering their genetic code, and identifying signatures; we sequenced the whole genome of Japanese encephalitis virus (JEV) isolated from equines. The virus genome of (10,977 nucleotides) revealed divergence with the other closely related JEV genomes around the globe. Phylogenetically, the virus was found belonging to genotype GIII. Continuing with unraveling of pathogen genomes, we also succeeded in sequencing the whole genome of recent pathogenic *Pasteurella multocida* buffalo isolate, recovered from a buffalo calf which had died of per-acute Haemorrhagic Septicemia, making this isolate of special significance to VTCC.

Equine influenza (EI) caused an epizootic in the country in 2008-09, and the disease was timely controlled with implementation of appropriate control strategies by State Animal Husbandry Departments with technical support of NRCE, and no new cases emerged from July, 2009 onwards. Further, work on characterization of EIVs was thus carried out this year for inter-se comparison among Indian isolates. For this, haemagglutinin (HA), matrix (M) and non-structural (NS) genes from rest of the viruses belonging to each geographical zone were analysed. Comparison of nucleotide and amino acid sequences of HA gene revealed two significant point mutations. It has been observed that variations exist in the susceptibility to EI within the same geographical area and even at the same organized farm. This natural resistance of indigenous breeds against infectious disease can be exploited to improve the disease control outcomes. In this regard, we are studying the Mx gene expression in EI-resistant and susceptible Marwari horses by its quantification through mRNA expression studies using real-time PCR of samples obtained from infected and control horses to elucidate the role of this gene in resistance to EI.

The Centre has always been in hot pursuit to develop state-of-the-art diagnostics for various viral and bacterial diseases of equines. The EI, Japanese encephalitis (JE), Equine infectious anaemia (EIA), Glanders, Piroplasmosis, and Trypanosomosis are some of the pressing problems in equines today, which are being investigated. We have developed the diagnostics for EI which includes HI assay, RT-PCR and real-time RT-PCR. However, the technologies for differentiation of infected and vaccinated animals (DIVA), a sandwich ELISA and a pen-side test for EIV antigen detection in nasal swabs is the need of the hour. An attempt is underway to develop a DIVA assay for differentiation of infected and vaccinated animals. Our previous results were inconsistent and thus new efforts are underway with a changed strategy that encompasses using recombinant NS1 protein. We have been successful to differentiate between the vaccinated and infected animal's serum by the new protein on a limited number of samples, however, further testing with large number of samples is underway. For development of sELISA and for antigenic characterization of EIVs, four viable secretory hybridoma clones were further subcloned. The viability and reproducibility of secretory hybridomas have been tested and subclass of MAbs determined by isotyping using ELISA test. Further, MAb production against recombinant nucleoprotein (rNP) for developing sELISA for EIV antigen detection was intensified. For this, full-length and N-terminal

portion of the NP genes have been cloned and expressed protein characterized. An immunoassay was standardized for screening of MAb and further experiment is in progress.

Till now, we are importing the antigen for EIA testing which requires foreign exchange besides continuous approvals. In order to ensure the continuous availability of diagnostic reagents, for sero-monitoring of EIA, a recombinant p26 protein antigen based ELISA and AGID assays employing synthetic gene technology were developed. The p26-ELISA was optimized and used to screen 4545 equine serum samples including horse (n=3648), mule (n=295), and donkey (n=602). In comparison, with AGID assay, relative diagnostic sensitivity and specificity of ELISA was 100% and 98.7%, respectively.

Glanders is a fatal infectious disease of equines caused by *Burkholderia mallei* with zoonotic significance. The disease erupted once again in March, 2012 and we could confirm cases with respiratory illness in mules in Uttar Pradesh by clinical (cutaneous as well as nasal forms) and serological examinations. NRCE is constantly monitoring the situation in collaboration with state authorities.

Trypanosomosis is a major haemoprotozoan disease afflicting equines. In order to effectively channelize the resources for development of diagnostic and immunoprophylactics against this disease, a major breakthrough is successful *in vitro* cultivation of the parasite. For *in vitro* cultivation of trypanosomes, five different media *viz.*, Iscove's Modified Eagle's medium (IMDM), HMI-9 medium, minimum essential medium, Alsevers' solution and Phosphate Buffered Saline supplemented with 1% glucose (PBS-G) were studied. The HMI-9 medium yielded best results in terms of adaptation, survivability and multiplication of *Trypanosoma evansi*. In the arena of development of immunodiagnostics against Trypanosomosis, three immuno-dominant antigens were identified previously. These antigens were applied successfully in detection of *T. evansi* antibodies in experimentally infected animals using immunoblot and ELISA. In evaluation of the sensitivity of sonicated and semi- purified proteins, we found that purified protein expressed in chronic stages of infection, is equally sensitive and specific as whole cell lysate (WCL) antigen.

Under the OIE Laboratory-Twinning Project on equine piroplasmosis, MASP culture of *Theileria equi* parasite was initiated and parasite could be observed from day 12-14 in culture. Recombinant EMA-1 and EMA-2 protein antigen-based ELISA for detection of *T. equi* was performed and both the proteins gave similar results. Further, an ICT-based assay developed as pen-side test by NRCPD, Japan was validated.

In DBT-sponsored project on 'Isolation and characterization of non-pathogenic adenoviruses from animals for their role in usage as vectors for delivery of protective antigens', 7 equine and 8 bovine adenoviruses were isolated. Sequence analysis confirmed 3 equine isolates to be of serotype 1 where as one bovine isolate was typed as serotype 8.

Continuous monitoring and surveillance of equine disease throughout the length and breadth of country is an arduous task, and we, in order to detect any unforeseen foci of EIV and to scotch any re-emergence of EI inside our borders, took out an adventure filled disease monitoring survey work in Middle Himalayas. A team of scientists visited various areas of Jammu and Kashmir including Srinagar, Sonmarg, Kargil, Drass, and Leh for surveillance and monitoring of equine influenza and other diseases in the region. The visit was important as the disease has been hypothesized to enter India from these frigid Northern borders earlier. The team collected serum, fecal, nasal, and blood samples from equines, donkeys and also double-humped camels of the region. None of the sample was positive for equine influenza. However, during the screening of the serum samples for other diseases a very high positivity for trypanosomosis (23.36%) was revealed.

One of the important services rendered by NRCE is contractual testing of samples from equine breeders and Race courses and Turf authorities. During the current year, Centre generated a revenue of ` 54,28,150 through testing of samples for various diseases including EIA, glanders, EI, EHV-1, EVA, CEM, *Theileria equi*, *Trypanosoma evansi*, *Trypanosoma equiperdum* and *Babesia equi*.

Further, scientists of the Centre collected samples from various regions during visits for health camps and animal fairs. These samples and samples received at the Center during 2011-12 were processed and investigated for various diseases of equines. Serum samples from various states were analysed during the period. There was no prevalence of the diseases like EIA, EVA, EI, brucellosis, CEM and *Salmonella* Abortusequi infection. Out of 9908 samples tested for glanders, six serum samples were found positive. Bacteriological analysis carried out on 189 samples yielded 48 isolates of *Rhodococcus equi*, *Streptococcus*, *Staphylococcus* spp., and *Escherichia coli*. Antibiotic sensitivity testing of clinical samples was also done and results were conveyed to various concerned quarters for moderating the treatment regimen accordingly. Out of 80

serum samples tested for EHV-1 under DI, 10 samples were found positive.

On the production front, we embarked on characterization of local non-descript donkeys in different geographic locations of the country as they are still relevant in arid regions and hilly terrains. In this endeavour, the biometric analysis of donkeys from two different geographical areas *viz.*, Himachal Pradesh, and Gujarat-in comparison with exotic French Poitu donkeys–revealed that in Spiti area, coat colour was quite different as most of these animals were dark brown with and without dark strip on back or grey and black, while, white was the prominent colour of donkeys from Gujarat. The comparative biometric analysis further revealed that donkeys from Spiti area of HP were the smallest one. Coat colour and biometric indices generated will serve as baseline information for defining and identifying these populations as different breeds. Limited work on molecular characterization of donkey has revealed that Rajasthan and Spiti donkeys are two different populations. Work on MHC profiling of exotic donkeys was further extended with PCR amplification and PCR-RFLP of DRB2 locus (276 bp) and DRB3 locus (379 bp) which indicated polymorphism in exotic donkeys. The results inferred that RFLP demonstrated potential to group the animals into different classes.

Equine chorionic gonadotropin (eCG) is an important hormone required for synchronization of estrus. It is also assayed for detection of pregnancy through non-invasive means. The work was thus initiated on the aspects of cloning of recombinant eCG for exploiting its usage for commercial production. In continuation with molecular characterization work, toll-like receptor 9 (TLR9) in Marwari horse were also characterized. Motifs of the TLR9 protein sequence of *Equus caballus* (Marwari horse) were detected using PROSITE tool. In order to achieve a cheaper, rapid and effective therapeutic response against an infectious agent, effective delivery of the biomolecules has been a challenge. To achieve a safe delivery mechanism, chitosan nanoparticles were synthesized to achieve sustained release of hydrophilic drug in the host. Preliminary studies showed that the synthesized nano-formulations are suitable for development of drug delivery module which can be evaluated for use in nano-based therapeutics in suitable animal models.

Artificial insemination (AI) is a practical method for horse breed improvement and breed conservation. The semen can be collected from stallion and stored for varying periods at low temperatures. However, the viability of semen is affected by various factors including media used for preservation. In this direction, a study on cryopreservation of equid semen using amides was carried out. Further, semen collected from three Zanskari and Marwari stallions were tested with Dimethyl Formamide (DMF), Dimethyl Sulfoxide, and Glycerol as cryoprotectants. No statistically significant difference was observed in sperm motility and sperm livability with the use of three different cryoprotectants in both pre-freeze semen and cryopreserved semen of Zanskari stallions and Marwari stallions. Similarly, physical and biochemical evaluation of semen of indigenous Jacks was done to assess their individual potential by studying their seminal characteristics for their further use in AI. Spermatozoa concentration in jack semen was 202.16±15.57x106 per ml. Among biochemical indices; GOT (IU), GPT (IU), LDH (IU) and glucose (mg/dl) were measured. Study was conducted to optimize glycerol concentration in freezing extender for cryopreservation of Indian jack semen and to evaluate the post-thaw motility. Significantly higher post-thaw motility was observed with addition of 5% glycerol. Studies on bacteriological quality of fresh and frozen Zanskari semen was carried out. Six Zanskari equine semen samples were processed for estimation of Standard Plate Count (SPC) and Total Viable Count (TVC) by selective plating on Sheep Blood Agar for identification of bacteria of pathogenic significance. The quantitative enumeration of aerobic bacteria showed an overall bacterial count range of 6.7 x 104 to 9x 105 cfu/ml in frozen semen and 1.5x 106 to 2.6 x 106 cfu/ml in fresh semen. Bacterial counts were lower in frozen samples as compared to fresh semen samples. It can be concluded that environmental contamination predominates the semen handling operation and warrants incorporation of sanitary measures in the process of semen collection and handling, especially discarding the practice of using dusty areas for semen collection. Potential venereal pathogens such as *Escherichia coli*, *Corynebacterium* spp., and *Streptococcus* spp. were isolated.

The use of animal draught energy is an important aspect of rural livelihood, and in order to optimize the usage and to take measures for animal welfare, understanding the physiological responses of equine from various load stimuli under different conditions is necessary. Under All India Co-ordinated Research Project (AICRP) studies for estimating optimum usage of mules and donkeys in different seasons such as summer, rainy season, and winter season; under

work-rest-work scheme for ploughing work; and use of donkeys in pair for ploughing and for sowing were performed. In mules, in ploughing during summer, winter, and rainy seasons, all the physiological responses increased significantly after three hours of work and values remained high even after a rest of 1 h except respiration rate. In use of mules in ploughing during winter season under work-rest-work scheme, using two furrow ploughs, the physiological responses did not come to normal after a rest of 1 h indicating animal's need for more rest. However, all the mules resumed to normal physiological conditions by the next morning. Similarly, in 3 h work -2 h rest -3 h work plan, animal was fully fatigued after 2.5 h of work in the second session and in the next morning also mules were reluctant to work. Hence, 2 h work -1 h rest -2 h work is suitable for mules. Indigenous donkeys in pair were used for ploughing and sowing for 3 h continuously using single-and-two furrow plough, respectively. A rest of 10 min was given after every hour of work. All the physiological responses increased significantly after three hours of work and values remained high even after a rest of 1 hour.

Literature on milk production and composition of the indigenous horse breeds in India is not available. Hence, work on milk composition was initiated and mare milk samples were collected during various stages of lactation. Average milk obtained during single milking was 522.0 ± 38.7ml (range 200-900 ml) and total milk produced during a day was 3.993±0.337 litre (range 1.2 - 7.2 litre). The milk samples were analyzed by automatic milk analyzer. The results indicated that mare milk is a naturally low fat milk.

Donkeys and mules are a source of livelihood for many underprivileged people in rural and peri-urban communities, who benefit directly from working equines. Donkeys and mules have often been associated with poverty (unlike cattle, camels or horses) and are mainly used as pack animals or transporting goods by cart. Therefore, a study on existing management systems and utilization of donkeys and mules for sustainable livelihood is underway. The survey data collected under the project on socio-economic status of equine owners, prevailing management practices and utilization pattern of donkeys and mules from Rajasthan, Uttarakhand, Haryana and Uttar Pradesh has been analyzed. We found that from Uttarakhand majority of respondents were from young age group and belonged to minority community. In Uttar Pradesh and Haryana, majority of respondents were from middle age group i.e., 36 to 50 years, and belonged to SC Category. Literacy level amongst respondents was higher in Uttarakhand as compared to Uttar Pradesh. Like UP, respondents from Rajasthan were also from middle age group i.e., 36 to 50 years, but belonged to minority community. In Uttarakhand, the mules were used by respondents in carts (80.65%) and also as pack animals (19.35%) as a source of livelihood. In Rajasthan, the ownership of donkeys was found higher (84.62%) as compared to mules (20.51%). In UP, the use of mules in carts for transportation of bricks at brick kilns was reported as a main source of livelihood. In Haryana, the donkeys and mules were used in cart transportation of agricultural produce, farm inputs, and construction material. It was found that deworming and vaccination was not a regular practice in all the three states.

The microbial repository at the NRCE for veterinary microbes - the Veterinary Type Culture Collection (VTCC) - made quick progress in increasing its culture collection. The activities of receiving samples from different livestock species across different geographical regions, acquisition of microbial isolates from different institutes/network units, characterization of the microbial isolates employing diverse molecular techniques and their preservation work continued and helped it enrich its culture collection diversity. The repository increased its collection from previous 358 (2010-11) numbers to present 546 (2011-12) of accessioned veterinary microbes including 440 bacterial and 106 viral cultures along with additional 180 accessioned recombinant clones. We did work towards the value addition to our collection by unraveling the genome of a novel pathogenic B:2 serotype of *Pasteurella multocida*. Previously, 3 isolates of *Pasteurella multocida* were isolated, and confirmed biochemically as well as by PCR and have been accessioned in repository vide Accn# VTCCBAA264, VTCCBAA265, and VTCCBAA266. We further enhanced the visibility of VTCC by including an isolate of newly christened genus viz., *Trueperella pyogenes* which often causes purulent infections, mainly pneumonia, arthritis, mastitis and subcutaneous abscess in cattle, buffalo, sheep, pigs and humans. VTCC also increased its culture collection diversity by isolation and preservation of *Rhodococcus equi* from double-humped camel from Leh-Ladakh. We further characterized *Bordetella bronchiseptica* the isolate by cloning, sequencing and analysis of bvgA, cyaA, fla and 16S rRNA genes. The higher sequence similarity of nucleotide and amino acid sequences indicates that these genes are highly conserved among *Bordetella*

species. In one of our studies done earlier, we came across biochemically atypical *Rhodococcus equi* isolates from equine samples. In order to further investigate the matter, these *Rhodococcus equi* isolates from Donkey, Camel, Pig and Horse were subjected to 16S rRNA gene sequence analysis. The global alignment of partial sequences showed that 5 isolates were of *Rhodococcus* genus; however the species could not be identified. Chemotaxonomic and ribotyping studies need to be performed in order to identify the isolates up to species level. In our investigations on Gram-positive rods collection from equines, camels and pigs, we present first report of *Enterococcus asini* from horse and *Exiguobacterium* spp. genus representatives from pig. We also report isolation and identification of hitherto unreported genus *Lysinibacillus fusiformis* from donkey and Bacillus cereus from horse. We also achieved isolation and identification of *Brevibacterium* spp. and *Brevibacillus* spp. bacteria isolated from equine feces and identified by homology analysis of 16S rRNA genes. *Brevibacillus* spp reclassified recently from *Bacillus* spp is used as biocontrol agent, and has also been shown to produce Gramicidin, a non-ribosomal antimicrobial peptide.

Further, molecular and phylogenetic analysis of buffalopox virus (BPXV) isolated from outbreak at Baatnor village of Meerut district in U.P. have been completed and preserved in the repository. Homology analysis of host-range genes (E3L, K3L, C7L & B5R) of BPXV isolates from buffalo, cattle and human revealed higher similarity of all these genes with *Orthopoxviruses* at both nt and aa levels. The phylogeny map based on concatenated sequences of these genes revealed that BPXVs are not as closely related to vaccine strain (Lister and Lister derived strain- LCm8), as hypothesized earlier; rather they are more closely related to other vaccinia and vaccinia-like viruses such as Passatempo and Aracatuba viruses found in Brazil. We further report first time detection of Parapox virus infection by employing semi-nested PCR and PCR-RFLP targeting the B2L envelop gene in samples received from milking cattle in UP as well as first isolation and identification of Newcastle disease virus (NDV) from donkey and sheep. Two NDV isolates (NDV/Donkey/1/Bikaner/2011 & NDV/Sheep/1/Bikaner/2011) recovered in cell culture from donkey and sheep plasma samples collected from Bikaner were further confirmed as genotype IV by sequence analysis of fusion gene.

Presently, the repository consists of viral isolates viz., equine influenza virus, camelpox virus buffalopox virus, goatpox virus, bovine herpes virus-1, equine herpes virus-1 & 4, Japanese encephalitis virus, bovine and human rotavirus; Newcastle disease virus and bacterial isolates *viz.*, *Bordetella bronchiseptica, Brucella melitensis, Brucella abortus, Actinobacillus* spp, *Citrobacter* spp, *Corynebacterium* spp, *Corynebacterium pseudotubeculosis, Trueperella pyogenes, Rhodococcus equi, E. coli, Streptococcus* spp., *Pasteurella* spp., *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp., *Klebsilella* spp., *Aeromonas* spp., dairy microbes *viz.*, *Lactobacillus* spp., *Lactococcus* spp. etc. and rumen microbes *viz.*, Methanogenic bacteria, *Pediococcus* spp., *Leuconostoc* spp., etc. *Listeria* isolates totalling more than 100 isolates including *Listeria monocytogenes* and *Listeria innocua* have been collected in repository.

कार्यकारी सारांश

अश्व प्रजाति में अनुसंधान के उद्देश्य से राष्ट्रीय अश्व अनुसंधान केन्द्र की स्थापना 26 नवम्बर 1989 में हरियाणा राज्य के हिसार में मुख्य परिसर निर्माण से हुई। वर्ष 1989 में बीकानेर, राजस्थान में अश्व प्रजनन परिसर की स्थापना की गयी। पिछली सदी में प्रारंभ किया गया यह अनुसंधान केन्द्र अश्व स्वास्थ्य एवं प्रजनन के कार्य में निरन्तर अग्रसर होकर नयी ऊंचाइयों को छू रहा है। प्रगति की राह में इस केन्द्र ने कभी पीछे मुड़ कर नहीं देखा। अश्व प्रजाति के पशुओं के समग्र विकास, अश्व व्याधि के निदान कार्य, सलाह और परामर्श सेवाएं प्रदान कर भूमिहीन एवं सीमांत अश्व पालकों के जीवन में यह केन्द्र अमूल्य परिवर्तन कर रहा है। केन्द्र के वैज्ञानिक, अश्व रोग के परीक्षण, निदान एवं चिकित्सा और जैविक विकास के क्षेत्र में अपना ध्यान केन्द्रित किए हुए है। इसके अलावा केन्द्र अश्व के लिए कार्योन्मुख, उत्पादन, उपयोग और देसी अश्व नस्लों के जर्मप्लाज्म के संरक्षण द्वारा महत्वपूर्ण भूमिका निभा रहा है। केन्द्र के विस्तार में पशु चिकित्सा प्रकार संस्कृति संग्रह (वी.टी.सी.सी.) की वर्ष 2006 में स्थापना हुई। वैज्ञानिकों के लिए रोगाणुओं की पहचान और उनके अनुवांशिक अन्वेषण के लिए जीवाणुओं का संग्रह एक चुनौतीपूर्ण जिम्मेवारी है। इन जीवाणुओं का लम्बी अवधि के लिए संवर्धन और अश्व एवं पशु विज्ञान के क्षेत्र से जुड़े अनुसंधानकर्ताओं में इनका जैविक प्रौद्योगिकी अनुसंधान कार्य के लिए वितरण हमारा मुख्य उद्देश्य है।

वर्ष 2011-12 में केन्द्र के भविष्य की योजना का उद्देश्य अनुसंधान की आधुनिक और तत्काल सेवा से अश्व पालकों और अनुसंधानकर्ताओं को बेहतर सेवाएं प्रदान करना और अश्व स्वास्थ एवं प्रजनन आदि विषयों पर विशेष ध्यान देना है। अश्वों में जापानी मस्तिष्क ज्वर की पहचान और विषाणु पृथककरण का कार्य किया गया। इस बिमारी के रोगाणुओं की बेहतर समझ के लिए डीएनए सिक्वन्स किया गया और इस सिक्वन्स की तुलना विदेशी जिनोम्स से की गई। इन तुलनाओं के अध्ययन से विश्वभर में जे.ई.वी. जिनोम्स के 27 अमिनो अम्ल फैलाव का पता चला। इसके अतिरिक्त वी.टी.सी.सी. कार्य के अंतर्गत वैज्ञानिकों ने एक भैंस-कटड़े से, जो तीव्र सैप्टिसीनिया से मर गया था, उससे पाश्च्युरैल्ला रोगाणु पृथक किया और उसका सफल डीएनए सिक्वन्स किया गया। यह कार्य वी.टी.सी.सी. के उद्देश्यों में से एक विशेष उपलब्धि है।

देश में अश्व-फ्लू (इक्वाइन एनफ्लूंजा) का दोबारा प्रकोप 2008-09 में हुआ था, जिसे केन्द्र के वैज्ञानिकों ने समय पर नियंत्रित कर लिया था। इस कार्य के लिए राज्यों के पशुपालन विभागों को राष्ट्रीय अश्व अनुसंधान केन्द्र से तकनीकी सहयोग और सही नियंत्रण की रणनीति एवं पारस्परिक सहयोग मिला, जिसके फलस्वरूप 2009 के बाद से अब तक इस बीमारी का पुन: प्रकोप नहीं हुआ है। संक्रमण के दौरान बीमारी का वायरस आयसोलेट किया गया। डीएनए कोडिंग कराने के बाद जीन अनुक्रमण विश्लेषणों के आधार पर पता चला कि यह फ्लोरिडा सब लाइन से मिलता जुलता है जबकि केन्द्र के द्वारा आइसोलेट्स के डीएनए कोडिंग करने से यह समूह अलग था, जिसे एशियाई क्लेड के रूप में केन्द्र ने निरूपित किया है। आगे इक्वाइन एनफ्लूंजा

वायरस के लक्षणों का अध्ययन केन्द्र में किया गया तथा भारतीय आइसोलेट का तुलनात्मक अध्ययन भी किया गया। इसके लिए हीमएग्लुटिनिन, मैट्रिक्स एवं न्यूरैमिनीडेज़ जीनों की अलग-अलग भौगोलिक क्षेत्रों से लेकर तुलना की गई।

एच.ए. जीन्स के न्यूक्लोटाइड्स और अमिनो अम्ल की तुलना करने पर दो महत्वपूर्ण बिन्दुओं पर बदलाव का पता चला। आइसोलेट्स के 278 स्थिति में भारतीय आइसोलेट्स में संवेदनशीलता देखी गई। विभिन्नता होने के बावजूद इन संक्रमण के प्रतिरोधन में इसके प्राकृतिक प्रतिरोध का उपयोग कर रोग के नियंत्रण के परिणामों में सुधार किया जा सकता हैं।

मारवाड़ी घोड़े इक्वाइन एनफ्लूंजा और इसके प्रतिरोधन में कितने संवेदनशील हैं? इस आशय की जानकारी हेतु संक्रमित घोड़ों के रक्त नमूने का रीयल टाईम पी.सी.आर. से प्रतिरोधकता के जीन्स को पहचानने का अध्ययन किया जा रहा है। यइ अत्यन्त संवेदनशील और परिणामोन्मुख है। इसके साथ इस जीन की अभिव्यक्ति का अध्ययन किया जा रहा है। जिससे इनके मध्य आपसी समानता का पता लगाकर रोग के निदान के लिए कारगर माध्यम बनाया जा सके। केन्द्र हमेशा जीवाणु और विषाणु रोगों की उच्चतर डायग्नोस्टिक किट्स, टीके समय-समय पर बनाने में सक्षम रहा है। केन्द्र आधुनिक तकनीकी का उपयोग कर वैक्सीन, डायग्नोस्टिक किट्स के विकास का कार्य बड़ी सुगमता से कर रहा है, जिस संदर्भ में हमारी प्राथमिकताएं इक्वाइन एनफ्लूंजा, इक्वायन इनफैक्शियस एनिमिया, ग्लैण्डर, पायरोप्लाज्मोसिस और ट्रीपैनोसोमोसिस हैं जो आज के घोड़ों के प्रमुख रोग हैं। इन पर विशेष ध्यान दिया जा रहा है जिसके लिए एचआई से आरटीपीसीआर, रियल टाईम-पीसीआर आदि तकनीकों का उपयोग कर इक्वायन एनफ्लूंजा के नए डायग्नोस्टिक किट का प्रयोग किया गया है।

संक्रमित (इनफेक्टिड) एवं टीकाकरण युक्त (वैक्सीनेटिड) पशुओं के नए परीक्षण के लिए सैंडविच एलाइजा का विकास किया गया है जिसमें नासिका नमूनों का उपयोग किया जाता है। यह तकनीकी आज की आवश्यकता है। इनफेक्टिड और वैक्सीनेटिड पशुओं की पहचान के लिए दिवा (DIVA) एक ऐसा परीक्षण है जिसको विकसित करने का प्रयास किया गया है। परिणाम सामान्य होने के कारण इसमें और प्रयास जारी हैं। इस कार्य के लिए एनएसवन प्रोटीन का उपयोग कर नई रणनीति के साथ कार्य किया जा रहा है। हम वैक्सीनेटिड (टीकाकरण युक्त) और इनफेक्टिड (संक्रमित) सीरम नमूने में अंतर की जांच करने के लिए ईज़ाद की गई तकनीक में केन्द्र को अपूर्व सफलता मिली है। यद्यपि इस नए प्रोटीन युक्त टैस्ट से कुछ नमूनों का परिक्षण किया गया है परन्तु अधिक मात्रा में नमूनों का टेस्ट जारी हैं।

अश्वों में ग्लैण्डर्स एक घातक संक्रामक रोग है जो बरखोलडेरिया मेलियाई रोगाणु के कारण होता है। इस रोग का मार्च 2012 में देश के कुछ प्रदेशों में पुन: प्रकोप हुआ। इस रोग की पहचान नेजल स्वैब और सीरम के नमूने द्वारा परीक्षण कर की गई। पिछले 5 वर्षों में ग्लैण्डर्स उत्तर प्रदेश के विभिन्न भागों से रिपोर्ट की गई। परीक्षण के बाद केन्द्र उत्तर प्रदेश के राज्य के अधिकारियों के साथ सहयोग करके इस पर नजर रखे हुए है।

घोड़ों पर ट्रीपैनोसोमोसिस रक्त में पाए जाने वाले परजीवी से होता है। यह बीमारी अश्वपालकों के लिए आर्थिक नुकसान का गम्भीर कारण है। इस रोग के पहचान के लिए केन्द्र में डाइग्नोस्टिक (परीक्षण किट) का विकास कर लिया है। इस परजीवी पर इनविट्रो

कल्चर में सफलतापूर्वक पांच अलग-अलग मीडिया में कल्चर किया गया है। प्रायोगिक चूहों में संक्रमित करने पर इनके लक्षण के परिणाम अच्छे आए हैं। ट्रीपैनोसोमा परजीवी के कल्चर का हिमशीतलन भी सफलतापूर्वक कर दिया गया है। इससे आगे के अध्ययन को सुचारू रूप से चलाया जा सकेगा। इस रोग के लिए रोग प्रतिरोध शक्ति आधारित नैदानिक विधि बनाने की दिशा में तीन प्रमुख इम्यूनोप्रतिजन की पहचान की गई है।

प्रयोगात्मक संक्रमित कारकों में रोग रोधक एंटीबॉडी का पता लगाने के लिए सफलतापूर्वक कार्य किया जा चुका है। ओ.आई.ई. प्रयोगशाला ट्विनिंग परियोजना के तहत घोड़ों में पाइरोप्लाजमीसिस परजीवी थिलेरिया इक्वाई का सफलतापूर्वक कल्चर किया गया है। रिकॉम्बीनेंट प्रोटीन पर आधारित एलायजा का सफल परीक्षण किया गया है। 37 सीरम नमूनों में दोनों विधियों से समान परिणाम पाए गए हैं। इसके आगे के अध्ययन में अध्याय जोड़ते हुए आईसी आधारित परीक्षण का विकास किया है।

एडिनो वायरस पशुओं में सुरक्षात्मक प्रतिजन के रूप में विद्यमान रहना है। पशुओं में पाए जाने वाले वायरस को मनुष्यों और पशुओं के लिए वैक्सीन बनाने में वेक्टर के रूप में उपयोग किया जाना है। डीबीटी प्रायोजित परियोजना के अंतर्गत एडीनो वायरस का अध्ययन/प्रयोग किया गया जिसमें 7 घोड़े एवं 8 भैंस प्रजाति के पशुओं से आइसोलेट किया गया। इनकी संरचना का अध्ययन किया गया और पाया गया कि घोड़े और भैंस प्रजातियों के पशुओं में पाए जाने वाले ये विषाणु अलग-अलग प्रकार के थे। घोड़े में 3 आइसोलेट एक सीरोटाइप के थे। वहीं भैंस प्रजाति के 8 आइसोलेट एक सीरोटाइप के थे।

इसी क्रम में इक्वाइन इन्फ्लूएंजा वायरस की निगरानी (मानिटरिंग) के लिए संपूर्ण भारत के यहां तक कि दुर्गम क्षेत्रों से रक्त नमूने, नेजल स्वैब एकत्र किए गए। इस कार्य के लिए वैज्ञानिक और तकनीकी टीम ने मध्य-हिमालय क्षेत्र में साहसिक सर्वेक्षण कार्य किया। वैज्ञानिक और उनकी टीम श्रीनगर, कारगिल, द्रास और लेह, जम्मू-काश्मीर क्षेत्रों का सर्वेक्षण के लिए दौरा किया। सीमाओं पर पशुओं में आर-पार आने से संपर्क के कारण संक्रमण का खतरा बना रहता है, जिसकी निगरानी आवश्यक है और रोग नियंत्रण का कारगर माध्यम है। वैज्ञानिकों की टीम ने इन क्षेत्रों से घोड़े, गधे, दो कूबड़ वाले ऊंटों से सीरम, मल, नेजल स्वैब एवं रक्त नमूने एकत्र करके इनका परीक्षण किया। किसी भी पशु में इक्वाइन इन्फ्लूएंजा का रोग नहीं मिला। अन्य रोग जैसे इक्वाइन ट्रिपैनोसोमोसिस के लिए रक्त नमूने की जांच से 23.36% की उच्च घनात्मकता संक्रमण का पता चला।

राष्ट्रीय अश्व अनुसंधान केन्द्र परीक्षण सेवाएं प्रदान करता है। इसके अंतर्गत व्यवसायिक अश्व पालकों, रेसकोर्स अश्वपालक के घोड़ों के नमूने अनुबंध परिक्षण किया जाता है। इसके अंतर्गत चालू वित्त वर्ष में 54,28,150 रुपये बतौर राजस्व प्राप्त किए। इस कार्य के लिए घोड़ों के नमूने से ईआईए, ग्लैण्डर्स, अश्व-फ्लू, ईएचवी-1, ईवीए, सीईएम, बबेसिया, थाइलिरिया, ट्रिपैनोसोमा बिमारियों का परीक्षण किया गया। इसके अलावा केन्द्र के वैज्ञानिकों द्वारा कई अश्व-स्वास्थ्य शिविरों का आयोजन किया गया और कई पशु मेलों में गए और घोड़ों के रक्त, मल, नासिका नमूने भी एकत्र किए। यह कार्य केन्द्र के वित्तिय संसाधनों द्वारा किया गया।

देश के विभिन्न राज्यों से वैज्ञानिकों ने भ्रमण पश्चात् रक्त के नमूने आदि एकत्रित किए जिनका परीक्षण अश्व रोगों के निदान हेतु किया गया। पन्द्रह से

अधिक राज्यों से एकत्रित सीरम का परीक्षण किया गया। अश्व रोग जैसे ई.आई.ए., ई.वी.ए., फ्लू, ब्रुसलोसिस, सी.ई.एम. एवं साल्मोनेला एबोट्रस इक्वाई परीक्षण में नहीं पाए गए। कुल 189 नमूनों का रोगाणु परीक्षण किया गया और कुल 48 रोगाणुओं को पृथक किया गया। इनमें से कुछ रोगाणु इस प्रकार हैं : रहोडोकाक्कस इक्वाई, स्ट्रैप्टोकाक्कस, स्टैफाईलोकाक्कस आदि, जिनका एंटीबायोटिक संवेदनशीलता परीक्षण भी किया गया। इक्वाईन हरपीज़ में 80 में 10 सीरम नमूने सही पाए गए।

