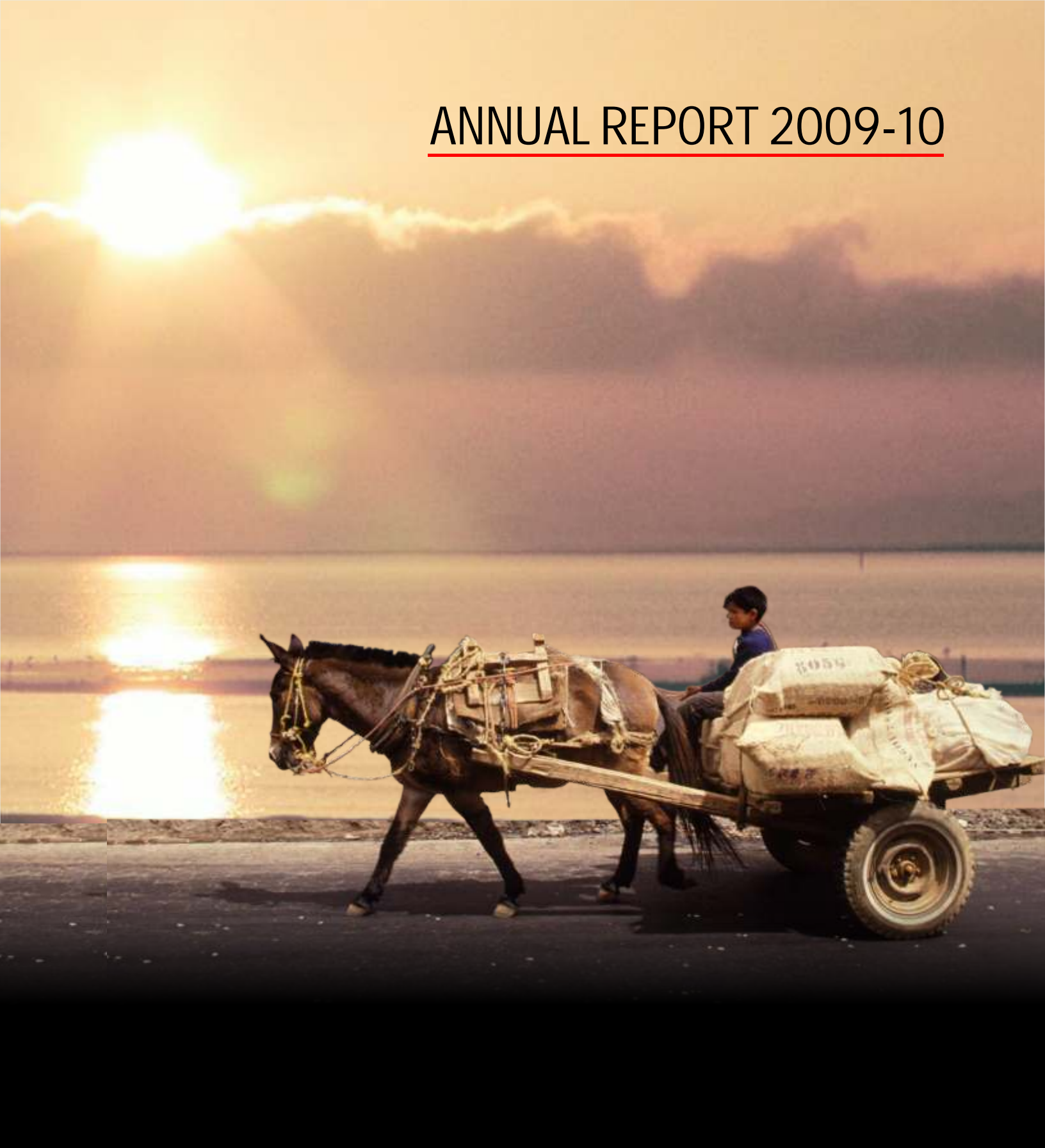


# ANNUAL REPORT 2009-10



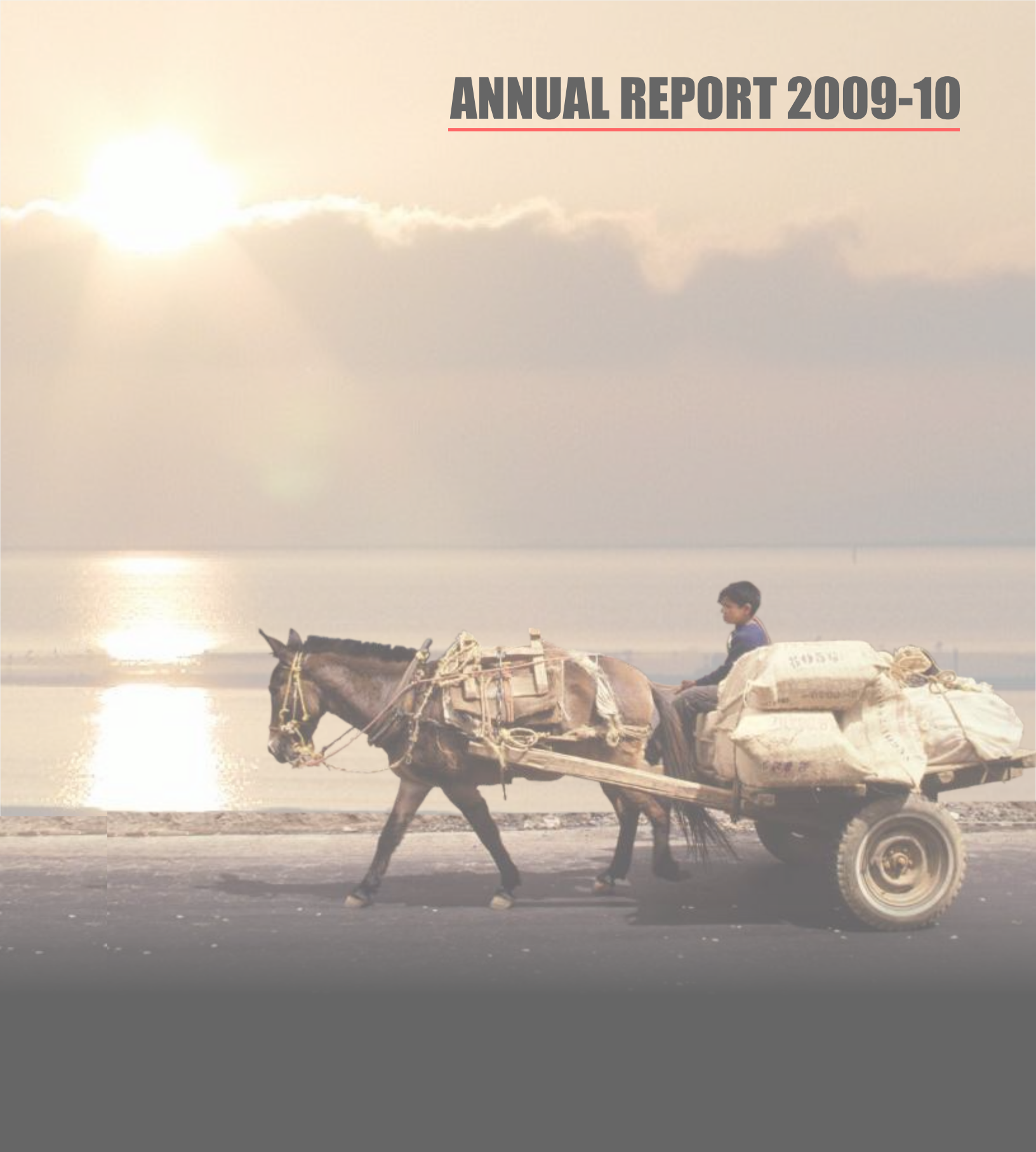
राष्ट्रीय अश्व अनुसंधान केन्द्र  
National Research Centre on Equines



