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National Research Centre on Equines

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Cover : Sindhi (Kutch) Horse in its home tract, Rann of Kutch, Gujarat (in water colour)

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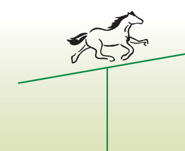


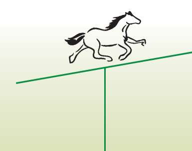
Zanskari Ponies in Ladakh

Director's Foreword

As we move along the comity of nations towards the dream of 21st century modern India, we see that the research itself is changing with the speed unforeseen. In our last report, I mentioned about the resurgent India's aspiration to regain our humble leadership in all spheres of human endeavor, however, we shall also have to see that although the economic development on industrial pedestal has given us the power to plan and move ahead, we are also beset with challenges of the future, like climate change and emerging diseases. In the process of the wholesome development and prosperity of the nation, an inclusive growth of our poor, economically and socially underprivileged, landless farmers and labor class is a must. The modern problems of environment and diseases, call for an innovation and excellence in research. However, this dream of reaching the zenith of research is not achievable, if we do not open our doors for cooperation, teamwork and funding. We have to be on our toes to forge new linkages spanning across institutions, organizations, communities, countries and scientific disciplines. In order to reach the ever evolving goal of excellence, we have to excel in our fields with a competitive zeal, and competitive grants!

We have a strong belief in the competitive edge of our scientific prowess, and our getting approval of external sourced funding from a large number of funding sources buttress our belief. In this, we can safely declare 2012 as NRCE's year of external funding. We have now running as many as 12 externally funded research projects. It is not only the national funding sources that we have been able to tap, but our scientists have also been able to get funding from International organizations such as Organization Internationale de Epizooties, Paris (OIE). We have been able to get funding grants from wide sources such as, Department of Biotechnology (DBT), Department of Science and Technology (DST), and Defense Research and Development Organization (DRDO) besides All India Coordinated Research Project (ICAR) and ICAR's National Fellow projects. Not only this, we have been also able to rope in private industrial funding towards ICAR-Pfizer contract research project on prevalence of bacterial and viral causes of calf scours. We also have been able to land a long term grant on Bioinformatics Instructional facility development project under BTISnet Programme of DBT.

In recognition of the reality of emerging diseases, such as glanders, piroplasmosis and equine influenza, NRCE striving towards forging links with Twinning of laboratories have bore sweet fruits. We have got three projects in these diseases with laboratories at Japan, Germany and United Kingdom. The programme of Piroplasmosis has already moved ahead with the visits of scientists, training and International training program conducted at NRCE.New innovative research by heavy water incorporation to test its effect in thermostabilization of recombinant p26 protein for sero-diagnosis of equine infectious anaemia by ELISA was undertaken. We have tested *In vitro* MASP culture system for maintaining *Theileria equi* parasite routinely in the laboratory. The genetic diversity of *T. equi* was also analysed. The other two scientific training programs are planned in near future. Our nationally funded research projects in cutting edge research areas include work on

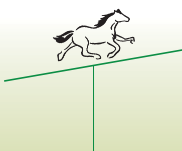


equine cytokines, equine stem cells, nanotechnology based drug formulations, development of molecular assays for diagnosis of *Trypanosoma evansi* and glanders, and biomarker for Trypanosomosis diagnosis by proteomic approach. Work on amniotic fluid-derived mesenchymal stem cells in equines holds promise for therapeutic use of stem cell in horses. The externally funded project on isolation of non-pathogenic adenoviruses from buffaloes and equines has potential of development of vectors with vaccine applications. An effective nano-based delivery system for trypanocidal drug quinapyramine sulphate is envisaged.

National Research Centre on Equines at Hisar made a humble beginning in last century. We achieved our silver jubilee in 2009-10, when we again took stock of our achievements. Helping in the conservation and improvement of the germplasm of indigenous equines breeds and the development of diagnostics/biological for major equine diseases, diagnosis, surveillance and monitoring of equine diseases, and providing diagnostic, advisory and consultancy services to equine owners and other stakeholders has been our forte. Our cutting edge research efforts for the development of diagnostics and biologicals for major equine diseases are continuing in right direction. Equine Influenza (EI) is single most important scourge to hit our equine population in 2008-09. We have been working on various aspects of disease diagnostic, control and prevention research of EI. In genetic and antigenic differentiation of EIVs, we have genetically analysed the 3 RNA polymerase genes. The sequences of polymerase genes of Indian isolates were compared and we observed significant point mutations in respect to other EIV isolates circulating globally. In order to ensure that a highly infectious and fast spreading disease like EI is detected rapidly and thus controlled effectively, we are working on development of Monoclonal antibodies (MAb) based diagnostic tests. We have been also putting our efforts in direction of antigenic differentiation of the isolates using MAbs and development of MAb- basedSandwich ELISA for early and rapid detection of EI. A TaqMan probe based qRT-PCR targeting nucleoprotein (NP) gene for detection of EI is under testing. We have also tested immunohistochemical method for detection of EIV antigen in MDCK monoloyers and in paraffin embaded tissue sections from BALB/c mice model developed for EI. Further, for evaluation of vaccine and pathogenesis study of EIV, BALB/c mice model for EI was developed.

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. In 2012-13, the maximum seroprevalence of Japanese encephalitis was reported in Gujarat & Haryana, whereas J & K reported nil seroprevalence. The dreaded glanders continued to emerge in the form of focal outbreaks in Uttar Pradesh in February-March, 2013. Lack of awareness among owners, compensation issues, lack in disease reporting, antibiotic treatment and subsequent relapse of the disease seems to be the major hurdles in glanders control, and we have to concentrate more on control of glanders in equines. During 2012-13, sero-survey was conducted on serum samples from 17 states of India. One EIA sample was positive by Coggin's test, whereas brucellosis and *Salmonella* Abortusequi (H antigen) tested were negative. Out of 7601 serum samples tested for glanders, 3 samples from UP were found positive. We also received and reported on 120 bacteriological samples from various states. Testing of samples from quarantine centre, and antimicrobial sensitivity results were conveyed. No new cases of EI were reported during the year. Samples were also tested for Equine herpevirus, *Theileria equi*, and trypanosomosis. In contractual services, Centre generated revenue of ₹ 4672647/- through testing of samples for various diseases.

Apart from viral diseases like EI, Japanese encephalitis, Equine Herpes Virus, the protozoal diseases like piroplasmosis and trypanosomosis also take a very heavy toll on general equine health and well-being. In continuation of our research activities on trypanosomosis, we raised experimental infection in ponies with *T. evansi*. Further, to develop sensitive and specific diagnostic test, three immuno dominant antigens have been identified, and evaluated for its immunodiagnostic potential. We have always given importance to scientists going to field for disease investigation, likewise, team visited Mysore and Bangalore for investigation of neurological disorders and



mortalities in horses, which was finally resolved as Trypanosomosis.

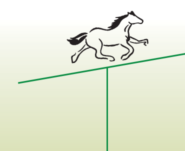
Donkeys, which constitute 37% of the total equines, are source of livelihood and transportation animals for small, marginal farmers and labourers of the country. On the production front, we continued our characterization of local non-descript donkeys from different geographic locations as these humble animals play a seminal role in livelihood enhancement of its keepers. Biometric analysis of donkeys from Leh and Maharashtra was done. Our scientists have also been working on cloning and characterization of eCG beta subunit, which will help in indigenous production of the hormones for various biomedical applications. We are on the verge of completion of project on characterization of equid semen, and its cryopreservation with the use of three different cryoprotectants i.e., glycerol, dimethyl formamide and dimethyl sulfoxide. Donkeys are important component in healthy mule production system. We have also analysed the Jack semen for its microbial load and quality. An AICRP project on "Increased utilization of animal energy with enhanced system efficiency" has evaluated the use of mules in ploughing and for chaffing green *bajra* (pearl millet) straw with the help of a rotary gear complex. Work on draughtability of indigenous donkeys is also underway.

In order to devise a safe, environmental friendly and innovative method of utilization of equine dung, we have used the technique for vermicomposting of the equine dung. The earthworms thus produced be utilized for another set of vermibeds to increase the production or can be sold for income generation. The vermicompost prepared have been utilised in our agricultural farms. We also utilized equine dung for preparation of simple compost. A pilot trial for production of mushroom production on mixture of equine dung and wheat straw was also successful. In order to increase the nutritive value of dry roughages/crop residues, silage production from dry roughages using molasses has been attempted. Preparation of molasses mineral block and molasses mineral mixture for feeding to equines has also been done.

Nutrition is the major part of equine management practice. Our works included effect of combinations of dry roughage feeding on digestibility and performance of horses in arid region of Rajasthan. For this, the feeding and digestibility trial was conducted on six pregnant Marwari mares. Innovation is an ingredient of research. In order to optimize the stallion semen freezing, and improve upon the present racks, in-house fabrication of two freezing racks and their study of efficiency for stallion semen cryopreservation on LN2 vapours were done.

Donkeys and mules have often been associated with poverty. These animals of drudgery are lifeline source of livelihood for a class of underprivileged peasantry in rural and peri-urban communities. A study on existing management systems and utilization of donkeys and mules for sustainable livelihood was undertaken in the states of Rajasthan and Haryana. Parameters of socio-economic status, prevailing feeding, housing management, and the utilization pattern of animals in different activities and the constraints faced by equine owners in earning their livelihood were important parameters of this study. The poor donkey owners face constraints like costly feed, lack of common pasture land, lack of good breeding stock, lack of social recognition, non-availability of bank loan, no insurance schemes for equines.

The VTCC which has a mandated aim of long-term preservation and distribution of veterinary dairy and rumen microbes, made one of its fastest increase in culture repository work in the year 2012-13. The repository increased its collection from previous 543 (2011-12) to present 751 (2012-13) of accessioned veterinary microbes including 627 bacterial and 124 viral isolate cultures along with additional 267 accessioned recombinant clones and 27 phage library. The repository has been strengthened with the addition of 187 veterinary bacteria, 21 virus, 45 rumen bacteria, 100 dairy microbes, 76 recombinant clones and 138 genomic DNA samples of bacteria during the current year. We are also concentrating on value addition of our accessioned isolates by whole genome sequencing of *Pasteurella multocida, Bordetella bronchiseptica, Salmonella* Gallinarum, *Trueperella pyogenes* and *Actinobacillus equilli* isolates. The challenging bioinformatic analysis of WGS results is underway. Significant new field isolations confirmed are *Nocardia otitidiscaviarum, Moraxella ovis*, *Delftia* spp, and methicillin-resistant *Staphylococcus sciuri* sub spp. *rodentium*. The species have been confirmed by



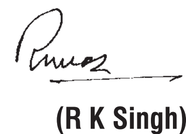
biochemical and 16S rRNA sequencing and phylogenetic analysis. Significant viral isolations include Rotavirus from foals and Sheep-pox virus. The Classical Swine fever outbreak from Haryana and Delhi was confirmed by PCR amplification of specific NS5B and E2 genes. We are also working on genetic characterization of buffalopox and camelpox isolates.

Our Agriculture section made a steady progress and continued high production of fodder crops at its farmland in Bikaner and Hisar for use of various equines kept for conservation at our farms. We have been able to sell excess fodder yield and Centre has been able to generate cash proceeds to the tune of Rs. 1486307/-. 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.

To increase the research output and meet the international standard, several infrastructural developments have been under taken. Info-equine museum was set up at ATIC which is the first of its kind in the country. Info-Equine Museum has comprehensive information about equines (horses, donkeys, mules, zebra, and hybrids) depicted through posters, translites, murals, storytelling board and information kiosk. The Microbial Containment Laboratory (MCL) at NRCE, Hisar, the first BSL-III laboratory in Haryana and VTCC building with functional laboratories were inaugurated and at the Centre. For beautification of the campus, land reclamation and plantation work have been done.

Apart from ICAR funded institutional research projects, as mentioned, we have bagged many national and international research grants. As a result of good research, our scientists have also published papers 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 as well as in India. An expert lecture was conducted under the auspices of CL Davis Foundation, USA and Indian Association of Veterinary Pathologists. We have been honoured by blessings and guidance by various distinguished dignitaries, guests and experts in various fields in our QRT and IRC meetings. We also organised interactive equine owners' meets, *Kisan Goshthis*, trainings and expert-lectures at NRCE.

NRCE gratefully acknowledges the kind guidance, support and encouragement from Dr. S.Ayyappan, Hon'ble Secretary, DARE and Director General, ICAR; Dr K M L Pathak, Deputy Director General (Animal Science); Dr Gaya Prasad, Assistant Director General (Animal Health); and expeditious help from Scientists and staff at ICAR Headquarters.



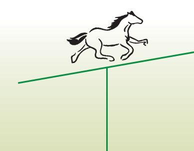
(R K Singh)

Executive Summary

National Research Centre on Equines (NRCE) first established 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 to various members of equine family including the donkeys and mules. The scientist 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, the Centre has been instrumental in bringing about action-oriented production, utilization, and conservation of the germplasm of indigenous equine breeds. We have kept up the pace of repositioning of Microbial genetic resources in the form of bacterial, viral cultures of veterinary, rumen and dairy origin, and have added significant novel isolates into our collection.

During the Year reported upon i.e., 2012-13, we continued to do our new useful researches with zeal and ardour befitting to the species we work with. The epidemic of equine influenza in the country in 2008-09 has put us on a path of being alert about its potential threat, and thus we have been working on various aspects of disease diagnostic, control and prevention research. In genetic and antigenic differentiation of equine influenza viruses, we have genetically analysed the RNA polymerase (PA, PB1 & PB2) genes. The sequences of polymerase genes of Indian isolates showed homology of 98-99.5% to Chinese and Mongolian isolates. Further, the specificity of four MAbs (1D12, 1G4, 5A7 and 5F4) raised against EIV in the previous year was tested employing indirect immunoperoxidase technique (IPT). EI virus infected MDCK cells gave positive immunoperoxidase reactions with all four MAbs and detected accumulation of immunizing antigen. EIVs (n=5) isolated from various parts of the country during 2008-09 epizootic and the isolate of 1987 outbreak were characterized antigenically for HA activity using two MAbs (1G4 & 5A7) employing HI assay. It was observed that MAb 1G4 recognized an epitope of 4 EI virus isolates/strains, while strains A/eq/Ludhiana/87(H3N8) and A/eq/Ahmedabad/1/09 were not recognised. However, 5A7 MAb recognised an epitope of all six EIV isolates. While Ludhiana/87 isolate has already been identified different from 2008-09 epizootic, the negative result for Ahmedabad isolate belonging to 2008-09 outbreak needs further studies which stimulate new dimension of research. The MAbs developed against EIV are being exploited to develop sandwich ELISA. The assay was standardized employing Clade 2 virus and the sensitive of the test was up to 0.025HA units of EIV detection. Further, EIVs from various lineages including Clade 2, American lineage, European lineage, Predivergent lineage and H7N7 gave positive results without any significant decrease in values indicating broad susceptibility of the MAb and primary antibody used in the assay. Moreover, nasal swabs spiked with antigens from various EIVs isolates were tested with encouraging results. Although molecular tests require sophisticated laboratory machinery, these tests are important for diagnosis and quantification of antigens. A TaqMan probe based qRT-PCR for detection of EI targeting nucleoprotein (NP) gene showed specific amplification curve for positive control as well as for NP gene, however, standardization of the test is underway.

For studies on vaccine and pathogenesis of EIV, mice model for equine influenza developed in BALB/c mice using EIV [A/eq/Jammu-Katra/08]. Mice inoculated with EIV showed no clinical signs except for slight ruffling of fur on 1 and 2 dpi and had no significant loss of body weight compared with control mice, however, on 1 and 2 dpi mice revealed patch of congestion in lungs. Histopathological



examination revealed lesions in trachea, and lungs from 2dpi onwards and maximum lesions could be seen on 3dpi. Viral antigen detected by immunohistochemical test in the nasal turbinates, trachea and lungs. Tissues from lungs as well as nasal turbinates showed positive results by RT-PCR till 5 dpi and 3 dpi respectively, while virus could be isolated on 1 dpi from lungs. Present investigations in BALB/c mice model demonstrated the virus replication in respiratory tract. These studies would help in developing a mice model for challenge studies prior to carrying out challenge studies in equines, thus ameliorating biosecurity and biosafety issues.

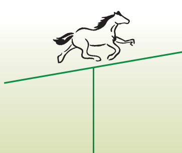
New innovative research by use of heavy water to test its effect in thermo-stabilisation of recombinant p26 protein for sero-diagnosis of equine infectious anaemia by ELISA was undertaken. The augmented strength of inter- and intra-molecular hydrogen bonds in D_2O has interesting consequences for biological macromolecules, as shown by reports on polio virus vaccine and lactate dehydrogenase enzyme. Thermostabilisation effect of heavy water was tested using various percentage of D_2O at different temperature range (37°C to 42°C) and the stabilization effect was observed in higher temperature at 80% D_2O. The findings will be useful in developing diagnostic assay and transporting the coated plate without cold chain requirement.

Sero-survey was conducted for various equine diseases in various states of the country. During this period, 7462 serum samples were examined for EIA which resulted in detection of one thoroughbred horse positive for EIA in September, 2012 in Haryana. The animal was eliminated as per policy guidelines. No new cases/ outbreaks of EI were reported during the year. Out of 3296 serum samples 21 samples were positive for equine influenza, however, none of the samples tested in pair showed rise in titres. None of 450 samples tested for EVA were found positive. Two samples yielded positive for EHV 1 in disease outbreaks attended. In 2012-13, 1153 equids from 6 states of India were tested for JEV antibodies and 45(3.9%) were positive. Maximum sero-prevalence was reported in Gujarat & Haryana, whereas none of the samples tested from J & K were found positive for JE antibodies. All of 1482 serum samples tested for brucellosis and *Salmonella* Abortusequi (H antigen) tested under S&M were negative.

7601 serum samples were tested for glanders, which included S&M (1482), disease investigation (1357) and contractual service (4762), in which 7 samples from UP were found positive. The dreaded glanders continued to emerge in the form of focal outbreaks in Uttar Pradesh in February-March, 2013. Outbreaks were detected from three different places in Uttar Pradesh namely Auriaya, Hardoi and Ganjdundwara block in Kasganj District. The affected equines exhibited respiratory and cutaneous lesions. Cutaneous and nasal forms were observed in Hardoi and Ganjdundwara whereas only respiratory form was observed in Auraiya. Lack of awareness among owners, compensation issues, lax disease reporting, antibiotic treatment and subsequent relapse of the disease seems to be the major hurdles in glanders control.

Bacteriological analysis of 120 samples including nasal swabs, vaginal swabs, uterine swab, rectal swab, lesion swab, tissues from PM, pus, blood, aborted foetus and contents and buccal cavity swab originating from Rajasthan, Haryana, U.P. and Uttarakhand yielded 48 isolates including *Streptococcus equi* subsp. *zooepidemicus* (5), *Streptococcus equi* subsp. *equi* (1), *Staphylococcus* sp.(1), *E. coli* (11), Gram-negative bacilli (6), Group C *Streptococcus* (16), *Nocardia asteroides* (6) and micrococci (2). Disease investigation through post-mortem examination and morbid material/ biopsy received from the field revealed cirrhosis of liver, volvulus leading to toxaemia, pulomanry nocardiosis, non-suppurative encephalitis due to trypanosomosis, non-suppurative encephalitis of unknown etiology. In parasitological studies, 1482 serum samples were tested for detection of *Babesia equi* antibodies by ELISA, with 34.6% positivity. *T. evansi* antibodies were positive in 75 out of 1482 serum samples.

One of the important services rendered by NRCE is contractual testing of samples from equine breeders and race courses. During the current year, Centre generated a revenue of ₹ 4672647/- through testing of samples for various diseases including EIA, glanders, EI, EHV-1, EVA, CEM, *Theileria equi*, *Trypanosoma evansi*, and *Babesia equi.*



The Mx protein confers resistance to Orthomyxo virus infection by modifying cellular functions needed along the viral replication pathway, which has been amply demonstrated in mice chicken and humans, but it is still unravelled in equines. In this direction, the Mx gene expression was studied both in unstimulated and stimulated PBMCs collected from Marwari horses by qRT-PCR. However, no discrete pattern Mx gene expression to differentiate EI infected and non-infected animals was observed. Amino acid sequence analysis revealed 99-100% sequence homology with thoroughbred horse but only 37-49% with human, canines and fishes. On phylogenetic analysis, the Mx sequences from Marwari horses formed separate clade with Thoroughbred Mx protein homolog.

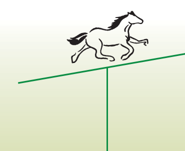
The protozoal diseases like piroplasmosis and trypanosomosis also take a very heavy toll on general equine health and well-being. In continuation of our research activities on trypanosomosis, experimental infection in ponies was raised with *T. evansi.* All the infected ponies developed sub-acute to acute disease within 56 dpi. The prepatent period of the infection was 5-7 days. Two ponies showed typical symptoms of trypanosomosis - edema in brisket, abdominal regions, staggering gait, and incoordination of hind-quarters. After treatment, ponies were found negative for parasitaemia by 48 hrs and all the animals recovered 10 weeks post-treatment. However, one of them showed acute neurological signs after 6 months, which was euthanized. The haematological studies indices indicate the gradual fall in values however, MCV values showed increasing trend after 14 dpi in all infected animals. The sensitivity of sonicated (WCL) and semi-purified antigens were comparatively evaluated using serum samples of experimental ponies. Both antigens detected *T. evansi* antibodies from 10-14 dpi onwards and shown rising trend till termination of experiment i.e. 56 by dpi. This suggests that predominant purified proteins, are equally sensitive and specific as WCL antigen in detection of T. evansi infection. The results of the Immuno blot recognizing polypeptide bands at 55 kDa region and dip stick ELISA detecting *T. evansi* antibodies at 14 dpi strongly suggestive of applicability of this test in field.

To investigate the neurological disorder and mortality in horses, a team from NRCE visited Mysore and Banglore race club in September, 2012 and the problem was diagnosed as *T. evansi* infection based on history, clinical signs, symptoms, test-reports and histo-pathological examination. Suitable control measures were suggested. Further confirmation was done by ELISA, immunoblot, PCR of blood, brain tissue & CSF, histopathgology and mass spectrometry based protein identification from the buffy coat of the infected horse which revealed presence of variable surface glycoprotein (VSG) and other Trypanosomal proteins. No concurrent infection of JEV, WNV and EHV-1 was observed with *T. evansi* seropositive horses.

On the production front, we continued characterization of local non-descript donkeys from different geographic locations as these humble animals play a seminal role in livelihood enhancement of its keepers. Biometric analysis of donkeys from two different geographical areas (Leh, Ladakh and Baramati, Pune) was carried out for generating systematic base line information. Fifteen biometric indices of 35 donkeys from Leh area (Ladakh, Jammu & Kashmir) and 59 donkeys from Baramati area, Pune, Maharashtra were recorded. Coat colour of most of donkeys at Baramati was grey, both light and dark with and without dark strip on back. Large white are mostly brought from Gujarat, and donkeys available at Leh were bay black. Comparative analysis clearly indicated that both the Indian populations are smaller in size than Poitu breed of donkeys. Further among both the Indian local populations, donkeys available at Baramati areas were also significantly taller and bigger in size than donkeys of Leh area.

The PCR-RFLP based genotyping of major histocompatibility complex class I genes in donkeys and mules revealed that digestion of ELA-A locus (800 bp) with Hha I and Dde I restriction enzymes resulted in different bands pattern showing polymorphism in donkeys and mules.

With larger objective of development of recombinant protein based diagnostics for detection of pregnancy in equines, beta-subunit of chorionionic gonadotropin (eCG) of mule was cloned and expressed in *E. coli.* The band of



about 30 kDa of recombinant protein was observed in SDS-PAGE and further biological characterization of the recombinant protein is under progress.

A new project was initiated for studying the endocrine, biochemical and gene expression profile of reproductive states in Marwari mares with an overall objective to analyse and improve the reproductive efficiency of mares. The adult mares and fillies (Between 1-2 Years of age) were selected to study the follicular dynamics during estrous cycle. The preliminary findings have revealed that the prevoulatory follicle grows to as large as 55 mm in the Marwari mares and the behavioural signs of estrus are more prominent as the prevoulatory follicle attains 35 mm diameter.

The work on characterization of equid semen, and its cryopreservation using amides revealed no statistically significant difference 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 and cryopreserved semen of indigenous jacks. The quantitative enumeration of aerobic bacteria in Jack semen showed bacterial count with range of 1.2×10^2 to 5.6×10^3 cfu/ml in fresh semen and 1×10^4 to 6×10^5 cfu/ml in frozen semen. In contrast to previous findings with the stallion semen, the bacterial counts were lower in fresh samples as compared to frozen semen samples. Out of bacterial isolates identified from Jack semen, the majority were identified as *Corynebacterium* spp. thus indicating that environmental contamination from air or soil has been the major contaminating source.

In order to devise a safe, environmental friendly and innovative method of utilization of equine dung, we have tailored the technique for vermicomposting of the equine dung. 35 quintals of vermicompost worth ₹17,500 was prepared during the year, which was utilized at farms in Bikaner and Hisar. We also utilized equine dung for preparation of simple compost as daily 20-25 quintals of dung is produced at Bikaner campus. Simple compost manure is produced in 4 months which is suitable to apply in field for crop production. To utilize equine dung for income generation of equine owners, a pilot trial for production of mushroom production on mixture of equine dung and wheat straw was also completed.

In order to increase the nutritive value of dry roughages/crop residues, silage production from dry roughages for feeding to equines was attempted. The nutritive value of dry roughages could be enhanced significantly in respect of crude protein, energy, aroma and palatability by ensiling. Unconventional method with supplementation of jaggery was followed for silage preparation. The problem of deficit in balance nutrition of equines can be solved to some extent by supplementing molasses mineral block or mineral mixture in the diet which was successfully tested. To study the effect of combinations of dry roughage feeding on digestibility and performance of horses in arid region of Rajasthan, 45 days experiment conducted in pregnant mares by feeding sewan grass kutar in dry roughage and premixed concentrate obtained from HAFED which resulted in increase in body weight.

A study on existing management systems and utilization of donkeys and mules for sustainable livelihood is being undertaken at NRCE. The survey conducted in Rajasthan, and Haryana indicated that majority of owners used their animals in cart transportation whereas 31.4 % were using donkeys as pack. The monthly income of most of the owners ranged between ₹ 4000 to 9000 from animals. The major constraints faced by donkey and mule owners were high cost of feed, competition with mechanized vehicle, lack of availability of common pasture land, non availability of good donkey stallion for breeding, lack of social recognition, non availability of bank loan/schemes for purchase of equines, no insurance schemes for equines.

It is a matter of great pride and satisfaction that our sustained efforts have fructified in the form of many externally funded projects. We here give a brief account of the Projects and the salient findings there in. In the Science & Engineering Research Board, DST, New Delhi funded project-"Eukaryotic expression of important equine cytokines and analysis of their biological activities", analyzed sequences of horse cytokines viz., IL-2, IL-4, IL-10 and IL-18 cytokines. Sequence and phylogenetic analyses of the four cytokine genes indicates that horse cytokines are closely related to suborder Suiformes (Pig) and Tylopoda (camel) in the Artiodactyla order. In another externally funded project, "Studies on *Burkholdderia mallei*

for rapid diagnosis of glanders in equines using molecular tools" sponsored by LSRB-DRDO, New Delhi, expressed and validated recombinant *Burkholderia mallei* TssB and Hcp1 protein based ELISA for development of sero-diagnostics of glanders among equines. The standardized indirect ELISA using recombinant TssB protein was used to test 1811 serum samples. The relative sensitivity, specificity, positive predictive value, and negative predictive value, of the ELISA were 100%, 99.72%, 92.45%, 100%, respectively in comparison to CFT. Cross-reactivity experiment of the truncated TssB protein with human meliodosis serum and equine serum was also done.

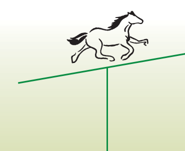
The Organisation Internationale de Epizooties (OIE) located in Paris is World Animal Health Organisation, which makes policies on prevention and control of animal diseases worldwide. The Centre has been able to win funding for laboratory twinning for Equine Piroplasmosis, Glanders and Equine Influenza at Japan, Germany and UK, respectively. In the OIE Laboratory Twinning Project on "Equine Piroplasmosis", In vitro MASP culture system was tested for maintaining *Theileria equi* parasite routinely in the laboratory. Blood samples collected from different locations were tested simultaneously in MASP in vitro system, ELISA/cELISA, and PCR/qPCR to demonstrate the presence of live *T. equi* parasites, anti-*T. equi* antibodies and parasite specific DNA to demonstrate *T. equi* live parasite or parasite specific DNA in antibody positive equids, which has been successfully illustrated. The genetic diversity of *T. equi* was also studied by sequencing of EMA-1 and 18sRNA gene of *T. equi* isolates. The 18s rRNA gene showed 98-99% similarity, whereas EMA-1 gene revealed 94% to 97% similarity to other strains of *T. equi* available in the NCBI. The phylogenetic analysis revealed three genotypic clades and Indian strain of *T. equi* falls with African and Texas strains. The qPCR test developed for detecting *Theileria equi* parasitic load using SYBR-Green chemistry which could detect as low as 200 copy number per μl. The OIE Twinning Programme on Equine Influenza with Animal Health Trust (UK) started in October, 2012. After the start, we have concluded online research discussions with AHT Scientists. Various aspects of the outbreak of equine influenza in India during 2008-2009 and facilities and surveillance infrastructure available in the two countries were discussed in details.

The DBT sponsored Project on "Isolation and characterization of amniotic fluid-derived mesenchymal stem cells in equines" holds potential for therapeutic use of stem cell in horses, especially, ligament and tendon injuries. As there is an interest in application of MSCs isolated from yet unexplored neonatal tissues, therefore, study was initiated to explore whether equine MSCs (eMSCs) can be harnessed from amniotic fluid at the time of foaling and to evaluate their tenogenic differentiation potential. Fibroblastic colonies have been isolated from mare amniotic fluid samples and studied for in vitro differentiation of AF-MSC towards osteoblasts, chondroblasts, adipocytes and tenocytes. The AF-MSCs were positive for expression of pluripotency markers.

In the DBT funded project entitled "Isolation and characterization of non-pathogenic adenoviruses from animals" the non-pathogenic adenoviruses were isolated and characterized from buffaloes and equines. During the year, the pathogenicity of two bovine adenovirus isolates (N-131 and N-134) and one equine adenovirus isolate (H-9) was studied in experimental mice and the two bovine and one equine adenovirus were non-pathogenic in mice. Seven equine and five bovine adenovirus isolates were clonally purified and the cloned isolates are being processed for RFLP analysis to collaborating laboratory.

In another DBT funded project "Development of biomarkers for diagnosis of Trypanosoma evansi infection in animals using proteomic approach", three clusters of immuno reactive proteins were identified using immunoblot and later purified by SDS-PAGE preparatory gel method. One cluster subjected to analysis by mass spectroscopy has revealed 5 proteins out of which one heat shock protein 70 (hsp70) gene has been cloned and expressed recombinant protein. The large scale purification of recombinant protein was made for further use in immunodiagnostic test.

In the Department of Science and technology (DST) sponsored project under Nano mission "Synthesis, characterization and evaluation of drug-loaded nano-formulations against *Trypanosoma evansi* in animal model", an effective delivery system for trypanocidal drug quinapyramine sulphate using drug-loaded sodium



alginate nanoparticles were formulated. The zeta potential values are adequate to form a stable nanoparticle suspension. The drug in this form has less cytotoxicity and is able to kill the parasites at much lower concentrations in vitro as well as in vivo in mice model of *T. evansi*.

The NRCE, Hisar has also been granted a Bioinformatics Infrastructure Project on "Bioinformatics infrastructure facility for biology teaching through Bioinformatics (BIF-BTBI)" under BTISnet program of DBT. The Infrastructure facility will be useful for availing and training for Bioinformatics, as it will be fully functional with high-speed net connected PCs and servers, with reprographic facility.

In the ICAR-Pfizer Contract research project, "Studies on prevalence of bacterial (*Escherichia coli* and *Salmonella*) and viral (Corona and Rota) causes of calf scours amongst dairy cattle in India", the presence of these infectious agents as a causative agent for diarrhoea in organized dairy farms was investigated. Rotavirus could be detected in 18.35% samples with G6 genotype predominating followed by G10. BCV in fecal samples was established by RT-PCR, nucleotide sequencing and phylogenetic analysis of three selected PCR products; however, isolation was not successful. HRT-18 cell-line for isolation was not available. Although the *E. coli* could be isolated from 64.3% swabs, only three the number of isolates found positive for K99 antigen by Serum agglutination test (SAT). Diagnostic PCR could not be employed for K99 strain detection due to non-availability of reference positive strain. In conclusion, data from this study showed that BCoV, BRV and *E. coli* infections are prevalent in Indian calf population.

An AICRP project on "Increased utilization of animal energy with enhanced system efficiency" has evaluated the use of mules in ploughing during winter season under work rest scheme. Mules were tested in ploughing under the scheme during July month using two furrow ploughs. A rest of 30 min was given after every 1.5 h of work. Physiological responses increased significantly after work and remained significantly high after the completion of work. Enzymes and glucose content in serum was observed statistically non-significant after work. The physiological responses which didn't come to normal after 1 hour rest became normal by the next morning. Deployment of mules in agro-processing could be an alternative option for their optimum utilization, therefore, study was conducted on use of mule power for chaffing green bajra straw with the help of a rotary gear complex, driven by a local mule of 350 kg body weight. The physiological indices of the mule increased significantly but mules did not exhibit physiological stress.

The Veterinary Type Cultures Collection (VTCC) has a mandated aim of long-term preservation and distribution of veterinary pathogens along with dairy and rumen microbes. VTCC made one of its best ever increase in Culture repository work in the year 2012-13. Many important virus, bacteria, recombinant clones and phage library have been characterized and reposited. During the period under report, the repository has been strengthened with the addition of 21 viral isolates, 187 pathogenic bacteria, 45 rumen bacteria, 100 dairy microbes, 76 recombinant clones and 138 genomic DNA of bacteria from different animal species. The repository has increased its accessioned culture collection to 1630 cultures. The repository increased its collection from previous 543 (2011-12) to present 751 (2012-13) of accessioned veterinary microbes including 627 bacterial and 124 viral isolate cultures along with 267 accessioned recombinant clones and 27 phage library.

Accessioned virus cultures reposited in VTCC include isolates of buffalopox virus, camelpox virus, goatpox virus, sheeppox virus, canine parvovirus, canine adenovirus, fowl adenovirus and street rabies virus among others. The important bacterial cultures accessioned include *Bordetella bronchiseptica*, *Actinobacillus equulli, Brucella melitensis* biowar I, *Brucella melitensis* biowar II, *Staphylococcus sciuri, Staphylococcus hyicus, Pseudomonas putida, Corynebacterium* spp, *Rhodococcus equi, Campylobacter coli, Citrobacter freuendii, Listeria monocytogenes, Moraxella ovis*, *Pasteurella multocida* sub spp. *multocida, Nocardia asteroides, Salmonella* Enteritidis, *Salmonella* Typhimurium, among others. VTCC is also maintaining eleven different cell lines along with one primary culture for isolation of different viruses in the repository.

To strengthen the R&D of rumen microbes, a total of 140 rumen microbes have been isolated, characterized,

accessioned and deposited in the VTCC repository. Bubaline rumen fungi such as *Anaeromyces* spp., *Orpinomyces intercalaris* and *Orpinomyces joyonii* and caprine isolates: *Piromyces* spp. and *Neocallimastix* spp. have been isolated and preserved. Several rumen bacteria such as *Bacillus licheniformis*, *Butyrivibrio* spp., *Eubacterium limosum*, *Megasphaera elsdenii*, *Prevotella* spp., *Streptococcus bovis*, *Strept. equinus*, *Strept. gallolyticus*, *Strept. lutetiensis*, *Strept. sanguinis* and *Veillonella parvula* have been isolated from cattle, goat and buffalo. Three rumen bacteria have also been isolated from camels. These isolates have been analyzed for useful biological activities such as fibre and protein degradation; urea hydrolysis; tannin degradation, bacteriocin production etc.

During the period, 89 dairy cultures were isolated and characterized, and a total of 490 bacterial cultures are available in the dairy microbes repository. Accessioned cultures include isolates belonging to *Lactococcus* spp., *Leuconostoc* spp., *Streptococcus*, *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Micrococcus*, *Kluyveromyces* and *Saccharomyces genus*.

The Centre has continued the value addition of culture collection by deciphering the genome of a B:2 serotype of *Pasteurella multocida* buffalo isolate from an outbreak of HS in buffalo. In this direction, the genome sequences of a Fowl typhoid isolate of *Salmonella* Gallinarum, and a equine respiratory *Bordetella bronchiseptica* isolate were carried out for value addition.

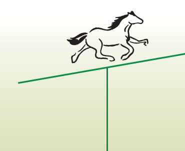
For strengthening the repository, 4 equine rotaviruses have been isolated from foal diarrhoea cases in Tohana, Hisar confirmed by amplification of the VP7 gene. Besides, one sheep pox virus could also be isolated in Lamb Testicle primary culture and the isolate has been confirmed by amplification of the B2L gene. Fifteen out of 26 samples collected from various outbreaks of Classical Swine fever from Haryana and Delhi were confirmed positive by PCR amplification of specific NS5B and E2 genes.

Lack of information on host tropism of buffalo pox virus (BPXV), led us to analyse the host range serpin 1 (SPI-1) gene of BPXVs isolated from previous outbreaks. Sequence analysis of SPI-1 gene revealed that BPXV isolates shared maximum homology among themselves as well as with Vaccinia virus (VACV) and exhibited closest homology with VACV isolates followed by other pox isolates. In this direction, sequences of six host-range genes viz., B5R, B7R, B9R, CBP, crmB & crmE of four isolates of CMLV isolated from camels in Delhi, Bikaner, Barmer and Jaisalmer were analyzed and found high similarity at aa level with CMLV and VARV isolates. However, B9R & crmE gene sequences were not observed in any VARV isolates, which indicates that inactivation of these two genes may increase the virulence of CMLV. Three significant point mutations were observed in CBP gene of Delhi isolates along with VARV.

A small survey was conducted to ascertain the status of PPR virus in which sixty biological samples from donkeys, sheep & goats from Bikaner were processed. None of the 11 donkey samples were found positive for PPR antigen or antibodies by sandwich cELISA, respectively, however sheep and goat samples gave positive tests by various antigen and antibody detection methods including sandwich cELISA and RT-PCR.

Four new bacteria confirmed by several biochemical tests and by cloning and sequencing of 16S rRNA have been isolated from clinical cases. These new bacterial isolates include *Nocardia otitidiscaviarum* from equine granulomatous pneumonia, *Moraxella ovis* from keratoconjunctivitis cases from sheep in J & K, *Bordetella bronchiseptica* isolate from pneumonic foal in Pushkar Rajasthan, and *Delftia* spp. from water sample from sheep watering hole in Gulmarg, J & K. All isolates were further confirmed by sequence comparison and phylogenetic analysis analysis of the 16S rRNA genes.

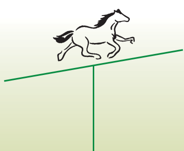
The investigation of saddle sore infection in ponies which are used to ferry pilgrims across to Amarnath shrine led to isolation of *Streptococcus equi* sub spp. *equi* and *Staphylococcus* spp. from wounds. Saddle sores are generally caused due to use of ill-fitting saddles. The animals were treated by debridement and drainage of wounds and application of topical povidone-iodine solution. Further, 3 strains of *Staphylococcus sciuri* sub spp. *rodentium* were isolated from goat milk. These isolates were resistant to penicillin, and were also found to



be carrier of mecA gene, as confirmed by PCR. What is interesting is that majority of *S. sciuri* isolates are generally fully susceptible to β-lactam antibiotics with mecA homologue, however, these isolates were detected for resistance by penicillin and methicillin resistance. The isolates have been reposited in VTCC.

Many bacteria have been identified and authenticated by cloning, sequencing and analysis of 16S rRNA genes. The BLAST homology analysis showed ~99.5% homology with the respective isolates. Some of the interesting isolates include *Bacillus pumilus*, *Bacillus licheniformis*, *Nocardia* spp. and *Moraxella ovis*.

For development of DNA and clone repository, good quality genomic DNA was purified from 138 bacteria of different genus and the DNA was stored in TE buffer and as ethanol precipitate at -80oC in the repository. Multiple copies of 76 recombinant clones of specific genes of various bacterial and viral isolates have been generated and are being maintained in the VTC repository. Further, ORF library has been developed by generating 13 entry clones of ORFs of buffalopox virus (12 ORFs) and equine influenza virus (1 ORF) in Gateway vector pDONR221. The targeted ORFs were 12 virulence associated genes viz., C3L, crmB, B28R, IL-18, C7L, ZFA, E3L, CBP, K1L, K3L, N1L, B29R of buffalopox virus isolate and nucleoprotein gene of equine influenza virus isolate. All entry clones preserved and accessioned in the repository.

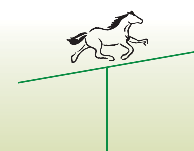


कार्यकारी सारांश

राष्ट्रीय अश्व अनुसंधान केन्द्र की स्थापना अश्व-प्रजाति के उत्तम स्वास्थ्य एवं उत्पादन के उद्देश्य से 26 नवम्बर, 1985 में हिसार में की गई थी, तत्पश्चात् वर्ष 1989 में अश्व उत्पादन परिसर की स्थापना बीकानेर में की गई। पिछली शताब्दी में जन्मे इस केन्द्र ने 2009-10 में अपने जीवन के 25 वसन्त पूरे किए और अब यह द्रुत गति से अश्व स्वास्थ्य एवं प्रजनन के कार्य में निरन्तर अग्रसर हो अनुसंधान में नित नए आयाम स्थापित कर रहा है। अश्व-प्रजाति के पशुओं के समग्र विकास, अश्व व्याधि के निदान कार्य, सलाह और परामर्श सेवाएं प्रदान कर भूमिहीन एवम् आर्थिक रूप से पिछड़े अश्व-पालकों के जीवन में यह केन्द्र अमूल्य परिवर्तन कर रहा है। राष्ट्रीय अश्व अनुसंधान केन्द्र के वैज्ञानिक अश्व रोगों की निगरानी के साथ-साथ प्रमुख रोगों के निदान, परीक्षण चिकित्सा और जैविकी विकास के क्षेत्र में अपना ध्यान लगा रहे हैं। इसके अलावा केन्द्र अश्व-उत्पादन, इस्तेमाल, एवम् भारतीय अश्व-नस्लों के जैविक-संरक्षण में भी कार्योन्मुख है। हमने अपने जीवाणु आनुवांशिक संग्रहण की चाल में चपलता से अग्रसर हैं और इसमें हमने पशु-चिकित्सा, रोमन्थी एवम् डेयरी जीवाणु एवम् विषाणुओं का संग्रहण किया है और नवीन महत्वपूर्ण जीवाणुओं का वियुक्तिकरण कर उन्हें अपने संग्रह में एकत्रित किया है।

सन् 2012-13 के वर्ष में हम पहले की तरह जोश एवम् उत्साह से अपना कार्य करते रहे हैं। विगत वर्ष 2008-09 में हुए अश्व-फ्लू प्रकोप के कारण हम आगामी भविष्यकाल में इस रोग के संभावित प्रकोप की आशंका के चलते इसके रोग-निदान एवम् रोकथाम के ऊपर अनुसंधान कर रहे हैं। हमने अश्व-फ्लू विषाणुओं के आनुवंशिक एवं अंतर्जनिक पृथक्कीकरण पर कार्य करते हुए राइबोन्यूक्लिक अम्ल पालीमरेस जीन्स का विश्लेषण किया है। भारतीय विषाणुओं के पालीमरेस जीन्स अनुक्रम चीनी एवं मंगोलियाई पालीमरेस जीन्स के अनुक्रमों से 98-99.5 प्रतिशत मेल खाते हैं। इसके अलावा, अश्व-फ्लू के विरुद्ध तैयार की गई चार मोनोक्लोनल प्रतिपिण्डों की विनिदर्शिता को नापने के उद्देश्य से उनका इम्यूनोपरआक्सीडेस परीक्षण किया गया। चारों मोनोक्लोनल्स ने अश्व-फ्लू संक्रमित एम.डी.सी.के. कोशिकाओं के साथ सकारात्मक परिणाम दिये। दो मोनोक्लोनल एण्टीबाडीज के द्वारा हमने 2008-09 रोग-प्रकोप के समय पृथक किए गए पाँच एवम् 1987 के एक विषाणु का हीमएग्लुटिनिन अतजनिक परीक्षण एच.आई. द्वारा किया। यह पाया गया कि 1जी4 मोनोक्लोनल द्वारा चार अश्व-फ्लू विषाणुओं पर केन्द्रित एपीटोप पहचाना गया परन्तु लुधियाना - 87 एवम् अहमदाबाद - 09 विषाणु एपीटोप का मोनोक्लोनल से मेल नहीं हुआ। हालांकि मोनोक्लोनल 5ए7 द्वारा सभी 6 अश्व-फ्लू विषाणुओं की पहचान सुस्पष्ट रूप से हो गई। लुधियाना - 87 विषाणु को तो पहले ही (2008-09 प्रकोप से) विभिन्न घोषित किया जा चुका है, परन्तु अहमदाबाद-09 विषाणु की विभिन्नता का विश्लेषण करना शोध का विषय है। इन मोनोक्लोनल्स का प्रयोग सैंडविच एलीसा तकनीक द्वारा रोग-निदान के रूप में किया जाएगा। इस परीक्षण का मानकीकरण भाग-2 क्लेड विषाणु के इस्तेमाल से हुआ है जो कि अति संवेदी है। मोनोक्लोनलज़ द्वारा सभी वंश के विषाणुओं की सही पहचान इसकी विस्तृत प्रामाणिकता दर्शाती है। इसके अतिरिक्त विभिन्न विषाणुओं से संक्रमित नासिका नमूनों के परीक्षण पर भी प्रभावी परिणाम मिले हैं। आण्विक परीक्षणों के लिए आधुनिक उपकरणों की जरूरत पड़ती है परन्तु यह परीक्षण अंतजन की प्रामाणिक जांच परिणाम के लिए जरूरी है। एक न्यूक्लियोप्रोटीन पर केन्द्रित टाक्मैन प्रोब आधारित आर.टी.पी. सी.आर. परीक्षण सही परिणाम दे रहा है परन्तु इसका मानकीकरण कार्य चल रहा है।

अश्व-फ्लू विषाणु एवम् रोग के टीके एवं रोगजनन के अध्ययन हेतु मूषक-माडल का प्रयोग कटरा - 09 विषाणु के साथ किया गया। चूहों में कोई रोग-लक्षण नहीं प्रकट हुए, अपितु 1-2 दिन पश्चात् बालों में हल्का रूखापन देखा गया। नियंत्रित मूषक समूह



की तुलना में संक्रमित चूहों का शारीरिक भार भी कम नहीं हुआ पर उनके फेफड़ों में रोग-घाव देखे गए। उत्तक परीक्षण पर श्वास नली एवम् फेफड़ों में 2 दिन पश्चात् रोग के लक्षण देखे गए जो संक्रमण के 3 दिन पश्चात् अधिकतम थे। नासिका, श्वास नली और फेफड़ों में विषाणु-अंतजन का इम्यूनोपरीक्षण द्वारा पता चला। संक्रमण-पश्चात् 5 एवं 3 दिन में फेफड़ों एवम् नासिका नमूने में आर.टी.पी.सी.आर. द्वारा रोग-अंतिजन परिलक्षित कर लिया गया। यह परीक्षण मूषक-माडल में विषाणु का बढ़ना दर्शाता है और इस मूषक-माडल के बन जाने से हम अश्वों में विरोध-परीक्षण करने से पहले टीका एवम् अन्य परीक्षण कर पाएंगे और जैव सुरक्षा नियमों का निस्तारण कर पाएंगे।

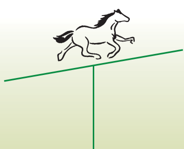
अश्व-संक्रमित रक्त-अल्पमतता (ई.आई.ऐ.) के निदान के लिए एलीसा परीक्षण में प्रयुक्त पी26 प्रोटीन का उष्णता-स्थायित्व परीक्षण सघन-जल द्वारा किया गया है। यह देखा गया है कि सघन-जल के अणुओं के घनत्व के कारण पोलियो विषाणु एवम् लेक्टेट डिहाइड्रोजिनेस किण्वक को उष्णता-स्थायित्व प्राप्त हुआ है। अलग-अलग तापमान (37 डिग्री से. से 42 डिग्री से.) एवम् सघन-जल की विभिन्न मात्राओं के परीक्षण से परिणाम स्वरूप 80% सघन जल का उच्च तापमान पर उष्णता-स्थायित्व प्रभाव देखा गया, जिसका इस्तेमाल प्रशीतलन बगैर ही एलीसा पट्टियों के परिवहन में किया जा सकता है।

देश के विभिन्न राज्यों से प्राप्त सीरम-नमूनों का परीक्षण किया गया। इस दौरान 7462 सीरम नमूनों का ई.आई.ए. परीक्षण किया गया जिसमें एक नमूने ने सकारात्मक परिणाम दिया। हरियाणा से प्राप्त इस अश्व का नीति-निर्धारित तरीके से विलोपन कर दिया गया। 3296 सीरम नमूनों में से अश्व-फ्लू के 21 नमूने सही पाए गए परन्तु कोई भी जोड़ा-सीरम-नमूने का अनुमापांक बढ़ता नहीं मिला। ई.वी.ए. का कोई नमूना सही नहीं मिला। एक रोग-प्रकोप से प्राप्त दो नमूनों से ई.एच.वी.-1 मिला है। जापानी मस्तिष्क ज्वर के परीक्षण में 1153 अश्व नमूनों में से 45 (3.9%) सही पाए गए एवम् गुजरात और हरियाणा में अधिकतम नमूने इस रोग के सही पाए गए। जम्मू-कश्मीर से कोई नमूना जापानी ज्वर के लिए सही नहीं मिला। ब्रुसैल्ला और साल्मोनैल्ला परीक्षण 1428 सीरम नमूनों पर किया गया और किसी भी नमूने से रोग परिलक्षित नहीं हुआ।