अश्व प्रजाति में गर्दभों की संख्या दिन प्रति दिन घटती जा रही है, अपितु इस पशु का छोटे एवं भूमिहीन किसानों, खानाबदोष पशुपालकों आदि की सामाजिक एवं आर्थिक जीविकोपार्जन में विशेष योगदान है। इस संदर्भ में हमने दो राज्यों के देसी नस्ल के गर्दभों के शरीर का माप-तोल किया। गुजरात एवं हिमाचल प्रदेश के गर्दभों का फ्रांसिसी गर्दभ पौयटू से तुलनात्मक अध्ययन किया गया। स्पीति क्षेत्र के गर्दभ गहरे भूरे रंग के पाए गए और उनकी पीठ पर काली पट्टी पाई गई, जबकि गुजराती गर्दभों का रंग सामान्यत: सफेद ही पाया गया। पौयटू प्रजाति के गर्दभ बड़े आकार के होते हैं। जबकि स्पीति गर्दभ सबसे छोटे आकार के थे। इनके शारीरिक अवयवों का माप-तोल इनकी प्रजाति एवं संख्या के विश्लेषण में मदद देगा। इन गर्दभों की एम.एच.सी. जीन का पी.सी. आर. द्वारा विश्लेषण किया गया एवं DRB2 बिन्दु एवं DRB3 बिन्दु ने पौयटू गर्दभों में भिन्नरूपता दर्शायी।

इक्वाइन गौनेंडोट्राफिन (ई.सी.जी.) हारमोन प्रजनन एवं ऋतु चक्र एकरूपता के लिए बहुत उपयोगी है। इसलिए इस हारमोन के जीन की क्लोनिंग पर कार्य शुरू किया गया है। मारवाड़ी अश्वों में टाल जैसे अभिग्राहकों (टी.एल.आर. 9) प्रोसाईट टूल द्वारा मारवाड़ी अश्व के टी.एल.आर. 9 प्रोटीन के मूल संस्करण की पहचान की गई। अश्वों के दवाई द्वारा इलाज के दौरान शरीर के भीतर औषधि की सही मात्रा सही स्थान पर पहुंचाने से उपयुक्त एवं किफायती ईलाज संभव है। अत: इसके लिए चीटोसैन नैनोकणों का निर्माण किया गया जिसके शुरूआती प्रयोग दर्शाते हैं कि इस तरह से औषधि प्रक्षेपण का अध्ययन माडल पशुओं में किया जा सकता है।

प्राकृतिक गर्भाधान के लिए अश्व वीर्य को इकट्ठा करने के पश्चात उसको ठण्डे तापमान पर विभिन्न समय के लिए संग्रहित कर लिया जाता है। हालांकि वीर्य की जीवनक्षमता पर असर डालने वाले कई कारक हैं, इसलिए अश्व वीर्य के हिमीकृत संग्रहण पर अमाईड के प्रभाव का अध्ययन किया गया। जंसकारी एवं मारवाड़ी नर अश्वों से एकत्रित वीर्य को डाईमिथाईल फार्मामाइड, डामिथाईल सल्फाक्साइड एवं ग्लिस्रौल द्वारा हिमीकृत किया गया और यह पाया गया कि इससे वीर्य के गुणों पर कोई असर नहीं पड़ता।

इसी तरह देशी नर गर्दभों के वीर्य के भौतिक एवं रासायनिक विश्लेषण किया गया ताकि उनके वीर्य का ए. आई. में इस्तेमाल हो सके। गर्दभों के वीर्य का समकेन्द्रीकरण $202.16\pm15.57\times10^6$ प्रति मि.ली. पाया गया। यह भी पाया गया कि 5% ग्लिस्रौल के उपयोग से हिमीकृत वीर्य के उपयोग के समय वीर्य की अधिक गतिशीलता प्राप्त होती है। इसके साथ ही वीर्य के एकत्रीकरण के समय जीवाणुओं का वीर्य में मिलना ए. आई. एवं वीर्य के निर्यात आदि व्यापार में बाधक हो सकता है। अत: जंसकारी अश्वों के वीर्य नमूनों का जीवाणु विश्लेषण किया गया। पाया गया कि एक हिमीकृत नमूनों में 6.7×10^4 से लेकर 9×10^5 और एक ताजे नमूने में 1.5×10^6 से 2.6×10^6 सी.एफ.यू. प्रति मि.ली. जीवाणु मिले। वीर्य एकत्रित करते समय साफ सफाई का

ध्यान अति आवश्यक है। मुख्य जनेन्द्रिय रोगाणु जैसे ई. कोबाई, स्ट्रैप्टोकाक्कस और कोराईनीवैक्टीरियस पाए गए।

ग्रामीण परिपेक्ष्य में पशुओं द्वारा बोझा एवं अन्य सामान आदि ढोने का कार्य ग्रामीणों की सामाजिक एवं आर्थिक उत्पादकता में सहायक है। इस संदर्भ में पशु कल्याण और उनके भार ढोने की क्षमता का दैहिक अध्ययन आवश्यक है। अखिल भारतीय समन्व्यन शोध परियोजना के अंतर्गत पशुओं की शक्ति का अधिक कुशलता से उपयोग शोध पर कार्य किया गया। जिसमें खच्चरों एवं गर्दभों द्वारा विभिन्न मौसमों में कार्य करने की क्षमता की जांच की गई। मौसम जैसे ग्रीष्म, शीत, वर्षा में गर्दभों की खेती में जुताई, एक गर्दभ जोड़े द्वारा जुताई एवं बीजारोपण कार्य का अध्ययन किया गया। खच्चरों में पाया गया कि सभी मौसम में तीन घंटे के कार्य पश्चात् इनके दैहिक कारकों में वृद्धि पाई जाती है जोकि एक घंटे के विश्राम के बाद भी सामान्य नहीं हो पाता है। यही विवरण खच्चरों में जुताईकार्य-आराम-फिर कार्य योजना में जाड़े में देखा गया। हालांकि सभी कार्य करने वाले खच्चर अगली सुबह तक सामान्य दैहिक अवस्था ग्रहण कर लेते हैं। देसी गर्दभों का जाड़े में जुताई के कार्य के लिए एवं बीजारोपण के लिए लगातार तीन घंटे तक उपयोग किया गया जिसमें उन्हें हर एक घंटे बाद 10 मिनट का आराम दिया गया। यह पाया गया कि दूसरा सारे दैहिक कारक 3 घंटे पश्चात् बढ़ जाते हैं और एक घंटे के आराम के बाद भी सामान्य नहीं होते हैं।

भारत के देसी नस्ल के अश्वों के दूध की उत्पादकता एवं उसके संयोजन का साहित्य उपलब्ध नहीं है। इसलिए इस आशय पर कार्य किया गया। एक बार के दुहन पर 522.0±38.7 मि.ली. दूध प्राप्त हुआ और एक दिन में कुल 3.993±0.337 लीटर दूध प्राप्त हुआ। दुग्ध के नमूनों का स्वचलित दुग्ध परीक्षण यंत्र द्वारा परिक्षित किया गया और औसतन वसा, एस.एन.एफ., प्रोटीन, लैक्टोस एवं ऐश प्रतिशत में तथा पी.एच. आदि 0.33%, 7.51%, 2.14%, 4.43%, 0.93% एवं 7.18 पाया गया। इससे ज्ञात होता है कि अश्व दुग्ध में वसा की मात्रा कम होती है। खच्चर एवं गर्दभ वर्ग के पशु आर्थिक एवं सामाजिक रूप से कमजोर वर्गों, भूमिहीनों, मजदूरों, खानाबदोशों, आदिवासियों आदि के लिए आय का एक महत्वपूर्ण साधन हैं। गर्दभों और खच्चरों को अधिकतम गरीबी के साथ नापा गया है और इनका मुख्य उपयोग बोझा ढोने और गधा और खच्चर गाड़ी द्वारा सामान लाने-ले जाने के लिए किया जाता है। इस विषय पर इनको रखने वालों का सामाजिक एवं आर्थिक विश्लेषण एवं उनके पालन-प्रबन्धन और उपयोग पर राजस्थान, उत्तराखंड, हरियाणा और उत्तर प्रदेश में एक अध्ययन किया गया। सर्वे में यह पाया गया कि उत्तराखण्ड से अधिकतम प्रत्याशी कम उम्र के थे यानि 36-50 वर्ष और यह एस. सी. वर्ग के थे। उत्तर प्रदेश की अपेक्षा उत्तराखण्ड के अधिकतर प्रत्याशी साक्षर थे। उत्तराखण्ड में खच्चरों का अधिक उपयोग (80.65) गाड़ी खींचने में और बोझ ढोने में (19.35%) होता है। राजस्थान में गधों का उपयोग अधिक है (84.62%) और खच्चरों का कम। उत्तर प्रदेश में खच्चरों का उपयोग ईंटें ढोने में अधिक होता है। हरियाणा में ज्यादातर खच्चरों और गर्दभों का उपयोग घोड़ा गाड़ी द्वारा खेती-उपज, खेती का सामान और मकान आदि बनाने के सामान लाने-ले जाने में होता है। अन्य रख रखाव के कार्यों जैसे साफ सफाई, जूता पहनाना आदि के विषय में भी जानकारी एकत्रित की गई।

वेटेरेनरी टाइप कल्चर्स कलैक्शन ने जीवाणुओं के संवर्धन कार्य में द्रुत गति से प्रगति की है। इस केन्द्र की मुख्य गतिविधियां विभिन्न भौगोलिक क्षेत्रों से पशुओं से रोगाणुओं को एकत्रित करना, विभिन्न आणिवक

तकनीकों द्वारा रोगाणुओं को अभिचिह्नित करके परिरक्षित करना है। वी.टी.सी.सी. ने अपने केन्द्र में पिछले वर्ष तक 358 (2010-11) जीवाणुओं से बढ़कर 546 (2011-12) जीवाणुओं की प्राप्ति की है, जिनमें 440 जीवाणु हैं और 106 विषाणु हैं। इसके अतिरिक्त वी.टी.सी.सी. भण्डार में 180 संयोजक जीन क्लोन प्राप्त हैं। हमने अपने जीवाणु भण्डारण की उपयोगिता बढ़ाने के लिए एक भैंस-कटड़े से पृथक किए गए पास्च्युरेल्ला मल्टोसिडा B:2 नामक रोगाणु को पूर्ण गुणसूत्र अनुक्रमीकरण किया। ऐसे ही तीन पास्च्युरेल्ला मल्टोसिडा रोगाणुओं को हमने अपने भण्डार गृह में प्राप्तांक क्रमांक वी.टी.सी.सी.बी.ए.ए. 264, 265 और 266 नम्बर पर परिसंचित कर रखा है। हमने एक नए रोगाणु टरूपिरैल्ला पायोजीनीज़ का पृथकीकरण कर उसे भण्डारीकृत किया जो कि गाय, भैंस, भेड़, सूकर आदि पशुओं में मवाद की बीमारी और थनैला रोग करता है। हमने अपने भण्डार में दो कूबड़ वाले लद्दाखी ऊंट से विच्छेदित रहोडोकाक्कस इक्वाइ रोगाणु प्राप्त किया और प्रतिरूपी रहोड़ोकाक्कस रोगाणुओं का विश्लेषण भी किया। गदर्भों, सूकर, ऊंट एवं अश्व से पृथक किए गए इन प्रतिरूपी रहोडोकाक्कस इक्वाई जीवाणुओं का 16S आर.एन.ए. आण्विक विश्लेषण क्लोनिंग एवं अनुक्रमीकरण किया गया जिससे उनकी प्रजाति पहचानी जा सके। पाक्षिक अनुक्रमीकरण को ब्लास्ट द्वारा मिलान करने पर वंश रहोडोकाक्कस तक का पुष्टिकरण हुआ लेकिन फिर भी प्रजाति का पता नहीं चल पाया। कुछ ग्राम-पॉजीटिव रोगाणुओं के अश्व, ऊंट और सूकर से पृथकीकरण के बाद उनकी पहचान की गई है और हम पहली बार अश्व से एन्टैरोकाक्कस एसिनाई और सूकर से एग्जीगुओबैक्टीरियम कीटाणुओं के पृथकीकरण की पुष्टि करते हैं। इसके अलावा अश्व मल से हम ब्रैवीबैसिलस और ब्रैवीबैक्टीरियम पृथकीकरण की पुष्टि करते हैं। इसके अलावा हमने भैंस चेचक का आण्विक एवं आनुवांशिक विश्लेषण किया जो कि मेरठ (उत्तर प्रदेश) के एक रोग प्रकोप नमूने से पृथक किया गया था और इसका भण्डारीकरण कर दिया गया है। मनुष्य भैंस एवं गौवंश से प्राप्त चेचक के इन विषाणुओं की परपोषी जीन का सादृश्य अन्वेषण करने पर यह ज्ञात हुआ है कि इनकी न्यूक्लियोटाइड एवं अमीनो अम्ल स्तर पर आर्थोपाक्स विषाणु से बहुत अधिक समानता है। इससे यह भी पता चलता है कि भैंस चेचक के टीके की विषाणु जाति प्रकार (लिस्टर आदि) के समीप नहीं है अपितु दूसरे वैक्सीनिया जैसे विषाणु वस्तुतः पासटैम्पो और आरकाटूबा विषाणु जो कि ब्राज़िलियन विषाणु हैं के करीब है। हम पैरापाक्स विषाणु की भी प्रथम दृष्टि पहचान की पुष्टि करते हैं जिसको हमने B2L जीन को सैमी-नेस्टड पी.सी.आर. और पी.सी.आर.-आर.एफ. एल.पी. से उत्तर प्रदेश के गौवंश पशु में पहचाना। हमने एक गर्दभ और एक भेड़ से एन.डी.वी. विषाणुओं का प्रथम पृथकीकरण किया है।

वर्तमान में वी.टी.सी.सी. भण्डार में निम्नलिखित विषाणु एवं रोगाणु संवर्धित हैं। विषाणुओं में बकरी चेचक, गौवंश हरपीज़ विषाणु 1 व 4, जापानी दिमागी बुखार विषाणु, गौवंशी एवं मानव रोटा विषाणु, रानीखेत रोग विषाणु एवं रोगाणुओं के अंतर्गत बोर्डीटैल्ला ब्रांकीसैप्टिका, ब्रूसैल्ला, एक्टिनोबैसीलस, सिट्रोबैक्टर, कोराइनीबैक्टिरियम, ट्रूपीरैल्ला, रोडोकाक्कस, इ-कोलाई, स्ट्रैप्टोकाक्कस, स्टैफाइलोकाक्कस, बैसीलस, सूडोमोनास, साल्मोनैल्ला, पास्च्युरैल्ला, ऐरोमोनास आदि शामिल हैं एवं डेयरी जीवाणुओं में लैक्टोबेसीलस, लैक्टोकाक्कस संवर्धित हैं। रूमन जीवाणुओं में पीडीओकाक्कस एवं ल्यूकोनोस्टोक हैं जो कि मिथेन गैस बनाते हैं। अभी हाल ही में हमने 100 से अधिक लिस्टिरिया रोगाणु जैसे लिस्टिरिया मोनोसाइटोजीन्स एवं लिस्टिरिया इनोकुआ प्राप्त किए हैं।

Introduction

Horse has been an indispensable partner of human beings since dawn of civilization. A peep into history tells whether it was Suryavanshi horse of Aswamegha Yagya or the Chetak of Maharana Pratap, fighting the Moghuls in the Haldi Valley, horse has been a fascinating animal and a source of inspiration to the human civilization. Might be that advancement of science has changed the situations and modern methods of transportation have become a substitute to this great animal, however, even in the era of mechanization the utility of the equines in difficult hilly terrains, arid and semi-arid regions as means of transport and source of livelihood for many cannot be ignored. In order to improve the health, production potential and conservation of the germplasm of indigenous equines breeds; National Research Centre on Equines (NRCE) was established on November 26, 1985 at Hisar (Haryana) under the aegis of the Indian Council of Agricultural Research. The main campus of NRCE is located at Hisar (Haryana) and has state-of-the-art laboratories and facilities for undertaking research in areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry and biotechnology. A sub-campus of NRCE was established in 1986 at Bikaner (Rajasthan) to undertake research on equine production, genetics and breeding, nutrition, reproduction and physiology. The research activities are supported by centralized services like animal and agriculture farms, experimental animal facility, BSLIII facility, ARIS cell, ATIC, library etc. The Centre has well maintained herds of Marwari, Kathiwari, Zanskari horses and indigenous and exotic donkeys at Equine Production Campus, Bikaner. Efforts of NRCE towards improvement in equine health, productivity, surveillance and monitoring of equine diseases in the country brought recognition to NRCE as premier research institute. NRCE is contributing significantly towards the upliftment of the underprivileged equine owners by helping in conservation and improvement of the germplasm of indigenous equine breeds, besides disseminating the technologies for the efficient and economically feasible equine production. Veterinary Type Cultures Collection (VTCC) was also established in the year 2005 at NRCE for collection and preservation of microbes of veterinary importance.

Mandate of NRCE

- To undertake research on health and production management in equines
- To develop diagnostics/biologicals for major equine diseases
- To act as national referral facilities 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 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.

Focus

- Strengthening of research in equine health on those diseases where NRCE has already succeeded, particularly on (i) refinement of diagnostic tests, assays, kits, reagents; (ii) vaccines and reagents to meet out the international requirements for diagnosis, prevention and control of infectious diseases of

equines; (iii) understanding pathogen evolution, emergence/re-emergence; (iv) emergency preparedness in terms of early diagnosis of disease and strategies for containment the disease. Emphasis will be given to clinical proteomics in disease diagnosis and pathogen characterization and nanonized molecule(s)- targeted drug/vaccine delivery.

- Use of bioinformatics and modern biotechnology tools in designing vaccines, drugs and stem-cell therapy approach for control of important equine diseases.
- A national policy on disease control, prevention and management is required to be developed by NRCE in respect of endemic, re-emerging and exotic equine diseases and this should be in compliance with OIE norms.
- To conduct epidemiological investigations especially in widely distributed working equine populations with a statistically based population sampling survey framework so as to formulate disease forecast and control measures.
- Establishment of equine sanctuary and *ex-situ* conservation of indigenous breeds of horses and donkeys by way of perfecting ETT technology.
- To devise indigenous breed conservation approaches and initiate immediate action plans with the respective state governments /NGO/ SAUs and, agencies/department approved by Government of India.
- To initiate research work on equine welfare issues *viz.* harness design, improving weight carrying capacity, shelter management etc.
- Database and validation of ITKs in equine production.
- Genetic improvement of mules, donkeys and ponies used for draught purposes.
- Promotion of research for enhancing nutritional quality of indigenous feed/fodder for formulation of ration for equids.
- Training of personnel including veterinarians and livestock assistants, educating equine breeders and farmers on training/adopting scientific equine practices for overall improvement of equine health and productivity.
- 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.
- 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 Animal Husbandry Departments.
- Converting biowaste arising out of equine husbandry to wealth for employment generation, augmenting income of the stakeholders including rural equine owners, ensuring animal/human health, and environmental sustainability.

MAJOR ACHIEVEMENTS

A) Equine Health

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 and 0.96, respectively. This assay has been validated by two external laboratories using bovine, sheep and equine rotavirus samples and detects rotavirus infection among different animals. A RT-PCR targeting VP6 gene 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 monoclonal antibody based sandwich ELISA for antigenic detection is under progress.

***Theileria equi*:** For serodiagnosis of *T. equi*, a recombinant antigen based-ELISA has been developed using a truncated gene segment of a merozoite surface protein, EMA-2. The DSp and DSn of this assay in comparison to OIE-approved CI ELISA kit was 0.97 and 0.96. 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 assay (HI) have been standardized for diagnosis of JE. Monoclonal antibodies against JEV have also been raised and are under trial for development of MAb-based capture ELISA.

Equine infectious anaemia: Coggin's test for EIA is routinely being used at the Centre. A recombinant protein of 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 re-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. Thus, the vaccine has been updated in 2010 incorporating epidemiologically relevant isolate {A/eq/Katra-Jammu/6/08 (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 were

detected and control measures were taken to prevent its further spread. Since, then there were no reports of glanders for two years from India. However, 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). In 2012, team of scientists from NRCE investigated the cases with respiratory illness and cutaneous lesions upon information from Veterinary Officer of Bulandshahar, Uttar Pradesh during March, 2012. Four mules in Ahmedpur village of Agotta Block (District Bulandshahar) and two mules in Shikarpur of the same district were found positive for glanders in clinical and serological examinations (CFT and ELISA). Cutaneous and nasal forms of glanders were observed in the affected mules. 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 that genotype 3 was responsible for causing the disease in equine and that the equine JEV isolate clustered with Vellore group of JE isolates responsible for JEV in humans in India.

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

***In vitro* culture of *Theilleria equi*:** *In vitro* culture of *T. equi* by MASP (Micro Aerophillic Stationary Phase) technique could be established from the blood of latently infected animal. 8-9% parasite could be seen in the culture by day 4 to 5.

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*, *Trypanosoma evansi* (3).
- NRCE has a number of hybridomas secreting monoclonal antibodies against equine herpes virus-1, equine rotavirus, Japanese encephalitis virus, West Nile virus.
- NRCE has a repository of more than 15000 equine serum samples collected from different geographical

locations in its Equine Serum Bank.

- NRCE has a collection of more than 100 recombinant plasmid clones with recombinant genes of pathogens including EIV, ERV, EHV-1, EHV-4, EI, JEV, EIAV, *R. equi, Burkholderia mallei, Trypanosoma evansi* and *Theileria equi.*

B) Equine Production

i) Establishment of Nucleus Herd

- **Exotic Donkeys:** Twenty jennies and 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 effort to conserve the true to breed equids, the Centre has also established a nucleus herd of Marwari horse at Equine Production Campus, 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 brought from Zanskar vally, Kargil, Ladakh, Jammu & Kashmir in November, 2009.
- **Indigenous donkey:** The Centre has initiated the establishment of nucleus heard of small grey and large white donkeys found in India

ii) Baseline data on different biochemical, physiological and hematological Indices in equines-

Different biochemical, physiological and hematological indices were evaluated to establish baseline data for different donkey populations (Local vs Poitu) and horse breeds (Spiti, Kathiawari, Thoroughbred) of either sex and different age groups in healthy and diseased animals (URTI, EIA, Chronic and acute hepatopahty, Trypanosomiosis, Equine Ataxia Cystitis Syndrome, Fatty liver in exotic donkey, Colic and Canker etc) for their further use in disease diagnosis and prognosis.

iii) Biochemical studies related to different stress conditions-

Different biochemical studies were conducted to evaluate stress due to routine use of drug (Ivermectin, Fenbendazole), vaccine (tetanus toxoid, influenza etc) and other natural stress conditions (short term and long term feed deprivation stress, heat stress, water deprivation stress)

iv) Equine work efficiency –

in terms of load carrying capacity of donkeys as pack animals with different loads have been evaluated for the benefit of poor equine owners. This study is based physiological, physical and biochemical indices with fatigue scores under different working hours with varied loads.

v) Indigenous breed characterization

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

All the six indigenous breeds of horses/ponies 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 of each breed were selected from their home tracts in India and fifteen different biometric indices were recorded for each equids. Significant differences among different biometric indices were observed due to breed as well as sex. 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 while Manipuri, Spiti, Zanskari and Bhutia breeds come under the category of Pony Breeds. Equids of Marwari and Spiti breed were observed to be significantly ($P>0.05$) taller and smallest respectively, among all the horse breeds. Almost similar pattern was observed in body length of all these breeds.

Genotypic characterization of Indian equine breeds

Genetic characterization is an important part of any breeding program, so an effort was made to study genetic diversity among different equine breeds available in India, using 55 different microsats. 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 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.

Improvement in production potential of equines

Poitu jacks semen for breeding purpose: Physical and

Biochemical Characterization of different Jack's Semen during different seasons, Influence of exercise on sexual and seminal characteristics in jacks, standardization of procedure and technique for cryopreservation of semen have been evaluated and perfected.

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.

Semen for breed improvement : Good quality semen of Poitu donkeys are made available to equine owners for good quality mule production. Likewise semen of Marwari horses are also available at facilities for collection of semen of elite horses at farmer's door. PMSG based pregnancy diagnostic facilities is available for poor equine owners.

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

Utilization of Animal Energy for agricultural operation has also been demonstrated successfully.

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

Patents

Granted

I. A method for preparation of a diagnostic kit useful forecasting Equine Herpes virus-I disease (Patent has been notified on 25.10.2003 and classified as 55E4-1891278).

II. A method for preparing complement fixation test based (Cofeb) kit For diagnosis of *Babesia equi* infection of equines (Patent has been granted 31.07.2009 and Patent No.196690)

Filed

I. COFEB Kit for diagnosis of *B. equi* infection (product) - 156/ Del/04 dated 03.02.2004.

II. A pregnancy diagnostic kit for equine based on detection of eCG by ELISA (Both process & product)- Application No. 15770 dated 16.03.2006.

III. Nano-drug delivery for quinapyramine sulphate (filed provisional application, No.2560/DEL/2011, dated 06.09.2011.

Joint Patent Applications filed

1. A recombinant protein for diagnosis of glanders – Application No.1328/DEL/2010. (DRDE Gwalior and NRCE, Hisar)
2. Polynucleotide sequence, composition and methods thereof- Application No. PCT/IB 2011/052475 (IIsc Bangalore and NRCE, Hisar)

Services

NRCE provides following services to the farmers and equine breeders:

- The Centre provides disease diagnostic services for various infectious and non-infectious equine diseases to equine owners, breeders, state animal husbandry departments, police and army horses.
- Artificial insemination to augment the production of superior quality Marwari horses, mules and donkeys.
- Quality jacks and jennies are supplied to various states, breeding societies and farmers, for production of superior quality mules and donkeys.
- NRCE is providing health certification for movement of equines within and outside the country. This facility has helped in promotion of export of horses.
- Assessment and transfer of technology to the end users using the latest know-how of information technology is done. 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.

C) Veterinary Type Culture Collection

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

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

b. Central storage of animal microbes from existing culture collection 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 centre focuses on the acquisition, authentication, production, preservation, development and distribution of standard reference microorganisms, cell lines and other microbial resources for research in Veterinary and life sciences.

Mandate

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

b. Identification, characterization and documentation of animal microbes.

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

d. Human Resource Development (HRD)

Milestone Achievements

a) Veterinary Microbes Component

- Whole Genome Sequencing of *Pasteurella multocida* sub spp. *multocida* B:2 serotype
- First isolation and molecular identification of *Trueperella pyogenes*
- First report of identification of *Enterococcus asini* from horse in the country
- First report of identification of *Exiguobacterium* spp. from pig in the country
- First isolation and characterization of *Bordetella bronchiseptica* from horse.
- First isolation and characterization of *Actionobacillus equilli* from foal.
- First isolation and characterization of *Staphylococcus hyicus* from pig.
- First isolation and characterization of *Corynebacterium pseudotuberculosis* and *Corynebacterium bovis* from horse.
- First detection of Methicillin-resistant Coagulase Negative *Staphylococcus sciuri* from pigs.
- Laboratory confirmed cases of Camelpox zoonosis-first report in the world.
- Isolation and characterization of camelpox virus (CMLV) from outbreaks (2009) in Delhi, Jaisalmer & Barmer.
- Isolation and characterization of zoonotic buffalopox virus (BPXV) from outbreak (2010) in Maharashtra.
- Isolation and characterization of buffalopox virus (BPXV) from outbreak (2011) in cattle, buffaloes and humans in Meerut, U.P.

(b) Rumen microbes component

- Isolation and characterization of seven tannin degrading bacteria-*Streptococcus gallolyticus*, from goat
- Isolation and characterization of fibre degrading bacteria includes *Ruminococcus flavefaciens, Prevotella* sp. and *Butyrivibrio* sp. from buffaloes and cattle.
- Isolation and characterization of nitrate reducing and cellulose degrading *E. coli* from buffalo.
- Two genera of archaea *viz.*, *Methanomicrobium mobile* and *Methanobrevibacterium ruminantium* were identified and preserved.
- Important rumen fungi isolated and preserved includes- *Anaeromyces* sp., *Orpinomyces intercalaris* and *Orpinomyces joyonii* from buffaloes; *Piromyces* spp. and *Neocallimastix* spp. From goats,
- Three important rumen bacteria *viz.*, *Streptococcus equinus*, *Streptococcus bovis* and *Streptococcus* sp. L10 from camels have been isolated, characterized and preserved.

(c) Dairy microbes component

- The important dairy microbes preserved in the repository includes *Lactobacillus* spp., *Lactococcus* spp., *Lactococcus lactis* ssp. lactis, *Lactococcus lactis*

ssp. *Cremoris*, *Lactococcus lactis* ssp. lactis bv. *Diacetylactis*, *Streptococcus thermophilus, Leuconostoc* spp., *Bifidobacterium* spp. *Bifidobacterium dentium*, *Bifidobacterium longum*, *Micrococcus* sp., *Kluyveromyces lactis* and *Saccharomyces bisporus*.

- Seven *Leuconostoc* isolates have flavour and EPS positive
- Two *L. lactis* isolates have characteristics of fast acidifier
- Combination of *L. lactis* ssp lactis-C12 and *Leuconostoc mesenteroides* ssp. mesenteroides is very suitable for dahi and lassi preparation.
- Two new species-*S. macedonicus* (SRC) and *S. infantarius* (HRL) have been identified
- Five different species of *Propionibacterium* spp. have been procured form DSMZ culture collection, Germany
- Six *Lactobacillus* spp. having phytase degrading potential and strong antifungal activity have been isolated form milk-cereal fermented products (Rabadi samples) and can be potentially used as starter cultures for preparation of milk-cereal fermented products with extended shelf life.
- One amylytic strain of *Pediococcus acidolactici* have been isolated and can be used as starter culture in preparation of milk-cereal fermented products.
- Diacetyl and EPS (15) producing strains of *Leuconostoc* spp. have been isolated and can be used as starter cultures for preparation of low fat fermented milks, lassi and other food and for use as natural bio-thickeners/ stabilizers in various food applications.

Present status of repository

- Veterinary microbes: Repository has been strengthened with 440 accessioned bacteria, 106 accessioned viruses, 180 accessioned recombinant clones and 27 accessioned recombinant bacteriophages.
- Rumen microbes: Repository has been strengthened with 7 accessioned anaerobic bacteria.
- Dairy microbes: Repository has been strengthened with 504 accessioned dairy microbes.

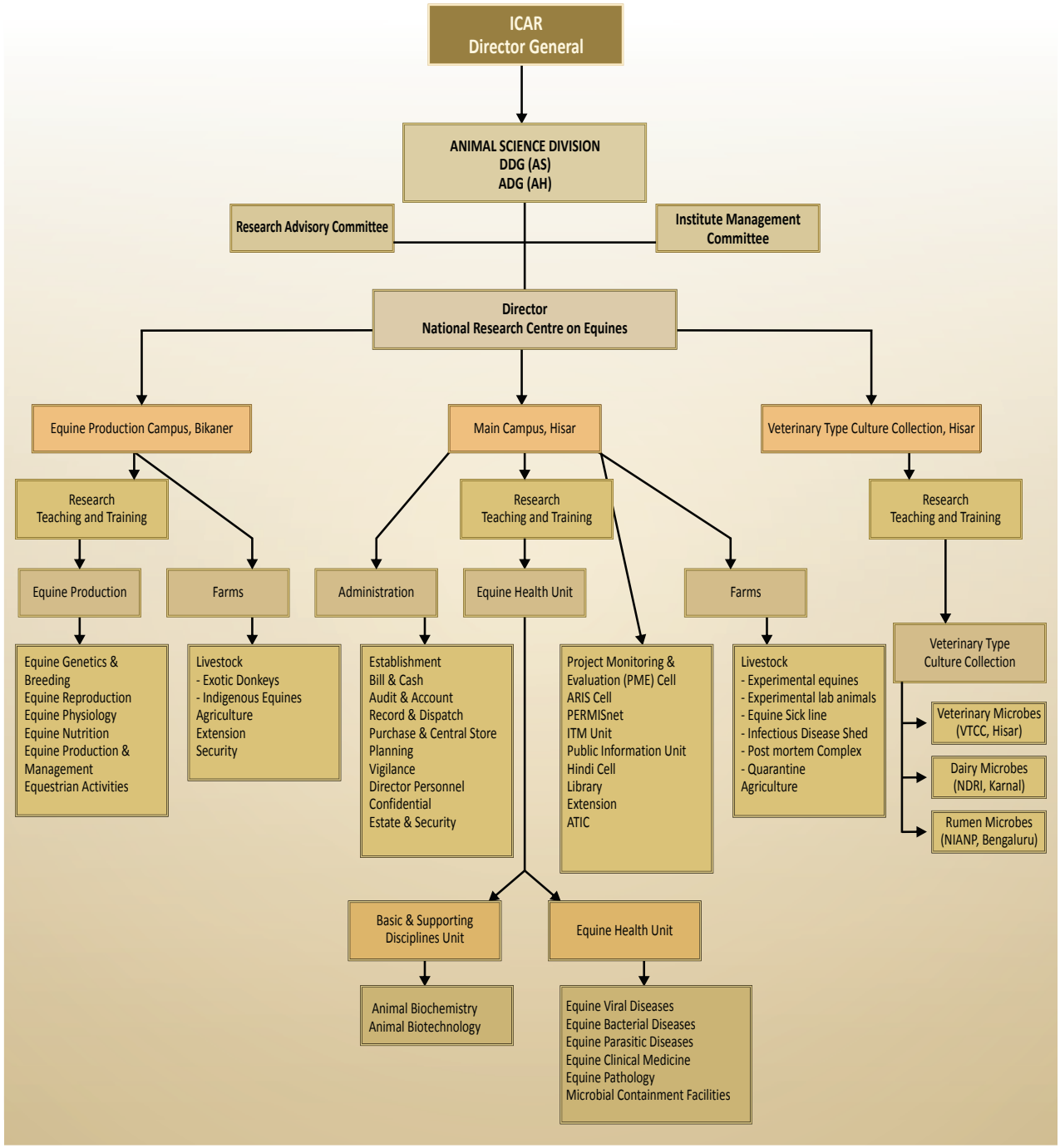
Staff position of NRCE and VTCC

Name of the post	NRCE			VTCC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	1	1	-	-	-	-
Scientific	26	23	3	10	9	1
Technical	23	22	1	1	1	-
Administrative	14	10	4	-	-	-
Supporting	22	20	2	-	-	-
Total	86	76	10	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 research on production 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 after 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 Thoroughbred 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 sELISA for detection of animal rotaviruses
2005	Establishment of Veterinary Type Culture Collection, at NRCE, Hisar
2006	Collection and cryopreservation of stallion semen at farmer's door using mobile laboratory
2006	World Organization for Animal Health declared India free of African horse sickness
2006	Outbreaks of glanders in equines
2008	Re-emergence of equine influenza
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 establishment at Bikaner
2009	First laboratory confirmed camel pox 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 *Staphlococcus hyicus* from pig
2009	First isolation of *Corynebacterium pseudotuberculosis* and *Corynebacterium bovis* from horse
2009	First isolation of Methicillin-resistant Coagulase Negative *Staphylococcus sciuri* from goats
2010	Equine sanctuary for conservation of indigenous breeds of horses
2010	A new clade designated as 'Asian Clade' of Equine influenza virus reported
2010	Award of OIE twining project on Equine Poroplasmosis between NRCPD, Japan and NRCE, India
2010	EIA-positive mule detected in indigenous equine
2010	Phenotypic characterization of all six indigenous equine breeds
2010	Re-emergence of glanders in Himachal Pradesh and Uttar Pradesh
2010	Standardization of AI using semen of Poitu donkeys & Marwari horses
2010	Zanskari stallion semen cryopreserved
2010	Started toll-free helpline no. 1800-180-1233 for advisory services to equine owners
2011	First laboratory confirmed report on BPXV causing disease in buffalo, human and cow in same time and space
2011	Whole genome sequencing of Indian strain of Japanese Encephalitis virus
2011	Whole genome sequencing of *Pasteurella multocida* B : 2 strain
2012	First isolation of *Rhodococcus equi* from Ladakhi double humped camel.
2012	First isolation of *Exiguobacterium* spp. from pig and *Enterococcus asini* from horse.