Equines support livelihood of the poorest-of-the-poor in several ways.....



# ANNUAL REPORT 2009-10



राष्ट्रीय अश्व अनुसंधान केन्द्र  
National Research Centre on Equines



**Published by****Dr. R.K. Singh, Director**

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The achievements and activities of the Centre from April 2009 to March 2010 are presented in this report. Mention of trademark, proprietary product, or firm in the text or figures does not constitute an endorsement and does not imply approval to the exclusion of other suitable products or firms.

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*Improving equine health & productivity is the priority of NRCE*

”



## Director's Foreword

We feel a sense of satisfaction and grandeur in realizing our existence for 25 glorious years in the service of equine owners and stakeholders in the areas of equine diagnosis, prophylaxis and production technologies, which have made a difference in the arena of equine husbandry of our country. Farmers, marginal landholders, landless poor and labourers rear equines (donkeys, ponies, mules and horses) to support their livelihood. Efforts towards improvement in health and productivity of these equines will help in achieving the goal of sustainable development with social justice for the poorest-of-the-poor farmers.

In our Silver Jubilee Year, we rededicate ourselves to the dream of Mahatma Gandhi "the dream of all-inclusive sustainable development" to serve our stakeholders, the richest-of-the-rich and poorest-of-the-poor equine keepers. We are already into the task of improving the existing diagnostics, vaccines and control measures for equine diseases and development of newer and cheaper biologicals for disease prevention and control. However, pathogens do not respect boundaries, nor do they respect social strata. In the year 2008-09, equine influenza hit the equines of poor animal keepers as well as horses kept by well-off people of the society. We, at NRCE, feel proud in sharing the success to control this disease, thus bringing succour to equine owners. While doing so, NRCE has also taken care of equines serving the sentinels of our borders. Early containment of equine influenza in the country could not have been possible without the active support of the state Animal Husbandry departments, equine breeders and farmers, NGOs, Turf Authorities of India and field veterinarians. NRCE owes a lot to all of them and craves for similar support in future. Apart from this, scientists at NRCE have also looked deeper into the genetic makeup of the equine influenza virus isolates, thus understanding the epidemiology of the disease. The disease surveillance and monitoring programs received renewed thrust so as to keep



an eye on the emerging health status of equines. For the sustenance of our equine genetic resources, we also endeavoured to evaluate different indigenous breeds of equines in India. Therefore, phenotypic characteristics of *Bhutia*, *Spiti* and *Zanskari* equids were recorded and analysed. A nucleus herd of *Zanskari* ponies has been successfully established at our Bikaner campus for *ex situ* conservation of the breed.

Towards strengthening of national repository of microorganisms of animal origin, a network programme was started with identification of sixteen institutes for collaborative efforts towards conservation of diversity of veterinary, dairy and rumen microbes. Successful field trials of EHV-1 and updated EI vaccines; extensive (internal and external) validation of ELISAs for detection of *Theileria equi* (Babesiosis) and *Trypanosoma evansi* antibodies; recombinant glycoprotein G antigen-based ELISA for differentiation of EHV-1 and EHV-4 infections; RT-PCRs for detection of EIV, EIAV, JE; PCR for *Rhodococcus equi* and glanders; development of EIAV gp26 recombinant protein antigen-based ELISA, etc are some of the R & D hallmarks of NRCE in the current year.





Our basic research endeavours - while on one hand - helped improve the existing diagnostics, they also led to develop new ones as well, notable being the recombinant gp26 protein antigen-based ELISA for detection of EIAV antibodies in clinical serum samples. Scientifically important new initiatives include research work on collaboration with Indian Institute of Science, Bangalore, on (a) clinical proteomics of *Trypanosoma evansi*, poxviruses (camelpox and buffalopox) and equine influenza virus to understand molecular pathogenesis of these organisms and also towards development of pre-clinical diagnostic assays, and (b) evaluating HSPs for their anti-trypanosomal activity in mouse model. The Piroplasmosis laboratory of NRCE was approved under OIE Twinning project to collaborate with National Research Centre on Protozoan Disease, Obihiro (Japan). This will pave the way – in coming years - for NRCE to become OIE Referral Laboratory on Piroplasmosis for this region.

The NRCE also makes it a point to deliver equine husbandry information, management practices and technologies to the farmers at their door steps. During 2009, sixteen farmer *gosthies*, nine equine health camps - farmer interactive meets and

equine husbandry awareness programmes were organized in Haryana, Rajasthan and Uttar Pradesh to provide solutions to equine husbandry problems. In the process, we received feedback that helped us to understand the contemporary research needs and requirements at the field level.

All our efforts have yielded fruits under the able guidance and support of Indian Council of Agricultural Research, New Delhi; particularly Dr. S. Ayyappan, Director General, ICAR and Secretary DARE, Dr KM Bujarbaruah, former DDG (AS), Dr. K.M.L. Pathak, DDG (AS), Dr. Lal Krishna, ADG (AH) and Dr. S.N. Prasad, ADG (ANP & PIMS). Vision, guidance and technical support provided from time to time by Hon'ble chairmen and members of QRT, RAC, IMC and experts of IRC has immensely helped NRCE to be in right direction and be very focused. Further support of scientists and other staff of Animal Science Division further enabled us to march ahead speedily.

I would like to congratulate and thank the members of the publication committee and other scientists who helped in bringing out this publication. My heartfelt appreciation goes to the dedicated team of NRCE family.