ग्लैण्डर रोग के 7601 सीरम नमूने जाँचे गए और उत्तर-प्रदेश से 7 नमूने रोग के लिए सकारात्मक प्राप्त हुए। यह नमूने 2013 फरवरी-मार्च में औरैया, हरदोई एवम् गंजडुड़वाड़ा से प्राप्त हुए। इनमें श्वास-प्रणाली एवम् त्वचा लक्षण देखे गए। ओरैया में केवल श्वास के लक्षण देखे गए। अश्व-पालकों का मुआवजा, रोग अनभिज्ञता, रोग सूचना ना पहुंचाना, एंटिबायटिक का प्रयोग और रोग का पुनरागमन इसके नियंत्रण में मुख्य बाधाएं हैं। एक सौ बीस नमूनों का रोगाणु-परीक्षण किया गया जिनमें नासिका, योनि, गर्भाशय, घाव, पोस्टमार्टम आदि के नमूने थे और राजस्थान, हरियाणा, उत्तर-प्रदेश और उत्तराखण्ड से नमूनों से 48 रोगाणु पृथक किए गए जिनमें स्टैप्टोकाक्कस, स्टैफाईलोकाक्स, ई. कोलाई, नोकाड्रिया और माइक्रोकाक्काई प्रमुख थे। पोस्टमार्टम द्वारा रोग-अन्वेषण और पशु-कोशिका परीक्षण से जिगर का सिरोसिस, आंतड़ियों के मुड़ने से ज़हर बनना, फेफड़ों का नोकाड्रिया संक्रमण, ट्रिपैनोसोमोसिस आदि रोगों का निदान किया गया। परजीवी परीक्षण में 1482 सीरम नमूनों को बबीसिया इक्वाई एलीसा द्वारा 34.6% सकारात्मक परिणाम पाया गया जबकि ट्रिपैनोसोमो इवैन्साई के 482 में से 75 नमूने सही मिले।

अश्व-प्रजननकर्ताओं और अश्व-दौड़ अश्वों के नमूनों की जांच एक कान्ट्रैक्ट-सेवा के अन्तर्गत की जाती है। इस सेवा से इस वर्ष हमारे केन्द्र को करीब छयालिस लाख बहत्तर हजार रुपए की वित्तीय प्राप्ति हुई जिसमें केन्द्र ने विभिन्न रोगों के लिए नमूनों का परीक्षण किया। एम.एक्स. जीन आर्थोमिक्सो विषाणु के विरुद्ध प्रतिरोध दर्शाती है और इसका अश्वों में परीक्षण और सत्यता प्रमाणित नहीं है। इसलिए उत्तेजित और अनउत्तेजित मोनोसाईट रूधिर कोशिकाओं को मारवाड़ी अश्वों से प्राप्त करके इन्हें क्यू. आर.टी.पी.सी.आर. द्वारा जांचा गया। परन्तु एम.एक्स. जीन की अभिव्यक्ति का सही प्रारुप प्राप्त नहीं हुआ।

चूंकि पाईरोपलास्मोसिस और ट्रिपैनोसोमोसिस परजीवी साधारणतया अश्व-स्वास्थ्य का बहुत नुकसान करते हैं इसलिए इन पर शोध के अन्तर्गत टट्टुओं पर टी.इवैन्साई का प्रायोगिक संक्रमण परीक्षण किया गया। संक्रमण के 56 दिन बाद सभी पशुओं में रोग लक्षण देखे गए। रोग 5-7 दिन में शुरू हुआ। दो पशुओं में रोग के असली अभिव्यक्त पशु-छाती पर पानी इकट्ठा



होने के लक्षण देखे गए। पशुओं का लड़खड़ा कर चलना और पृष्ठ भाग का लड़खड़ाना देखा गया। उपचार पर सभी पशु ठीक हो गए पर 6 महीने पर एक पशु में स्नायुतिक लक्षण मिले। परजीवी के डब्ल्यू. सी.एल. और अर्द्ध–परिशोधित अंतजन का इन टट्टुओं के सीरम नमूने से परीक्षण किया गया। दोनों अंतजन संक्रमण के 10–14 दिन पश्चात् रोग निदान कर लेते हैं और एण्टीबाडीज़ का बढ़ना 56 दिन तक दर्शाते हैं। इम्यूनोब्लाटिंग द्वारा 55 के.डी.ए. क्षेत्र में पालीपैप्टाईड का मिलना और डिप–स्टिक एलीसा तकनीक द्वारा 14 दिन डी.पी. आई. सकारात्मकता इन तकनीकों की रोग–निदान प्रयोगरूपता दर्शाती है।

केन्द्र की एक टीम मैसूर और बैंगलोर में अश्व–स्नायिक विकार और मृत्यु की जांच के लिए गई और इस रोग का निदान टी. इवैन्साई संक्रमण के रूप में हुआ। रोग के लक्षणों, इतिहास, परीक्षण–रिपोर्ट और कोशिका परीक्षण के पश्चात् रोग का पक्का निदान इसी रोग के रूप में हुआ। अलग–अलग आण्विक रूधिर, प्रोटीन पहचान, इम्यूयानोब्लाट परीक्षण पश्चात् वी.एस.जी. ग्लाइकोप्रोटीन की पहचान हो गई। दूसरे रोगों के कारक जैसे जापानी मस्तिष्क ज्वर, पश्चिमी नील, इ.एच.वी. 1 की पुष्टि नहीं हुई।

उत्पादन के क्षेत्र में हम स्थानीय गदर्भों के विश्लेषण कार्य में रत हैं। यह प्राणी गरीब अश्व–पालक की रोज़ी–रोटी का साधन है। शरीरिक अवयवों का माप–तोल दो विभिन्न स्थानों जैसे लेह, लद्दाख और बारामती के गदर्भों का किया गया। लेह से 35 गदर्भों का 15 शारीरिक मानकों का और बारामती क्षेत्र से 59 गदर्भों का शारीरिक मानकीकरण किया गया। बारामती के गदर्भ अधिकतर भूरे रंग के थे, हल्के और गहरे भूरे, और पीठ पर काली पट्टी भी देखी गई जो कि सब में नहीं थी। बड़े सफेद प्रकार के गदर्भ गुजरात में मिलते हैं जबकि लेह में भूरे काले रंग के गदर्भ मिले। भारतीय गदर्भ पोइटू से छोटे ही देखे गए हैं। इसके अलावा स्थानीय भारतीय गदर्भों में देखा गया कि बारामती से प्राप्त पशु लेह के गदर्भों से अधिक ऊंचे और बड़े थे।

गदर्भों और टट्टुओं के प्रमुख–हिस्टोकमपैटीबिलिटी काम्पलैक्स अव्यवों का पी.सी.आर., आर.एफ.एल.पी. द्वारा विश्लेषण किया गया। प्रतिबंध किण्वकों द्वारा विश्लेषण इनमें बहुरूपिता दर्शाता है।

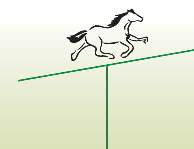
अश्व कोरियानिक गौनेडोट्रापिन हार्मोन का अश्व गर्भावस्था परीक्षण में उपयोग के लिए पुन: संयोजक प्रोटीन उत्पादन के लिए इसके बीटा–सबयूनिट की क्लोनिंग और अभिव्यक्तिकरण किया गया। एस.डी.एस. पेज में पुन: संयोजक प्रोटीन 30 के.डी. के का देखा गया और इस पर आगे का कार्य जारी है।

मारवाड़ी घोड़ियों में गर्भावस्था एन्डोक्राईन, बायोकेमिकल और जीन अभिव्यक्ति के शोध का कार्य शुरू किया गया ताकि घोड़ियों की प्रजनन दक्षता बढ़ाई जा सके। व्यस्क मादा और बछेड़ी घोड़ियों को इसके लिए चुना गया। देखा गया कि अड़ उत्सर्जन से पूर्व का झिल्ली–कूप 55 एमएम आकार तक बढ़ जाता है और 35 एमएम के कूप के आकार पर ही घोड़ी द्वारा गर्म होने के व्यवहारिक लक्षण प्रकट होने लगते हैं।

अश्व–वीर्य के विश्लेषण कार्य के अन्तर्गत देखा गया कि एमाइड के प्रयोग से वीर्य–गीतशीलता और जीवन–क्षमता पर कोई खास असर नहीं पड़ता है। यही बात ग्लिस्रौल, और डाईमियाईल सल्फौक्साइड के हिमीकृत संग्रहण के लिए प्रयोग पर भी देखी गई। नर–गदर्भों के ताजे और शीतल वीर्य का जीवाणु मात्रा अध्ययन भी किया गया। जीवाणुओं की पहचान करने पर कोराईनीबैक्टीरियम नामक जीवाणु अधिकतम पाया गया जो कि दर्शाता है कि सम्भवत: वीर्य का संदूषण वातावरण से प्राप्त हुआ है।

अश्व–मल/गोबर आदि के सुरक्षित, प्रभावशाली, उपयोगी और अभिनव तरीके से इस्तेमाल के लिए हमने वर्मीकमपोस्टिंग तरीके का प्रभावी इस्तेमाल किया है। वर्ष में 35 कुंतल वर्मीकम्पोस्ट तैयार की गई। इसका इस्तेमाल हिसार एवम् बीकानेर के चारा उत्पादन कृषि में किया गया। अश्व–मल का साधारण कम्पोस्ट खाद बनाने में भी उपयोग किया गया। साधारण खाद 4 महीने में तैयार हो जाती है। अश्व–गोबर का इस्तेमाल अश्व पालने वालों की आय के स्रोत में करने के लिए एक प्रायोगिक परीक्षण इसका खुम्बी उत्पादन में भी किया गया।

शुष्क चारे/फसल अवशेषों के पोषक तत्वों को बढ़ाने हेतु अश्वों के लिए साइलेज उत्पादन का परीक्षण भी किया गया। कच्चे प्रोटीन, ऊर्जा, पोष्टिकता और स्वाद आदि बढ़ाने के लिए शुष्क चारे आदि की गुड़ के शीरे से मिलाई के पश्चात् इसको गढ्ढों में



भरकर तैयार किया जाता है। अश्वों के संतुलित चारे की कमी को पूरा करने के लिए गुड़ के शीरे के ब्लाक, खनिज-ब्लाक अथवा खनिज-मिश्रण आदि का परीक्षण किया गया। राजस्थान जैसे शुष्क स्थल पर सूखे चारे का ग्याभिन घोड़ियों के चारा पाचन और शारीरिक परफार्मैंस पर असर का परीक्षण 45 दिन तक किया गया जिसमें उन्हें सेवन घास कुत्तर और पूर्व-मिश्रित कन्सन्ट्रेट दिया गया था। इनसे उनके शारीरिक भार में बढ़ोतरी दर्ज की गई।

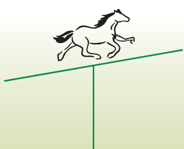
खच्चरों और गधों की मौजूदा पालन प्रबन्धन प्रणाली और उनके उपयोग संबन्धी जानकारी का प्रयोग इनके पालनकर्ताओं की जीविकोपार्जन पर पड़ता है। अत: इसका अध्ययन किया जा रहा है। हरियाणा और राजस्थान में किए गए सर्वेक्षण के अनुसार अधिकतर पालक इनका उपयोग गाड़ी खींचने में करते हैं और 31.4% इनका उपयोग बोझा ढोने में करते हैं। इन पालकों की मासिक आय 4000/- से 9000/- रुपये आंकी गई है। इनकी अधिकतर मुश्किलें चारे की महंगी दर, मशीनी वाहनों से प्रतिस्पर्धा, चारागाहों का ना होना, अच्छे नर गधों की कमी, बैंक लोन, बीमा आदि का अभाव है।

यह बहुत गौरव और संतुष्टि की बात है कि हमारे सतत प्रयासों के फलस्वरूप हमारे केन्द्र को इन वर्षों में बाहरी वित्तीय-पोषित अनुसंधान परियोजनाएं प्राप्त हुई हैं। अश्वों के साइटोकाईन अभिव्यक्ति और इनके जैविक-विश्लेषण की शोध परियोजना में अश्व के आई. एल. 2, 4, 10 और 8 का अनुक्रमिकरण और वंशावली विश्लेषण किया गया है। इससे यह ज्ञात हुआ कि अश्व साईटाकाईन सूकर और उष्ट्र साईटोकाईन के ज्यादा करीब है। एक अलग, ग्लैण्डर के द्रुतगति आण्विक निदान के शोधकार्य में बरखोलडेरिया मैलियाई के 2 प्रोटीनों की क्लोनिंग, अभिव्यक्तिकरण एवं अनुक्रमीकरण किया गया जिनका उपयोग एलीसा पद्धति द्वारा निदान में किया गया। इसके द्वारा 1811 सीरम नमूनों की जाँच की गई। यह सी.एफ.टी. से कई मानकों से इसका तुलनात्मक अध्ययन हुआ। इसका मनुष्य के मैलीडियोसिस सीरम नमूने से प्रतिक्रियाशीलता का अध्ययन भी किया गया।

फ्रांस के पैरिस शहर में स्थित ओ.आई.ई. पूरे संसार के लिए पशुओं में फैलने वाले रोगों और प्रकोप की रोकथाम और नियंत्रण पर नीति निर्धारित करते हैं। केन्द्र ने ओ.आई.ई. से अश्व पाइरोप्लासमोसिस, ग्लैण्डर और अश्व फ्लू पर प्रयोगशाला जुड़ाव के अंतर्गत वित्तीय-पोषित परियोजनाएं प्राप्त की हैं जिसमें जापान, जर्मनी और ब्रिटेन की प्रयोगशालाओं के साथ केन्द्र की तीन प्रयोगशालाएं जुड़ेंगी। अश्व पाइरोप्लासमोसिस पर बाहरी एम.ए.एस.पी. संवर्द्धन प्रणाली का प्रयोग करके इसे थिलेरिया इक्वाई परजीवी को प्रयोगशाला में रखा जा रहा है। अलग-अलग स्रोतों से प्राप्त रूधिर नमूनों का उपयोग परजीवी का पता लगाने में किया गया। एलीसा और पी.सी.आर. का प्रयोग भी किया गया। ई.एम.ए.-1 जीन और 18 एस जीन का अनुक्रमीकरण कर इसका आनुवांशिक अध्ययन भी किया गया। 18 एस जीन की अपेक्षा इ. एम.ऐ.-1 जीन में अधिक विविधता पाई गई। भारतीय टी. इक्वाई परजीवी अफ्रीकी और टैक्सास समूह के साथ मेल खाते हैं। अश्व-फ्लू के प्रयोगशाला जोड़ का कार्यक्रम अक्तूबर 2012 में शुरू हुआ है और हमारे और ब्रितानी वैज्ञानिकों के बीच इंटरनेट के माध्यम से वार्तालाप हुआ है।

अश्व के उल्विक-द्रव्य से मिसनकाईमल स्टैम कोशिकाओं के पृथक्कीकरण और विश्लेषण का शोधकार्य अश्व जाति पशु की चोट की चिकित्सा के लिए उपयोगी होगा। इसके लिए अश्व-शावक के जन्म के समय उल्विक-द्रव्य का संग्रह किया गया और उनसे मिसनकाईमल कोशिकाएं निकाली गई।

पशुओं में रोग न करने वाले एडीनो विषाणुओं के पृथक्कीकरण का शोध कार्य भैंस और अश्व में किया जा रहा है। इस दौरान 2 भैंसों से और एक अश्व से मिले एडीनो विषाणु का चूहों में रोग उत्पन्न करने का अध्ययन किया गया। यह रोग करते नहीं पाए गए। इसके अलावा अश्वों के 7 और गायों के 5 विषाणुओं को भी परिशुद्धित किया गया जिसका आण्विक विश्लेषण जारी है। एक अलग शोध परियोजना में अश्वों के ट्रिपैनोसोमा इवैन्साइ परजीवी से इस रोग निदान के लिए प्रोटिओमिक्स द्वारा जैवचिह्नित प्रोटीन का पता लाया जा रहा है। इस कार्य के अन्तर्गत प्रोटीन के तीन रोग प्रतिरोधी क्षमता के समूहों की पहचान की गई है। इम्यूनोब्लॉट एवं एस.डी.एस. पेज तकनीक द्वारा इन प्रोटीन समूहों को पहचाना गया है। सामूहिक स्पैक्ट्रोस्कोपी द्वारा एक समूह के विश्लेषण से पांच प्रोटीनों का पता चला है जिसमें से एक उष्ण-शाक प्रोटीन 70 जीन थी जिसकी क्लोनिंग के पश्चात् उसे पुन: बन्धित प्रोटीन के रूप में अभिव्यक्त करा लिया गया है। जिसका रोग प्रतिरोधी नैदानिक कार्य के लिए प्रचुर मात्रा में उत्पादन किया गया।

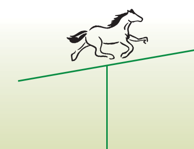


नैनो तकनीक के शोधकार्य के अन्तर्गत डी.एस.टी. की एक परियोजना स्वीकृत हुई जिसमें पशु माडल में ट्रिपैनोसोमा परजीवी की चिकित्सा के लिए दवायुक्त नैनोयुक्तिकरण कणों का निर्माण, विश्लेषण एवं मूल्यांकन किया जा रहा है। सोडियम अलजिनेट के नैनोकणों में क्विनपाइरामिन सल्फेट दवा की युक्ति की गई, ऐसी दवा प्राणी के लिए कम विषाक्त होगी और यह कम मात्रा में भी परजीवी को शरीर के भीतर और बाहर मार पाएगी। राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार को जैव प्रोद्योगिकी विभाग, भारत सरकार के सौजन्य से जैक्सूचना विषय पर बी.टी.आइ.एस. नैट का जीव विज्ञान का जैव सूचना माध्यम से अध्यापन पर भी एक प्रयोगशाला निर्माण पर धन प्राप्त हुआ है। इसमें द्रुत गति इंटरनेट पर जैव सूचना विषय से अध्यापन, प्रशिक्षण और शोध कार्य किया जा सकेगा।

भारतीय कृषि अनुसंधान परिषद् एवं दवा कम्पनी फाईज़र के करार के अंतर्गत गाय-भैंस के बछड़ों में जीवाणु (ई कोलाई एवं सालमोनैल्ला) और विषाणु (रोटा एवं कोरोना विषाणु) द्वारा दस्त की बिमारी के कारकों के रूप में प्रसार एवं पृथक्कीकरण पर शोध कार्य पूर्ण किया गया। 18.35% नमूनों में जी6 रोटा विषाणु का प्रकोप मिला, और फिर जी10 प्रकार का। आर.टी.पी.सी.आर. द्वारा गौवंशी कोरोना की पुष्टि हुई परन्तु एच.आर.टी. 18 कोशिकाओं के अभाव में इस विषाणु का पृथक्कीकरण नहीं किया जा सका। ई कोलाई 64.3% नमूनों में मिला परन्तु इनमें से 3 ही के-99 युक्त थे, अतः रोटा, कोरोना और ई कोलाई संक्रमण गौवंशी बछड़ों में विद्यमान हैं।

एक अखिल भारतीय समन्वयन शोध परियोजना के अन्तर्गत खच्चरों का जुलाई माह में जुताई के कार्य क्षमता के लिए कार्य विश्राम विधि द्वारा मूल्यांकन किया गया। दो कुंडी के हलों पर खच्चरों को जोता गया और हरेक 1.5 घंटे के कार्य पश्चात् आधे घंटे का विश्राम खच्चरों को दिया गया। कार्य के पश्चात् खच्चरों के शारीरिक अवयवों/मापदण्डों में चढ़ाव दर्जा हुआ जो कि कार्य समाप्त होने के पश्चात् भी ऊँचाई पर रहा। कार्य पश्चात् रूधिर-शर्करा एवं अन्य कण्विकों में महत्वपूर्ण बदलाव नहीं देखा गया। कार्य के एक घंटे के बाद भी शारीरिक मापदण्ड साधारण स्तर पर नहीं पहुँचा जो कि अगली सुबह जाकर साधारण स्तर पर पहुंचा। खच्चरों की शक्ति का हरी बाजरा की कटाई में भी रोटरी-गियर प्रणाली द्वारा प्रयोग किया गया। इसमें एक स्थानीय 350 किलो शरीर-भार के खच्चर को लगाया गया। इस कार्य से खच्चर के शारीरिक अवयवों मापदण्डों में महत्वपूर्ण बदलाव दर्ज हुआ पर खच्चर में शारीरिक तनाव नहीं देखा गया।

वेटेनरी टाईप कल्चर्स कलैक्शन (वी.टी.सी.सी.) को लम्बे समय तक के लिए जीवाणु परिरक्षण एवं वितरण का जनादेश प्राप्त है। सन् 2012-13 में वी.टी.सी.सी. द्वारा कल्चर परिरक्षण कार्य में अभूतपूर्व वृद्धि दर्ज की। कई प्रकार के महत्वपूर्ण विषाणु, जीवाणु, पुनःबन्धित क्लोन और फाज कोषालय का विश्लेषण एवं संवर्धन किया गया। इस वर्ष के दौरान जीवाणुकोष में 21 विषाणु, 187 रोगाणु, 45 रोमन्थी जीवाणु, करीब 100 डेयरी जीवाणुओं, 76 पुनःबन्धित क्लोन एवं 138 जीवाणु गुणसूत्रसार का विभिन्न पशुओं से प्राप्ति पश्चात् संवर्धन किया गया। इस प्रकार जीवाणुकोष में अब 1630 कल्चर संवर्धित हैं। पिछले 543 कल्चर्स की अपेक्षा इस वर्ष हमने 751 कल्चर्स का संवर्धन किया है। अब यहां कुल 124 विषाणु, 627 जीवाणु, 267 पुनःबन्धित क्लोन और 27 फाज कोष संवर्धित हैं। वी.टी.सी.सी. में परिग्रहित विषाणु कल्चर्स में भैंस चेचक, उष्ट्र चेचक, बकरी चेचक, भेड़ चेचक, कुक्कर पार्वो, कुक्कर एडीनो, मुर्गी एडीनो एवं जंगली रैबीज़ प्रमुख हैं। मुख्य जीवाणुओं में बौरडिटैल्ला, एक्टीनोबैसिलस, बुसैल्ला, स्टैफाइलोकाक्कस, सुडोमोनास, कोराइनीबैक्टिरियम, रहोडोकाक्कस, कैम्पाइलोबैक्टर, सिट्रोबैक्टर, लिस्टीरिया, मौराक्सैल्ला, पास्चुरैल्ला, नोकार्डिया, साल्मोनैल्ला आदि विद्यमान है। वी.टी.सी.सी. में 11 कोशिका समूहों का भी विषाणु पृथक्कीकरण कार्य के लिए संवर्धन किया गया है। रोमन्थी जीवाणुओं के शोध और विकास की दिशा में बढ़ते हुए 140 रोमन्थी जीवाणुओं का पृथक्कीकरण, विश्लेषण, परिग्रहण और संवर्धन किया गया है। भैंस के रूमन की फंफूद जैसे अवेरोमाइसिस, औरपिनोमाइसिस, बकरी से पाइरोमाइसेस, नियोकैलीमास्टिक्स को अलग और संवर्धित किया गया है। गौवंश, बकरी और भैंस रूमन से बैसिलस, ब्यूटाइरीविब्रियो, यूबैक्टिरियम, मैगास्फिरा, प्रैवोटैल्ला, स्ट्रैप्टोकाक्कस और वैलोनैल्ला प्राप्त कर जीवाणुकोष में संवर्धित किए गए हैं। उष्ट्र से भी तीन रूमन जीवाणु मिले हैं। इन जीवाणुओं का विभिन्न जैविक विधियों में जैसे तन्तु और प्रोटीन अवक्रमण, यूरिया

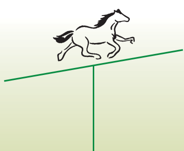


जलापघटिकर्ण, टैनिन अवक्रमण और बैक्टीरियोसिन उत्पादन में प्रयोग किया गया। इस दौरान 89 डेयरी जीवाणुओं भी प्राप्त और विश्लेषित किए गए। वर्तमान में कुल 490 डेयरी कल्चर्स जीवाणु कोष में शीत परिग्रहित हैं जैसे लैक्टोकाक्कस, ल्यूकोनास्टाक, स्ट्रैप्टोकाक्कस, लैक्टोबैसिलस, बाइफिडोबैक्टिरियम, माइक्रोकाक्कस, क्लूव्यरोमाइसिस और सैक्हैरोमाइसिस। केन्द्र में कल्चर कोष मूल्यावृद्धि हेतु हमने पास्चुरैल्ला मल्टोसिडा बी.2 भैंस रोगाणु का पूर्ण गुणसूत्र अनुक्रमिकरण किया है और साथ-साथ मुर्गी पीलिया रोग का रोगाणु साल्मोनैल्ला गैलीनेरम एवं एक अश्व श्वास रोग रोगाणु बौरडिटैल्ला ब्राँकीसैप्टिका का भी पूर्ण गुणसूत्र अनुक्रमिकरण कर लिया है।

वी.टी.सी.सी. जीवाणु कोष में हमने 4 अश्व रोटा विषाणु डाले हैं जिनको टोहाना, हिसार के एक अश्व फार्म में अश्व-शावकों के दस्त से पृथक किया गया था। भेड़ अण्ड-कोष कोशिका में एक भेड़-चेचक विषाणु भी पृथक किया गया। क्लासिकल सूकर ज्वर के 26 नमूनों को दिल्ली और हरियाणा से एकत्रित करने के पश्चात् पी.सी.आर. द्वारा जांचा गया जिसमें 15 नमूने सकारात्मक नतीजे वाले प्राप्त हुये। भैंस चेचक विषाणु और किन-किन पशुओं में संक्रमण में सक्षम है। इस आशय पर शोध के लिए इनमें विद्यमान सरपिन जीन का विस्तृत अध्ययन किया गया है। इनसे पता चला है कि सभी भैंस चेचक विषाणुओं में आपस में और वैक्सीनिया विषाणु के साथ भी समीप का मेल है। इसके अलावा दिल्ली, बीकानेर, बाड़मेर और जैसलमेर के ऊँटों से पृथक किए गए चेचक विषाणुओं के 6 होस्ट-रेंज जीनों का अनुक्रमिकरण करने पर ज्ञात हुआ कि यह विषाणु अमीनो अम्ल के स्तर पर आपस में और वैरिमोला विषाणुओं के वंश समीप हैं। दिल्ली के एक उष्ट्र चेचक विषाणु में महत्वपूर्ण बिन्दु उत्परिवर्तन देखा गया।

बीकानेर से गदर्भो, भेड़ों और बकरियों से 60 नमूनों की पी.पी.आर. रोग की जांच में गदर्भो में नकारात्मक और भेड़-बकरियों में सकारात्मक नतीजे मिले। इनका सैंडविच एलीसा और आर.टी. पी.सी.आर. परीक्षण किया गया।

कई बायोकैमिकल एवं 16 एस राइबोज़ोम जीन पी.सी.आर. अनुक्रमिकरण द्वारा 4 नए जीवाणु का पता लगाया गया जिनको बिमार पशुओं से पृथक किया गया था। इनमें से मुख्य हैं- अश्व-फेफड़ा न्यूमोनिया से नोकारडिया औटाइटिडिस्कावैरियम, जम्मू-कश्मीर के एक भेड़ रेवड़ की भेड़ों की आंखों से मौरैक्सैल्ला ओविस, पुष्कर राजस्थान मेले से एक न्यूमोनिया ग्रसित अश्व-शावक से बौरडिटैल्ला ब्राँकिसैप्टिका और भेड़ के पानी पीने वाले खुए से डैल्फटिया। सब जीवाणुओं का अनुक्रमिकरण और वंशावली अध्ययन द्वारा पक्का पहचान लिया गया है। अमरनाथ यात्रा में प्रयोग होने वाले खच्चरों में काठी के घाव से स्ट्रैप्टोकाक्कस एवं स्टैफाइलोकाक्कस को पृथक किया गया। बिमार चोटग्रस्त पशुओं के घावों को साफ करके उन्हें धोया गया और मरहम पट्टी की गई। बकरी के दूध से पृथक किए गए 3 जीवाणुओं की पहचान स्टैफाइलोकाक्कस स्यूरी प्रकार रौडैन्शियम के रूप में की गई जोकि मैथीसिलिन प्रतिरोधी है और विभिन्न प्रकार के जीवाणुओं की पहचान प्रमाण 16 एस जीन क्लोनिंग, अनुक्रमिकरण और विश्लेषण द्वारा की गई। इंटरनेट जैव सूचना ब्लास्ट कार्यक्रम पर इनकी 99.5% समीपता दिखी। नए जीवाणुओं से कुछ महत्वपूर्ण हैं जैसे बैसिलस प्यूमिलस, बैसिलस लिचैनीफारमिस, नोकार्डिया आदि क्लोन एवं गुणसूत्र कोष तैयार करने के लिए 138 जीवाणुओं से उच्च गुणवत्ता वाला गुणसूत्र डी.एन.ए. परिष्कृत किया गया और उसे टी.ई. बफर में ईथनाल के साथ -80° C पर संरक्षित किया गया। 76 प्रकार के पुन:बन्धित खास जीनों के क्लोन्स को जीवाणु और विषाणुओं स्रोत से बनाया गया है और इन्हें वी.टी.सी.सी. प्रयोगशाला में संरक्षित कर लिया गया है। भैंस-चेचक विषाणु से 13 आगन्तुक क्लोन्स तैयार किए गए जिनका ओ.आर.एफ. कोष विकसित किया जा रहा है। इसी तरह अश्व-फ्लू का भी ओ.आर.एफ. कोष तैयार किया गया। यह ओर.आर.एफ. कोष भैंस-चेचक के 12 रोगकारक जीन्स के हैं और एक क्लोन अश्व-फ्लू के न्यूक्लियोप्रोटीन जीन का भी है। सभी आगन्तुक क्लोन्स को वी.टी.सी.सी. प्रयोगशाला में संरक्षित एवं परिग्रहित कर लिया गया है।



Introduction

The domestication of the horse was the important development in the history of human civilization. The term “horse power” is a reminder of horses' ability to perform hard work, day after day, in myriad of situations. Every great civilization, ancient or modern, was founded from the back of a horse. In ancient times, horse was crucial to warfare and it was the fastest and most reliable form of land transport. Over the years, the equines have played significant role in the rural economy and it also contributes towards urban transport. The equines have been important in ensuring the livelihood of underprivileged people. Even in the era of mechanization, working equines serve as means of transport and source of livelihood for many marginalised farmers in difficult hilly terrains, arid and semi-arid regions and urban and rural communities. In order to improve the health, production potential and conservation of the germplasm of indigenous equine 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, pathology, parasitology, immunology, 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, BSL-3 facility, ARIS cell, ATIC, Library and Info-Equine Museum. The Centre has well maintained pack of Marwari, Kathiwari, Zanskari horses and indigenous and exotic donkeys at Equine Production Campus, Bikaner.

The centre's efforts have been concentrated to understand infectious diseases confronting equines and surveillance and monitoring of equine diseases in the country directed towards improvement in equine health which have been appreciable. The research and scientific achievements of the Centre since its inception- continue to improve health and reduce disease burden in equines in India. The NRCE-with its research activities and scientific research achievements- has always been acknowledged at national and international level. The vision of the Centre is the enhanced utilization of equines for agricultural and transport purpose through equine development programmes in order to elevate socio-economic status of the under-privileged owners while encouraging the Thorough Bred industry and Marwari Horse sector in breeding and export. Veterinary Type Culture Collection was also established in the year 2005 at NRCE for collection and preservation of microbes of animal 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.

Thrust Areas

- Surveillance and monitoring of important equine diseases including emerging and existing diseases with special emphasis on foal mortality and production losses.
- Development of effective, affordable and preferably field-based diagnostics against major equine diseases threatening equine health and production in India.
- Development of effective, affordable and potent immunoprophylactics against important equine infectious diseases threatening equines in India.
- Development of effective plant-based products for management of some economically important equine diseases and to enhance performance in equines.
- To provide diagnostic and consultancy services for beneficiaries particularly equine farmers and breeders.
- Propagation of sustainable and economically viable AI technology for mule production in India using cryopreserved jack semen for use at farmers' door.
- Perfection and propagation of artificial insemination techniques in horse and pony production using frozen semen of true to breed indigenous stallions for the consortium of threatening breeds in India.
- Breed characterization and *in situ* conservation of various indigenous breed of horses.
- Effective Utilization of equine draught power for economically weaker section of the society.
- Explorative research for value addition of equine products and by-products namely blood/serum, dung, urine, milk, placenta and hair.
- Extension activities through information technology and institute development programmes for the upgradation of the indigenous breeds of equids in the different parts of the country in collaboration with the State departments.

MAJOR ACHIEVEMENTS

Diagnostics for equine diseases

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 protein for detection of equine rotavirus (ERV) from stool samples. This assay has been validated by two external laboratories using bovine, sheep and equine rotavirus samples and detects rotavirus infection among different animals. A RT-PCR using VP6 gene primers was also developed and its results were compared with the s-ELISA. The RT-PCR was found to be equally sensitive as s-ELISA.

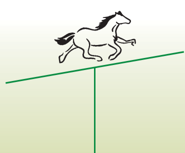
Equine influenza virus (EIV): EIV is routinely diagnosed by haemagglutination inhibition assay. RT-PCR for equine influenza diagnosis and typing has also been developed. Furthermore, real-time RT-PCR based assay targeting M gene has also been developed for diagnosis of EIV. Additionally, development of 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 developed using whole cell lysate antigen of *Trypanosoma evansi*. RoTat 1.2 gene-specific PCR has also been standardized for sensitive detection of surra.

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

Equine infectious anemia: Coggins test for EIA is routinely being used at the Centre. A recombinant protein (26kDa) of a synthetic gene 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 has 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 belonging to Clade 2 of Florida Sublineage was isolated which was different from the 1987 isolates. It was thus imperative that 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/06/08 (H3N8)] responsible for equine influenza outbreaks during 2008-09. The updated vaccine is safe and efficacious as evident by the protective immune response generated by the vaccine in equines in a limited experimental trial as well as in field trials. Further, a new cell culture-based inactivated equine influenza vaccine is being developed by the Centre.

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

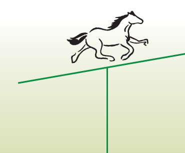
Monoclonal antibodies: Monoclonal antibodies have been developed for diagnosis and characterization of EHV-1, equine rotavirus, equine influenza virus and Japanese encephalitis virus.

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 The World Organization of Animal Health (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 again confirmed from Chandpur area of Bijnor district on the basis of clinical symptoms, agent isolation and identification, PCR and serological tests (CFT and ELISA). In 2012, team of scientists from NRCE investigated the cases with respiratory illness and cutaneous lesions in Bulandshahr, Uttar Pradesh during March, 2012. Four mules in Ahmedpur village of District Bulandshahr and two mules in Shikarpur of the same district were found positive for glanders. 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. In Feb-March, 2013 outbreaks of glanders were once again noticed in U.P. in the areas of Auriaya, Hardoi and Ganjdundwara districts. Both cutaneous and nasal form were observed in Hardoi and Ganjdundwara where as in Auriaya only nasal form was observed.
- NRCE diagnosed equine influenza (EI) in India in July, 2008 from Jammu region that subsequently affected equines in 14 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 July, 2009 when last case confirmed for EI was reported from Uttarakhand.



- NRCE has continuously been screening equines for equine infectious anemia from 1998. One mule has been found seropositive during 2009-10 and in year 2012 one thorough bred horse was found positive in Haryana.

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. Further PA, PB1 & PB2 genes and NS1 & NP have also been sequenced and where found to be clustering with clade 2 EIV isolates.

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

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

In vitro culture of *Trypanosoma evansi*

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

Biological resource Bank

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

- Pathogenic isolates (viruses, bacteria and parasites) of equine origin available with NRCE include EHV-1 (6 isolates), EHV-4 (14), equine rotavirus (29), equine influenza (11), Japanese encephalitis virus (2), West Nile virus (1), *Rhodococcus equi, Streptococcus equi, S. Zooepidemicus, Burkholderia mallei*, *Salmonella Abortusequi*, *S. equisimilis*, *Enterobacter aerogenes*, *E. coli, Staphylococcus aureus*, *Trypanosoma evansi* (3).
- NRCE has a number of hybridomas secreting monoclonal antibodies against equine influenza, EHV-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 equine influenza virus, equine rotavirus, EHV-1, EHV-4, EI, JEV, EIAV, *R. equi*, *Burkholderia mallei*, *Trypanosoma evansi* & *Theileria equi.*, etc.

Indigenous breed characterization

Phenotypic characterization of Indigenous breeds

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

- Marwari and Kathiawari had wither height equivalent to 150 cm or more and as such both these breeds come under the category of horse breeds. It is well established that equids having height less than 150 cm are termed as Ponies. Both mares and stallions of Kathiawari and Marwari breeds were at par as far their height at wither is concerned. Equids of Manipuri (129.04 cm), Spiti (123.54 cm), Zanskari (126.32 cm) and Bhutia (126.94 cm) breeds had their mean wither height < 150 cm and come under the category of Pony

Breed. Spiti stallions (117.80cm) were observed to be significantly (P<0.05) small among both the sexes of all the breeds.

- Equids of Marwari and Spiti breed were observed to be significantly (P>0.05) tallest and smallest, respectively, among all the horse breeds. Almost similar pattern was observed in body length of all these breeds.
- Differences in heart girth among both horse breeds (Kathiawari and Marwari) were also significant (P<0.05). Heart girth in Spiti, Zanskari and Bhutia animals were at par with each other but significantly (P<0.05) higher than Manipuri equines. Canon length of Kathiawari equids was significantly (P>0.05) higher than Marwari, Zanskari and Bhutia breeds. Further, Bhutia ponies had longer canon than Zanskari ponies.
- Ear length and width were recorded to be maximum and significantly (P>0.05) higher in Spiti and Manipuri animals, respectively than all other breeds. Among Kathiawari and Marwari horses, both these indices were significantly (P>0.05) higher in Marwari animals. Ear width of Spiti equines was significantly (P>0.05) lower but with maximum ear length than rest of the breeds.
- Among all the breeds, gap between ears (pole) was significantly (P>0.05) low in the animals of Kathiawari breeds than other breeds. Maximum pole was observed to be in Manipuri breed.
- Among all the six breeds, face of Manipuri ponies was observed to be the broadest. Face length also varied significantly (P>0.05) among all the breeds with minimum in Spiti ponies and maximum value in Kathiawari animals.
- Both fore and hind leg lengths were significantly (P<0.05) higher in Marwari animals with lowest values in Spiti ponies. Like leg length, height at hock and knee height were maximum and significantly higher in Marwari animals than rest of the five breeds. No significant difference, due to sex, was observed in fore and hind leg lengths in mares and stallions of all the breeds.
- Hoof length was observed to be maximum and significantly higher both in Marwari and Bhutia equids, however as far as hoof width is concerned, it was maximum and significantly higher in Marwari animals than rest of the equines breeds.

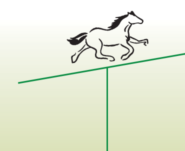
Genotypic characterization of Indian equine breeds

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

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

Establishment of Nucleus 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. The elite herd of these jacks and jennies is still being maintained. Poitu jack semen is most preferred for mule production.
- **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. These ponies are doing well in hot desert conditions as well.
- **Indigenous donkey:** The Centre has initiated the establishment of nucleus heard of small grey and large white donkeys found in India. A total of small grey (14 male) and large white (5 male and 7 female) have been inducted in sanctuary in 2010-11.

Improvement in production potential of equines

- **Semen cryopreservation and Artificial Insemination (AI):** In order to conserve the germplasm of indigenous equine breeds, the technique for cryopreservation of semen of Marwari, Kathiawari stallions and donkeys have been optimized. The technique of AI using frozen semen for production of superior quality Marwari horses, Zanskari ponies, superior mules and donkeys has been perfected. The pure germplasm of endangered indigenous breeds of horses is being conserved using this technology.
- **Early pregnancy diagnosis:** Pregnancy diagnosis between days 14 and 18 post-insemination has been achieved using ultrasonography in donkey and horse mares.
- **Donkey fibre has been used to produce carpets** by mixing with sheep fibres in the ratio of 40:60.
- **Kit for pregnancy diagnosis:** An eCG s-ELISA kit.

International and National Collaborations, linkages, recognitions

OIE Twinning Laboratory Projects (Three)

- Equine Piroplasmosis with National Research Centre on Protozoan Diseases (NRCPD), Obihiro University, Obihiro, Hokkaido Island, Japan (May, 2010 to June, 2013).
- Glanders with Frederick Loeffler Institute (FLI), Jena, Germany (July, 2012 to June, 2015).
- Equine Influenza with Animal Health Trust, UK (October, 2012 to September, 2015).

National Referral Lab status

- NRCE has been recognized by DAHD&F (GoI) as National Referral Laboratory for diagnosis of 10 equine diseases)
- NRCE has also been recognized by Ministry of Health and Family Welfare (GoI) as National Referral Laboratory for diagnosis of Human Glanders

No. of patents filed and granted

Patents Granted (Two)

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

Patents Filed (Three)

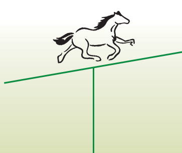
- COFEB Kit for diagnosis of *B. equi* infection (product) - 156/ Del/04 dated 03.02.2004
- A pregnancy diagnostic kit for equine based on detection of eCG by ELISA (Both process & product)- Application No. 15770 dated 16.03.2006
- A highly sensitive kit for detection of antibodies against *Theileria equi* in serum of equids- Application No. 2763/DEL/2012 dated 06.09.2012

Joint Patent Applications Filed (Three)

- A recombinant protein for diagnosis of glanders – Application No.1328/DEL/2010. (DRDE Gwalior and NRCE, Hisar)
- Polynucleotide sequence, composition and methods thereof- Application No. PCT/IB 2011/052475 (IISc Bangalore and NRCE, Hisar)
- Nano-drug delivery for quinapyramine sulphate– Application No. 2560/DEL/2011, dated 06.09.2012 (GJUST, Hisar 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.
- NRCE also participated in National level exhibitions, fairs and sales meetings.
- Assessment and transfer of technology using the latest know-how of information technology is also given due importance to extend the technologies to the end-users. The scientific and technical staff provides clinical and diagnostic (including pregnancy diagnosis) services and consultancy to the farmers on-demand in the areas of equine health and production. Farmers and veterinarians are imparted trainings and supplied education materials for equine management, production and health.
- **Extension activities:** To promote awareness on equine husbandry and management and to receive feedback on constraints faced by the equine owners, various activities like equine health camps, awareness camps, *Kisan Goshthis*, scientist-farmer interactive meets etc., are organized on regular basis in different areas of the country. NRCE also participates in National level exhibitions, *Kisan mela*, animal fairs with display of NRCE technologies and information on various aspects of equine husbandry and management for the benefit of equine owners.

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.

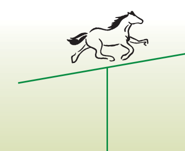
b. Identification, characterization and documentation of animal microbes.

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

d. Human Resource Development (HRD)

Milestone Achievements

- 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.
- The first isolations of: *Nocardia otitidiscaviarum* from equine granulomatous pneumonia, *Moraxella ovis* from sheep, *Delftia* spp. from water sample.
- Whole genome sequencing of *Trueperella pyogenes, Bordetella bronchiseptica, Pasteurella multocida, Actinobacillus equuli* and *Salmonella* Gallinarum.
- Laboratory confirmed cases of Camelpox zoonosis-first report in the world.



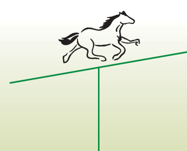
- Isolation and characterization of camelpox virus (CMLV) from outbreaks (2009) in Delhi, Jaisalmer & Barmer.
- Isolation and characterization of zoonotic buffalopox virus (BPXV) from outbreak (2010) in Maharashtra
- Isolation and characterization of buffalopox virus (BPXV) from outbreak (2011) in cattle, buffalo and humans in Meerut, U.P.
- Strengthening of repository with Veterinary microbes during 2012-13:
  - ❖ Bacteria accessioned : 187
  - ❖ Virus accessioned : 21
  - ❖ Recombinant clones accessioned : 76
  - ❖ Genomic DNA : 138
- The repository owns 1630 accessions comprised of veterinary microbes (751 nos.), rumen microbes (140 nos.), dairy microbes (307 nos.), recombinant clones (267 nos.), genomic DNA (138 nos.) and phage library (27nos).

The repository has been strengthened with the addition of viral isolates from different animal species *viz.*, bovine, ovine, equine, canine and poultry. Important viral isolates present in the repository are: buffalopox virus, camelpox virus, goatpox virus, sheeppox virus, equine influenza virus, equine rotavirus, Japanese encephalitis virus, bovine and human rotavirus, bovine herpes virus-1, and equine herpes virus-1 & 4, Newcastle Disease virus, canine parvovirus, canine adenovirus, Fowl adenovirus and street rabies virus.

Significant new bacterial isolations have been made from various geographic locations, including isolation of a *Nocardia otitidiscaviarum* from equine granulomatous pneumonia case, *Moraxella (Branhamella) ovis* from ovine keratoconjunctivitis in sheep, *Bordetella bronchiseptica* from pneumonic case and a rare isolate of *Delftia* spp. from water sample from sheep watering hole. Staphylococcal isolates (3nos.) have been identified as *Staphylococcus sciuri* (methicillin resistant, *mec*A gene sequenced), *Bacillus licheniformis*, *Actinobacillus equuli* sub spp *hemolyticus*, *Rhodococcus coprophylus* from camel in Ladakh, and *Bacillus subtilis* from soft tick after completion of molecular identification. *Salmonella* spp, *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Bacillus* spp. were isolated from cattle calf diahhorea cases; *Escherichia coli*, *Bacillus*, and *Streptococcus* spp. were isolated from buffalo diarrhea. From vasrious pathological samples: *Corynebacterium spp, Bacillus spp, Streptococcus equi, Staphylococcus spp, Escherichia coli, Enterobacter* were isolated. Other important bacterial isolates in VTCC include: *Listeria monocytogenes, Listeria innocua, Klebsiella pneumoniae, Campylobacter jejuni, Campylobacter coli, Corynebacterium pseudotuberculosis, Bacillus cereus, Streptococcus equi, Actinobacillus equuli, Proteus* spp, *Lysinibacillus fusiformis, Brucella abortus, Brucella melitensis, Escherichia coli, Citrobacter freundii, Branhamella, Alcaligenes, Salmonella Typhimurium, Salmonella enterica* sub spp. *enterica* serovar Gallinarum,Weltreverden, Agona, Dublin, Essen etc. and preserved over 500 unidentified isolates by cryopreservation.