Organizational Set-Up



Summary of Expenditure & Revenue Generation

(` in lacs)

Summary of Expenditure		2010-11	2011-12
	Non-plan		
1.	Establishment charges including LSP/PF, wages, OTA	498.98	508.92
2.	Travelling allowances	3.03	3.50
3.	Others charges including equipments & recurring charges	143.81	318.98
4.	Works	17.39	–
	Total Non-Plan Expenditure	663.21	831.4
	Plan		
1.	Establishment charges including LSP/PF, wages, OTA	–	–
2.	Traveling allowances & HRD	8.48	18.10
3.	Others including equipments & recurring charges	426.92	687.77
4.	Works	26.63	258.00
	Total Plan Expenditure	**462.03**	963.87
	Total Expenditure (Plan-Non Plan)	**1,125.24**	**1,795.27**
Summary of Revenue Generation			
1.	Sale of farm produce	2,45,640.00	11,32,224.00
2.	Sale of livestock	70,000.00	3,79,500.00
3.	Sale of publication and advertisements	2,160.00	52,600.00
4.	License fee	94,926.00	60,924.00
5.	Interest on loans and advances	99,027.00	24,406.00
6.	Interest on short term deposits	51,760.00	5,27,308.00
7.	Income from internal resource generation	44,49,000.00	42,02,092.00
8.	Receipt from services	–	–
9.	Other misc. receipts	14,59,826.00	24,30,079.00
	Total Revenue	**64,72,339.00**	**88,09,333.00**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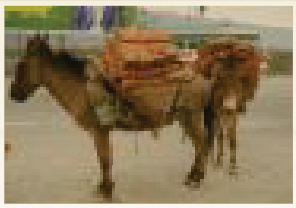
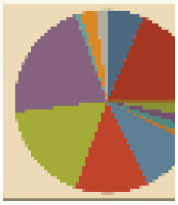
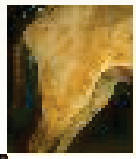
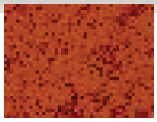
Position in alignment







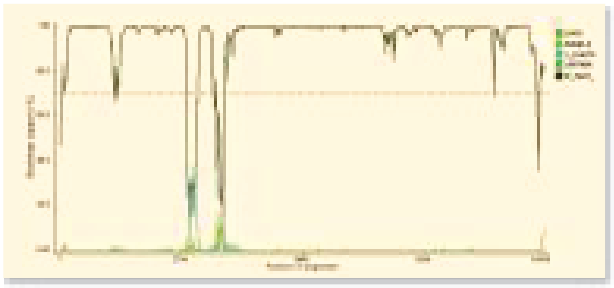


96
100

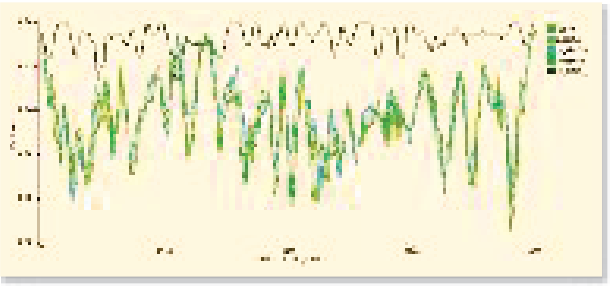
Research Achievements

Whole Genome Sequencing of Japanese encephalitis virus isolated from equines in India

Japanese encephalitis (JE) is a mosquito-transmitted viral disease of human and horses caused by the JE virus (JEV) belonging to the genus *Flavivirus* and family *Flaviviridae*. To decipher the genetic characteristics, complete genome of JEV isolated from a foal exhibiting neurological signs (JE/eq/India/H225/2009) was sequenced. The virus genome is 10,977 nucleotides in length with an ORF of 10,299 nucleotides, flanked by 5'- and 3'-NCRs of 95 and 583 nucleotides, respectively. Comparison of assembled genome with sequence of 45 other JEV strains available in GenBank revealed that JEV/H225 had 97% (B58, China) to 89% (SH17M-07, China) nucleotide identity, and 99% (JaGAR 01, Japan) to 91% (XZ0934, China) identity at amino acid sequence level (Fig. 1). Phylogenetic trees constructed on the basis of the C/prM and E genes as well as whole genome sequences selected across JEV genotypes revealed that it belongs to genotype GIII. Comparison of JEV/H225 genome with the closely related JEV genome, revealed divergence of 74 nucleotides and 27 amino acids. Majority of the amino acid changes (6) were observed in envelope protein, followed by non-structural proteins [NS5 (5) and NS3 (4) proteins]. Both



(A)



(B)

Fig. 1: Boot scan (A) Simplot (B) comparison of complete genome sequences of JEV/Eq/H225, GP78, 014178, 057434 and 04940-4. The x-axis indicates the nucleotide position, and the y-axis, the percent bootscan support and distance between the sequence of the putative recombinantion site of JEV strain at the midpoint of the window. The window size was 200 bp, and the increment was 50 bp.

3' and 5' NTR, NS1, NS2a, NS2b, NS4a and NS4b were found to be highly conserved among JEV strains. The study confirmed that JEV genotype III is circulating among equines and is associated with clinical cases in equines in India.

(BR Gulati, BK Singh, H. Singha and Nitin Virmani)

Antigenic and genetic differentiation of equine influenza viruses

Influenza viruses, due to the lack of proof reading by viral RNA polymerase, allow the replication errors which result in point mutations and continuous antigenic drift. Since the first isolation of H3N8 virus (Miami/63), these viruses have diversified into many lineages and clades. Initially, H3N8 viruses were grouped - according to geographical distribution – into American and Eurasian lineage. Later, the American lineage was further subdivided into Argentina, Kentucky and Florida sublineages. EIVs of Florida sublineage have been provisionally split into clade 1 and 2 viruses. This high level of diversification of EIVs warrants genetic and antigenic characterization of all the isolates. Work on genetic and antigenic analysis was further

expanded and monoclonal antibodies were also produced for use in antigenic analysis of newly emerging EIVs.

Genetic characterization of equine influenza viruses

For the purpose of the detection of genetic variation in different genes of EIVs, the left over isolates viz., (A/eq/Katra/2/2008; A/eq/Leh/1/08; A/eq/Ahmedabad/2/09; A/eq/Mysore/2/09; A/eq/Gopeshwar/09 & A/eq/Uttarkashi/2/09) from outbreaks during 2008-09 were selected. All these isolates were propagated in embryonated hen's egg. Three genes (HA, M and NS) of (A/eq/Katra/2/2008; A/eq/Leh/1/08; A/eq/Ahmedabad/2/09; A/eq/Mysore/2/09; A/eq/Gopeshwar/2/09 & A/eq/Uttarkashi/1/2/09) and three genes (PA, PB1 & PB2) of A/eq/Katra/2/08 and A/eq/Gopeshwar/09 isolates were amplified and cloned into pTZ57R/T vector. Sequencing data of the HA1 gene of A/eq/Katra/2/08, A/eq/Ahmedabad/2/09, A/eq/Leh/1/08 and A/eq/Mysore02/08 isolates were obtained and results for other genes are awaited. Comparison of nt & aa sequences revealed two significant point mutations: one at position 211 (Glutamine to Lysine in A/eq/Katra/2/08, A/eq/Leh/1/08 and A/eq/Ahmedabad/2/09 isolates) and another at position 278 (Valine to Alanine in all Indian isolates). Phylogenetic analysis of HA1 genes grouped the Indian EIVs with Clade 2 isolates (Fig. 2).

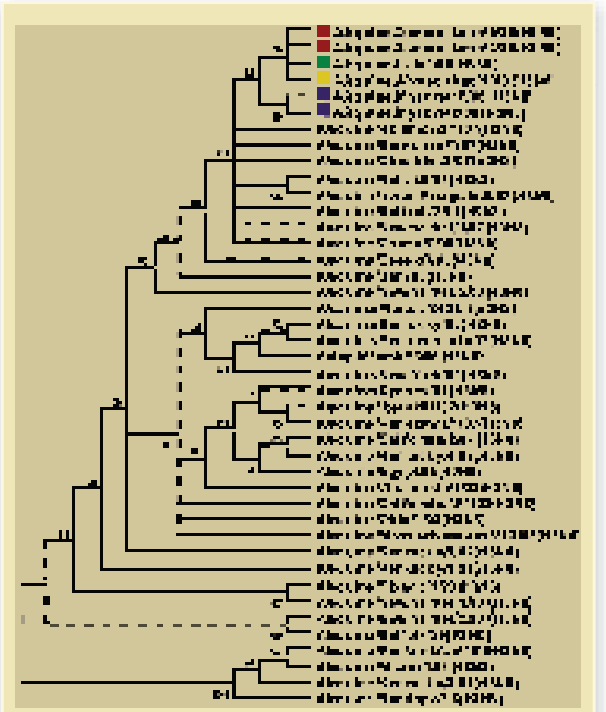


Fig. 2: Phylogenetic analysis of HA1 genes of H3N8 isolates.

Production and characterization of monoclonal antibodies (MAbs) against equine influenza (EI) viruses

With an objective of developing MAb-based ELISA for detection of EIV, four viable secretory hybridoma clones were further subcloned. These secretory clones were further subcloned using 2% 50X HAT in growth medium. The supernantant was tested by ELISA and it was found that four clones (1D12, 1G4, 5A7 and 5F4) were viable (Table 1).

Table 1: Viability, reproducibility and cloning of secretory hybridomas.

Sr. No.	Name of Clone	Viability Status	Cloning Status
1.	1D12	Viable	Cloned
2.	1G4	Viable	Cloned
3.	5A7	Viable	Cloned
4.	5F4	Viable	Cloned
5.	3C7,5G7,4D9 &2G5	Non-viable	Not applicable

The class/subclass of MAbs was determined by isotyping using ELISA test. Specificity analysis of MAbs was done using indirect ELISA, and HI test. MAbs were isotyped by ELISA with a panel of rabbit antisera to mouse immunoglobulin sub-class. The isotypes of MAbs, ELISA titre and HI activity of MAbs are depicted in Table 2. HI activity of MAbs against A/eq/02/08 was found in 2 out of 4 ascitic fluids (Table 2).

Table 2: Isotyping, ELISA titres and HI activity of MAbs against Equine influenza

Sr. No.	Clone secreting MAbs	Isotype	Reciprocal ELISA titre	Recognized HA Epitope
1	1D12	IgG1	1024000	Negative
2	1G4	IgM	256000	Positive
3	5A7	IgM	32000	Positive
4	5F4	IgG1	512000	Negative

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

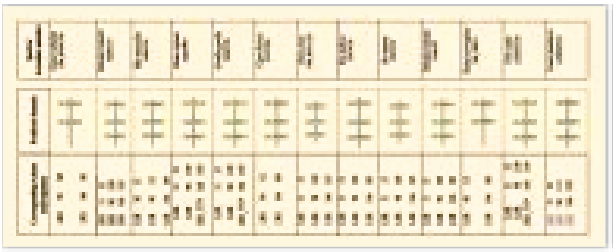
Functional motifs and protein domain structures of toll-like receptor 9 in Marwari horse

Toll-like receptors (TLRs) constitute an ancient superfamily of proteins that are expressed in both vertebrate and invertebrate species. In mammals, 13 TLRs (TLRs 1-13) have been identified. TLR9 has been characterized as a receptor that can recognize unmethylated CpG motif and trigger a pro-inflammatory cytokine response that influences both innate and adaptive immunity. Significant structural differences in the extracellular domain of TLR9 account for species-specific recognition of CpG ODN sequences. Motifs of the TLR9 protein sequence of *Equus caballus* (Marwari horse) were detected by scanning the protein sequence in PROSITE scanner. Distinct functional motifs were identified as shown in Fig 3. Comprehensive characterization of the agonists in terms of how these structural parameters correlate with the activation of unique cellular responses is further required.

TLRs contain multiple repeats that are protected by special LRR-N terminal end and LRR-C-terminal end motifs. The online utility SMART (http://smart.embl-heidelberg.de/) 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* (Table 3). Comparative prediction of TLR9 protein domain architectures for Mus musculus, Homosapiens, 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 3. Functional motifs of TLR9 Marwari breed *Equus caballus*

Table 3. Three predicted leucine rich repeats (LRR) patterns towards N-terminal sequence of TLR9.



(Anju Manuja, Balvinder Kumar and H. Singha)

Relative quantification of Mx gene expression in Equine Influenza resistant and susceptible Marwari horses

Differences in innate immune mechanisms have been shown to be critical in host susceptibility to infection. The Mx protein confers resistance to *Orthomyxovirus* infection by modifying cellular functions needed along the viral replication pathway. It has been observed that variations exist in the susceptibility to Equine Influenza within the same geographical area and even in same organized farm. In the present study, the quantization of Mx gene expression in EI resistant and susceptible Marwari horses was performed. Blood samples were collected from Marwari horses declared positive for equine influenza and in contact animals with history of no clinical signs. To assess the expression of Mx gene, the blood samples, were treated with selected doses of IFN alpha. The samples induced with 2080 IU of IFN alpha exhibited marked increase in Mx gene expression. Total RNA was extracted from these induced blood samples of EI resistant and susceptible animals and reverse transcribed. Mx gene expression was measured by relative quantitation, which compared the threshold cycle (Ct) of Mx gene to the Ct generated by a reference sample referred to as the calibrator i.e. β actin gene using Taqman chemistry. The probes for Mx gene and housekeeping gene labeled at the 5'-end with the reporter dye 6FAM (6-carboxyfluorescein) and at the 3'-end with the quencher dye TAMRA (6-carboxytetramethyl-rhodamine) were used. Fig. 4 is a representative example of relative quantification of Mx gene expression from EI resistant and susceptible phenotypes showing higher expression of Mx gene in in EI infected animals. On the basis of relative quantification, no discrete pattern was observed to differentiate between EI resistant and susceptible animals on the basis of Mx expression but in some animals, Mx expression was higher in Equine influenza susceptible Marwari horses than resistant one. However, nucleotide and deduced amino acid sequence analysis using suitable bio-informatic algorithms

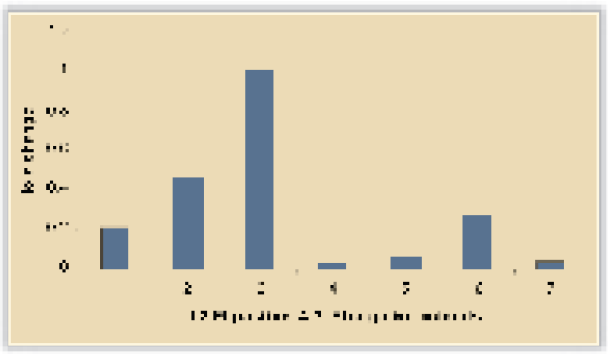


Fig. 4: Relative Mx expression in Equine infuenza resistant and susceptible Marwari horses

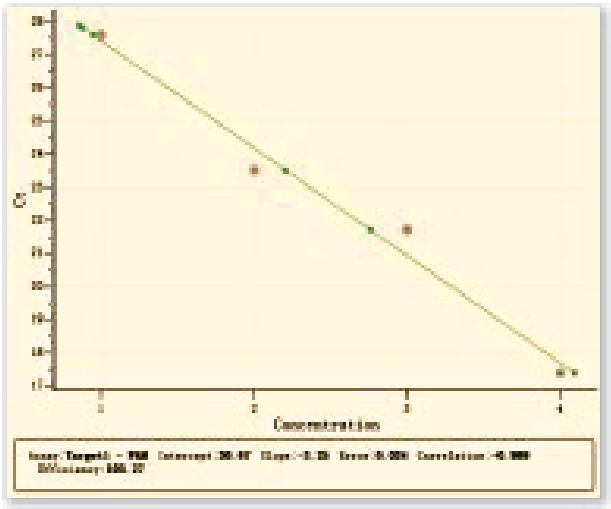


Fig. 5: Standard curve generated by real time PCR using tenfold dilutions of Mx plasmid with 98.9% correlation

would further provide insight to the functional diversity within Mx proteins. Absolute quantitation of Mx gene using q-PCR was standardized with 98.9% correlation for further studies (Fig. 5).

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

Development of recombinant NS1 (rNS1) protein based immunoassay for differentiation of infected and vaccinated animals

The rNS1 C-terminal protein with thioredoxin tag was not able to distinguish between infected, vaccinated and control serum as it was giving positive reaction in negative controls as well. This led us to devise alternate strategy for production of rNS1 with His-tag. For this purpose, full-length and C-terminal portion of the NS1 gene were amplified using new set of primers having specific restriction enzymes (RE) (*BamHI* & *SalI*) sites and cloned into prokaryotic expression vector pQE30 vector. The recombinant proteins were expressed as histidine tag- identified by SDS-PAGE- and western blotting–as 22 kDa & 12 kDa proteins corresponding to full length and C-terminal of NS1 protein, respectively. Immunoblot was standardized using serum samples from known vaccinated, infected and normal animals. The assay was modified using different concentrations of blocking with various blocking agents. Optimum results could be seen with casein and skimmed milk at concentration of 7.5%. Serum dilution was standardized at 1:3200 while anti-Horse HRPO conjugate was used at the dilution of 1:1000. The C-terminal proteins could differentiate between the vaccinated and infected serum, in limited number of samples, however, further testing with large number of samples is under process.

Development of MAbs against recombinant nucleoprotein (rNP) for developing sELISA for EIV antigen detection

Full-length and N-terminal portion of the NP gene were amplified, cloned into prokaryotic expression vector pET32a, transformed recombinant construct into BL-21 cell, and the recombinant proteins expressed as fusion protein (~34kDa) with thioredoxin- tagged. The full-length protein is not getting expressed. The N-terminal rNP protein, after purification using Nickel column was used for standardization of Western blot for detection of EIV anti-NP antibodies in serum samples. Serum samples from known infected and known uninfected animals were utilized for standardization of the test which gave optimal results with serum dilution 1:6400 and anti- Horse HRPO conjugate dilution of 1:1000.

An immunoassay was standardized using rNP for screening of monoclonal antibodies. The optimum concentration of rNP was standardized using standard checker board procedure and the assay was finally established employing mice serum. For production of monoclonal antibodies, BALB/c mice were immunized with rNP protein and further experiment is under progress.

(Nitin Virmani, B.C. Bera, Shanmugasundaram, K. [till January, 2012], B.K. Singh and B. R. Gulati)

Development of sensitive and specific diagnostic test for detection of *T. evansi*

To develop sensitive and specific diagnostic test for detection of *T. evansi*, three immuno-dominant antigens have been identified. Out of which, the cluster of polypeptides (62-66 kDa) were purified in large quantity and applied successfully in immuno blot and ELISA detection of *T. evansi* antibodies in experimentally infected animals. The sensitivity of sonicated whole cell lysate (WCL) and semi-purified antigen was comparatively evaluated using infected serum samples of equines. Both antigens detected *T. evansi* antibodies in donkeys from 2nd week onwards and shown rising trend and reached at peak by 5-7 weeks PI, there after maintained the plateau with high antibody titre till 280 days PI. This suggests that purified protein, which predominantly expressed in chronic stages of infection, is equally sensitive and specific as WCL antigen in detection of *T. evansi* antibodies. Immuno blot studies further revealed that all the infected donkey serum samples strongly recognized polypeptide bands from 2nd week PI onward in the MW range of 66-55 kDa. Likewise, all horses infected with *T. evansi* also recognized antibodies using this semi-purified protein. The inference of results of antibody detection in horses and donkeys even during chronic stages of infection indicates this ELISA can be used for diagnosis of *T. evansi* in clinical as well as chronic stages of infection.

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

Evaluation of Chitosan-coated nanoparticles for nanobased therapeutics in equines

Effective delivery of therapeutic molecules has been a major challenge to achieve the desired therapeutic response against the disease causing agent. Some of the drugs reach therapeutic levels quickly but can cause local and systemic reactions in the host. They can also have adverse physiological effects at higher doses. In order to reduce its dose and side effects, chitosan nanoparticles were synthesized to achieve sustained release of hydrophilic drug in the host. Nanosuspension was formulated by ionotropic gelation method and characterized by zetasizer, TEM, SEM and differential scanning calorimetry for evaluation of process parameters *viz.,* size, stability, morphology, functional groups etc. *In vitro* cytotoxicity and cell viability studies were performed by metabolic assay i.e resazurin assay at different concentrations of chitosan nanoparticles in animal cell line and peripheral blood mononuclear cells of horse. Preliminary studies showed that the synthesized nanoformulations are suitable for development of drug delivery module which can be evaluated for use in nanobased therapeutics in suitable animal models.

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

Glanders strikes again in Uttar Pradesh (2012)

Glanders is a fatal infectious disease of equines with zoonotic significance, caused by *Burkholderia mallei*. Team of scientists from NRCE investigated the cases with respiratory illness (Fig. 6A) and cutaneous lesions (Fig. 6B) in Bulandshahar, Uttar Pradesh during March, 2012. Four mules in Ahmedpur village of Agotta Block (Bulandshahar) and two mules in Shikarpur of the same district were found positive for glanders in clinical and serological examinations (CFT and ELISA). Cutaneous and nasal forms of glanders were observed in the affected mules. In December 2010, glanders was also reported in Chandpur (Bijnor) and Babugarh area (Ghaziabad) of Uttar Pradesh, wherein ponies and mules were affected. During the last five years (2007-12), glanders has repeatedly been reported from various parts of Uttar Pradesh. Continuous follow up surveillance of the respective regions and other areas should be of utmost priority for effective control of the disease. Efforts on the part of NRCE in coordination with Central and State Animal Husbandry (AH) Deptt. authorities could contain the disease effectively. More concerted efforts

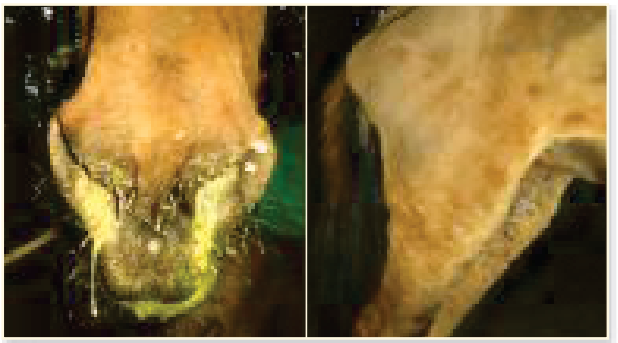


Fig.6: (A) Nasal form of glanders with highly infectious yellowish green mucopurulent exudates discharge from ulcerated nasal septum. (B) Cutaneous glanders with the typical glanders nodules in hind limbs of a mule.

by State AH authorities like regular blood sampling and serological testing of the equines, regulation of movement of equines in the state as well as inter state movement of animals and reasonable compensation to the farmers of the glanders- affected animals with the technical support of NRCE may lead to its eradication.

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

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

During the period under report (2011-12), sero-survey was conducted on serum samples received/ collected from various States/UTs of India, namely Maharashtra, Rajasthan, Chandigarh, Delhi, Haryana, Punjab, Tamil Nadu, Uttar Pradesh, Karnataka, Andhra Pradesh, Uttarakhand, Madhya Pradesh, Gujarat, Chhattisgarh, Himachal Pradesh, Manipur and West Bengal (Table 4).

Testing of 5475 serum samples from thoroughbred as well as indigenous equines for EIA and 1437 serum samples tested for Brucellosis and *Salmonella* Abortusequi (H antigen) revealed that none of the samples tested was positive (Table 4). Testing of 9908 serum samples for glanders, which included 1437 samples under S&M, 803 samples under disease investigation and 7668 samples under contractual service were in six serum samples out of 803 samples collected during diseases investigation from UP were found positive for glanders. Further, a total of 350 samples from Animal Quarantine Centres including 317 vaginal swabs and 33 preputial swabs tested were negative for CEM.

Bacteriological analysis was carried out on 189 samples originating from Rajasthan, Haryana, U.P, Gujarat, Uttarakhand and Maharashtra including nasal swabs, vaginal swabs, uterine swab, skin swab, lesion swab, tissues from PM, faecal and milk yielded 48 isolates Viz., *Rhodococcus equi* (13), *Streptococcus equi* subsp. zooepidemicus (6), *Streptococcus equi* subsp. equi (7), *Staphylococcus* sp.(2), *E.coli* (13), unidentified Gram negative bacilli (3), Group C *Streptococcus* (2), Group G *Streptococcus* (1) and Group F *Streptococcus* (1) (Table 5). Antibiotic sensitivity testing of clinical samples also done and results were conveyed to various concerned quarters.

Outbreaks of equine influenza were reported from several states of the country during previous years. Follow up action continued affected States. No new cases/ outbreaks of EI were reported during the year. Out of 2621 serum samples tested for equine influenza, 100 serum samples were found positive, however, none of the samples tested in pair showed rise in titres indicating that there were no fresh cases of EI in all the states from which serum samples were tested. Similarly out of 80 samples tested for EHV-1 under DI, 10 samples were found positive which were vaccinated as per the case history records. None of 26 samples tested for EVA by VNT were found positive.

Disease investigation through post-mortem examination and morbid material/ biopsy received from the field revealed important conditions including enteritis (2), endometritis and toxaemia (1), acute encephalitis (3), bronchopneumonia (1) and suppurative bronchopneumonia due to *R. equi* with typhilitis (1).

Table 4. Seroprevalence of important equine diseases

State	EIA	Glanders	*T evansi*	EHV-1	*B equi*	JE	*S* Abortusequi	Brucellosis
Rajasthan	0/970	0/970	68/970	41/970	350/836	23/970	0/970	0/970
Gujarat	0/125	0/125	25/125	1/82	32/125	18/125	0/125	0/125
Haryana	0/128	0/128	10/128	6/128	48/119	5/128	0/128	0/128
Uttarakhand	0/27	0/27	11/27	2/27	8/27	0/27	0/27	0/27
J & K	0/107	0/107	25/107	13/107	16/107	0/107	0/107	0/107
U. P.	0/23	0/23	2/23	0/23	9/23	0/23	0/23	0/23
H.P.	0/57	0/57	0/57	6/57	32/57	0/57	0/57	0/57
Total	0/1437	0/1437	141/1437 (10%)	69/1394 (5%)	495/1394 (36%)	46/1437 (3%)	0/1437	0/1437

Table 5. Isolates recovered and their origin

Isolate	Number	Nature of sample	From
Streptococcus equi subsp. equi	7	Nasal swab (3), PM Tissues (2), SM Lesion Swab (2)	Rajasthan (3), Haryana (4)
Streptococcus equi subsp. zooepidemicus	6	Nasal swab (5), SM Lesion Swab (1)	Haryana (6)
Rhodococcus equi	13	Nasal swab (6), Faecal swab (1), PM Tissue (6)	Rajasthan (13)
E. coli	13	PM Tissues (10), Faecal (3)	Rajasthan (11), Haryana (2)
Staphylococcus spp	2	PM Tissues (2)	Rajasthan (2)
Unidentified Gram-negative bacilli	3	Nasal Swab (2), PM Tissues (1)	Haryana (3)
Group C *Streptococcus*	2	Vaginal swab *(2)*	Haryana (2)
Group G *Streptococci*	1	PM Tissues (1)	UP (1)
Group F *Streptococci*	1	PM Tissues (1)	UP (1)
Total	48	Nasal Swab (16), PM Tissues (23), SM Lesion Swab (3), Faecal Swab (4), Vaginal Swab (2)	Rajasthan(29), Haryana (17), UP (2)

Disease monitoring by team of scientists from NRCE in J&K including Leh & Ladakh as a part of Indian Emergency Preparedness for EI

A team of Scientists (B.K.Singh, Nitin Virmani and R.K.Vaid) visited various areas of Jammu and Kashmir including Patnitop, Srinagar, Sonmarg, Kargil, Drass and Leh for surveillance and monitoring of equine influenza and other diseases in the region. The visit was important in the wake of ongoing fresh equine influenza outbreaks in Mongolia since April 2011.In Leh, the team visited Government Equine Breeding Farm, Chuchot; where Zanskari ponies were sampled. The team also collected the samples from Donkey Sanctuary at Leh. In 2008, the epizootic of equine influenza which covered fourteen states in the country and caused huge economic losses to the equine industry, came from Mongolia via China to India through northern borders. The path of travel of disease through northern borders was confirmed by virus isolation and its phylogenetic analysis which revealed that all the Indian isolates of EIVs belonged to clade 2 virus of Florida sublineage with specific changes found in the virus from Mongolia onwards.

The team collected one hundred and seven serum samples and thirty nasal swabs from various areas from J&K. None of the sample was positive for equine influenza indicating that the country is still safe. However, screening of the serum samples for other diseases revealed a very high positivity for Trypanosomosis (23.36%), EHV1 (12%), Piroplasmosis (15%) and *Rhodococcus equi* was isolated from double -humped camel.

(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)

Phenotypic characterization of local non-descript donkeys in different geographic locations

As per latest livestock census (2007), India has about 4,23,779 indigenous donkeys in different parts of the country. These are local non-descript donkeys which serve as a source of livelihood for their poor owners either as a cart animal or as pack animal for carrying the load. Donkey population has decreased drastically from 1992 to 2007 from 1 million to 0.42 millions. Though this animal is very useful, yet very little is known about their genetic traits inspite of large variations in phenotypic characters. In order to improve health and reproduction efficiency of these animals and their better utilization, there is a dire need for systematic and scientific evaluation of donkeys with respect to breeding, health, production and management practices and conservation plans.

Biometric analysis of donkeys from two different geographical areas *viz.,* Himachal Pradesh, which is a cold northern hilly mountainous tract and Gujarat, which is an arid and coastal region on west was carried out in comparison with exotic French Poitu donkeys, for generating systematic baseline information on indigenous non-descript donkey populations. Various biometric indices of 96 donkeys from Spiti area (Himachal Pradesh), 55 donkeys from Gujarat and 29 donkeys of Poitu breeds were recorded for assessing their phenotypic characteristics (Table 6).

In Spiti area, coat colour was quite different as most of these animals were dark brown (44%) with and without dark strip on back (22 and 20%, respectively), grey (33%) and black (25%) while white was the prominent colour of donkeys from Gujarat area. Poitu donkeys were brown black in colour.

Comparative biometric analysis revealed that donkeys of Poitu breed were significantly taller than the indigenous donkeys of Himachal Pradesh and Gujarat while donkeys from Spiti area of HP were the smallest one. Among both the indigenous donkey populations; canon, face length and width as well as hoof length and width were at par with each other while rest of the biometric indices were significantly higher in donkeys from Gujarat areas indicating that donkeys from Spiti areas were smaller among the two. Coat colour and biometric indices generated will serve as baseline information for defining and identifying these populations as different breeds.

(A.K. Gupta, Yash Pal, Anuradha Bharadwaj, Sanjay Kumar and Mamta Chauhan)

Amplification and cloning of eCG alpha subunit

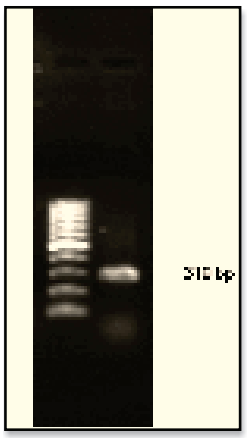


Fig. 7: PCR amplification of eCG alpha subunit.