(R.K. Singh)







## Executive Summary

National Research Centre on Equines (NRCE) was established on 26<sup>th</sup> November 1985 at Hisar (Haryana) and sub-campus at Bikaner in 1989 under the aegis of the Indian Council of Agricultural Research. The mandate of the Centre is to undertake research on health, production and management in equines; development of diagnostics/biologicals for major equine diseases; diagnosis, surveillance and monitoring of equine diseases; and providing diagnostic, advisory and consultancy services to equine owners and other stakeholders. A brief account of efforts to accomplish the mandated targets of NRCE in the year 2009-10 is outlined below:

Equine influenza epizootic occurred in India in 2008-2009 in eleven states of the country. The epizootic was caused by H3N8 subtype of the virus. Sequencing of haemagglutinin gene of Indian EIV isolates characterised the viruses belonging to clade 2 of Florida sublineage of American lineage, that is quite different from the one isolated from EI outbreak of 1987. We updated an inactivated equine influenza vaccine using the EIV isolated from the 2008 outbreak of equine influenza (A/eq/Jammu-Katra/6/08/H3N8). The potency of both egg-adapted and MDCK-based EIV/Katra vaccines were tested in the guinea pigs and results were compared with the previous vaccine (Ludhiana/87). The updated vaccine was further tested in horses for safety and immunogenicity and was found to be safe and protective. The vaccine was further evaluated in 150 indigenous horses in a field trial. None of the animals showed any adverse reactions following primary and booster vaccination and showed protective antibody titres.

Equiherpabort, an inactivated Equine Herpes Virus-1 (EHV-1) vaccine developed by the Centre, was tested in field in pregnant mares. The vaccine was inoculated in 58 pregnant mares of 5 month gestation and 9 mares were kept as unvaccinated control. The animals were given booster vaccination in 7<sup>th</sup> month of gestation. There was no untoward effect after vaccination. The vaccine generated protective immune response in mares.

Development and refinement of diagnostics for major equine diseases is frontier area of research at the Centre. Efforts are being made for the development of RT-PCR based diagnostic tests targeting influenza A virus-specific genes and recombinant protein based immunoassays for detection of antigen as well as differentiation of infected and

vaccinated animals (DIVA). Using RT-PCR; Matrix (M), haemagglutinin (HA, N and C-terminal) and full-length neuraminidase (NA) genes were amplified for diagnosis and subtyping of equine influenza virus. NS1 protein was cloned and 13.5 kDa histidine-tagged protein of C-terminal segment of NS1 is being further explored for development of DIVA (differentiation of infected and vaccinated animals) assay. For diagnosis of glanders, an indirect ELISA using one of the recombinant antigens (protein B) was developed that has sensitivity and specificity of 100% and 99.3%, respectively in comparison to conventionally used complement fixation test. The test is safe, rapid, reproducible, specific and sufficiently sensitive for use in field.

To develop a field oriented kit for the diagnosis of equine infectious anemia (EIA), synthetic gene of the EIAV gag encoding the protein p26 was cloned into pQE30 expression vector. The rp26-AGID test was carried out and compared with commercially available EIA-AGID kits. The test was found to be specific and sensitive.

*Rhodococcus equi* is a very important pathogen of equines causing pneumonia in foals resulting in high mortality in endemic farms. The disease is difficult to diagnose and treat. PCR-based diagnostics were developed using five different primers. Further, twenty isolates were also characterized using these PCRs.

During the year, previously developed single-dilution plate ELISA and immune dipstick kits for differentiation of EHV-1 & EHV-4 and recombinant antigen based ELISA kit for detection of *Theileria equi* antibodies were validated.

Japanese encephalitis (JE) is a mosquito-transmitted disease caused by JE virus (JEV) causing disease in equines, pigs and humans and is prevalent in eastern and southern Asia. JEV was isolated for the first time in equines in India from a clinical case of JE in Hisar, Haryana. The envelope gene (1500 bp) of the isolated virus (JE/eq/India/H225/2009) was sequenced. The phylogenetic analysis indicated that the isolate belonged to genogroup III of JEV and that the equine isolate clustered together with Vellore group of JEV isolates from India.

Trypanosomiasis is an economically important haemoprotozoan disease of equids. Preliminary studies revealed that *Trypanosoma evansi* releases infection-specific cysteine proteinases during course of acute infection, and could be a possible putative target for





immunodiagnosis. A specific cysteine proteinase inhibitor E-64 inhibited *T. evansi* cysteine proteinases in whole cell lysate of *T. evansi* antigen. Some of these cysteine proteinases were also found reactive in western blot with experimentally/field infected serum sample of equines. Zymography profile of *T. evansi* donkey isolates appears to be similar with camel isolate. To identify potential diagnostic markers and drug targets, clinical proteome profile of *T. evansi* using mass spectrometry (MS) was studied. MS revealed over 160 proteins expressed by *T. evansi* in mice infected with camel isolate, including drug targets such as oligopeptidases, kinases and cysteine proteinases.

For diagnosis of *T. evansi* infection, an indirect ELISA for antibody detection was developed and employed for seroprevalence study. Out of 1005 serum samples tested, 115 (11.44%) were positive for antibodies against *T. evansi* in northern region of India. Multiplex PCR for simultaneous amplification of specific gene fragments from *Theileria equi* and *T. evansi* has also been standardized.

During 2009-10, equine disease sero-survey was conducted in 8 States/UTs of India, viz. Rajasthan, Haryana, Punjab, Uttar Pradesh, Uttarakhand, Madhya Pradesh, Chhattisgarh and Sikkim. A total of 176 (16.84%) out of 1045 equines had antibodies to equine influenza virus while 28 (2.67 %) were positive for EHV-1 and 107 (10.43%) were positive for antibodies to Japanese encephalitis virus. Antibodies to *Theileria equi* were detected in 320 (38.09%) of 840 equines, while *T. evansi* antibodies were detected in 49 (5.40%) out of 906 equines tested. None of the 1045 equines tested showed antibodies to *Brucella* and *Salmonella* Abortusequi (H antigen).

During the year, NRCE scientists attended disease outbreaks in various parts of India including, equine influenza (Uttarakhand, Rajasthan and Gujarat), equine infectious anemia (Uttarakhand), camelpox (Rajasthan), buffalopox (Maharashtra), glanders (Chhattisgarh) and rhodococcosis (Rajasthan). In addition, Centre provided consultancy services to various stakeholders (equine owners, field veterinarians, equine breeders, NGOs) in management of problems faced by them from time to time in the area of equine health and production.

Besides this, serum samples from private organizations, quarantine stations and other establishments were tested for various diseases. A total of 4510 equine serum samples for EIA and 3543 samples for Glanders were tested. A total of 1684 samples from animal quarantine centres including 1599 vaginal swabs and 85 preputial swabs tested for CEM were found negative.