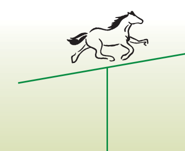
Staff position of NRCE and VTCC (as on 31.03.2013)

Name of the post	NRCE			VTCC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	1	1	-	-	-	-
Scientific	26	21	5	10	8	2
Technical	24	22	2	1	-	1
Administrative	14	12	2	-	-	-
Supporting	22	20	2	1	1	-
Total	87	76	11	12	9	3

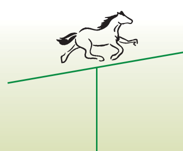


Major Landmarks

1985	NRCE established at Hisar with Prof. P. K. Uppal joining as Founder Director
1987	Outbreak of Equine Influenza in Northern India
1989	Sub Campus of NRCE established at Bikaner for research on production in equines
1989	Occurrence of Equine Infectious Anaemia in India
1990	Exotic donkey germplasm with Poitu blood introduced from France
1991	Artificial insemination (AI) initiated in equines using fresh extended liquid semen
1991	Early pregnancy diagnosis (15 days post insemination) using ultrasonography
1995	Ciq-ELISA developed for detection of circulating immune complexes in EIA-infected horses
1995	Development of field-oriented immune-stick ELISA kit for detection of EHV-1 latent infection in Throughbred horses
1995	Cryopreservation of Jack semen and technology of AI perfected using frozen semen with 40% conception rate
1996	Establishment of a nucleus herd of Marwari horses at Bikaner campus
1996	Crystal structure of mare milk lactoferrin deduced by crystallography
1996	New carpet fabric developed by blending of donkey and sheep hair (Assheep)
1997	Equine Influenza vaccine using indigenous isolate (A/Equi-2/Ludhiana/87) released
2001	Patent for complement fixation test based diagnostic (COFEB)
2003	An Indian patent granted to a diagnostic kit for forecasting EHV
2005	Mab-based sELISA for detection of animal rotaviruses
2005	Establishment of Veterinary Type Culture Centre, at NRCE, Hisar
2006	Collection and cryopreservation of stallion semen at farmer's door using mobile laboratory
2006	World Organization for Animal Health declared India free of African horse sickness
2006	Outbreaks of Glanders in equines
2008	Re-emergence of Equine Influenza after 1987
2008	Equine Herpes Virus-1 diagnosis kit released
2008	ELISA based pregnancy diagnosis kit (Pregmare kit) for pregnancy diagnosis in mares released
2009	Development of Equine Herpesvirus-1 vaccine
2009	A nucleus herd of Zanskari ponies established at Bikaner
2009	First loboratory confirmed Camelpox zoonosis in the world
2009	Japanese Encephalitis Virus isolated from equines in India
2009	Re-emergence of Glanders in Chhattisgarh
2009	Updation of Equine Influenza vaccine
2009	First isolation of *Bordetella bronchiseptica* from horse, *Staphlococcus hyicus* from pig, *Corynebacterium pseudotuberculosis* and *Corynebacterium bovis* from horse & Methicillin- resistant Coagulase Negative *Staphylococcus sciuri* from goats



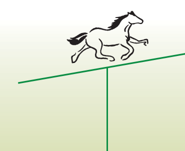
2010	Equine sanctuary for conservation of indigenous breeds of horses and indigenous donkeys initiated
2010	A new clade designated as 'Asian Clade' of Equine Influenza Virus reported
2010	Award of OIE twining project on Equine Poroplasmosis between NRCPD, Japan and NRCE, India
2010	EIA-positive mule detected in Haldwani: Re-emergance of EIA after 1998
2010	Phenotypic characterization of all six indigenous equine breeds
2010	Re-emergence of glanders in Himachal Pradesh and Uttar Pradesh
2010	Standardization of AI using semen of Poitu donkeys & Marwari horses
2010	Zanskari stallion semen cryopreserved
2010	Started toll-free helpline no. 1800-180-1233 for advisory services to equine owners at NRCE Hisar
2011	First laboratory confirmed report on BPXV causing disease in Buffalo, human and cow in same time and space
2011	Whole genome sequencing of Indian strain of Japanese Encephalitis virus
2011	Whole genome sequencing of *Pasteurella multocida* B : 2 strain
2011	First isolation of *Trueperella pyogenes* from buffalo, *Enterococcus asini* from horse & *Exiguobacterium* spp. from pig and *Brevibacterium* spp. and *Brevibacillus* spp. from Equine
2011	Indigenous donkeys (Small grey & Large white) inducted in Equine Sanctuary at EPC, NRCE, Hisar
2012	MOU with NRDC for commercialization of technologies generated by NRCE
2012	OIE twinning proposals for Equine Influenza and Glanders with Animal Health Trust, UK and Friedrich Loeffler Institute, Germany initiated
2012	Re-emergence of Equine Infectious Anaemia in Thoroughbred Polo horse in Haryana
2012	Started toll-free helpline no. 1800-180-6225 for advisory services to equine owners at EPC Bikaner
2012	Isolation of *Rhodococcus equi* from double-humped camel of Leh & Ladakh
2012	Development of recombinant protein -based ELISA kits for Glanders and Equine Piroplasmosis
2012	Development of EIA virus p26 synthetic protein -based ELISA for diagnosis of Equine Infectious Anaemia
2012	Whole genome sequencing of *Trueperella pyogenes, Bordetella bronchiseptica, Pasteurella multocida, Actinobacillus equuli, Salmonella gallinarum and EHV-1*
2012	Single donkey/mule use ploughs and double donkey/mule use ploughs developed
2012	Work-Rest-Cycle established for indigenous donkeys/mules for ploughing/sowing
2012	Technique for Vermi-composting using equine dung developed
2013	Microbial Containment Laboratory (BSL-3 facility), Phase 1 of Veterinary Type Culture Collection (VTCC) Laboratory Complex, ATIC and Info-Equine Museum at NRCE dedicated to nation inaugurated by Dr S. Ayyappan, Secretary DARE and DG ICAR
2013	Foundation stone of BSL-3 Facility of VTCC laid by Dr S. Ayyappan, Secretary DARE and DG ICAR
2013	Isolation of a rare isolate of *Delftia* spp. from water sample from sheep watering hole
2013	First isolation of a *Nocardia otitidiscaviarum* from equine granulomatous pneumonia case and *Moraxella (Branhamella) ovis* from ovine keratoconjunctivitis in sheep
2013	Re-emergence of Glanders in Uttar Pradesh



Summary of Expenditure & Revenue Generation

(₹ in lacs)

Summary of Expenditure		2011-12	2012-13
	Non-plan		
1.	Establishment charges including LSP/PF, wages, OTA	508.92	560.30
2.	Travelling allowances	3.50	3.99
3.	Others charges including equipments & recurring charges	318.98	406.90
4.	Works	0	0
	Total Non-Plan Expenditure	**831.4**	**971.19**
	Plan		
1.	Establishment charges including LSP/PF, wages, OTA	0.00	0.00
2.	Traveling allowances & HRD	18.10	20.16
3.	Others including equipments & recurring charges	687.77	668.37
4.	Works	258.00	233.06
	Total Plan Expenditure	963.87	921.59
	Total Expenditure (Plan & Non Plan)	**1795.27**	**1892.78**
Summary of Revenue Generation			
1.	Sale of farm produce	1132224.00	1486307.00
2.	Sale of livestock	379500.00	250100.00
3.	Sale of publication and Advertisements	52600.00	2020.00
4.	License fee	60924.00	58822.00
5.	Interest on loans and advances	24406.00	232490.00
6.	Interest on short term deposits	527308.00	1047019.00
7.	Income from internal resource generation	4202092.00	4672647.00
8.	Receipt from services	0.00	0.00
9.	Other misc. receipts	2430079.00	1971139.00
	Total Revenue	**8809333.00**	**9720544.00**



Organizational Set-Up

ICAR Director General

↓

ANIMAL SCIENCE DIVISION
DDG (AS)
ADG (AH)

- Research Advisory Committee
- Institute Management Committee

Director
National Research Centre on Equines

Equine Production Campus, Bikaner

- Research Teaching and Training
  - Equine Production
    - Equine Genetics & Breeding
    - Equine Reproduction
    - Equine Physiology
    - Equine Nutrition
    - Equine Production & Management
    - Equestrian Activities
- Farms
  - Livestock
    - Exotic Donkeys
    - Indigenous Equines
  - Agriculture
  - Extension
  - Security

Main Campus, Hisar

- Administration
  - Establishment
  - Bill & Cash
  - Audit & Account
  - Record & Dispatch
  - Purchase & Central Store
  - Planning
  - Vigilance
  - Director Personnel
  - Confidential
  - Estate & Security
- Research Teaching and Training
  - Research Unit
    - Basic & Supporting Disciplines Unit
      - Animal Biochemistry
      - Animal Biotechnology
    - Equine Health Unit
      - Equine Viral Diseases
      - Equine Bacterial Diseases
      - Equine Parasitic Diseases
      - Equine Clinical Medicine
      - Equine Pathology
      - Microbial Containment Facilities
- Project Monitoring & Evaluation (PME) Cell
- ARIS Cell
- PERMISnet
- ITM Unit
- Public Information Unit
- Hindi Cell
- Library
- Extension
- ATIC
- Farms
  - Livestock
    - Experimental equines
    - Experimental lab animals
    - Equine Sick line
    - Infectious Disease Shed
    - Post mortem Complex
    - Quarantine
  - Agriculture

Veterinary Type Culture Collection, Hisar

- Research Teaching and Training
  - Veterinary Type Culture Collection
    - Veterinary Microbes (VTCC, Hisar)
    - Dairy Microbes (NDRI, Karnal)
    - Rumen Microbes (NIANP, Bengaluru)

Research
Achievements

Genetic and antigenic differentiation of equine influenza viruses

Genetic analysis of RNA polymerase (PA, PB1 & PB2) genes

The RNA polymerase genes comprising PA, PB1 & PB 2 genes of influenza A virus - known to function in many aspects of viral replication and to interact with host factors - play a critical role during viral pathogenesis. Many reports suggested that the mutations in these genes are responsible for virulence of the virus. In order to find out the genetic changes in these genes of equine influenza virus (EIV) isolates recovered from epizootic during 2008-09. Three polymerase genes (PA, PB1 & PB2) were cloned and sequenced. Sequence analysis revealed that polymerase genes encode nucleotide sequences of 2151 bp for PA, 2274 bp for PB1 and 2280 bp for PB2 genes which inturn encode proteins of 716, 757 and 759 amino acid sequences for PA, PB1 & PB2 proteins, respectively. The nucleotide and deduced aa sequences of polymerase genes of Indian isolates were compared with other EIV isolates circulating globally. All three genes showed similarity of 98-99.5% at nt and aa level to Chinese and Mongolian EIV isolates. Comparison of aa sequence of A/eq/Katra/06/08 with other isolates circulating globally revealed three point mutations (His146Leu; Ala414Val & Glu604Gly) in PA; seven mutations (Val3Ala; Met92Thr; Leu212Ser; Lys215Met; Ile317Val; Ile525Val & Ala643Val) in PB1 and eight mutations (Lys140Glu; Pro302Ser; Arg380Lys; His437Tyr; Val606Ile; Gly651Asp; Glu681Asp & Asn701Ser) in PB2 genes in comparison to the Chinese and Mongolian EIV isolates.

Phylogeny of the three genes (Fig. 1A, B, C) showed similar branching pattern with closest clustering of Indian isolate with Florida Clade 2 isolates of EIVs. Indian isolates grouped together with the Chinese and Mongolian isolates.

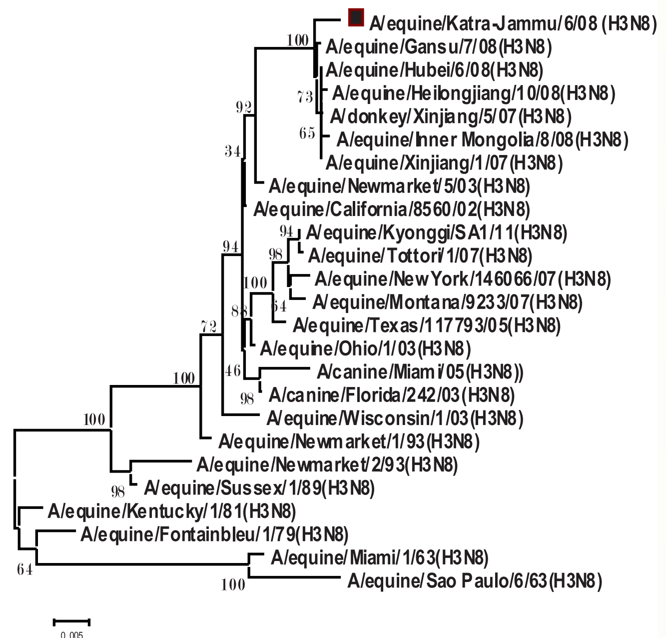


Fig. 1A : Phylogenetic tree of PA gene

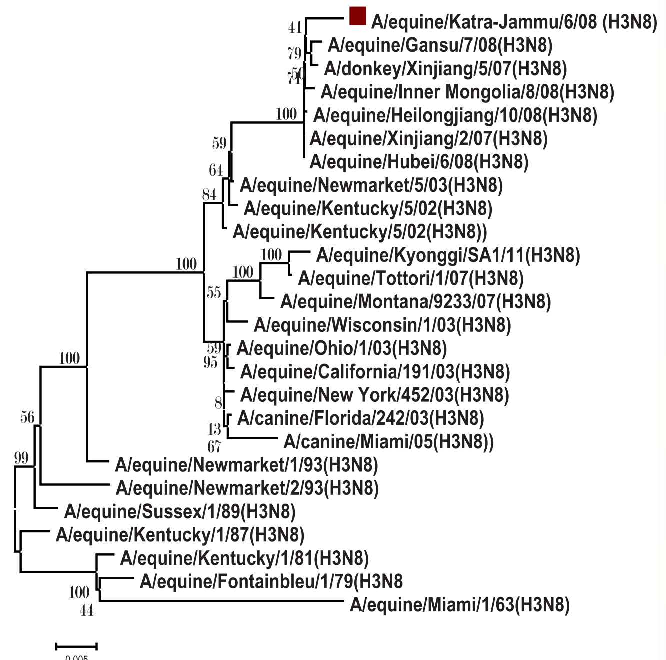


Fig. 1B : Phylogenetic tree of PB1 gene

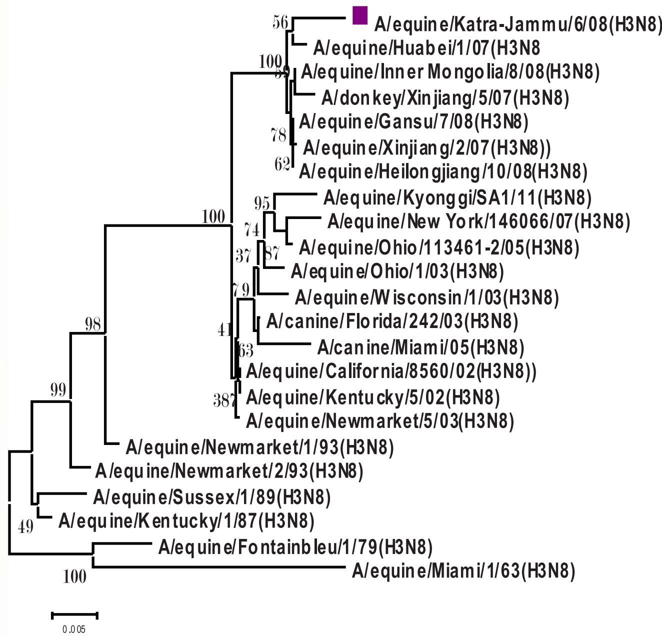


Fig. 1C : Phylogenetic tree of PB2 gene

Characterization of MAbs raised against HA protein of EIV

Specificity of four monoclonal antibodies (1D12, 1G4, 5A7 and 5F4) developed against HA protein of EIV was tested against equine influenza virus (EIV) using Immunoperoxidase test (IPT), Complement dependent virus neutralization test (CDVNT) and Western blotting (WB). Equine influenza virus (EIV) infected MDCK cells gave positive immunoperoxidase reactions with all four MAbs. All four MAbs detected accumulation of immunizing antigen (A/eq/Jammu-Katra/06/08 (H3N8) in infected cells (Fig. 2).

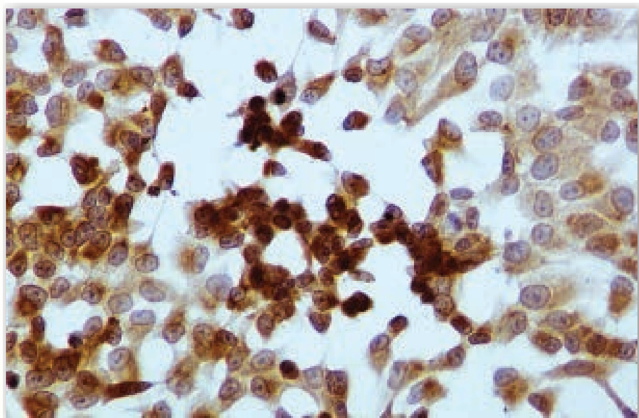


Fig. 2 : EIV infected MDCK cells reacted with 1D12 MAb (1:50 dilution) and stained using IPT. Infected cells showing brownish colour.

In vitro neutralization assays (CDVNT) of two MAbs (1G4 & 5A7) having haemagglutination inhibiting (HAI) characteristics were tested for their neutralizing activity in presence as well as in absence of guinea pig complement in MDCK cells. None of these MAbs showed neutralizing activity against EI virus. Earlier literature also indicates that MAbs with HAI activity may or may not react during virus neutralization against EI virus indicating that the epitope(s) for HAI & VN characteristics may or may not be closely resembling to each other.

Western blotting analysis using 1:100 to 1:1000 dilution of MAbs (1G4 & 5A7) raised in BALB/c mice and anti-mouse HRPO conjugate did not revealed positive reaction of these MAbs with EI virus.

Antigenic differentiation of six EIV isolates using MAbs by HAI test

Six Equine Influenza virus isolates were tested against two monoclonal antibodies (1G4 and 5A7) using HAI tests (Table 1) for antigenic differentiation of EIV isolates/strains.

In HAI test, the MAb 1G4 recognized an epitope of 4 EIV isolates [A/eq/Jammu-Katra/06/08 (H3N8), A/eq/Gopeshwar/1/09 (H3N8), A/eq/Uttarkashi/1/09 (H3N8) & A/eq/ Mysore/12/08 (H3N8)], while none for other two EIV isolates [A/eq/Ludhiana/87(H3N8) & A/eq/Ahmedabad/1/09 (H3N8)]. However, 5A7 MAb recognised an epitope on all the six EIV isolates. It is interesting that A/eq/Ludhiana/87(H3N8) isolate has already been identified different than 2008 epidemic isolate at genetic level. Recognition of A/eq/Ahmedabad/1/09 (H3N8) isolate as a different virus isolate than other EI isolates of 2008 epidemic needs further genetic analysis which might reveal some interesting facts on the possibility of circulation of two antigenically different strains during 2008 EI epidemic. A/eq/Ludhiana/87(H3N8) isolate reacted with 1G4 MAb giving reciprocal HAI titre 8 while A/eq/Ahmedabad/1/09 (H3N8) isolate did not react at all with 1G4 MAb in HAI test indicating the possibilities of antigenic difference with other isolates.

Table 1. Antigenic differentiation of 6 EIV isolates using MAbs by HAI test

Sl No	Virus isolate	Place of Origin	Year of isolation	Clinical manifestation (Respiratory Infection)	Reciprocal of HAI titre of MAbs	
					1G4 (IgM)	5A7 (IgM)
1	A/eq/Ludhiana/87	Ludhiana	1987	yes	Negative (8*)	128
2	A/eq/Jammu-Katra/06/08	Jammu-Katra	2008	yes	512	128
3	A/eq/ Mysore/12/08	Mysore	2008	yes	128	64
4	A/eq/Gopeshwar/1/09	Gopeshwar	2009	yes	128	32
5	A/eq/Uttarkashi/1/09	Uttarkashi	2009	yes	64	16
6	A/eq/Ahmedabad/1/09	Ahmedabad	2009	yes	Negative	64

* = reciprocal HAI titre 16 is considered positive.

(BK Singh, Nitin Virmani, BC Bera and BR Gulati)

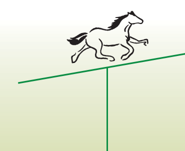
Development of diagnostics for equine influenza

Monoclonal antibody based Sandwich ELISA for detection of equine influenza

Equine influenza, an OIE listed disease of equines caused by either H7N7 or H3N8 influenza A viruses is an acute, highly contagious viral respiratory disease that caused a huge epizootic in India in 2008-09. For faster and precise diagnosis of EI on the penside and in less sophisticated laboratories, enzyme immunoassays are being utilized world over. In the present study, sandwich ELISA using MAbs against HA antigen and polyclonal serum raised against EIV isolate [A/eq/Jammu-Katra/06/08 (H3N8)] was developed for detection of EI. For development of antigen capture ELISA, 96 well microtiter plates format was used. The cut-off level was estimated and the specificity of the assay was tested by using viral antigen from various lineages of EIV (four EIV from 2008-09 outbreak, Clade 1 EIV-South Africa/03, Ludhiana/87, predivergent lineage EIV-Kentucky/81, prototype virus antigen-Miami/63, Prague/56 of H7N7 subtype). For a 99% confidence interval, the cut off was defined as follows: the mean of the negative serum OD_{450} values + three SDs = $0.068 \pm 3 \times 0.0085 = 0.213$. EIV viruses across the lineages and H7N7 subtype gave positive results with same OD without any significant decrease in values indicating broad susceptibility of the MAbs and primary antibody used in the assay. The OD_{450} values for all the controls were lower than the cutoff value and could indicate that the assay could differentiate EIV when compared to the other pathogens. EHV-1 and 4 were chosen as control as they also cause respiratory disorders and can give false positive results in the nasal swabs. The sensitivity limit of the assay was upto 0.025HA units. Further, nasal swabs were spiked with antigen from various EIVs isolated from 2008-09 outbreak, Ludhiana/87 and Miami/63 virus and similar results were obtained.

TaqMan probe - based qRT-PCR for detection of EI

TaqMan probe - based qRT-PCR test is being standardized targeting nucleoprotein gene of the equine influenza virus. For standardization of the test, TaqMan® Single- Step RT-PCR master Mix & TaqMan® Exogenous Internal Positive Control Reagents both from Applied Biosystem and Dual-labeled Probe, 5' FAM/3' BHQ-1 against NP gene were used in Step One Real-time PCR machine, Applied Biosystem. Known quantity of the RNA isolated from the purified EIV was used for reaction set up. The qRT-PCR test showed specific amplification curve for positive control as well as for NP gene. Further standardization for detection of copy numbers of EIV is underway.



Recombinant non-structural (rNS1) protein - based DIVA assay

The rNS1, full - length as well as C-terminal protein cloned into prokaryotic expression vector pQE30 and expressed earlier were utilized for screening serum samples from confirmed infected (unvaccinated) and vaccinated (non - infected) animals. Full - length protein was not giving adequate results with the serum samples and thus C-terminal rNS1 was utilized. Western blot assay standardized for the purpose was used on confirmed infected (n=30) and vaccinated (n=20) animals. The assay could not differentiate between the true infected and vaccinated animals. Serum from vaccinated animals showed positive results (n=18) (confirmed seropositivity by HI assay and titres 64-256), while serum from infected animals was giving results as per expectations (n=26). This is because the non - structural proteins are present in the vaccine preparation. This indicates that for development of DIVA assay using NS1 protein, the pre-requisite should be NS1 protein - free vaccine preparation.

Immunohistochemistry for Equine Influenza in tissue sections

Immunohistochemical method employing indirect immunoperoxidase method was standardized for detection of equine influenza viral antigen in MDCK monoloyers infected with EIV and in paraffin sections from BALB/c mice experimentally challenged in another experiment in EI Vaccine project. MDCK monolayers infected with EIV/ tissue sections were treated with a primary mouse monoclonal antibody to H3 HA influenza A antigen and anti - mouse HRPO conjugate was used as secondary antibody followed by detection using DAB as substrate and haematoxylin as counter stain. Intracytoplasmic brown staining could be seen in the MDCK monolayers infected with EIV (Fig. 3) and in lung/trachea sections from BALB/c mice.

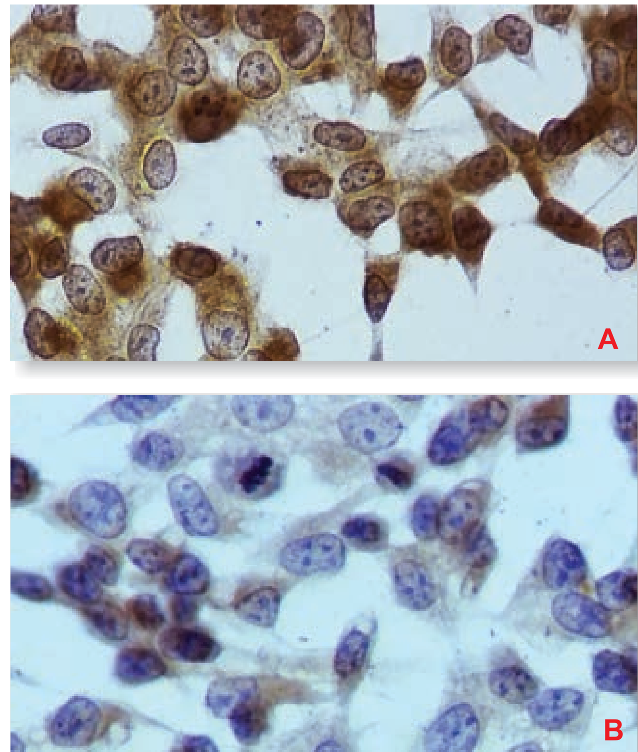


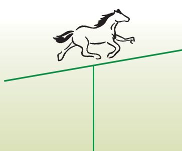
Fig. 3 : Indirect IPT showing intracytoplasmic brown stain of EIV infected MDCK cells (A). Negative control (B)

(Nitin Virmani, BC Bera, BR Gulati and BK Singh)

Evaluation and updation of equine influenza vaccine

Experimental studies on establishment of equine influenza (H3N8) infection in BALB/c mice model

The mice model for equine influenza was developed using BALB/c mice which could be used for further studies on vaccine and pathogenesis of EIV. A dose of 10^7TCID50 / 25μl of the EIV isolate {A/equine/Jammu-Katra/06/08 (H3N8)} was used to establish infection in mice model. Infected mice did not show clinical signs except for slight ruffling of fur on 1 and 2 dpi and had no significant loss of body weight compared to control. On 1 and 2 dpi, mice revealed patch of congestion in lungs which was not observed on 3dpi onwards. Histopathological examination revealed lesions in trachea included moderate focal necrosis in epithelial lining along with infiltration of lymphocytes and neutrophils in the epithelium and lamina propria from 1dpi and the intensity increased till 3 dpi followed by decrease in severity. In lungs, several lesions including bronchiolar epithelial necrosis along with epithelial hyperplasia at places were observed from 2dpi onwards and maximum lesions observed on 3dpi. Few bronchioles were filled with eosiniphillic exudates and mild peribronchial and perivascular lymphocytic infiltration was observed in many places at 3dpi. Inflammatory changes and epithelial necrosis in lungs were most severe from 3 to



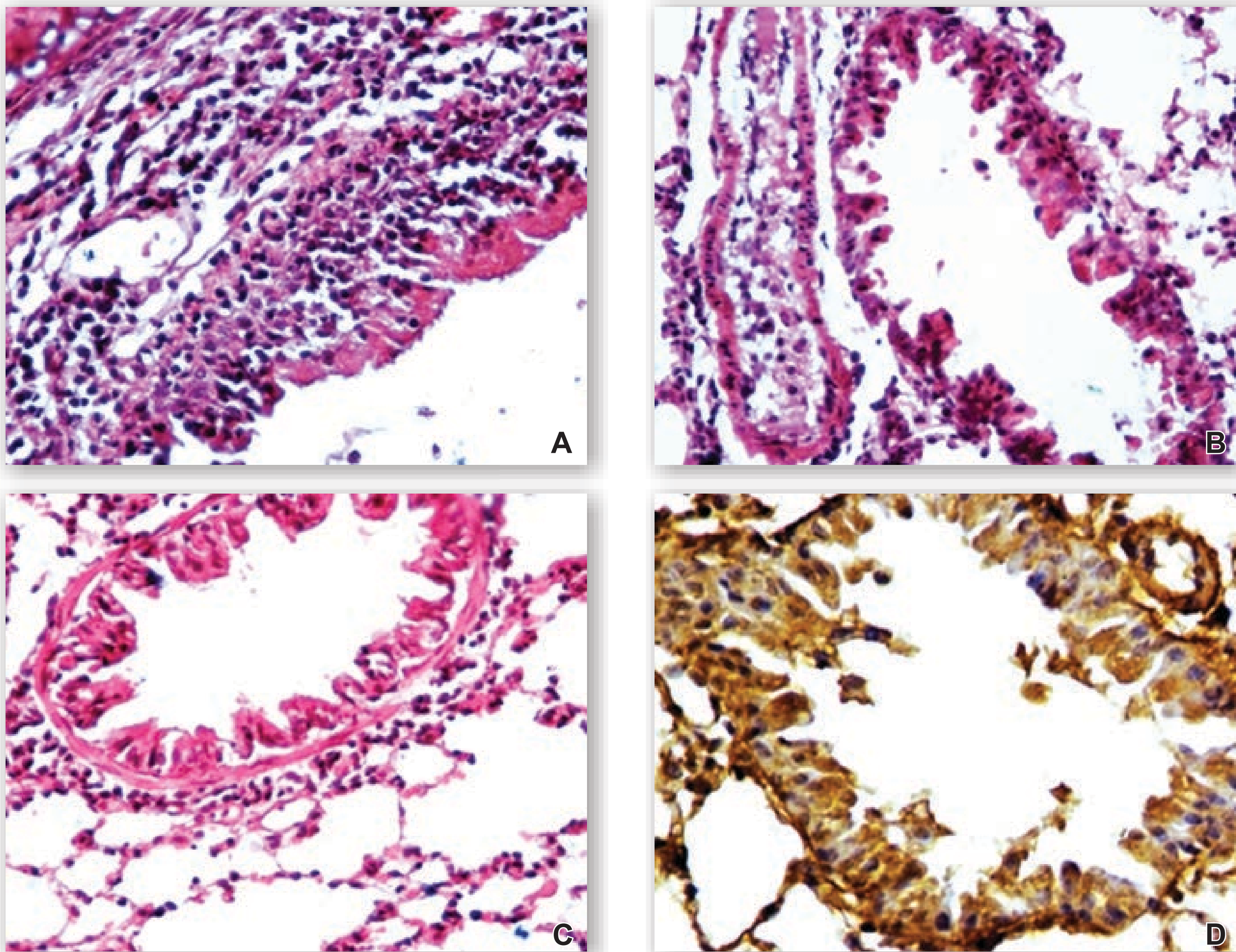


Fig. 4: Experimental EIV infection in BALB/c mice- (A) Trachea-2dpi-Epithelium necrosis and lymphocytic infiltration, (B) Lungs-3dpi-Bronchial epithelium necrosis and hyperplasia at places, swelling of epithelium cells (C) Lungs-3dpi-Peribronchial lymphocytic cuffing (D) Lungs-Bronchial epithelium-Intracytoplasmic brown antigen

5 dpi.

Indirect immunoperoxidase technique was used for detecting viral antigen in paraffin sections and its distribution in different organs at various days post inoculation using MAbs against H3. Nasal turbinates and trachea showed presence of specific intracytoplasmic brown staining in the epithelial cells from 1 dpi to 3dpi (Fig. 4). However, lungs were positive for presence of influenza virus antigen on 2 and 3 dpi only (Fig. 4). Maximum viral antigen could be detected in bronchiolar epithelium and alveolar parenchyma at these intervals (Fig. 4). Infectious virus was not detected beyond one day in lung tissues from mice, however, RT-PCR could detect viral nucleic acid in tissues from lungs as well as nasal turbinates till 5dpi and 3dpi, respectively.

Present investigations in the BALB/c model could establish the replication of the virus and its reaching to lungs. These studies would help in developing a roadmap for challenge studies in mice model prior to carrying out the challenge in equines which in itself is complicated and tedious process in terms of issues related to ethics, biosecurity and biosafety.

(Nitin Virmani, BR Gulati and BK Singh)

Raising of experimental infection of *Trypanosoma evansi* in ponies

Experimental infection of *T.evansi* was successfully set up in six ponies (9-12 months age). The serum/blood samples were collected weekly until termination of experiment i.e. 63 dpi. During this period, three ponies died respectively on 59, 60 & 61 dpi due to symptoms of acute trypanosomosis. The remaining 3 ponies showed acute clinical signs *viz.*, unable to stand/ walk and anorexia along with other symptoms *viz.*, intermittent fever, dullness, weakness, emaciation, anemia, anorexia and incoordination in hind quarters at terminal stage of infection. Two ponies showed edema in brisket, abdominal regions, staggering gate, and incoordination in hind quarters.

The prepatent period of infection was 5-7 days and body temperature rose up to 102-107^{0}F. Haematological studies indicated a gradual fall in haemoglobin, HCT, and RBC from 14 dpi, onward and lowest was recorded at termination of experiment with an average of 4.83 g/dl, 24.28% and 6.27 million/ml, respectively. However, MCV values showed increasing trend after 14 dpi in all infected animals. The uninfected control animals showed normal values of all the indices studied in the experiment. After treatment with quinapyrimine sulphate at the end of experiment, ponies were monitored for parasitaemia and were found negative after 48 hrs. All the animals recovered 10 weeks post-treatment, showed normal haematological indices and maintained good health. However, one pony showed acute neurological signs-tilting of head, circling motion, hyper excitability, recumbence and peddling of legs six month after treatment.

T. evansi antibodies in experimental ponies were detected by developed ELISA from 10-14 dpi onwards and showed rising trend till termination of experiment.

(SC Yadav, Rajender Kumar and Sanjay Kumar)

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

The Mx protein confers resistance to Orthomyxovirus infection by modifying cellular functions needed along the viral replication pathway. Mx gene expression was studied both in unstimulated and stimulated PBMCs collected from Marwari horses by qRT-PCR. The samples were categorized on the basis of Mx gene expression as well as on the basis of history of influenza infection. Results did not reveal discrete pattern of Mx gene expression to differentiate EI infected and non-infected animals. Full-length and partial overlapping gene fragments from representative samples of both the groups were amplified and sequenced. Amino acid sequence analysis revealed 99-100% homology with thoroughbred horse. Two substitutions at position 31 (proline to leucine) and at 166 (glycine to glutamic acid) were observed. On phylogenetic analysis, the Mx sequences from Marwari horses formed separate clade with *Equus caballus* (Thoroughbred) (Fig. 5).

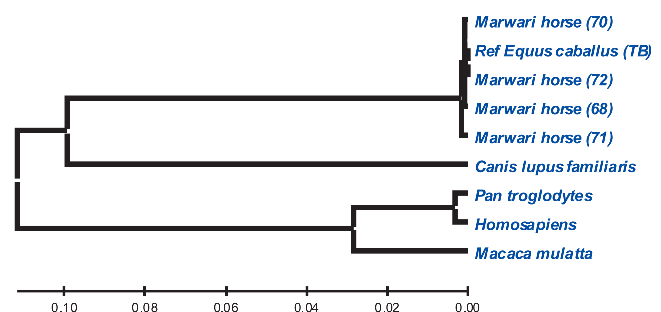
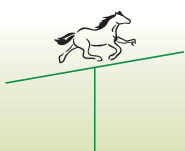


Fig. 5: Evolutionary relationships of *Mx* gene of Marwari horse with other species

(BK Manuja, Anju Manuja and RC Sharma)

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

During the period of report, 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. The processing of samples and seroprevalence of important equine diseases are depicted in table 2 & 3. For EIA, 7462 serum samples from thoroughbred as well as indigenous equines were examined by Coggins test under S&M (1482), disease investigation (709) and contractual service(5371). Out of 7462 samples tested, one sample from Delhi was found positive for EIA. Testing of 1482 serum samples for Brucellosis and *Salmonella* Abortusequi (H antigen) revealed no positive samples. 7601 serum samples were tested for glanders, which included S&M (1482), disease investigation (1357) and contractual service (4762). Seven serum samples from UP were found positive for glanders antibodies, out of 1357 samples tested under disease investigation.

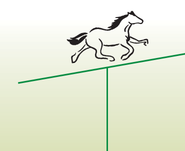
Outbreaks of equine influenza were reported from several states of the country during previous years. Follow up action is continuing in affected States. No new cases/ outbreaks of EI were reported during the year. HAI test for EI was conducted on 1482 samples under serosurveillance from indigenous equines which yielded antibodies in 21 (1.41%) serum samples. Further, 3296 serum samples were tested for equine influenza and 21 samples were found positive. However, none of the samples tested under serosurveillance and disease investigation categories showed any rise in titres in paired samples thus indicating

Table 2 : Seroprevalence of important equine diseases (2012 - 2013)

Sl. No.	State	EIA	EI	Glanders	*T.*evansi	EHV-1	*T.equi*	JE/WNV	*S.*abortusequi	Brucellosis
1.	Rajasthan	0/450	5/450	0/450	9/450	8/450	227/450	26/450	0/450	0/450
2.	Uttrakhand	0/155	0/155	0/155	11/155	10/155	47/155	0/155	0/155	0/155
3.	Haryana	0/100	5/100	0/100	2/100	0/100	48/100	13/100	0/100	0/100
4.	Maharashtra	0/328	11/328	0/328	6/328	33/328	48/328	0	0/328	0/328
5.	J&K	0/366	0/366	0/366	29/366	41/366	94/366	0/366	0/366	0/366
6.	UP	0/71	0/71	0/71	17/71	4/71	36/71	4/71	0/71	0/71
7.	Gujarat	0/12	0/12	0/12	1/12	1/12	11/12	2/12	0/12	0/12
	Total	**0/1482**	**21/1482**	**0/1482**	**75/1482**	**97/1482**	**513/1482**	**45/1153**	**0/1482**	**0/1482**

Table 3 : Number of samples processed and bacteria recovered

	Uttarakhand	Rajasthan	Haryana	Uttar Pradesh	Total
Nasal Swab	12	10	2	15	**39**
Vaginal Swab	0	5	0	0	**5**
Buccal Cavity Saliva/Swab	0	4	0	0	**4**
Blood	0	1	0	0	**1**
PM Tissues/Swabs	0	40	9	0	**49**
Pus	0	1	0	0	**1**
Uterine Swab	0	1	2	0	**3**
Aborted Foetus and Contents	0	12	2	0	**14**
Rectal Swab	0	2	0	0	**2**
Lesion Swab	0	0	0	2	**2**
Total	**12**	**76**	**15**	**17**	**120**



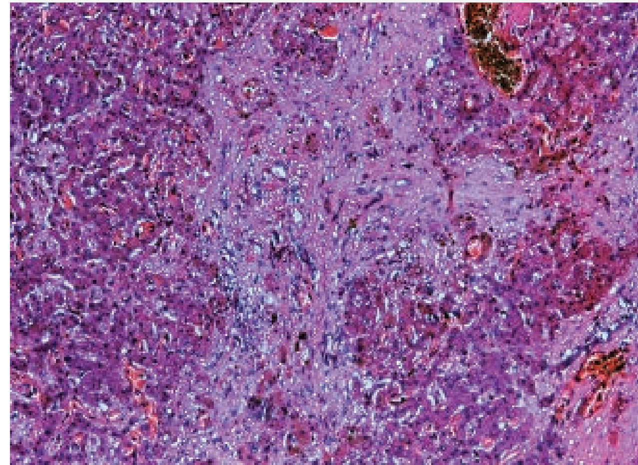
residual antibody titre in positive animals from past infection.

Similarly, 83 samples out of 805 samples tested for EHV-1 under DI were found positive. None of 450 samples tested for EVA were found positive. An outbreak of abortions was attended by team of Scientists at an organized stud farm where 12 mares aborted. EHV-1 virus could be isolated from two aborted fetuses during the outbreak. A total of 1482 serum samples from indigenous equines from various parts of the country were tested serologically and 97(6.54%) samples were positive.

In parasitological studies, 1482 serum samples (all from indigenous equines) were tested for detection of *Babesia equi* antibodies by ELISA and 513 samples were found positive having a rate of infection of 34.61%. *T. evansi* antibodies were positive in 75 samples out of 1482 serum samples showing a prevalence of 5.06%. Serosurveillance of JEV antibodies showed 45 (3.90 %) samples positive out of 1153 serum samples tested.

Disease investigation through post-mortem examination and morbid material/ biopsy received from the field and important conditions recorded on the samples received from the field included Cirrhosis of liver (3), anoxia (1), Volvulus leading to toxaemia (1), Nocardiosis (1), Non suppurative encephalitis due to Trypanosomosis (7), non suppurative encephalitis (reason not established) (1), equine infectious anaemia (1).

Bacteriological analysis done on 120 samples originating from Rajasthan (76), Haryana (15), U.P. (17) and Uttarakhand (12) including nasal swabs, vaginal swabs, uterine swab, rectal swab, lesion swab, tissues from PM, pus, blood, aborted foetus and contents and buccal cavity swab yielded 48 isolates including *Streptococcus equi* subsp. *zooepidemicus* (5), *Streptococcus equi* subsp. *equi* (1), *Staphylococcus* sp. (1), *E. coli* (11), Gram negative bacilli (6), Group C *Streptococcus* (16), *Nocardia asteroids* (6) and Micrococci (2) (table 4). 235 samples from animal quarantine Centres including vaginal swabs and preputial swabs tested for CEM were negative.



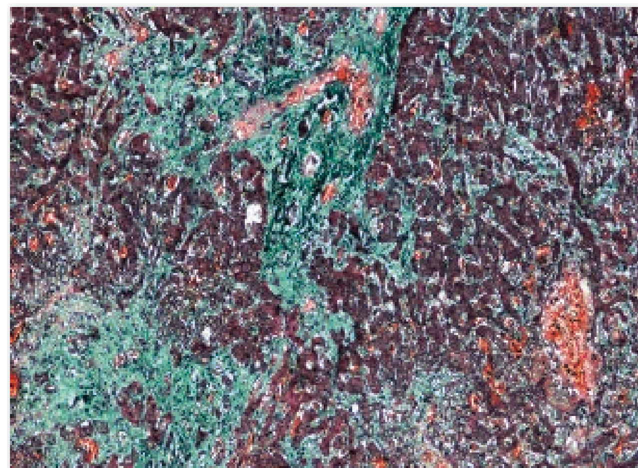


Fig. 6: A. Cirrhosis in liver- Islands of hepatocytes separated by thick mature fibrous tissue leading to loss of hepatic architecture, B. Fibrous tissue proliferation as demonstrated by green coloured collagen employing Massons Trichrome stain

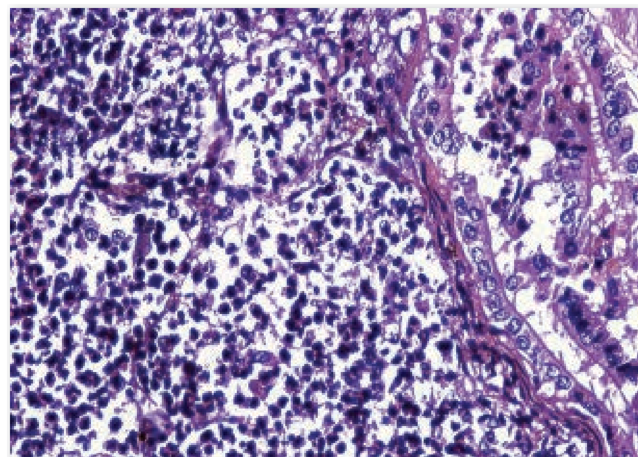


Fig. 6: C. Section of Lungs-Nocardiosis-PMN cell infiltration, necrosis in parenchyma and connective tissue proliferation

Table 4 : Details of bacterial isolates from samples of various origin from equines

Bacterial isolate	No.	Sample	State
Nocardia asteroides	6	PM Tissues (5), Pus (1)	Rajasthan (6)
E.coli	11	Nasal Swab (2), Vaginal Swab (1), Rectal Swab (1), Oral Swab (1), PM Tissue (6)	Rajasthan (9), UP (2)
Streptococcus equi	1	Nasal Swab (1)	Haryana (1)
Streptococcus zooepidemicus	5	Rectal Swab (1), PM Tissue (4)	Rajasthan (5)
C' Group Streptococci	16	Nasal Swab (12), Vaginal Swab (2), Rectal Swab (1), Oral Swab (1)	Rajasthan (5), UP (11)
Staphylococcus sp.	1	Nasal Swab (1)	Haryana (1)
Micrococci	2	PM Tissues (2)	Rajasthan (2)
Gram negative bacilli	6	PM Tissues (5), Aborted material (1)	Rajasthan (5), Haryana (1)
Total	**48**		

The trends of seroprevalence of Japanese Encephalitis *B.equi* and EHV-1 during past years as indicated in figures 7 & 8.

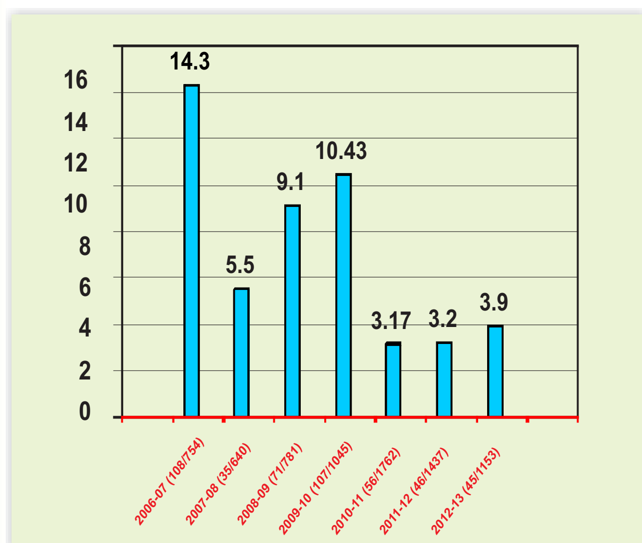


Fig. 7A : Percent seroprevalence of Japanese Encephalitis (Trends)

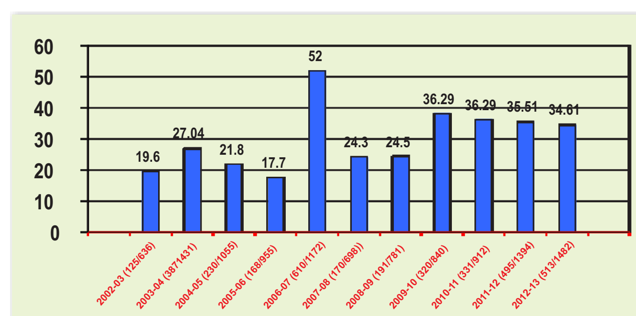


Fig. 7B : Percent seroprevalence of *B.equi* (Trends)

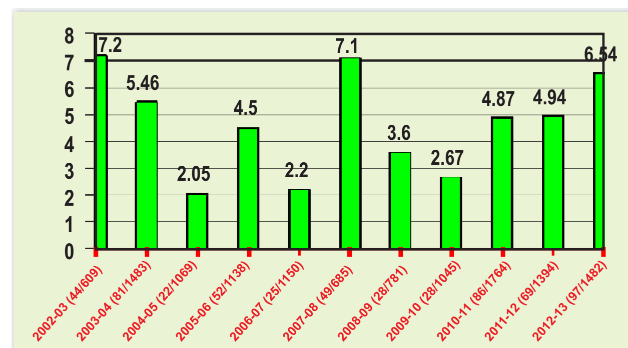
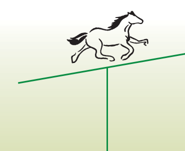


Fig. 8 : Percent seroprevalence of EHV-1 (Trends)

(SK Khurana, RK Singh, BK Singh, SC Yadav, BR Gulati, Praveen Malik, Rajender Kumar, Nitin Virmani, Sanjay Kumar, Sanjay Barua, Rajesh Vaid, H Singha and Anju Manuja)



Neurological trypanosomosis: an emerging disease trend in horses in India

Trypanosomosis caused by *T. evansi* is persistent in domestic and wild animals in several states in northern, eastern and also in semi-arid deserts of Rajasthan state of India. The disease is most severe and frequently diagnosed in horses, however, neurological or meningoencephalitis forms were occasionally recognized. Recently, many cases of trypanosomosis in horses, showing neurological disorder followed by mortality are being reported in India.

During March 2012, approximately 98 horses, aged two years, were transported from organised stud farm located near New Delhi to seven race courses located at different parts of India. Of these transported horses, twelve horses stabled at Pune, Bombay and Bangalore exhibited incoordination of hind legs, circling motion, hyperexitability, blindness, finally death, showing acute neurological signs in span of 5 month (Fig. 9). All the horses, which were brought from organised stud farm stabled at these places, were treated with quinapyramine sulphate. National Research Centre on Equines (NRCE) after this episode, received 49, and 85 serum samples, respectively during August/September, 2012 from RWITC for testing against West nile virus (WNV), Equine herpes virus (EHV-1), Japanese encephalitis (JE) viruses, along with *T. evansi*. Besides, a total of (271) serum samples from organised stud farm near Delhi, horses were tested regularly from Sept., 2012 to Jan., 2013 for above ailments.

None of the quinapyramine sulphate treated animal showed parasite in wet blood film and stained blood smear examination. The first batch of 49 serum samples were subjected to ELISA using Whole Cell Lysate (WCL) and exo-antigen of *T. evansi*, together with reference positive and negative controls. Of them, 8 horses were found sero positive with both antigens for *T. evansi* antibodies. These serum samples were subsequently subjected to immunoblot, which further confirmed positivity of ELISA positive samples – as three strong bands at the molecular wt ranges of 66, 55, 43 kDa were observed which recognize the *T. evansi* infection. Immunoblot with exo-antigen also revealed reactivity at 66 kDa region. The representative samples of blood, brain tissue, CSF were also tested by PCR with three set of primers and revealed *T. evansi* specific amplification. Further, mass spectrometry based protein identification from the buffy coat of the infected horse blood sample revealed presence of variable surface glycoprotein (VSG) and other Trypanosomal proteins. The results supported serological findings indicating *T. evansi* infection in horses. Further, screening of 85 serum samples from these stud farms revealed 8 more animals positive for *T. evansi*. During the episode, an overall 16 horses were detected sero-positive. Of them, 6 horses which were already declared sero positive, died due to similar neurological disorder in the span of over 6 month and rest 11 horses recovered. No new horse became sero positive for *T. evansi* in any of the areas where infection was noticed earlier.

Gross lesions in brain in one horse where PM was conducted by NRCE showed liquifactive necrosis and haemorrhage in mid brain and thalamus (Fig. 10A). Histopathology of the brain from severe animals revealed moderate to severe multifocal perivascular cuffing of blood vessels in almost all the sections. Virchow Robbin space showed aggregation of lymphocytes, plasma cells and macrophages (Fig. 10B). Blood vessels and capillaries were severely congested. Widespread degeneration and necrosis of neurons was discernible along with central chromatolysis. Proliferation of glial cells could be seen in the neuropil. Satellitosis and neuronophagia around the necrosed neurons is present. Moderate presence of fat laden macrophages (gitter cells) could be seen in many areas in the sections. These findings further supported the diagnosis as neurological disease due to Trypanosomosis has been reported previously to cause such kind of lessons in brain.

No concurrent infection of JEV, WNV and EHV-1 viruses was observed in *T. evansi* sero positive horses. However, it is interesting to note that none of 271 serum samples of the horses stabled at stud farm near Delhi had any positivity of *T. evansi,* JE, WNV or EHV-1. Also there was no mortality in the mentioned stud farm during the entire episode.

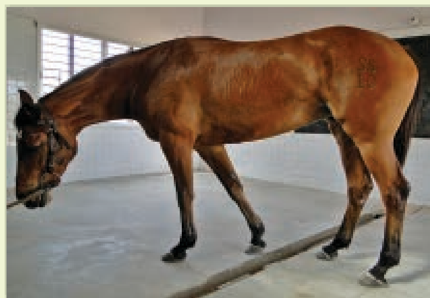
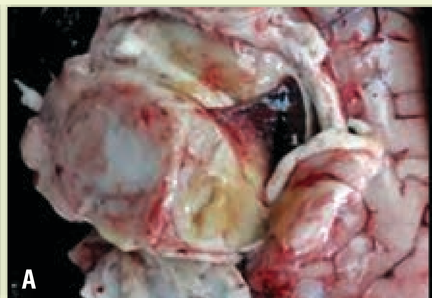


Fig. 9: Neurological sign exhibited in horse infected with *T. evansi*.



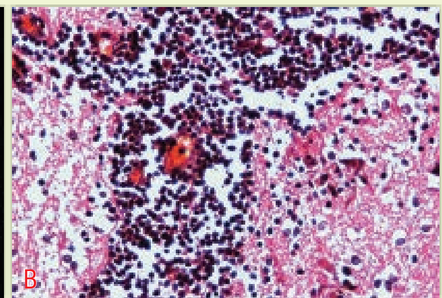
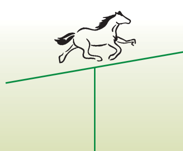


Fig. 10:A. Haemorrhages and necrosis in mid brain, B. Section of brain showing severe perivascular cuffing around blood vessel

(SC Yadav, R Kumar, BR Gulati, N Virmani, SK Khurana, BK Singh, U Tatu, SK Kulkarni, SV Chinmulgund, and M Rajasekhar)



Focal outbreaks of glanders in Uttar Pradesh in 2013

Glanders is a fatal infectious disease of horses, donkeys, and mules and also communicable to man and other animal species. The disease is caused by the Gram-negative nonmotile bacillus *Burkholderia mallei*, and is characterized by nodular lesions of the lungs and other organs as well as ulcerative lesions of the skin and mucous membranes of the nasal cavity and respiratory passages. Following reporting of cases suspecting glanders during February-March 2013, focal outbreaks were detected from three different places in Uttar Pradesh namely Auriaya, Hardoi and Ganjdundwara block in Kasganj District. Team of scientists from NRCE investigated the affected equines showing respiratory illness and cutaneous lesions (Fig. 6). Four horses in Mawaiya village of Hariyawan Block (District Hardoi), two horses stabled in Amar brick kiln near Khanpur village (District Auraiya) and one horse stabled in Bombay brick kiln Ganeshpur, Ganjdundwara block of Kasganj District were found positive for glanders in clinical and serological examinations (CFT, WB, iELISA and dot-ELISA). Cutaneous and nasal forms of glanders were observed in the affected horses in Hardoi (Fig. 11A,B) and Ganjdundwara (Fig. 12) whereas only respiratory form of glanders was observed in Auraiya. In previous years, glanders was also reported in Bulandshahr, Chandpur and Babugarh area of Uttar Pradesh during the same time periods. Dry winter season (Jan-March) seems to favour incidence of glanders outbreak in this state. Lack of awareness among equine owners about the disease, compensation issues, reluctance in disease reporting, antibiotic treatment of affected animals (temporary healing of nodules) and subsequent relapse of the disease seems to be the major reason behind continuous presence of glanders as well as stumbling block towards its eradication from the country. Continuous follow up surveillance of the respective regions and other areas should be of utmost priority for effective control of the disease. More concerted efforts by State Animal Husbandry authorities in the form of organizing awareness camps, regular seromonitoring of the equines, regulation of movement of equines to and from affected zones, and providing reasonable compensation to the owners of the glanders-affected animals, with technical support from NRCE are to be put together with the aim of making the country glanders free.