Equine chorionic gonadotropins (eCG) is a glycoprotein hormone composed of two subunits: alpha and beta. The alpha subunit is common to all glycoprotein hormones (LH, FSH, TSH, CG). Purification of the equine pituitary gonadotropin has lagged behind purification of those from other species because of a relative scarcity of horse pituitaries and technical difficulties. In the present study, we obtained mare's pituitary from which total RNA was isolated, cDNA was synthesized using Oligo-dT primer, and amplification of the alpha subunit was done with polymerase chain reaction with specific primer pairs (Fig. 7). For amplification of alpha subunit, reported primers from literature were selected. The amplified PCR product (~306 bp) was cloned in TOPO-TA vector and custom sequenced. The analysis for ORF and sequence similarity studies revealed 96-99% similarity with other reference sequences.

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

Table 6. Comparative analysis of various biometric indices of local donkeys (Spiti, H.P. and Gujarat) with Poitu donkey breed

Population	Body Length	Wither Height	Heart Girth	Fore Leg Length	Height at Knee	Canon	Hind Leg Length	Height at Hock	Ear Length	Ear Width	Face Length	Face Width	Pole	Hoof Length	Hoof Width
Spiti	97.094^{a}	91.885^{a}	104.698^{a}	57.281^{a}	27.990^{a}	18.344^{a}	60.385^{a}	35.620^{a}	19.948^{a}	14.359^{a}	48.844^{a}	14.318^{a}	11.646^{c}	7.818^{a}	6.234^{a}
Gujarat	101.18^{b}	98.95^{b}	108.10^{b}	68.89^{c}	30.80^{b}	17.55^{a}	73.32^{b}	40.82^{b}	21.96^{b}	19.40^{b}	49.96^{a}	14.23^{a}	10.02^{a}	7.35^{a}	6.36^{a}
Poitu	138.79^{c}	130.38^{c}	150.72^{c}	90.62^{d}	41.00^{c}	23.53^{b}	94.76^{c}	51.83^{c}	28.79^{c}	24.24^{c}	60.83^{b}	21.78^{b}	10.69^{b}	11.60^{b}	8.72^{b}

Studies on class I and II genes of Major Histocompatibility Complex in donkeys

For studying MHC profile in exotic donkeys, PCR was done on DNA isolated from 72 blood samples of exotic donkeys belonging to Army Stud farm, Hisar and NRCE, Bikaner. The PCR conditions for amplification of DRB3 and DRB2 loci were optimized and respective fragments of 309 bp and 276 bp were amplified using prescribed primers, *viz.,* LA31 & LA32 and DRB2a & DRB2b, respctively. The PCR-RFLP analysis revealed amplified fragment of DRB2 locus (276 bp) digested with *Hinf* I and *Hae* III restriction enzymes resolved different fragments of varied lengths that showed polymorphism in exotic donkeys. The digestion with restriction enzyme *Msp* I resolved homozygous status in the above samples. The RFLP analysis of ELA-DRB3 locus was also carried out in indigenous donkeys with three different restriction enzymes *viz., Hinf* I, *Hae* III and *Msp* I. Digestion of amplified fragments of DRB3 locus (309 bp) with theses enzymes resolved different cutting sites and revealed polymorphism. The results inferred that RFLP showed its potential to group the animals into different classes and genotypes. Further work envisages sequencing of these genes in different groups of animals to study diversification among donkey population.

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

Cryopreservation of equid semen using amides

Semen was collected using artificial vagina from three Zanskari stallions (a total of 17 ejaculates) and six Marawari stallions (a total of 44 ejaculates) filtered through gauge to remove gel fractions and seminal characteristics in fresh semen were noted. The gel-free semen was mixed with primary extender (Citrate EDTA) in equal volume and centrifuged to get sperm pellet at the base of centrifuge tubes. The semen plasma was removed and sperm pellet was re-suspended in secondary (Lactose 11%) extender containing Glycerol, Dimethyl Formamide (DMF) and Dimethyl Sulfoxide as cryoprotectants at the rate of 2% to the total volume. Methyl formamide was discontinued as cryoprotectant due to previous poor results of post thaw sperm motility (below 10%). The diluted semen was equilibrated in cooling cabinet and cryopreserved. The seminal parameters observed in fresh semen, at pre-freeze stage and in cryopreserved semen for Zanskari stallion and Marwari stallion are listed below in Table 7 and 8.

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 pre-freeze semen and cryopreserved semen of Zanskari stallions and Marwari stallions.

(Thirumala Rao Talluri, S.K. Ravi, Yash Pal, S. K. Khurana and R. K. Vaid)

Table. 7. Seminal characteristics of stallion in fresh semen

Stallion	Total Semen vol	Gel vol	Gel free vol	pH	Initial Motility (%)	Progressive Motility (%)	Conc. ($\times 10^6$)	Live ability in fresh semen
Zanskari	30.56 ± 3.82	9.93 ± 2.42	22.18 ± 2.41	7.15 ± 0.03	80 ± 3.06	75 ± 3.06	284.25 ± 15.69	79.55 ± 2.89
Marwari	77.13 ± 5.82	28.61 ± 5.07	48.52 ± 3.53	7.26 ± 0.03	74.02 ± 1.49	68.17 ± 1.60	223.76 ± 10.19	77.39 ± 1.69

Table. 8. Seminal characteristics of stallion pre-freeze and in cryopreserved semen

Stallion No.	Pre-freeze Motility			Post-thaw Motility			Live ability in Pre-freeze Semen			Liveability in Post-thaw Semen		
	Glycerol	DMSO	DMF	Glycerol	DMSO	DMF	Gly	DMSO	DMF	Gly	DMSO	DMF
Zanskari	65 ± 2.18	68.33 ± 2.41	68.63 ± 2.24	39.09 ± 3.15	38.5 ± 2.69	42.5 ± 3.00	73.21 ± 2.44	75.49 ± 1.80	78.1 ± 1.92	52.83 ± 4.25	60.02 ± 4.83	69.43 ± 3.08
Marwari	61.66 ± 3.34	65 ± 5.40	65 ± 5.40	28.33 ± 1.66	30 ± 2.89	38 ± 4.41	73.08 ± 1.97	71.86 ± 1.71	73.20 ± 2.16	43.23 ± 2.83	45.23 ± 2.87	51.84 ± 3.08

Development of Intra-vaginal device for estrus control in mares

(Inter Institutional Project work in collaboration with CSWRI, Avikanagar).

Two trials were conducted with non-medicated intravaginal sponges for their retention. Similar trial was conducted with medicated (progesterone impregnated) sponges in six mares for a period of 13 days. Blood samples were taken from mares inserted with non-medicated and medicated intra-vaginal sponges on day 0th, day 5th, day 10th and day 12th of sponge insertion. Serum was separated and analyzed for steroid hormone profile (estrogen and progesterone) using RIA kits. The estrogen and progesterone concentration in non-medicated sponge insertion (control) group mares at day 0th, day 5th, day 10th, day 12th of sponge insertion were found to be 15.03±2.28, 9.42±4.36, 16.92±6.05, 22.03±7.51 picogram (pg) and 5.10±1.95, 9.58±5.34, 9.33 ±2.06 and 1.65±0.81 nanogram (ng), respectively. The estrogen and progesterone concentration in progesterone sponge medicated group mares day 0th, day 5th, day 10th and day 12th of sponge insertion were found 64.04±34.32, 66.99±30.76, 42.70±21.89 and 52.13±7.70pg; 6.85±5.27, 3.37±1.65, 2.88±0.44 and 2.14±1.20ng, respectively. All values recorded for estrogen and progesterone concentration on similar day was not statistically significant between mares inserted with non-medicated and medicated sponge. This could be due to less number of samples and need further verification. (Project was prematurely terminated as expected results were obtained in equines).

(CSWRI, Avikanagar: S.M.K. Naqui and Sejian; EPC, Bikaner: T.R. Talluri and S. K. Ravi)

Semen characterization and cryopreservation

Scrotal biometry

Since no work was ever undertaken on indigenous jack semen, no data on scrotal biometry and indigenous jack semen is available. We, at NRCE, make first attempt on generation of data on scrotal biometry as well as characterization and cryopreservation of semen from indigenous jacks. Study on body weight and scrotal biometry of Indigenous Jacks with various age were carried out. The mean age of the indigenous Jacks was 62.78±4.10 months (range 43-65 months) and body weight was 114.78±4.65 kg (326-384 Kg). The average scrotal circumference of the Indigenous Jacks was found to be 27.72 ±0.84 cm (24 – 33 cm). The mean length, width and height of right and left testes were recorded as 6.47±0.22, 3.3±0.17, 4.57±0.14 cm and 6.49±0.21, 3.79±0.15, 4.58±0.15 cm, respectively (Table 9). There is positive correlation which is not significant was observed between the age, body weight and scrotal circumference. This observation may be due to less number of replications or samples.

Study of seminal characteristics and the first attempt at semen cryopreservation of indigenous jacks

Physical and biochemical evaluation of semen of indigenous Jacks was done to assess their individual potential by studying seminal characteristics for further use in artificial insemination. Semen samples were collected from ten donkey stallions (n=30) by AV at alternate day for a week in the morning hours before feeding during June. Mean total semen volume, gel free semen volume, pH, initial and progressive motility were observed as 57.87±5.85 (range 12-175), 40.97±3.89 (range 15-100), 7.18±0.019 (range 7-7.5), 86.17±1.142 (range 70-95) and 80.33±1.333 (range 60-90), respectively. Spermatozoa concentration in jack semen was 202.16±15.57x106 per ml (range 85-330x106). Mean live and dead spermatozoa were 86.92±0.785 (range 74.5-92.5%) and 13.12±0.785 (range 6.5-24.5%), respectively. Abnormal spermatozoa were 4.07± 0.265 (range 2-5.5%). Among biochemical indices: GOT (IU), GPT (IU), LDH (IU) and glucose (mg/dl) were observed as 182.92±11.45, 12.77±1.23, 2342±195 and 25.33±2.386, respectively.

Study was conducted to optimize glycerol concentration in freezing extender for cryopreservation of Indian jack semen

Table 9. Age, Body weight and Scrotal biometry of Indigenous Jacks

No. of Jacks	Average Age (Months)	Body wt. (kg)	Scrotal circum-ference (cm)	Right testes			Left testes		
				Length (cm)	Width (cm)	Height (cm)	Length (cm)	Width (cm)	Height (cm)
14	62.78 ± 4.10	114.78 ± 4.65	27.72 ± 0.84	6.47 ± 0.22	3.3 ± 0.17	4.57 ± 0.14	6.49 ± 0.21	3.79 ± 0.15	4.58 ± 0.15

and to evaluate the post-thaw motility. Pre-freeze and post-thaw motility of indigenous jack semen with 2, 3 and 5% glycerol were 63.70±1.85%, 65.32±1.84%, 67.74±1.95%, 27.25±1.39%, 30.96±1.64% and 33.96±1.72%, respectively. Post-thaw live sperm with 2, 3 and 5% glycerol were 78.41±0.95, 79.54±0.877 and 80.14±0.94%, respectively. Significant and higher post-thaw motility was observed with addition of 5% glycerol compared to 2%. All other values differed non-significantly between the groups. It is concluded that addition of 5% glycerol resulted in higher post-thaw motility compared to 2% glycerol level.

Effect of dietary supplementation of alpha tocoferol acetate, lycopene and lutein on raw semen characteristics of jacks was evaluated. Ten adult healthy jacks (Fig. 8) with normal fertility were divided into two groups: a control group (CG), in which standard diet was provided, and a treated group (TG),

Fig. 8: A pack of large white indigenous jacks at EPC, Bikaner

in which the standard diet was supplemented with alpha tocoferol acetate, lycopene and lutein in soya bean oil for a period of one month orally once a day in the morning. Semen ejaculates were evaluated for total semen volume, gel-free semen volume, pH, semen motility (initial and progressive motility), sperm concentration, live and dead count and percent abnormal spermatozoa. Mean values observed in control versus treatment group were 58.08 and 57.71ml; 42.92 and 39.47ml; 7.16 and 7.19; 84.62 and 87.35%; 78.46 and 81.76%; 204.23 and 200.59x106; 83.68 and 87.32%; 13.54 and 12.79%, 4.0 and 4.12%, respectively. No significant changes in the seminal parameters were observed in control and treatment group.

(S.K. Ravi, Yash Pal, R.A. Legha, R.K. Dedar, T.R. Talluri, S.K. Ravi and R.A. Legha)

All India Co-ordinated Research Project on Increased utilization of animal energy with enhanced system efficiency

a. Use of mules in ploughing during summer

Mules were used in ploughing continuously for 3 hours during summer (May & June) using two furrow plough. A rest of 10 min was given after every hour of work. All the physiological responses (PR, RR and RT) increased significantly ($P<0.05$) after three hours of work and values remained high even after a rest of 1 hr except RR (Table 10). Average area ploughed was 0.387±0.013ha. PCV and TLC increased significantly after work, whereas Hb and TEC did not change significantly. The meteorological parameters for the months are given (Table 11).

Table 10. Changes in physiological indices

Parameters	Control	3 h work	1 h Rest
PR/min	33.0±1.30	60.78±2.36	40.78±1.64
RR/min	23.0±1.54	45.89±4.38	23.0±1.45
RT/min	99.64±0.24	102.01±0.25	101.73±0.17

Table 11. Meteorological observations during May and June

Month	Temperature (°C)		R.H. (%)		Total rainfall (mm)	Rainy days	Wind speed (kmph)	Evaporation (mm/day)	BSSH
	Max.	Min.	RH1	RH2					
May	43.3	27.8	52	16	11.6	1	12.1	15.1	9.6
June	41.9	29.0	57	25	27.0	1	12.0	13.6	5.5
Average	42.6	28.4	54.5	20.5	19.3	1	12.05	14.35	7.55

b. Use of mules in ploughing during rainy season

Mules were used in ploughing continuously for 3 hours during rainy season (July & August) using two furrow plough. A rest of 10 min was given after every hour of work. The physiological responses (PR, RR and RT) increased significantly after three hours of work and values remained high even after a rest of 1 hr except RR (Table 12). Average area ploughed was 0.376±0.009 ha. The meteorological parameters for the months are given (Table 13).

Table 12. Changes in physiological indices

Parameters	Control	3h work	1 h Rest
PR/min	31.20 ±0.70	62.40±1.49	40.70±1.56
RR/min	22.0±0.98	46.10±3.53	24.80±0.91
RT/min	99.71±0.11	102.02±0.17	101.75±0.16

Table 13. Meteorological observations during July and August

Month	Temperature (°C)		R.H. (%)		Total rainfall (mm)	Rainy days	Wind speed (kmph)	Evaporation (mm/day)	BSSH
	Max.	Min.	RH_1	RH_2					
July	38.7	27.7	67	39	82.8	2	9.9	8.8	6.2
August	35.6	26.3	80	56	100.6	6	7.4	6.5	7.3
Average	37.15	27	73.5	47.5	91.7	4	8.65	7.65	6.75

c. Use of mules in ploughing during winter season

Mules were used in ploughing continuously for 3 hours during winter season (December & January) using two furrow plough in sandy soils (Fig. 9). A rest of 10 min was given after every hour of work. All the physiological responses (PR, RR and RT) increased significantly after three hours of work and values remained high even after a rest of 1 h except RR (Table 14). Average area ploughed was 0.423±0.024 ha. Speed of operation was 2.51±0.31 km/h. The winter months meteorological observations are given (Table 15).

Table 14. Changes in physiological indices

Parameters	Control	1h Work	2h work	3h work	1h Rest
PR/min	26.42 ± 1.19	50.58 ± 0.78	54.83 ± 0.86	57.67 ± 0.96	35.20 ± 2.90
RR/min	20.50 ± 0.94	42.0 ± 2.23	48.92 ± 1.29	53.08 ± 1.12	25.20 ± 1.39
RT/min	97.30 ± 0.30	100.27 ± 0.25	101.19 ± 0.19	101.90 ± 0.17	99.44 ± 0.45

Ploughing in pair

Table 15. Meteorological observations during December and January

Month	Temperature (°C)		R.H. (%)		Total rainfall (mm)	Rainy days	Wind speed (kmph)	Evaporation (mm/day)	BSSH
	Max.	Min.	RH_1	RH_2					
December	24.7	6.5	72	27	000.0	0	3.1	2.4	8.3
January	19.2	5.4	76.5	30.75	0	0	3.8	1.75	7.75
Average	21.95	5.95	74.25	28.87	0	0	3.45	2.07	8.02

Fig. 9: Use of mule in ploughing

d. Use of mules in ploughing during winter season under "work-rest-work" scheme

2 hours work-1 hour rest-2 hours work: Mules were used in ploughing under the scheme (2 hours work -1 hour rest -2 hours work) during winter season (December) using two furrow plough. A rest of 10 min was given after every hour of work. Pattern of change in all the physiological responses (PR, RR and RT) have been shown in the Table 16. Average area ploughed was 0.261±0.02 ha during 1st session and 0.249±0.01 ha during 2nd session of 2 hour. Total area ploughed by the mules in 4 hour work was 0.511±0.03 ha. Speed of operation was 2.07±0.16 Km/h and 1.95±0.08 km/h during 1st and 2nd session, respectively. The physiological responses didn't come to normal after a rest of one hour indicating more rest is required. But, all the mules resumed to normal physiological conditions by the next morning.

3 hours work -2 hour rest -3 hours work: Mules were used in ploughing under the scheme (3 hours work -2 hour rest -3 hours work) during winter season using two furrow ploughs in sandy soils. A rest of 10 min was given after every hour of work. But, animal was fully fatigued after 2.5 h of work in the second session and in the next morning also mules were reluctant to work. Hence, 2 hours work -1 hour rest -2 hours work is suitable for mules.

e. Use of donkeys in pair for ploughing

Indigenous donkeys in pair were used for ploughing for 3 h continuously using single furrow plough. A rest of 10 min was given after every hour of work. All the physiological responses (PR, RR and RT) increased significantly after three hours of work and values remained high even after a rest of 1 hour (Table 17). Average area ploughed was 0.184±0.021 ha.

Table 17. Changes in physiological indices

Parameters	Control	3h work	1h Rest
PR/min	34.0±1.05	50.78±2.22	38.17±0.69
RR/min	20.67±0.47	45.44±2.17	27.0±0.58
RT/min	99.08±0.10	100.74±0.14	100.57±0.15

f. Use of donkeys in pair for sowing

Indigenous donkeys in pair were used for sowing bajra (pearl millet) crop for 3 hr continuously using two furrow plough (Fig. 10). A rest of 10 min was given after every hour of work. All the physiological responses (PR, RR and RT) increased significantly and remained high during the entire period of work (Table 18). The physiological values didn't come to normal level even after a rest of 1 hour. Average

Table 16. Changes in physiological indices

Parameters	Control	1h Work	2h Work	1h Rest	1h Work	2h Work	1h Rest
PR/min	26.0a±0.5	46.22±1.47	54.0±0.93	35.56±1.31	48.22±1.08	54.44±0.83	35.0 b±1.48
RR/min	20.22a0.67±	39.44±2.50	48.67±2.57	26.78±1.56	41.33±2.35	50.11±2.86	26.22 b±1.67
RT/min	96.94a±0.28	99.38±0.24	100.96±0.08	99.22±0.24	100.61±0.24	101.31±0.17	100.04b±0.22

Fig. 10. Use of donkeys in pair for sowing

area sowed was 0.338±0.007 ha.

Table 18. Changes in physiological indices parameters

Parameters	Control	3h work	1h Rest
PR/min	28.0±0.60	57.62±2.44	32.14±0.52
RR/min	21.25±0.56	46.0±2.78	27.5±0.63
RT/min	99.07±0.16	101.7±0.08	100.6±0.13

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

Composition of mare milk

Effect of breed, type of feed, stage of lactation on milk composition is well established. Also, literature on milk production and composition of the indigenous horse breeds in India is not available. Hence, work on milk composition was initiated and milk samples from ten mares of Marwari breed were collected during various stages of lactation. The mares were separated from their foals for three hours before milking. Average milk obtained during single milking was 522.0±38.7ml (ranged 200-900 ml) and total milk produced during a day was 3.993±0.337 litre (range 1.2 - 7.2 litre). The milk samples were analyzed for the composition by automatic milk analyzer. The average values (±SEM) observed for fat%, SNF%, density, protein%, lactose%, ash%, pH and conductivity were 0.33 ±0.06, 7.51± 0.04, 27.04± 0.22, 2.14± 0.014, 4.43±0.025, 0.93±0.005, 7.18± 0.057 and 2.56±0.074, respectively. The results indicated that since the mare milk is naturally defatted, it could be the best milk for rehabilitation of patients of coronary health disease.

(Yash Pal, R.A. Legha and R. Singh)

Bacteriological analysis of Zanskari semen

In order to evaluate the semen bacteriologically, 6 Zanskari equine semen samples received (3 frozen and 3 fresh) were processed for estimation of Standard Plate Count (SPC) on Nutreint Agar and isolation on Sheep Blood Agar. Plates were incubated aerobically and at 5% CO_2 atmosphere. Plates were also prepared on MLA for Enterobactericeae family.

On MLA, colonies were counted separately as Lactose Non-fermentors (LNF) and Lactose fermentors (LF). The quantitative enumeration of aerobic bacteria showed an overall bacterial count range of $6.7x10^4$ to $9x10^5$ cfu/ml in frozen semen and $1.5x10^6$ to $2.6x10^6$ cfu/ml in fresh semen. As in the previous study, it was observed that- in general the bacterial counts were lower in frozen samples as compared to fresh semen samples, where counts consistently touched 10^6 cfu/ml in all samples. On examination of MLA plates, it was observed that MLA plates consistently yielded LNF bacteria with arrange of counts between 5×10^2 to 2.5×10^4 cfu/ml.

Of the 6 samples examined, none was found to be sterile, while the rest yielded a predominantly mixed flora. Out of 6 equine semen samples, 10 isolates were picked up, purified and subjected to biochemical identification. Out of 10 isolates, 6 are Gram-positive and 4 are Gram-negative. Potential venereal pathogens isolated from samples included *Escherichia coli* (1 sample). Other bacteria include 2 of *Corynebacteria* and one of *Streptococcus spp,* however further identification is underway. Glucose non-fermenter group is also represented by stout Gram negative rods. Isolation of bacteria from semen of stallions indicates that the environment contamination predominates the semen handling operation, which warrants incorporation of sanitary measures in the process of semen collection and handling.

(R.K. Vaid, S.K. Ravi and T.R. Talluri)

Outsourcing of semen to field veterinarians for AI in mares in field conditions

A total of 60 frozen semen doses of our farm Marwari stallions were given to Dr. A.K. Goel, Veterinary Officer at Gulabewala, Shri Ganganagar for AI in field mares. He did AI in 24 mares and six mares conceived out of which two has given birth to healthy foals (Fig. 11&12). This extremely low percentage of success is due to lack of training of field veterinarians in AI in mares and the lack of facilities like ultrasound for observation of foliculogenesis which helps in

deciding the right time of insemination. Further, AI in mares requires 3 inseminations on consecutive 3 days, which means veterinarians has to attend the mare continuously for 3 days which is not happening in the field. The way forward is creating AI facilities in equine dominated areas and training of field veterinarian for performing AI in mares. Such demands are being sustainably received from Rajasthan and Gujarat

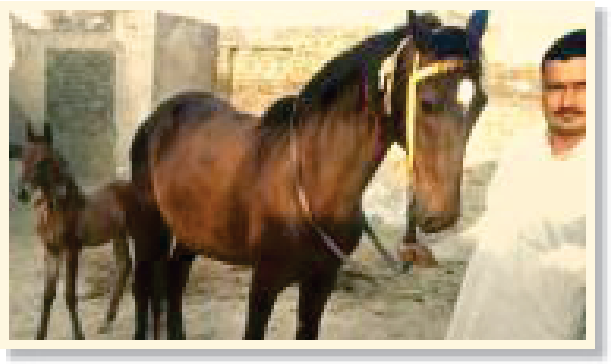


Fig. 11: Foal produced by AI with frozen semen of stallion Paras in field

Fig. 12: Foal produced by AI with frozen semen of stallion Chaman in field

Cryopreservation of semen from elite Marwari stallion in field at farmer's door

During the period of April, 2011 to March, 2012, a total of 102 semen doses of six elite Marwari stallions were cryopreserved for our use at EPC and in mares of the farmers.

Semen stock in semen bank at EPC/NRCE

During April, 2011 to March, 2012, 63, 65 and 20 semen doses of Marwari and Zanskari stallions and Exotic jacks, respectively, were cryopreserved for future use in farm and field. Ten Marwari and 3 Zanskari mares were successfully impregnated through artificial insemination by the use of cryopreserved semen.

(T.R. Talluri and S.K. Ravi)

A study on existing management systems and utilization of donkeys and mules for sustainable livelihood

Donkeys and mules are a source of livelihood for many underprivileged people in rural and peri - urban communities benefitting directly from working equines. Donkeys and mules have often been associated with poverty (unlike cattle, camels or horses) and are mainly used as pack or transporting goods by cart as draft and pack animals in India. They are immensely useful due to their ability to travel through small narrow lanes and tracts, mountains and hilly areas, kutcha roads and even muddy places where other vehicles cannot ply efficiently. The donkeys and mules are mostly used in transportation of agricultural produce, farm inputs like fertilizer, seeds, timber and firewood, construction material etc. They are also found working in brick kilns and constructions sites. Use of donkey and mule in transport and as pack not only reduces drudgery, it also makes major economic contribution to the household income, supports livelihood and contributes to national production and economic development. The majority of working equines are owned by individuals who use them as their sole means of income to sustain their large and extended families. The donkey is the work animal which has the most to offer in assisting rural people and alleviating poverty and providing sustainable livelihood. This is particularly true in the difficult circumstances of the arid and semi-arid tropics to which the donkey is naturally well-adapted. Donkeys are excellent work animal and are generally inexpensive, have low maintenance cost, easy to train, resistant to disease (in dry zones) and of low risk and are seldom stolen.

The survey data collected under the project on socio-economic status of equine owners, prevailing management practices and utilization pattern of donkeys and mules was tabulated for analysis and includes respondents from Rajasthan (n=39), Uttarakhand (n=31), Haryana (n=24), and Uttar Pradesh (n=18).

Table 19: Socio-economic status of the donkey and mule owners

Sr. No.	Characters	Category	Uttarakhand (n1=31)		UP (n2=18)		Rajasthan (n3=39)		Haryana (n3=24)	
			F	(%)	F	(%)	F	(%)	F	(%)
1	Age	Young	20	64.52	5	27.78	8	20.51	7	29.17
		Middle-aged	7	22.58	13	72.22	29	74.36	16	66.67
		Old	4	12.9	0	0	2	5.13	1	4.17
2	Category	SC	9	29.03	10	55.56	13	33.33	24	100.00
		OBC	22	70.97	8	44.44	26	66.67	0	0.00
3	Education	Illiterate	13	41.94	11	61.11	7	17.95	2	8.33
		literate	11	35.48	4	22.22	16	41.03	4	16.67
		Primary	6	19.35	3	16.67	9	23.08	9	37.50
		Middle	0	0	0	0	7	17.95	6	25.00
		High school	1	3.23	0	0	0	0	3	12.50
4	Family size	Small (< 7)	5	16.13	3	16.67	12	30.77	3	12.50
		Medium (7 to 10)	23	74.19	11	61.11	21	53.85	17	70.83
		Large (>10)	3	9.68	4	22.22	6	15.38	4	16.67
5	Experience in	Low (<11)	10	32.26	3	16.67	6	15.38	6	25.00
	equine husba-	Medium (11 to 20)	12	38.71	11	61.11	20	51.28	11	45.83
	ndry (in years)	High (> 20)	9	29.03	4	22.22	13	33.33	7	29.17
6	Monthly Income	Up to Rs. 3000	4	12.90	0	0.00	4	10.26	9	37.50
		Rs. 3001-5000	16	51.61	15	83.33	30	76.92	15	62.50
		Above Rs. 5000	11	35.48	3	16.67	5	12.82	0	0.00

A perusal of Table 19 & Fig. 13 indicates that majority of respondents in Uttarakhand, were from young age group (64.52%) upto 35 years, belonging to minority community (70.97%). Literacy level among the respondents was found to be 58.06 percent. More than one third of respondents (38.71%) were having experience of 11 to 20 years in equine husbandry and had a family size of 7 to 10 members per family (74.19%). In Uttar Pradesh, majority of respondents (72.22%) were from middle age group i.e. 36 to 50 years, belonging to SC Category (55.56%). Literacy level was 38.89 percent and majority of respondents (61.11) were having medium level of experience 11 to 20 years in equine husbandry with a medium family size (61.11%) i.e. of 7 to 10 members per family. In Rajasthan majority of respondents (74.36%) were from middle age group i.e. 36 to 50 years, belonging to minority community (66.67%). Literacy level was found to be 41.03 percent. Majority of respondents (51.28%) were having medium level of experience 11 to 20 years in equine husbandry and medium family size (53.85%) i.e. of 7 to 10 members per family with monthly income between ` 3000 to 5000. Whereas in Haryana, majority of respondents (66.67%) were from middle age group i.e., 36 to 50 years, belonging to belonging to SC Category and most of the respondents were literate. 45.83 percent respondents were having medium level of experience 11 to 20 years in equine husbandry and medium family size (70.83%) i.e., of 7 to 10 members per family having monthly income between ` 3000 to 5000.

Fig. 13: Socio-economic profile of respondents

Usage pattern in different states

The usage pattern of donkeys & mules in different states is depicted in Fig. 14. In Uttarakhand, the mules were used by respondents in carts (80.65%) and also as pack animals (19.35%) as a source of livelihood. The data collected shows that the utilisation of pack animals was mainly seen in the hilly terrain state like Uttrakhand, however, this was not observed in Uttar Pradesh, Rajasthan and Haryana. The small animals were used as pack whereas sturdy animals were used in cart transportation by equine owners. In Rajasthan, the ownership of donkeys was found high (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. In Uttar Pradesh, the mules were used by respondents in carts for transportation of bricks at brick kilns as a main source of livelihood. In Haryana, the donkeys and mules were used in cart transportation of agricultural produce, farm inputs and construction material.

Regarding management practices daily cleanliness of animals was higher in Uttar Pradesh (77.78%), followed by Haryana (66.47) and Rajasthan (46.15%) (Table 20). Remaining respondents were cleaning their animals twice a week. Grooming practices were followed by 61.11 percent respondents in Uttar Pradesh and the percentage of respondents following grooming practices was lowest in Uttarakhand. With regard to shoeing of working equines the mules in all the three states were shoed regularly but shoeing was not observed in case of donkeys. Deworming and vaccination for any disease was not a regular practice in all the four states (Table 20). In none of the states the routine recommended managemental practices were being followed by the equine owners.

Fig. 14: Use of Donkey and mule in transporting construction material

Table 20: Utilization and Management Pattern of Working Equines

Parameter	Category	Uttarakhand		Uttar Pradesh		Rajasthan		Haryana	
		F	%	F	%	F	%	F	%
Ownership	Donkey	0	0.00	0	0.00	33	84.62	9	37.50
	mule	31	100.00	18	100.00	8	20.51	18	75.00
Utilization	pack	6	19.35	0	0.00	0	0.00	0	0.00
	cart	25	80.65	18	100.00	39	100.00	24	100.00
Cleaning	Daily	6	19.35	14	77.78	18	46.15	16	66.67
	Twice/week	25	80.65	4	22.22	21	53.85	8	33.33
Grooming	Regularly	5	16.13	11	61.11	6	15.38	9	37.50
	Sometimes	26	83.87	7	38.89	29	74.36	15	62.50
Shoeing	Yes	31	100.00	18	100.00	8	20.51	16	66.67
	No	0	0.00	0	0.00	31	79.49	8	33.33
Deworming	Regularly	3	9.68	1	5.56	0	0.00	6	25.00
	Sometimes	17	54.84	6	33.33	16	41.03	5	20.83
	Never	11	35.48	10	55.56	23	58.97	13	54.17
Vaccination	Never	31	100.00	18	100.00	39	100.00	24	100.00

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

VTCC Accomplishment

Veterinary Type Culture Collection has a mandated aim of long-term reposition and distribution of well characterized Veterinary, Dairy and Rumen microbes. This will serve as a Microbial genetic resource center, which will provide necessary impetus to development of the livestock sector. This distribution of characterized microbes to the different stake-holders of the country will help in further research and development in the field of microbiology, taxonomy, biotechnology, epidemiology and vaccinology. During the year 2011-12 VTCC repository increased its

Table 21. Cultures reposited during the period April 2011- March 2012

Microbial Resource	Accessioned	Under accessioning	To be deposited*	Total
Veterinary Microbes				
Bacteria	185	339	96	620
Virus	3	4	-	7
Fungus	-	13	80	93
Recombinant clones	70	12	-	82
Phage library	-	-	-	-
Total	258	368	176	802
Rumen Microbes				
Anaerobic bacteria	-	63	-	63
Fungi/Yeast	-	94	-	94
Methanogenic archae	-	8	-	8
Total	-	165	-	165
Dairy Microbes				
Bacteria	178	66	51	295
Grand Total	436	599	227	1262

Table 22. Present strength of the repository

Microbial Resource	Accessioned	Under accessioning	To be deposited	Total
Veterinary Microbes				
Bacteria	440	339	96	875
Virus	106	4	-	110
Fungus	-	13	80	93
Recombinant clones	180	12	-	192
Phage library	27	-	-	27
Total	753	368	176	1297
Rumen Microbes				
Anaerobic bacteria	7	63	-	70
Fungi/Yeast	-	94	-	94
Methanogenic archae	-	8	-	8
Total	7	165	200	172
Dairy Microbes				
Bacteria	504	66	51	621
Grand Total	1264	599	427	2090

collection from previous 358 (2010-11) numbers to present 546 (2011-12) of accessioned veterinary microbes including 440 bacterial and 106 viral cultures along with 180 accessioned recombinant clones. Details of the cultures reposited during the period April, 2011- March, 2012 are depicted (Table 21). The present strength of the repository mentioned in Table 22. Year wise accessioning of veterinary, rumen and dairy microbes are presented (Table 23, Fig 15). The VTCC repository has been strengthen with reposition of many bacterial species (Fig 16) covering large numbers of host species (Fig 17) and different pathogenic virus isolates (Fig 18). The cultures deposited in the repository are from the different states of the country (Fig 19).