A total of 5385 equines from different parts of the country were tested for glanders. Glanders re-emerged in Chhattisgarh during November 2009-March 2010 and 13 new cases were detected in equines from Raipur city of Chhattisgarh. Two isolates of *Burkholderia mallei* were recovered from nasal swabs of these equines.

Toll-like receptor 9 (TLR9) has been characterized as a receptor that can recognise unmethylated CpG motifs within bacterial DNA. Signalling by the receptor triggers pro-inflammatory cytokine response that influences both innate and adaptive immune responses. Expression of TLR 9 was studied in peripheral blood mononuclear cells of *Equus caballus* (Marwari breed) and *Equus asinus*. Estimates of evolutionary divergence between the deduced amino acid sequences of TLR9 revealed that *E. caballus* (Marwari breed) and *E. asinus* differ from human TLR9 by 19% and 16%, respectively. In phylogenetic analysis, TLR9 proteins from *E. caballus* (Marwari breed) and *E. asinus* clustered together, while human, cattle, dog, sheep, mice, and buffalo formed separate clades. The analysis indicated conserved pattern and close association of TLR9 proteins within species and high divergence with other species of animals.

The Mx protein confers resistance to *Orthomyxovirus* infection by modifying cellular functions needed for viral replication. Mining for variations in Mx genes in resistant and susceptible animals/different breeds of horses may provide insight to the functional diversity within Mx proteins. RT-PCR was standardized and full-length Mx gene was amplified from vaccinated Marwari horse to explore its potential in resistance to equine influenza infection.

Bhutia equids - also known as Bhutia or Bhotia ponies - originated in the Himalayan region of India, and are now found in countries including Bhutan and India (Sikkim and Darjeeling). Bhutia ponies were characterized phenotypically. Fifteen different biometrical indices of these animals were recorded for their phenotypic characterization. Spiti ponies have been declared endangered breed and there is urgent need to conserve this breed. Survey of Spiti valley (HP) were conducted to study socio-economic status of equine owners, feeding, housing, healthcare, general management, and indigenous technical practices in vogue in the area.

With an aim to standardize cryopreservation of embryos for conservation of Marwari horses, the procedures for non-surgical embryo collection and transfer were standardized. Six embryos were recovered out of twenty one flushings (6-8 days post ovulation) and transferred to suitable recipient





mares. To further improve the fertility of frozen semen, secondary (Lactose 11%) extenders containing glycerol, methyl formamide (MF), dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) were studied as cryoprotectants.

A repository of characterized isolates of viral pathogens of different livestock species has been established at Veterinary Type Culture Centre (VTCC) for conservation and distribution. The activities entail collection of samples from different livestock species across different geographical regions, acquisition of microbial isolates from different institutes, characterization of the microbial isolates employing diverse molecular techniques and their preservation. In VTCC, camelpox virus (CMLV) outbreaks in camel and its zoonosis on the basis of clinical and epidemiological features was studied in three human patients in a Border Security Force (BSF) camp at Barmer. This is the first laboratory confirmed case of camelpox zoonosis. Buffalopox virus from outbreaks in Maharashtra in 2009-2010 was also isolated, characterized, and repositied.

Various isolations of bacteria associated with animal disease viz *Actinobacillus equuli* and *Serratia marcescens* from vegetative growth on endocardium of horse, and *Pseudomonas aeruginosa* from intestinal contents, *Klebsiella pneumoniae*, *Escherichia coli* and *Edwardsiella* sp from equine abortion cases were characterized from a taxonomical point of view and preserved under -20°C and -70°C conditions.

Under transfer of technology to end users, different extension programmes such as animal health camps, artificial insemination camps, problem solving meets, training-cum-workshops, *Kisan Goshthies* and fields visits were organized by the Centre. During 2009-10, sixteen *Kisan Goshthies*, interactive meets and equine husbandry awareness programmes were organized in Haryana, Rajasthan and Uttar Pradesh. The equine owners were educated and supplied literature related to equine husbandry. Centre organized nine Health Camps in Haryana, Rajasthan and Uttar Pradesh, and participated in animal fairs organized at various places of Haryana, Punjab, Rajasthan and Uttar Pradesh, and displayed technologies developed by the Centre.

A survey was conducted in Jalore, Hanumangarh and Haldi Ghati (Rajasthan) and Spiti Valley (Himachal Pradesh) to record equine management practices and indigenous traditional knowledge (ITKs) followed by equine owners in these regions. In addition, existing donkey and mule production systems in Haryana, Rajasthan and Uttar

Pradesh were also studied. The lack of awareness regarding scientific breeding, feeding practices and lack of financial assistance was mentioned as the major constraints by 97.55 and 96.74% of the respondents, respectively.

In view of re-emergence of equine influenza in India during 2008-2009, a short course on 'Equine Influenza Diagnosis and Control' was organized to update the knowledge and skills of field veterinarians in equine influenza diagnosis, control and emergency preparedness. Another short-course on "Equine Health and Management" was organized at NRCE, Hisar for veterinarians from Seema Surksha Bal and Gujarat State Animal Husbandry department. The trainings emphasized on 'hands-on' practice on equine disease diagnosis, use of techniques like endoscopy, ultrasonography etc. to detect internal diseases and optimum management system for the sound health of equines.

During the year, the Piroplasmiosis Laboratory of NRCE was approved under OIE Twinning project to collaborate with National Research Centre on Protozoan Disease, Obihiro (Japan). NRCE was recognized as one of the Centres of 'All India Co-ordinated Research Project on Increased Utilization of Animal Energy with Enhanced System Efficiency' to take up research on utilization of equine energy since April 2009.

The scientists of the Centre published 24 original research articles in international and national journals and presented 23 research papers in different conferences and symposia. Sixteen post-graduate students from state universities were provided 4-6 months trainings by the scientists of the Centre. Two compendia were published for ready reference of the trainees comprising important lectures on Equine health and production.

Under infrastructure development activities, thirty acre of saline and water logged land was reclaimed and used for cultivation of fodder crops. During the year, 2362 quintal of green fodder, 205 quintal of dry fodder and 127 quintal of grains of different fodder crops were produced and supplied to animals.

The revenue for the Centre is generated through many activities. During the year, revenue to the tune of Rs. 64.89 lakhs was generated by the Centre. The contractual diagnostic services contributed Rs 42.88 lakhs through testing of samples for various diseases including EIA, glanders, CEM, EHV-1, piroplasmiosis, dourine, trypanosomosis, equine influenza, and *Salmonella* Abortusequi.