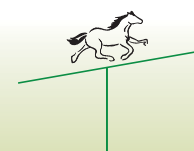
Fig.11: Cutaneous (A) and nasal form (B) of glanders observed in horses in Mawaiya village of Hardoi, UP.

Fig. 12. The typical glanders nodules in hind limbs of a horse stabled at Ganjdundwara, UP.

(Praveen Malik, H Singha and SK Khurana)

Surveillance of Japanese encephalitis among equines in India

Japanese encephalitis (JE) is a mosquito-transmitted viral disease of human and horses in Asia caused by the JE virus (JEV) belonging to the genus *Flavivirus* and family *Flaviviridae*. With a view to monitor the disease status in equines during 2012-13, a total of 1153 equid samples from 6 states of India were tested for JEV antibodies and 45 equuid samples (3.9%) were detected positive. Maximum sero-prevalence was reported in Gujarat (16.7%), followed by Haryana (13.0%). None ot the equid samples from the hill state of J&K and Uttarakhand was found positive for JEV antibodies.



Detection of EIA seropositive horse in Haryana in 2012

Equine infectious anemia (EIA) is a retroviral disease caused by EIA virus (EIAV), a macrophage tropic lentivirus belonging to subfamily *Orthoretrovirinae* under family *Retroviridae.* EIA is a chronic, debilitating disease of all equidae, including horses, mules, and donkeys. EIA infection is widely reported in several countries in European and American continent. Previous studies suggested that EIAV infections in thoroughbred equines had been endemic in certain states of India during 1987 to 1999. A single case of EIA positive mule was detected in January 2010 from Uttarakhand. Following detection of EIA positive reactor, surveillance was heightened in the country. In September 2012, one thoroughbred horse showing presence of EIAV antibody was detected in Haryana state. Seropositivity for EIA has been indicated by immunoassays *viz.,* Coggins test and cELISA, rp26 protein based western blot and iELISA. Attempts to isolate the EIAV and PCR amplify the EIAV-specific gene sequences using published primer failed. Following immediate quarantine, the animal was eliminated and surveillance is being followed rigorously as per National Policy on EIA. It is a very important and alarming finding in view of absence of the disease in the country for quite long period. Similarly, in recent past, the disease incidence was detected in South-Eastern Asia eg., China, Japan, Mongolia, Thailand, Uzbekistan, Philippines and Malaysia after a long interval. The presence of hematophagous vectors and hot-humid climate remains the most important risk factors for EIA in this region. Repeated sampling and more concerted statewise sero-surveillance is recommended to know the actual EIA status among equine populations in India.

(Praveen Malik, H Singha and SK Khurana)

Equine Health Camp in J&K by team of NRCE

A team of Scientists (Dr BK Singh, Dr Nitin Virmani, Dr RK Vaid and Dr AA Raut) from NRCE, visited Sonmarg, Baltal, Gulmarg, and Chandanbari of J&K w.e.f July, 18-27, 2012 for organizing Equine Health Camp and also for disease surveillance and monitoring. Organizing, Equine Health Camp in J & K during this period was important because Shri Amarnathji Yatra was going on during this period. Large numbers of equines from all over the state gathered at this occasion for carrying loads and pilgrims at this holy event. Veterinary specialists from different disciplines participated in this camp. State Animal Husbandry Deptt., (Kashmir-Srinagar), Govt of J& K and Shri Amarnathji Shrine Board supported to get this Equine Health Camp grand success. A total of four camps (Sonmarg, July 20; Baltal, July 21-22; Gulmarg, July 24 and Chandanwari, July 25) were organized (Fig 13) and 233 equines (Baltal-32; Sonmarg-93; Gulmarg-43 and Chandanwari-65) were examined. Relevant bio-samples (serum, nasal swabs etc.) were collected from these animals. Ponies, mules etc. were critically examined in the health camps. Prompt disease diagnosis was given based on spot clinical examination of animals. Sick animals were given treatment. Equine owners were provided basic health coverage like anthelmentics (antiparasitic drugs) along with mineral mixtures (for nutritional propose), first aid, relevant medicines to their animals. Serum samples were negative for equine infectious anaemia, equine influenza, *Salmonella abortus equi* and Brucella antibodies. However, 10.72% (25/233) equines were seropositive for trypanosoma antibodies, 9.4% (22/233 for equine herpesvirus-1 and 8.58% for babesiosis. During these health camps cases of foot rot and pink eyes were also noticed in sheep. *Moraxella bovis was* isolated from pink eye cases of sheep. Information bulletins and pamphlets detailing the various aspects of equine health were also distributed to equine owners. Their knowledge for proper upkeep of equines was also updated.

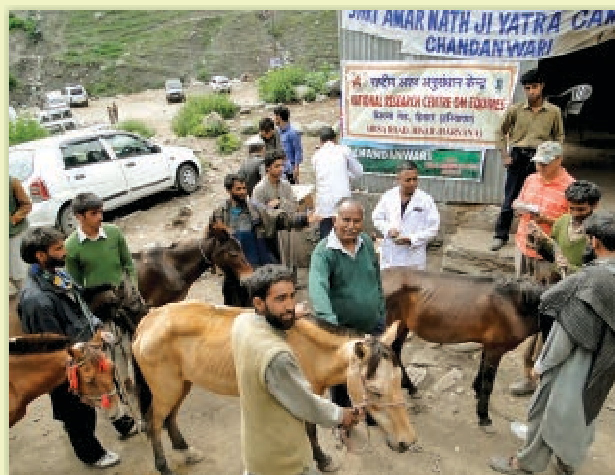
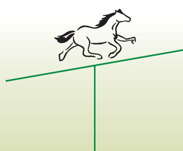


Fig. 13: Equine health camp at Chandanwari



Characterization of indigenous, non-descript and geographically distinct donkeys

As per 18th Livestock Census (2007), total equine population in India is about 1.18 millions and donkeys constitute 37% of the total equines. Donkeys, available in different parts throughout India, are known as non-descript local donkeys as no attempt was made to characterize them on the basis of their phenotypic and genetic characteristics. A need was felt for the systematic and scientific evaluation for their better utilization and improvement in health and reproduction efficiency. At present, it is not possible to predict their exact requirement in future but in view of decreasing fossil fuel resources, donkeys as source of animal power will certainly be the requirement of poor section of the society.

Biometric analysis of donkeys from two different geographical areas (Leh, Ladakh and Baramati, Pune) was carried out for generating a systematic base line information on Indigenous non-descript donkey populations as well as for their comparative analysis with Poitu breed of exotic donkeys. Leh has cold desert conditions (temperature varied between 30°C to -40°C during summer and winter) and soil is loamy mixed with stones and gravels while Baramati has hot summer season (March, April and May with temperature ranging from 32°C to above 40°C) with scanty rainfall (June to September) and winters from the months of December to February (temperature 8°C to 25°C).

Fifteen biometric indices namely body length (BL), height at wither (HW), heart girth (HG), fore leg length (FLL), height at knee (HK), Canon (can), hind leg length (HL), height at hock (HH), ear length (EL), ear width (EW), face length (FL), face width (FW), gap between base of ears (pole), hoof length (HoL) and hoof width (HoW) of 50 donkeys from Leh area (Ladakh, Jammu & Kshmir) and 59 donkeys from Baramati area, Pune, Maharashtra were recorded for assessing their phenotypic characteristics and comparison with Poitu donkeys.

Coat Colour: Coat colour of most of donkeys at Baramati was grey (both light and dark) (59%) with and without dark strip on back (22 and 20 % respectively), large white (39%), mostly brought from Gujarat and brown (2%) while donkeys available at Leh were bay black in colour.

Biometric indices

At Baramati, both BL and HW of donkeys were almost same (91 – 128 cm) with average wither height as 101.00 ± 0.726 cm. Average values for other biometric indices were: HG (108.86 ± 1.003 cm), FLL (64.22 ± 0.57 cm), HK (31.26 ± 0.442 cm), can (18.92 ± 0.308 cm), FL (48.27 ± 0.422), FW (19.05 ±0.250 cm) and pole (12.02 ± 0.177). Donkeys at Leh had BL ranging from 79 to 112 with an average of 95.76 ± 1.545 cm. Average values of other indices were: HW (88.30 ± 1.262), HG (98.52 ± 1.607), FLL (61.91 ± 1.118), FL (46.55 ± 0.800) and FW (16.44 ± 0.376).

Comparative analysis (Fig. 14, A, B) clearly indicated that all biometric indices were significantly higher in Poitu breed of donkeys than local donkey populations available at Leh and Baramati areas indicating that both the Indian populations are smaller in size than Poitu breed of donkeys. Further, among both the Indian local populations, donkeys available in Baramati area were significantly taller and bigger in size than donkeys at Leh. This information along with genotyping information will be quite useful in finally defining and identifying the local population as separate clusters or breed.

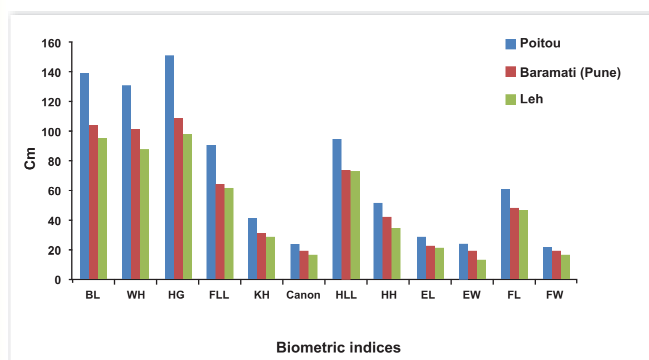


Fig. 14: Biometric indices of donkeys from Leh and Baramati along with exotic Poitu donkeys

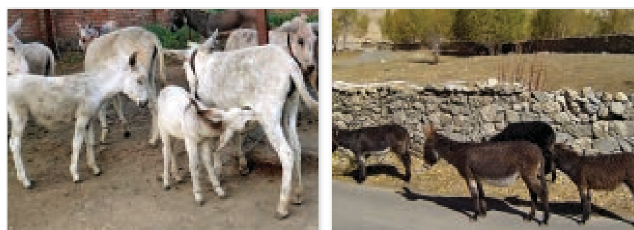


Fig. 14 A : Donkey's from Baramati

Fig. 14 B : Donkey's from Leh town

(AK Gupta, Yash Pal, RC Sharma, Anuradha Bhardwaj and Sanjay Kumar)

Sindhi (Kutch) Horses of Great Rann, Gujarat

A Team of scientists from NRCE visited Banni grasslands, a belt of arid grassland ecosystem on southern edge of Rann of Kutch, to assess the prevailing condition of locally available equids there. These grasslands are currently legally protected under the status as a Protected Forest in India. Banni grassland has Sindhi speaking Maldhari people. Most of these people are dependent on livestock for their livelihood. Livestock in this area is totally maintained on open grazing. Horses plays an important role in the Maldhari life styles by helping them to manage their livestock while grazing and providing entertainment through horse racing and riding.

NRCE team found some typical phenotypic features distinct to the other horse breeds of the country in these horses. Maldharis explain the origin of their horses from the Sindh province of Pakistan so they call these horses Sindhi. Most prevalent colors in the Sindhi horses are bay and chestnut. Most distinct phenotypic character of this breed is the typical shape of its nose- raised at its middle and dropping sharply at end of nostrils - parrot nose due to its shape.

Biometry was conducted on 10 horses by Scientists at NRCE while organizing Equine Health Camp during Banni Pashu Mela, Kutch, Gujarat on February 19-20, 2013. Average height of these horses was found to be 149 cm (Range 139-156 cm) which is slightly less than Marwari horses. Average ear to ear distance was 12.5 cm, that is more than Marwari and Kathiawadi horses and ears are straight and make about 45 to 60^0 angle to the poll. Broad chest and heart girth 167 cm, short body length 88.8 cm, straight back and long legs makes these horses

A sindhi stallion, having all qualities for a light racing horse

good race animals. Already these horses are famous among farmers of north western Indian states for their *Rewal Chaal* (a typical gait while racing)- ambling gait-making this breed a gaited horses like Marwari. Rewal in itself a different version of trot, and is faster and comfortable for the rider. As per the belief of breeders it runs rewal faster than the Marwari horses. Sindhi horses can also be seen at famous equine fair of Tilwara Rajasthan. Some good quality Sindhi horses are also found in Jaisalmar and Barmer districts of Rajasthan. Distinct phenotypic features and racing potentials of Sindhi horse, makes it essential to study its genotype to conserve it as a quality race and transport horse.

Typical nose and ears of sindhi horse

Biometric analysis of Sindhi horses of Kachh region

Biometric parametrs	Centimeters	Range
Height at wither	149.25±1.44	139-156
Heart girth	167±2.23	157-176
Body length shoulder point to pin bone	88.8±1.28	82-96
Face length	52.1±.46	50-55
Ear to ear distance	12.5±.94	5.5-15.5
Chest	30.35±1.06	25-37
Neck length	51.9±1.25	46-58
Fore leg length	87.6±.94	83-92
Fore leg canon bone length	14.9±.38	12-17
Ear to mandible base distance	26.6±.58	24-30
Ear length outer side	19.7±.30	19-22
Ear length inner	15.8±.13	15-16

Sindhi horse in rewal (typical gait)

(RK Dedar, Nitin Virmani, RK Vaid and RA Legha)

Genotyping of major histocompatibility complex (MHC) class I genes of donkeys and mules

For genotyping of MHC class-I, 315 genomic DNA samples (105 indigenous donkeys + 105 samples of exotic donkeys + 105 samples of mules) of ELA-A gene of donkeys and mules were analysed. The ELA-A locus of 800bp length were amplified using primers ELA2F & ELA3R. The PCR-RFLP analysis of the amplicons using *Hha* I and *Dde* I restriction enzymes revealed different band pattern that showed polymorphism in donkeys and mules (Fig. 15). The PCR-RFLP pattern showed its potential to group the animals into different classes and genotypes. Further, sequencing of the amplicons need to be carried out to know band pattern and diversification among donkey and mules population.

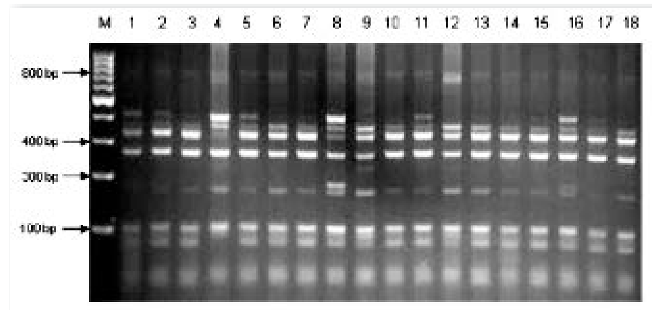


Fig. 15 : MHC I-ELA-A: Digest with *Dd*eI in exotic donkeys

(RC Sharma, Balvinder Kumar and AK Gupta)

Cloning and expression of recombinant equine chorionic gonadotropin (eCG) beta subunit

PCR amplification of eCG of mule

Equine chorionic gonadotropin (eCG) is a glycoprotein hormone composed of two dissimilar subunits: alpha and beta. The alpha subunit is common to all glycoprotein hormones (LH, FSH, TSH, CG) while the beta subunit is specific and responsible for receptor binding. However, in the equids (horses, donkeys, zebras), the Chorionic Gonadotropin have the same beta subunit as the Luteinizing Hormone. In the present study, eCG of equine was amplified by two step RT-PCR using total RNA isolated from mare's pituitary. The cDNA was synthesized from the RNA using Oligo-dT primer and beta subunit was amplified by PCR with gene-specific primer pairs (Fig. 16). The amplified PCR product (~450 bp) was cloned in TOPO-TA vector and the insert was confirmed by PCR. The recombinant plasmid was custom-sequenced and sequence data was analyzed for ORF and sequence similarity with other reported sequences. The nt sequence data showed 46-55% similarity with other reference sequences.

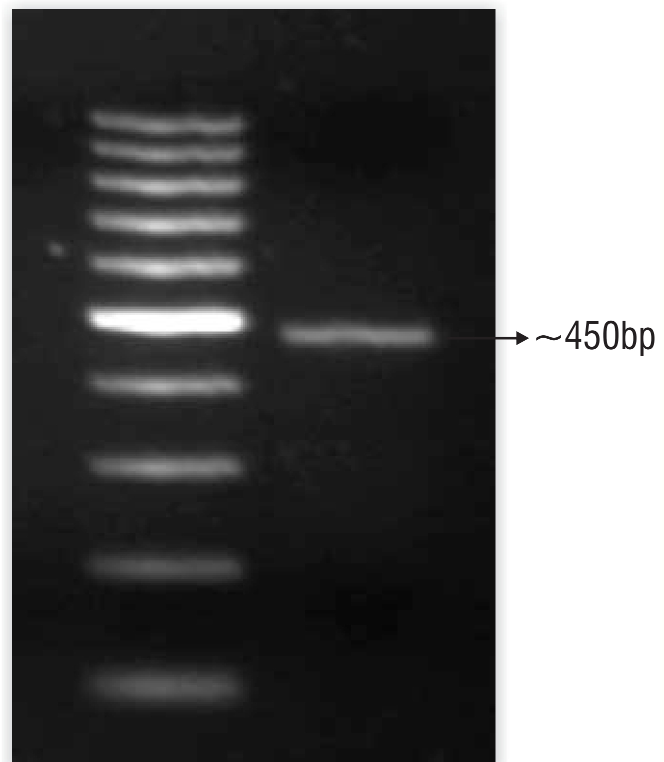


Fig. 16 : PCR amplification of eCG of mule

Expression of recombinant eCG in E coli

The gene of eCG was sub-cloned into pET 32a expression vector in *E. Coli DH5-alpha* cells. The positive clones were selected and the plasmids were isolated and checked for the presence of desired insert by PCR. The recombinant plasmids were then transformed in *E. Coli BL-21C* cells and expression of recombinant protein was carried out with 1mM IPTG after 3hrs. The expression of recombinant protein was confirmed by detecting band of about 30 kD in SDS-PAGE (12%) (Fig.17). Further characterization of the recombinant protein is in progress.

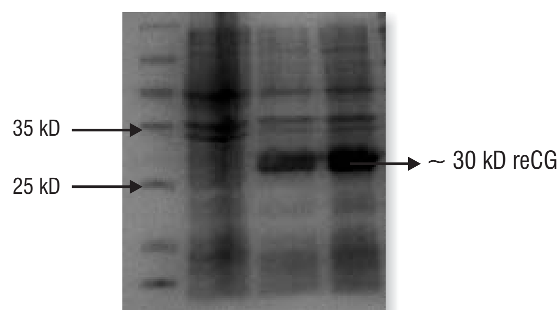


Fig. 17 : Expression of recombinant eCG.

(Anuradha Bhardwaj, AK Gupta, Sanjay Kumar and Varij Nayan)

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

The endocrine, biochemical and gene expression profile of reproductive states of Marwari mares was studied with an overall objective to analyse and improve the reproductive efficiency of mares. The adult mares and fillies (between 1-2 years of age) were selected to study the follicular dynamics during estrous cycle. The adult mares were monitored by ultrasonography during estrous cycle. The behaviour of estrous was also noted. The preliminary findings have revealed that the prevoulatory follicle grows to as large as 55 mm in the Marwari mares and the behavioural signs of estrus are more prominent as the prevoulatory follicle (Fig. 18) attains 35 mm diameter. The fillies are being trained gradually for monitoring through ultrasonography. Blood plasma is being collected twice a week and at period near ovulation, and stored at -20ºC for analysis of reproductive hormones and biochemical parameters. Further studies on RNA preparation and insemination of mares for studying the pregnancy stages are in progress.

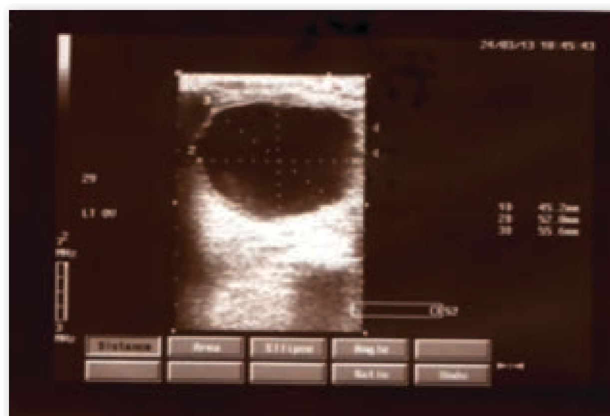


Fig. 18 : A preovulatory follicle in Marwari Mare

(Vijay Kumar, RK Dedar, SK Ravi and Jitendar Singh)

Cryopreservation of equid semen using amides

A total of 31 ejaculates from ten indigenous jacks were collected using artificial vagina. The ejaculates were filtered through gauge to remove gel fractions and fresh semen parameters were recorded (Table 5). Primary extender (citrate EDTA) was added in equal volume to gel free semen and centrifuged to obtain sperm pellet. After removal of supernatant; sperm pellet was re-suspended in secondary extender containing glycerol, dimethyl sulfoxide and dimethyl formamide as cryoprotectant at the rate 2% to its total volume. The diluted semen was kept in cooling cabinet at equilibration cum cooling period for 2 hrs and cryopreserved. Seminal parameters were observed at pre freeze stage and after freezing as given in table 6.

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 and cryopreserved semen of indigenous jacks.

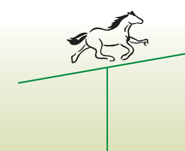


Table 5 : Fresh semen characteristics of Indigenous jacks

Total semen volume	Gel volume	Gel free volume	pH	Initial Motility (%)	Progressive Motility (%)	Conc ($\times10^6$)	Livability in fresh semen (%)
51.29	17.58	33.54	7.45	85.16	79.51	255.16	79.55
±3.70	±2.09	±2.25	±0.03	±1.52	±1.71	±9.08	±2.89

Table 6 : Pre-freeze and frozen semen characteristics of Indigenous jacks

Pre freeze motility (%)			Post thawMotility (%)			Livability in pre-freeze semen (%)			Livability in post- thaw semen (%)		
Gly	DMSO	DMF	Gly	DMSO	DMF	Gly	DMSO	DMF	Gly	DMSO	DMF
68.70	67.58	68.61	34.80	34.58	36.87	73.21	75.49	78.1	48.73	47.22	49.25
±1.76	±2.67	±2.18	±1.16	±0.99	±1.34	±2.44	±1.80	±1.92	±1.42	±1.35	±2.38

(SK Ravi, Yash Pal and RK Vaid)

Successful utilization of equine dung for preparation of vermicompost

To overcome the problem of equine dung disposal, vermicomposting in trenches was initiated and the vermicompost was prepared successfully in readymade vermibeds (Fig. 19). The vermibed units were installed under the tree shade or green net for optimum climatic conditions for vermicomposting. Sufficient slope was provided to the vermibeds so that vermiwash could be harvested easily. Before filling partially decomposed dung in vermibeds, a thin layer of neem leaves and wheat straw was spread to get good quality vermiwash from beds. After filling partially decomposed dung, the vermiculture (6-8 kg, about 800 worms/ kg vermiculture) per bed (11x4x2 feet) was mixed in upper layer. The beds were covered with jute bags or tree leaves to save the earthworms from predators and provide darkness. Vermicompost was removed from upper layer at every 10-15 days interval. It took about 90-100 days for converting equine dung in vermicompost.

During the present year, vermicompost was prepared in four portable commercially available vermibes. A total of 35 quintals of vermicompost was prepared out of which 9 quintals was supplied to NRCE, Hisar for plantation and rest was utilized at EPC, Bikaner, in trees and plants. The value of vermicompost would be ₹ 17,500 (₹ 5-/ kg) and value of multiplied earthworms is about ₹ 28,000 (₹ 300-/ Kg) if sold. All the four beds were again filled with partially decomposed equine dung and earthworms obtained from previous batch of vermicompost. Keeping in view the success of project, the activity of vermicomposting has also been started at NRCE, Hisar.

Fig. 19 : Vermicompost beds

(RA Legha and Yash Pal)

Production of mushroom (Dhingari) on mixture of equine dung and wheat straw

To utilize equine dung for income generation of equine owners, a trial for production of mushroom (dhingari) using equine dung was conducted. Spawn of Dhingari mushroom was received from SKRAU, ARS Sri Ganga Nagar. The trial was conducted in three groups *viz.* fresh equine dung, fresh equine dung and wheat straw mix (Fig. 20) and neem leaves. The medium was treated in hot water and after cooling equal quantity of spawn was mixed in each group. It

was observed that after one week spawn started spreading in all medium but growth was much faster in fresh equine dung followed by neem leaves and fresh dung. The bags were opened after two weeks. Mushroom buds started growing in group 2 from all sides of bag after 4-6 days. In group 1 and 3, growth was very slow and stunted. The cultivated dhingari mushroom was harvested, sun-dried and packed in polythene bags (Fig. 21). The study indicates that dhingari mushroom can be successfully grown on equine dung in combination with wheat straw.

Fig. 20 : Dhingari (mushroom) on mixture of equine dung and wheat straw

Fig. 21 : Sun-dried mushroom in polythene packets

(RA Legha and Yash Pal)

Preparation of silage using dry roughages for equines

In India, availability of green fodder round the year for feeding to livestock is a serious problem. The livestock has to be maintained on dry roughages during lean period when the green fodder is not available. However, the nutritive values of crop residues especially cereal crops is very poor. The livestock fed with dry roughages do not get sufficient energy, protein and other nutrients for maintenance and production purposes and animals remain deficient in major nutrients. This problem can be solved to some extent by preparing silage from dry roughages after adding some supplement for enrichment of dry roughages for feeding to equines. Generally for making unconventional silage for ruminants from dry roughages, it is soaked in a solution of 3-4% urea and 8-10% molasses is added for enrichment. However, for making unconventional silage from dry roughages for feeding to equines, the treatment of straw with urea is not advisable because of equines being the monogastric animals and it may cause toxicity. For this reason, silage was prepared by treating dry roughages with 8-10% molasses/jaggery for enhancing palatability and nutritive value (Fig. 22 & 23). The crude protein content in unconventional silage treated with urea and molasses is about 12-14%.

Fig. 22 : A Soaked wheat straw sprinkled with gur solution

Fig. 23 : Treated straw filled in silo

(RA Legha and Yash Pal)

Preparation of molasses mineral block and molasses mineral mixture for feeding to equines

In arid regions of India, equines are not getting balanced ration round-the-year because of non-availability of green fodder and good quality hay/ dry roughages. This problem can be solved to some extent by supplementing molasses mineral block or molasses mineral mixture in the diet of equines especially for working equids depending upon the availability of green fodder and good quality hay for equine feeding. Feeding of molasses mineral block or molasses mineral mixture, according to stage of animals will be beneficial for equines growth, reproduction, treatment of metabolic disorders etc. Ingredients that have been used for preparation of molasses mineral block and molasses mineral mixture include Molasses/ Gur: 28 kg, Wheat bran: 50 kg, Common salt: 3 kg, Salt sendha: 2.5 kg, Mineral mixture: 2.5 kg, Dolomite/ DCP: 5 kg & Methi powder: 10 kg (Fig. 24 & 25)

Fig. 24 : Molasses mineral mixture filled in airtight polythene bags

Fig. 25 : Molasses mineral mixture blocks

(RA Legha and Yash Pal)

Effect of combinations of dry roughage and concentrate feeding on digestibility and performance of horses (young stock) in arid region of Rajasthan

The feeding and digestibility trial using different combination of dry roughages and concentrate was conducted on pregnant Marwari mares (6 nos.) to study the voluntary feed intake and digestibility of nutrients under existing feeding practices at EPC, Bikaner. The animals were fed sewan grass kutar in dry roughage and premixed concentrate obtained from HAFED. The trial was conducted for 45 days. The mares were tested for total dry matter intake and digestibility of the feeds (Table 7 & 8).

Table 7: Effect of feeding on body weight

Mare's name	Animal ID	Initial Body weight of animals (kg)	Final Body weight of animals (kg)
Hashmita	57	360	398
Rajnigandha	29	418	425
Hemlata	42	405	415
Razia	11	371	372
Kajal	44	346	365
Payal	22	410	422
Maya	67	355	367

Table 8: Feed combination of dry roughages and concentrate

Mare's name	Concent-rate Intake (kg)	Roughage Intake (kg)	Total Dry matter intake (kg)	DMI (%) bwt. (kg)
Hashmita	3.62	4.50	8.12	2.26
Rajnigandha	2.95	5.81	8.77	2.10
Hemlata	3.62	5.38	9.00	2.22
Razia	0.99	6.43	7.41	2.00
Kajal	3.21	4.50	7.71	2.23
Payal	3.62	5.38	9.00	2.19
Maya	2.26	5.29	7.55	2.13

(RA Legha, PA Bala and NV Patil)

Survey on management systems and utilization of donkeys and mules for sustainable livelihood

Working equines mainly donkeys and mules play a fundamental role in improvement of livelihood as these animals transport everything from people, farm produce, construction materials, market products, water, clay, fodder, manure and fuel. Moreover, donkeys are the cheapest option of power for draft purpose and transportation, able to survive heat and dehydration and can obtain nutrients and energy from poor quality foods making them suitable for harsh environment condition in arid, semi-arid regions and difficult working conditions like hilly terrain. A survey was undertaken to analyse the socio-economic status of these working donkey and mule owners. The survey was conducted using pre-tested structured interview schedule. The survey data was collected from respondents in Rajasthan (31 nos.), Haryana (36 nos.) on socio-economic status of equine owners, prevailing management practices and utilization pattern of donkeys and mules.

A perusal of socio-economic status (Table 9 & Fig. 26) indicates that majority of respondents (66.53%) were from middle age group between 26-46 years, belonging to SC category (85.48%). Literacy level was found low as 53.81% respondents were illiterate. Most of the respondents (69.53%) were having medium level of experience in equine husbandry and were keeping equines since 8 to 21 years. With regard to family size 72.76% respondents were having medium family size (4 to 8 members). Majority of respondents (65.37%) were having monthly income between ₹ 4000 to 9000.

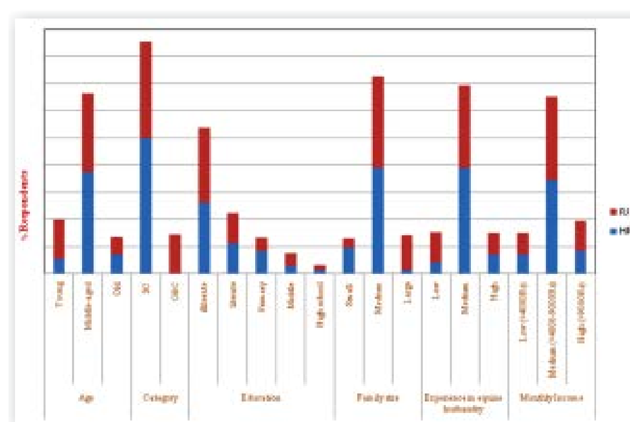


Fig. 26 : Socio-economic profile of respondents

Table 9 : Socio-economic status of the donkey and mule owners

Sl. No.	Characters	Category	Percentage of respondents		
			Haryana (n1=36)	Rajasthan (n2=31)	Total (N=67)
1	Age	Young	11.11	29.03	20.07
		Middle-aged	75.00	58.06	66.53
		Old	13.89	12.90	13.40
2	Category	SC	100.00	70.97	85.48
		OBC	0.00	29.03	14.52
3	Education	Illiterate	52.78	54.84	53.81
		Literate	22.22	22.58	22.40
		Primary	16.67	9.68	13.17
		Middle	5.56	9.68	7.62
		High school	2.78	3.23	3.00
4	Family size (member/family)	Small (<4)	19.44	6.45	12.95
		Medium (4-8)	77.78	67.74	72.76
		Large (>8)	2.78	25.81	14.29
5	Experience in equine husbandry (Years)	Low (<8)	8.33	22.58	15.46
		Medium (8-22)	77.78	61.29	69.53
		High (>22)	13.89	16.13	15.01
6	Monthly Income	Low (< ₹ 4000)	13.89	16.13	15.01
		Medium (< ₹ 4000-9000)	69.44	61.29	65.37
		High (> ₹ 9000)	16.67	22.58	19.62

Ownership and utilization pattern of donkeys and mules

The majority of owners (68.64%) were using their mules and donkeys in cart transportation whereas 31.36 % were using donkeys as pack (Fig.27). The mules and donkeys were used in different activities as depicted in Table 10 viz. transport of farm produce and inputs (45.34), construction material (59.09%), market products (53.85), water and clay (10.39%), fodder (31.33%), manure (13.89%) and fuel wood (11.11%). The donkeys used as pack were mostly utilized for transportation of clay and construction material Fig. 27 & 28.

The mule cart is used to transport 800-12000kg of goods at one time and charges about ₹ 25-35 for 50 kg bag for transportation of goods and market produce. The daily earnings of mule cart owners ranges between ₹ 500-800. Whereas donkey cart is used to transport 200-500 kg load at ₹ 20-35 for 50 kg of goods depending upon distance and goods. Daily earnings of mule cart owners ranges between ₹ 300-500. The respondents who were using donkeys as pack animals were charging money depending on the amount of material and distance of place where it is to be transported. The pack donkey owners daily earnings ranges between ₹ 200-300 per donkey. The per day earnings of mule owners was found higher as compared to daily earning of donkey owners.

Table 10 : Use of donkeys and mules in different activities

Use in activity	Haryana (%) (n1=36)	Rajasthan (%) (n1=31)	Total (%) (N=67)
Construction material	47.22	70.97	59.09
Farm produce & inputs	77.78	12.90	45.34
Water & clay	11.11	9.68	10.39
Manure	27.78	0.00	13.89
Fuel wood	22.22	0.00	11.11
Fodder	52.78	9.68	31.23
Market products	72.22	35.48	53.85

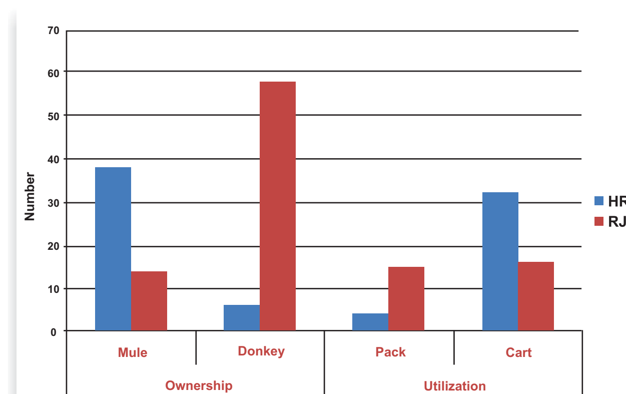


Fig. 27 : Ownership & utilization pattern

A: Donkeys carrying stones

B: Family moving with their donkeys and goods

C : Donkeys ready for work at brick kiln

D : Donkey cart carrying goods from market

E : donkeys carrying construction material

F : Donkeys dumping construction material

Fig. 28 : Utility of donkeys in different activities

Management practices: Grooming practices were followed regularly by most of the respondents. With regard to shoeing of working equines the mules were shoed regularly but shoeing was not observed in case of donkeys. Deworming was not a regular practice. The animals were not vaccinated by the respondents in the study area.

Feeding and Housing Pattern: It was found that mostly donkeys were allowed to graze in open; however working mules and donkeys in some areas were provided locally available dry fodder and concentrates. Housing was in temporary *kaccha* thatched roof (Fig. 29). The feeding of donkeys is based on grazing on community pastures and on roadside verges. Donkeys survive due to their tremendous capacity to utilize foods of low quality. The feed provided included groundnut *bhusa*, wheat *bhusa*, *gram bhusa* in Rajasthan. Whereas in Haryana wheat straw,

A : Housing in temporary brick shed (Jodhpur, Rajasthan)

B : Housing in brick wall with tin shed at brick kiln (Pali, Rajasthan)

C : Feeding of green fodder in shed (Bhani Chanderpal, Haryana)

D : Feeding of fodder in feed trough in shed (Madina, Haryana)

E : Housing with thatched roof with guny bags (Maham, Haryana)

F : Kaccha housing of donkeys (Bhani Chanderpal, Haryana)

Fig. 29: Housing, feeding and management practices

wheat barley and barn mainly as dry fodder and dhoob grass, berseem was feed as green fodder. The equine owners were also providing additional supplements like Jaggery occasionally and oil in winter season to their animals in both the states.

Constraints faced by the respondents: The major constraints faced by donkey and mule owners in equine husbandry and livelihood were high cost of feed and concentrates, lack of adequate employment, competition with mechanized vehicle, lack of interest in keeping donkeys and mules by children, lack of availability of common pasture land, non availability of good donkey stallion for breeding, lack of social recognition, non availability of bank loan/ schemes for purchase of equines, no insurance schemes for equines.

The survey indicated that the working equines are mostly overworked, undernourished, and not well cared which leads to low work outputs and reduces their work efficiency and life span. The situation is often compounded by a lack of accessible and affordable animal health services. There is a need of intervention to improve the welfare of equines needs to address these issues with the active involvement of equine owners in participatory manner. The adoption of good equine health, welfare, and working practices is an important means for traditional equine owning communities to secure their income and sustainable livelihood for their family.

(AA Raut, Yash Pal, RA Legha, Ramesh Dedar and Jitendar Singh)

VTCC
Accomplishment

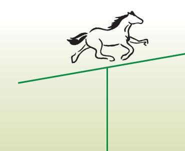
Culture Collection: At a glance

The Veterinary Type Culture Collection (VTCC) was established at NRCE campus in 2004 as a national repository of animal microbes including dairy and rumen microbes aimed at (i) exploration and collection of microorganisms of animal origin/significance/relevance, (ii) central storage of animal microbes from existing culture collection centers, institutions and universities, (iii) characterization, documentation and digitization of microbial database of cultures of animal microbes, (iv) development of a National Microbial Gene Bank for conserving the biodiversity of animal microbes, and (v) conservation and utilization of microorganisms. This will serve as a microbial genetic resource centre, which will provide necessary impetus to development of the livestock sector. 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 further research and development in the field of microbiology, taxonomy, biotechnology, epidemiology and vaccinology. Detailed achievements are described as below-

a) **Veterinary microbes component:** Veterinary microbe component of VTCC characterized and reposited many important virus, bacteria, recombinant clones and phages as mentioned below-

(i) Viral isolates: The repository has been strengthened with the addition of twenty one viral isolates from different animal species viz., bovine, ovine, equine, canine and poultry. At present, one hundred and twenty four (124) viral isolates of different livestock species have been accessioned and preserved in freeze dried form in the repository. Accessioned cultures include viral isolates *viz.,* buffalopox virus, camelpox virus, goatpox virus, sheeppox virus, equine influenza virus, equine rotavirus, Japanese encephalitis virus, bovine and human rotavirus, bovine herpes virus-1, and equine herpes virus-1 & 4, Newcastle Disease virus canine parvovirus, canine adenovirus, Fowl adenovirus and street rabies virus.

(ii) Bacterial isolates: A total of 627 economically/ scientifically important bacterial isolates are accessioned & stored at -70°C. A total of 187 bacterial cultures have been accessioned during the period under report. More than 300 bacterial isolates obtained have been preserved. Phenotypic/genotypic information including microscopic cell morphology photographs, 16S rRNA and other specific gene sequences and clones of isolates were generated. Accessioned cultures include *Bordetella bronchiseptica, Actinobacillus equulli, Brucella melitensis* biovar I, *Brucella melitensis* biovar II, *Pseudomonas aeruginosa, Bacillus spp., Streptomyces spp., Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Staphylococcus sciuri, Staphylococcus hyicus, Shigella spp, Pseudomonas putida, Flavobacterium* spp., *Enterobacter* spp, *Micrococcus* spp., *Corynebacterium* spp, *Rhodococcus equi,* Atypical *Rhodococcus equi, Salmonella* spp., *Aeromonas* spp, *Serratia marcescens, Bacillus subtilis, Edwardsiella tarda, Enterobacter cloacae, Aeromonas hydrophila, Campylobacter coli, Citrobacter freundii, Enterobacter aerogenes, Listeria monocytogenes, Moraxella (Branhamella) bovis, Moraxella (Branhamella) catarrhalis, Moraxella (Branhamella) osloensis, Pasteurella multocida* subspp. *multocida, Pasteurella*



pneumotropica, Yersinia enterocolitica, Nocardia asteroids, Salmonella enteritidis, Salmonella typhimurium, Streptococcus spp., *Pasteurella* spp., *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp., *Klebsilella* spp. and *Aeromonas* spp.

(iii) Fungi/Yeast: Many fungi and yeast have been isolated from the clinical cases and deposited in the repository. Around 93 fungi and yeast cultures available in different Network Units of the repository includes-*Candida albicans, Dermatophytes, Trichophyton sp. , Microsporum sp., Epidermophyton sp., other keratinophilic fungi-Aphanoascus fulvescens, Nattrassia mangifera, Non-dermatophytes, Aspergillus niger, Fusarium spp., Curvularia spp., Bipolaris spp., Syncephalastrum spp., and Sepedonium spp. Malassezia pachydermatis.*

(iv) Recombinant clones and Phage library: Specific genes of viral and bacterial isolates cloned, sequenced and validated by homology searching with available sequences. At present, 267 recombinant clones and 27 bacteriophages have been accessioned and preserved in the repository, out of which 76 clones were reposited during current period under report and 15 clones are ready to be deposited.

b) **Rumen microbes component:** Important rumen microbes (140 Nos.) have been isolated, characterized, accessioned and reposited in the VTCC repository. Important bubaline rumen fungi such as *Anaeromyces* spp., *Orpinomyces intercalaris and Orpinomyces joyonii* and caprine isolates: *Piromyces* spp*. and Neocallimastix* spp. have been isolated and preserved. Several rumen bacteria such as *Bacillus licheniformis, Butyrivibrio* spp., *Eubacterium limosum, Megasphaera elsdenii, Prevotella* spp., *Streptococcus bovis, Streptococcus equines, 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* spp. These isolates have also been further analyzed for useful biological activities such as fibre and protein degradation (*Prevotella* spp., *Butyrivibrio* spp.); urea hydrolysis (*Megasphaera spp.); tannin degradation (Streptococcus spp.);* bacteriocin production *(Bacillus)* etc. Nineteen (19) anaerobic bacteria and seventy six (76) fungi have been accessioned and preserved in the repository.

c) **Dairy microbes component:** A total of 490 bacterial cultures/isolates are available in the dairy microbes repository, out of which 307 cultures are accessioned of which 89 cultures were isolated, characterized and accessioned during the period. Accessioned cultures include- *Lactococcus* spp, *Leuconostoc* spp, *Streptococcus thermophilus and Lactobacillus* spp, *Lactococcus lactis* ssp *lactis, Leuconostoc lactis, Lactobacillus fermentum, Bifidobacterium dentium, Bifidobacterium longum, Leuconostoc mesenteroids* ssp *mesentroids* , *Leuconostoc mesenteroids* ssp *cremoris, Leuconostoc* spp, *Leuconostoc mesenteroids* ssp *para mesentroids, Lactococcus lactis* ssp *diacetylactis, Micrococcus lactis, Lactococcus lactis* ssp *cremoris, Lactococcus lactis* ssp *lactis bv. Diacetylactis, Streptococcus thermophilus, Kluyveromyces lactis, Saccharomyces bisporus* etc.

During the year 2012-13, the viral repository increased its accessioned culture collection to 1630. The repository has been strengthened with the addition of twenty one viral isolates, 187 pathogenic bacteria, 45 rumen bacteria, 100 dairy microbes, 76 recombinant clones and 138 genomic DNA of bacteria from different animal species *viz.*, bovine, ovine, equine, canine and poultry. Year wise accessioning of veterinary, rumen and dairy microbes are presented (Table 11, Fig. 30). The VTCC repository has been strengthened with reposition of different pathogenic virus isolates (Fig. 31) and several bacterial species (Fig. 32) covering large number of host species (Fig. 33). The cultures deposited in the repository are from the different states of the country (Fig 34).

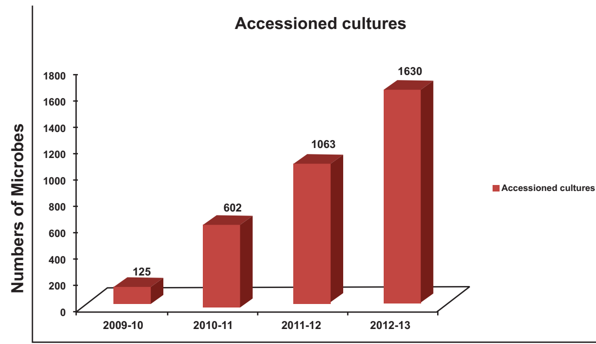


Fig 30. Year wise accessioning of cultures

Table 11: Status of Accessioning of Cultures

Microbial Resource	2009-10	2010-11	2011-12	2012-13
Veterinary Microbes	24	347	543	751
Rumen Microbes	0	0	95	140
Dairy Microbes	40	118	207	307
Recombinant clones	34	110	191	267
Genomic DNA	0	0	0	138
Phage library	27	27	27	27
Grand Total	125	602	1063	1630

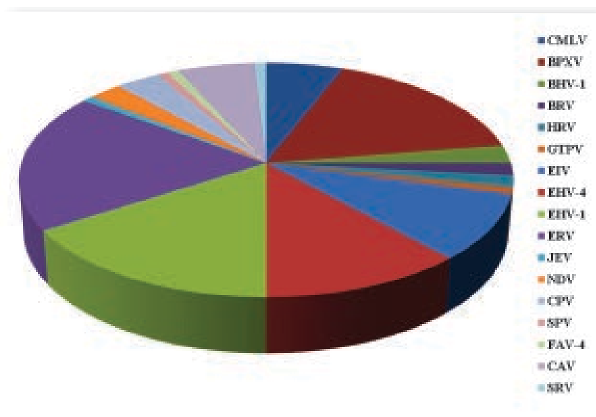


Fig 31. Distribution of viral isolates

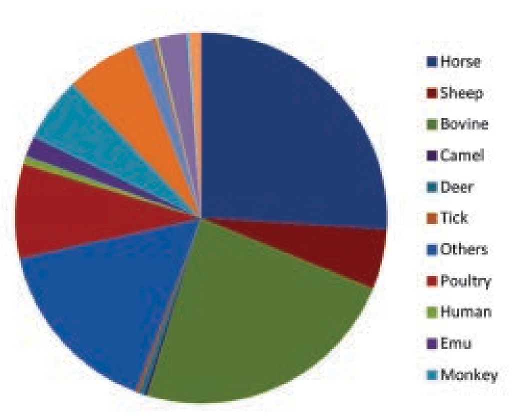


Fig 33. Host-wise distribution of bacteria

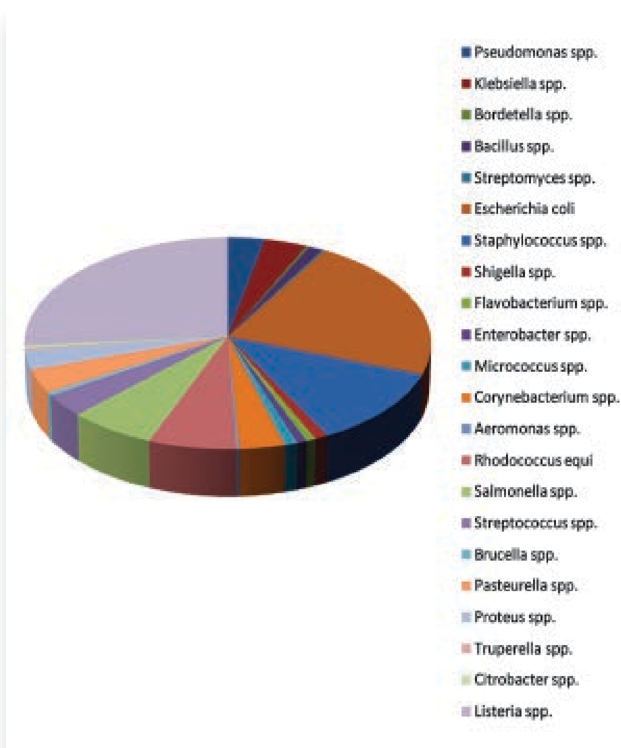
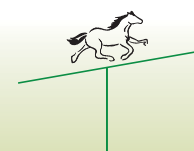


Fig 32. Genus-wise distribution of bacteria

Fig 34. State-wise distribution of cultures reposited in VTCC



Isolation and Cryopreservation of Viruses in the repository

Four equine rotaviruses have been isolated in MA-104 cell line from foal diarrhea cases in Tohana, Hisar. The virus was also confirmed by amplification of the VP7 gene of rotavirus. One sheep pox virus could also be isolated in Lamb Testicle primary culture from sample received from Palampur, H.P. SPV was further confirmed by amplification of the B2L gene. More than 1200 ampoules of different virus isolates *viz*., EHV-1, NDV, BPXV, BRV, HRV, SPV, ERV and CAV isolates have been preserved in freeze dried form as well as in -80^0 C deep freezers in the repository.

Detection of Classical swine fever (CSF) in field outbreaks

Classical swine fever (CSF) is a highly contagious viral disease of swine caused by classical swine fever virus (CSFV), a member of the genus Pestivirus within the family Flaviviridae. The disease is prevalent in many parts of the globe including in India with majority of the outbreaks being reported from North Eastern states. A total of 26 samples collected from various outbreaks of Haryana and Delhi were tested for CSFV targeting NS5B and E2 genes. Specific amplicons of 424 and 309 bp were observed in positive samples for NS5B and E2 genes respectively. Out of 26 samples tested, 15 were positive for CSFV. Sequencing of the positive samples is to be carried out for ascertaining the genotype of the currently circulating virulent virus.

Fig. 35 Amplification of CSFV

Marker 100 bp, Lane1 = Lymphnode, Lane2 = Spleen, Lane 3 = Positive control (IVRI challenge virus), Lane4 = Negative control

Molecular identification/characterization of viral isolates/field samples

A number of viral isolates *viz.*, canine adenovirus, canine parvovirus, fowl adenovirus, equine influenza and street rabies virus were identified by amplification of virus-specific region by PCR for authentication and subsequently preserved in the repository.