Table 23. Year wise accessioning of cultures

Microbial Resource	2009-10	2010-11	2011-12
Veterinary Microbes	212	358	546
Rumen Microbes	0	7	7
Dairy Microbes	296	326	504
Recombinant Clones	34	110	180
Phage Library	27	27	27
Grand Total	569	828	1264
		45.5% increase	52.7% increase

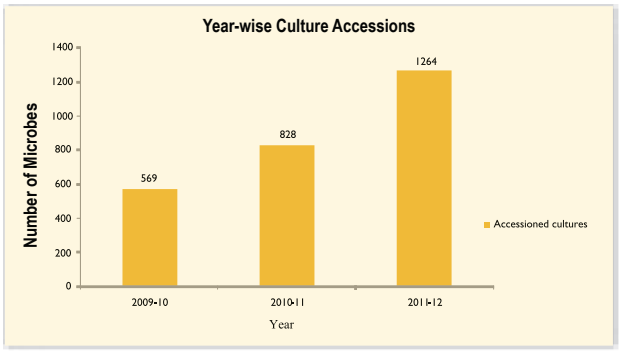


Fig 15. Year wise accessioning of cultures

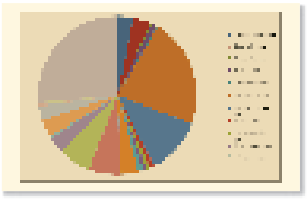


Fig 16. Genus-wise distribution of bacteria

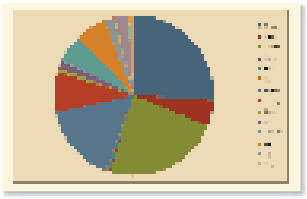


Fig 17. Host-wise distribution of bacteria

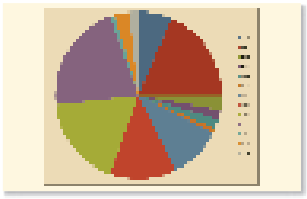


Fig 18. Distribution of viral isolates

Fig 19. State-wise distribution of cultures

First detection of Parapoxvirus (Pseudocowpox) zoonosis in cattle and human

Parapoxvirus was detected by semi-nested PCR and PCR-RFLP targeting B2L envelop gene in samples collected from cattle and a human from Modipuram area in Meerut in U.P. in 2011 involving 5 cattle, a community milker and as animal owner. Lesions were observed only in milking cows. The lesions consisted of vesicles, scab and ulcers on udder and teats (Fig.20A). No lesions could be seen on other parts of the body. There was no mortality. Further investigations revealed vesicle and scab formation on fingers, fore arm and hands of a community milker and the animal owner (Fig.20B). Clinical samples were collected from the affected animal and human cases. The Parapoxvirus infection was confirmed by employing semi-nested PCR and PCR-RFLP on the samples targeting the B2L envelop gene (Fig. 21). Virus isolation could not be achieved due to difficulty in adaptation of Parapoxvirus in lamb testicle and MDBK cell cultures. However, bovine foetal muscle cell line is required for isolation of Parapoxvirus.

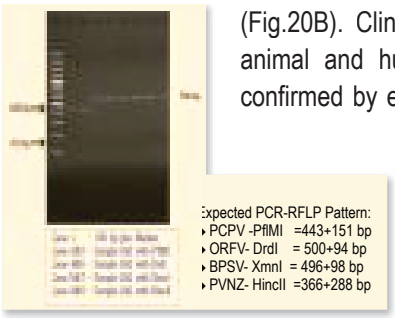


Fig. 21: PCR-RFLP confirmation of PCPV

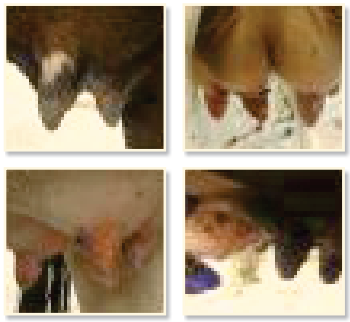


Fig. 20A: Lesions on the teats in Cattle

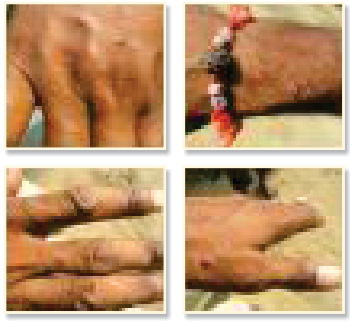


Fig. 20B: Lesions on the hand and fingers

Molecular characterization of host-range and structural genes of BPXV isolates for reposition

To add to the passport information of the reposited buffalopox viruses isolated from cattle, buffalo and human, different host-range and structural genes were sequenced and analyzed. This will help to elucidate the host-tropism of poxvirus infecting un-natural hosts like cattle and humans. Among the host-range genes, E3L, K3L & C7L are essential for virus replication by preventing interferon resistance, whereas B5R is essential for spread of the virus and evasion from the host's immune response as in VACV. The genetic differences between host-range genes of BPXV isolates from buffalo, cattle and human were elucidated. Four host-range genes viz., E3L, K3L, C7L & B5R of buffalopox virus (BPXV), from buffalo (BPXV/buffalo/Baatnor/2011), cattle (BPXV/cattle/Baatnor/2011) and human (BPXV/human/Baatnor/2011) were PCR amplified, cloned and sequenced. Homology analysis revealed high similarity (~97 to 99% at nt and ~95 to 99% at aa level for E3L; ~98 to 99% at nt and ~97 to100% at aa level for K3L; ~98 to 98% at nt and ~97% at aa level for C7L; and ~97 to 99% at nt and ~96 to 99% at aa level for B5R genes) with vaccinia virus (VACV). The phylogeny constructed based on concatenated gene sequences revealed that BPXVs are not as closely related to vaccine strain (Lister and Lister derived strain- LCm8), as hypothesized earlier, rather they are more closely related to other vaccinia viruses like Duke, Ankara and modified Ankara strains (Fig 22). This information aids in deciphering the molecular pathogenesis of poxvirus in spillover hosts, which will help to develop control strategies.

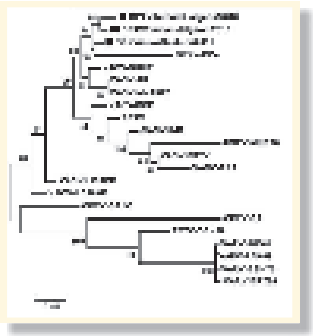


Fig. 22: Maximum-likelihood tree constructed using concatenated alignment of nucleotide sequences of E3L, K3L, C7L and B5R genes of BPXV isolates

Molecular characterization of Newcastle Disease virus (NDV) from Sheep and Donkey for reposition

The newly isolated Newcastle Disease virus (NDV) reposited in VTCC collection were authenticated by sequence analysis of F gene of the NDV. Isolations were made from plasma samples collected from spillover hosts-sheep and Donkey from Bikaner (Rajasthan). The natural host of NDV is primarily avian species. This virus has not been isolated from naturally infected non-avian and non-human hosts; however, a report of NDV isolation from cattle in 1952 and recently from sheep in 2012 is also reported. Here, we report the first isolation and identification of NDV from sheep and donkey. Fusion protein genes of two NDV isolates (NDV/Sheep/1/Bikaner/2011 & NDV/Donkey/1/Bikaner/2011) isolated from plasma samples collected from Bikaner, were PCR amplified using published primers, cloned and sequenced to ascertain their genotype. Sequence analysis revealed that these isolates belonged to the genotype IV of Newcastle disease virus under the genus Avulavirus of *Paramyxoviridae* family. Homology analysis showed ~95% similarity with Chicken/87 and Peacock/08 isolates from Tamil Nadu. Phylogeny of the nucleotide sequences (Fig. 23) revealed close clustering with Indian isolates. The findings suggest that this virus be included in the screening of viruses from non-avian hosts.

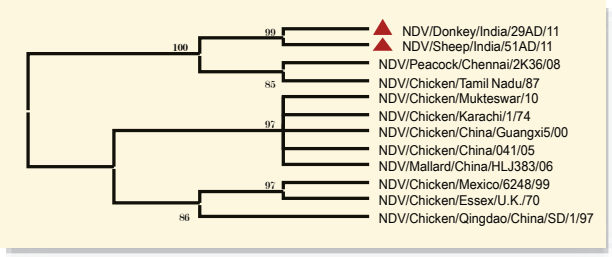


Fig. 23: Phylogenetic analysis of F genes of NDV isolates from sheep and donkey.

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

Whole Genome Sequencing of *Pasteurella multocida* sub spp. *multocida* B:2 serotype

Many isolates of *Pasteurella multocida* are available in the repository. To strengthen the passport information of the isolates, the genome of a novel pathogenic B:2 serotype of *Pasteurella multocida* isolated from a buffalo in an outbreak of Haemorrhagic Septicemia was sequenced employing pyrosequencing technique.

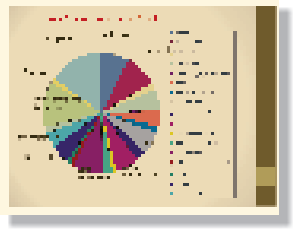


Fig. 24: RAST analysis of *Pasteurella multocida* genome.

Previously, 3 isolates of *Pasteurella multocida* were isolated from that outbreak, and confirmed biochemically as well as by PCR. The isolates have been accessioned in repository with the accession nos.VTCCBAA264, VTCCBAA265 & VTCCBAA266. The sequence analysis revealed that the genome of *Pasteurella multocida* (VTCCBAA264) consisted of 2,07,386 bp nucleotide sequence distributed in 953 contigs. The contigs were analysed using online RAST platform and annotated different genes related to biochemical pathways (Fig.20). The genome contained 52 RNA operons comprising of 6 rRNA operons and 46 tRNAs. The contigs of *Pasteurella multocida* VTCCBAA264 have been submitted to GenBank, NCBI on March, 2012 under BioProject No. PRJNA89423. The detailed analysis of the genome will decipher the information on genomic characteristics and pathogenic island(s) of the virulent isolate of *Pasteurella multocida*. The availability of a sequenced bacterial culture in the VTCC culture collection not only enhances the value of collection, but also opens up new vistas of research on a pathogen which is an important cause of economic losses in ruminants in our country.

First isolation, molecular identification and reposition of microbes

Trueperella *pyogenes* from buffalo

Trueperella *pyogenes* is a representative of new Genus, which has been recently recognized taxonomically. It often causes, purulent infections, mainly pneumonia, arthritis, mastitis and subcutaneous abscess in cattle, buffalo, sheep, pigs and humans. We isolated *T. pyogenes* from pus sample of subcutaneous abscess of a buffalo from an organised dairy farm. The Gram-positive isolate (Fig. 25 & 26), which grew in CO_2 atmosphere slowly was identified biochemically and than by 16S rRNA sequencing. It has been preserved at -80°C in glycerol stock with Accession No. VTCCBAA267.

Fig. 25 : Dew drop colonies of *Trueperella pyogenes*

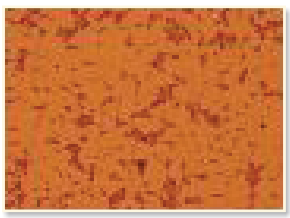


Fig. 26: Gram positive *Trueperella pyogenes* after 48 hours incubation at 5% CO_2 on SBA

Rhodococcus equi from double-humped camel of Leh-Ladakh

A set of faecal samples collected from double-humped camel from a Government Camel Farm where the elusive double-humped camel (Fig. 27) are being kept led to isolation of *R. equi*. The samples were processed by Selective Plating on NANAT media and isolated *Rhodococcus equi* with atypical colony colour (Fig. 28 & 29). The isolates were subjected to choE, vapA and tra genes specific-PCR, which confirmed isolates as commensal *Rhodococcus equi* isolates. The isolates have been preserved as a rare isolates from doubled-humped camel in the repository.

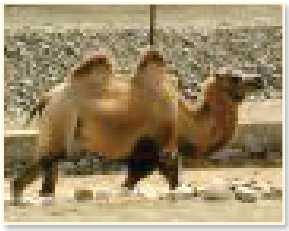


Fig. 27: Double humped camel

Fig. 28: *Rhodococcus equi* with atypical colony colour

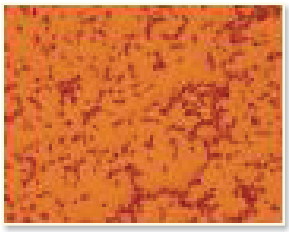


Fig. 29: Gram's staining of *Rhodococcus equi* isolate

Bordetella bronchiseptica from a thoroughbred horse

The *Bordetella bronchiseptica* isolate (Accession No. VTCCBAA1)-previously isolated (Fig. 30) from thoroughbred horse in Tohana (Haryana) which constituted first laboratory report of *B. bronchiseptica* isolation from nasal swab of a thoroughbred horse suffering from respiratory tract infection in India-was further characterized.

Bordetella bronchiseptica was further confirmed by 16S rRNA PCR and sequence analysis. Amplification of 16S rRNA, bvgA, cyaA and flaA genes using DNA isolated from purified culture yielded 1522bp, 768bp, 1185bp and 748bp products, respectively. Upon comparison of 16S rRNA

Fig. 30: Gram-negative bacillary rods of *Bordetella bronchiseptica*

sequences, Indian isolate shared similarity of 99.74% with nucleotide sequences of *Bordetella bronchiseptica* available in GenBank. The bvgA, cyaA and flaA genes showed identity of 98 to 99% at nt level, respectively among compared isolates of *Bordetella* spp. The higher sequence similarity of nucleotide and amino acid sequences indicates that these genes are highly conserved among *Bordetella* species. The topologies of the phylograms of all three genes (Fig. 31a, b, c) followed similar branching pattern with clustering of *B. bronchiseptica* isolates in a single group.

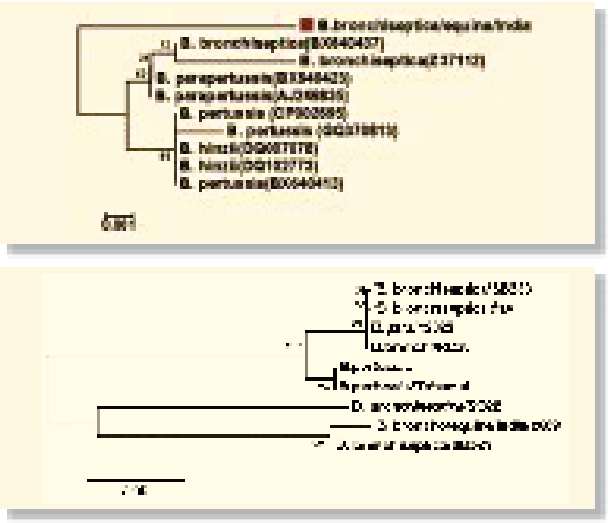


Fig. 31: Phylogenetic trees of (a) *CyaA* gene; (b) *fla* gene of *B. bronchiseptica*

Enterococcus asini from horse & *Exiguobacterium* spp. from pig

Different corynebacterial and other Gram-positive isolates previously isolated from different animal species like horse pig and donkeys feces and available in the repository were subjected to 16S rRNA gene analysis. Upon sequence analysis, *Enterococcus asini* (Fig. 32) isolated from horse was confirmed. Homology analysis using BLAST revealed >90% homology with sequences available in the database.

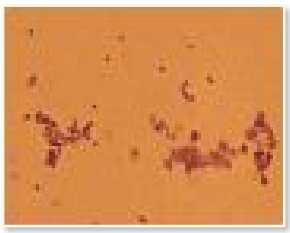


Fig. 32: Gram staining of *Enterococcus asini.*

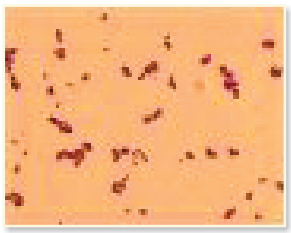


Fig. 33: Gram staining of *Exiguobacterium* spp.

The isolated bacterium from pig feces was identified as *Exiguobacterium* spp (Fig. 33) by homology analysis of nucleotide sequence data of the 16S rRNA gene which was cloned and sequenced.

Brevibacterium spp. and *Brevibacillus* spp. from Equine

In order to investigate the normal microflora of healthy equines including horse and donkeys feces samples were subjected to selective plate screening and Gram-positive rods and cocci were isolated and subjected to biochemical testing. Further screening and sequence analysis of 16S rRNA of the bacteria isolated from equine faeces led to identification of *Brevibacterium* spp. (Fig. 34) and *Brevibacillus* spp. bacteria (Fig. 35). These microorganisms are opportunistic pathogens and have been previously reported to be involved in human clinical cases. *Brevibacillus* spp-reclassified recently from *Bacillus* spp. - is

Fig. 34: Gram staining of *Brevibacterium* spp.

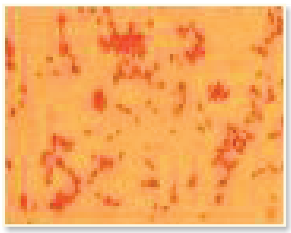


Fig. 35: Gram staining of *Brevibacillus spp.*

used as bio-control agent, has been isolated from AIDS patients, and clinical cases of peritonitis in humans. It has also been shown to produce Gramicidin, a non-ribosomal antimicrobial peptide.

Molecular Characterization of atypical *Rhodococcus equi* isolates from donkey, camel, horse and pig

Rhodococcus equi, the Gram-positive pleiomorphic rod that is a free-living saprophyte with widespread distribution, causes chronic granulomatous pneumonia, lung abscesses and occasional enterocolitis in foals and is a common isolate from cervical lymph nodes in swine. Its importance as zoonotic pathogen has been increasing, particularly in immunocompromised hosts. Due to the emergence of many corynebacteria as animal and human pathogens, rigorous biochemical and molecular tools have increasingly been

applied for identification and epidemiological studies of *R. equi*. Previously, isolates of *R. equi*, isolated from different animal species and subjected to biochemical identification revealed biochemically atypical isolates. Seven of these atypical *Rhodococcus equi* isolates from Donkey, Camel, Pig and Horse were subjected to 16S rRNA gene sequence analysis. The global alignment (Table 24) of partial sequences showed that 5 isolates were of *Rhodococcus* genus; however the species could not be identified. Chemotaxonomic and ribotyping studies need to be performed in order to identify the isolates up to species level. The present study indicates prevalence of *Rhodococcus* in donkey, pig and camel population. The presence of typical and atypical biotypes of these actinomycetes indicates inadequacy of biochemical characterization in confirmation of taxon and virulence properties.

Table 24: Identification of bacteria by analysis of 16S rRNA sequences.

Sl. No	Culture ID.	Host Animal	Identification
1	D7BD	Donkey	*Rhodococcus* spp. dasan
2	C1BD	Camel	*Rhodococcus* spp. PK10
3	D2AD	Donkey	*Rhodococcus* spp. ARG-BN062
4	D7AD	Donkey	*Lysinibacillus fusiformis*
5	C1A	Camel	*Rhodococcus* spp. WT123
6	E2JD	Horse	*Bacillus cereus*
7	P6BD	Pig	*Rhodococcus* spp. BF4

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

Rumen Microbes Component

Isolation and characterization of tannin degrading, nitrate reducing and cellulose degrading bacteria and archae and their reposition

Several biologically active ruminal bacreia have been isolated from different livestock species. Seven tannin degrading bacteria- *Streptococcus gallolyticus* from goat, fibre degrading bacteria- *Ruminococcus flavefaciens*, *Prevotella* sp. and *Butyrivibrio* sp. from buffaloes and cattle and nitrate reducing & cellulose degrading *E. coli* from buffalo have been isolated. They have also been characterized morphologically and by sequencing of 16S rRNA and sodA genes. Two genera of archaea *viz.*, *Methanomicrobium mobile* and *Methanobrevibacterium ruminantium* were identified, characterized and preserved.

Reposition of important rumen fungi and bacteria

Important bubaline rumen fungi such as *Anaeromyces* sp., *Orpinomyces intercalaris* and *Orpinomyces joyonii* and caprine isolates: *Piromyces* sp. and *Neocallimastix* spp. have been isolated and preserved. Several rumen bacteria such as *Bacillus licheniformis*, *Butyrivibrio* sp., *Eubacterium limosum*, *Megasphaera elsdenii*, *Prevotella* sp., *Streptococcus bovis*, *Streptococcus equinus*, *Streptococcus gallolyticus*, *Streptococcus lutetiensis*, *Streptococcus sanguinis* and *Veillonella parvula* have been isolated from cattle, goat and buffalo. These isolates were characterized by sequence analysis of ITS and LSU regions of the genome. Three important rumen bacteria *viz.*, *Streptococcus equinus*, *Streptococcus bovis* and *Streptococcus* sp. L10 have also been isolated from camels. These isolates have also been further analyzed for use ful biological activities such as fibre and protein degradation (*Prevotella* spp, *Butyrivibrio* spp.); urea hydrolysis (*Megasphaera* spp.); tannin degradation (*Streptococcus* spp.); bacteriocin production (*Bacillus*) etc.

Dairy Microbes Component

Important dairy microbes preserved in the repository

Dairy microbes includes *Lactobacillus* spp., *Lactococcus* spp., *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *Cremoris*, *Lactococcus lactis* ssp. *lactis* bv. *Diacetylactis*, *Streptococcus thermophilus*, *Leuconostoc* spp., *Bifidobacterium spp. Bifidobacterium dentium, Bifidobacterium longum, Micrococcus sp., Kluyveromyces lactis and Saccharomyces bisporus* etc. have been isolated

from different sources such as fermented milks, vegetables, fruits, fermented foods like dahi, lassi, chhas, shrikhand, idli batter, dosa batter, jalebi batter *etc*.

Practical utility and commercial potential of isolated dairy cultures

Different varieties of dairy products such as curd, shrikhand and butter milk are preferred by consumers because of their flavour, nutritive value and health benefits. These functional properties of the products depend on the characteristics of the starter cultures of the dairy microbes. In this direction, the isolated cultures have been evaluated for such properties. *Seven Leuconostoc* isolates have flavour and EPS positive characters. Two *L. lactis* isolates have characteristics of fast acidifier. Combination of *L. lactis* ssp *lactis*-C12 and *Leuconostoc mesenteroides* ssp. *mesenteroides* is very suitable for dahi and lassi preparation. Six *Lactobacillus* spp. with phytase degrading and antifungal activities, and a *Pediococcus acidolactici* isolated form milk-cereal fermented products (Rabadi samples) can be potentially used as starter cultures for preparation of milk-cereal fermented products with extended shelf life. Diacetyl and EPS producing strains of *Leuconostoc* spp. have the potential for preparation of low fat fermented milks, lassi and as natural bio-thickeners/ stabilizers. Thirty fast acidifying *Lactococcus lactis* and ten galactose positive *Streptococcus thermophilus* can be used for various fermented milk products.

Important microbial isolates available in the repository

Some important veterinary, rumen and dairy microbial isolates are listed in table 25.

Table 25: List of some veterinary, rumen origin and dairy microbial isolates in repository collection

Microbe	Importance	Accession No.
Bordetella bronchiseptica	First equine isolate from horse in India	VTCCBAA1
Pseudomonas aeruginosa	Horse semen isolate	VTCCBAA4
Serratia marcescens	Reference Strain	VTCCBAA89
Bacillus spp.	Soft tick isolate	VTCCBAA3
Klebsiella pneumoniae	Foal diarrhea isolate	VTCCBAA6
Edwardsiella tarda	Reference Strain	VTCCBAA92
Klebsiella pneumoniae	Mare abortion isolate	VTCCBAA7
Escherichia coli	Mare abortion isolate	VTCCBAA8
Aeromonas hydrophila	Reference Strian	VTCCBAA95
Staphylococcus sciuri	Methicillin resistant isolates from goat	VTCCBAA22VTCCBAA25
Moraxella bovis	Reference Strain	VTCCBAA101
Staphylococcus hyicus	Pig skin pathogenic isolates	VTCCBAA32VTCCBAA32
Pseudomonas putida	Equine Vegetative Endocarditis isolate	VTCCBAA39
Rhodococcus equi	Foal pneumonia isolate VapA postive	VTCCBAA61
Nocardia asteroides	Reference strain	VTCCBAA110
Salmonella spp.	Lamb diahhorea isolate	VTCCBAA116
Escherichia coli O116	Calf diahrea isolate	VTCCBAA115
Escherichia coli O60, O71	Monkey isolates	VTCCBAA112VTCCBAA113
Escherichia coli	Emu isolates	VTCCBAA139VTCCBAA140
Salmonella spp.	Bovine isolate	VTCCBAA154
Salmonella spp.	Emu isolate	VTCCBAA155
Bacillus subtilis	Canine isolate	VTCCBAA166
Bacillus subtilis	Bovine isolate	VTCCBAA180
Salmonella Typhimurium	Sheep isolate	VTCCBAA208
Brucella melitensis Biovar I	Biovar I of goat abortion isolate	VTCCBAA227
Brucella melitensis Biovar III	Biovar III of goat abortion isolate	VTCCBAA228
Pasteurella multocida	Cattle isolate	VTCCBAA229
Pasteurella multocida	Calf nasal isolate	VTCCBAA232
Pasteurella multocida	Rabbit nasal isolate	VTCCBAA233
Pasteurella multocida	Buffalo lung isolate	VTCCBAA234
Pseudomonas aeruginosa	Canine fecal isolate	VTCCBAA238
Pseudomonas aeruginosa	Hog deer isolate	VTCCBAA239
Escherichia coli	Sambhar deer isolate	VTCCBAA243

Rhodococcus equi	Mare abortion isolate	VTCCBAA234
Rhodococcus equi	adult mesenteric LN rare isolate	VTCCBAA259
Trueperella pyogenes	First Genus representative of *Trueperella* from pus of buffaloes	VTCCBAA267
Pasteurella multocida	Whole genome sequenced Biovar II buffalo isolate	VTCCBAA264
Actinomyces spp.	Buffalo abortion isolates	VTCCBAA331
Exiguobacterium spp.	First isolate of Genus representative from Pig	VTCCBAA317
Bacillus cereus	Horse dung	VTCCBAA443
Corynebacterium pseudotubeculosis	Goat isolate	VTCCBAA442
Listeria monocytogenes	Fresh water isolate	VTCCBAA336
Listeria innocua	Animal clinical isolate	VTCCBAA336
Listeria monocytogenes	Human clinical isolate	VTCCBAA338
Listeria innocua	Seafood isolate	VTCCBAA362
Listeria monocytogenes	Vegetable isolate	VTCCBAA341
Listeria monocytogenes	Meat isolate	VTCCBAA353
Listeria monocytogenes	Seafood isolate	VTCCBAA363
Citrobacter freundii	Calf diahhorea	VTCCBAA309
Anaeromyces spp.	Rumen fungi from buffalo	HQ263340HQ703466
Orpinomyces intercalaris	Rumen fungi from buffalo	JF974135HQ703471
Orpinomyces joyonii	Rumen fungi from buffalo	HQ263324HQ263325
Piromyces spp.1	Rumen fungi from goat	JF974089JF974094
Piromyces spp.2	Rumen fungi from goat	JF974103JF974117
Neocallimastix spp.	Rumen fungi from goat	JF974107JF974121
Olsenella spp.	Rumen bacteria from goat	JF709905
Megasphaera elsdenii	Buffalo rumen bacterial isolate	JF709902
Eubacterium limosum	Goat rumen bacterial isolate	JF709903
Megasphaera elsdenii	Goat rumen bacterial isolate	JF709896
Streptococcus bovis	Buffalo rumen bacterial isolate	JF709892
Streptococcus equinus	Buffalo rumen bacterial isolate	JF709893
Streptococcus gallolyticus	Goat rumen bacterial isolate	JF709907
Streptococcus lutetiensis	Buffalo rumen bacterial isolate	JF709891
Streptococcus sanguinus	goat rumen bacterial isolate	JF709904
Veillonella parvula	goat rumen bacterial isolate	JF709906
Lactococcus lactis ssp. *Lactis*	Dairy microbe	NCDC 154
Lactococcus lactis ssp. *Lactis*	Dairy microbe	NCDC 161
Leuconostoc mesenteroides ssp. *mesenteroides*	Dairy microbe	NCDC 153
Lactobacillus rhamnosus	Rabadi isolate from Rajasthan	HQ008217
Lactobacillus fermentum	Rabadi isolate from Rajasthan	HQ008219
Lactococcus lactis	Isolate from beans	GU992398
Lactococcus lactis	Isolate from crepe jasmine	GQ267535
Lactococcus lactis	Isolate from White Rose	GQ267536
Lactococcus lactis	Isolate from Kefir	GQ267537
Lactococcus lactis	Isolate from dahi	GQ267538
Lactococcus lactis	Isolate from spinach	GQ267541
Lactococcus lactis	Isolate from Green chilly	GQ267542
Lactococcus lactis	Isolate from carrot	GQ267543
Lactococcus lactis	Isolate from Cheddar cheese	GU056806
Streptococcus thermophilus	Isolate from market dahi	GQ253961
Streptococcus thermophilus	Isolate from market dahi	GQ253961
Lactococcus lactis	Isolate from Yak milk	GU056862
Lactobacillus helvetisuc	Human vaginal isolate	GQ253959
Lactobacillus helvetisuc	Human intestinal tract isolate	GQ253960
Propionibacterium freudenreichiii subsp. *freudenreichii*	Reference Strain	DSM 20271
Propionibacterium freudenreichiii subsp. *shermanii*	Reference Strain	DSM 20270
Propionibacterium acidipropionici	Reference Strain	DSM 20272
Propionibacterium jensenii	Reference Strain	DSM 20279
Propionibacterium thoenii	Reference Strain	DSM 20277

Externally Funded Projects

Inter-Institutional and Externally-funded projects

1. OIE Laboratory-Twinning Project on Equine Piroplasmosis

In vitro MASP culture of *Theileria equi*

In vitro MASP culture of *Theileria equi* was initiated in double gas (5%CO_2 and 5%O_2) incubator using M199 medium supplemented with L-glutamine and 40% horse serum. The blood samples from *T. equi* antibody positive horses were collected in vaccutainer (EDTA) and culture was started. The parasites were observed on the 12-14th day of culturing (Fig. 36). Subsequently, subculturing was done and culture was maintained. Further, Plan is initiate MASP culture from more number of *T. equi* positive horses.

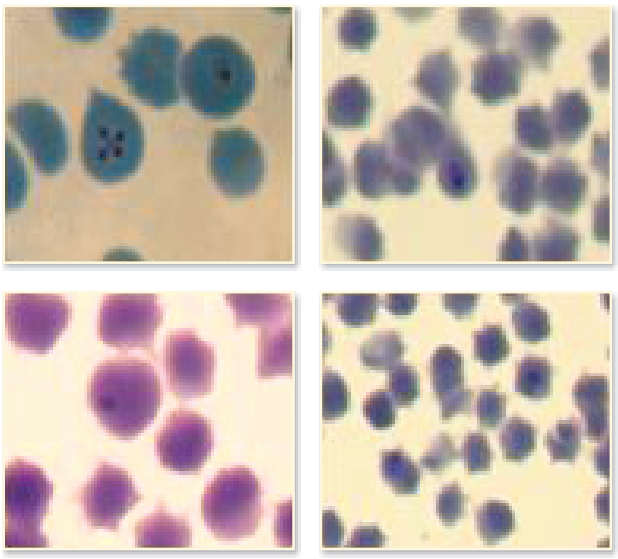


Fig. 36: Kinetics of *T. equi* development in MASP culture initiated from ELISA positive horses

PCR and Nested-PCR

Blood samples were collected from fifty randomly selected equines from endemic areas around Hisar and Karnal districts of Haryana. DNA was isolated from these samples for use in PCR. The primers were designed targeting EMA-2 gene of *T. equi* (AB013725) using online software (Primer 3). The two sets of primers were designed for nested PCR. Primer sequences for first PCR were: forward 5'- CGCCG ATGAGGCACCAAAGGT-3' & reverse 5'- AGAGCT TTCCCTCCTTCAAGTGAGT-3' and for nested-PCR, primers were: forward-5'- CATTGACCACGTAACCGTTG -3' and reverse – 5'- CTTGGGGCATCTACCTTCAA -3'. The first PCR condition was standardized with annealing temperature 58°C and subsequently subjected to second round nested-PCR. The samples tested with nested-PCR were compared with recombinant antigen based ELISA (r-ELISA) (Table 26). Result showed that the nested-PCR was more sensitive than r-ELISA.

Recombinant EMA-1 and EMA-2 protein based ELISA for detection of *T. equi*

To develop diagnostics for detection of *T. equi*, recombinant EMA-1 and EMA-2 proteins expressed previously were used in ELISA test and results were compared with serum samples collected from field. These recombinant antigens gave optimum results in ELISA when diluted to 5 µg/ml concentration. A set of serum samples collected from experimentally infected horses (available at NRCPD, Japan and samples collected up to 37 days post-infection) were tested in ELISA using these two r-Ags and significant rise in OD was observed 9 days post-infection onward (Fig. 37). The results obtained by these two antigens on experimental

Table 26: Comparison of results on field samples by PCR, nested-PCR & r-ELISA

Location	No of samples examined	r-ELISA positive samples	PCR positive (%)	Nested-PCR positive (%)
Kalrum village, Karnal	18	14 (77.77)	14 (77.77)	14 (77.77)
Madhuban, Karnal	19	12 (63.15)	12 (63.15)	12 (63.15)
TVCC HAU, Hisar	13	2 (15.38)	2 (15.38)	4 (30.76)
Total	**50**	**28 (56)**	**28 (56)**	**30 (60)**

serum samples were nearly similar.