# कार्यकारी सारांश

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, ykbtztk fodfl r fd; k x; kA bl fof/k dk mi ; ksx l hje  
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# Introduction

National Research Centre on Equines (NRCE) was established on 26<sup>th</sup> November 1985 at Hisar (Haryana) under the aegis of the Indian Council of Agricultural Research. Since its inception, NRCE has strived hard for bringing in improvements in health and production of equines in India. A sub-campus at Bikaner – established in 1989 – is contributing significantly for upliftment of the landless and marginal farmers by helping in conservation and improvement of the germplasm of indigenous equines breeds, besides disseminating the technologies for the efficient and economically feasible equine production.

In a short span, NRCE has been recognized as a premier research Centre in the area of equine health and production. The main campus of NRCE is located at Hisar (Haryana) with state-of-the-art laboratories for undertaking research in areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry and biotechnology. In addition, NRCE has sub-campus at Bikaner (Rajasthan) where research laboratories for genetics and breeding, reproduction, physiology and nutrition are established to undertake research on equine production. Veterinary Type Culture Centre has also been established in the year 2005 at NRCE for collection and preservation of microbes of animal origin.

## MANDATE

- ❑ To undertake research on health and production management in equines;
- ❑ To develop diagnostics/biologicals for major equine diseases;
- ❑ To act as National Referral Facility for diagnosis, surveillance and monitoring of equine diseases
- ❑ To provide diagnostic, advisory and consultancy services.

## OBJECTIVES

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

## MAJOR ISSUES

- ? Achieving freedom from dreaded equine diseases through development of modern diagnostics & vaccines.
- ? Transfer of technology for superior mule & true-to-breed indigenous horse production in their home tracts using artificial insemination and embryo transfer technology with an aim to establish embryo bank of Marwari/Kathiawari horses to enhance export.
- ? Enhancing performance of working equids especially in arid, semi-arid & mountainous regions.
- ? Income generation through market intelligence activities.

## THRUST AREAS

- ? Surveillance and monitoring of important equine diseases including emerging and existing diseases with special emphasis on foal mortality and production losses.
- ? Development of effective, affordable and preferably field-based diagnostics against major equine diseases threatening equine health and production in India.
- ? Development of effective, affordable and potent immunoprophylactics against important equine infectious diseases threatening equines in India.
- ? Development of effective plant-based products for management of some economically important equine diseases and to enhance performance in equines.
- ? To provide diagnostic and consultancy services for beneficiaries particularly equine farmers and breeders.
- ? Propagation of sustainable and economically viable AI technology for mule production in India using cryopreserved jack semen for use at farmers' door.
- ? Perfection and propagation of artificial insemination techniques in horse and pony production using frozen semen of true to breed indigenous stallions for the consortium of threatening breeds in India.
- ? Breed characterization and *in situ* conservation of various indigenous breed of horses.





- ? Exploiting importance of equine draught power for economically weaker section of the society.
- ? Explorative research for value addition of equine products and by-products namely blood/serum, dung, urine, milk, placenta and hair.
- ? Extension activities through information technology and institute development programmes for the upgradation of the indigenous breeds of equids in the different parts of the country in collaboration with the State departments.

## MAJOR ACHIEVEMENTS

### Diagnosics for equine diseases

The Centre has been recognized as national referral centre for diagnosis of important equine infectious diseases by Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture (Government of India). The Centre has developed and refined diagnostics including immunodiagnosics and molecular diagnostics against various equine diseases.

**Equine herpes virus-1 (EHV-1):** A highly sensitive and specific neutralizing monoclonal antibodies-based diagnostic kit named Equiherpes B-ELISA was developed by the Centre for diagnosis of EHV-1 antibodies. This kit tests the serum sample using single dilution (1:250). This kit was formally released by Hon'ble DG, ICAR on August 20, 2008.

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

**Equine Rotavirus:** A sandwich enzyme-linked immunosorbent assay (s-ELISA) was developed employing a monoclonal antibody (mAb) raised against VP6 of rotavirus, for detection of equine rotavirus (ERV) from stool samples. The diagnostic sensitivity (DSn) and specificity (DSp) of ELISA was 1.0 (0.8076-1.0) and 0.96 (0.8541-0.9932), respectively. ART-PCR using VP6 gene primers was also developed and its results were compared with the s-ELISA. The s-ELISA was equally sensitive as RT-PCR.

**Equine influenza virus (EIV):** EIV is routinely diagnosed by

haemagglutination inhibition assay. RT-PCR for equine influenza diagnosis targeting HA, NA, M genes have been developed. RT-PCR for EIV diagnosis and typing has been developed that is under internal validation.

***Theileria equi:*** For serodiagnosis of *T. equi*, a recombinant antigen based-ELISA has been developed using a truncated gene segment of a merozoite surface proteins, 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 also been validated both internally and externally.

**Trypanosomosis:** An indirect ELISA has been standardized using whole cell lysate antigen of *Trypanosoma evansi*. Out of 1005 samples tested for *T. evansi* antibodies using this ELISA, 115 (11.44%) were detected positive. 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 (HAI) standardized for diagnosis of JE. The sensitivity of HAI was 96.29% and specificity 100% in comparison to SNT. RT-PCR employing primers targeted against E-gene and 3'NTR has been standardized and is under internal validation. mAb-based ELISA for Japanese encephalitis is also being developed.

**Equine infectious anemia:** Coggins test for EIA is routinely being used at the Centre. In addition, r-protein based ELISA for equine infectious anaemia is being developed.

**Equine viral arteritis:** Virus neutralization for equine viral arteritis is 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 incorporating indigenous strain (Hisar-90-7) of EHV-1 has been developed by the Centre. Experimental as well as field trials of equine herpesvirus-1 inactivated vaccine have been completed. The vaccine generates protective immune response, which is comparable to that of commercial imported Pneumabort 'K' vaccine in pregnant mares. No EHV-1 associated abortions or adverse reactions were observed in vaccinated mares. Validation of vaccine in field animals is being done.

**Equine influenza vaccine:** The Centre developed equine influenza vaccine using indigenous isolate (A/equi-2/Ludhiana/87). The vaccine has been updated in 2010 incorporating epidemiologically relevant isolate responsible







for equine influenza outbreaks during 2008-09. This updated vaccine is safe and efficacious as is evident by the protective immune response generated by this vaccine in equines in a limited experimental trial. A new cell culture-based inactivated equine influenza vaccine has also been developed and is under validation.

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

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

Kits for disease diagnosis: HERP kit & Equiherpes B-ELISA kit (for EHV-1 diagnosis) and COFEB kit (for diagnosis of piroplasmiasis) were 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 World Organization for Animal Health (OIE).