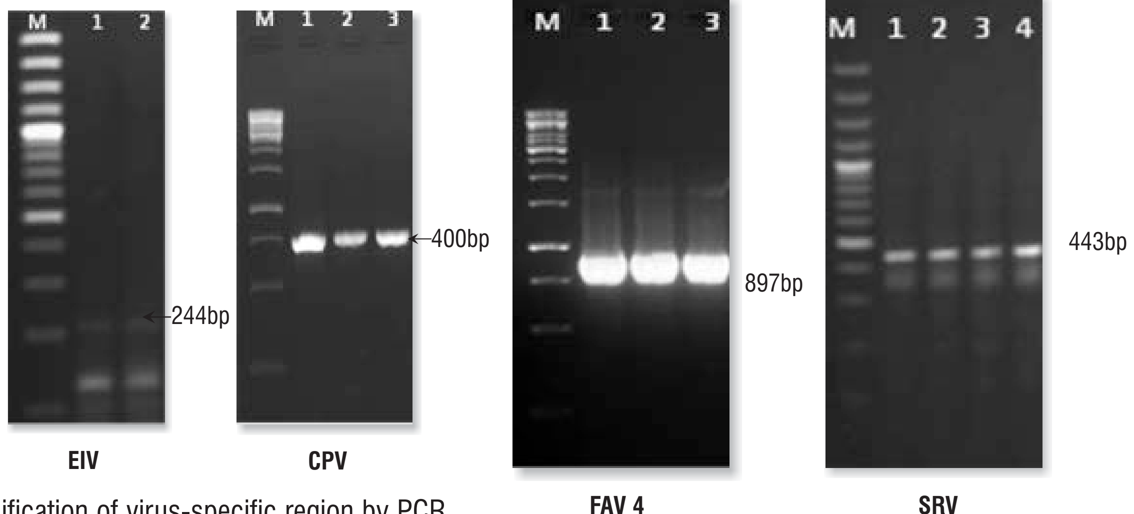


Fig. 36. PCR amplification of specific-genes of different virus isolates

Sequence analysis of serine protease inhibitor 1 (SPI- 1) gene of buffalopox virus isolated from buffaloes, cattle and human

Buffalopox is an emerging contagious viral zoonosis of domestic buffaloes (*Bubalus bubalis*) which also infects cattle and humans. The disease is caused by buffalopox virus (BPXV) - a close variant of vaccinia virus (VACV)

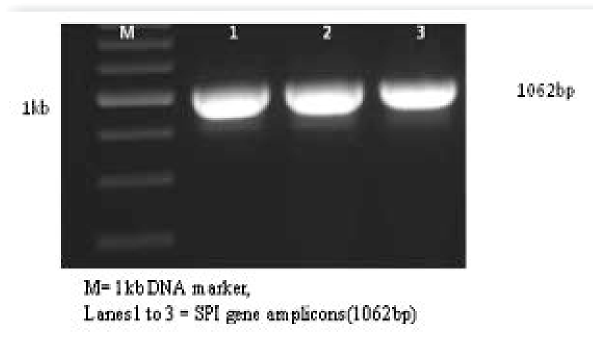


Fig. 37. PCR amplification of serpin 1 (SPI -1) gene of BPXV isolates from buffalo, cattle and human.

being recognized as an occupational zoonosis due to the naive population against orthopoxviruses. Lack of information on host tropism of BPXV, led us to analyze the host range serpin-1 (SPI-1) gene of BPXVs isolated from outbreaks (2010 & 2011) in buffaloes, cattle and human in Maharashtra and Uttar Pradesh. The encoded protein of this gene is expressed in the early stages of infection and acts as anti-apoptosis factor in vaccinia virus. The virus was earlier isolated in Vero cells from infected scabs collected from animals and humans and the extracted viral DNA was subjected to PCR amplification of serpin 1 gene (Fig. 37). The amplicons were cloned in pTZ57R/T vector and sequenced commercially. An open reading frame (ORF) nucleotide sequence homology search was carried out using the NCBI BLAST. Sequence analysis revealed that BPXV isolates (buffalo, cattle and human) shared maximum

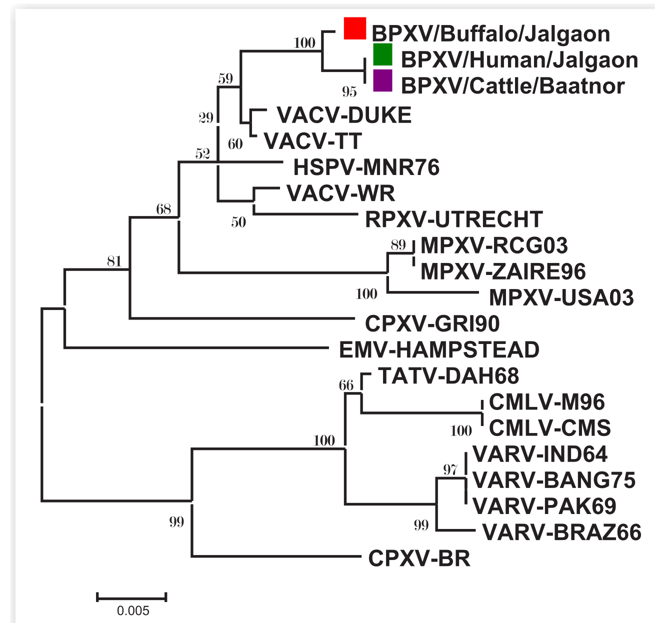
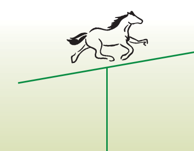


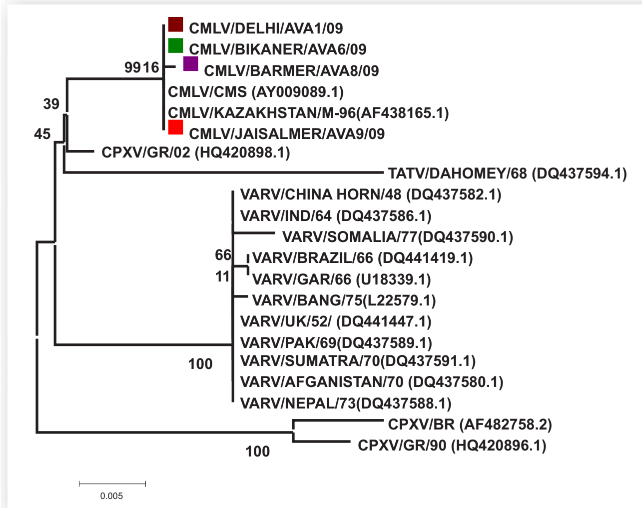
Fig. 38. Phylogenetic of SPI-1 gene of BPXVs

homology (99% at nt and 98.5% at aa level) among themselves as well as with VACV. Furthermore, phylogenetic analysis exhibited closest homology with VACV isolates followed by RPXV, CPXV, HSPV, Contagalo virus, MPXV and VARV (Fig. 38). The high degree of sequence similarity and close evolutionary relationship with VACV and other poxviruses indicates the conserved nature of the gene among Orthopoxviruses which could play a role similar to VACV in viral pathogenesis. This is the first report of genetic analysis of anti-apoptosis gene of buffalopox virus which will be useful in elucidating the host antiviral response.

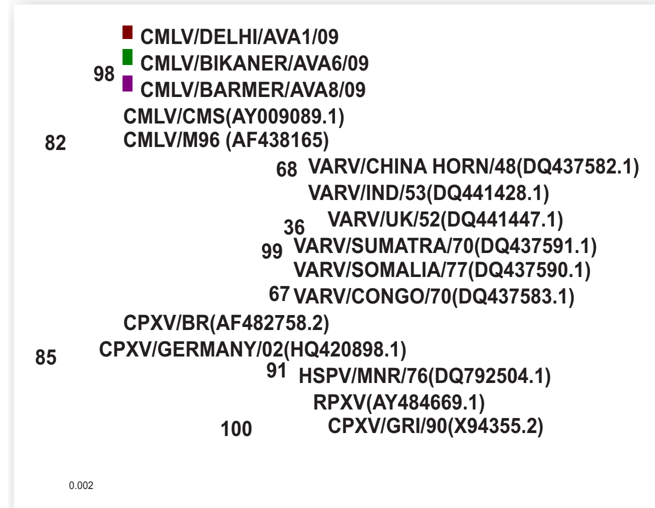
Cloning, Sequencing and phylogenetic analysis of host -range genes of camelpox virus (CMLV) isolates from outbreaks in Delhi, Bikaner, Barmer and Jaisalmer, Rajasthan

Six host - range genes viz., B5R, B7R, B9R, CBP, crmB & crmE of four isolates of CMLV from camels (CMLV/camel/Delhi/09, CMLV/camel/Bikaner/09, CMLV/camel/Barmer/09 & CMLV/camel/Jaisalmer/09) were PCR amplified, cloned and sequenced. Homology analysis revealed high similarity (~95- 100% at nt and ~92-100% at aa level) with CMLV and VARV isolates. However, B9R & crmE gene sequences were not observed in any VARV isolates, which indicates that inactivation of these two genes may increase the virulence of CMLV. Three significant point mutations (Q52K; I66N & S83F) were observed in CBP gene of Delhi isolates along with VARV. One mutation N63S was observed only in Delhi isolate. Analysis of phylogenetic trees revealed clustering of CMLV isolates together with VARV. This is the first report of genetic analysis of host - range genes of Indian CMLVs which will be useful in elucidating the host antiviral response.

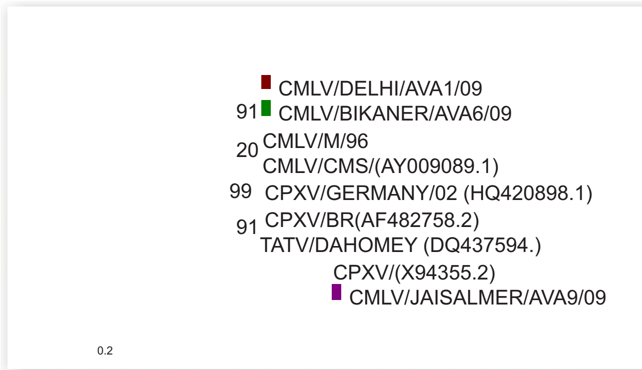




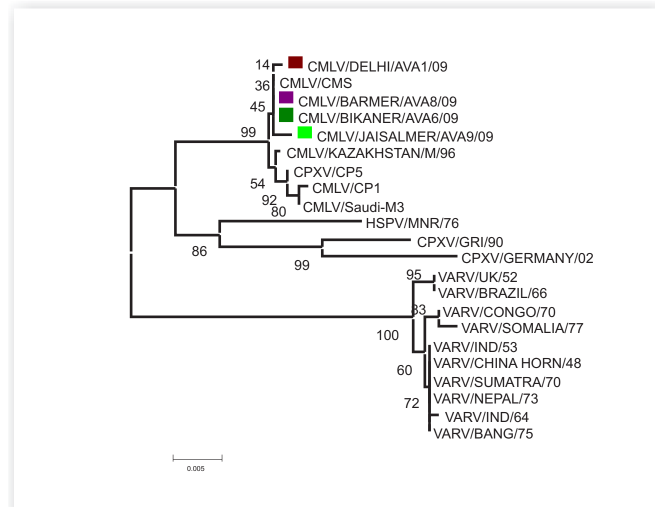
A : Phylogenetic tree of B5R gene



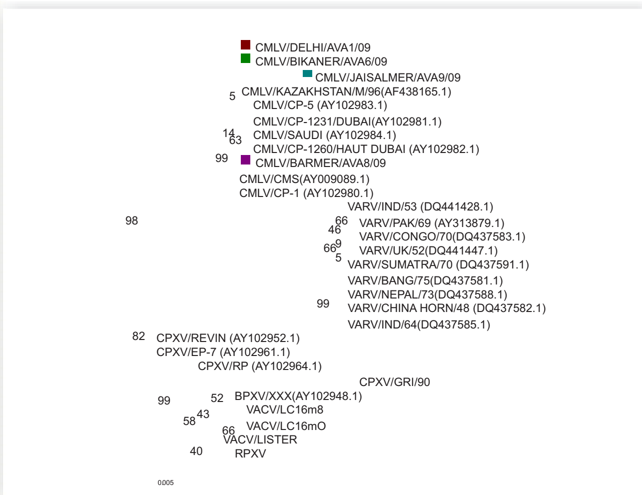
B : Phylogenetic tree of B7R gene



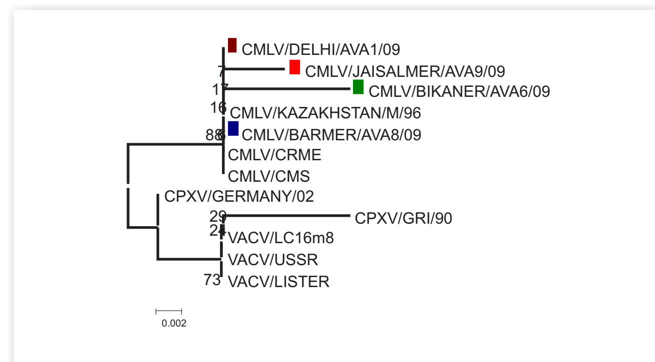
C : Phylogenetic tree of B9R gene



D : Phylogenetic tree of crmB gene



E : Phylogenetic tree of CBP gene



F : Phylogenetic tree of crmE gene

Fig. 39. Phylogenetic trees of host-range genes of camelpox virus isolates.

Investigation of PPRV in donkey, sheep and goat samples from villages surrounding Bikaner

A small survey was conducted to ascertain the status of PPR virus and its antibodies in field animals living in close proximity, in the aftermath of the recent infections occurring in unrelated animal species. In this context, sixty biological samples from donkeys, sheep & goats collected from villages surrounding Bikaner were processed for detection of PPR virus antigen and antibodies. None of the 11 donkey samples were found to be positive for PPR antigen or antibodies by sandwich and competitive ELISA, respectively. Out of the 28 sheep samples, 5 were positive for PPRV antigen by sandwich-ELISA as well as by RT-PCR targeting the N-gene, while 19 were positive for PPRV antibodies by c-ELISA. Analysis of the 21 goat serum samples, yielded 5 positive samples for PPR antigen while only 4 were positive for PPRV antigen by RT-PCR targeting the N-gene (Fig. 40). Besides, 16 goats were positive for PPRV antibodies by c-ELISA. Six animals [Sheep (3) & Goats (3)] were positive for both PPR antigen and antibodies. Although none of the donkey samples were positive for PPRV, the presence of PPRV antigen and antibodies in small ruminants, (both sheep and goats) establishes them to be the primary foci of PPRV infection which could act as reservoirs for the transmission of the virus to other unrelated animals living in close proximity in the field conditions.

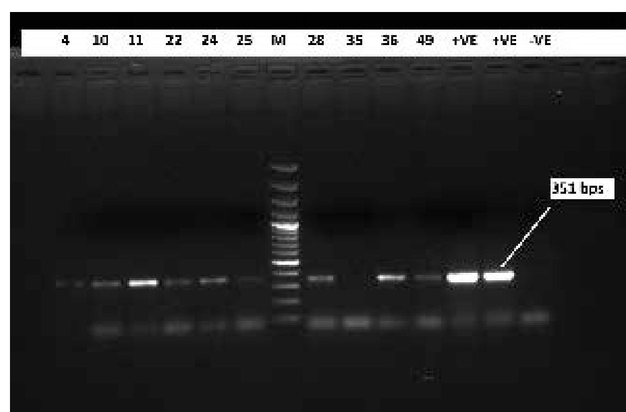


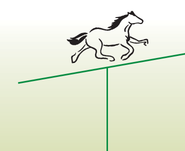
Fig. 40. RT-PCR for detection of PPRV targeting N-gene. Lanes: Samples: 4, 10, 11, 22 24, 25, 28, 36, 49- PPRV positive samples; Sample: 35 – negative

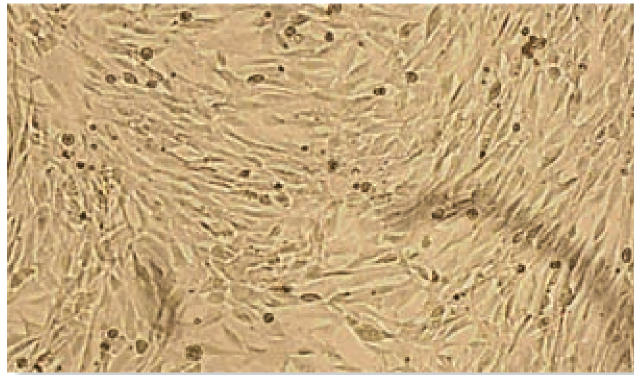
Cell lines/Primary culture maintained/ preserved in the repository

The eleven different cell lines along with one primary culture are being maintained for isolation of different viruses in the repository. Furthermore, these have also been preserved in liquid nitrogen for future use. Details of the different cell lines has been depicted in table no. 12 and figure 41.

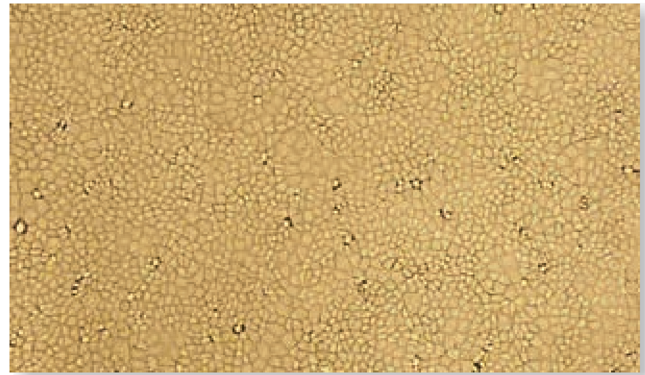
Table 12. Different cell lines present in VTCC repository.

Sl No	Cell line	Name	Host	Origin tissue
1	VERO	African Green Monkey Kidney	Monkey	Kidney epithelium
2	MDBK	Madin Darby Bovine Kidney	Bovine	Kidney Epithelium
3	MDCK	Madin Darby Canine Kidney	Dog	Kidney Epithelium
4	BHK21	Baby Hamster Kidney	Hamster	Kidney Fibroblast
5	RK13	Rabbit Kidney	Rabbit	Kidney tissue
6	HELA	Human Epidermoid Carcinoma	Human	Cervical cancer Epithelium
7	PK15	Porcine kidney	Pig	Kidney Epithelium
8	HEP2	Human Negroid cervix carcinoma	Human	Cervix carcinoma Epithelial
9	MRC5	Human foetal lung	Human (foetal)	Lung Fibroblast
10	MA104	Embryonic Rhesus Monkey kidney	Monkey	Embryonic kidney Epithelium
11	PS	Porcine Stable	Pig	Kidney tissue
12	LT	Lamb Testicle	Lamb	Testicle tissue

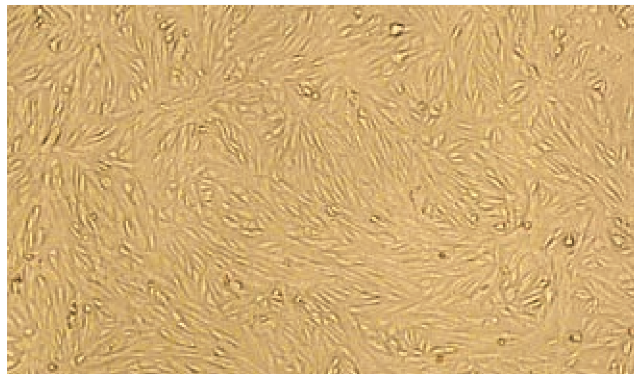




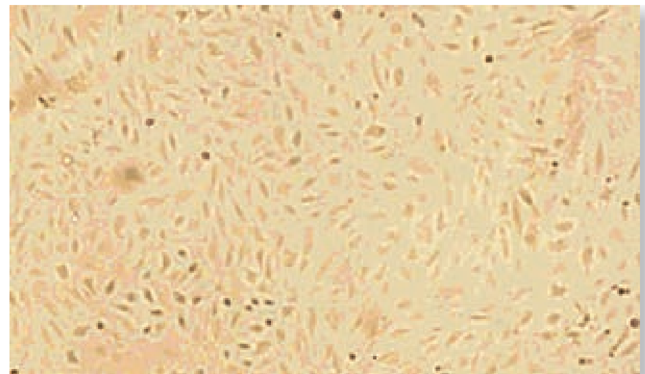
BHK cell line



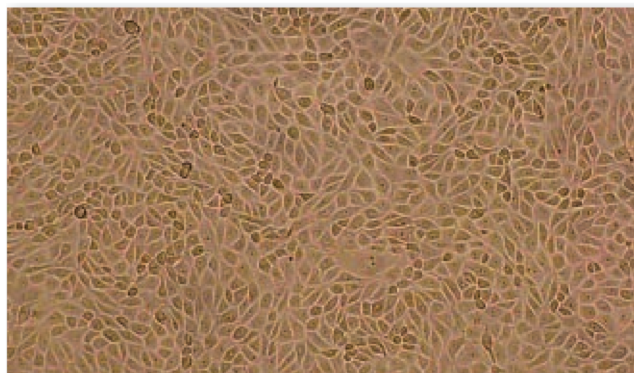
MDCK cell line



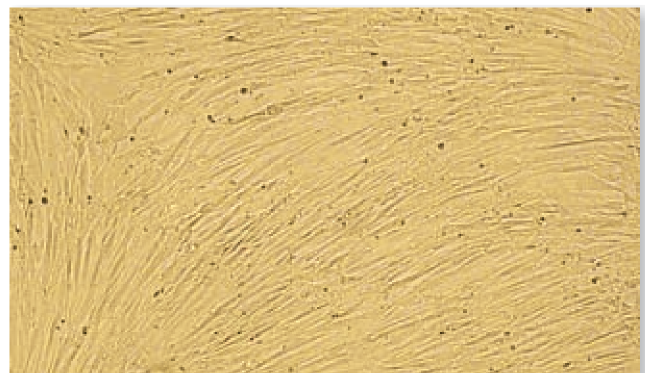
VERO cell line



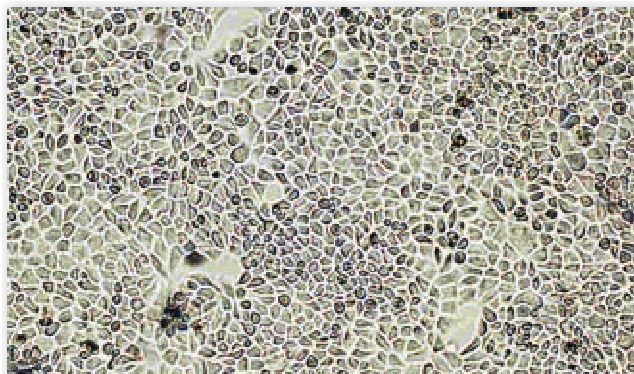
LT cells



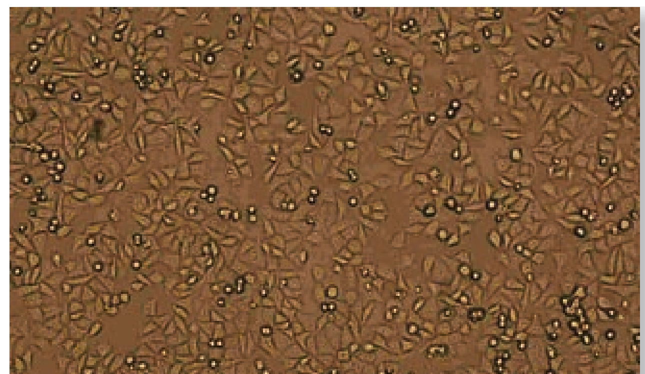
NLBK cell line



MRC5 cell line

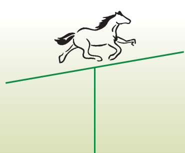


HELA cell line



HEP2 cell line

Fig. 41. Different cell lines reposited in the VTCC respiratory



Development of recombinant clones library of different genes of animal viruses

Forty three (43) recombinant clones of specific genes of camelpox virus, equine influenza virus and buffalopox virus have been generated by cloning into different vectors. The generated clones were accessioned, preserved and are being maintained in the VTCC repository.

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

Isolation and Identification of new bacteria

Four new bacteria have been isolated from samples collected from different geographical regions of the country. The isolates were confirmed by several biochemical tests and by cloning and sequencing of 16S rRNA genes of the isolates. The new isolates include:

i. *Nocardia otitidiscaviarum* from equine granulomatous pneumonia,

ii. *Moraxella ovis* from sheep from J & K,

iii. *Bordetella bronchiseptica* isolate from pneumonic foal in Pushkar, Rajasthan,

iv. *Delftia* spp. from water sample from sheep watering hole in Gulmarg, J & K.

First isolation of *Nocardia* spp. from Equine Pulmonary Nocardiosis

Significant new isolations have been made of *Nocardia asteroides* complex from equine granulomatous pneumonic lungs from case of Equine pulmonary nocardiosis. The isolate has been identified as *Nocardia otitidiscaviarum* after phenotypic, biochemical studies and 16S rRNA gene sequencing. This is the first report of *Nocardia otitidiscaviarum* from horse in India, although *N. otitidiscaviarum* has been identified in horses affected by pleuritis and pneumonia, however a worldwide reference search has been elusive. The isolation was made from PM lung sample, which grew classical minute, white, powdery non-hemolytic embedded colonies on Sheep Blood Agar (Fig. 42). Nocardiae are present in most environments and

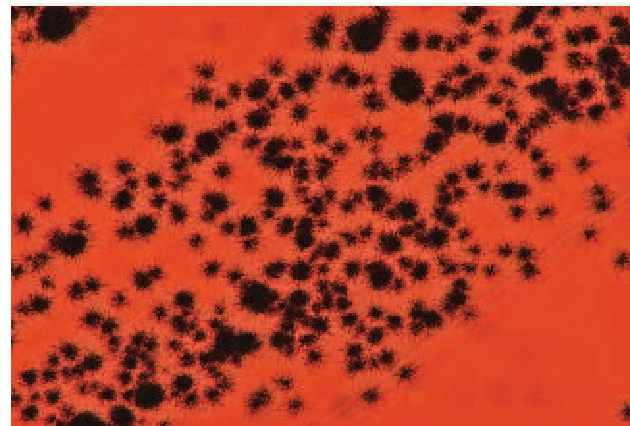


Fig. 42. 17 hr *Nocardia asteroides* on MSA see star like projections of individual colonies, 4x

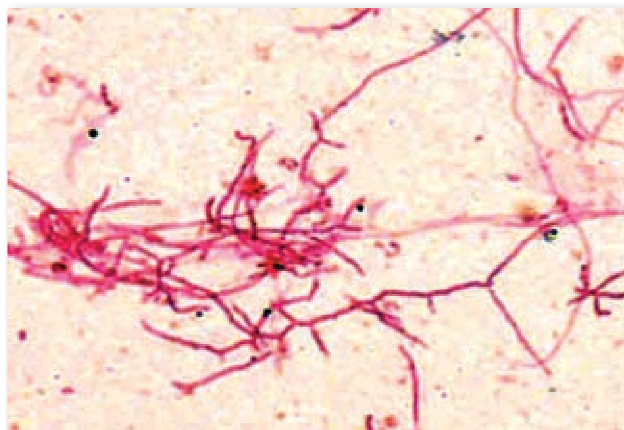


Fig. 43. Acid fast *Nocardia* spp. showing branching filaments (mod. AF stain 1000x)

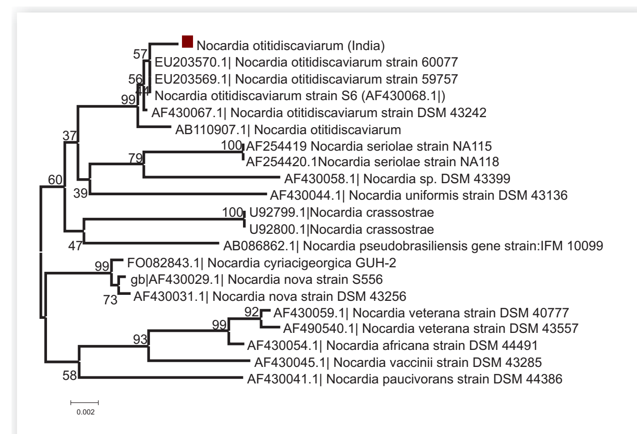
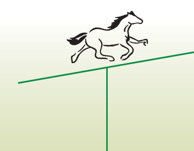


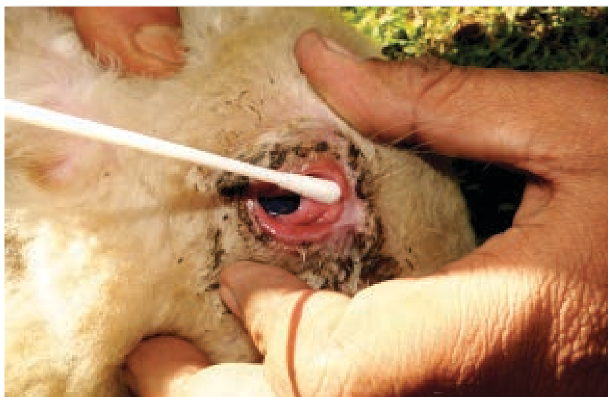
Fig. 44. Phylogenetic analysis of *Nocardia otitisdiscaviarum*



is extensively distributed in water and soil. The infection may have been acquired through inhalation, and in the horse this opportunistic infection appears to be as a result of some constitutional or immune-suppressive disturbances. Gram stains from tissue revealed gram-positive branching filament, which were confirmed by modified acid-fast staining (Fig. 43). Phylogenetic analysis of the 16S rRNA sequence grouped Indian isolates with other isolates of *N. otitidiscaviarum* available in the database (Fig. 44). The isolate has been reposited in VTCC repository.

Isolation and identification of *Moraxella (Branhamella) ovis* from Infectious keratoconjunctivitis in sheep

Ovine infectious keratoconjunctivitis, commonly known as pinkeye, is a disease of small ruminants, responsible for economic losses as a result of low weaning weights or blindness and the value of stock decreases. The disease can be identified by redness in the outer edges of the eye, squinting, tearing and clouding of the cornea (Fig. 45). Pinkeye-associated bacteria are opportunistic members of the normal ocular flora and cause disease following injury to the cornea by UV rays, dirt, insects and feed particles. The disease ovine infectious keratoconjunctivitis is caused by *Moraxella* (*branhamella*) *ovis* (Lindquist 1960) and other members of the genus. The *Moraxella ovis* was isolated from cases of keratoconjunctivitis in sheep from a nomadic herd in remote Gulmarg valley (J & K). *Moraxella* are short, plump, Gram-negative rods, characteristically observed in pairs (Fig. 46). The plump rods almost look like cocci (Fig. 46). The isolate was further confirmed by sequence comparison and phylogenetic analysis of the 16S rRNA gene (Fig. 47). The Oa3A strain accessioned as VTCCBAA498, and deposited in the VTCC repository.



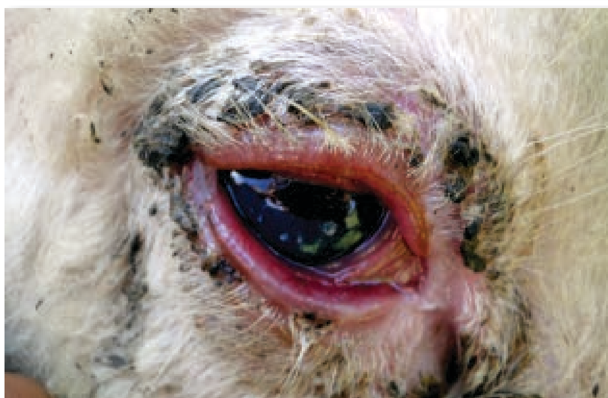


Fig. 45. Infectious bovine keratoconjunctivitis (IBK) infection in sheep showing redness in the outer edges of the eye, squinting, tearing and clouding of the cornea.

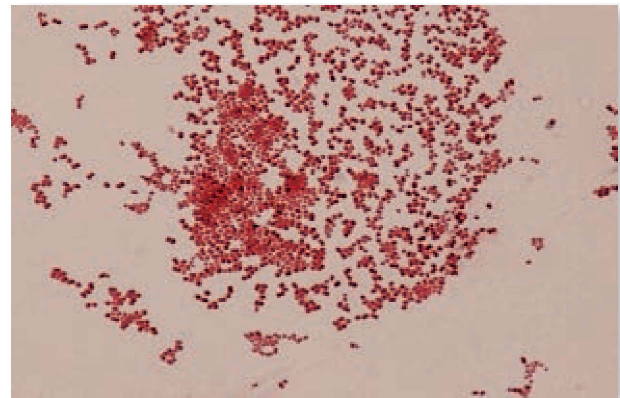


Fig. 46. *Moraxella ovis* are short, plump, Gram-negative rods look like cocci, characteristically observed in pairs.

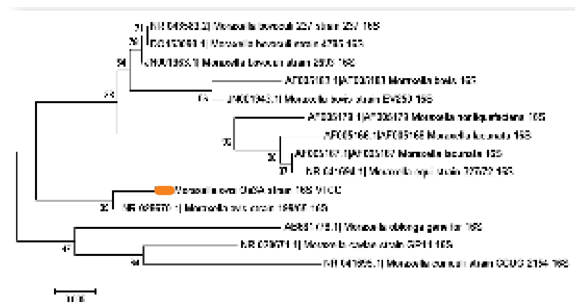


Fig. 47. Phylogenetic analysis of *Moraxella (Branhamella) ovis*

Isolation of *Streptococcus equi* from Saddle Sores cases in Amarnath, Jammu & Kashmir

Disease caused by *Streptococcus equi* sub sp. *equi* in horses is commonly referred to as "strangles" which is a respiratory disease of ancient antecedents. The gram-positive coccoid bacterium typically appears in pairs of cocci or chains. Colonies on SBA are mucoid and cause a wide zone of beta hemolysis. Although the disease afflicts in the form of respiratory tract infections, pneumonia, guttural pouch infection, and metastatic spread of agents in lymph node leading to abcessation. We came across cases of saddle sore in ponies (Fig. 48) which are used to ferry pilgrims across to Amarnath shrine. Saddle sores are generally caused due to use of ill-fitting saddles. The wounds were sampled and bacteriological examination revealed isolation of *Streptococcus equi* sub sp. *equi* (Fig. 49 & 50) and *Staphylococcus* spp. from wounds. The animals were treated by debridement and drainage of wounds and application of topical povidone-iodine solution.

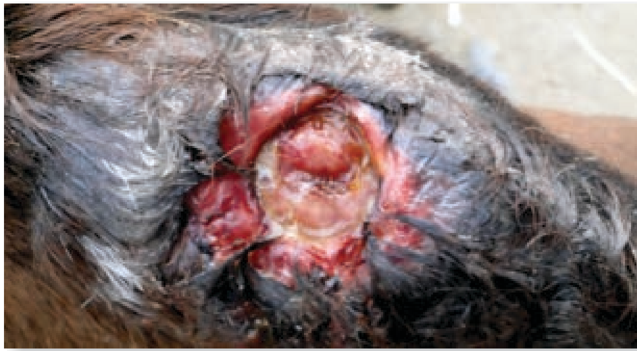


Fig. 48. Saddle Sores in ponies

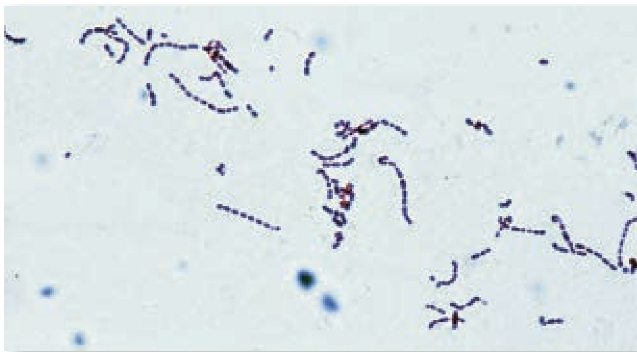


Fig. 49. *Streptococcus equi*

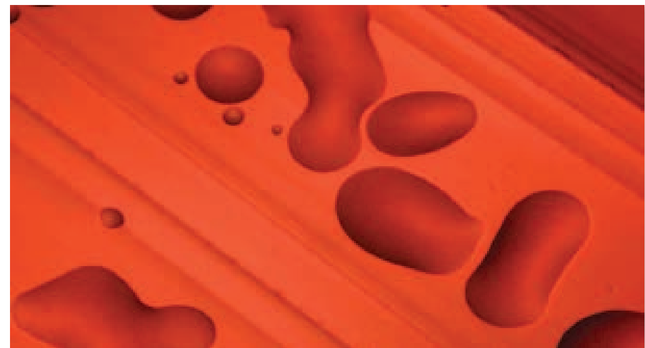


Fig. 50. Mucoid coalescing colonies of *Streptococcus equi* on SBA (10x)

Isolation and Characterization of *Delftia* spp. from sheep water hole

Delftia spp. was isolated from water sample from sheep watering hole in Gulmarg, J & K (Fig. 51). Gram-negative bi-polar rods of *Delftia* spp. (Fig. 52) identified by 16S rRNA sequence comparison with available sequences in the public domain by BLAST homology search.

Fig. 51. Sheep watering hole in Gulmarg, J & K.

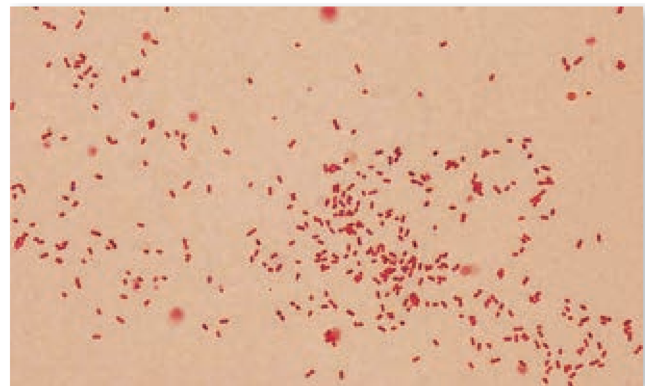


Fig.52. Gram-negative bi-polar rods of *Delftia* spp

Detection and sequence confirmation of methicillin-resistant *Staphylococcus sciuri* sub spp. *rodentium* isolates from goat milk

Three strains of *Staphylococcus sciuri* were isolated and identified from goat milk, with *in vitro* resistance to penicillin, and these were also found to be carrier of *mec*A gene, as confirmed by PCR. *mecA* gene was cloned and sequenced , and confirmed the identity and the presence of methicillin resistance in *S. sciuri* strains from goats. The phylogenetic analysis clearly shows that *Staphylococcus sciuri* is divided into sub species (Fig. 53), even with respect to *mec*A gene, which is a phenotypic character of *S. sciuri*, one of the most primitive *Staphylococcus* species. Methicillin resistance is a worldwide emerging anti-microbial resistance problem in isolates of *Staphylococcus* spp, especially *Staphylococcus aureus*, (MRSA). Acquiring the methicillin resistance cassette (*mec*A) gene results in generation of strains with resistance to last line of potential drug action, as *S. aureus* causes severe animal diseases, such as suppurative disease, mastitis, arthritis, and urinary tract infection, that are associated with numerous virulence factors. The β-lactam resistance gene *mecA* has been proposed to be acquired by natural reservoir of *mec*A gene, like *Staphylococcus sciuri*. What is interesting is that majority of *S. sciuri* isolates are generally fully susceptible to β-lactam antibiotics with *mecA* homologue, however our isolates were detected for resistance by penicillin and oxacillin resistance. The isolates have been reposited in VTCC.

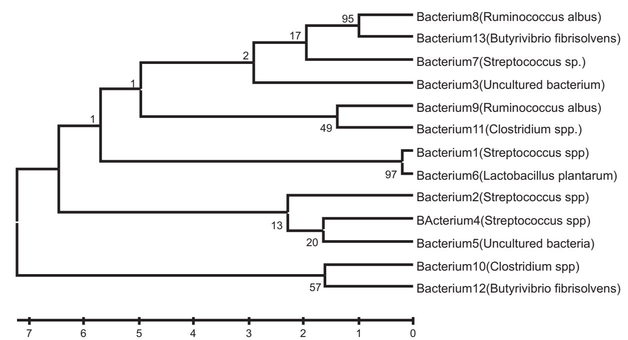


Fig. 53. Phylogenetic analysis of *Staphylococcus sciuri* sub sp. *rodentium*

Whole genome sequencing of bacterial isolates

The access to whole genome sequence of bacteria is revolutionizing the fields of bacteriology and infectious diseases. Globally, by the end of 2012, the number of complete bacterial genomes is nearing 2500. Therefore, we at Veterinary Type Culture Collection, in collaboration with AAU, Anand, Gujarat, have also embarked upon a programme of adding value to our culture collection by WGS of chosen bacterial isolates, by utilizing the pyrosequencing platform. We have been able to achieve good coverage and quality genome sequence data for *Bordetella bronchiseptica, Pasteurella multocida* and *Salmonella* Gallinarum, and the sequence quality parameters of other two genomes is under analysis (Table 13). Previously completed WGS of *P. multocida* strain VTCCBAA264 isolated from an outbreak of HS in buffalo has also been submitted to Genbank with Accession No. ALYC01000001-ALYC01000936. The metadata of the isolate is available in Genome Online database No. Gi16627. The detailed bioinformatic analysis of WGS results is underway by using various online tools like RAST and MAKER. Such huge amount of genomic data can lead to advances in pathogen diagnosis, genotyping, detection of virulence, and detection of resistance to antibiotics. The pathogens which have been sequenced represent important pathogenic isolates causing Hemorrhagic

Table 13. Details of the bacteria used for whole genome sequencing.

ACC. NO.	DID	ID	SOURCE	GENOME SIZE
BAA1	Eq24E	*Bordetella bronchiseptica*	Nasal swab	5,264,383 bp
BAA267	Bu5	*Trueperella pyogenes*	Buffalo pus	NA
BAA264	Bu1	*Pasteurella multocida*	Buffalo Intestine	2,073,865
BAA445	Eq28B	*Actinobacillus equilli*	Nasal swab foal	2,295,342
BAA614	Sal40	*Salmonella* Gallinarum	Poultry Fowl Typhoid	4,809,037

septicemia, respiratory diseases in horse and dogs, mastitis pathogen, etiological agent of sleepy foal disease in horses and Fowl typhoid. The *Salmonella Gallinarum* isolate has been deposited by Dr. N. Jindal from Veterinary College Hisar. This genomic information will make an impact on the research for microbial diseases related on animal and human health. Pure quality genomic DNA was purified from five important bacteria and sequenced whole genomes of those bacteria by pyro-sequencing. Good quality sequence data have been generated with 10-15X genome coverage. The generated contig data are being assembled and annotated using online software(s).

RAST genome analysis of *Salmonella* Gallinarum

The genome sequence data has been assembled and analysed using RAST software. The genome sequence data has been assembled into 92 contigs. Upon analysis it was observed that the genome of *Salmonella enterica* subsp. *enterica* serovar Gallinarum contained 4.6×10^6 bp nucleotide, 567 subsystems, 4634 coding sequences and 72 RNA coding sequences. Different sub-systems of the organism are depicted in the figure 54.

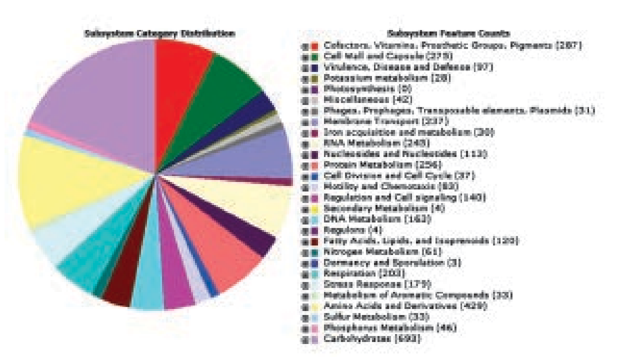


Fig. 54. Subsystem analysis of *Salmonella Gallinarum*

RAST analysis of *Pasteurella multocida*

The genome sequence data has been assembled and analysed using RAST software. The genome sequence data has been assembled into 97 contigs. Upon analysis it was observed that the genome of *Pasteurella multocida* subspp. *multocida* Bu 1 contained 2.28×10^6 bp nucleotide, 415 subsystems, 2130 coding sequences and 52 RNA coding sequences. Different sub-systems of the organism is depicted in the figure 55.

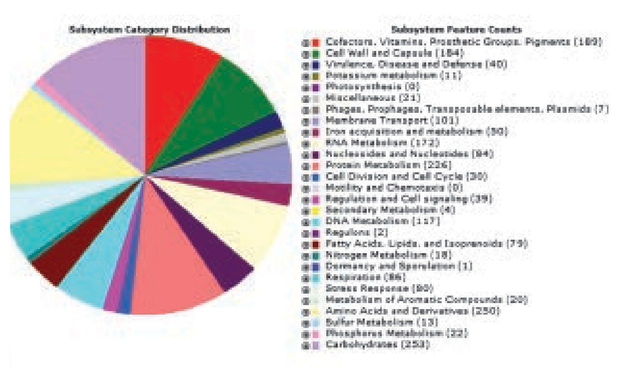


Fig. 55. Subsystem analysis of *Pasteurella multocida*

Cloning and sequencing of 16S rRNA genes of bacteria

Many bacteria have been identified and authenticated by cloning, sequencing and analysis of 16S rRNA genes. The BLAST homology analysis of the sequence data showed ~99.5% homology with the respective isolates. The details of the bacterial isolates are mentioned in the table 14.

(RK Vaid, BC Bera, Taruna Anand, T. Riyesh and S. Barua)

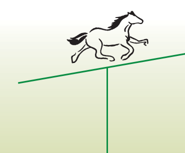


Table 14.16S rRNA sequencing of bacteria

Culture No.	16S rRNA ID
A1 from Soft tick	*Bacillus subtilis*
M5 Goat	*Staphylococcus sciuri mecA positive*
M44 Goat	*Staphylococcus sciuri mecA positive*
M45 goat	*Staphylococcus sciuri mecA positive*
P3 Pig	*Staphylococcus* spp.
P4 Pig	*Staphylococcus* spp.
Oa3A Sheep	*Moraxella ovis*
162A	*Bacillus spp.*
Eq106 semen	*Streptomyces* spp.

Culture No.	16S rRNA ID
Culture No	*16S rRNA ID*
Eq102B	*Bacillus pumilus*
Oa3H20a	*Acinetobacter spp.*
Eq116D	*Bacillus licheniformis*
Oa4A	*Pseudomonas spp.*
Dr1	*Rhodococcus coprophilus*
Eq116	*Nocardia* spp.
Oa3H2	*Delftia* spp.
Eq28B	*Actinobacillus equilli* ssp. *haemolyticus*

Development of DNA and recombinant clone libraries of bacteria

Purified good quality genomic DNA of 138 bacteria of different genus has been stored in TE buffer and as ethanol precipitate at -80°C in the repository. Multiple copies of twenty two (22) recombinant clones of specific genes of various bacterial isolates viz., *Bacillus subtilis* , *Staphylococcus sciuri mecA* positive (3 Nos.), *Staphylococcus* spp., *Moraxella ovis, Bacillus* spp., *Streptomyces* spp., *Bacillus pumilus, Acinetobacter* spp., *Bacillus licheniformis, Pseudomonas* spp., *Rhodococcus coprophilus, Nocardia* spp., *Delftia* spp., *Actinobacillus equilli sub* spp. *haemolyticus,* etc., have been generated and are being maintained in the VTCC repository.

Development of ORF library of virulence associated genes of zoonotic buffalopox and equine influenza viruses

Entry clones of ORFs of buffalopox virus (BPXV-12 ORFs) and equine influenza virus (EIV- 1 ORF) were generated by cloning into Gateway vector pDONR221, Invitrogen Bioscience by homologous recombination based cloning strategy. Primers have been designed targeting the selected ORFs of buffalopox virus and equine influenza virus using ORF-specific primer designing software and sequence of phage lamda attachment site (att) was manually added at the end of the each primer to facilitate homologous recombination based cloning. Two rounds of PCRs were employed to incorporate complete sequence of att site into the amplicons. The recombinant clones were generated on the basis of suicidal action of the non-recombinant clones due to the presence of suicide gene (ccd) in the vector at recombinational site. The clones were further confirmed by colony PCR and sequencing. A total of 13 entry clones (BPXV-12 ORFs & EIV- 1 ORF) were generated. The targeted ORFs were 12 virulence associated genes viz., C3L, crmB, B28R, IL-18, C7L, ZFA, E3L, CBP, K1L, K3L, N1L, B29R of buffalopox virus isolate (BPXV/buffalo/Meerut/2011) and nucleoprotein (NP) gene of equine influenza virus isolate (A/eq/Katra-Jammu/06/08). ORFs of all clones were validated by BLAST homology analysis of sequences. Five clones of each gene were preserved as glycerol stock at -80°C and accessioned in the VTCC repository (Table 15).

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

Table 15. Accessioning of ORF library in the VTCC repository

Gene	Clone ID	Accession number
B28R	BPXV/B28R/pDONR221	VTCCMBA285
crmB	BPXV/B28R/pDONR221	VTCCMBA286
IL-18	BPXV/B28R/pDONR221	VTCCMBA287
CBP	BPXV/B28R/pDONR221	VTCCMBA288
C7L	BPXV/B28R/pDONR221	VTCCMBA289
B29R	BPXV/B28R/pDONR221	VTCCMBA290
ZFA	BPXV/B28R/pDONR221	VTCCMBA291
N1L	BPXV/B28R/pDONR221	VTCCMBA292
K1L	BPXV/B28R/pDONR221	VTCCMBA293
E3L	BPXV/B28R/pDONR221	VTCCMBA294
K3L	BPXV/B28R/pDONR221	VTCCMBA295
C3L	BPXV/B28R/pDONR221	VTCCMBA296
NP	BPXV/B28R/pDONR221	VTCCMBA297

Research achievements of Rumen Microbes component of VTCC

The three main groups of rumen microbes include bacteria - carry out most of the digestion of poly and monosaccharides, proteins; fungi - comprising of a small fraction of the rumen microbial population - play an important function by digesting the outer layers of the plant cell wall thereby exposing the inner portion of the fibre for bacterial digestion and protozoa that ingest and digest bacteria, starch granules, and fibre. Rumen bacteria were isolated from domestic ruminants (cattle, buffalo, sheep and goats), characterized these bacteria microbiologically and biochemically, amplified the gene encoding 16S rRNA, cloned amplicons in suitable vectors and sequenced. Moreover, 30 samples of bacterial isolates from CSWRI isolated from sheep were also taken up for characterization. Out of the 30 samples, DNA was extracted from 18 pure cultures and was used for downstream processing. It was found that all these cultures belonged to *Streptococcus sp.* The various bacteria that have been identified and added to our repository include: *Streptococcus infantarius subsp. infantarius, Streptococcus lutetiensis, Streptococcus bovis, Streptococcus equines, Clostridium bifermentans, Escherichia coli, Clostridium botulinum A3* and *Clostridium* spp. Three live anaerobic cultures were submitted by Anand Agricultural University corresponding to *Bacilllus nealsonii* (VTCCRM000020B), *Clostridium beijerincki* (VTCCRM000021B), *Clostridium sartagoforme* (VTCCRM000022B). All the three bacteria have been found to be gram positive and these were identified by Anand Agricultural University based on whole genome sequencing. A total of 30 rumen bacterial cultures have been accessioned in the current financial year. The phylogenetic analysis of a few bacterial isolates obtained in the studies is given in Fig. 56. A few bacterial isolates corresponded to uncultured bacteria reported in the GenBank.