Separately, we tested 48 serum samples with ELISA based on r-Ags, (EMA-1 or EMA-2) and with CI ELISA (recommended by OIE). Of these 48 serum samples; 38, 35, 35 were detected positive and 10, 13, 13 were detected negative in CI ELISA and ELISAs using r-Ag of EMA-2 and EMA-1, respectively. Testing of field samples by these recombinant antigens based ELISA is in progress.

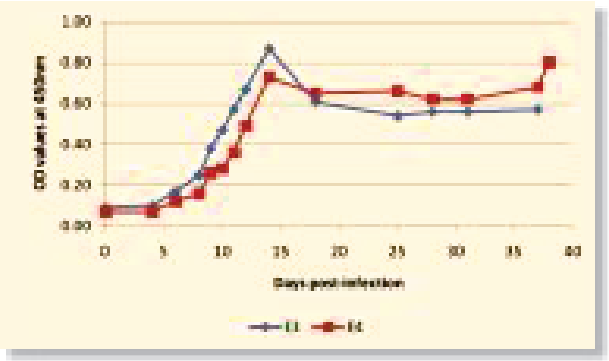


Fig. 37: Testing of serum samples using r-Ag based ELISA

Immuno-chromatographic Test (ICT) for detection of *T. equi*

NRCPD develops ICT strips as a pen-side diagnostic test. This test is very simple to use in the field and no extraordinary skill is required for carrying out this test. Just a drop of serum would be required and result can be read in 10 minutes. We tested suitability of test on field serum samples (34) collected from an endemic area (Hanumangarh, Rajasthan). These samples were tested on ICT strips (Fig. 38) and results were compared with ELISA (based on recombinant antigen) (Table 27). Out of 34 serum samples tested on ICT strips and ELISA two samples were negative for the presence of *T. equi* specific antibodies in both the assays (Table 27). Two serum samples reacted very weakly in ICT while these samples recorded low antibody titre (as

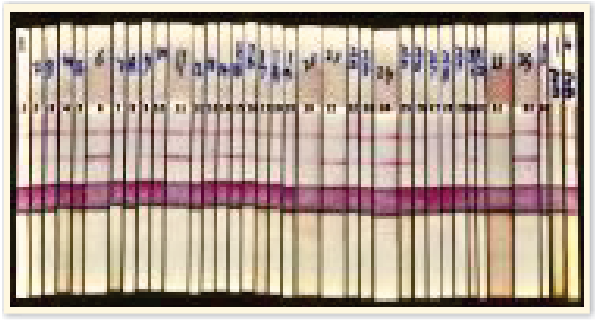


Fig. 38: ICT strips immunoreacted with serum samples collected from field

low OD value was observed) in ELISA. Efforts are on to confirm the presence of parasite in these low titre serum samples by initiating MASP culture and demonstrating *T. equi*.

Table 27: Comparison of results obtained on serum samples tested with ICT strips and ELISA

Status	ELISA	ICT
High OD/Clear positive line	12	12
Low OD/low positive line	7	8
Positive at margin/faint positive line	12	9 + 2(±)
Negative	2	2
Total		34

(NRCE, India; Sanjay Kumar, Rajender Kumar and R.K. Singh; NRCPD, Japan: Prof. I. Igarashi and N. Yokoyama)

Besides above twinning project, two more twinning projects have been approved by OIE, as mentioned below :

- The twining programme on Glanders is approved and will commence from July, 2012 for a period of three years.
- The twinning programme on Equine Influenza has been approved and is listed in OIE website as "Approved and to commence".

UK-India partnering Award granted by BBSRC (UK)

UK-India Partnering Award has been granted by BBSRC (UK) with sanction of a financial outlay of £ 25000. This award is between Institute for Animal Health (Pirbright, UK), Project Directorate on FMD (Mukteswar, India), and VTCC/NRCE (Hisar, India). Under this award, the scientists from UK and Indian labs which are participating in his project will be able to have exchange visits to Indian and UK labs to explore possibilities of identifying potential areas for research collaboration - on "Smart Partnership" basis - and developing research project proposals which are mutually beneficial.

2. DBT-funded inter-institutional project on Isolation and characterization of non-pathogenic adenoviruses from animals

Adenoviruses from buffaloes and horses can be used as vectors for delivery of protective antigens to develop recombinant vaccines for humans as well as animals. In order to use bovine and equine adenoviruses as vectors, non-pathogenic adenoviruses from buffaloes and equines were isolated and characterized. During the year, non-pathogenic equine adenoviruses were isolated by passaging in Equine Fibroblast cells from 7 PCR-positive nasal swabs collected from healthy foals. The virus isolates were confirmed by PCR (Fig. 37). The PCR products were cloned and sequenced from 3 isolates (H-9, H20 and H-24). Sequence analysis confirmed type of the isolates as serotype 1.

Fig: 37. PCR of EAdV1 isolated in EF (L 1:blank; L 2 and 3: H-24P3; L 4 & 5: H-24P3; Lane 6: 100 bp ladder)

In addition, nasal and stool samples from 156 healthy buffalo calves were screened for bovine adenovirus by nested-PCR (nPCR) and 16 nasal and one stool samples were found positive. Bovine adenovirus could be isolated by adapting to grow in BU cells (two samples) and SJMRF cells (6 samples). The PCR product (430 bp) from nPCR was cloned and sequenced. The isolated virus F-74 was typed as bovine adenovirus serotype 8.

(Sudhansu Vrati, B.R. Gulati, M.K. Kumanan, M. Partihiban, Amarjit Singh and Ramnek)

3. ICAR funded National Fellow Project on Development of sensitive and specific diagnostic assays for detection of *Trypanosoma evansi* infection in animals using modern molecular tools

a) Comparative efficacy of different media for *in vitro* cultivation of *Trypanosoma evansi*

For *in vitro* cultivation of trypanosomes, five different media were used *viz*., (1) Iscove's Modified Eagle's medium (IMDM), (2) HMI-9 medium (supplemented with 0.1mM bathocuproinedisulphonic acid, 1.5 mM l-cysteine, 1 mM hypoxanthine, 0.2 mM 2-mercaptoethanol, 1 mM pyruvate, 0.16 mM thymidine, 0.06M Hepes and 20% FBS) (3) Minimum essential medium (supplemented with 1mg/ml glucose, 2.2mg/ml NaHCO3, 10mM HEPES, 2mM sodium pyruvate, 0.2mM mercaptoethanol, 0.1mM hypoxanthine and 20% heat inactivated horse serum), (4) Alsever's solution and (5) Phosphate Buffered Saline supplemented with 1% glucose (PBS-G). In Alsever's solution trypanosomes survived for 24h, in PBS-G trypanosomes survived for 36h, in IMDM and MEM trypanosomes survived for 48h only. In HMI-9 medium trypanosomes survived and there is no increase in number up to 8th day. On 9th day, there was exponential increase in number of trypanosomes up to 15th day. From 16th-23rd day there was no significant increase in number. On 24th day onwards, constant increases in number of actively growing parasites was observed and were subcultured daily up to 55th day (Fig. 38). These trypanosomes were also cryopreserved (1 x 10^6/ml) in LN_2

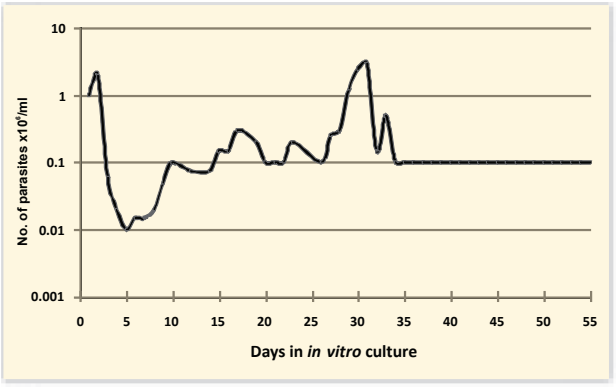


Fig. 38: Growth curve of *Trypanosomaevansi*in HMI-9 medium

for further studies. Out of five media, HMI-9 medium yielded best results in terms of adaptation, survivability and multiplication. The parasites retained their morphological characteristics and infectivity to mice.

b) Genetic diversity analysis of vsg gene (Ro Tat 1.2) of *Trypanosoma evansi*

A wide variety of biochemical and molecular typing systems are in use in the field of trypanosomosis. These tools help to elucidate genetic diversity, the relationships among different species and subspecies. Several genetic markers are being used for phylogenetic analysis and/or characterization of polymorphisms in *T. evansi* populations. In earlier studies it is established that the Ro Tat 1.2 (vsg) is a predominant vsg and thought to be expressed in majority of *T. evansi* stocks during early stage of infection. Our recent studies indicated that antibodies against this vsg persist even in chronic stage of infection in susceptible host. Majority of diagnostics have been developed using this vsg. In the present study phylogenetic analysis of vsg gene (Ro Tat 1.2) of *Trypanosoma evansi* isolates of different hosts and geographical zones was carried out to detect genetic diversity, if any. Full/partial length sequencing was done of four isolates viz., T.ev-India-NRCE-camel1 (Bikaner), T.ev-India-NRCE-Donkey1 (Hardoi), T.ev-India-NRCE-Donkey2 (Junagarh) andT.ev-India-NRCE-Horse1 (Hisar).For phylogenetic analysis, Maximum Likelyhood (ML) method was employed. Nucleotide sequence data were aligned against homologous sequences deposited in Genbank.

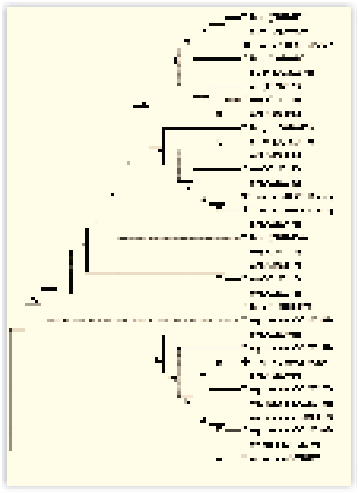


Fig 39: Phylogenetic relationships of *Trypanosoma evansi* Rotat vsg gene using Maximum Likelihood method

The sequence analysis and comparison by BLAST search showed a very high similarity (> 90%) with the sequences of Ro Tat 1.2 gene of other known salivarian *Trypanosoma* species/strains (*T. evansi*, *T. equiperdum*, *T. bruceibrucei*, *T. bruceirhodesiense*, *T. bruceigambiense*) Phylogenetic analysis of the Ro tat vsg sequences by ML method (Fig. 39) showed that T. ev-India-NRCE-Donkey1 (Hardoi), T.ev-India-NRCE-Donkey2 (Junagarh) are very closely related whereas T.ev-India-NRCE-camel1 and T.ev-India-NRCE-Horse1 isolates were clearly separated in one branch among other *T. evansi* indicating slight heterogeneity among these isolates of the obtained sequences with those in database. The data will be useful for development of uniform diagnostic assay against trypanosomosis for different geographical zones in India.

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

Technology Developed and Assessed

Validation of recombinant protein (p26) antigen based AGID/ELISA for equine infectious anaemia

Equine infectious anaemia (EIA) is a chronic, debilitating retroviral disease of equids. Control of EIA is based on identification of inapparent carriers by detection of antibodies to EIA virus (EIAV) by internationally accepted standard serologic tests, generally the agar gel immunodiffusion (AGID) test. In order to ensure the continuous availability of diagnostic reagents, and to serve as a national resource for its availability to disease diagnostic laboratories for sero-monitoring of EIA; recombinant p26 protein antigen-based ELISA and AGID assays were developed during the past year. The p26-ELISA was optimized and used to screen 4545 equine serum samples including horse (n= 3648), mule (n=295), and donkey (n=602). Calculated cut-off value of the assay was found at 0.22 (Fig. 40). Since prevalence of EIA is less in India, further 0.1 value was added to cut-off value to increase the specificity and maximize the benefit of the assay in consideration to economic and social consequences of misdiagnosis and the prevalence of the disease. All of the test samples were also simultaneously tested with commercial AGID test kit and were found negative for the presence of EIAV antibody. In comparison, with AGID assay, relative diagnostic sensitivity and specificity of ELISA was 100% and 98.7%, respectively.

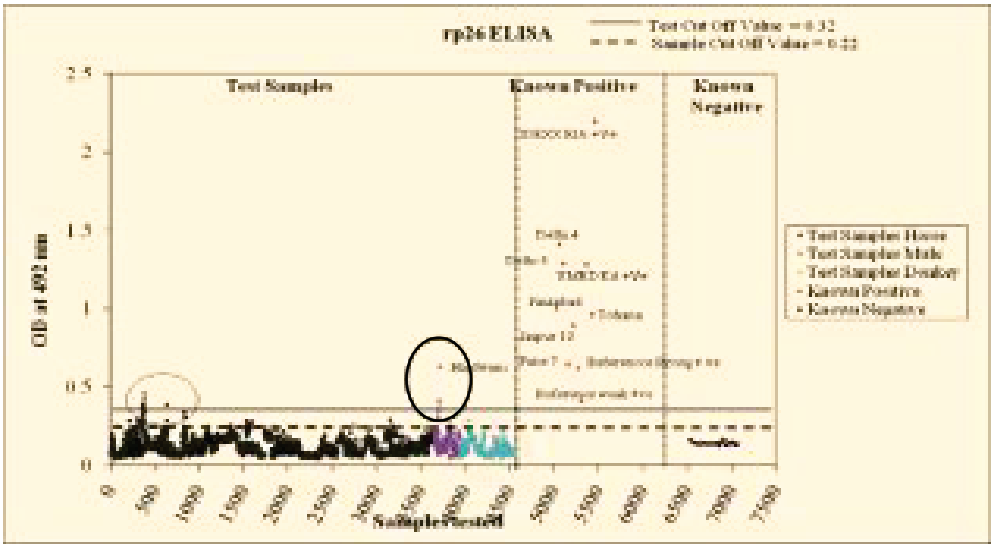


Fig. 40: Distribution of optical densities (OD) of positive, negative and test serum samples tested by rp26-ELISA. False positive samples are indicated in circle. Haldwani sample wasfound positive both by reference agar gel immunodiffusion (AGID) test and ELISA.

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

Number of patents filed and granted

1. Nano-drug delivery for quinapyramine sulphate (filed provisional application, No.2560/DEL/2011, dated 06.09.2011)
2. Polynucleotide sequence, composition and method thereof- Application No. PCT/IB 2011/052475 (IISc, Bangalore and NRCE, Hisar)


EQUINE INFLUENZA VACCINE
Virus strain used: A/eq/Katra-Jammu/08/08 (H3N8) Killed Virus
Batch : Experimental
Dose: 1ml Intramuscularly
Date of Manufacture:
Caution: Store at 4°C
Date of Expiry:
Mfd. by :
National Research Centre On Equines
Sirsa Road, Hisar-125 001, Haryana (India)


EQUIHERPES B-ELISA KIT


EQUINE HERP KIT
RUSH !
PERISHABLE BIOLOGICALS
STORE AT+4°C
ON ARRIVAL


Theileria equi Antibody Detection ELISA kit

Consultancy & Commercialization of Technology

Consultancy

One of the mandates of NRCE is to act as National Referral Centre for equine disease diagnosis, and to provide the diagnostic, advisory and consultancy services to various stake-holders for disease investigation and testing for health certification in the country. As part of this programme, experts from the Centre conduct on-farm/field visits to different parts of the country for attending disease occurrences and outbreaks amongst equines. Besides, samples are also submitted by State Disease Diagnostic Laboratories (SDDL) and Regional Disease Diagnostic Laboratories (RDDL), Regional Animal Quarantine and Certification Stations, Polo Associations, Equestrian Federation of India, field veterinarians and equine owners for testing in the labs for various diseases. The results along with the expert and technical advice are communicated to the respective agencies for further necessary action at their ends. If required, the Animal Husbandry Authorities of State or Central Government are informed accordingly to initiate the action for containment and formulating control strategies and/or notification.

During the current year, the Centre generated a revenue of ` 54,28,150 through testing of samples for various diseases including EIA, glanders, equine influenza, EHV-1, EVA, CEM, *Theileria equi*, *Trypanosoma evansi*, *Trypanosoma equiperdum*, *Babesia equi*, *Salmonella* Abortusequi. A total of 5475 samples were tested for equine infectious anaemia (EIA) by Coggins test and all were found negative for EIA. The Centre also tested 9908 equine serum samples for glanders using complement fixation test (CFT). Similar to previous year, none of the 2621 samples tested for equine influenza gave positive titres in the paired serum sample, however, the residual titres from 2008-09 outbreak could be seen in 100 cases. Out of 1474 samples tested for EHV-1, 79 were found positive. None of the 27 samples tested for EVA was found positive. The testing of 350 swabs from mares for Contagious Equine Metritis through agent identification revealed negative results in all the swabs tested.

Commercialization of Technologies

National Research Development Corporation - A premier Government of India Enterprise-invited NRCE to attend meeting of Expert Panel on Life Sciences on January 13, 2012 and deliberate regarding the technologies developed at NRCE for their suitability for commercialization. A team of Scientists comprising Dr B.K. Singh, Dr N. Virmani and Dr Sanjay Kumar attended the meeting and discussed about the kits and vaccine developed at this Centre for commercialization purpose. The technologies discussed during the meet with potential for commercialization included Equiherpes B-ELISA Kit for diagnosis of EHV-1 infection/immune status in equines, Equiherpabort vaccine for control of abortions in mares, Equine Influenza vaccine for control of Equine Influenza, r-Ag based ELISA Kit for detection of Theileria antibody in equines, and eCG-ELISA Kit for pregnancy diagnosis in mares. The panel of the experts included Dr P.K. Ghosh, Ex Vice President, Cadilla; Dr Devi Sarkar, Gene Therapy Specialist; Dr J.N. Verma, Managing Director, Life Care; Dr Amitabh Mishra, Dy Manager (Bio Tech), NRDC, New Delhi besides other members. Further to deliberations with NRCE scientists, NRDC granted approval for uptake of technologies for commercialization. A MoU for commercialization between two Institutions is to be signed soon.

Technologies developed by NRCE and ready for commercialization

Sr. No.	Technology
1	Updated Equine Influenza Vaccine
2	Equi Herpes B-ELISA Kit
3	Equine herpes virus-1 vaccine (Equiherpabort)
4	MAb- ELISA kit for diagnosis of rota virus infection in equines
5	Recombinant antigen ELISA kit for *Babesia equi* diagnosis
6	Pregmare kit for pregnancy diagnosis in mares
7	Cryopreservation of equine semen
8	Recombinant protein based ELISA for diagnosis of EIA

Education & Training

Annual Scientific Review Meet of Network Project of Veterinary Type Culture Collection (VTCC)

Annual scientific review meeting of VTCC Network Project was held on September 23, 2011 at NRCE, Hisar. The meeting was chaired by Prof. Gaya Prasad, ADG (AH), ICAR, at NRCE, Hisar. Director, NRCE coordinated the meet and presented the PC's report and the outline of the future research priorities for XII Plan. PI's from co-ordinating units and network units presented the major accomplishments under this network project. Unit-wise progress was also evaluated on the basis of their inputs. Dr Rameshwar Singh, Principal Scientist, NDRI, Karnal and Technical Coordinator, Dairy Microbes Component, presented the overall performance of the components while Dr D. N. Kamra, Principal Scientist, IVRI and Technical Coordinator, Rumen Microbes Component presented the progress report of the units. All the Nodal Officers of the Network Units of Dairy, Rumen and Veterinary Microbes presented their progress report. Prof. Gaya Prasad, ADG (AH) in his concluding remarks suggested to expand this programme in new agro-climatic zones involving new livestock species. He emphasized on the need of further strengthening of repository with novel microbes having unique characters.

Annual Scientific Review Meet of VTCC

ICAR sponsored-short training course on application of Nanotechnology in Animal Sciences

A ten days training course on "Application of Nanotechnology in Animal Sciences" sponsored by ICAR was organized at NRCE during February 01-10, 2012 in collaboration with Guru Jambheshwar University of Science and Technology, Hisar. This short training course was attended by 12 participants from different ICAR Institutes. The training comprised of lectures and hands-on practical training to the participants. Bedsides, NRCE and GJUS&T, Guest Faculty from reputed institutes like IVRI, Izatnagar; NDRI, Karnal; CIRCOT, Mumbai; IIT, Delhi; IGIB, Delhi; PAU, Ludhiana and MDU, Rohtak presented lectures on different topics. Dr S. C. Yadav, Principal Scientist, NRCE was the Course Director of the training course. Dr Anju Manuja, Senior Scientist, NRCE and Dr Neeraj Dilbagi, Associate Professor and Chairman, Department of Bio & Nano Technology, GJUS&T, Hisar acted as Course

Chief Guest addressing participants during training course

Coordinators. A series of lectures and practical demonstrations covered the basics of nanotechnology, synthesis and characterization of nano-drugs, *in vitro* and *in vivo* safety and toxicity testing and applications in treatment of animal diseases and diagnosis. A total of 18 lectures were delivered by the experts covering various aspects on synthesis, characterization and application of nano-materials during the training course, apart from the practicals organized at NRCE and nano-biotechnology laboratories at GJUS&T, Hisar. The participants were also given opportunity to visit Punjab University, Chandigarh for characterization/ demonstration of the nano material synthesized by them including carbon nanotubes using SEM and TEM. Dr M.L. Ranga, Vice-Chancellor GJUS&T, Hisar was the Chief Guest on the occasion of valedictory function of short training course on application of Nanotechnology in Animal Sciences. Dr R. K. Singh, Director, NRCE congratulated Course Director (Dr S. C. Yadav) and Course Coordinators (Dr Anju Manuja and Dr Neeraj Dilbagi) for successful organization of the training course. Chief Guest in his speech emphasized on the role of livestock in our society. The participants of the course also gave their feedback in terms of organization of course, practicals, hands-on training and the arrangement and schedule of training course.

Training programme on Integrated Farming and Animal Husbandry Management

Five days training programme for the farmers of Jhunjhunu (Rajasthan) on "समन्वित कृषि एवं पशुपालन प्रबंधन" sponsored by Agriculture Technology Management Agency (ATMA) Jhunjhunu, Rajasthan was organized at Equine Production Campus, Bikaner from February 21-25, 2012. The training was attended by 20 farmers from Rajasthan. The training was inaugurated by Dr N. V. Patil, Director, NRCC, Bikaner and organized by Dr Yash Pal, Dr R. A. Legha and Dr R. K. Dedar from EPC, Bikaner. Subject matter specialists were also invited for the training as guest faculty from CAZRI, Bikaner; CIAH, Bikaner; CSWRI, Bikaner; SKRAU, Bikaner; RAJUVAS, Bikaner; NRCC, Bikaner and State Animal Husbandry Deptt., Rajasthan to cover the topic of training course. A compendium was released on the occasion of valedictory function by Dr. R.K. Beniwal, Former Head, RRS, CAZRI, Bikaner.

Training programmes on "Integrated Farming and Animal Husbandry Management" and "Horticulture and Animal Husbandry Management"

Training Programme for Farmers on Horticulture and Animal Husbandry Management

Two days training programme for farmers on **"बागवानी एवं पशुपालन प्रबंधन"** sponsored by Agriculture Technology Management Agency (ATMA) Bikaner, Rajasthan was organized at Equine Production Campus, Bikaner from March 19-20, 2012. The training was attended by 30 farmers from various villages of district Bikaner that included Gadwala, Kilchu, Gigasar and Surdhana. Training was organized by Dr Yash Pal, Senior Scientist and Incharge, EPC and Dr R. A. Legha, Senior Scientist, EPC, NRCE Bikaner. Subject matter specialists for the training were also invited from CAZRI, Bikaner, CIAH, Bikaner, RAJUVAS, Bikaner and NRCC, Bikaner to cover the topic of training programme.

Trainings/Workshops/Seminars organized

- Training on "Lifestyle Disorders and Stress Management" organized by "Sanjeevani" Bangalore at NRCE, Hisar on April 15, 2011.
- XX Annual Convention and a Society on "Managing Emerging and Re-emerging Plant, Animal, Human and Aquatic Viral Diseases: One Health Perspective" held at National Research Centre on Equines, Hisar from December 29-31, 2011.

Expert Lectures at NRCE

- Dr R. K. Singh, Director, NRCE delivered a lecture on "Significance of World Veterinary Day and Role of Veterinarians" on the occasion of World Veterinary Day on April 30, 2011.
- Dr Sanjay Kumar, Senior Scientist, NRCE delivered a lecture on "History of Veterinary Science" on the occasion of World Veterinary Day on April 30, 2011.
- Dr R. K. Vaid, Senior Scientist, NRCE delivered a lecture on "Veterinary Science as a Career Option" on the occasion of World Veterinary Day on April 30, 2011.
- Dr S. K. Khurana, Senior Scientist, NRCE delivered a lecture on "Rabies" on the occasion of World Veterinary Day on April 30, 2011.
- Mr D. C. Gabriel from M/s K&S Partners, Gurgaon delivered lecture on "Overview of IP and Patents" and "Patenting of Biotechnology Research-Current Scenario" on May 27, 2011.
- Dr S. C. Yadav, Principal Scientist, NRCE delivered a lecture on "Equine Trypanosomosis - Recent Advances in Diagnosis with Special Reference to Secretome" on May 28, 2011.
- Dr B. R. Gulati, Principal Scientist, NRCE delivered a lecture on "Risk Assessment of Exotic Equine Viruses" on June 25, 2011.
- Dr S. K. Khurana, Senior Scientist, NRCE delivered a lecture on "Global Warming and Infectious Diseases" on July 30, 2011.
- Dr Rajender Kumar, National Fellow, ICAR, NRCE delivered a lecture on "Important International treaties Interfacing IP Regime- Current Scenario" on August 27, 2011.
- Dr P. Malik, Principal Scientist, VTCC delivered a lecture on "Integrating Biosafety and Biosecurity" on October 29, 2011.
- Dr Anju Manuja, Senior Scientist, NRCE delivered a lecture on "Nanobased Drug Delivery Systems" and "Biopharmaceutical characterization of drug-loaded nanoformulations" during Short course on "Applications of Nanotechnology in Animal Sciences" at NRCE, Hisar from February 1-10, 2012.
- Dr Balvinder Kumar and Anju Manuja, Senior Scientist, NRCE delivered a lecture on "Nanotechnology: Current Research and Potential Applications in Animal Sciences" during Short Course on "Applications of Nanotechnology in Animal Sciences at NRCE, Hisar from February 1-10, 2012.

Expert Lectures Outside

- Dr R.K. Vaid, Senior Scientist participated and presented invited lecture in "2nd National Conference on

Antimicrobial Resistance at Sam Higginbottom Institute of Agriculture Technology and Sciences, Allahabad, February 6-8, 2012.

- Dr Rajender Kumar, National Fellow, ICAR, NRCE delivered a lecture on "Challenges associated with control and prevention of Surra in India" in the Symposium organized by Brooke India at Ghaziabad on March18, 2012.
- Dr Anju Manuja, Senior Scientist, NRCE delivered a lecture on "Real-time PCR: A method for quantitation of nucleic acids", in Refresher Course in Life Sciences, GJUS&T, Hisar on December 9-29,2011.
- Dr S.K. Khurana, Senior Scientist, NRCE delivered a lecture on "An Overview of Common Zoonotic Diseases" in Refresher Course in Life Sciences, GJUS&T, Hisar on December 9-29, 2011.
- Dr S.C. Yadav, Principal Scientist, NRCE delivered a lecture as guest speaker on "Immunological approach in diagnosis of animal trypanosomosis using defined antigens" during Refresher Course in Life Science, GJUS&T, Hisar on January 28, 2012.
- Dr R. K. Singh, Director, NRCE delivered keynote lecture on "Climate Change: Disease Emergence & Food Safety" during National Seminar on "Challenges in Combating Diseases: Cause to Cure" at MDU, Rohtak on March 23, 2012.

NRCE in News

गर्दभ सप्ताह में हुई विभिन्न प्रतियोगिताएं

पहला अश्व व गर्दभ पार्क बीकानेर में

Virologist conferred KS Bhargava Oration Award

जानवरों को बीमार करने वाले वायरस और बैक्टीरिया का बैंक तैयार

अनुसंधान केंद्र में वैक्सीन कल से

Rafi Ahmed Kidwai Award for Hisar scientist

यमुना बेल्ट में जापानी इंसेफ्लाइटिस का खतरा

वायरस पर मंथन को जुटे वैज्ञानिक

कैंप में गधों को बीमारियों का उपचार

RAC, IRC & IMC Meetings

XIV Research Advisory Committee Meeting, 2010-11

XIV Research Advisory Committee (RAC) meeting was held under the chairmanship of Dr A.T. Sherikar, former Vice-Chancellor, Maharashtra Animal and Fishery Sciences University, Nagpur, on April 25, 2011 to review various research projects of the Centre. The RAC members included Col (Dr) B. Raut, Dr R.C. Katoch, Dr S.N. Maurya, Dr D.V. Rangnekar, Col (Dr) Umaid Singh Rathore, Prof. Gaya Prasad (ADG, AH) and Dr R.K. Singh (Director, NRCE). The RAC discussed on various aspects of ongoing research projects in the area of equine production, health, extension and veterinary type cultures facility and also approved one new research project to be under taken by the Centre. The RAC emphasized on the basic work in the areas of equine nutrition, breeding and production in a very concerted manner for the benefit of equine owners. The Chairman RAC congratulated the Director addressing an overview of research progress of the Centre executed during the period and praised the scientists for their sincere contribution in the various fields of research work and other developmental activities of the Centre. The Chairman also emphasized that the diagnostics developed by the Centre should reach to the end-users for effective disease management and augmenting equine productivity, thereby, ensuring livelihood of poor equine owners and also uplifting the socio-economic status of stakeholders through timely intervention of the experts. He also suggested for strengthening the extension work in collaboration with similar Institutes/Universities and to develop the ideas for making equines more useful for farmers and other stakeholders to enhance the status of equines in the country, in the changing scenario.

New Research Projects Approved by XIV RAC

- Development of protein expression clone repository of virulence associated genes of zoonotic buffalopox virus.

XV Research Advisory Committee Meeting, 2011-12

XV Research Advisory Committee meeting of the Centre was held on February 25, 2011 under the Chairmanship of Dr S.K. Dwivedi, Ex-Director, NRCE, Hisar. The RAC was reconstituted for three years subsequent to completion of tenure of previous committee. The RAC members who participated in the meeting included Dr G. Dhinakar Raj, Dr Arun Varma, Col (Dr) Devender Kumar, Dr S.K. Agarwal, Dr R.K. Singh, Director (NRCE) and Col (Dr) Umaid Singh Rathore.

The Chairman RAC apprised the house that the RAC is constituted to advise on how to facilitate and harmonize the research and extension activities of the Centre. Generation of demand-driven technologies for the benefit of equine fraternity should be the priority agenda of the Centre. The

RAC meeting in progress

Chairman also expressed concern over the dwindling equine population and emphasized that there is a need to develop interventional strategies to stop or slow down the

pace of decline in equine population. He further opined that the NRCE should identify the challenges being faced by the equine sector and work towards providing solutions to these challenges in the XII plan.

The Chairman and other members emphasized that NRCE has to contribute significantly in the area of *in-situ* conservation of true-to-breed indigenous horses and ponies which requires extensive effort in semen collection, preservation and AI in collaboration with State Governments and dedicated NGOs. The committee further stressed upon the need of strengthening equine clinical medicine through development of modern diagnostics and treatment of the systemic disorders of equines and also to work on equine nutrition, especially in the area of alternate sources of feed for equines such as exploiting the use of crop residues as source of nutrition. The committee suggested to work on utilization of equine byproducts and ancient literature may be retrieved for delivering the quality byproducts through collaborative studies with other animal sciences and human health institutes.

New Research Projects Approved by XIV RAC

- Development of vaccine against Japanese encephalitis virus for animals (April, 2012-March, 2014)

33rd and 34th Institute Management Committee Meetings

The 33rd meeting of Institute Management Committee (IMC) was held on September 24, 2011 at NRCE, Hisar. The meeting of the IMC was held under the Chairmanship of Dr R.K. Singh, Director, NRCE. The esteemed members of NRCE IMC such as Prof. (Dr) Gaya Prasad (ADG(AH), ICAR, New Delhi); Dr G.K. Singh (Dean, Vety. College, GBPUA&T; Pantnagar); Dr Parmod Kumar (Dy. Director, Animal Husbandry, Himachal Pradesh-Representative of the Director, Animal Husbandry, HP); Dr S.C. Mehta (Principal Scientist, NRCC, Bikaner); Sh. V.K. Sharma (F&AO, IASRI, New Delhi); Dr Yash Pal (Sr. Scientist & I/c EPC, Bikaner) and Sh. R.B. Saxena (Admn.Officer, NRCE, Hisar) attended the meeting. The IMC adopted and confirmed the proceedings of 32nd IMC meeting. The committee discussed about the various issues of the Centre and approved the agenda items like Interior furnishing of VTC building at NRCE, Hisar; constitution of Grievances Committee of NRCE, Hisar; naming of the BSL-III facility as Microbial Containment Laboratory (MCL); sharing of resource generated through EIA/CEM testing under contractual service, etc. The IMC emphasized upon purchase of equipments approved in the SFC upto Dec., 2011 positively. They also recommended for election of representative from Administrative Category for Constitution of Grievances Committee of the Centre.

The 34th meeting of the IMC was also held at NRCE, Hisar under the Chairmanship of Dr R.K. Singh, Director, NRCE on February 25, 2012. The members of the committee who attended the meeting were Dr G.K. Singh (Dean, Vety. College, GBPUA&T, Pantnagar); Dr R. Sanwal (Principal Scientist, ARCCS & WRI, Bikaner); Sh. V.K. Sharma (F&AO,IASRI, New Delhi); Dr Yash Pal (Sr. Scientist & I/c EPC, Bikaner), and Sh. R.B. Saxena (Admn. Officer, NRCE, Hisar). The IMC confirmed the proceedings of 33rd IMC meeting. The committee was apprised regarding various issues of the Centre that require immediate attention. The IMC agreed to all the agenda items such as replacement of items against those approved equipments in XI Plan, creation of duplicate off-site remote repository of Veterinary Microbes, approval for commercialization of technologies through NRDC, etc.