Outbreaks of equine glanders in country during 2006-07 were timely detected and its control measures were taken to prevent its further spread. After that, there were no reports of glanders for two years from India. However, from November 2009 to March 2010, ten fresh cases of glanders have been diagnosed from equines in Raipur (Chhatisgarh). The Centre is working in collaboration with the Directorate of Veterinary Services, Chhatisgarh for surveillance and containment of the disease.

NRCE diagnosed the equine influenza (EI) in India in 2008 from Jammu region (July 2008) that subsequently affected equines in 11 different states. The biosecurity measures were implemented in collaboration with various state animal husbandry departments. No new cases of EI have been reported from India since May 2009.

NRCE has continuously been screening equines for equine infectious anemia in India. No case of EIA has been reported since 1998. However, one mule has been found seropositive during 2009-10.

Zoonotic camel pox virus outbreak: We reported a camelpox virus outbreak and its zoonoses in three human in camels in Barmer district (Rajasthan).

#### 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 equi/2 (H3N8) subtype and that Indian isolates were identical to the Clade 2 of American lineage of H3N8 subtype.

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 indicated that genotype 3 is responsible for causing disease in equine.

*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 (3), equine rotavirus (29), equine influenza (7), Japanese encephalitis virus (2), West Nile virus (1), *Rhodococcus equi*, *Streptococcus equi*, *S. zooepidemicus*, *Burkholderia mallei*, *Salmonella Abortusequi*, *S. equisimilis*, *Enterobacter aerogenes*, *E. coli*, *Staphylococcus aureus*, *Trypanosoma evansi*(3).
- ? NRCE has a number of hybridomas secreting monoclonal antibodies against equine herpes virus-1, equine rotavirus, Japanese encephalitis virus, West Nile virus.
- ? NRCE has 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, JEV, EIAV, *R. equi*, *Burkholderia mallei*, *Trypanosoma evansi*, *Theileria equi*.

#### Indigenous breed characterization

**Marwari:** This breed of horses constitutes an elite group of indigenous horses, which are known for their sturdiness, swiftness, elegance and beauty. A total of 114 true-to-breed Marwari horses comprising of 98 mares and 16 stallions from seven different locations were evaluated. DNA polymorphism studies revealed high level of heterozygosity and low level of heterozygosity deficit in the Marwari horse population which reflect high genetic variability in Marwari equine population. The Centre in collaboration with Department of Animal Husbandry, Dairying & Fisheries (DAHDF), Government of India has finalized the breed descriptor for the Marwari breed of horses for entry of these animals in to the stud book. The regions of MHC class-II (DRB-2a and 2b) gene in Marwari horses exhibited polymorphism in 48.39% genotypes.

**Kathiawari:** Genotyping of Kathiawari breeds of horses employing 30 fluorescent-labeled microsat pairs in multiplex PCRs showed high heterozygosity which clearly indicated that there is adequate genetic diversity among these equines.

**Bhutia:** Characterization of true to breed animals of Bhutia breed ponies (n=35) in Sikkim revealed their mean heights at withers as 126.5 cm. In these ponies, bay was the most common colour (69%) followed by chestnut (23%), grey and other colours. Average body length and heart girth were recorded as 129 and 148.5 cm, respectively without any significant difference due to sex.

**Spiti:** In India, distribution of this breed is confined to Lahoul & Spiti, Kinnaur, Kullu, Mandi, limited areas of Kangra and Shimla district in Himachal Pradesh; Ladakh division of J & K and Uttarakhand but the true breeding tract is confined to 15 villages of two Panchayats (Kungri and Sagnam) in Pin valley of Spiti sub-division of Distt. Lahoul & Spiti. The total population of Spiti ponies in H.P. is approximately 4000. True-to-breed phenotypic characters of this breed have been recorded by NRCE.

**Manipuri:** The phenotypic characterization of Manipuri ponies indicated that height at wither in both the sexes of

Manipuri ponies ranged from 119-134 cm. In addition, heart girth (143.6 cm), hind leg length (82.3 cm), fore leg length (78.4 cm) and height at knee (39.4 cm), etc were also recorded.

**Zanskari :** Biometric indices of true-to-Zanskari breed of horses were recorded in their home tract in and around Leh, Laddakh (Jammu & Kashmir). Average height at wither of Zanskari breed was 126 cm. Average animal height was slightly higher in stallions ( $127.21 \pm 7.57$ ) than mares ( $125.45 \pm 4.74$  cm) but the differences were non significant. Beside this, average body length (123.07 vs 129.5 cm), heart girth (144.4 vs 148.9 cm), hind leg length (80.11 vs 79.95 cm), canon length (16.18 vs 15.80 cm), height at knee (37.57 vs 36.95 cm), face length (53.79 vs 53.75cm), face width (15.68 vs 15.25 cm), etc. were almost same in both stallions and mares, respectively without any significant difference. A nuclear herd of Zanskari ponies at our Bikaner Campus, after translocating these animals from their native herd at Ladakh in J&K.

#### Improvement in production potentials of equines

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

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

**Kit for pregnancy diagnosis:** An eCG s-ELISA kit (Pregmare kit) has been developed by the Centre.

#### Patents

Patent has been granted by the Patent Office, Government of India entitled "A method for preparation of a diagnostic kit useful for forecasting Equine Herpes Virus-1 disease".

A patent has been filed for "COFEB-Kit for diagnosis of *Babesia equi* infection in equines".

A patent has been filed for "A method for preparing complement fixation test based (COFEB) kit for the diagnosis of *Babesia equi* infection in equines".





The Centre has filed a patent for "A kit for detection of pregnancy in equines and assay thereof".

### Services

NRCE provides following services to the farmers and equine breeders:

- ? The Centre provides disease diagnostic services for various infectious and non-infectious equine diseases to equine owners, breeders, state animal husbandry departments, police and army horses.
- ? Artificial insemination to augment the production of superior quality Marwari horses, mules and donkeys.
- ? Quality jacks and jennies are supplied to various states, breeding societies and farmers, for production of superior quality mules and donkeys.
- ? The Centre is also providing pregnancy diagnostic services based on serum and ultrasound scanning facilities.
- ? NRCE is providing health certification for movement of equines within and outside the country. This facility has helped in promotion of export of horses.
- ? Assessment and transfer of technology using the latest know-how of information technology is also given due importance to extend the technologies to the end-users. The scientific and technical staff provides clinical and diagnostic (including pregnancy diagnosis) services and consultancy to the farmers on demand in the areas of equine health and production. Farmers are imparted trainings and supplied education materials for equine management, production and health.

- ? Extension activities: To receive feedback from the equine owners, various activities like health camp, awareness and farmers meets are organized on regular basis in different areas of the country.