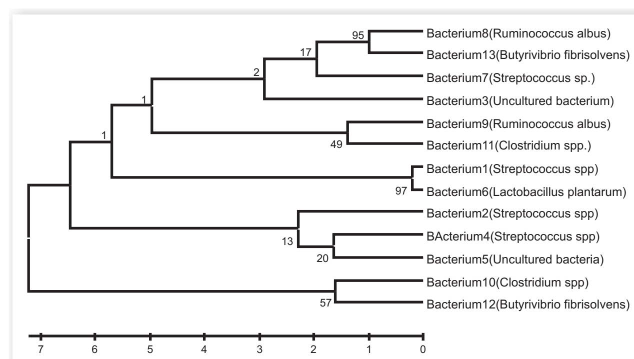
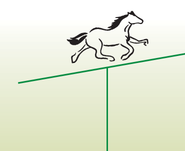


Fig. 56. Phylogenetic analysis of few bacteria isolated in the present study.

The present strength of rumen microbes is 140 accessioned microbes. These microbes have been isolated, characterized, accessioned and reposited in the VTCC repository. Important bubaline rumen fungi such as *Anaeromyces* spp., *Orpinomyces intercalaris and Orpinomyces joyonii* and caprine isolates: *Piromyces* spp. *and Neocallimastix* spp. have been isolated and preserved. Several rumen bacteria such as *Bacillus licheniformis, Butyrivibrio* spp., *Eubacterium limosum, Megasphaera elsdenii, Prevotella* spp., *Streptococcus bovis, Streptococcus equines, 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* spp. These isolates have also been further analyzed for useful biological activities such as fibre and protein degradation (*Prevotella* spp., *Butyrivibrio* spp.); urea hydrolysis (*Megasphaera spp.);* tannin degradation *(Streptococcus spp.);* bacteriocin production *(Bacillus)* etc. Nineteen (19) anaerobic bacteria and seventy six (76) fungi have been accessioned and preserved in the repository.

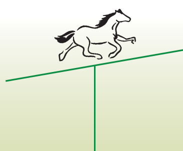


Research Achievements of Dairy component of VTCC

Different isolates of *Lactococcus* spp., *Leuconostoc* spp., *Streptococcus thermophilus* and *Lactobacillus* spp. have been isolated from mixed strains cultures and village dahi samples; identified and characterized on the basis of morphological, biochemical and molecular features. A total of 490 bacterial cultures/isolates are available in the dairy microbes repository, out of which 307 cultures are accessioned of which 89 cultures isolated and characterized and accessioned during the period. Accessioned cultures include- *Lactococcus spp., Leuconostoc spp., Streptococcus thermophilus and Lactobacillus spp Lactococcus lactis ssp.lactis, Leuconostoc lactis, Lactobacillus fermentum, Bifidobacterium dentium, Bifidobacterium longum, Leuconostoc mesenteroids spp mesentroids , Leuconostoc.mesenteroids ssp.cremoris , Leuconostoc sp., Leuconostoc mesenteroids spp para mesentroids , Lactococcus lactis ssp.diacetylactis, Micrococcus sp., lactis, Lactococcus lactis ssp. Cremoris, Lactococcus lactis ssp. lactis bv. Diacetylactis, Streptococcus thermophilus, Kluyveromyces lactis, Saccharomyces bisporus* etc.

The important dairy microbes along with their properties are given below-

- *Leuconostoc* isolates (D6, D14, D54, D50, D51, D52, D55) - 7 flavour and EPS positive
- *L. lactis* isolate (C12 and C16) - fast acidifier
- Combination of C12 (*L. lactis* ssp *lactis*) and D54 (*Leuconostoc mesenteroides* ssp. *mesenteroides*) is very suitable for dahi and lassi preparation.
- Two technological valuable strains (fast acid production proteolytic acivity) A9 and E13 are for the preparation of dahi/ curd.
- 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.
- EPS (15) and mannitol (4) producing strains of *Leuconostoc* spp. have been isolated and can be used as starter cultures for preparation of low caloric and low fat lassi and also for production of mannitol and EPS for other food applications.
- Fast acidifying *Lactococcus lactis* (30), *Streptococcus thermophilus* (100) and galactose positive *Streptococcus thermophilus* (10) have been isolated can be used for various fermented milk products.



Externally Funded Projects

Inter – institutional and externally funded projects

OIE Laboratory Twinning Project on "Equine Piroplasmosis"

Validation of MASP *in-vitro* culture

In-vitro MASP culture system has been developed for maintaining *Theileria equi* parasite routinely in the laboratory. A total of 114 samples (blood in EDTA vacuutainer/tube for serum isolation) were collected from Animal Shed, NRCE, Hisar; EPC, Bikaner; Rajli, Hisar; Julana, Jind; Meham, Haryana and Hanumangarh, Rajasthan. These samples were tested simultaneously in MASP *in-vitro* system, ELISA/cELISA, and PCR/qPCR so as to demonstrate the presence of live *T. equi* parasites, anti-*T. equi* antibodies and parasite specific DNA (Table 16). Few ELISA antibody positive samples could not demonstrate the presence of *T. equi* parasite by MASP *in-vitro* cultivation system or specific DNA in PCR. ELISA is a very sensitive assay and able to detect very low level of circulating specific antibodies. In the present study also ELISA was found to be more specific than other tests/assay. The aim of this experiment was to demonstrate *T. equi* live parasite or parasite specific DNA in antibody positive equids, which has been successfully illustrated.

Table 16:Testing of samples for *T. evansi* by various assays

Tests/Assay	Number of samples positive			
Sampling Area (*n*)	MASP *In-vitro* culture (*)	ELISA	cELISA	PCR
NRCE, Hisar (23)	14 (10)	16	16	15
EPC, Bikaner (30)	20 (12)	23	23	18
Rajli, Haryana (23)	16 (10)	15	15	16
Julana, Haryana (20)	12 (8)	14	14	10
Meham, Rohtak (18)	13 (7)	14	14	11

*Samples were further sub-cultured and cryopreserved

This has validated the results as obtained in ELISA.

Genetic Diversity

The previous sero-surveillance studies at NRCE indicated endemic status of *Theileria equi* infected among Indian equine population. With a step forward, to know the genetic diversity of this parasite in different endemic zones of India, cloned and sequenced EMA-1 and 18s rRNA gene of *T. equi* (Indian strain) collected from different places in Haryana and Rajasthan. The 18s rRNA gene showed 98-99% similarity, whereas EMA-1 gene revealed 94% to 97% similarity to other strains of *T. equi* available in the NCBI data base. The phylogenetic analysis revealed three genotypic clades and Indian strain of *Theileria equi* falls with African and Texas strain of *T. equi* in clade A (Fig. 58).

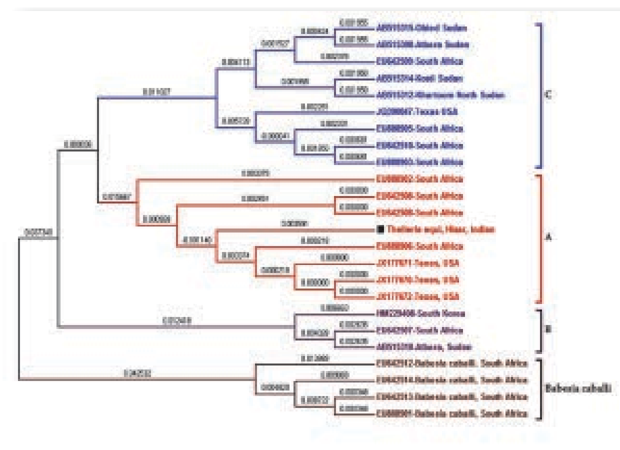
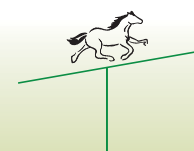


Fig. 58. Phylogram of *Theileria equi* based on 18s rRNA sequence, showing evolutionary relationship with other strain of the parasite.

qPCR for detecting *Theileria equi* parasitic load

The parasitic load in latently infected equids was estimated by qPCR using SYBR-Green chemistry (Fig. 59). The



sensitivity and specificity of qPCR was determined on DNA extracted from blood sample with known per cent parasitaemia. It could detect as low as 200 copy numbers per μl. As in field equids, the circulating per cent parasitaemia is very low and not detectable by routine blood smear examination. Detecting parasitic load by this technique is a novel aid in pin-pointing parasitic load in latently infected animals.

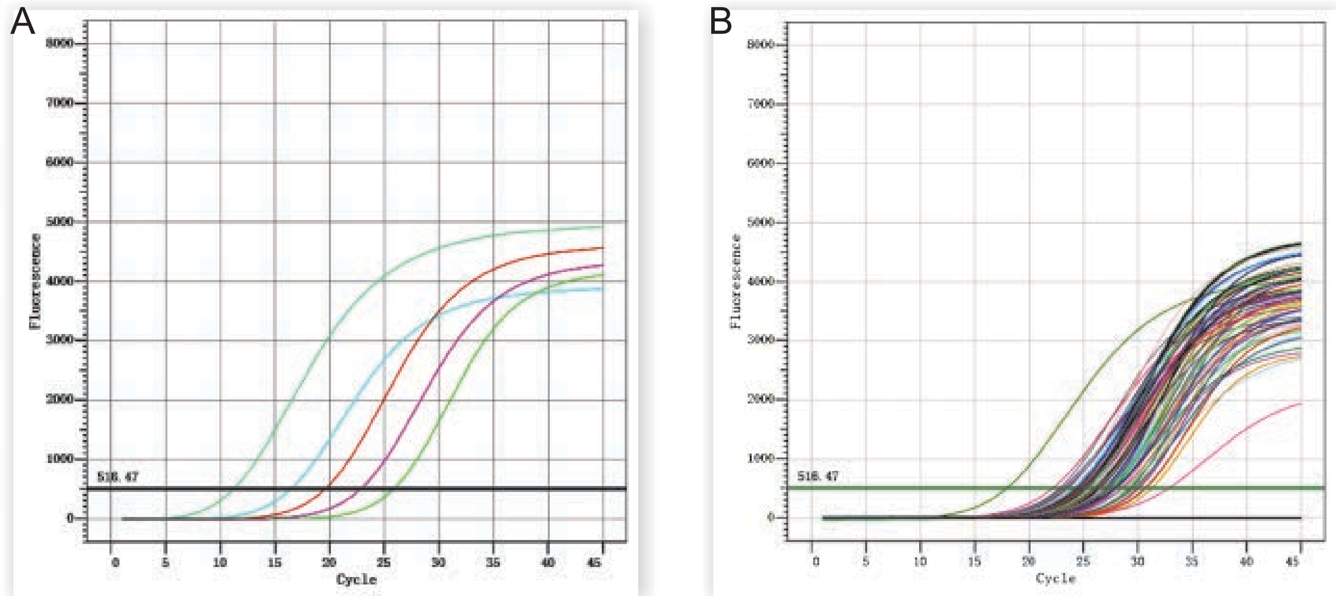


Fig.59. qPCR with standard *T. equi* DNA (A) and unknown field DNA from horses (B), for quantification of parasitic load

(Sanjay Kumar, Rajender Kumar, RK Singh, I Igarashi and N Yokoyama)

OIE Twinning on Glanders with Freiderich-Loeffler-Institute, Jena, Germany

The twinning project was initiated on July 2012 with the objectives of capacity building of National Research Centre Equines, Hisar, India so that it can act as a 'OIE reference laboratory for Glanders' in the Indian sub-continent with particular reference to SAARC countries. The financial subcontract between both partners has been finalized and signed in February 2013. Therefore, it was not possible to start the project till February 2013 and consequently an interim report cannot be provided. However,the success of the project will not be affected by the delay in starting the project.

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

OIE Laboratory Twinning Project on "Equine Influenza with Animal Health Trust (UK) & National Research Centre on Equines (India)

The Twinning project started in October, 2012 and contact primarily involved email exchanges to discuss how best to manage the project and refine the areas that NRCE wishes to concentrate on from a training perspective. During 2013, we have progressed to joint laboratory meetings, carried out using Skype (cost free for both parties). Our first meeting was held on 2nd April and included 6 participants from the two laboratories. AHT provided an overview of the surveillance and diagnostic programmes carried out in the UK and a second speaker covered the approaches used to determining complete genome sequences at low cost. NRCE discussed the outbreak of equine influenza in India in 2008-2009. The meeting involved two hours of active discussion and allowed for a comparison of facilities and surveillance infrastructure available in the two countries. Planning was carried out for a second lab meeting to be held in May.

(Nitin Virmani, RK Singh, BC Bera and RK Vaid)

DBT funded project: "Isolation and characterization of amniotic fluid-derived mesenchymal stem cells in equines"

The therapeutic use of stem cell in horses has recently been realized in the treatment of equine orthopedic diseases, with particular attention to ligament and tendon injuries. The injuries to musculoskeletal tissues in horses (e.g. osteoarthritis, injury to superficial digital flexor tendon) have limited capacity for complete functional repair, making the horses worthless. India has elite horses in Government and Private Sectors, which can be benefited by application of stem cell research in the field of regenerative medicine. Although equine adipose and bone marrow-derived mesenchymal stem cells (MSCs) have been preferred in equine therapy, there is an increasing interest in application of MSCs isolated from various neonatal tissues (extra-embryonic sources, including amniotic fluid, a source of not much explored. Therefore, a DBT-funded study was initiated to explore whether equine MSCs (eMSCs) can be harnessed from amniotic fluid at the time of foaling and to evaluate their tenogenic differentiation potential.

Isolation of eMSCs: About 50-60 ml of amniotic fluid (AF) was collected aseptically at full term delivery from the amniotic sac protruding from the vulva before its spontaneous rupture from 17 Thoroughbred mares and successful fibroblastic colonies were isolated from 11 samples.

Characterization of eMSCs: Amniotic fluid-derived MSCs (AF-MSCs) were studied for their morphology and growth kinetics. The cells were analyzed for expression genes for pluripotency (OCT4, SOX-2 and NANOG) and MSC-associated cell surface markers (CD14, CD34, CD45, CD73, CD90 and CD105). The cells were also evaluated for expression of surface markers by immunophenotyping and

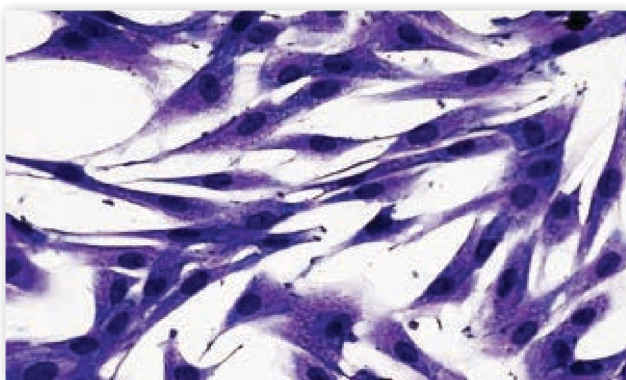


Fig. 60. Giemsa stained eMSC colony showing typical spindle shape with long cytoplasmic processes

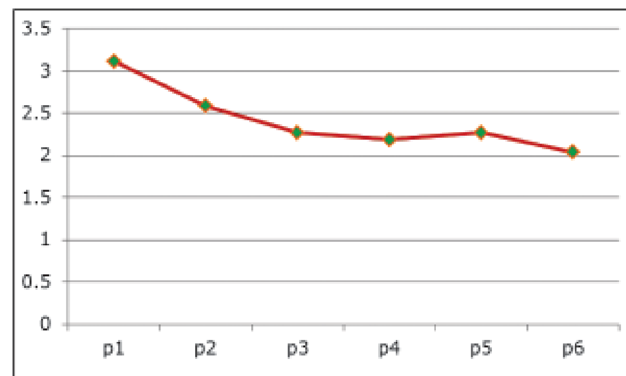


Fig. 61. AF-MSC colony showing plating efficiency at different passages

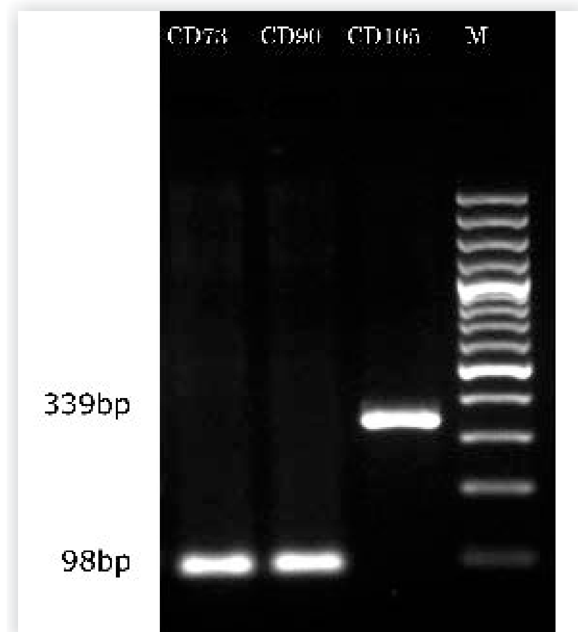


Fig. 62. Amniotic fluid derived eMSCs at passage 3 showing expression of positive cell surface markers by RT-PCR

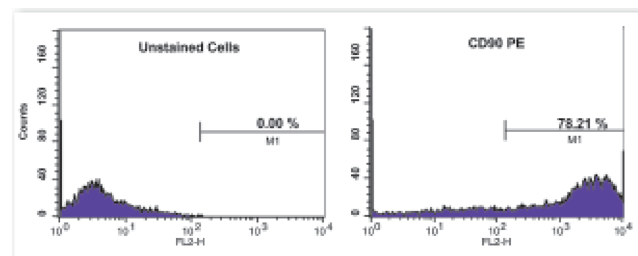
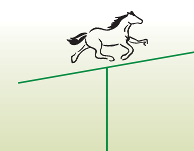


Fig. 63. Immunophenotypic analysis of eMSCs derived from amniotic fluid. Histogram representing Unstained eMSCs (Left panel) and the flow cytometry performed on the eMSCs in the 5th passage using CD90 PE marker (right panel panel).



flow-cytometry. The *in vitro* differentiation of AF-MSC towards osteoblasts, chondroblasts, adipocytes and tenocytes was also done.

The results of the study indicated that amniotic fluid derived eMSCs were plastic adherent and had typical spindle shape with long cytoplasmic processes (Fig. 60). The AF-MSCs have mean cell doubling time of 43.34±1.05 h and plating efficiency of 2.46±0.158%. Both cell doubling time and plating efficiency decreased as the number of passages advanced (Fig. 61). The AF-MSCs isolated in this study were positive for expression of pluripotency markers, *viz.* OCT4, SOX-2 and NANOG and expressed CD73, CD90 and CD105 and were negative for CD14, CD34, and CD45 by RT-PCR (Fig. 62). In Flow-Cytometry, the AF-MSCs were found to express CD90 and were negative for CD34 and CD45 (Fig. 63).

Trilineage Differentiation: Trilineage differentiation is one of the minimum criteria set for characterization of MSCs. The AF-MSCs induced to osteogenic differentiation stained positive for Von Kossa and Alizarine Red S and showed expression of Runx2 (Fig. 64), osteopontin, osteonectin and osteocalcin genes by RT-PCR. The AF-MSCs induced to adipogenic differentiation stained positive for Oil Red O and expressed genes for Adiponectin and leptin and PPAR-γ. The AF-MSCs induced to chondrogenic differentiation stained positive for Alcian blue (Fig. 65) and showed expression of Col2α by RT-PCR. In addition, we could

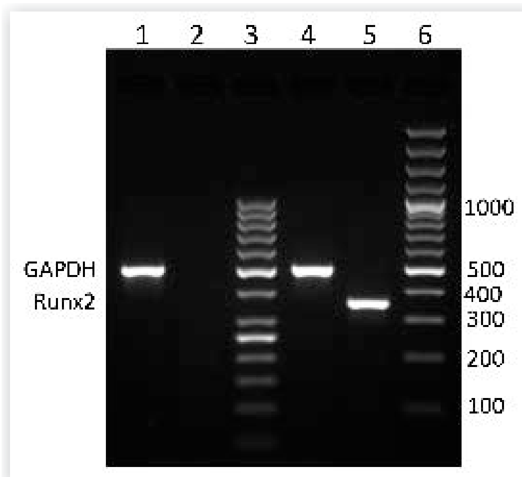
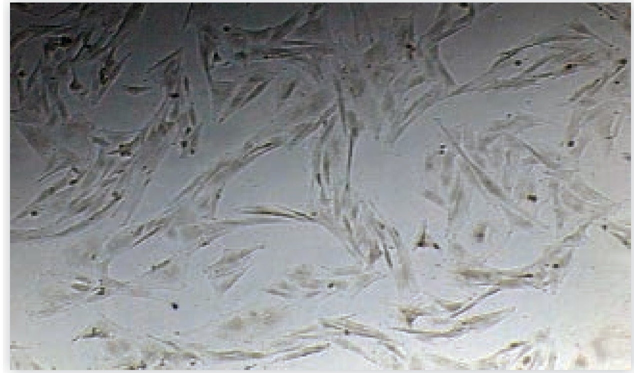


Fig. 64. Amniotic fluid derived eMSCs at passage 3 showing amplified products of GAPDH and Runx2 gene in osteogenic differentiation induced eMSCs. Undifferentiated eMSCs showing expression of GAPDH (lane 1), but negative for Runx2 (lane 2). The differentiated cells expressed GAPDH (lane 4) and Runx2 (Lane 5) genes. Lane 3: 50bp DNA ladder and Lane 6: 100bp DNA ladder



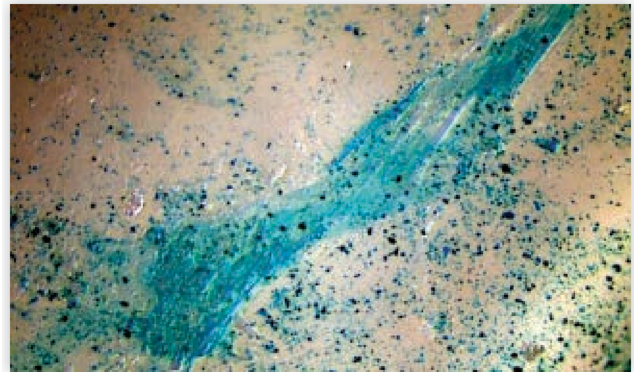
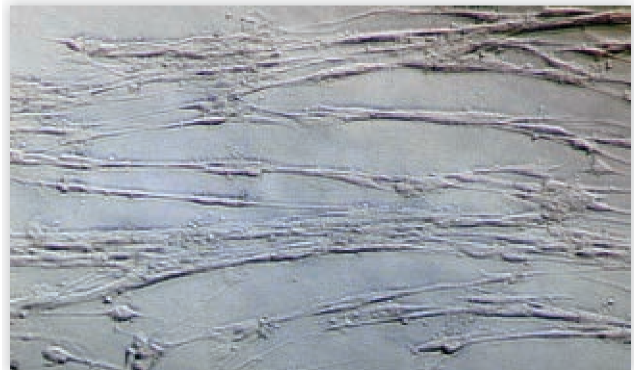


Fig. 65. Amniotic fluid derived eMSCs induced towards in vitro chondrogenic differentiation at passage 5 were confirmed by Alcian blue staining on 21st day of induction



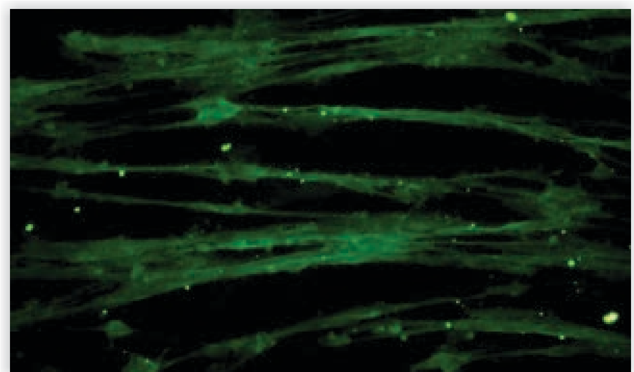


Fig. 66. Amniotic fluid derived eMSCs induced to tenogenic differentiation at passage 5 were confirmed by immunocuochemistry on 21st day of induction for expression of tenocytes marker Tenomodulin. A: Brightfield and B: Fluorescent view.

successfully induce tenogenic differentiation of AF-MSCs as confirmed by change in morphology, RT-PCR analysis and immunocytochemistry by showing the expression of Decorin and Tenomodulin (Fig. 66). The isolated AF-MSCS were subsequently cryopreserved at different passages and re-evaluated for their mesenchymal properties after cryopreservation and revival.

(BR Gulati, BK Singh, Taruna Anand, Pawan Kumar, PS Yadav)

DBT funded project : "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 view of that the non-pathogenic adenoviruses were isolated and characterized from buffaloes and equines.

During the year, the pathogenicity of two bovine adenovirus isolates (N-131 and N-134) and one equine adenovirus isolate (H-9) was studied in experimental mice. For this, Swiss Albino mice (4 week of age) were inoculated by intraperitoneal and intranasal routes and observed for 10 days for clinical signs and at regular interval, mice were killed for observation of gross and histological changes. None of the three isolates produced clinical signs, gross or histopathological changes in infected mice as compared to control. This indicates that two bovine and one equine adenovirus are non-pathogenic in mice as well.

Seven equine adenovirus isolates (H-9, H-20, H-24, H-25, H-31, H-36 and H-51) and five bovine adenovirus isolates (N-126, N-131, N-132, N-134, N-138) were clonally purified and the cloned isolates are being processed for RFLP analysis to collaborating laboratory. In addition, the clonally purified BAdV N-134 was bulk cultivated and submitted for genome sequencing.

(Sudhanshu Vrati, BR Gulati, Minakshi, K Kumanan, M Parthiban, Amarjit Singh and Ramnek)

DBT funded project: "Development of Biomarker(s) for diagnosis of *Trypanosoma evansi* infection in animals using proteomic approach"

For development of diagnostic specific biomarkers of *T.evansi,* three cluster of immuno reactive proteins (62-66kDa, 52-55kDa and 41-43kDa were identified using immunoblot and later purified by SDS-PAGE preparatory gel method (Fig. 67). Of them, 62-66kDa cluster was subjected to analysis by mass spectroscopy which revealed 5 proteins. Out of 5 protein, gene encoding heat shock protein 70 (hsp70) has been amplified and cloned in cloning and expression vector i.e. (pTZ57R/T) and (pET32a), respectively. The kinetics of expression studies were standardized and large scale purification of recombinant protein (Fig.68) were made for further use in immunodiagnostic test.

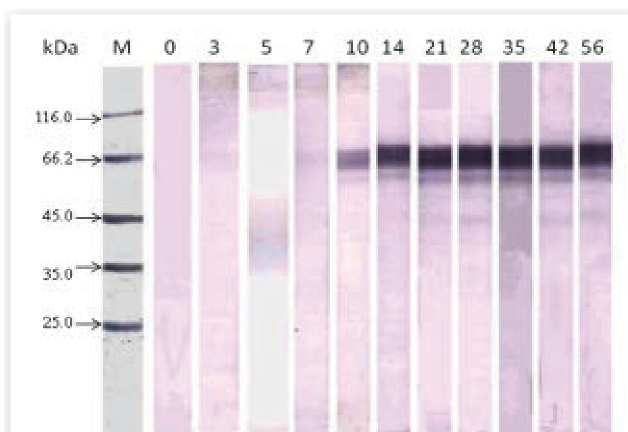


Fig. 67. Immunoblot with pooled sera of exp. infected ponies using semi-purified protein, M- Marker unstained; 0: 0 day serum; 3-56: 3-56 days post infection (DPI) sera

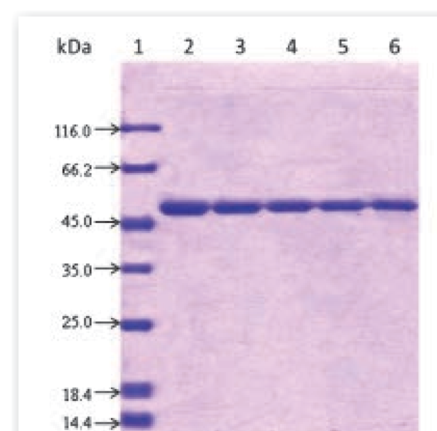
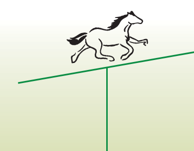


Fig. 68. Recombinant HSP70 (C-terminal) protein purified by Ni-NTA agarose column, L1: Marker unstained; L2: Elution 1; L3: Elution 2; L4: Elution 3; L5: Elution 4; L6: Elution 5

(U. Tatu, SC Yadav, Rajender Kumar and BC Bera)



DST funded project under Nanomission : "Synthesis, characterization and evaluation of drug-loaded nano-formulations against *Trypanosoma evansi* in animal model"

The present work aims to provide an effective delivery system for trypanocidal drug quinapyramine sulphate. Formulated quinapyramine sulphate- loaded nanoparticles (QS-NPs) were well formed and regular in shape with small particle size (less than 100nm), high drug encapsulation efficiency (96.48 %), and a well-dispersed state. The images of quinapyramine sulphate-loaded sodium alginate nanoparticles were viewed under Surface electron microscope (SEM) (Fig. 69A) and Atomic Force Microscope (AFM) (Fig. 69B) Three-dimensional view of AFM image (Fig. 69C). The zeta potential values (-40.5 mV) of QS-NPs are adequate to form a stable nanoparticle suspension. The drug in the form of QS-NPs has remarkably less cytotoxicity as compared to conventional quinapyramine sulphate drug. QS-NPs were highly effective against parasite *Trypanosoma evansi* and shown to kill the parasites at much lower concentrations *in vitro* as well as *in vivo* in mice model of *T. evansi.* Nanoformulation (QS-NPs) is nontoxic, biocompatible, biodegradable, and physicochemically stable and is highly effective against parasite *T. evansi* at highly reduced dose.

(Anju Manuja, Neeraj Dilbaghi, Sandeep Kumar, Rajender Kumar, Balvinder Kumar and SC Yadav)

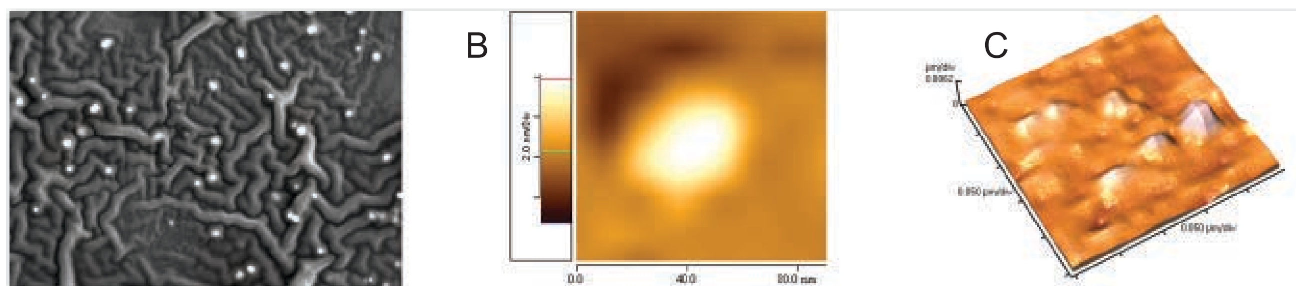


Fig.69. Images of quinapyramine sulphate-loaded sodium alginate nanoparticles were viewed under Surface electron microscope (SEM) (A) and Atomic Force Microscope (AFM) (B) and Three-dimensional view of AFM image (C).

ICAR-Pfizer contract research project: "Studies on prevalence of bacterial (*Escherichia coli* and *Salmonella*) and viral (Corona and Rota) causes of calf scours amongst dairy cattle in India"

Bovine rotavirus (BRV), Bovine coronavirus (BCoV) and *Escherichia coli (E.coli*) are regarded as most important causes of diarrhoea in newborn calves throughout the world. A study was carried out to investigate the presence of these infectious agents as a causative agent for diarrhoea in organized dairy farms. A total of 523 stool samples from diarrhoeic calves below one month of age from nine states of India were examined for the presence of viruses (Rotavirus and Coronavirus). A total of 476 diarrhoeic stool swabs collected from these calves were also tested for *E. coli* K99.

Rotavirus could be detected in 96 of 523 (18.35%) samples tested. Genotyping indicated that majority of samples were positive for G6 followed by G10. Genotyping also revealed that G6 [P11] and G10 [P11] group A rotaviruses circulating in cow and buffalo herds of organized farms in India. In addition, unique genetic/antigenic repertoire of rotaviruses i.e. G10P[3] was also observed in the study. The information is of significance in the context of the development of an effective vaccine aimed to control diarrhoea in buffalo calves. Novel genomic constellations of group A rotavirus also emphasize the need for

continuous monitoring of genotypes of group A rotaviruses.

Presence of BCV in fecal samples was established by RT-PCR targeting the nucleoprotein gene of BCV. Further, the etiology was confirmed by nucleotide sequencing and phylogenetic analysis of three selected PCR products. On phylogenetic analysis, the BCV circulating in India showed close similarity with various strains such as Kakegawa (Japan), V270 (Germany), Mebus (USA) and Quebec (Canada). The attempts to isolate the virus in NLBK cells did not yield result, however, isolation could not be tried in HRT-18, due to lack of availability of the cell line (HRT-18). It is further iterated that virus isolations in case of BCV is very difficult and rare.

Although, the E. coli could be isolated from 306 samples out of 476 samples tested (64.3%), the number of isolates found positive for K99 antigen by Serum agglutination test (SAT) was three. Diagnostic PCR could not be employed for K99 strain detection due to non availability of reference positive strain. Although higher prevalence of K99 bearing *E. coli* has also been reported previously from India in diarrheic calves, the low prevalence of K99 in the present study might be due to low sensitivity of SAT.

In conclusion, data from this study showed that BCoV, BRV and *E. coli* infections are prevalent in Indian calf population, especially in winter months. The economic impact of unabated calf scours to the dairy farmer or dairy industry cannot be underestimated. The wide distribution and prevalence of BCoV, BRV and *E.coli* infections in organized farms warrants immediate attention and preventive measures to be developed and implemented, including prophylactic vaccination of dams to reduce the incidence of diarrhoea associated with BRV, BCoV and *E. coli*.

(RK Singh, BR Gulati, Praveen Malik, RK Vaid, BC Bera and T Riyesh)

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

Expression of recombinant *Burkholderia mallei* TssB and Hcp1 protein for development of sero-diagnostics of glanders among equines

Glanders is a highly contagious and often fatal zoonotic disease of solipeds, including horses, mules, and donkeys. The development of adequate diagnostic tests for identification of infected animals, particularly asymptomatic animal, is very essential for glanders control. Development of a highly specific serological test with defined immuno-dominant protein still remains elusive target. Therefore, the objective of present study was to express recombinant TssB, and Hcp1 proteins of *B. mallei* and evaluating their specificity for detecting the *B. mallei* antibody in equine serum by western blot and indirect ELISA.

Two immunogenic proteins namely TssB and Hcp1of type six secretion system clusters of *B. mallei* were expressed and purified. The 600 bp fragment of *tss*B gene (locus tag BMAA0743) and 500 bp *hcp*1 gene (locus tag BMAA0742) were selected for recombinant protein production. Codons of the target sequence were optimized according to codon usage in *E. coli* K-12 strain. Then the customized tssB gene was commercially synthesized and cloned in pUC57 vector. Identity of nucleotide and deduced amino acid sequence was determined by online BLASTn and BLASTp services provided by NCBI (http://www.ncbi.nlm.nih.gov). The gel

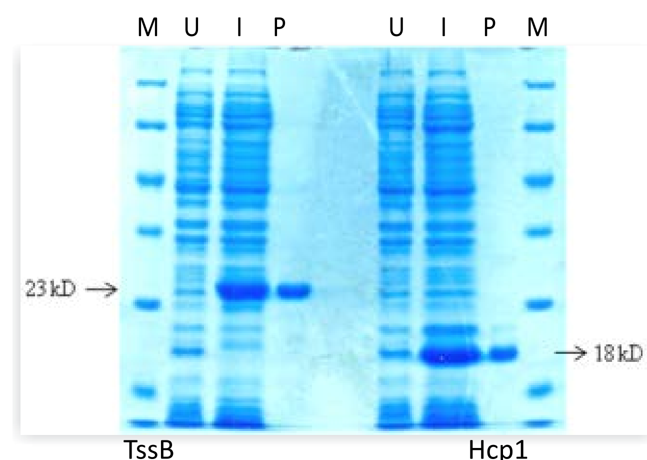


Fig.70. Expression and purification of *tss*B and *hcp*1 protein of *B. mallei* in *E. coli*. Lane M=Protein molecular weight marker, U=uninduced cells, I=induced cells, P=purified protein

purified gene fragments was ligated to *Bam*HI and *Hind*III restricted pQE30 prokaryotic expression vector and ligated product was transformed in to chemically competent *E. coli* M15 cells. Transformants producing recombinant proteins were screened by SDS-PAGE. Molecular weight of the expressed proteins was 23 kDa and 18 kDa for TssB and Hcp1, respectively (Fig. 70). Recombinant proteins were validated by western blotting (Fig. 71) with known positive equine serum (n=49), known negative serum (n=30), and other equine disease control serum (strangles=5).

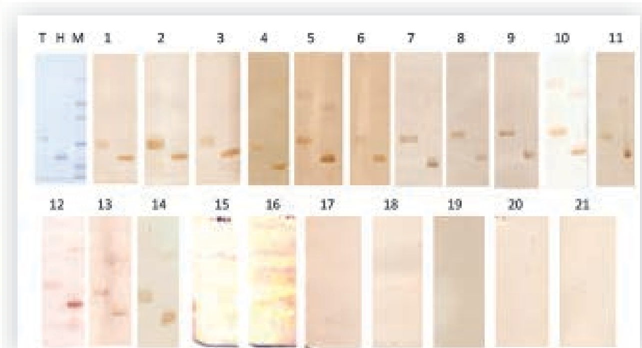


Fig. 71: Representative figure of western blot. T= TssB protein, H= Hcp1 protein and M= protein molecular weight marker. Glanders positive equine serum samples (1-14). Negative control serum showed no reactivity (15-18). Positive serum samples collected from equines affected with strangles (19-21) did not show any cross-reaction.

After verifying the authenticity of the proteins by western blot, standardization of indirect ELISA using recombinant TssB protein was attempted. Optimum concentration of ELISA reagents were determined by checkerboard titration analysis using known glanders positive (n=49) and negative (n=30) equine serum. The optimum cut-off value of the ELISA was determined by receiver operative curves (ROC curves) analysis using normalized OD_{492} values (PP%). After optimization of reagents, 1811 test serum samples were assayed in duplicate by indirect ELISA

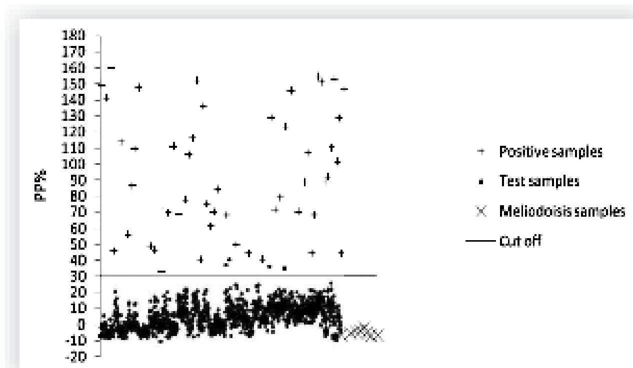


Fig. 72. Distribution of PP% values of equine serum samples as determined by iELISA. PP% was calculated by the formula: Percent positivity (PP%)= [(OD_{492} sample serum- OD_{492} negative control)/ [(OD_{492} positive control - OD_{492} negative control)]x 100%. The PP % value of 30 was calculated as diagnostic cut-off.

according to standard method (Fig. 72). Relative sensitivity, specificity, positive predictive value, and negative predictive value, of the ELISA were 100%, 99.72%, 92.45%, 100%, respectively in comparison to CFT. Cross-reactivity experiment of the truncated TssB protein with human meliodosis serum (n=10) and equine serum obtained from unrelated bacterial infection (n=15) showed the protein is highly specific to *B. mallei* antibodies.

In conclusion, recombinant truncated TssB and Hcp1 encoding the immune-dominant epitopes was expressed, purified, and used it as the antigen to detect *B. mallei*-specific antibodies. The indirect ELISA method using the recombinant truncated TssB more rapid and efficient means of serodiagnosis of glanders. Future studies should be directed towards validation of the immunoassays using these recombinant proteins as well as devising alternative immunoassay (eg. Dip-stick assay, ICT flow through assay etc.) for on-site diagnosis of glanders.

(Praveen Malik, H Singha, SK Goyal, SK Khurana and RK Singh)

DST funded project: "Eukaryotic expression of important equine cytokines and analysis of their biological activities"

Cloning and sequence analysis of cDNAs encoding horse IL-2, IL-4, IL-10 and IL-18 cytokines

Cytokines are indicators of innate and adaptive immunity and have multiple stimulating and regulatory functions during immune responses. The principal limitations in the field of equine immunology include the lack of knowledge in areas cytokine biology, MHC and TCR diversity, and study of host-pathogen immune response. Till now, no sequence information of cytokines of Indian Marwari horse is available. Thus, in the present study, cDNAs encoding IL-2,

IL-4, IL-10 and IL-18 cytokines of Marwari horse were PCR amplified (Fig. 73), cloned in pGEMT-easy vector, sequenced and subsequently compared with related sequences from other mammalian species including, thoroughbred horse, zebra, donkey, cattle, buffalo, sheep, goat, camel and pig available in GenBank database.

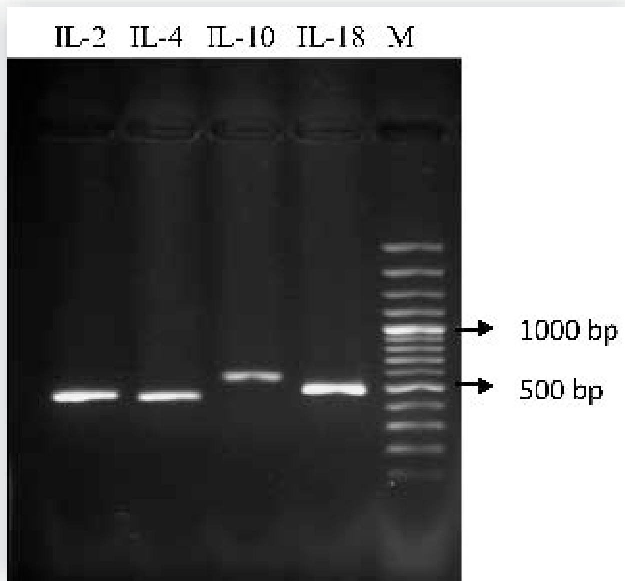


Fig . 73. PCR amplification of cytokine cDNAs of Marwari horse. Lanes are indicated with corresponding cytokines IL-2 (450 bp), IL-4 (414 bp), IL-10 (537 bp), and mature IL-18 (474 bp). Lane M: 1 kb DNA ladder plus.

The sequences were deposited in the GenBank and assigned accession number JQ432544 (IL-2), JQ432546 (IL-4), JQ432545 (IL-10), and JQ432547 (IL-18). The nucleotide and deduced amino acid sequences of cytokines obtained from Marwari horse were aligned and phylogenetically compared with the existing sequences from other mammals. Since the phylogenetic tree clustering patterns constructed with these four cytokines

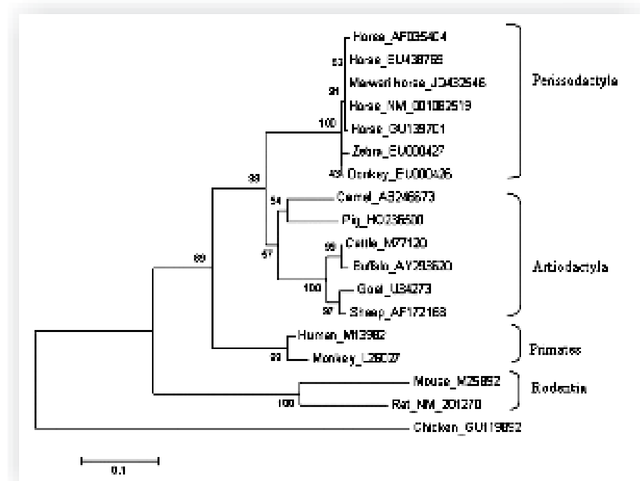


Fig. 74: Phylogenetic tree based on the nucleotide sequences of IL-4 cytokines.

were similar, only that of IL-4 phylogenetic tree is shown in Fig. 74. Horse IL-10 and IL-18 shows high percentage of sequence homology (~90%) both at nucleotide and amino acid level than IL-2 and IL-4 (~65-80%) with major livestock animal species. Further, Marwari horse IL-10 and IL-18 cytokine showed greatest homology (91.5%) to the published camel and pig sequences, respectively. Cysteine residues, potential N-linked glycosylation sites, and myristoylation sites were conserved within the order Perrisodactyla. Nucleotide and amino acid sequences identity and phylogenetic analyses of the four cytokine genes in the present study indicates that horse cytokines are closely related to suborder Suiformes (Pig) and Tylopoda (camel) in the Artiodactyla order. The present work contributes to the assessment of existing structure and sequence diversity of Marwari horse IL-2, IL-4, IL-10, and IL-18 cytokines in relation to other animals.

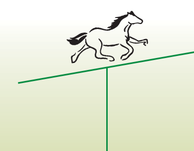
(H. Singha and Praveen Malik)

ICAR National Fellow project: "Development of PCR/Loop-Mediated Isothermal Amplification (LAMP) assays for diagnosis of *T. evansi*"

Standardization of PCR for diagnosis of *T. evansi* using gold standard TBR primers

Polymerase chain reaction (PCR) has been developed in order to overcome the problems faced with conventional and serological techniques for detection of *T. evansi*. TBR1/2 primers (designed) were used to amplify a 164 bp highly repeated sequence of mini-chromosome satellite DNA. PCR assay was evaluated for detection of *Trypanosoma evansi* infection in experimentally infected mice and naturally infected horses using gold standard TBR primers as described by OIE.

The Swiss albino mice were inoculated with *T. evansi*



isolate (T.ev-India-NRCE-Horse1/Hisar/Haryana) maintained and cryopreserved in the laboratory. To check the sensitivity of PCR assay with these primers, DNA was extracted from serially diluted blood collected from infected mice having parasitaemia 10^6parasites/ml. The PCR assay was employed on genomic DNA sample of different *T. evansi* isolates. The DNA was also extracted from whole blood, CSF and tissue samples of horses obtained in the laboratory for testing of *T. evansi* infection. In positive DNA samples, TBR primers amplified 164 bp DNA fragment. The PCR assay employed on genomic DNA sample of different *T. evansi* isolates revealed specific amplification of 164 bp size along with non-specific bands (Fig.75). The PCR results of blood, CSF and tissue samples submitted for testing of *T. evansi* infection showed 100 % correlation with ELISA results. The higher sensitivity (detection limit 10 to 1 parasite) and repeatability confirm that TBR1/2 may be used as a gold standard for the detection of trypanozoon DNA.

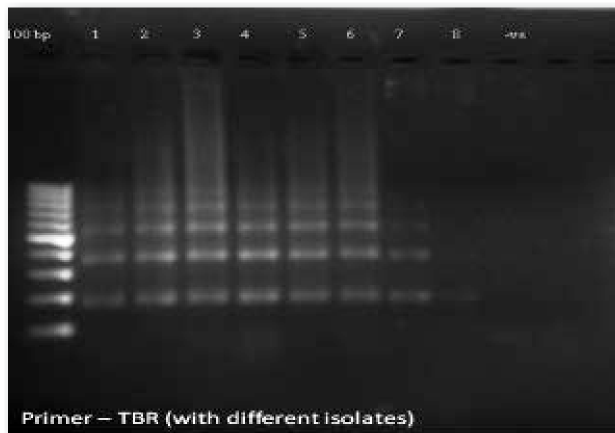


Fig.75: PCR amplification of different *T. evansi* isolates using TBR primers.

Standardization of Loop-Mediated Isothermal Amplification (LAMP) method for detection of *Trypanosoma evansi* infection

The Loop-Mediated Isothermal Amplification (LAMP) technique is a novel DNA amplification method to amplify from a few copies of DNA to 10^9 copies in less than an hour under isothermal conditions. This assay relies on autocycling strand displacement DNA synthesis by a *Bst* DNA polymerase. LAMP is simple and easy to perform, highly sensitive and specific DNA amplification technique suitable for diagnosis of an infectious disease both in well equipped laboratories and in field situations.

Designing of LAMP Primers and standardization of PCR

Previously, primers were designed using sequence of the Ro Tat 1.2 gene (VSG gene) of *T. evansi* (Accession no. AF317914) through Primer Explorer software. Many aspects of the technique still needed further evaluation in terms of repeatability of test, range of incubation time, etc. In the present study, 18S rRNA gene was chosen as target gene and dsigned primers. For PCR, each reaction mixture (total volume 25 µl) contained: 12.5 µl of reaction buffer, 1 µl (8 units) of *Bst* DNA polymerase, 0.9 µl primer mix (FIP and BIP at 40 pmol each and F3 and B3 at 5 pmol each), 2 µl of template DNA and 8.6 µl of distilled water. The reaction mixture was incubated in a heat block at 60-66 °C for 10 min - 1h and then at 80 °C for 2 min to terminate the reaction. The LAMP products were electrophoresed in a 1.5 % agarose gel. Gels were stained with ethidium bromide solution (1µg/ml). The primer set amplified their target sequences in 18S rRNA gene of *T. evansi* at 64 °C and 66°C and the LAMP products appeared as a ladder of multiple bands (Fig. 76). This amplification pattern is characteristic of the LAMP reaction and indicates that stem-loop DNAs with inverted repeats of the target sequence were produced. For specificity, *Theileria equi* genomic DNA was used as negative control.