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 3rd, 5th and 7th December, 2011 for appraisal of the research achievements of the ongoing projects and to consider new research project proposals for the year 2011-2012. The IRC reviewed the progress of ongoing research projects in the area of equine production, health, extension and Veterinary Type Culture Collection and also discussed the shortcomings and future work plan. Chairman discussed on various aspects of the new research proposals and approved projects to be taken up by the Centre.

IRC meeting in progress

Workshop Seminar & Institutional Activities

Annual Conference of Indian Virological Society (VIROCON-2011) at NRCE, Hisar

XX Annual Convention of Indian Virological Society (VIROCON-2011) and National Conference on topic entitled "Managing Emerging and Re-emerging Plant, Animal, Human and Aquatic Viral Diseases: One Health Perspective" was held at National Research Centre on Equines, Hisar on December 29-31, 2011. The conference was organized with great success and nearly 200 participants from 18 states covering all the corners of the country attended the conference. The conference was inaugurated by Prof. (Dr) M.L. Madan, former DDG (AS). Prof Kameshwar Sahai Bhargava Oration Award was given to Prof. (Dr) P.K. Uppal. Diversified research works in the field of Plant Virology, Animal Virology, Medical Virology, Biotechnology etc. were presented during the conference.

Inauguration of Annual Conference of Indian Virological Society

There were 109 oral presentations of research papers (21 in Plant virology, 52 in Animal Virology, 23 in Medical Virology & 13 others) and 69 poster (33 on plant viruses, 24 on animal viruses & 12 medical viruses) presentations. The special sessions on Influenza and Heavy Water were held keeping in mind the importance in public and animal health and vaccinology. Various prizes were given to the best research work in different sections. During the conference, expert lectures from diversified fields were delivered to discuss the burning issues related to "One Health" perspectives. The local organizing committee included Dr R.K. Singh, (Chairman), Dr B.K. Singh (Organising Secretaries) and Dr B.R. Gulati & Dr Sanjay Barua (Co-organising Secretary). Prof. (Dr) A.K. Gahlot, Vice Chancellor, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan was the Chief Guest of the plenary session. The sponsors for this conference were Department of Biotechnology, Delhi; NRDC; Influenza Foundation; Heavy Water Board, Mumbai; Shree Krishna Traders, Hisar; Labline Scientific Corporation, Hisar; Spectrum Technologies, Hisar; The Bharat Instruments and Chemicals, Hisar; Panacea Instruments Pvt. Ltd., Delhi; Tarsons Products Pvt. Ltd, Kolkata; Himedia, Mumbai; Rajat Enterprises, Hisar; Pfizer Pharmaceuticals India Pvt. Ltd., Mumbai; Shara Saher, Lucknow; Eppendorf India Ltd., Delhi; HAFED, Haryana, and HSCC India (Ltd.), Noida.

International Donkey Week Celebrated

To boost the awareness about importance of working donkeys, International Donkey Week was celebrated by NRCE during May 3-10, 2011. During this period, a health camp was organized at Nirankari Bhawan area, Hisar for benefit of working donkey owners. In the camp, NRCE experts provided advises in various aspects of donkey production and health management and carried out health check-up, treated the animals, and distributed various medicines etc. including deworming tablets, mineral mixture, anti-septic dressing. Director NRCE was also

International Donkey Week celebration by NRCE

At NRCE, Hisar

At Spiti valley

present on this occasion. On the eve of International Donkey Week celebration, various donkey competitions including donkey cart race, best donkey, and best donkey foal was organized at NRCE, Hisar on April 5, 2011. Donkey owners from Hisar and nearby villages participated in this competition.

During International Donkey Week, donkey competition including cart race and health camp was also organized at Ratangarh in Rajasthan by team of scientists and staff from EPC NRCE, Bikaner and Hisar on May 7, 2011. During the camp, donkey owners were trained about feeding of balanced rations, hoof management, making suitable harnesses and halters for animals in loading carts and health management of their animals. Farmers were supplied literature and extension bulletins about health and management practices published by the Centre.

Similarly, a Donkey Owners Meet was organized at Spiti Valley in Himachal Pradesh on May 10-12, 2011. The

At Rajgarh

donkey owners were trained about feeding of balanced rations, hoof management, making suitable harnesses and halters for animals in loading carts and health management of their animals. Farmers were supplied literature and extension bulletins about health and management practices published by the Centre.

World Veterinary Day Celebrated

World Veterinary Day function was organized on April 30, 2011 at NRCE, Hisar. On this occasion, students from Thakurdas Bhargava Sr. Secondary Model School were invited to attend the function. The students were educated in various aspects of the equines such as different breeds of equines in India and the utility of equines in different activities along with the various field outreach programmes like organization of health camps, kisan goshthis, equine owners meet and participation in animal fairs, exhibition for benefit of equine owners. The students visited the animal shed complex and equine exhibition at Agricultural

World Veterinary Day Celebrated at NRCE

Technology Information Centre (ATIC) of the Centre. Chief Guest of the function Dr S. K. Kalra (Ex-Prof. & Head, Deptt. of Microbiology, CCSHAU, Hisar) enlightened the audience about the role played by veterianarians in animal welfare. Director, NRCE addressed the students and staff of NRCE and delivered an overview on World Veterinary Day. Scientists of the Centre also apprised the audience about History of Veterinary Science (Dr Sanjay Kumar, Sr. Scientist), Career Option in Veterinary Science (Dr R.K. Vaid, Sr. Scientist), and Rabies (Dr S.K. Khurana, Sr. Scientist).

The year 2011 was celebrated as World Veterinary Year to mark the 250th anniversary of veterinary education and veterinary profession. The World Veterinary Association selected special theme on Rabies for the year 2011 with the idea to educate and raise awareness among public regarding prevention and control of rabies. On the occasion of World Veterinary Day, students and people were educated about dreaded disease rabies and about its cause, prevention, and control.

Celebration of Communal Harmony Week

Communal Harmony Week was celebrated at NRCE during September 19-25, 2011 with organization of expert lectures on Communal Harmony and National integration. On this occasion; Sri Himanshu (Editor, Dainik Bhaskar, Hisar) delivered a Lecture on "Communal Harmony and National Integration". Shri Rakesh Kranti (Bureau Chief, Dainik Bhaskar, Hisar) also enlightened on diverse aspects of the communal harmony. The Director, NRCE in his speech emphasized on unity, integrity and hormony among people. He stated that every individual must work towards communal harmony which will help to maintain peace and development in nation.

Vigilance Awareness Week at the Centre

Vigilance Awareness Week was observed during October 31-November 5, 2011 and various programmes and activities were organized. As part of Vigilance Awareness Week celebration, programme on “Awareness and Publicity against Corruption” was organized on November 5, 2011. On this occasion, Shri Naveen Jain (Comptroller, CCSHAU, Hisar) delivered a lecture on “Vigilance and Finance” and Prof. Sudama Agrawal gave a lecture on “The Evolution of Right to Information Act”. Director, NRCE solemnized pledge to the scientists and exhorted the staff of NRCE for their sincere contribution towards betterment of the equines in the country. Director, NRCE in his message also stressed on coordinated efforts for eliminating the menace of corruption from the society.

Bhagidar Sammelan-An Interactive meet of Equine Owners organised at NRCE

On the occasion of NRCE Foundation Day on November 21, 2011; Bhagidar Sammelan- An Interactive meet of Equine Owners was organised at NRCE, Hisar. On this occasion, more than eighty equine owners from Haryana and Rajasthan participated in this Sammelan. Prof. I. Igarashi of the OIE Reference Laboratory on Equine Piroplasmosis NRCPD, Obihiro, Japan was the Chief Guest of the programme. Dr B.K. Singh, (Principal Scientist, NRCE) briefed the participants and equine owners about the purpose of organizing this interactive meet. Dr R.K. Singh, (Director, NRCE) invited suggestions from equine owners

Bhagidar Sammelan at NRCE

regarding research priorities and extension activities for forthcoming XII[th] Five Year Plan. Chief Guest of the programme, Prof. I. Igarashi in his address briefed equine owners about management of Equine Piroplasmosis. During the interaction with equine owners, scientist at NRCE briefed the equine owners about preventative measures to control equine diseases and effective management of equines. The equine owners asked questions and queries related to equine heath and management which were responded by the experts.

Stakeholder Meet organized at NRCE

Stakeholder Meet organized at NRCE, Hisar on December 10, 2011 to discuss and seek views on equine welfare. Equine owners from Harayana, Rajasthan and senior veterinary officers from different states participated in the meet. Dr A.K. Gupta, (Principal Scientist, NRCE) briefed participants about the purpose of oraganizing the stakeholder meet. Dr R.K. Singh, (Director NRCE) briefly reviewed the ongoing research and extension activities of the Centre. Director NRCE also informed stakeholders about the proposed research and extension activities at NRCE and seek out suggestions from equine owners and equine stakeholders. Chief Guest also appealed equine owners to promote equestrian events, riding, and equine veterinary officers from different states about their views and concerns on equine welfare. The Chief Guest of the programme, Prof. (Dr) A.K. Gahlot, (Vice Chancellar, Rajasthan University of Veterinary and Animal Science, Bikaner) expressed happiness on research programmes, equine welfare activities organized by NRCE, and the future programmes to be proposed keeping in view the needs of

Stakeholder Meet organized at NRCE

MoU Signed for Construction of BSL-III Laboratory at VTCC

A Memorandum of Understanding was signed on December 9, 2011 between NRCE, Hisar and HSCC India Limited (Undertaking of Health and Family Welfare Ministry, Govt. of India) for construction of new Microbial Containment Laboratory (BSL-III facility) at Veterinary Type Culture Collection for handling hazardous microbes. Mr H.A. Osmani, CGM (DC), Mr S. Mukharjee, DGM (Civil), Mr Shri Tapas Nath, Senior Manager (BME) represented HSCC India Ltd. and Dr R.K. Singh, Director NRCE, Dr Praveen Malik, Principal Scientist and Incharge VTCC and Mr R.B. Saxena, Administrative Officer represented NRCE. The proposed Microbial Containment Laboratory at VTCC will be used for Veterinary Type Culture Collection requiring high bio-safety and bio-security measures for handling various pathogens of known and unknown origin.

MoU signed between GJUS&T, Hisar and NRCE for academic cooperation

A Memorandum of Understanding for academic cooperation was signed between GJUS&T, Hisar and NRCE on March 4, 2011. Prof. (Dr) M.L. Ranga, Vice-Chancellor, Dr R.S. Jaglan, Resgistrar, Dr A. Chaudhary, Professor & Chairman, Department of Bio & Nano Technology, represented GJUS&T whereas Dr R.K. Singh, Director NRCE, Dr A.K. Gupta, Principal Scientist and In-charge PME and Dr B.C. Bera represented NRCE. Under the MoU; GJUS&T, Hisar recognized NRCE, Hisar as a research centre at par with the academic departments of GJUS&T, Hisar and NRCE, Hisar faculty at par with GJUS&T, Hisar for academic work. NRCE, Hisar and GJUS&T shall allow their infrastructure and facilities to be used by the staff and students.

MOU signing between NRCE and GJUS&T

MoU signed between ICAR (NRCE) and Pfizer Pharma. India Pvt. Ltd. for Contractual Research Project

An MoU was signed between ICAR (NRCE) and Pfizer Pharmaceutical India Pvt. Ltd. on April 29, 2012 R&D on Epidemiology of Bovine Rotavirus (BRV), Bovine Corona Virus (BCV), *E. coli* and *Salmonella* in partnership mode between ICAR (NRCE) and Pfizer Pharmaceutical India Private Limited. Under this, a research project entitled "Studies on prevalence of bacterial (*Escherichia coli* and *Salmonella*) and viral (Corona and Rota) causes of calf scours amongst dairy cattle in India" was sanctioned to NRCE with a budgetary sanction of ` 45,55,831/- for 18 month period.

Interactive meet with members of Turf Authority of India

In relation to the agenda item decided for the year 2011-12; Director, NRCE attended a meeting with members of Turf Authority of India on April 12, 2012. A one to one meeting was held between Director, NRCE and Dr K.M. Srinivasa Gowda, Chairman, Turf Authority of India wherein Chairman, Turf Authority of India exulted the NRCE for giving timely result of samples sent for diagnosis. Further, a separate interactive meet was held with Secretary, Banglore Turf Club, Dr. Rajshekhar, Technical Director, RWITC and Veterinary Officers of various race clubs of the country to discuss various issues regarding equine health and how NRCE's technical expertise can be utilized in a better way by the stake holders. The members attending the meet also showed keen interest in visiting NRCE to have hands-on experience of various expertise at the Centre and to discuss their issues with the scientists.

राष्ट्रीय अश्व अनुसंधान केंद्र, हिसार में हिंदी सप्ताह का आयोजन

राष्ट्रीय अश्व अनुसंधान केन्द्र में 26 सितम्बर से 30 सितम्बर 2011 तक हिन्दी सप्ताह का आयोजन किया गया। इस अवसर पर केन्द्र में विभिन्न प्रतियोगिताएं आयोजित की गई। हिसार स्थित केन्द्रीय कर्मचारियों ने एवं रा.अ.अनु. केन्द्र के कर्मचारियों ने प्रतियोगिताओं में बढ़-चढ़ कर भाग लिया।

दिनांक 30 सितम्बर 2011 को केंद्र में काव्य गोष्ठी का आयोजन किया गया। केंद्र के निदेशक डॉ. राज कुमार सिंह व कार्यक्रम के मुख्य अतिथि डॉ. ए.के. परूथी, डीन, पशु चिकित्सा महाविद्यालय, ललुवास,हिसार द्वारा दीप प्रज्ज्वलित कर आज के कार्यक्रम (हिंदी काव्य गोष्ठी) का शुभारंभ किया। काव्य गोष्ठी में राधेश्याम शुक्ल, रघुवीर अनाम, महेन्द्र जैन, नरेश शर्मा, औमप्रकाश दिलबर, डॉ. चंद्रशेखर, प्रद्युमन भल्ला एवं डॉ. इंद्रजीत जैसे कवि मौजूद थे। कार्यक्रम के अंतर्गत प्रख्यात कवियों ने अपनी रचनाओं से सभागार में बैठे सभी श्रोताओं को अपनी ओर आकर्षित किया।

हिंदी सप्ताह के अवसर पर काव्य गोष्ठी का आयोजन

Interactive meet with Professor Igarashi from NRCPD, Japan

Prof. I. Igarashi from NRC on Protozoan Diseases, Obihiro University, Hokkaido, Japan (OIE Reference Laboratory on Equine Piroplasmosis) during his visit regarding OIE-sponsored Laboratory-Twinning project on "Equine Piroplasmosis" with NRCE, participated in the interaction meet with equine owners on November 26, 2011. Prof Igarashi also visited ICAR, Delhi and had a meeting with Dr S. Ayyappan, Secretary (DARE) & Director General (ICAR); Prof. (Dr) K.M.L. Pathak, Deputy Director General (Animal Science); and Prof. (Dr) Gaya Prasad, Assistant Director General (AH) along with Director and scientists of NRCE.

Equine Health Camps and Kisan Goshthis

NRCE organizes equine health camps and kisan goshthis to create awareness about equine heath and management and provide timely help and support to the underprivileged equine owners. During 2011-12, twelve health camps and kisan goshthis were organized. At the health camps, the animals were examined for various ailments by the experts

Equine Health Camp at Julana

Equine Health Camp at Hanumangargh

from NRCE. Free medicines and treatment was provided to diseased animals at the camp. Pregnancy diagnosis was done during the camps. Deworming tablets and mineral mixture was provided to equine owners free of cost. During Kisan Goshthis, equine owners interacted with scientists on various aspects of equine husbandry and management. The interaction was useful to equine owners in terms of knowledge gain and information sharing about deworming schedule, prevention and management of colic and lameness in equines.

Equine Health Camps and Kisan Goshthis organized during 2011-12

Sr.No.	Place	Date
1.	Meham, Haryana	April 13, 2011
2.	Hisar, Haryana	May 3, 2011
3.	Ratangarh, Rajasthan	May 7, 2011
4.	Spiti, Himachal Pradesh	May 10-12, 2011
5.	Rajli, Haryana	May 13, 2011
6.	Jodhpur, Rajasthan	August 29, 2011
7.	Julana, Haryana	September 8, 2011
8.	Sriganganagar, Rajasthan	November 14, 2011
9.	Pirkamria, Rajasthan	November 21, 2011
10.	Guda Balotan (Jalore), Rajasthan	December, 24-25 2011
11.	Hanumangarh, Rajasthan	February 20-21, 2012
12.	Balotra, Rajasthan	March 16-19, 2012

Participation in Exhibitions and Animal Fairs

During 2011-12, NRCE participated in seven exhibitions and animal fairs at national level with NRCE exhibition stall. Exhibits and extension material on various aspects of equine husbandry were displayed for the benefit of equine owners. Exhibition stall also displayed different technologies developed at NRCE. NRCE participated at CAZRI Kisan Mela, Jodhpur, Rajasthan (August 29, 2011); IVRI Kisan Mela, Izatnagar, Uttar Pradesh (November 18-20, 2011); Maharana Pratap Ashwa Mela, Sri Ganganagar, Rajasthan (November 14, 2011); CIRB Buffalo Mela, Hisar,

J&K Chief Secretary Madav Lal with Dr B Mishra VC SKUAST Jammu Visiting NRCE Stall at SKUAST Kisan Mela

NRCE stall during CIRB buffalo Mela

Haryana (February 01, 2012); Bhatner Ashwa Mela, Hanumangarh, Rajasthan (February 20-21, 2012); Pusa Krishi Vigyan Mela , IARI, New Delhi (March 1-3, 2012) and SKUAST Kisan Mela, Jammu (March 19-20, 2012). During these exhibitions, animal fairs and equine owners meet, & interaction among scientists and equine owners was also organized. Information in the form of extension literature was provided to visitors, farmers and equine owners on different aspects of equine husbandry and management.

Shri Harish Rawat and Shri Charan Das Mahant, Union Minister of State for Agriculture at NRCE stall at Pusa Krishi Vigyan Mela

Students Educational Tours and Exposure Visit of Farmers

During 2011-12, visitors including students from schools, SAUs, farmers, equine owners, officers and trainees from different places visited NRCE as part of educational tour and exposure visit. During the visit, the visitors were briefed about the different activities of the Centre, field extension activities, services provided to equine owners. The visitors also visited ATIC and Animal Shed Complex.

Visit of Dignitaries

- Dr D.K. Arora (Director, NBAIM) visited NRCE on July 6, 2011 in relation to seeing the facilities at Veterinary Type Culture Collection. He appreciated the Culture collection activities at the Centre and state-of-the-art laboratories being developed for the purpose.
- ICAR Governing Body member Shri Kuldeep Dhaliwal visited NRCE on September 7, 2011. On this occasion, he visited different laboratories of the Centre. Director NRCE briefed him about the various ongoing research activities at NRCE. Shri Kuldeep Dhaliwal also visited the ATIC, BSL-III, VTCC, and Animal Shed Complex at NRCE. He appreciated the research and infrastructure facilities available at NRCE.
- Expert Validation Committee comprising of Dr Gaya Prasad (ADG (AH) ICAR); Dr H.K. Pradhan (WHO (India) Consultant); Dr S.C. Dubey (JD, HSADL, Bhopal); Dr V.A. Srinivasan (Research Director, Indian Immuno-logicals, Hyderabad); Dr R.K. Singh (Director, NRCE, Hisar); Sh Tapas Nath (HSCC Representative) and Sh Anwar Khan (Klenzaids) visited NRCE on 22.10.2011 for inspection of BSL-III facility as a step towards validation. The experts thoroughly examined different facilities at the site and witnessed various validation tests. After the on-site visit, the experts deliberated on the facility and suggested some modifications before the validation of the facility.
- Dr K. Pradhan, (Ex. VC RAU/OUAT), Dr N.V. Patil (Director, NRCC) and Dr S.S. Kundu (Head DCN, NDRI) visited NRCE on November 11, 2011 to participate in Animal Nutrition Group meeting with NRCE scientists.
- Prof. I. Igarashi from NRC on Protozoan Diseases, Obihiro University, Hokkaido, Japan visited NRCE, Hisar under OIE-sponsored Laboratory-Twinning project on "Equine Piroplasmosis" between NRCPD (Parasitology Lab) and NRCE (Host Lab). He visited different Laboratories, Animal Shed Complex and EPC, Bikaner. During his visit, he had interaction meets with scientists of NRCE, Hisar and equine owners on November, 26, 2011 at NRCE, Hisar.
- Dr A.K. Gahlot, Vice Chancellar, Rajathan University of Veterinary and Animal Science, Bikaner visited NRCE on December 10, 2011 as Chief Guest for One-day Stakeholder Meet organized at NRCE.
- VIROCON 2011 attracted many dignitaries at NRCE including Dr M.L. Madan (Former DDG (AS)), Dr Pradeep Sethy (President, Research Foundation, Gurgaon), Dr A.K. Prasad (Chairman, Influenza Foundation, India), Dr Sobha Broor (Professor & Head, Deptt of Microbiology, AIIMS). Prof. M.P. Yadav (Former Director, IVRI); Prof. P.K. Uppal (Former Director, NRCE); Prof. Narayan Rishi (Amity University, Noida); Prof. K.S. Palaniswamy (TANUVAS); Prof. G.D. Raj (TANUVAS) and Dr A.K. Gahlot (VC, RAJUVAS) from December 29-31, 2012.
- Dr M.L. Ranga, Vice-Chancellor, GJUS&T, Hisar visited NRCE on February 10, 2012 as Chief Guest on the occasion of valedictory function of ICAR-sponsored short training course on "Application of Nanotechnology in Animal Sciences".
- A team of officials from National Informatics Centre, New Delhi and Hisar visited NRCE on March 16, 2012 to discuss collaboration with NRCE regarding National Animal Disease Reporting System (NADRES) for diseases in Equines. Various modules like Scheme Monitoring, Disease Reporting, Block MIS, NADRES Portal and Animal Disease Diagnostic Labs workflow

NIC Officials interacting with NRCE Scientists

NIC Officials interacting with NRCE Scientists

were presented by the NIC officials. Scientists of NRCE expressed their views and provided relevant suggestions for effective development of the modules.

- Dr Gurbachan Singh (Hon'ble Chairman, ASRB) visited NRCE, Hisar on March 26, 2012. He had Joint interaction meet with scientists from CIRB and NRCE at CIRB, Hisar. Dr R.K. Sethi (Director, CIRB) and Dr R.K. Singh (Director, NRCE) were also present on this occasion. The Hon'ble Chairman, ASRB Dr Gurbachan Singh visited different laboratories of NRCE/VTCC, BSL-III Facility, ATIC, stud farms and agriculture farm at

Hon'ble Chairman (ASRB) interacting with scientists

NRCE and appreciated the infrastructure facilities and ongoing research & extension activities at NRCE.

At EPC (NRCE), Bikaner

- Mrs. Chanda Nimbkar, Member, Planning Commission, GoI visited EPC (NRCE), Bikaner on August 6, 2011 and interacted with scientists regarding their ongoing research work and difficulties faced by them. Plantation was also done on this occasion.

Plantation by Mrs Chanda Nimbkar

Mrs Chanda Nimbkar milking a mare

- A team comprising of a score of dignitaries including former Director-cum-VC, President NAVS (Prof. M.P. Yadav); Former ADG (AH), ICAR (Dr Lal Krishna); ADG (AH), ICAR and Secretary General NAVS (Prof. Gaya Prasad); Former Director (Acting); (Dr Nem Singh), Ex Director, NRCE (Prof. P.K. Uppal); Ex Director CIRB (Dr N.N. Pathak); Director NRCC (Dr. N.V. Patil); Vice President NAVS, Dr (Col.) V.K. Bhatnagar) and Prof. P.P. Gupta visited EPC, NRCE, Bikaner on November 12, 2011. Plantation was done on this occasion by all the dignitaries.

Plantation by Dr M P Yadav

Infrastructure & Developmental Activities

Veterinary Type Culture Collection

The first phase of developmental works of VTCC has been completed and the laboratory building has become fully functional. The internal furnishing of the individual laboratories viz., Bacteriology, Virology, Biotechnology, Pathology, along with the Microbial repository and other support facilities have also been completed. Furthermore, the facility of setting up of internet, LAN, EPABX etc. for the laboratories is underway.

A glimpse of VTCC laboratory

The agreement for the development of BSL-III laboratory for VTCC has been signed with the Hospital Services Consultancy Corporation (under Ministry of Health & Family Welfare, GOI) with a provision of about ` 8.80 crores. The facility will pave the way for research on emerging animal pathogens. Besides, the process for the development of second phase of VTCC building has also been initiated.

Validation of BSL-III Laboratory

The validation committee comprising of Dr Gaya Prasad, ADG (AH), ICAR; Dr. H.K. Pradhan, WHO (India) Consultant; Dr S.C. Dubey, Ex- JD, HSADL, Bhopal, and Dr V.A. Srinivasan, Research Director, Indian Immunologicals, Hyderabad inspected the facility on Oct. 22, 2011 and suggested some minor points for rectification. The facility is expected to be due for final validation once the corrective measures suggested by the committee are addressed.

Inspection of BSL III facility by expert committee

Agriculture Farm Production

Crop Production

During the period, about ninety five acres of land was used rotationally for cultivation of different types of crops. In spite of the high water table and salinity in bulk 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 yield of grain is also as source of revenue generation. A sum of ` 8,27, 974.00 (Eight lac twenty seven thousand nine hundred seventy four only) was generated through the sale

of 195 Qt. oat grains & 174 Qt. mustard grains.

During the period under report no green fodder was purchased from the local market and livestock requirements of EPC, Bikaner was met out from on farm produce. Towards reclamation, about 20 hectares of land was levelled and improved to bring more area under cultivation. A new tubewell was also installed to meet out the increasing demands of water for the livestock, agricultural farms and campus.

Earlier approach to the office building and residence was through the animal shed. To enhance the biosafety measures, kutchcha road (murad road) of about 300m has been developed away from the animal sheds.

Fodder grown at EPC, Bikaner

Crop production at Agriculture farm, NRCE, Hisar (2011-12)

Name of Crop (Green Fodder)	Cultivated Area (Acre)	Production quantity (Qt.)
Oat + Berseem	2	465
Oat		108
Sorghum sudan grass + Cowpea	15	990
Sorghum sudan grass		372
Lucerne	3	261
Total Green Fodder		2196
Grain Production		
Oat	35	195
Mustard	40	174
Oat Straw	35	Not yet ascertained

Crop production at Agriculture farm, EPC/ NRCE, Bikaner (2011-12)

Name of crop	Cultivated area (ha)	Production quantity (q)
Total dry fodder (rain fed)	12	135
Total green fodder	4	1110

Road for main building at EPC, Bikaner

Standing mustard crop in the field

Land reclamation and Development at NRCE, Hisar and EPC, Bikaner

About fifty acres of land near the pond was weeded out and developed through JCB for tractor operation & future planning of crop cultivation. The lawns were developed with suitable grass spp. at animal shed, along the road side & exercise ground within the NRCE campus for improving the environmental condition and beautification of the premises. Pop-up & spray-head irrigation systems were also installed in the lawns of main building for judicious use of water in the maintenance of grass & other plants. As a result, there was saving & proper distribution of water with salinity reclamation. A tube well was also installed in the VTCC premises for regular arrangement of water supply for the laboratories, lawns and plantation.

Besides, different species of plants were propagated and maintained in the backyard nursery of guest house and Director's residence. These plants were utilized for plantation work at NRCE Campus and EPC, Bikaner. Different species of flowering, ornamental and shady plants were also planted to improve the environmental condition of the campus.

Land reclamation at EPC, Bikaner

Land preparation for sowing

Livestock

Herd Strength at Equine Production Campus, Bikaner (2011-12)

Category	Horses		Ponies				Donkeys				Mules		Total
	Marwari		Zanskari		Indigenous		Exotic		Indigenous				
	M	F	M	F	M	F	M	F	M	F	M	F	
Stock as on 1.4.2011	22	44	7	7	—	3	12	17	14	7	3	2	138
Births during the year	6	6	1	1	—	—	2	1	—	—	—	—	17
Purchased during the year	—	—	—	—	—	—	—	—	4	1	—	—	5
Deaths during the year	—	4	—	1	—	—	—	1	—	1	—	—	7
Sold during the year	4	4	—	—	—	—	4	1	—	—	—	—	13
Balance as on 31.3.2012	24	42	8	7	—	3	10	16	18	7	3	2	140

Herd Strength at NRCE Main Campus, Hisar (2011-12)

Category	Horses		Ponies		Donkeys		Mules		Teaser	Total
	Marwari		Indigenous		Exotic					
	M	F	M	F	M	F	M	F		
Stock as on 1.4.2011	2	18	1	3	3	3	2	-	1	33
Births during the year	1	4	-	1	-	-	-	-	-	6
Purchased during the year	-	-	-	-	-	-	-	-	-	-
Deaths during the year	-	1	-	-	-	-	-	-	-	1
Sold during the year	-	-	-	-	-	-	-	-	-	-
Balance as on 31.3.2012	3	21	1	4	3	3	2	-	1	38

Ongoing Research Projects (2011-12)

Equine Health

Sr. No.	Title	Team	From	To
1.	Surveillance, Monitoring and Control of Emerging and Existing Diseases of Equines	S.K. Khurana *, B.K. Singh, S.C. Yadav, Baldev R. Gulati, Rajender Kumar, P. Malik, Nitin Virmani, Sanjay Kumar, Sanjay Barua, Rajesh Kumar Vaid, A. Arangasamy, H. Singha and Ramesh Dedar	April, 1995	Continuous Service Project
2.	Cysteine proteinase, a defined antigen of *T. evansi* for control of trypanosomosis	S.C. Yadav*, Rajender Kumar, Sanjay Kumar and A.K. Gupta	Sept. 2008	March 2012
3.	Genetic and antigenic differentiation of equine influenza viruses	B.K. Singh*, Nitin Virmani, B.C. Bera, B.R. Gulati and K. Shanmugasundaram	Sept. 2009	Aug. 2012
4.	Development of diagnostics for equine influenza	Nitin Virmani*, Bidhan, C. Bera, Baldev. R. Gulati, Shanmuga-sundaram K. and B.K.Singh	Sept. 2009	Aug. 2012
5.	Evaluation and Updation of the inactivated equine influenza virus vaccine	Nitin Virmani*, Baldev R. Gulati, A.K. Gupta and B.K. Singh	Oct. 2009	Sept. 2012
6.	Diversity of Mx gene and association of polymorphic markers with susceptibility vis-à-vis resistance against Equine Influenza	Balvinder Kumar*, R.C. Sharma and Anju Manuja	Sept. 2009	Aug. 2012
7.	Development of monoclonol antibodies and recombinant antigens based assays for detection of *Trypanosoma evansi* infection in equine	Rajender Kumar*, S.C. Yadav, Sanjay Kumar and Baldev R. Gulati	Sept. 2010	Sept. 2012

8.	Characterization of Toll-like receptor 9 and its role in CpG immuno-modulation in equines	Anju Manuja*, Balvinder Kumar, Sanjay Kumar and H.S. Singha	Oct. 2010	Sept. 2013
9.	Development of recombinant protein-based immune-diagnostic kit for equine infectious anemia (EIA)	Praveen Malik* and H.S. Singha	Sept. 2010	Aug. 2012
10.	Development of targeted drug release therapeutics using nanoparticles in Equine Medicine	Anju Manuja*, Neeraj Dilbaghi, Sandeep Kumar, Rajender Kumar, Balvinder Kumar and S.C. Yadav	Oct. 2010	Oct. 2013

Equine Production

Sr.No.	Title	Team	Date of Start	Date of Completion
1.	Characterization of indigenous non-descript and geographically distinct donkeys	A.K. Gupta*, Yash Pal, R.C. Sharma, Anuradha Bhardwaj, Sanjay Kumar and Mamta Chauhan	Aug. 2010	July 2014
2.	Enhancing reproductive efficiency in horses through semen cryopre-servation and embryo transfer technologies	A. Arangasamy*, T.R. Talluri, Sanjay Kr. Ravi, Yash Pal, S.K. Khurana and R.K. Vaid	Sept. 2009	Aug. 2011
3.	Studies on class I and II genes of Major Histocompatibility Complex in donkeys	R.C. Sharma*, Balvinder Kumar and A.K. Gupta	Apr. 2010	March 2013
4.	Draughtability studies and utilization of equine (mule and donkey) energy in agricultural operations including transport (AICRP)	Yash Pal*, R.A. Legha and A.K. Gupta	April 2009	March 2012
5.	Cloning, expression and characterization of equine chorionic gonadotropin (eCG)	Anuradha Bhardawaj*, A.K. Gupta, Sanjay Kumar and Varij Nayan	Dec. 2010	Nov. 2013

Extension

Sr.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	A.A. Raut*, Yash Pal and R.A. Legha	Sep 2009	March 2013

VTCC

Sr. No.	Title	Team	Duration	To
1.	Isolation, maintenance and characterization of bacterial pathogens and their molecular identification	Rajesh Kumar Vaid*, Sanjay Barua, Shanmugasundaram K., B.C. Bera and Taruna Anand	Jun. 2007	March 2013
2.	Isolation, molecular characterization and reposition of viruses of animal origin	Sanjay Barua*, B.C. Bera, R.K. Vaid, K. Shanmugasundaram, B.R. Gulati, Sarita Yadav, Riyesh T. and Taruna Anand	Sept. 2009	March 2013
3.	Development of protein expression clone repository of virulence associated genes of zoonotic buffalopox and equine influenza viruses	B.C. Bera*, Sanjay Barua, Nitin Virmani, Taruna Anand, Riyesh T. and Sarita Yadav	Jan. 2012	March 2013