### Veterinary Type Cultures Facility at NRCE

Indian Council of Agricultural Research entrusted NRCE the responsibility of establishing Veterinary Type Culture Centre (VTCC) during the X plan period. The VTCC became functional in June 2005 for establishing national repository of microorganisms of animal origin including recombinant cultures and plasmids; and identification, characterization, conservation, maintenance and utilization of microorganisms.

#### MANDATE OF VTCC

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

Approximately 250 bacterial isolates and 25 viral isolates have been collected in VTCC. Repository of VTCC includes viral isolates (camel poxvirus, buffalo poxvirus, bovine herpes virus-1, rotavirus), bacterial cultures (*R. equi*, *E. coli*, *Bordetella*, *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Pseudomonas*, *Enterobacter* sp). This repository is ever expanding day by day. In addition, a phage display library of single-domain antibodies of Indian desert camel and the twenty seven antigen-binder clones selected from the library have been deposited in the VTCC

### STAFF POSITION OF NRCE AND VTCC

Name of the post	NRCE			VTCC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	1	1	-	-	-	-
Scientific	25	24	1	10	7	3
Technical	23	22	1	1	1	0
Administrative	11	10	1	0	0	0
Supporting	22	21	1	0	0	0
Total	82	78	4	11	8	3





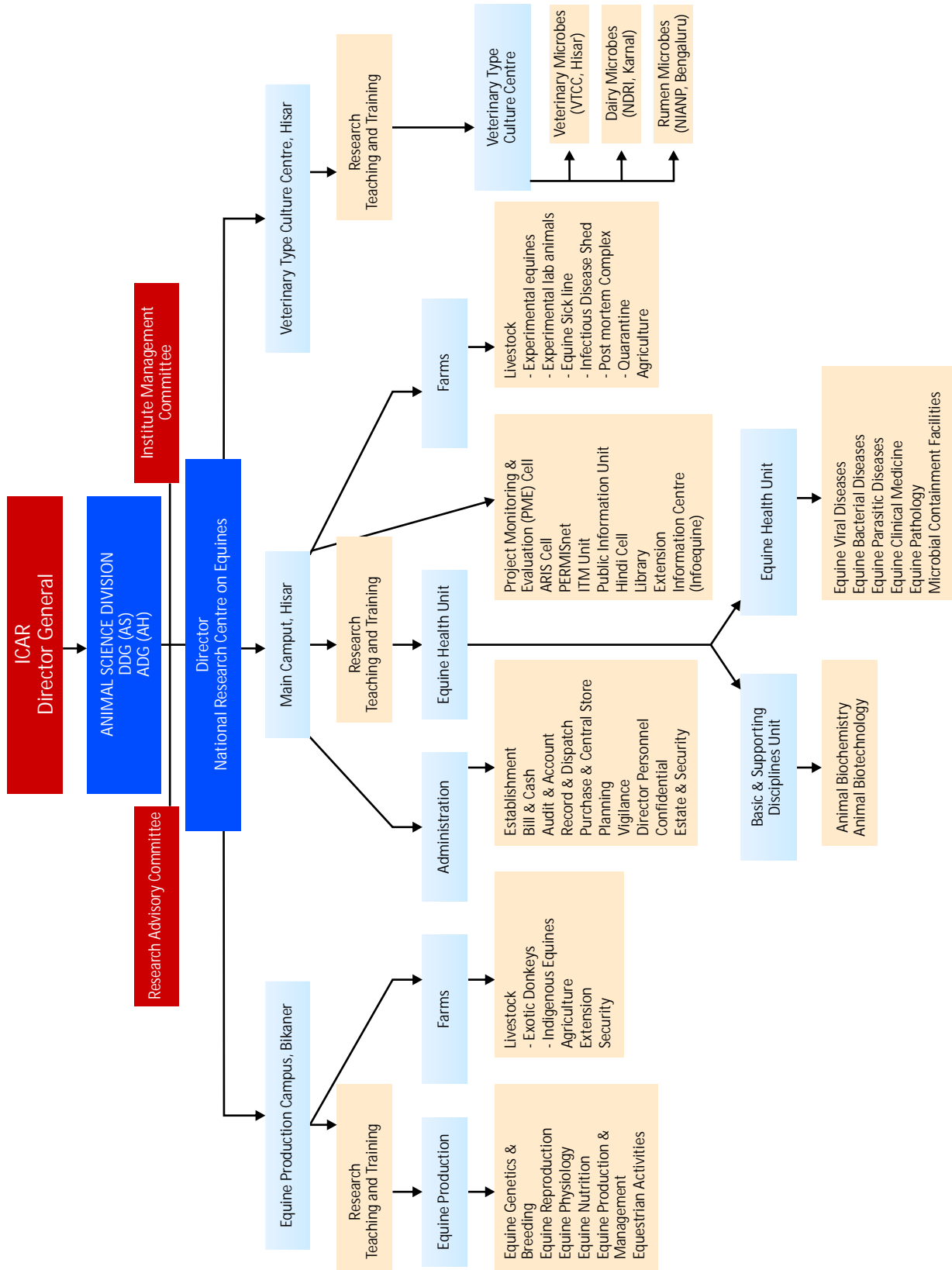
## Major Landmarks

1985	NRCE established at Hisar with Prof. P. K. Uppal as Founder Director
1987	Outbreak of equine influenza in Northern India
1989	Sub-campus of NRCE established at Bikaner for research on production in equines
1990	Exotic donkey germplasm with Poitu blood introduced from France
1991	Artificial insemination (AI) initiated in equines using fresh extended liquid semen
1991	Early pregnancy diagnosis (15 days post insemination) using ultrasonography
1995	Ciq-ELISA developed for detection of circulating immune complexes (CICs) in EIA-infected 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 (Asssheep)
1997	Equine Influenza vaccine using indigenous isolate (A/Equi-2/Ludhiana/87) released
2001	Patent for complement fixation test based diagnostic (COFEB-kit) filed
2003	An Indian patent granted to a diagnostic kit for forecasting EHV-1 disease
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	Re-emergence of glanders in equines
2008	Re-emergence of equine influenza
2008	Equine Herpes Virus-1 diagnosis kit released
2008	ELISA based pregnancy diagnosis kit for pregnancy diagnosis in mares released
2009	Development of equine herpesvirus-1 vaccine
2009	A nucleus herd of Zanskari ponies established at Bikaner
2009	First report of laboratory confirmed camelpox virus zoonosis
2009	First isolation of Japanese Encephalitis Virus from equines in India
2010	Equine influenza vaccine developed incorporating A/equi/Jammu-Katra/6/08/H3N8
2010	EIA detected in an equine after a gap of 11 years in India