(Rajender Kumar)

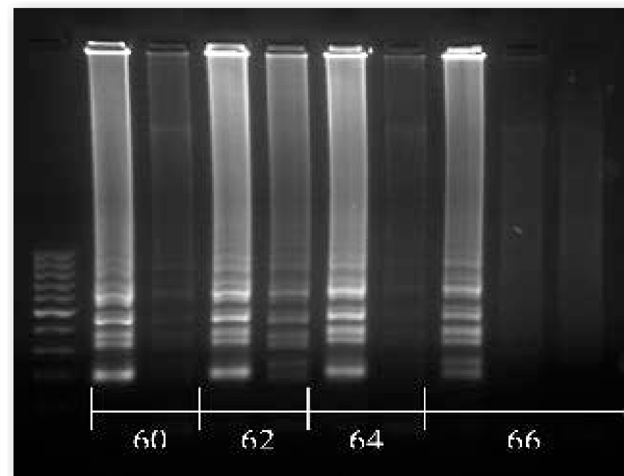


Fig. 76: LAMP amplification of DNA showing multiple bands (ladder like pattern) with 18S rRNA gene primer set.

AICRP project on "Increased utilization of animal energy with enhanced system efficiency"

Use of mules in ploughing during winter season under work rest scheme

Mules were tested in ploughing under the scheme (1.5 h work -0.5h rest -1.5 h work -0.5h rest -1.5 h work -1h rest) during July month using two furrow plough (Fig. 77). A rest of 30 min was given after every 1.5 h of work. Pattern of change in all the physiological responses (PR, RR and RT) has been shown in the Table 17. All the physiological responses increased significantly after work and remained significantly high than the control values even after in between rest of 30 min and 1 hour rest after the completion of work. Total area ploughed by the mules in 4.5 h work time was 0.610±0.024 ha. Speed of operation was 2.18±0.07 Km/h. Enzymes (SGPT, SGOT & ALP) and glucose content in serum was observed statistically non-significant after work. 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.

Fig. 77: Use of mules in ploughing.

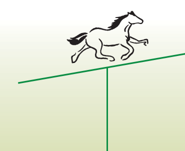
Sustainable utilization of Mule power for chaffing operation through mechanical gear system

In India, use of mules for agricultural operations is limited to transportation only. They remain idle for some time on daily basis creating economic burden to the farmer. Deployment of mules in agro-processing could be an alternative option for their optimum utilization. This study was conducted on use of mule power for chaffing green bajra straw with the help of a rotary gear complex, driven by a local mule of 350 kg body weight (Fig. 78). The operation was performed for 45 min (9.30-10.15 AM) in a cool and comfortable environmental condition. The physiological response of the mule at the end of the operation, exhibited significant escalation in pulse rate, respiration rate and body temperature as depicted in the table 18, but mules did not exhibit physiological stress. No significant changes were observed in the blood sugar, urea, cholesterol and lactate dehydrogenase (LDH) contents of working mules where as content of total protein and albumin increased significantly after work in mules. The average output capacity of chopped bajra straw in rotary mode chaff cutter was 660 kg/h. But, it may not be as economic as electric chaff-cutter

Fig. 78: Use of mules for chaffing operation.

Table 17: Changes in physiological indices

Parameters	Control	1.5h Work	0.5h Rest	1.5h Work	0.5h Rest	1.5h Work	1h Rest
PR/min	27.67[a]±1.2	50.67[b]±3.41	39.33[b]±2.23	59.67[b]±1.96	43.0[b]±2.57	60.0[b]±2.37	36.67[b]±1.43
RR/min	21.33[a]±0.99	53.67[b]±5.69	34.33[b]±3.20	66.33[b]±2.27	40.67[b]±4.58	66.67[b]±2.04	30.33[b]±1.50
RT/min	98.47[a]±0.51	100.87[b]±0.46	99.53±0.71	101.43[b]±0.45	100.53[b]±0.47	101.87[b]±0.36	100.7[b]±0.27



for chaffing operation. However, due to unavailability/ shortage of electric power in rural remote areas, it would be helpful and eco-friendly to utilize those mules/ equines in rotary mode operations during idle hours, which are reared by the farmers for other purposes i.e. transportation, pack load, riding etc. Also, work of chaff cutting is mainly done by rural ladies. Use of mules in chaff cutting will definitely help to reduce their burden. It would compensate the maintenance cost of the mules during idle period. Hence, deployment of mules for operating a chaff cutter in rotary mode of operation is a viable option for sustainable utilization of equine power.

Table 18: Changes in physiological responses during sowing operation

Parameters/time	Control	45 min Work	1.5 h Rest
RR (per min)	22.33±0.76[a]	43.67±1.11[b]	21.33±2.11[a]
PR (per min)	30.67±0.42[a]	52.0±1.26[b]	38.67±1.52[c]
RT (^{0}C)	97.27±1.52[a]	102.4±0.32[b]	100.3±0.11[c]

Draughtability studies on indigenous donkeys

India has nearly 1.18 million equine population, out of which donkeys constitute 37% (0.64 million). Donkeys are mainly concentrated in Uttar Pradesh (24%) and Rajasthan (22%) The donkey population has been declining (1.30 million in 1951) due to rapid mechanization. However, in Rajasthan, the donkey is still an integral part of goat and sheep farming system, goods/materials transport in market, brick-kilns and construction sites in cities and villages of western Rajasthan. Although the donkey pack-transport service has enormous opportunities, the enterprises and the donkeys face various constraints. The donkeys are usually overloaded and suffer from wounds related to overwork. Thus work on draughtability of indigenous donkeys with different loads is very important. In view of above facts we conducted initial trials on indigenous donkeys with 10% draft applied in loading car. The changes in physiological indices during 10% of draft load in donkeys are depicted in table 19. All the physiological responses increased significantly (P>0.01) after a work of 2 hour in loading car during 10% draft (Table 19). Even after 2 hour of rest all the values remained significantly (P>0.01) high indicating a rest of 2 hour was not sufficient to bring all the physiological responses normal.

Table 19: Changes in physiological responses in donkeys during 10% draught

Parameters/time	Control	2 h Work	2 h Rest
RR (per min)	25.6± 0.44[a]	47.53± 0.55[b]	28.53± 0.65[c]
PR (per min)	36.77± 0.40[a]	50.17± 0.98[b]	42.00± 0.82[c]
RT (^{0}C)	98.11± 0.109[a]	100.64± 0.087[b]	99.01± 0.112[c]

The biochemical changes in donkeys during 10% draught depicted in Table 20. Activity of creatine kinase (CK) increased significantly (P>0.1) after a work of 2 hour in loading car during 10% draft and came to normal after a rest of 2 hour. Elevation of CK is an indication of damage to muscle. Contents of lactate increased and glucose decreased significantly (P>0.1) after work and returned to normal after a rest of 2 hour while there was no effect of work on cholesterol content. Content of glucose decreased as it was utilized during work. During this study lactate content increased after during work. Lactic acid may play a role in fatigue its supposed role in muscle soreness has been disproved and it is now being recognized as more of a positive player in metabolism and lactic acid is a key substance used to provide energy. High levels of lactic acid were to meet out the energy demand of the working donkey. Cholesterol levels remained unchanged.

(Yash Pal and RA Legha and AK Gupta)

Table 20: Changes in biochemical indices in donkeys during 10% draught

Parameters/time	Control	2 h Work	2 h Rest
CK (IU)	66.75± 3.9[a]	82.03± 4.95[b]	68.56± 4.35[a]
Lactate (mmol/l)	0.239± 0.009[a]	0.439± 0.058[b]	0.252± 0.027[a]
Cholesterol (mmol/l)	3.068± 0.087[a]	3.004± 0.076[a]	2.94± 0.013[a]
Glucose (mmol/l)	4.67± 0.098[a]	4.00± 0.124[b]	4.65± 0.154[a]

Consultancy and Commercialization of Technologies

Consultancy

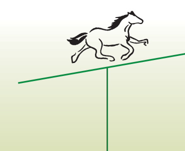
One of the mandates of NRCE is to act as National Referral Centre for equine disease diagnosis, and to provide the diagnostic, advisory and consultancy services to various stake-holders for disease investigation and testing for health certification in the country. As part of this programme, experts from 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 ₹ 46,72,647 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.

Technologies developed at National Research Centre on Equines geared up for commercialization

National Research Centre on Equines, Hisar is actively involved in research on equine health and production since its inception. Many diagnostics kits, vaccines and packages of practices have been developed by the dedicated team of NRCE scientists for stakeholders and these technologies are ready for transfer and commercialization. National Research Development Corporation (NRDC), New Delhi- a Govt of India Enterprise, having expertise in the area of technology transfer and commercialization has collaborated with NRCE, Hisar and a Memorandum of Agreement for technology transfer and commercialization of technologies has been signed between NRDC and NRCE on August 7, 2012. Dr Rita Kumar, Chairman & Managing Director NRDC; Shri A Pradhan, Head, Business Development and Operation; Shri D. C. Joshi, Head, Business Plan Development & Design Engineering; Shri Amitabh Mishra, Deputy Manager; Dr. Ashish Kumar Srivastava Scientific Officer represented NRDC while Dr R. K. Singh, Director NRCE, Dr B. K. Singh, Principal Scientist & Incharge Equine Health Unit, Dr Rajender Kumar, National Fellow & Incharge ITMU and Dr Sanjay Kumar, Sr Scientist represented NRCE. Under the MOA, necessary steps will be taken for management, development, promotion and commercial exploitation of technologies developed by NRCE in complementary mode, so that the technologies reach to the stakeholders.

Signing of MOA between NRCE, Hisar & NRDC, New Delhi



List of technologies under MoA

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

No. of patents filed and granted

Patents Granted (Two):

- 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);
- 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)

Patents Filed (Three):

- COFEB Kit for diagnosis of *B. equi* infection (product) - 156/ Del/04 dated 03.02.2004,
- A pregnancy diagnostic kit for equine based on detection of eCG by ELISA (Both process & product)- Application No. 15770 dated 16.03.2006;
- A highly sensitive kit for detection of antibodies against *Theileria equi* in serum of equids- Application No. 2763/DEL/2012 dated 06.09.2012.

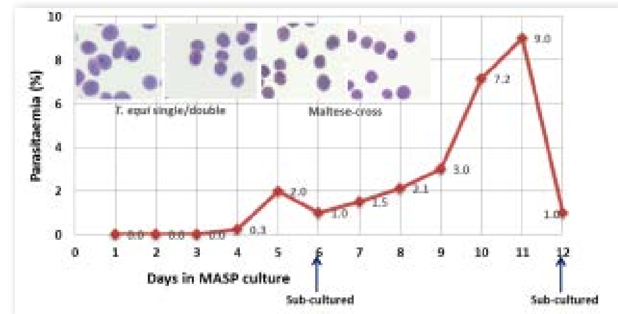
Joint Patent Applications filed (Three):

- A recombinant protein for diagnosis of glanders – Application No.1328/DEL/2010. (DRDE Gwalior and NRCE, Hisar),
- Polynucleotide sequence, composition and methods thereof- Application No. PCT/IB 2011/052475 (IISc Bangalore and NRCE, Hisar):
- Nano-drug delivery for quinapyramine sulphate – Application No. 2560/DEL/2011, dated 06.09.2012 (GJUST, Hisar and NRCE, Hisar).

Technologies
Developed and Assessed

MASP *in-vitro* cultivation technique for *Theileria equi* (Indian strain)

Development of MASP cultivation system is a major breakthrough in *Theileria equi* research as it helped the researchers – i) in replacing the animal experimentation system for production of antigen, for various purposes; ii) for maintenance of parasite in laboratory system and; iii) testing the battery of drugs in *in vitro* culture system before attempting in vivo experiments with most potent drug. The blood samples for cultures were collected by venipuncture into sterile Vacutainer tubes containing EDTA, plasma and buffy layer was discarded. RBC pellet was washed (three times) with Vega Y Martinez (VYM) phosphate buffered saline. The cultivation medium was prepared - M199 medium supplemented with L-glutamine, antibiotic, hypoxanthine and 40% normal horse serum. The RBCs are suspended in the above medium (10% of total volume) and cultures are initiated in double gas incubator (5%CO_2 and



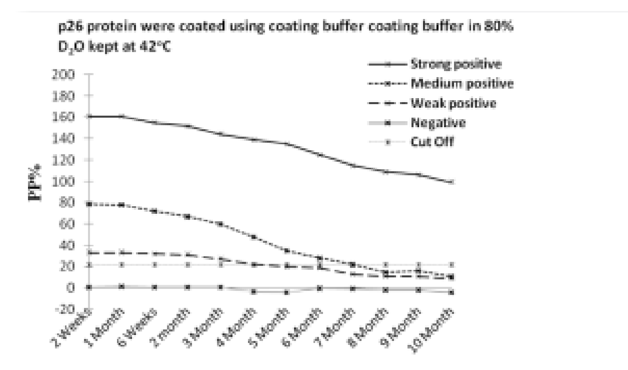
In-vitro* MASP cultivation of *T. equi

2%O_2 and 92% N). This MASP cultivation system initiated intra-erythrocytic development of *T. equi* parasite. The parasites were observed 6-10th day of culture. The culture was subculture subsequently and maintained. By this system a maximum parasitaemia of 10-11% was achieved.

(Sanjay Kumar, Rajender Kumar & RK Singh)

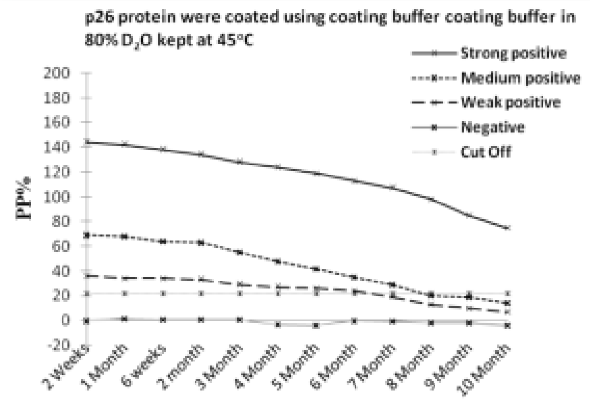
Thermostabilization of recombinant p26 protein using heavy water for sero-diagnosis of equine infectious anaemia by ELISA

Heavy water, formally called deuterium oxide or ^2H_2O or D_2O, is a form of water that contains the hydrogen isotope deuterium (also known as "heavy hydrogen"). Deuterium (D or ^2H) nuclei are heavier than normal hydrogen nuclei which contributes to a greater strength of deuterium than hydrogen bonds thus conferring stability to protein against denaturation. D_2O-mediated stabilization phenomenon has been reported for polio virus vaccine and lactate dehydrogenase enzyme. The purpose of present investigation was to study the protective thermostable effect of D_2O on rp26 protein with the aim to develop thermostable ELISA for diagnosis of EIAV antibodies in equine serum. For this purpose, the carbonate-bicarbonate coating buffer prepared in 60% and 80% D_2O was used for coating ELISA plates with rp26 protein and kept at different temperatures *viz.* 4°C, 37°C, 42°C and 45°C for various time points (2 weeks to 1 year). A set of serum panel with varying titre strength of known EIAV positive equine and known negative equine serum were assessed in ELISA. The



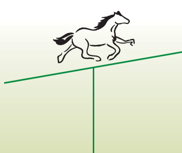
Diagnostic cut-off of ELISA using 80% D_2O at 42°C for detection of EIAV.

assay was compared with freshly coated p26- ELISA plate using coating buffer prepared in natural water. The absorbance data analysis revealed a protective effect of heavy water on coated protein p26. Improvement in thermostability effect of heavy water was observed in higher temperature at 80% D_2O. The effective diagnostic cut-off of ELISA using 80% D_2O at 37°C, 42°C, 45°C could be obtained upto 6 weeks, 2 months, and 3 months, respectively (Fig. 1-3). However, no significant difference in terms of protective thermostable activity of protein at 60% D_2O was observed. The findings will be useful in developing diagnostic assay and transporting the coated plate without cold chain requirement.



Diagnostic cut-off of ELISA using 80% D_2O at 45°C for detection of EIAV.

(H Singha, Praveen Malik, SK Goyal and RK Singh)



Education and Training

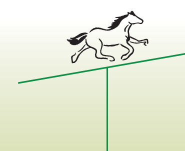
Annual Scientific Review Meet of Network Project of Veterinary Type Culture Collection (VTCC)

The Third Annual Review meet of VTCC Network Project, NRCE, Hisar was held in NASC Complex, New Delhi on May 2, 2012. The meeting was presided over by Dr Gaya Prasad, Hon'ble ADG (Animal Health). ADG exhorted the network participants to be self-critical about their achievements, and performance. ADG further emphasized that by pursuing the goals and mandate of VTCC, our visibility will be more in future. During discussion, two more Centers for Dairy Component i.e., North East Hill University and NIFTM (National Institute for Food Technology Management, Sonepat) were proposed to be included while many Centers were proposed to be added to the Veterinary Microbes Component. ADG also drew his attention towards initiation of distribution of Cultures for teaching and research, which is an important component for fulfillment of VTCC mandate. There was discussion about the classification of Cultures which are being deposited by Network Units and many Network Units are submitting cultures as Safe deposit. However ADG clarified and said that there should not be any confusion and all cultures being deposited by Network Units are General deposits, meant for distribution for education and research purpose. It was, however, agreed that any IPR emanating from a culture will be protected. ADG commented that working on rumen microbes is quite different than veterinary microbes and their contributions can be invaluable if they could obtain proper guidance and help from NDRI, IVRI and NIANP. To wrap up the meeting, Dr G Prasad, ADG concluded by mentioning the importance of the project. He emphasized on focusing on finding own shortfalls and work hard to achieve the targets.

Training on "Artificial insemination and pregnancy diagnosis in equines"

Imparted six days training to inseminators entitled "Training on Artificial Insemination and Pregnancy Diagnosis in Equines" from May 21-26, 2012 at EPC, NRCE, Bikaner. Training was organized by Dr Yash Pal, Dr R A Legha, Dr S.K. Ravi and Dr Jitender Singh. The topics covered during training programme includes teasing and heat detection, breeding soundness of stallions, semen collection, evaluation and cryopreservation, pregnancy diagnosis, infertility in mares and general health problems of horses and their remedies. Four inseminators participated in training on payment basis. The matter of training was also compiled in the form of compendium and provided to trainees.

Training programme on Artificial insemination and pregnancy diagnosis in equines



Training course conducted on "Artificial insemination in horses and their conservation"

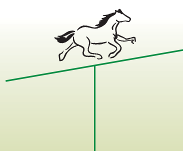
A ten days training course on "*Artificial insemination in horses and their conservation"* was organized during January 22-31, 2013 at EPC, Bikaner. The training course was fully sponsored by Directorate of Animal Husbandry, Government of Gujarat. At the inaugural function of the training course, Prof. A. K. Gahlot, Vice-Chancellor, RAJUVAS, Bikaner emphasized the utility of said training course in genetic improvement of field animals at faster rate in addition to other benefits. The program was organized at EPC, Bikaner by Dr R.C. Sharma as Course Director with Dr S.K. Ravi, Dr Vijay Kumar and Dr Jitendar Singh as coordinators. Ten Veterinary Officers from different regions of Gujarat and one Livestock Assistant from Jhunjhunu, Rajasthan participated in the said training course. The participants were provided 'Hands On' training on various techniques like per rectal palpation of genitalia, artificial insemination, ultrasonography for ovarian examination and pregnancy detection etc. A total of 21 theory lectures on various aspects of equine production besides practicals/ demonstrations were delivered by NRCE faculty and other guest speakers during the said training course. A compendium was published which included 21 chapters on different aspects such as artificial insemination, semen cryopreservation, associated techniques of breeding, reproduction and conservation strategies in equines, reproductive problems, common diseases, nutrition and appropriate management practices of equines. The compendium was made available to the participants and other concerned. During the valedictory function on January 31, 2013, Dr R.K. Singh, Director, NRCE while addressing the participants stressed upon utilizing the techniques they have learnt during the ten days period and asked them to come forward in disseminating the improved germ plasm in the field for the benefit of equine owners and conservation of equines. At this occasion, participants provided their feedback and expectations from NRCE for the welfare of equine fraternity. Dr N.V. Patil, Director, NRC on Camel, Bikaner along with Dr R.K. Singh distributed the certificates to the trainees during the valedictory function.

Group photo of trainees with Director and staff of EPC, Bikaner

Successful AI of Mare by NRCE trained VLDA

During short course for the inseminators at EPC Bikaner, one inseminator (VLDA) from UP was trained for AI in mares. Presently he is working in Lucknow - Barabanki area of UP state. After successful completion of training at EPC NRCE also provided him semen doses of our horse stallion (45 Nos.) and poitou jacks (10 Nos.) He had inseminated a mare on sixth day of estrus (06.05.2012) with horse stallion cryo-preserved semen, and now that mare has foaled a male foal on 08.03.2013.

Foal produced by AI



Training on "Optimal use of equids in agricultural operations"

Imparted two days training for equine owners on the topic "Training on optimal use of equids in agricultural operations" during March 30-31, 2013 at EPC, Bikaner. Dr Yash Pal and Dr R A Legha organized the training. Fifteen equine owners of Bikaner district participated in the said training. They were demonstrated about the use of equids for various agricultural operations *viz*., ploughing, sowing, chaff cutting, leveling and carting. The importance of good quality harness and its impact on health of working equids were discussed with the trainees. Feed and general management of working equids as well as their welfare issues were also discussed.

Training on use of donkeys in ploughing

Training on chaff - cutting

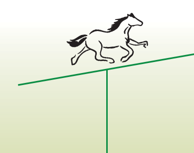
International Workshop for SAARC countries participants under OIE Twinning Program on Equine Piroplasmosis at NRC on equines, Hisar, India

The OIE – The World Organisation for Animal Health (Paris, France) – initiated OIE Laboratory Twinning Programme with the aim to create opportunities for developing and in-transition countries to develop laboratory diagnostic methods based on the OIE Standards. Each Twinning project is a partnership between an OIE Reference Laboratory and a Candidate Laboratory. In this endeavor, National Research Centre on Equines (NRCE) initiated the OIE-sponsored twinning project on Equine Piroplasmosis with National Research Centre for Protozoan diseases (NRCPD), Japan (2010-2013). OIE Laboratory Twinning Program on Equine Piroplasmosis is the first such project awarded by the OIE to the country.

Inauguration of International Workshop on "Equine Piroplasmosis for the SAARC Country Participants"

Under this OIE Twinning Project, NRCE organized an International Workshop for the participants from SAARC countries on *"Molecular Diagnosis of Equine Piroplasmosis"* from November 29 to December 8, 2012. This workshop was attended by one participant each from Afghanistan, Nepal, Bhutan, Bangladesh and Sri Lanka, and seven participants from state animal husbandry department of Haryana, Rajasthan, Gujarat; DAHD&F (Quarantine Officers) and Turf Club Authority of India. Prof.

Participants of International Training under OIE twining for equine piroplasmosis



I. Igarashi from NRCPD, Japan facilitated this International Workshop at NRCE as an OIE Expert on Equine Piroplasmosis. Eventually development of these state-of-the-art diagnostic facilities and capabilities will pave the way for NRCE in applying to the OIE for Reference Laboratory on equine piroplasmosis.

CL Davis Foundation Lecture at NRCE on "Pathology of Aquatic Animals, Farmed and Laboratory Fish including Integrated Aquaculture and Waste Management"

National Research Centre on Equines (NRCE) organized one-day satellite seminar - CL Davis Foundation Lecture- under the auspices of Indian Association of Veterinary Pathologists on November 8, 2012. The topic of the seminar was "Pathology of aquatic animals, farmed and laboratory fish including integrated aquaculture and waste management". The distinguished speaker for the seminar was MAJ Eric D. Lombardini VMD, MSc, DACVP, DACVPM; Chief, Divisions of Comparative Pathology & Veterinary Medical Research, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. Dr R.K. Singh, Director NRCE was patron of the seminar while Dr Nitin Virmani and Dr. R.K. Vaid worked as Co-organizing secretary for the event. The 8 hour seminar provided information on important diseases of farmed fish and integrated macroscopic and microscopic pathology of aquatic animals, piscine models of human and animal disease and fish as laboratory species. The gathering of the eminent scientists from the field of aquatic fauna was useful to highlight the aims and objectives as well as activities of VTCC, besides understanding the futurology of piscine model of animal diseases as well as understanding pathobiology of diseases/pathogens of aquatic animals.

Participants of CL Davis Foundation Lecture

Lecture delivered by MAJ Eric D. Lombardini, Chief, Divisions of Comparative Pathology & Veterinary Medical Research, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand

Expert Lectures at NRCE

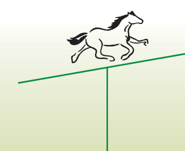
- Dr Satish Ayyar, Secretary, Indian Stud book Authority visited NRCE on June 12, 2012 and delivered lecture on the topic entitled "DNA Typing in Equines".
- Dr El Harrak Mehdi, Head of R&D Department, MCI Animal Health, Morocco visited NRCE on September 06, 2012. He delivered a lecture on "West Nile VIrus.
- Dr Satya Parida, Head of Vaccine Differentiation Group, Pirbright Laboratory, Institute of Animal Health, UK visited NRCE on October 10, 2012. He delivered a lecture on "Development of Recombinant FMDV and PPRV marker (DIVA) Vaccines using Reverse Genetics Technique.
- Mr Praveen Pasricha delivered lecture on "Quality Management System as per ISO 9001: 2008 on February 21, 2013.

Dr El Harrak Mehdi delivering a lecture at NRCE

Dr Satya Parida delivering a lecture at NRCE

Expert Lectures Outside

- B. K. Singh, Principal Scientist, NRCE, Hisar delivered Guest Lecture on the topic "Management of Herpes Virus infection in Horses: Diagnosis and vaccination strategies" in the CAFT Training course on "Control of Infectious Diseases of Animals" organized by Deptt. of Veterinary Microbiology, ICAR Centre for Advance Faculty Training (CAFT) at LLRU, Hisar w.e.f. September 18 to October 8, 2012.
- B. K. Singh, Principal Scientist, NRCE, Hisar delivered invited lecture on the topic "Equine Herpesvirus 1 (EHV-1): the Indian Scenario" in XXI National Conference of Indian Virological Society "VIROCON-2012" at Indian Veterinary Research Institute, Mukteshwar, Nainital w.e.f November 8-10, 2012.
- B. R. Gulati, Principal Scientist, NRCE, Hisar participated and presented an Invited Paper entitled 'Japanese encephalitis virus among horses in India: perspective on epidemiology and intervention'. In XXI National Conference on Immunobiology and Management of Viral Diseases in 21st Century "VIROCON-2012", Indian Veterinary Research Institute, Mukteswar, November 8-10, 2012.
- B. R. Gulati, Principal Scientist, NRCE, Hisar participated and presented an Invited Paper entitled 'Mesenchymal stem cells: application in equine medicine' in National Workshop on Stem Cell Research and Therapeutics: Current Status and Future Strategies, Division of Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar (U.P.), September 28-29, 2012.
- Nitin Virmani, Principal Scientist, NRCE, Hisar, participated and presented a lead paper on "Molecular characterization and diagnosis of equine influenza- a current perspective" at XXIX Annual Conference of Indian Association of Veterinary Pathologists and National Symposium on "Challenges in diagnostic pathology in domestic pet, wild and aquatic animals" and National Seminar on "Emerging trends in diagnosis and control of poultry diseases" held at College of Veterinary Sciences, LLRUVAS, Hisar from 5-7 Nov. 2013.
- B.C. Bera, Scientist, VTCC, NRCE, Hisar delivered lecture on the topic " Storage of Nucleic acid: basic to advance approach" to the trainees attended in the short course entitled "Recombinant DNA Tools and cell culture Techniques" on October 13, 2012 at Department of Animal Biotechnology, LLRUVAS, Hisar, Haryana.
- R. K. Vaid, Senior Scientist, VTCC, NRCE, Hisar presented Invited Paper on "Advanced techniques in bacterial Identification" in XXIX Annual Conference of Indian Association of Veterinary Pathologists & National Symposium in "Challenges in Diagnostic Pathology in domestic, pet, wild and Aquatic animals" & National Seminar on Emerging trends in diagnosis & control of poultry diseases", November 5-7, 2012 at College of Veterinary Sciences, LLRUVAS, Hisar.



RAC, IRC & IMC Meetings

Quinquennial Review Team (QRT) Meeting

The Quinquennial Review Team (QRT) constituted by the Director General, Indian Council of Agricultural Research vide letter no. 24-3/2012-IA-I dt 30.07.2012 convened six meeting to review the work done by NRC on Equines, Hisar for the period from April, 2007 to March 2013. The meeting was chaired by Dr R.N. Sreenivas Gowda, Ex-Vice Chancellor, KVAFSU which was attended by members - Dr D.N. Garg, Ex-Dean, Hisar; Dr H.K. Pradhan, Ex-Director, HSADL, Odisha; Dr K.C. Varshney, Prof & Head, Deptt of Pathology, Rajiv Gandhi College of Vety. & Animal Sciences, Kurumbapet, Puducherry; Brig. (Dr) N.M. Singhvi, Jodhpur; Col. (Dr) B. Raut, Ex-Director, DIHAR (Leh), Consultant, DRDO Field Research Laboratory, Chandigarh; Dr G. Butchiah, Ex-Dean, Rajiv Gandhi College of Veterinary Science, Hyderabad and Dr A.K. Gupta, Pr. Scientist, NRC on Equines, Hisar as Member Secretary.

To review the work done by NRCE during 2007-2013, the QRT had six meetings at Main Campus, Hisar and Equine Production centre (sub-campus), Bikaner between Sept., 2012 and May, 2013. The committee visited all the research laboratories and had thorough discussion with individual scientists regarding their research projects and problems, if any, faced by them during course of their work. QR Team also had an opportunity to interact with equine owners during health camp organized at Bikaner. The committee strongly recommended the need of increasing the strength of regular administrative, technical and supporting staff both at Hisar and Bikaner. Establishment of regional stations of NRCE in equine populated areas and separation of VTCC from NRCE were other important recommendations. The committee emphasized that there is an urgent need to do more systematic work in the field of equine production as very good work is already being done in equine health.

QRT meeting in progress

Institute Research Committee Meeting

The annual meeting of Institute Research Committee (IRC) was held under the chairmanship of Director, NRCE, Hisar on May 28-29, 2012 at NRCE, Hisar and June 30, 2012 at EPC, Bikaner for appraisal of the research achievements of the ongoing projects and to consider new research project proposals for the year 2012-2013. The IRC reviewed the progress of ongoing research projects in the area of equine production, health, extension and Veterinary Type Culture Collection. Thorough discussion was done on each project regarding outcomes, shortcomings and future course of work. Chairman emphasized that more progress has to be done in terms of the output of the project to uplift the status of the Centre at the national as well as international level.

Presentation during IRC meeting

Workshop, Seminar and Institutional Activities

World Veterinary Day Celebrated at NRCE

XIII World Veterinary Day was celebrated at National Research Centre on Equines, Hisar on April, 28, 2012. World Veterinary Day was instigated by World Veterinary Association in 2000. The selected theme for World Veterinary Day for 2012 was "Antimicrobial Resistance". On this occasion Dr V. A. Srinivasan, Research Director, Indian Immunological Limited, Dr H. K. Pradhan, Consultant, WHO (India), Dr Gaya Prasad ADG (AH) ICAR and Dr R. K. Sethi Director CIRB were present as the Guest of Honour.

Dr V. A. Srinivasan emphasized on the role of veterinary profession and their services to mankind, while Dr H. K. Pradhan opined that veterinarians play an important role to serve the society with purpose to increase the production and productivity of animals. Veterinarians have developed different vaccines and diagnostic kits for different diseases and now we are not dependent on imported diagnostic kits. Dr Gaya Prasad in his speech stated that World Veterinary Day is a symbolic recognition for veterinarians and those who are related with animal husbandry, biochemistry,

Farm Innovators Day at EPC Bikaner

genetics, breeding, nutrition and other related disciplines. He stated that, in recent past, drug resistance in animals and poultry was a major concern and thus "Antimicrobial Resistance" was selected as theme for 13th World Veterinary Day. In his message to veterinarians, he said veterinary professionals are major users of antimicrobial drugs and he appealed to Veterinarians to minimize the use of antibiotics and use antibiotics only then when no other option is available.

Farm Innovators Day organized at EPC Bikaner

Farm Innovators Day was organized at the Sub-Campus, Bikaner on October 15, 2012 in which 48 progressive equine owners and representatives of horse breeding society of Rajasthan viz., Sh. Narayan Singh Manaklao, Ex-MP and Col. Umaid Singh from Jodhpur also participated in meeting. Donkey Show/ Equine Health Camp was also organized on the same day in which 17 donkey farmers along with their 18 animals participated. Scientists of the Centre and Hon'ble QRT members interacted with the equine owners.

Veterinary Day celebration at NRCE

हिन्दी पखवाडा का आयोजन

केन्द्र में 1-12 अक्टूबर 2012 को हिन्दी पखवाड़ा आयोजित किया गया। इस अवसर पर केन्द्र में हिन्दी के अधिकाधिक प्रयोग हेतु हिन्दी भाषा से संबंधित विभिन्न स्पर्धाओं का आयोजन किया गया जिसमें केन्द्र के अधिकारियों, कर्मचारियों व केन्द्र सरकार से संबंधित अन्य संस्थाओं के कर्मचारियों ने बढ़-चढ़ कर हिस्सा लिया। 1 अक्टूबर 2012 को हिन्दी पखवाड़ा उद्घाटन समारोह आयोजित किया गया। इस कार्यक्रम में केन्द्र के निदेशक डा० राजकुमार सिंह, डा० राधे श्याम शुक्ल, सेवानिवृत प्राध्यापक; डा० मधुसूदन पाटिल, प्रोफेसर, जाट कालेज; डा० वंदना पाण्डे, जन संपर्क विभागाध्यक्ष, गुरू जम्भेश्वर विश्वविद्यालय ने दीप प्रज्जवलित करके कार्यक्रम का शुभारम्भ किया। कार्यक्रम के शुभावसर पर डा० मधुसूदन पाटिल ने हिन्दी भाषा की सांविधानिक स्थिति पर प्रकाश डाला। डा. वंदना पाण्डे ने हिन्दी की दशा व दिशा बताते हुए कहा कि हिन्दी केवल भाषा नहीं बल्कि एक सांस्कृतिक परम्परा बन चुकी है जो अन्य कई भाषाओं को अपने अन्दर समाहित कर बहुत सशक्त बन चुकी है। डा. शुक्ल ने हिन्दी ही क्यों विषय पर अपने विचार व्यक्त किए और हिन्दी के महत्व की विस्तार से चर्चा की। इस अवसर पर निदेशक ने केन्द्र में हिन्दी के उपयोग को अधिक कारगर बनाने के लिए कई सुझाव दिए तथा केन्द्र में हिन्दी की प्रगति की वर्तमान स्थिति से अवगत कराया। हिन्दी पखवाड़ा के दौरान निबन्ध प्रतियोगिता, वाद-विवाद प्रतियोगिता, सुलेख एवं श्रुतलेख प्रतियोगिता, भाषण प्रतियोगिता आदि में केन्द्रीय कार्यालयों के कर्मचारियों एवं केन्द्र के सभी वर्गो के कर्मचारियों ने भाग लिया। निबन्ध प्रतियोगिता में श्रीमती पार्वती शर्मा प्रथम, श्रीमती शम्मी त्यागी द्वितीय व कुमारी रजनी दलाल तृतीय स्थान पर रहे। वाद-विवाद प्रतियोगिता में श्रीमती शम्मी त्यागी, श्रीमती वंदना कपूर, श्रीमती पार्वती शर्मा ने क्रमशः प्रथम, द्वितीय, व तृतीय स्थान प्राप्त किया। हिन्दी अनुवाद प्रतियोगिता में श्री के.एस. मीणा, श्री रमेश बाबू सक्सेना, श्रीमती अनुराधा एवं शम्मी त्यागी ने क्रमशः प्रथम, द्वितीय, व तृतीय स्थान प्राप्त किया। सुलेख एवं श्रुतलेख प्रतियोगिता में श्री रमेश बाबू सक्सेना प्रथम, श्रीमती अनुराधा भारद्वाज द्वितीय तथा श्रीमती पार्वती शर्मा ने तृतीय पुरस्कार प्राप्त किया। श्रीमती तरूणा आनन्द प्रथम, श्रीमती पार्वती शर्मा द्वितीय व श्री प्रीतम यादव तृतीय भाषण प्रतियोगिता में अव्वल रहे।

हिन्दी पखवाडा में भाषण प्रतियोगिता का आयोजन

Belgian team visits EPC, Bikaner

Team from Belgium visited EPC, Bikaner to record demonstration of NRCE activities including collection of semen and its freezing for promotion of breed conservation for documentary film on October 25, 2012. Belgium Team, accompanied by Sh. Raguvendra Singh, Dundlod, visited different facilities at EPC, Bikaner and interacted with scientists who briefed them about different activities of the NRCE. The team praised the upkeep of animal farm and the campus.

Belgium Team along with Sh. Raguvendra Singh and staff of EPC, Bikaner

NRCE CUP function at BTC, Bengaluru

NRCE Cup function at Bangalore Turf Club was organised on 26 January, 2013 at Bengaluru. The NRCE Cup function, is conducted every year by BTC. A total of eight races were planned on "NRCE CUP" date including "the Kimmane Bangalore Derby" race. This race was for 1400 Meters and 13 horses participated. There were five prizes for winner: The first prize winner was given NRCE CUP and a cash prize of Rupees two lakh sixty four thousand (₹ 2, 64, 000/) only, followed by 2nd, 3rd, 4th and 5th prizes with cash prize of ₹ 1,32,000/-, ₹ 66,000/-, ₹ 39,600/- and ₹ 13,200/-, respectively. NRCE CUP was presented to first winner by Dr B.K. Singh, Principal Scientist & I/c Equine Health Unit on behalf of Director, NRCE. Other dignitaries present at this occasion were Chairman, Secretary and Staff members of BTC, Bengaluru.

NRCE CUP function at BTC, Bengaluru

Vigilance Awareness Week at the Centre

Vigilance Awareness Week was observed from 29th October to 3rd November, 2012. As part of Vigilance Awareness Week celebration Director, NRCE has administered pledge to the scientists and staff of NRCE for honesty and transparency in public life. Director, NRCE in his message also stress on coordinated efforts for eliminating the menace of corruption from the society and work collaboratively towards betterment of the equines in the country.

Foundation Day Lecture at NRCE

On the occasion of Foundation Day of NRCE on November 26, 2012, the Foundation Day Lecture was organized at NRCE wherein Prof. D. K. Mitra, Department of Transplant Immunology, AIIMS, New Delhi delivered a lecture on "Emerging Human Health Issues". Brigadier Desh Raj, Commandent, Equine Breeding Stud, Hisar and Dr R. K. Sethi, Director CIRB were present on this occasion the Guest of Honour. On this occasion, the plantation was also done by the Chief Guest and other dignitaries near animal shed complex at NRCE.

Foundation Day Lecture at NRCE

Secretary DARE and DG ICAR inaugurates sophisticated laboratories and Info-Equine Museum at NRCE, Hisar

Dr S. Ayyappan, Secretary DARE and DG ICAR inaugurated a Microbial Containment Laboratory (BSL-3 facility), Veterinary Type Culture Collection (VTCC) Laboratory Complex (Phase I), and Info-Equine Museum at National Research Centre on Equines, Hisar on March 9, 2013. This MCL facility of Hisar is the first BSL-3 laboratory in Haryana while VTCC is only of its own kind not only in India but in the world. VTCC is concentrating on microbial culture collection of animal origin (disease causing microbes including zoonotic pathogens, rumen microbes, and dairy microbes) but other collections around the world concentrate on either of human/animal pathogens, dairy microbes or rumen microbes unlike VTCC. The Info-Equine Museum has comprehensive information about equines (horses, donkeys, mules, zebra, and hybrids) depicted through models, murals, diorama, audio-visuals like Press Button Board, Storey Telling Board, Interactive Information Kiosk, etc. The Museum also has collection of statuettes/sculptures (camel bone, Terracotta, wooden, velvet, different metals/alloys, etc), equine-related accessories, and different types of regional feed/fodder/supplements. This museum is informative and will be an attraction for people from all walks of life. On this occasion, the Honorable Secretary DARE and DG ICAR also laid foundation stone for BSL-3 facility of VTCC. This occasion was also graced by Prof. KML Pathak, Deputy Director General (Animal Science), ICAR, New Delhi.

Secretary DARE and DG ICAR inaugurating Microbial Containment Laboratory

Secretary DARE and DG ICAR inaugurating ATIC and Info-Equine Museum

Secretary DARE and DG ICAR at ATIC Info-Equine Museum

Secretary DARE and DG appreciating horse models at Info-Equine Museum

Secretary DARE and DG ICAR inaugurating VTCC Laboratory Complex (Phase I)

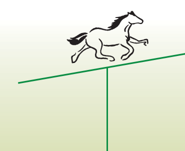
Secretary DARE and DG ICAR laying Foundation Stone for BSL-3 facility of VTCC

Equine Health Camps & *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 2012-13, forteen health camps and *kisan goshthis were* organized in Haryana, Rajasthan, Gujarat, Maharashtra and Jammu & Kashmir; Amarnath Yatra Route, Leh and Laddakh. During the health camps, animals were examined for various ailments by the experts from NRCE. Free medicines and treatment was provided to diseased animals at the camp. Pregnancy diagnosis was done during the camps. Deworming tablets and mineral mixture was distributed 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 goshtis organized

<table>
<tr><th>Sr. No.</th><th>Place</th><th>Date</th><th>Activities</th></tr>
<tr><td>1.</td><td>Rajli, Haryana</td><td>June 16, 2012</td><td rowspan="14">During equine health camps, the animals were examined for various ailments by the experts from NRCE. Basic treatment was provided to diseased animals and medicines, deworming tablets and mineral mixture was provided for equine owners free of cost at the camp. Pregnancy diagnosis was done during the camps. During Kisan Goshthis, Equine Owners interacted with experts from NRCE on various issues related to health, production and management of equine and the constraints faced by them in equine husbandry.</td></tr>
<tr><td>2.</td><td>Sonmarg, Jammu and Kashmir</td><td>July 20, 2012</td></tr>
<tr><td>3.</td><td>Baltal, Jammu and Kashmir</td><td>July 21-22, 2012</td></tr>
<tr><td>4.</td><td>Gulmarg, Jammu and Kashmir</td><td>July 23, 2012</td></tr>
<tr><td>5.</td><td>Chandanwadi, Jammu and Kashmir</td><td>July 25, 2012</td></tr>
<tr><td>6.</td><td>Maham, Haryana</td><td>September 28, 2012</td></tr>
<tr><td>7.</td><td>Jodhpur, Rajasthan</td><td>September 30, 2012</td></tr>
<tr><td>8.</td><td>Maham, Haryana</td><td>October 10, 2012</td></tr>
<tr><td>9.</td><td>Bikaner, Rajasthan</td><td>October 15, 2012</td></tr>
<tr><td>10.</td><td>Pushkar, Rajasthan</td><td>November 23-24, 2012</td></tr>
<tr><td>11.</td><td>Sahpini , Rajasthan</td><td>November 30, 2012</td></tr>
<tr><td>12.</td><td>Pirkamariya, Rajasthan</td><td>December 10, 2012</td></tr>
<tr><td>13.</td><td>Jodhpur, Rajasthan</td><td>January 10, 2013</td></tr>
<tr><td>14.</td><td>Kutch, Gujarat</td><td>February 19-20, 2013</td></tr>
</table>



Equine health camp at Sahpini

Equine health camp at Jodhpur

Equine health camp at Chandanwari

Equine health camp at Maham

Equine health camp at Pushkar

Kisan Goshthi at Sahpini

NRCE Participation in Exhibitions and Fairs

Sr. No.	Place	Date	Activities
1.	Pandusar, Lunkaransar, Bikaner, Rajasthan	August, 24, 2012	Exhibits and extension material on various aspects of equine husbandry and management were displayed for the benefit of Equine Owners. NRCE video film "*Aswa Gatha*" was played during the exhibitions. Exhibition stall also displayed different technologies developed at NRCE. Equine owners met and interacted with scientists on various aspects of equine husbandry.
2.	Pushkar Animal Fair, Rajasthan	November 23-24, 2012	
3.	Agriculture Education Day at CIRB, Hisar, Haryana	November 27, 2012	
4.	CAZRI, Jodhpur	September 12, 2012	
5.	Kisan Diwas, NDRI Karnal	December 23, 2012	
6.	National Livestock Championship Muktasar, Punjab	January 8-12, 2013	
7.	Lunkaransar, Bikaner Rajasthan	March 17, 2013	
8.	CSWRI Avikanagar, Rajasthan	March 23, 2013	

NRCE Exhibition Stall at Pandusar

Sh. Virender Beniwal, Hon'ble State Minister for Home & Transport, Rajasthan at NRCE Stall at Lunkaransar

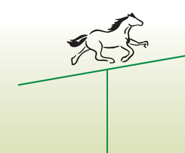
NRCE Exhibition Stall at CAZRI, Jodhpur

NRCE Exhibition Stall at Muktasar

Exposure Visit of Farmers/ Educational Tours of students/ Study tours & visits during April 2012 to March 2013 at NRCE

During 2012-13 following visitors from different places including farmers, students from SAU's, schools and other visitors visiting NRCE. During visits visitors were briefed about the research activities and different extension and field activities of NRCE for benefit of equine owners and various on-going programmes of NRCE.

Sr.No.	Details of Visitors	Date	No of visitors
1.	Study tour of Rawandian Artisans from Republic of Rawanda	August 30, 2012	07
2.	Study tour of Officers undergoing National Diploma in Equine Husbandry Medicine and Surgery (NDEHMS) course at RVC Meerut	August 30, 2012	09
3.	Officers from Ministry of Agriculture, Irrigation and Livestock (MAIL), Government of Afghanistan	September 12, 2012	16
4.	Exposure visit of Farmers from Rajasthan	March 6, 2013	41
5.	Exposure visit of Farmers from Haryana	March 15, 2013	16



NRCE in News

इंटरनेशनल कार्यशाला में घोड़ों को बीमारियों से बचाने पर मंथन शुरू

राष्ट्रीय रिसर्च सेंटर में शुरू हुए दस दिवसीय कार्यक्रम में मौजूद प्रशिक्षु व अधिकारी।

योजना : मार्च तक हिसार बन जाएगा एशिया की रेफरल लैब

अब एशियन गेम्स में जा सकेंगे भारतीय घोड़े

'ग्लैंडर्स व इक्वाइन एन्फ्लुएंजा के लिए रेफरल लैब की भी है तैयारी'

डॉ. आरके सिंह

हिसार जागरण

हिसार, 16 नवंबर 2012

अश्व अनुसंधान के प्रधान वैज्ञानिक आईवीएस अवार्ड से सम्मानित

जागरण संवाददाता, हिसार : इंडियन वायरोलॉजिकल सोसायटी (आईवीएस) की तरफ से 21वां राष्ट्रीय सम्मेलन उत्तराखंड के भारतीय पशु चिकित्सा अनुसंधान संस्थान मुक्तेसर में हुआ। इसमें सिरसा रोड स्थित राष्ट्रीय अश्व अनुसंधान केंद्र के प्रधान वैज्ञानिक डॉ. बीरेंद्र कुमार सिंह आईवीएस के सम्मान से नवाजा गया। वैज्ञानिकों में इस सम्मान को सबसे बड़ा माना जाता है।

आईवीएस की तरफ से मुक्तेसर में मेडिकल, वेटनरी, कृषि और फिशरिज के 250 वैज्ञानिकों को बुलाया गया था। तीन दिन तक चले सम्मेलन में अनेक विषयों पर चर्चा हुई। आईवीएस की तरफ से हर साल इन फिल्ड में से देश में एक वैज्ञानिक को सम्मानित किया जाता है। इस बार यह सम्मान हिसार के अश्व अनुसंधान के प्रधान वैज्ञानिक डॉ. बीके सिंह को मिला। डॉ. सिंह को यह सम्मान उनके द्वारा घोड़ों के लिए हरपीस वायरस पर किए गए शोध पर दिया गया है। अनुसंधान के निदेशक डॉ. राजकुमार सिंह ने कहा कि डॉ. सिंह का फैलो ऑफ इंडियन वायरोलॉजिकल सोसायटी की तरफ से सम्मानित होना अनुसंधान के लिए सम्मान की बात है।

सेमिनार : अश्व रोग पर मंथन, भारत के साथ जापान के भी आएंगे वैज्ञानिक

बेशकीमती घोड़ों को 'मलेरिया'

क्या होता है बीमारी में

29 को जुटेंगे सात देश के वैज्ञानिक

प्रशिक्षुओं ने हनुमानगढ़ से घोड़ों के खून के सैंपल लिए

- राष्ट्रीय रिसर्च सेंटर इक्वाइन की इंटरनेशनल कार्यशाला का दूसरा दिन

टेस्ट के बारे बताया जाएगा

बाहर आना है तो 40 लीटर पानी से नहाना होगा

हाईटेक लैब : घोड़ा फार्म में साढ़े तीन करोड़ से बनी हाईटेक लैब, बैक्टीरिया को बाहर आने से रोकने का उपाय।

राष्ट्रीय अश्व अनुसंधान केंद्र में बनी लैब।

कल होगा शुभारंभ

लैब में है तीन विंग

भूतल पर लगी हैं मशीनें

पानी का होगा ट्रीटमेंट

हिसार भास्कर

हरपीस पर शोध का डॉ. बीके को इनाम

हिसार में होगा घोड़ों के 'मलेरिया' का इलाज

शोध व सुविधा : घोड़ों में चीचड़ जनित रोग के लक्षण होते हैं मलेरिया रोग जैसे, अश्व केंद्र में दो से छह घंटों में मिलेगी टेस्ट रिपोर्ट

यहां से पहुंचे प्रतिनिधि

यूं फैलती है ईपी

अश्व केंद्र में होगा टेस्ट

हिसार जागरण सिटी

घोड़ियों का अब नहीं हो सकेगा गर्भपात

Visit of Dignitaries

- A team of dignitaries comprising of Dr V. A. Srinivasan, Research Director, Indian Immunological Limited, Dr H. K. Pradhan, Consultant, WHO (India), Dr Gaya Prasad, ADG (AH), ICAR visited NRCE on April 28, 2012 on the occasion of World Veterinary Day Celebration.
- Dr M. Rajasekhar, Technical Director, Diagnostic Research Laboratories, RWITC, Race Course, Pune visited NRCE on May 10, 2012. He visited ATIC, BSL-3 Laboratory, Animal Shed complex, VTCC, and different labs at the Centre.
- Dr Satish Ayyar, Secretary, Indian Stud book Authority visited NRCE on June 12, 2012 and delivered lecture on the topic entitled "DNA Typing in Equines".
- Dr El. Harrak Mehdi, Head of R&D Department, Dr Khalid Onari Tadlaoui, MCI, Animal Health, Morocco and Amitabh Mishra, NRDC, New Delhi visited NRCE on September 06, 2012. Dr Mehdi delivered a lecture on "West Nile VIrus." On this occasion, plantation was also done by Dr El Harrak Mehdi at NRCE.
- Dr Satya Parida, Head of Vaccine Differentiation Group, Pirbright Laboratory, Institute of Animal Health, UK visited NRCE on September 12, 2012. He delivered a lecture on "Development of Recombinant FMDV and PPRV marker (DIVA) Vaccines using Reverse Genetics Technique."