Other Projects

Sr.No.	Title	Team	Date of Start	Date of Completion
1.	Isolation & characterization of animal adenoviruses & development of a novel viral vector for vaccine delivery (DBT)	Sudhanshu Vrati*, Baldev R. Gulati, Minakshi, K. Kumanan, M. Parthiban, Amarjit Singh and Ramnek	June 2010	May 2013
2.	Studies on *B. mallei* for rapid diagnosis of glanders in equines using molecular tools (DRDO)	Praveen Malik*, S.K. Khurana, H.S. Singha and R.K. Singh	Aug. 2010	July 2013
3.	OIE Twining Laboratories Project on Equine Piroplasmosis (Japan)	NRCE, India: Sanjay Kumar*, Rajender Kumar and R.K. Singh, NRCPD Japan: Prof. I. Igarashi and N. Yokoyama	June 2010	May 2013
4.	Development of Intravaginal Device for estrus control in Mares	S.M.K. Naqvi*, Sajjan Singh, T.R. Talluri and Sanjay Kr. Ravi	July 2010	July 2012
5.	Development of biomarker(s) for diagnosis of *Trypanosoma evansi* infection in animals using proteomic approach	Prof. Utpal Tatu*, S.C. Yadav, Rajender Kumar and B.C. Bera	June 2011	May 2014
6.	ICAR funded National Fellow Project on Development of sensitive and specific diagnostic assays for detection of *Trypanosoma evansi* infection in animals using modern molecular tools	Rajender Kumar	April 2011	March 2016
7.	Synthesis, characterization and evaluation of drug loaded nano-formulation against *Trypanosoma evansi* in animal model	Anju Manuja*, Neeraj Dilbagi, Sandeep Kumar, Rajender Kumar, Balvinder Kumar and S.C. Yadav	March 2012	March 2015

* Principal Investigator

Research Publications

List of Published Papers

1. Barua, S., Bera, B.C., Shanmugasundaram, K., Anand, T., Riyesh, T., Vaid, R.K., Virmani, N., Bansal, M., Shukla, B.N. and Singh, R.K. 2011. Molecular appraisal of host-range K1L gene of buffalo pox virus isolates from an outbreak (2010) in Maharashtra, *Journal of Immunology and Immunopathology*. 13(2) (Accepted).
2. Barua, S., Bera, B.C., Shanmugasundaram, K., Anand, T., Riyesh, T., Vaid, R.K., Virmani, N., Bansal, M., Shukla, B.N. and Singh, R.K. Sequence and phylogenetic analysis of Ankyrin gene of Camelpox virus from an outbreak in Rajasthan, *Journal of Immunology and Immunopathology*. 13(2) (Accepted).
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. 2011. Zoonotic cases of Camelpox infection in India. *Veterinary Microbiology* 152:29-38.
4. Chauhan, M., Gupta, A. K., Sharma, Y., Bhardwaj, A., Sharma, P. 2012. Efficacy of nine microsatellite markers in parentage testing of horse breeds. *Indian Vet J* (Accepted).
5. Dedar, R.K., Yash Pal, Kumar, S., Ghurai, S.K., Legha, R.A., and Singh, R.K. 2011. Therapeutic Evaluation of Ivermectin against endoparasites of donkeys, *Vet Practitioner* 12(1) 86-87.
6. Dedar, R.K., Yash Pal, Kumar, S., Legha, R.A., and Singh, R.K. Anthelminitic Activity of a Traditional Herbal Mixture in Horses in North Western Rajasthan. *Vet Practitioner (Accepted).*
7. 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. *J Vet Sci*: 12(4):341-345.
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11. Khurana, S.K., Srivastava, S.K. and Prabhudas, K. 2012. Seroprevalence of bovine brucellosis in Haryana by Avidin-Biotin serum ELISA and its comparison with RBPT and SAT. *Indian J Anim. Sci* (Accepted)
12. Khurana, S.K., Srivastava, S.K., Prabhudas, K. 2012. Serosurveillance of Infectious Bovine Rhinotracheitis (IBR) in Southern Districts of Haryana state by avidin-biotin ELISA. *Indian Vet. J* (Accepted)
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पृष्ठ 79-80 प्रकाशक, राष्ट्रीय अश्व अनुसन्धान केन्द्र, बीकानेर।

- राम अवतार लेघा एवम् यश पाल, 2012 अश्व रख-रखाव, पृष्ठ 125-136 जनजातीय क्षेत्र में समग्र पशुधन विकास, प्रकाशक, राष्ट्रीय अश्व अनुसन्धान केन्द्र, बीकानेर।
- राम अवतार लेघा एवम् यश पाल, 2012 अश्वों को लंगड़ापन के खतरे से कैसे बचायें, पृष्ठ 137-139 जनजातीय क्षेत्र में समग्र पशुधन विकास, प्रकाशक, राष्ट्रीय अश्व अनुसन्धान केन्द्र, बीकानेर।
- यश पाल एवम राम अवतार लेघा, 2012 खच्चर उत्पादन लाभदायक व्यवसाय, पृष्ठ 140-144 जनजातीय क्षेत्र में समग्र पशुधन विकास, प्रकाशक, राष्ट्रीय अश्व अनुसंधान केन्द्र, बीकानेर।
- राम अवतार लेघा, यश पाल, आर.के. देदड़ एवम् रमेशचन्द्र शर्मा, 2012 बागवानी एवं पशुपालन एवं पशुपालन प्रबन्धन मार्च 19-20, 2012 प्रकाशक राष्ट्रीय अश्व अनुसंधान केन्द्र, बीकानेर।
- यश पाल, राम अवतार लेघा एवम् आर.के. देदड़, 2012 अश्व नवजात के जन्म के समय रखी जाने वाली सावधानियां एवं देखभाल बागवानी एवं पशुपालन एवं पशुपालन प्रबन्धन मार्च 11-20, 2012 प्रकाशक राष्ट्रीय अश्व अनुसंधान केन्द्र, बीकानेर।
- रमेशचन्द्र शर्मा, यश पाल एवम् राम अवतार लेघा, 2012 अश्वों की नस्ले एवम् उनके सुधार की रणनीति समन्वित कृषि एवं पशुपालन प्रबंन्धन पृष्ठ 81-84 प्रकाशक, राष्ट्रीय अश्व अनुसन्धान केन्द्र, बीकानेर।

Submissions to GenBank

1. Anand, T., Bera, B.C., Shanmugasundaram, K., Vaid, R.K., Sharma, G., Bansal, M., Shukla, B.N., Virmani, N. and Singh, R.K. *Rhodococcus equi* strain SNP89 virulence associated protein (*VapD*) gene, complete cds. JN990997. (Nov., 2011).
2. Anand, T., Bera, B.C., Shanmugasundaram, K., Vaid, R.K., Sharma, G., Bansal, M., Shukla, B.N., Virmani, N. and Singh, R.K. *Rhodococcus equi* strain BBG163 virulence associated protein (*VapD*) gene, complete cds. JN990998. (Nov., 2011).
3. Anand, T., Bera, B.C., Vaid, R.K., Shanmugasundaram, K., Bansal, M., Shukla, B.N., Virmani, N. and Singh, R.K. *Rhodococcus equi* strain SNP85 virulence associated protein (*VapA*) gene, complete cds. JN710453. (Sept. 2011).
4. Anand, T., Bera, B.C., Vaid, R.K., Shanmugasundaram, K., Bansal, M., Shukla, B.N., Virmani, N. and Singh, R.K. *Rhodococcus equi* strain SNP89 virulence associated protein (*VapA*) gene, complete cds. JN710454. (Sept. 2011).
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8. Anand, T., Bera, B.C., Vaid, R.K., Shanmugasundaram, K., Sharma, G., Virmani, N., Bansal, M., Shukla, B.N. and Singh, R.K. *Rhodococcus equi* strain BBG163 virulence associated protein (*VapC*) gene, complete cds. JN990994. (Nov., 2011).
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10. Anand, T., Bera, B.C., Vaid, R.K., Shanmugasundaram, K., Virmani, N., Sharma, G., Bansal, M., Shukla, B.N. and Singh, R.K. *Rhodococcus equi* strain SNP85 virulence associated protein (*VapG*) gene, complete cds. JQ001825 Nov., 2011.
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47. Gupta, A.K., Tandon, S. N., Bhardwaj, A., Chauhan, M., Sharma, P. and Goyal, L. *Equus caballus* mitochondrial partial tRNA-Thr gene, tRNA-Pro gene and D-loop, isolate Kathiawari (23). HE580440 to HE580462.

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64. Vaid, R.K., Anand, T., Bera, B.C., Shanmugasundaram, K., Virmani, N., Bansal, M., Shukla, B.N. and Singh, R.K. *Rhodococcus equi* strain Eq21C virulence associated protein (*VapA*) gene, complete cds. JN990991. (Nov., 2011).

65. Virmani, N., Bera, B.C., Shanmugasundaram, K. Singh, B.K., Vaid, R.K. and Singh, R.K. Influenza A virus A/equine/Katra-Jammu/7/2008(H3N8) Z-segment 6 neuraminidase (NA) gene, complete cds. JN674066. (Sept. 2011).

66. Virmani, N., Bera, B.C., Shanmugasundaram, K., Singh, B.K., Vaid, R.K. and Singh, R.K. Influenza A virus A/equine/Mysore/12/2008(H3N8) segment 6 neuraminidase (NA) gene, complete cds. JN674068. (Sept. 2011).

Participation in Trainings, Workshops, Conferences and Symposia

(a) Participation in Trainings

1. A.K. Gupta, Principal Scientist and I/C PME attended one day ICAR-CII industry meet at NAS Complex, New Delhi and also acted as rapporteur for session-III on High end- Research on May 23, 2011.
2. R.K. Vaid, Senior Scientist and Harisankar Singha, Scientist attended hands on training of "GS-FLX: A pyro-sequnecer based 454 technology for high throughput sequencing technology" at Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University from July 18-25, 2011.
3. A.K. Gupta, Principal Scientist attended one day meet with Kisan Ayog Board "Working group on development of Animal Husbandry in Haryana" under the leadership of Dr. M.L. Madan, Ex-DDG (ICAR), on July 23, 2011, at CCSHAU, Hisar.
4. Balvinder Kumar, Senior Scientist, attended WHO sponsored workshop on "Laboratory Biosafety and Biosecurity" at HSADL, Bhopal from August 19-21, 2011.
5. S.C. Yadav, Principal Scientist, participated in short training course on "Bioinformatics in Agriculture" from August 29- September 7, 2011 at IASRI, New Delhi.
6. A.A. Raut, Scientist, participated in two days workshop on "Installation and Upgradation of SAS Package" held at NDRI, Karnal from November 8-9, 2011
7. Harisankar Singha, and Riyesh T., Scientists, participated in the 24th CAFT course on "The Molecular and Cellular Immunology Techniques for Animal Health" organized by Department of Veterinary Microbiology at Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar from November 3-23, 2011.
8. S.K. Khurana, Senior Scientist, attended training course on "Molecular diagnostics and bioinformatics tools" organized by Department of Biotechnology, at Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar from November 8-28, 2011.
9. Anju Manuja, Senior Scientist, participated in National dialogue for "Application of Nanotechnology in Agriculture" from November 11-12, 2011 at TNAU, Coimbatore.
10. S.C. Yadav, Principal Scientist, participated in short training course on "Synthesis and Characterization of Nanomaterials and their Application in Agriculture" from November 16-29, 2011 at Central Institute for Research on Cotton Research, Mumbai.
11. A.K. Gupta, Principal Scientist and I/C PME attended Web Page and Web Portal meeting at ICAR under chairmanship of DDG (AS) on November 28, 2011.
12. Anju Manuja, Senior Scientist, acted as Chairperson in Indo-Taiwan Workshop, 2011 from December 14-15, 2011, ISF College of Pharmacy, Moga.
13. A.K. Gupta, Principal Scientist and I/C PME attended one day "Sensitization-cum-Training Workshop for the Officer-in-charge of PME Cells of ICAR institutes" on March 3, 2012 at IASRI, New Delhi.
14. Ramesh Kumar Dedar, Scientist, participated in "Farriery Course 1" organized by Indigenous Horse Society Dundlod, District Jhunjhunu (Rajasthan) from March 22-24, 2012.

(b) Participation in Conferences, Workshops and Symposium

1. R.K. Singh attended and acted as Panelist in the "Plenary Session" during the XXV Annual Convention

of IAVMI and International Conference on "Energizing animal health for better livestock production" under WTO regime held at Veterinary College, Hebbal, Bangalore on 08-11th June, 2011.

2. Parveen Malik and Yash Pal attended a meeting-cum-workshop of Heads of the Divisions and Regional Stations/Centres held at CIAE, Bhopal during June 14-15, 2011.
3. Yash Pal attended one day workshop on "Inventorisation and documentation of location specific problems requiring Science and Technology Intervention" organized by Department of Science & Technology on June 24, 2011 at Science Centre Regional Office, Bikaner.
4. R.K. Singh attended Directors' Conference and also received Rafi Ahmed Kidwai Award for outstanding research in Agricultural Sciences-2010 on 16th July, 2011.
5. R.K. Singh attended Biosecurity Workshop for semen stations and progeny testing/indigenous breed development projects at NDDB, Anand on 27.07.2011.
6. Meena K. S., T-6, participated in seminar on "Multicut sorghum for sustaining milk production & productivity in buffaloes" on July 27, 2011 sponsored by Mahyco Ltd. at CIRB, Hisar.
7. Praveen Malik attended the WHO workshop on 'Laboratory Biosafety' at HSADL, IVRI, Bhopal during 17-19 August 2011.
8. R.K.Singh, Yash Pal, R.A. Legha, Balwinder Manuja and Sanjay Kumar attended 16th World Conference on Clinical Nutrition and 6th ICCD organized by International College of Nutrition & International College of Cardiology held at New Delhi from September 12-14, 2011.
9. Praveen Malik attended the meeting of ICAR scientists and officers of MoA, chaired by Hon'ble Union AM at NASC Complex,New Delhi on 8 November 2011.
10. Yash Pal, R.A. Legha and S.K. Ravi participated in National Symposium on "Biotechnologies for Augmenting Fertility and Conservation of Animal Species with Special Reference to North Eastern Hill Region" & XXVII Annual Convention of the Indian Society for the Study of Animal Reproduction (ISSAR) held at Central Agricultural University, Selesih, Aizawl, Mizoram from September 27-29, 2011.
11. A.K. Gupta, S.C. Yadav, Praveen Malik, Yashpal, Nitin Virmani, Sanjay Kumar, and R.K. Vaid participated in "12th Congress of World Equine Veterinary Association" held at Hyderabad from November 2-5, 2011.
12. A.A. Raut participated in INSEE International Conference 2011 on "Innovative Approaches for Agricultural Knowledge Management: Global Extension Experiences" 2011 by the International Society of Extension Education held at New Delhi from November 9-12, 2011.
13. R.K. Vaid, Sanjay Kumar and S. Barua, Senior Scientists participated in 3 day Meet of NARS Scientist trained through "International Trainings in Frontier areas of Agricultural Sciences NAIP, 28-30th November, 2011, at A P Shinde Memorial Symposium hall, NASC, N Delhi.
14. R.K. Singh, Yashpal, R.A. Legha, Ramesh Dedar, Talluri Rao, S.K. Ravi, R.K. Singh and P. Mallik participated in Annual Convention and Convocation 2011 of National Academy of Veterinary Science (India) and Seminar on "Veterinary Profession: Challenges and Opportunities under WTO Regime" organized by RAJUVAS, Bikaner during November 12-13, 2011.
15. R.K. Singh attended the VI Uttarakhand State Science & Technology Congress- 2011 from 13-15 Nov., 2011.
16. R.K. Singh attended International Symposium and expert consultation on "Strengthening the Veterinary Profession in India" jointly organized by ICAR & CABI at NASC, Pusa, New Delhi on 25.11.2011.
17. B.K. Singh attended Symposium on "Sustainable Livestock and Poultry Development in Jharkhand" held at Veterinary College, Birsa Agricultural University, Ranchi. from November 29-30, 2011.
18. Harisankar Singha participated in XVIII Annual Convention of Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and National Symposium on "Effective utilization of translational research plat forms for animal biotechnology". Organized by Dept. of Animal Biotechnology, College of Vety. Sc. And animal husbandry, Sardarkrushinagar Dantiwada Agricultural University, Gujarat, December 12-14, 2011.
19. S.C.Yadav attended and participated in International conference on "Nanomaterials and Nanotechnology" held at the Conference centre, University of Delhi, Delhi, India during December 18-21, 2011.

20. R.K. Singh, B.K. Singh, B.R. Gulati, Praveen Malik, Sandip Khurana, Rajender Kumar, Nitin Virmani, Sanjay Kumar, S. Barua, R.K. Vaid, Balvinder Kumar, Anju Manuja, B.C. Bera, Harisankar Singha, Shanmugasundaram, K., Sarita Yadav, Riyesh, T., and K.S. Meena participated in XX Annual Convention of IVS and National conference on "Managing Emerging and Re-emerging Plant, Animal, Human and Aquatic Viral Diseases: One health perspective". Organized by National Research Centre on Equines, Hisar, during December 29-31, 2011.

21. R.A. Legha and Ramesh Kumar Dedar participated in International Conference on "Emerging Frontiers & Challenges in Radiation Biology" organized by Department of Zoology Govt. Dungar college Bikaner in collaboration with MGS University, Bikaner January 24-25, 2012 held at Department of Zoology. Govt. Dungar College Bikaner.

22. R.K. Vaid, attended 2nd National Conference on "Antimicrobial Resistance: A cause of global concern" at Department of Microbiology Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Science (Deemed University), Allahabad, Feb 6-8: 2012, and presented invited paper on "Antimicrobials and their proper use in livestock".

23. R.K. Singh attended Workshop on Livestock Health organized by Deptt. of Biotechnology, Ministry of Science & Technology GoI in collaboration with BBSRC, UK at National Institute of Immunology, New Delhi from 07-08.02.2012.

24. Meena K.S., T-6, participated in the seminar on "New Perspectives in Aromatic and Medicinal Plants" at CCSHAU, Hisar organized by Department of Genetics and Plant breeding & Directorate of arecanut and spices development board, Calicut held on February 8-9, 2012.

25. R.K. Singh attended International Conference on "Scientific developments & technical challenges in the progressive control of FMD in South Asia" held at NASC, Delhi as member of the stage operation committee from 11-15 Feb, 2012.

26. R.K. Singh attended Directors' Conference held at NASC, New Delhi from 16-18 February, 2012.

27. Yash Pal, R.A. Legha and Ramesh Kumar Dedar attended International Congress on "Modern concepts in Canine Health and Diseases of Human Concern" and 9th Convention of Indian Society for Advancements of Canine Practice organized by RAJUVAS, Bikaner and Indian Society for Advancements of Canine Practice held at RAJUVAS, Bikaner, Rajasthan from February 9-11, 2012.

28. Praveen Malik attended the Launch Workshop for Half Yearly progress Monitoring (of Scientists) system/software at IASRI, New Delhi on March 3, 2012.

29. R.K. Singh attended and delivered keynote lecture on "Climate Change: Disease Emergence & Food Safety" during National Seminar on "Challenges in Combating Diseases: Cause to Cure" at MDU, Rohtak on 23.03.2012.

International Trainings and Visits Abroad

- Dr R.K. Vaid, Senior Scientist, VTCC, NRCE, underwent NAIP sponsored training at George Mason University, Manassas, Virginia, USA w.e.f. 17.01.2011 to 16.04.2011.
- Dr R.K. Singh, Director, NRCE supervised and reviewed the training component under OIE Twinning concept project at National Research Centre for Protozoan Diseases (NRCPD) Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Hokkaido, Japan w.e.f. 18.02.2011 to 24.02.2011.
- Dr Rajender Kumar, National Fellow and Dr Sanjay Kumar, Sr. Scientist NRCE underwent training under the OIE Twinning concept project at National Research Centre for Protozoan Diseases (NRCPD) Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Hokkaido, Japan w.e.f. 11.1.12 to 25.1.12.
- Dr B.R.Gulati, Principal Scientist, NRCE attended FAO sponsored training at the Australian Animal Health Laboratory (AAHL), Geelong, Victoria, Australia w.e.f. 14.11.2011 to 18.11.2011.
- Dr Sanjay Barua, Senior Scientist, VTCC, NRCE attended a FAO sponsored training at the Australian Animal Health Laboratory (AAHL), Geelong, Victoria, Australia w.e.f. 14.11.2011 to 18.11.2011.
- Dr. R.K. Singh (Director, NRCE)- as ICAR Representative- attended the Inaugural Global PPRV Research Alliance Meeting organized at Wellcome Convention Centre, London and visited the Institute for Animal Health (IAH), UK w.e.f. 07.03.12 to 11.03.12.

Personnel Milestones

New Joining

- Dr Rajender Kumar joined as National Fellow ICAR w.e.f. April 8, 2011.
- Shri R.B. Saxena, joined the Centre as Administrative Officer on promotion from IVRI, Izatnagar, Bareilly, UP on 18.04.2011.

Promotions

- Shri K.S. Meena, T-5, has been promoted to T-6 (Farm Manager) vide assessment w.e.f. 19.02.2011 onwards.
- Shri Narender Chauhan, T-4, has been promoted to T-5 (Farm Technician) vide assessment w.e.f. 13.09.2010 onwards.
- Shri Joginder Singh, T-3, has been promoted to T-4 (Lab Technician) vide assessment w.e.f. 10.11.2012 onwards.
- Shri S.N. Paswan, T-2, has been promoted to T-3 (Livestock) vide assessment w.e.f. 31.05.2011 onwards.
- Shri Sajjan Kumar, T-3, has been promoted to T-4 (Driver) vide assessment w.e.f. 29.06.2011 onwards
- Shri Suresh Kumar, T-3, has been promoted to T-4 (Driver) vide assessment w.e.f. 29.06.2011 onwards
- Shri Rajender Singh, T-2, has been promoted to T-3 (Lab technician) vide assessment w.e.f. 23.08.2010 onwards
- Shri Raghbir Singh, T-1, has been promoted to T-2 (Driver) vide assessment w.e.f. 05.05.2010 onwards

Study leave

- Dr Shanmugasundaram K., Scientist, VTCC, NRCE was granted study leave w.e.f. 09.01.2012 to 08.01.2015 for pursuing Ph.D in Veterinary Patholgy at Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Canada.
- Dr T.R. Talluri, Scientist, EPC, Bikaner, NRCE was granted study leave w.e.f. 16.02.2012 to 15.02.2015 for pursuing Ph.D in Animal reproduction at Veterinary Research and Animal Biology Centre in University of Veterinary Medicine, Hannover, Germany.
- Dr Ramesh Kumar Dedar, Scientist EPC, Bikaner, NRCE joined back his duties after completing Ph.D in Veterinary Medicine (on study leave) on 12.10.2011.

Transfers

- Dr A. Arangasamy, Scientist (Sr Scale) EPC, Bikaner, NRCE was relieved from the Centre on 31.10.2011 (A/N) subsequent to his transfer to NIANP, Bengaluru.
- Dr Balvinder Kumar, Sr. Scientist joined Main campus, NRCE Hisar subsequent to his transfer from EPC, Bikaner on 01.09.2011.

Obituary

Shri. Hawa Singh, AAO (12.2.1955-13.12.2011)

It is with great sadness that the NRCE, Hisar announces the sudden demise of our dear office colleague, **Shri Hawa Singh ji**, after a brief illness and hospitalization, on December 13, 2011, at the age of 56 years.

Shri Hawa Singh, S/o Shri Hari Singh was born on February 12, 1955 at Village Dhatrat, Tehsil and District Jind, Haryana. Hawa Singh joined ICAR services as Messenger in 1978 at CSSRI, Karnal and he rendered his services at Karnal for a long time before joining at NRCE, Hisar. Hawa Singh was a diligent, soft spoken and religious man, who was liked by all. In recognition for his services, he was recently promoted to the post of AAO at NRCE, Hisar. Shri Hawa Singh leaves behind his wife, two sons and a daughter, with whom our office staff joins in solemn condolences. The cremation was performed at his native village which was attended by NRCE staff. Shri Hawa Singh ji will be lovingly remembered by all NRCE staff who pray for peace of the dear departed soul.

Awards and Recognition

Awards

1. **Dr R.K. Singh, Director, NRCE receives Rafi Ahmad Kidwai Award**

   In recognition to the outstanding contribution made in the field of Animal Sciences Dr R.K. Singh, Director NRCE, received prestigious Rafi Ahmed Kidwai Award carrying a cash prize of ` 5 lac and a citation. The award was presented to Dr R.K. Singh by Hon'ble Union Minister for Agriculture Shri Sharad Pawar for his significant contribution in development of vaccines, diagnostic tests, assays and kits. These technologies will help to minimize the use of both capital and human resources. He has also been instrumental in generating baseline data on sero and molecular epidemiology of sheep pox, goat pox, buffalo pox, camel pox, equine influenza, glanders and equine infectious anemia.

2. **Agriculture Leadership Award 2011 for Dr R.K. Singh, Director, NRCE**

   Agriculture Today, a National Agriculture Journal has presented the Agriculture Leadership Award 2011 to Dr R.K. Singh, Director NRCE for his contribution especially on development of diagnostics & vaccines, generation of baseline data on many animal diseases of economic importance, human resource development in animal biotechnology and microbiology, Institution building at IVRI, Izatnagar and NRCE, Hisar. Vaccines and diagnostics developed by him and his group have empowered the nation in launch of national animal disease control programmes for PPR, sheeppox, goatpox, orf, and buffalopox. Molecular diagnostic tests and kits developed are already in use in the various laboratories in the country and have led to substantial import substitution.

3. Dr R.K. Singh was conferred Fellowship of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases (IAVMI) and Indian Virological Society during the VIROCON-2011 for his outstanding contribution in the field of virology, animal vaccines and diagnostics.

4. Dr B.C. Bera, Scientist, received Young Scientist Award at XX National Conference of Indian Virological Society on "Managing Emerging and Re-emerging Plant,

Director NRCE receiving Rafi Ahmad Kidwai Award.

Director NRCE conferred Fellowship of IAVMI

Dr B.C. Bera receiving Young Scientist Award during VIROCON 2011

Dr H. Singha bestowed Young Scientist Award during VIROCON 2011

Animal, Human and Aquatic Viral Diseases: One Health Perspective" held at National Research Centre on Equines, Hisar from December 29-31, 2011 for the research work by the authors Bera, B.C., Virmani, N., Shanmugasundaram, K., Singh, B.K., Gulati, B.R., Vaid, R.K., Barua, S., Shukla, B.N. and Singh, R.K. entitled "Molecular epidemiology of equine influenza virus isolates from 2008-09 outbreaks in India".

5. Dr H. Singha, Scientist, received Young Scientist Award at XX National Conference of Indian Virological Society on "Managing Emerging and Re-emerging Plant, Animal, Human and Aquatic Viral diseases: One Health Perspective" held at National Research Centre on Equines, Hisar from December 29-31, 2011 for the research work by the authors Singha H., Gulati B.R., Kumar P., Virmani N., Singh B.K. and Singh R.K., on Complete genome analysis of a Japanese encephalitis virus isolated from a horse in India"

Recognition

- Dr Shanmugasundaram, K., Scientist and Dr Thirumala Talluri Rao, Scientist received ICAR International fellowship and proceeded for Ph.D at University of Guelph, Ontario, Canada and University of Veterinary Medicine, Hannover, Germany, respectively.
- Dr Rajender Kumar, National Fellow, ICAR was nominated Member IMC, Central Sheep and Wool Research Institute, Avikanagar for three years w.e.f. 02.01.2012 by the Council.

Staff at NRCE

Director : Dr. R.K. Singh

Scientists at NRCE, Hisar Campus

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

National Fellow (ICAR), NRCE, Hisar

1. Dr Rajender Kumar, National Fellow, Veterinary Parasitology

Scientists at EPC (NRCE), Bikaner Campus

1. Dr Yash Pal, Senior Scientist, Animal Physiology
2. Dr R.C. Sharma, Senior Scientist, AG&B
3. Dr R. A. Legha, Senior Scientist, LPM
4. Dr P.A. Bala, Scientist, Animal Nutrition
5. Dr T. Rao Talluri, Scientist, Veterinary Reproduction & Gynecology
6. Dr Ramesh Dedar, Scientist, Veterinary Medicine
7. Dr Sanjay Kr. Ravi, Scientist, Animal Reproduction

Scientists at VTCC, NRCE, Hisar

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

Technical Staff at NRCE, Hisar

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

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-5, Farm Manager
5. Sh Om Prakash, T-3, Tractor Driver
6. Sh S.N. Paswan, T-3, Livestock Assistant
7. Sh Rajendra Singh, T-3, Lab. Technician
8. Sh Gopal Nath, T-1, Vehicle Driver

Technical Staff at VTCC, NRCE, Hisar

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

Administrative Staff at NRCE, Hisar

1. Sh. R.B.Saxena, AO
2. Smt. Shammi Tyagi, AF&AO (Additional Charge)
3. Hawa Singh, AAO
4. Sh Ram Pal, AAO
5. Sh S.P. Kaushik, Assistant
6. Sh Subhash Chander, Assistant
7. Sh Pratap Singh, Assistant
8. Sh Ashok Arora, Personal Assistant
9. Sh D.D. Sharma, UDC
10. Sh Om Prakash, UDC
11 Sh Deepak Kumar, LDC

Administrative Staff at EPC,Bikaner

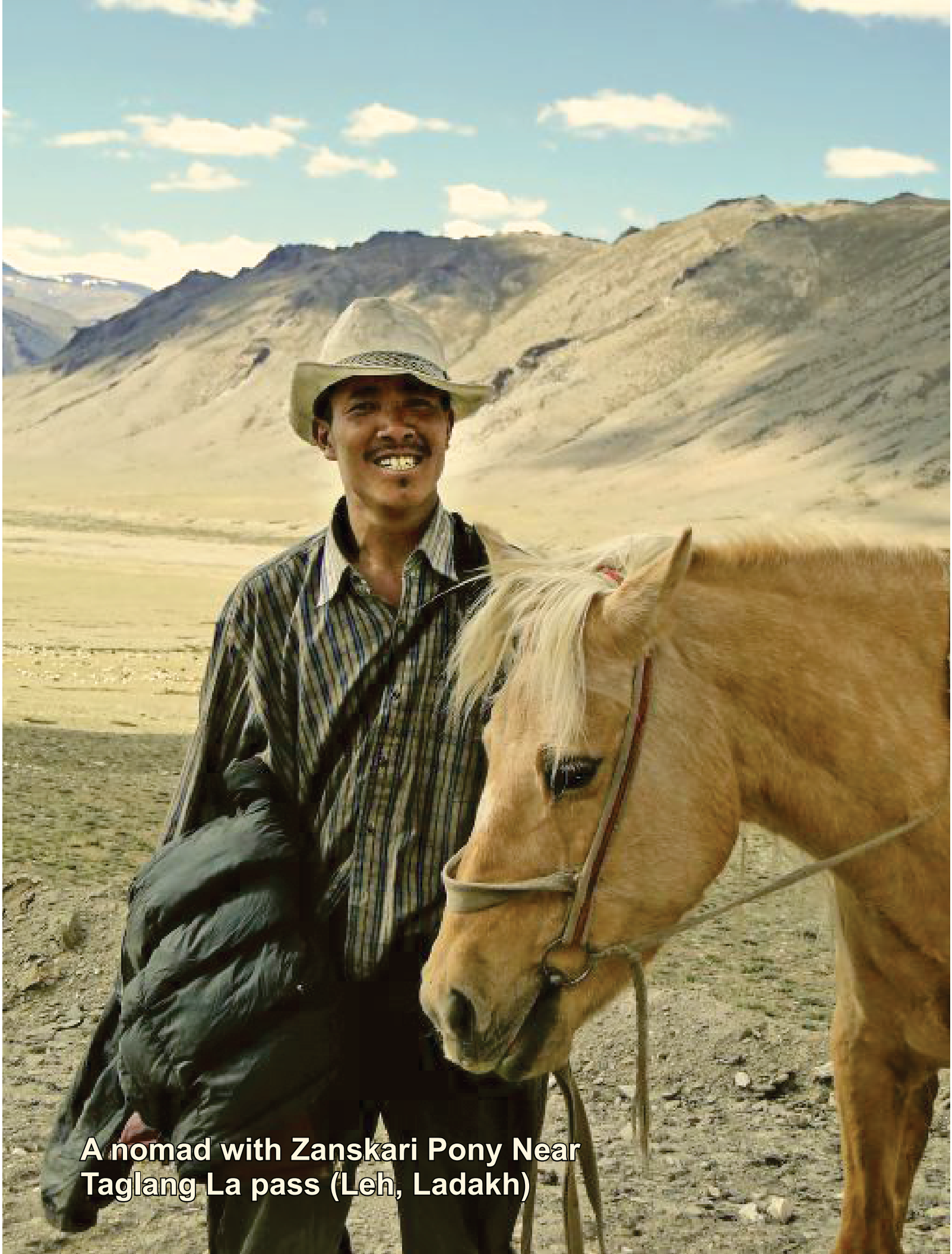
1. Sh Mahender Singh, LDC

Supporting Staff at NRCE, Hisar

1. Sh Ishwar Singh
2. Sh Guru Dutt
3. Sh Jai Singh
4. Sh Ramesh Chander
5. Sh Mardan
6. Sh Mahabir Prasad
7. Sh Desh Raj
8. Sh Ishwar Chander
9. Sh Om Prakash
10. Sh Satbir Singh
11. Sh Hanuman Singh
12. Sh Subhash Chander
13. Sh Ishwar Singh
14. Sh Ram Singh
15. Sm. 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



A nomad with Zanskari Pony Near Taglang La pass (Leh, Ladakh)


Improving equine health & productivity is the priority of NRCE
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