Dr El Harrak Mehdi interacting with NRCE scientists

Dr Satya Parida delivering lecture at NRCE

- Dr C. Balachandran, Registrar, TANUVAS visited NRCE on November 3, 2012. He visited ATIC, BSL-3 Laboratory, Animal Shed Complex, VTCC and different labs in the Centre. Director, NRCE briefed him about different ongoing research activities in the NRCE. He appreciated the research and infrastructure facilities available at NRCE.

Dr C. Balachandran visiting NRCE laboratory

- Dr MAJ Eric D. Lombardini, Chief, Divisions of Comparative Pathology & Veterinary Medical Research, Armed Forces Research Institute of

Medical Sciences (AFRIMS), Bangkok, Thailand delivered lecture on "Pathology of aquatic animals, farmed and laboratory fish including integrated aquaculture and waste management"on November 8, 2012.

Dr MAJ Eric D. Lombardini delivering lecture at NRCE, Hisar

- Brigadier Desh Raj, Commandent, Equine Breeding Stud, Hisar visited NRCE, Hisar on the occasion of Foundation Day celebration on November 26, 2012.
- Prof. D. K. Mitra, Department of Transplant Immunology, AIIMS, New Delhi visited NRCE to deliver a lecture on NRCE foundation day on November 26, 2012.
- Prof. I. Igarashi (NRCPD, Japan), Obihiro, Japan visited NRCE, Hisar on November 29, 2012 as an OIE Expert on Equine Piroplasmosis during International Workshop for SAARC countries participants under OIE Twinning Program on Equine Piroplasmosis at NRCE. During his visit, he also visited Sahpini village in Rajasthan on 30 November, 2012 to attend Kisan Gosthi and Equine Health Camp along with NRCE scientists and participants of training programme.

Prof. I. Igarashi Interacting with equine owners

- Dr D. S. Shivdekar, Former Director, Institute of Animal Health and Veterinary Biologicals & Scientific Advisor, Indovax Pvt. Ltd. visited NRCE on January 15, 2013. He visited different laboratories and facilities at NRCE and VTCC.
- Dr S. Ayyappan, Secretary, DARE and DG, ICAR inaugurated a Microbial Containment Laboratory (BSL-3 facility), Veterinary Type Culture Collection (VTCC) Laboratory Complex (Phase I), and Info-Equine Museum at National Research Centre on Equines, Hisar on March 9, 2013.
- Prof. K.M.L. Pathak, Deputy Director General (Animal Science), ICAR; Dr D.K. Sharma, Director, CSSRI, Karnal, and Dr Indu Sharma, Project Director, DWR, Karnal visited NRCE on March 9, 2013 during the inauguration of various facilities at NRCE.
- Dr M.P. Yadav, President, NAVS and Sh. Kuldeep Dhaliwal, Member, GB, ICAR visited NRCE, Hisar on March 9, 2013 during the inauguration of various facilities at NRCE.
- Dr A.K. Dahama, Vice-Chancellor, Swami Keshwanand Rajasthan Agricultural University, Bikaner visited NRCE on March 10, 2013. He visited ATIC Info-Equine museum, VTCC, and Animal Shed complex at NRCE.

Dr A.K. Dahama, Vice-Chancellor, Swami Keshwanand Rajasthan Agricultural University, Bikaner at Animal Shed Complex at NRCE, Hisar

Plantation by Dr S. Ayyappan, Secretary, DARE and DG, ICAR

At EPC, Bikaner

- Sh. Chander Bhan, Commissioner of Customs, Jaipur visited the Sub-Campus on May 22, 2012 and appreciated the management of animals at the Centre.
- Dr K.M.L. Pathak, Hon'ble DDG (Animal Sciences) ICAR, New Delhi visited the Sub - Campus on September 27, 2012. The management of campus and upkeep of equines breeds at the Centre was well praised and asked to carry out good research and conservation activities.

Dr. K.M.L. Pathak, DDG (Animal Science) visiting EPC, Bikaner Centre

- QRT of NRCE headed by Dr. R.N. Sreenivas Gowda, Former Vice Chancellor of KVASFU, Bidar visited EPC, Bikaner from October15-16, 2012 and interacted with the scientists of the Centre. The QRT team also interacted with the equine owners and discussed the practical problems of the equine owners and also took feedback from them with regard to the kind of services that is expected from NRCE.

QRT of NRCE at Bikaner campus

- A Team from Belgium visited EPC, Bikaner on October 25, 2012 to record demonstration of NRCE activities including semen freezing etc. for documentary film on breed conservation. They interacted with the scientists and praised the upkeep of the animal farm and the campus.
- Smt. Margaret Alva, Hon,ble Governor of Rajasthan visited our Animal Show organised at NRCC, Bikaner on November 7, 2012. She appreciated our precious germplasm being raised at EPC Bikaner and shown keen interest in the activities regarding propagation and conservation of equine species undertaken by NRCE.

Smt. Margaret Alva, Hon'ble Governor of Rajasthan at EPC Bikaner

- Dr A.M. Shekh, Vice-Chancellor, AAU, Anand, Gujarat visited the Centre on November 11, 2012 along with Director of Research and Director of Extension. They all appreciated the ongoing

research and development activities being carried out at the Centre.

- Dr S.P.S. Ahlawat, Former Director, IVRI, Izatnagar along with QRT team of NRCC, Bikaner visited EPC, Bikaner on January 31, 2013 and interacted with the scientists of the Centre. They all appreciated the campus including the animal and agriculture farm.
- Dr S.M.K. Naqvi, Director, CSWRI Avikanagar visited EPC, Bikaner on February 11, 2013 and interacted with scientists and shown keen interest in the ongoing research and development activities of the sub-campus at Bikaner.

Dr S.P.S. Ahlawat with team of NRCC QRT at EPC Bikaner

Dr S.M.K. Naqvi, Director, CSWRI Avikanagar at EPC, Bikaner

Infrastructure Development and Developmental Activities

Development of Info-Equine Museum at ATIC, NRCE

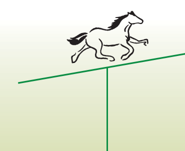
Info-equine museum at ATIC, NRCE is the first of its kind in the country. The museum portrays the evolution of equines over a period of time from Hyracotherium or Eohippus that lived about 55 million years ago to *Equus* which is the only surviving genus in the once diverse family of horses. The coat colour variation in horses over time Pleistocene to Copper, Bronze and Iron Age depicting variation in colours of horses over a period of time. Museum displays the information on important breeds of horses and population scenario of equines in India and the World. Info-Equine Museum has comprehensive information about equines (horses, donkeys, mules, zebra, and hybrids) depicted through posters, translites, murals, storytelling board and information kiosk. Info equine museum also have models of different breeds of horses and press button board for locating the breeding tracts of equines on map of India.

The museum also has the collection of terracotta horse statuette, wooden horse statuette, handcrafted camel bone horse statuettes, brass, metal alloy, fibre and velvet horse figuring collected from different parts of the country. The utility of equines in different activities like transportation, agricultural and postharvest operations, equestrian events, brick kilns and utility in hilly areas has been depicted in form of translite and diorama. The murals depicting the importance of equines in our culture and utility in different activities are also been portrayed in the ATIC.

The NRCE gallery of Info-equine museum gives the glimpses of research and development activities at the centre. It also displays the various technologies developed including diagnostic kits, vaccines, information on different equine diseases. The Information Kiosk in museum provides the information on different aspects of equine husbandry and management in user friendly touch screen interface.

Inside view of Info-equine Museum

The equine accessories section of museum has the collection of equine-related accessories which includes different types of horse shoes, stirrupps, whips and bits. The various equine related accessories like saddles, horse grooming set, ferrier set, AI kit, polo stick and different types of regional feed/fodder/supplements are also displayed in the museum. The museum also has a collection of horse carriages and carts meant for transportation. The Info-Equine Museum was inaugurating by Dr S. Ayyappan, Secretary DARE and DG ICAR at National Research Centre on Equines, Hisar on March 9, 2013. This museum is very informative and will be an attraction for people from all walks of life.



Secretary DARE and DG ICAR inaugurating ATIC and Info-Equine Museum

Different types of horse shoes and bits

DG ICAR & Secretary DARE & other dignitaries in front of antique horse carriage

Inauguration of Microbial Containment Laboratory

Microbial Containment Laboratory (MCL) at NRCE, Hisar is the first BSL-3 laboratory in Haryana. Dr S. Ayyappan, Secretary DARE and DG ICAR inaugurated a Microbial Containment Laboratory (BSL-3 facility), on March 9, 2013. This occasion was also graced by Prof. KML Pathak, Deputy Director General (Animal Science), ICAR, New Delhi. Access to the laboratory is restricted, and the Standard Operating Protocols based on Standard Microbiological Practices for Biosafety Level 3 have been developed and are rigorously followed. This laboratory will cater to the needs of the centre for working on highly infectious equine diseases that can spread rapidly from animal-to-animal or even to human beings. The facility will allow handling indigenous or exotic agents including bacteria, parasites and viruses which may cause serious or potentially lethal disease to human beings.

Microbial Containment Laboratory (MCL) at NRCE, Hisar

Secretary DARE and DG ICAR inaugurating Microbial Containment Laboratory

Inauguration of Veterinary Type Culture Collection facility

Dr S. Ayyappan, Secretary DARE and DG ICAR inaugurated Veterinary Type Culture Collection (VTCC) Laboratory Complex (Phase I) on March 9, 2013. VTCC- the national repository of animal microbes is one of its kind not only in India but in the world. The repository is proactively involved in conserving microbial diversity of animal origin (including zoonotic pathogens, rumen microbes, and dairy microbes) unlike other collections around the world which are single either human/animal pathogens, dairy microbes or rumen microbes unlike VTCC.

Secretary DARE and DG ICAR inaugurating VTCC Laboratory Complex (Phase I)

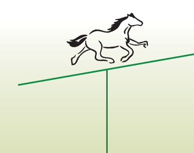
Construction of 2nd phase building of Veterinary Type Culture Collection

The first phase of construction of VTCC building has been completed and the laboratory building has become fully functional. The internal furnishing of the individual laboratories, including the Microbial repository and other support facilities has also been completed. Besides the entrance lobby of the VTCC building has been renovated to include a Reception & Seminar/Conference room. Furthermore, the internet and LAN facility has also become operational. The biosecurity aspect of the VTCC complex has been put in place with the installation of CCTV cameras and an automatic access system for ensuring security of the valuable microbial resources within the repository. The foundation stone of the BSL-III laboratory for VTCC has been laid by the Hon'ble DG ICAR on March 9, 2013. The second phase construction of the VTCC building is also in full swing which would help in augmenting the current research activities.

Secretary DARE and DG ICAR laying foundation stone for BSL-III facility of VTCC

Reception of VTCC Main Building

1st phase VTCC Building



Agriculture Farm Production

Crop production at a Glance (QRT period 2007-2013)

Overall status of fodder/grain production and land reclamation is given in Table.

Crop production at agriculture farm, NRCE, Hisar

S. No.	Name of produce	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14
1.	Green fodder	932 Qt.	754 Qt.	1278 Qt.	1926 Qt.	2196 Qt.	1806.50 Qt.	-
2.	Dry fodder	70 Qt.	18 Qt.	-	205 Qt.	130 Qt.	84 Qt.	322.5 Qt.
3.	Oat grain	-	13.90 Qt.	-	127 Qt.	194.69 Qt.	206.42 Qt.	273.66Qt.
4.	Mustard grain	-	-	-	-	174 Qt.	178.45 Qt.	142.4 Qt
5.	Area under cultivation	17 Ac.	17 Ac	30 Ac.	70 Ac.	80 Ac.	82 Ac.	-
6.	Area under development	-	-	50 Ac.	25 Ac.	30 Ac.	30 Ac.	30 Ac.
7.	Revenue generation	-	-	-	2,45,640	8,27,974	9,31,987	-
	(in ₹)				.00	.00	.00	

Crop production at agriculture farm, NRCE, Bikanera

S. No.	Name of produce	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14
1.	Green fodder	1439.65 Qt.	1001 Qt.	1084 Qt.	1446.90 Qt.	1110 Qt.	2502.23 Qt.	-
2.	Dry fodder	-	-	20 Qt.	400 Qt.	13 Qt.	325.23 Qt.	170 Qt.
3.	Oat grain	-	-	-	-	4.75 Qt.	06.00 Qt.	51.5 Qt.
4.	Area under cultivation	-	10 Ac.	15 Ac.	30 Ac.	50 Ac.	70 Ac.	-
5.	Area under	-	-	05 Ac.	10 Ac.	40 Ac.	15 Ac.	-
	development							
6.	Revenue generation	-	-	-	-	-	-	-
	(in ₹)							

Crop production at NRCE, Hisar

During the period under report, about ninety three acre land rotationally was used for growing of different types crops. In spite of high water table and salinity in most of the farm areas, vigorous efforts were made to produce maximum feed & fodders. A total of 1806.5 Qt of green fodder, 37Qt of Oat and 38 Qt of Mustard have been produced during this period. The efforts put in this activity not only resulted in self sufficiency of the centre in terms of fodder requirement but yield was also surplus which is being used for revenue generation.

Production of crops at NRCE, Hisar (2012-13)

Sr. No.	Crop	Area (Acre)	Production (Qt.)
1.	Oat+ Berseem	6	500.5
2.	Sorghum sudan grass + Cowpea	13	450
3.	Sorghum sudan grass	-	452
4.	Lucern	3	210
5.	Maize + Cowpea	2	194
		Total Green Fodder	**1806.5**
Other Crops			
1.	Oat	37	
2.	Mustard	38	

Crop production at EPC, Bikaner

During the period under report, a total of 2502.23 Qt. of green fodder (Lucerne 441.06 Qt. + Oat 321.75 Qt. + Millet 1739.42 Qt.) and 300.23 Qt. dry fodder of Millet was produced at the agriculture farm of EPC Bikaner and provided to the animals. The Centre has saved the purchase of chaffed Sewan grass to the tune of more than 1100 Qt. by producing above mentioned fodder (2802.46 Qt.) of different kinds. 25 Qt. of Guar fodder and 6 Qt. of guar Grain have also been produced. About 100 Qt. of Oat grain and 250 Qt. of dry fodder of Oat are likely to be chaffed in near future. During the rabbi season (Nov-Dec, 2012) Oat in 35 acres of land (maximum ever) and Lucerne in 5.50 acres has been sown after reclamation of 7-8 acres land at the sub campus Bikaner.

Oat field at EPC Bikaner

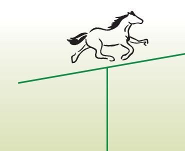
Land Reclamation and development of field at NRCE, Hisar & EPC, Bikaner

The lawn was developed with suitable grass spp. at ATIC and VTCC for improving environmental condition and beautification of premises. Underground Pipelines installed in lawn & plantation area of ATIC, MCL, HSDC wall side & VTCC building to save water & proper maintenance of grass & plants. The efforts put in this activity resulted in saving & proper distribution of water with salinity reclamation. A tube well also installed in ATIC premises for arrangement of water for lawn, plants & building.

A lawn of 60 x 250 feet size along with main road side at EPC, Bikaner was developed after levelling and removing of bushes. Different species of ornamental and shady plants in addition to flowering were also planted for making the environment more pleasant and beautification of premises.

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

Photo of Mustard crop at NRCE, Hisar



Landscape and plantation work

Different spp. of plants were prepared and maintained in nursery at the back side of the guest house and Director's residence area. The efforts put in this activity resulted in plantation work at NRCE Campus. Different species of flowering, ornamental and shady plants were planted to improve environmental condition of the campus. The plantation around animal sheds & farm area will provide shelter to the farm people and animals.

Plantation by Secretary DARE and ICAR DG

Plantation by DDG (AS)

Livestock strength at NRCE, Hisar & EPC, Bikaner

Herd Strength at Equine Production Campus, Bikaner (2012-13)

Category	Marwari Horse		Zanskari Horse		Pony		Exotic Donkey		Ind. Donkey		Mule		Total
	M	F	M	F	M	F	M	F	M	F	M	F	
Stock as on 01.04.12	24	42	08	07	00	03	10	16	18	07	03	02	140
Birth during the year	04	07	01	00	00	00	01	02	00	00	00	00	15
Purchase during the year	00	00	00	00	00	00	00	00	00	00	00	00	00
Sold during the year	00	00	00	00	00	00	00	00	00	00	00	00	00
Transfer from NRCE Hisar	00	05	00	00	00	00	00	00	00	00	00	00	05
Death during the year	01	02	00	00	00	00	01	00	00	00	00	00	04
Balance as on 31.03.13	27	52	09	07	00	03	10	18	18	07	03	02	156

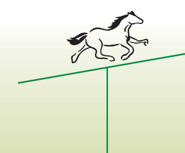
Herd Strength at NRCE Main Campus, Hisar (2012-13)

Category	Marwari Horse		Ind. Horse	Pony		Exotic Donkey		Mule		Total
	M	F	M	M	F	M	F	M	F	
Stock as on 01.04.12	03	21	01	01	04	03	03	02	00	38
Birth during the year	03	01	00	00	00	00	01	00	00	05
Purchase during the year	00	00	00	00	00	00	00	00	00	00
Sold during the year	03	01	00	01	02	02	01	01	00	11
Transfer from NRCE Hisar	00	05	00	00	00	00	00	00	00	00
Death during the year	00	00	00	00	00	00	00	00	00	00
Balance as on 31.03.13	03	16	01	00	02	01	03	01	00	27

On-going
Research Projects (2012-13)

Equine Health

Sr. No.	Title	Team	From	To	PIMS Code
1.	Surveillance, Monitoring and Control of Emerging and Existing Diseases of Equines	S.K. Khurana*, B.K. Singh, S.C. Yadav, Baldev R. Gulati, Rajender Kumar, Praveen Malik, Sanjay Kumar, Nitin Virmani, Sanjay Barua, Rajesh Kr. Vaid, Anju Manuja, H. Singha and Ramesh Dedar	April, 1995	Continuous Service Project	IXX00257
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	Aug. 2010 Extended up to March. 2013 in XV RAC & IRC 2011-12	IXX00258
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 Extended up to March. 2013 in XV RAC & IRC 2011-12	IXX00259
4.	Development of diagnostics for equine influenza	Nitin Virmani*, B. C. Bera, Baldev. R.Gulati, and B.K.Singh	Sept. 2009	Aug.2012 Extended up to March. 2013 in XV RAC & IRC 2011-12	IXX00260
5.	Evaluation and Updation of the inactivated equine influenza virus vaccine	Nitin Virmani*, Baldev R. Gulati, and B.K. Singh	Oct 2009	Sept. 2012 Extended up to March. 2013 in XV RAC & IRC 2011-12	IXX00261
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,	Oct. 2009	Sept. 2012 Extended up to March. 2013 in XV RAC & IRC 2011-12	IXX00262



Sr. No.	Title	Team	From	To	PIMS Code
7.	Characterization of Toll-like receptor 9 and its role in CpG immuno-modulation in equines	Anju Manuja*, Balvinder Kumar, and H.S. Singha	Oct. 2010	Sept. 2013	IXX00276
8.	Development of recombinant protein-based immune-diagnostic kit for equine infectious anemia (EIA)	Praveen Malik* and H.S. Singha	Sept. 2010	Aug. 2013 (XV RAC recommended the project may be completed by March 2013)	IXX08120

* Principal Investigator

Extension

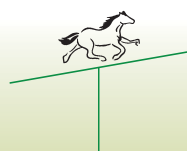
Sr. No	Title	Team	Date of Start	Date of Completion	PIMS Code
1.	Studies on existing management systems and utilization of donkeys and mules for sustainable livelihood	Ajay Raut*, Yash Pal and R.A. Legha, R.K. Dedar and J. Singh (From 29th May 2012)	Sept., 2009	Aug 2011, Extended up to March. 2013 in XV RAC & IRC 2011-12	IXX00268

*** Principal Investigator**

VTCC

Sr. No.	Title	Team	Duration	To	PIMS Code
1.	Isolation, maintenance and characterization of bacterial pathogens and their molecular identification	Rajesh Kumar Vaid*, Sanjay Barua, B.C. Bera, Taruna Anand (from July, 2010), T.Riyesh (from January, 2012) and Sarita Yadav (upto 30th Nov. 2012)	June, 2007	May, 2010 Extended up to March. 2013 in XV RAC & IRC 2011-12	IXX00269
2.	Isolation, molecular characterization and reposition of viruses of animal origin	Sanjay Barua*, B.C. Bera, R. K. Vaid, B.R. Gulati, T. Riyesh, Sarita Yadav (upto 30th Nov. 2012) and Taruna Anand (from 3rd Dec. 2011)	Sept. 2009	Aug. 2012, Extended up to March. 2013 in IRC 2011-12	IXX00270
3.	Development of protein expression clone repository of virulence associated genes of zoonotic buffalopox and equine influenza viruses	B.C. Bera*, Sanjay Barua, Nitin Virmani, Taruna Anand, Riyesh T. and Sarita Yadav (up to 30 Nov. 2012)	Jan. 2012	Dec. 2015	IXX07760

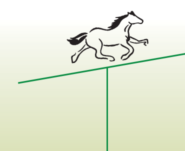
*** Principal Investigator**



Equine Production

Sr. No.	Title	Team	Date of Start	Date of Completion	PIMS Code
1.	Studies on class I and II genes of major histocompatibility complex in donkeys	R. C. Sharma*, Balvinder Kumar	April, 2010	March 2013, XV RAC and IRC (2011-12) recommended, this project completed by March 2013	IXX00265
2.	Cryopreservation of equid semen using amides	S. K. Ravi (PI from Feb. 2012)*, Yash Pal and R.K. Vaid (Co-PI from Feb., 2010)	Oct., 2009	Sept. 2011, Extended up to Sept. 2012 in IRC-2010-11.	IXX00266
3.	Characterization of indigenous non-descript and geographically distinct donkeys	A.K. Gupta*, Yash Pal, R.C. Sharma, Anuradha Bhardwaj and Sanjay Kumar	Aug. 2010	March 2014, XV RAC recommended: this project completed latest by March 2014	IXX00274
4.	Cloning, Expression and Characterization of equine chorionic gonadotropin (eCG)	Anuradha Bhardwaj*, A.K. Gupta, Sanjay Kumar and Varij Nayan	Dec. 2010	Nov. 2013	IXX02769
5.	Characterization of donkey milk with emphasis on important milk proteins	Yah Pal*, Raghvendra Singh, Sanjay Kumar and Mamata Chauhan	June 2012	March 2015	IXX07761
6.	Effect of feeding various combinations of dry roughages available in arid region of Rajasthan on growth and nutrient utilization in growing horses	R A Legha*, P A Bala, Vijay Kumar and N V Patil	June 2012	March 2015	IXX07762
7.	Therapeutic and performance enhancing capacity of antioxidants in equines	Ramesh Kr. Dedar*, Vijay Kumar, Jitender Singh and A.P. Singh	July 2012	March 2015	IXX09641
8.	Endocrine, biochemical and gene expression profiling of reproductive states in Marwari Mares	Vijay Kumar*, Sanjay Kr. Ravi, R.K. Dedar, Raghvendra Singh and J. Singh	Oct., 2012	March, 2015	IXX09663

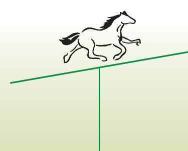
*** Principal Investigator**



Externally Funded Research Projects (2012-13)

Sr. No.	Title	Team	Date of Start	Date of Completion	Status	PIMS Code
1.	National Fellow Scheme-Development of sensitive and specific diagnostic assays for detection of *Trypanosoma evansi* infection in animals using modern molecular tools	Rajender Kumar*	April 2011	April 2016	Ongoing	OXX01431
2.	All India Co-ordinated Research Project on "Increased Utilization of Animal Energy with enhanced system efficiency" (AICRP on UAE)	Yash Pal* and R A Legha	April, 2009	March 2015	Ongoing	OXX00486
3.	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	Ongoing	OXX00393
4.	Studies on *B. mallei* for rapid diagnosis of glanders in equines using molecular tools	Praveen Malik*, S.K. Khurana, H.S. Singha and R.K. Singh	Aug. 2010	July 2013	Ongoing	OXX00394
5.	OIE Twinning Laboratories Project on Equine Piroplasmosis (Japan)	Sanjay Kumar*, Rajender Kumar and R.K. Singh (NRCE, India) Prof. I. Igarashi and N. Yokoyama (NRCPD: Japan)	June 2010	May 2013	Ongoing	OXX01557
6.	Development of biomarkers 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	Ongoing	OXX01616
7.	Synthesis, characterization and evaluation of drug loaded nano-formulation against *Trypanosoma evansi* in animal model	Anju Manuja*, Neeraja Dilbahgi, Sandeep Kumar, Rajender Kumar, Balvinder Kumar and S.C. Yadav	March 2012	March 2015	Ongoing	OXX01526
8.	Isolation and characterization of equine mesenchymal stem cells from amniotic fluid	B. R. Gulati*, Pawan Kumar, Prem Singh Yadav, Taruna Anand and B.K. Singh	April 2012 (for 18th Months)	Sept. 2013	Ongoing	OXX02186
9.	Eukaryotic expression of important equine cytokines and analysis of their biological activities	H.S. Singha*	Oct. 2012	Sept. 2015	Ongoing	OXX02228
10.	OIE Twinning program for Glanders	Praveen Malik*, H. S. Singha and R.K. Singh	July 2012	June, 2015	Ongoing	OXX02428
11.	OIE Twinning program on Equine Influenza	Nitin Viirmani*, R.K. Singh, B.C. Bera and R.K. Vaid	Oct. 2012	Sept. 2015	Ongoing	OXX02429
12.	Bioinformatics Infrastructure facility for Biology teaching through Bioinformatics (BIF-BTBI) under BTISnet at NRCE, Hisar	R. K. Vaid* and Sanjay Kumar	Jan. 2013	-	Ongoing	-

***Principal Investigator**



Research Publications

List of Published Papers

1. Arangasamy, A., Rao, T. T., Singh, R. K., Yash Pal and Tandon, S.N. 2012. Unilateral cryptorchidism in Marwari stallion – A case study. *Indian Veterinary Journal,* (Accepted).
2. Anand, T., Bera B. C., Vaid, R. K., Shanmugasundaram, K., Sharma, Gautam., Virmani, N., Shukla B. N., Bansal. M., Riyesh. T., Barua, S., Malik, P. and Singh, R. K. 2013. Molecular characterization of virulence-associated protein (Vap) family genes of pathogenic *Rhodococcus equi* isolates from clinical cases of Indian equines. *Indian Journal of Biotechnology*, (Accepted).
3. Bera, B. C., Shanmugasundaram, K., Barua, S., Anand, T., Riyesh, T., Vaid R K, Virmani, N., Bansal, M., Shukla, B. N., Malik, P and Singh, R. K. 2012. Sequence and phylogenetic analysis of host-range (E3L, K3L & C7L) and structural protein (B5R) genes of buffalopox virus isolates from buffalo, cattle and human in India. *Virus Genes*. 45(3):488-98.
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5. Chauhan, M., Gupta, A. K., Sharma, Y.P., Bhardwaj, A. and Sharma, P. 2012. Efficacy of nine microsatellite markers in parentage testing of horse breeds. *Indian Veterinary Journal, (*Accepted).
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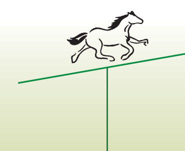

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Review articles

1. Bhanuprakash, V., Hosamani, M., Venkatesan, G., Balamurugan, V., Yogosharadhya, R. and Singh, R.

K. 2012. Animal poxvirus vaccines: A comprehensive review. *Expert Rev. Vaccines* 11(11): 1355-1374.

2. Bhardwaj, A., Nayan, V., Parvati, Mamta and Gupta. A. K. 2012. Inhibin: A role for fecundity augmentation in farm animals. *Asian Journal of Animal and Veterinary Advances,* 7(9):771-789.

3. Kumar, D., Anand, T., Yadav, P.S. and Sethi, R.K. 2011. Clinical ans therapeutic application of skin cells in domestic animals. *Indian J Comp Microbiol Immunol and Infect. Dis.* Vol 32 (Online published on 9th Oct. 2012)

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5. Thakur, S. D., Vaid, R. K., Panda, A. K. and Saini, Y. 2012. Marine mammal brucellosis: a new dimension to an old zoonosis, *Current Science,* 103:902-910.

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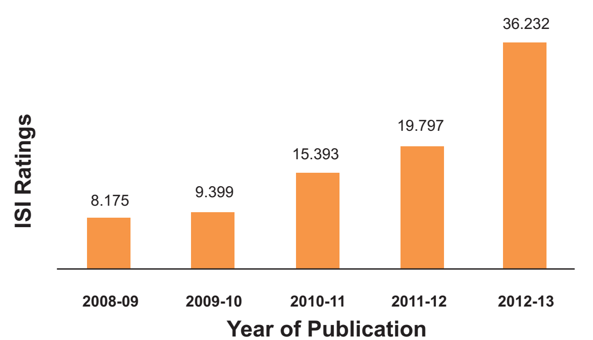
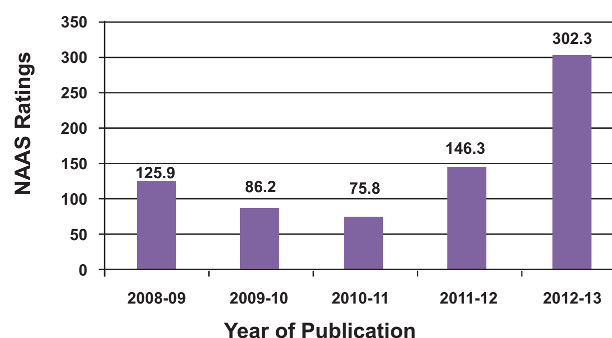
Table: Year wise impact factor of NRCE publications as per NAAS and ISI rating

Year	NAAS Rating	ISI impact factor
2007-08	67.8	10.477
2008-09	125.9	8.175
2009-10	86.2	9.399
2010-11	75.8	15.393
2011-12	146.3	19.797
2012-13	302.3	36.232



Year wise impact factor of NRCE publications as per ISI rating



Year wise impact factor of NRCE publications as per NAAS rating

Abstracts in Symposia/ Conferences

1. Gulati, B.R. presented an invited Paper 'Japanese encephalitis virus among horses in India: perspective on epidemiology and intervention', In XXI National Conference on Immunobiology and Management of Viral Diseases in 21st Century "VIROCON-2012", Indian Veterinary Research Institute, Mukteshwar, November 8-10, 2012.

2. Bera, B.C., Barua, Sanjay Riyesh T., Shanmugasundaram K., Vaid, R.K., Anand, Taruna, Virmani, Manish Bansal, Praveen Malik and R.K.Singh. (2013). Adaptive Evolution of Buffalopox and Camelpox Viruses in Spill Over Hosts. MS450: *In*, The Proceedings of 1st International Conference on Bio-Resource and Stress management on February 3-9, 2013, Science City, Kolkata organized by Ratikanta Maiti Foundation. Pp 8.6.

3. Singh, B.K., presented paper on "Neurological Disorders in horses reported by RWITC: Sequential Steps Taken by NRCE to conclude its diagnosis" at technical Seminar related to mortality of RWITC horses (2 yrs old) at RWITC, Pune on February 7, 2013.

4. Singh, B.K. presented research paper entitled "Equine herpes virus 1(EHV-1): the Indian scenario' XXI in National Conference during "VIROCON-2012" held at Indian Veterinary Research Institute, Mukteshwar, Nainital w.e.f November 8-10, 2012.

5. Gulati, B.R. presented an Invited Paper entitled 'Mesenchymal stem cells: application in equine medicine' in National Workshop on Stem Cell Research and Therapeutics: Current Status and Future Strategies, Division of Physiology and Climatology, Indian Veterinary Research Institute,

Izatnagar (U.P.), September 28-29, 2012, p20.

6. Barnela, M., Manuja, A., Saini, R., Kaur, H., Kumar, S., Chopra, M., Yadav, S.C., Kumar, R., Kumar, B., Dilbaghi, N. 2012. Drug loaded nanoparticles for sustained delivery of trypanocidal drug for use in animals. In The Proceedings of "Current and Future scenarios in drug development and delivery", Aug 11-12 , 2012.

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9. Bhardwaj, A., Chauhan, M., Sharma, P., Nayan, V., Kumar, S. and Gupta, A.K. 2012. Reproductive hormones in vitro: molecular cloning, characterization and expression analysis. In "International Conference on Biotechnology Emerging Trends (ICB-2012) at Chaudhari Devi Lal University, Sirsa (Haryana), India from Sept. 18-20, 2012.

10. Bhardwaj, A., Nayan, V., Kumar, S., Chauhan, M., Yash Pal Sharma, Jitendra Singh and A.K. Gupta. "Equine Pituitary Gonadotropins: cloning and characterization of alpha subunit" in World congress of biotechnology, Bright Conference. Hyderabad May 4-6, 2012.

11. Bhardwaj, A., Nayan, V., Kumar, S., Chauhan, M., Yash Pal, Singh, J. and Gupta, A.K. 2012. Equine pituitary gonadotropins: cloning and sequence analysis of alpha subunit. In World Congress on Biotechnology with a theme of "Harnessing recent and novel trends in the field of Life Sciences" held during May 4-6, 2012, Hyderabad.

12. Dedar, R., Singh, J., Kumar, S., Legha, R.A., Yash Pal and Singh R.K. 2012. Prevalence of various clinical ailments of indigenous horses in Rajasthan. In International Conference on Extension Education in the Perspective of Advances in Natural Resource Management in Agriculture (NaRMA-IV) held at SKRAU, Bikaner during December 19-21, 2012 Abstract No. D-130.

13. Gupta, A.K., Chauhan, M., Bhardwaj, A. and Kumar, S. 2012. Genetic diversity and relationship between Marwari, Kathiawari and Thoroughbred horses. In World Congress on Biotechnology with a theme of "Harnessing recent and novel trends in the field of Life Sciences" held during May 4-6, 2012, Hyderabad. Pp

14. Gupta, A. K., Yash Pal, Kumar, S., Bhardwaj, A., Sharma, P., Sharma, R.C., Legha, R.A., and Raut A. 2013. Biometric analysis and different managemental practices for donkey rearing in different geographic areas in India. In X National Symposium on "Integrated Development of Vast Biodiversity of Indigenous Livestock for Long Term Rural Livelihood Security" held at Deptt of Animal Genetics & Breeding, CoV & Anim Sciences, G.B. Pantnagar University of Agriculture and Technology, Pantnagar from Feb 7-8, 2013. Pp254-255.

15. Kumar, R., Singh, R., Singh, J., Kumar, S. and Yadav S.C. 2012. Genetic diversity analysis of invariant surface glycoprotein gene in *Trypanosoma evansi* isolate of different host from different geographical regions of India. XXIII National Congress of Veterinary Parasitology and National Symposium held at Guwahati from Dec. 12-14, 2012.

16. Kumar R, Singh, R, Singh J, Kumar S and Yadav SC. 2012.Comparative efficacy of different media for *in-vitro* cultivation of different *T. evansi isolate.* XXIII National Congress of Veterinary Parasitology and National Symposium held at Guwahati from Dec. 12-14, 2012.

17. Kumar, V., Chaudhary, A. and Yadav, S.C. 2012. Exo-antigen of *Trypanosoma evansi*: A new approach for detection of *T. evansi* infection in donkey using

Western blot. XXIII National Congress of Veterinary Parasitology and National Symposium held at Guwahati from Dec. 12-14, 2012.

18. Legha, R.A., Yash Pal and Dedar, R.K. 2012. Indigenous technical know-how (ITK's) practiced by equine owners in Rajasthan. In International Conference on Extension Education in the Perspective of Advances in Natural Resource Management in Agriculture (NaRMA-IV) held at SKRAU, Bikaner during December 19-21, 2012 Abstract No. B-46.

19. Legha, R.A., Yash Pal and Gupta, A.K., 2013. Ploughing efficiency of indigenous donkeys using single furrow and double furrow plough. In National seminar on "New Paradigms in Livestock Production: From Traditional to Commercial Farming and Beyond" held at NDRI, Karnal during January, 28-30, 2013. (Abstract no. F51)

20. Legha, R.A., Yash Pal, Dedar, R.K. and Vaid, R.K. 2012. Successful utilization of equine dug for preparation of vermicompost. In International Conference on Extension Education in the Perspective of Advances in Natural Resource Management in Agriculture (NaRMA-IV) held at SKRAU, Bikaner during December 19-21, 2012 Abstract No. B-45.

21. Mohanty, M., Gulati, B.R., Kumar, R., Gera, S. and Gupta, S., 2013. Characterization and differentiation of equine umbilical cord blood-derived mesenchymal stem cells. In: International Symposium of in Stem Mouse Embryology Workshop: Stem Cells to Organogenesis, Institute for Stem Cell Biology and Regenerative Medicine, Bangalore, March 10-12, 2013 p30.

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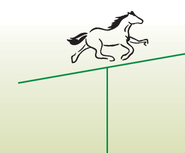

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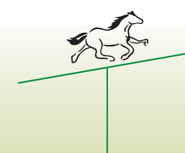

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Submissions to GenBank

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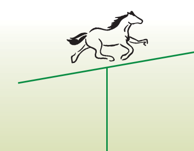

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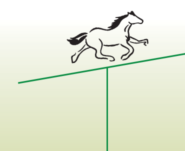
Participation in Training, Workshop Conferences and Symposia

Participation in Trainings

1. Dr B. R. Gulati participated in Bio-risk Management Training at HSADL, Bhopal during 17-21 April, 2012. The training included visit to containment areas (Laboratories, Animal Wings, ETP). In addition, construction, maintenance and operation details were elaborated during the training process.
2. Dr Anuradha Bhardwaj attended training on "Phenomic and Genomic tools for analysis of livestock genome" organized at NBAGR from 14-23 June, 2012.
3. Dr Vijay Kumar attended NAIP sponsored National Training programme on "Assessment of Microbial Diversity by Next Generation Sequencing (NGS) for taxonomic and Metabolic reconstruction of the gut microbes" at NIANP Bangaluru from 22 August to 4 Sept., 2012)
4. Dr B. R. Gulati participated in Basic Course in "Flow Cytometry" organized by BD FACS Academy, Jamia Hamdard University, New Delhi from 6-8 August 2012.
5. Harisankar Singha participated in Training for "National Animal Disease Reporting System" at National Informatic Centre, CGO Complex, New Delhi from 26-30 November, 2012.
6. Dr S. C. Yadav participated in short training course on "CPCSEA nominee training" from 14-18 January, 2013 at National Institute of Animal Welfare, Ballabhgarh.

Participation in Conferences, Workshops and Symposia

1. Anju Manuja participated in National Seminar on "Recent trends of nanotechnology in Pharmaceuticals" organized by Department of Pharmaceutical Sciences, GJUS&T, Hisar held on 2nd March, 2013.
2. Anuradha Bhardwaj participated in World Congress of Biotechnology, Bright Technologies, Hyderabad, held from 4-6 May, 2012.
3. Anuradha Bhardwaj participated in International Conference on "Biotechnology: Emerging Trends (ICB 2012)" at CDLU, Sirsa, held from 18-20 September, 2012.
4. Harisankar Singha participated in International Conference on "Biotechnology: Emerging Trends (ICB 2012)" Organized by Dept. of Biotechnology, CDLU, Sirsa (Haryana) from 18-20 September, 2012.
5. B. R. Gulati participated in XXI National Conference on "Immunobiology and Management of Viral Diseases in 21st Century "VIROCON-2012", organized at Indian Veterinary Research Institute, Mukteswar, from 8-10 November, 2012.
6. B. R. Gulati in participated in National Workshop on "Stem Cell Research and Therapeutics: Current Status and Future Strategies" at Division of Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar (U.P.) from 28-29 September,2012.
7. Balvinder Kumar participated in National Seminar on "Recent trends of nanotechnology in Pharmaceuticals" organized by Department of Pharmaceutical Sciences, GJUS&T, Hisar held on 2 March, 2013.
8. B. K. Singh attended XXI National Conference "Immunobiology and Management of Viral Diseases in 21st Century "VIROCON-2012", organized at Indian Veterinary Research Institute, Mukteswar, from 8-10 November, 2012.
9. BC Bera participated in XXI National Conference of Indian Virological Society, VIROCON-12 on "Immunobiology and Management of Viral Diseases in 21st Century", held at Indian Veterinary Research Institute, Mukteswar Campus, Nainital from 8-10 November, 2012.



10. B. K. Singh attended technical seminar related to mortality of RWITC horses (2 yrs old) at RWITC, Pune on 7 February, 2013. Also acted as Technical member in the panel during this conference.
11. Nitin Virmani presented a lead paper on "Molecular characterization and diagnosis of equine influenza-a current perspective" at XXIX Annual Conference of Indian Association of Veterinary Pathologists and National Symposium on "Challenges in diagnostic pathology in domestic pet, wild and aquatic animals" and National Seminar on "Emerging trends in diagnosis and control of poultry diseases" held at College of Veterinary Sciences, LLRUVAS, Hisar from 5-7 Nov. 2013.
12. R.C. Sharma participated in International Conference on "Extension Education in the Perspectives of Advances in Natural Resource Management in Agriculture (NaRMA-IV)" held at SKRAU, Bikaner Rajasthan, India from 19-21 December, 2012.
13. R.A. Legha attended National seminar on "New Paradigms in Livestock Production: From Traditional to Commercial Farming and Beyond" held at NDRI, Karnal from 28-30 January, 2012.
14. R.A. Legha attended the 8th Biennial ANAC, 2012 held at RAJUVAS, Bikaner from 28-30 November, 2012.
15. R.A. Legha attended National seminar on "Formulation of strategies for Disaster Management Technologies for Animals" organized by RAJUVAS, Bikaner, Rajasthan from 21-22 March, 2013.
16. R.K. Vaid attended the 1st International Conference on Bio-Resource and Stress management on February 3-9, 2013 at Science City, Kolkata in Livestock and Aquaculture Session, Organized by Ratikanta Maiti Foundation, Kolkata.
17. S.C. Yadav attended XXIII National Congress of Veterinary Parasitology and National Symposium held at Guwahati from 12 -14 December, 2012.
18. Yashpal, R.A. Legha, R. K. Dedar, P. Bala, R.K. Vaid, J. Singh, S. Kumar and R.K. Singh, participated in International Conference on "Extension Education in the Perspective of Advances in Natural Resource Management in Agriculture (NaRMA-IV)" held at SKRAU, Bikaner during 19-21 December, 2012.
19. S. K Ravi Participated in exhibition on "Livestock Management in Arid Zone: A Challenge" organized by Central Wool Development Board, Jodhpur in collaboration with RAJUVAS, Bikaner at Pandusar, Lunkaransar, Bikaner on 24 August, 2012.
20. S. K Ravi Participated in exhibition on "Farmer's Fair cum Farm Innovation Day" organized by CAZRI at Jodhpur on 12 September, 2012.
21. R.K.Singh attended expert consultation on Managing Transboundry Diseases of Agricultural Importance in Asia Pacific at New Delhi on 9 August, 2012.
22. R.K.Singh attended the Knowledge Meet organized by ICAR with the Vice-Chancellors of CAU/SAUs and Directors of ICAR institutes at NASC, New Delhi on 21-22 August, 2012.
23. R.K.Singh attended the Annual Convocation and National Seminar of NAVS and presented a lead paper on 'IPR issues in livestock' at Veterinary University, Mathura from 1-2 November, 2012 and also acted as Chairman in the Plenary Session of the Seminar.
24. R.K. Singh attended the ILRI-ICAR partnership workshop on livestock, research and development in India at NASC, Pusa Institute, New Delhi on 7 November, 2012.
25. Attended the XXI National Conference of Indian Virological Society at IVRI, Mukteshwar as Vice President from 8-11 November, 2012.
26. R.K.Singh attended the Multi Stakeholders Consultancy workshop at Agri Biotech Foundation, Hyderabad from 18-19 January, 2013.
27. R.K. Singh attended the 3rd meeting of the National Advisory Board for Management of Genetic Resources at NBAGR, Karnal on 5 March, 2013 as Special Invitee.

International Trainings and Visits Abroad

1. Dr Sanjay Kumar and Dr R. K. Goel attended two weeks training at National Research Centre for Protozoan Diseases, Obihiro, Hokkaido, Japan under OIE Twinning Project on Equine Piroplasmosis to learn the technique of "Lateral flow assay" and experiments on qPCR for *T. equi.*
2. Dr R.K. Singh visited Brussels, Belgium to attend the "Info Day and Brokerage Event Call FP7-KBBE-7-2013" organized by European Commission in collaboration with Biocircle-2 followed by Biocricle-2 meeting and training from 16-18 July, 2012.

Personnel
Milestones

New Joining

- Dr Vijay Kumar joined the Centre as Scientist (Sr.Scale) on transfer from NIANP, Bangalore on 14.05.2012
- Smt. Shammi Tyagi joined the Centre as A.F. & A.O. on transfer from CIRB, Hisar on 07.03.2012.
- Sh. Sunil joined as Assistant at the Centre on 19.05.2012.

Promotions

- Dr. BR Gulati Principal Scientist has been promoted to the post of Principal Scientist w.e.f. 01.01.2009.
- Dr. Yash Pal Sr. Scientist has been promoted to the post of Principal Scientist w.e.f. 01.01.2009.
- Dr. Rajender Kumar National Fellow has been promoted to the post of Principal Scientist w.e.f. 22.03.2011.
- Dr. Nitin Virmani Sr. Scientist has been promoted to the post of Principal Scientist w.e.f. 14.10.2011.
- Dr. Sanjay Barua Sr. Scientist has been promoted to the post of Principal Scientist w.e.f. 27.03.2012.

Transfer

- Dr Sarita Yadav, Scientist was relieved from the Centre on 31.11.2012 subsequent to her transfer to CIRB, Hisar.
- Shri R.K. Chaturvedi, T-6 (Technical Officer) was relieved from the Centre on 02.02.2013 subsequent to his transfer to CICR, Nagpur.

Superannuation

- Shri Satbir Singh, Skilled Support Staff retired from NRCE upon superannuation on 31.10.2010

Awards and Recognition

Awards

- **Dr Birendra Kumar Singh**, Principal Scientist, NRCE, Hisar was elected ***Fellow of Indian Virological Society (FIVS)*** for the year 2012 for his outstanding contribution in Animal Virus Research. He was conferred FIVS award during XXI National Conference of Indian Virological Society **"VIROCON-2012"** held at Indian Veterinary Research Institute, Mukteshwar, Nainital w.e.f 8-10 November, 2012.

Dr B.K. Singh receiving Fellow of Indian Virological Society award

- **Dr R.K. Singh**, awarded Tata Innovation Fellowship (2012-2013) by Department of Biotechnology, Government of India, New Delhi, on account of his scientific achievements in the field of Animal Biotechnology. Dr Singh has been awarded a research project under this fellowship entitled "Thermostabilization of recombinant protein antigens in diagnostic assays/kits using Heavy Water"
- **Best poster award**: Barnela, M., Manuja, A., Saini, R., Kaur, H., Kumar, S., Chopra, M., Yadav, SC,

Kumar, R., Kumar, BK, Dilbaghi, N. 2012. Drug-Loaded chitosan nanoparticles for sustained delivery of trypanocidal drug for use in animals. In: AICTE sponsored International onference "*On Current and Future Scenarios in Drug Development and Delivery*" at J DM ollege of pharmacy, Sirsa, Haryana, August 11-12, 2012.

- **Dr R.K. Singh** awarded Fellowship of National Academy of Agricultural Sciences (NAAS) w.e.f. 01.01.2013.
- **Best Oral Presentation**: Awarded to Dr S.C. Yadav for research work on "Exo antigen of *Trypanosoma evansi*: A new approach for detection of *T.evansi* infection in donkey using western blot" in XXIII National Congress of Veterinary Parasitology and National Symposium held at Guwahati w.e.f. December 12-14, 2012.

Recognition

- **Dr Rajender Kumar and Dr Sanjay Kumar**, completed one year Post-Graduate Diploma in Technology Management in Agriculture (PGDTMA) with distinction, jointly awarded by National Academy of Agricultural Research and Management, and University of Hyderabad, Hyderabad under Centre for Distance and Virtual Learning.

Obituary

Smt. Ramkali, (01.12.1972-08.08.2012)

It is with great sadnesss that the NRCE, Hisar announces the sudden demise of our dear colleague, Smt. Ramkali after brief illness and hospitalization on 08.08.2012 at the age of 40 years. Smt. Ramkali was born on 01.12.1972 and joined the service at NRCE as Skilled Support Staff on 15.03.2005. Smt. Ramkali leaves behind three sons, with whom NRCE staff joins in solemn condolences. The cremation was performed at Hisar which was attended by NRCE staff.

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 Yash Pal, Principal Scientist, Animal Physiology
6. Dr Nitin Virmani, Principal Scientist, Veterinary Pathology
7. Dr S.K. Khurana, Senior Scientist, Veterinary Public Health
8. Dr Sanjay Kumar, Senior Scientist, Veterinary Medicine
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 R.C. Sharma, Senior Scientist, AG&B
2. Dr R. A. Legha, Senior Scientist, LPM
3. Dr P.A. Bala, Scientist, Animal Nutrition
4. Dr T. Rao Talluri, Scientist, Veterinary Reproduction & Gynecology
5. Dr Ramesh Dedar, Scientist, Veterinary Medicine
6. Dr Sanjay Kr. Ravi, Scientist, Animal Reproduction
7. Dr Vijay Kumar, Scientist Sr. Scale, Animal Physiology

Scientists at VTCC, NRCE, Hisar

1. Dr Praveen Malik, Principal Scientist, Veterinary Microbiology
2. Dr Sanjay Barua, Principal Scientist, Veterinary Microbiology
3. Dr R.K. Vaid, 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 Taruna Anand, Scientist, Animal Biotechnology
8. Dr Riyesh T., Scientist, Veterinary Microbiology

Technical Staff at NRCE, Hisar

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

Administrative Staff at NRCE, Hisar

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

Administrative Staff at EPC,Bikaner

1. Sh Mahender Singh, LDC

Supporting Staff at NRCE, Hisar

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

Supporting Staff at EPC, Bikaner

1. Sh Raju Ram
2. Sh Mahabir Prasad

Kachchi Horse (Sindhi) in Banni Pasture Lands, Hodka Kachch

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