# Organizational Set-Up





## Summary of Expenditure & Revenue Generation

Rs. in lacs

Summary of Expenditure	2008-09	2009-10
<b>NON-PLAN</b>		
1. Establishment charges including LSP/PF, wages, OTA	319.37	484.34
2. Traveling allowances	2.88	3.70
3. Others charges including equipments & recurring charges	116.84	103.88
4. Works	20.00	9.27
<b>Total Non-Plan Expenditure</b>	<b>459.09</b>	<b>601.19</b>
<b>PLAN</b>		
1. Establishment charges including LSP/PF, wages, OTA	23.09	0.00
2. Traveling allowances & HRD	3.95	6.56
3. Others including equipments & recurring charge	205.57	236.59
4. Works	260.49	117.69
<b>Total Plan Expenditure</b>	<b>493.10</b>	<b>360.84</b>
<b>Total Expenditure (Plan and Non-Plan)</b>	<b>952.19</b>	<b>962.03</b>

1. Sale of farm produce & auction of dry trees	600.00	250.00
2. Sale of livestock	4,07,800.00	0
3. Sale of publication and advertisements	9,200.00	3540
4. License fee	75,904.00	1,37,379.00
5. Interest on loans and advances	1,62,286.00	43,599.00
6. Interest on short term deposits	5,88,876.00	2,77,487.00
7. Income from internal resource generation	35,14,763.00	42,88,867.00
8. Receipt from services	54,300.00	0
9. Other misc. receipts	12,19,495.00	17,37,752.00
<b>Total Revenue</b>	<b>60,33,224.00</b>	<b>64,88,874.00</b>



## Research Achievements

### Development of diagnostics for equine influenza

Equine influenza is an acute viral disease characterized by watery-to-thick mucopurulent nasal discharge, dry hacking cough, high fever and rapid spread of respiratory infection in horses and other equine species. Early diagnosis of the disease is essential to control the spread of EI. Confirmatory lab diagnosis may be made using a number of tests including virus isolation, detection of viral antigen or specific antibodies, or detection of viral genetic material by reverse transcription-polymerase chain reaction (RT-PCR). Efforts are being made for the development of the RT-PCR based diagnostic tests targeting influenza A virus-specific genes and recombinant protein based immunoassays for detection of antigen as well as differentiation of infected and vaccinated animals (DIVA).

#### RT-PCR for detection and typing of EI

For the purpose of the development of RT-PCR-based diagnostics of EI from field samples, primers targeting matrix (M), hemagglutinin (HA) and neuraminidase (NA) genes were used. RT-PCR showed amplification of the expected products of 244 bp (M gene) (Fig. 1A), 981 bp (N-terminal portion of HA gene), 950 bp (C-terminal portion of HA gene) (Fig. 1B) and 1413 bp (full length NA gene). Forty nasal

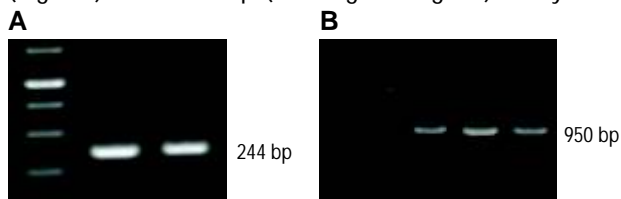


Fig 1: RT-PCR amplification M gene (A) and HA gene (B)

swabs were tested with standardized RT-PCR for detection of equine influenza virus. Five samples were found positive for Influenza A virus by M-gene based RT-PCR. These were then subjected to typing RT-PCR targeting HA and NA genes and were typed as H3N8 subtype. The RT-PCR results also corroborated with the results of virus isolation in embryonated chicken eggs and HA typing of the isolate by haemagglutination inhibition assay as H3.

#### Cloning and expression of NS1 gene for DIVA assay

NS1 protein is known to be useful in differentiation of infected and vaccinated animals as it is expressed only in animals exposed to the live influenza virus. For expression



Fig 2: RT-PCR amplification of NS1 gene

of recombinant, full length NS1(673 bp) gene was amplified (Fig 2) and cloned in pQE30 vector. The recombinant protein was expressed in M15 cells and analysed in SDS-PAGE. The 13.5 kDa histidine-tagged protein of C-terminal segment of NS1 was confirmed and is being further explored for development of this DIVA assay.

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

### Antigenic and genetic characterization of equine influenza viruses

Antigenic and genetic characterization of influenza A viruses is essential, as the change in the structure of proteins, especially the surface protein haemagglutinin makes the vaccines less effective. Equine influenza viruses (EIVs) belonging to H3N8 subtype have been classified into two lineages and several sublineages based on antigenic and genetic drift in the haemagglutinin gene. An epizootic of equine influenza in India occurred in year 2008-09, that started from Jammu and Kashmir (Katra) and subsequently

spread to other parts of the country affecting equines in 11 states.

EIV (H3N8) was isolated from nasal swabs obtained from clinical cases in embryonated (9-11 days) chicken eggs from various locations of the country including Katra (Jammu and Kashmir), Mysore (Karnataka), Ahmedabad (Gujarat) and Gopeshwar & Uttarkashi (Uttarakhand). The antigenic and genetic characterization of the EIV isolates was done during the year.





### Antigenic characterization of the EIV isolates

EIV isolates were assayed by HI test using standard serum against H3N8 of various lineages (A/eq/Newmarket/1/93, A/eq/Newmarket/2/93, A/eq/Kentucky/1/81, A/eq/Miami/63) and EIV H7N7 (A/eq/Prague/56), obtained from NIBSC, UK (Table 1). Indian isolates gave maximum HI titres with the serum against A/eq/Newmarket/1/93 indicating that the Indian EIV isolates were antigenically similar to A/eq/Newmarket/1/93 and belong to American lineage.

In cross-HI assay, representative convalescent serum samples (n=4 from each region) of EIV infected equines from seven different regions of the country were tested using

antigen of standard and native isolates (Table 2). EI infected serum samples gave highest titres with homologous antigens of Indian isolates of current outbreak (A/eq/Jammu-Katra/6/08, A/eq/Mysore/08 and A/eq/Ahmedabad/09) and A/eq/Newmarket/1/93 antigen).

Equine serum samples did not react with Prague/56 antigen and titres with A/eq/Kentucky/81, A/eq/Ludhiana/87, A/eq/Miami/63 and A/eq/Newmarket/2/93 antigens ranged between  $8 \pm 0$  and  $192 \pm 37$ . The findings established that the EI epizootic of 2008-09 in India was due to EIV that is antigenically closer to EIV isolates of American lineage like A/eq/Newmarket/1/93.

Table 1: Haemagglutination inhibition titres of Indian isolates with standard serum obtained from NIBSC, UK

Virus Reference virus antigen	Standard serum			
	A/eq/New market/1/93 Serum	A/eq/New market/2/93 Serum	A/eq/ Kentucky/ 81 serum	A/eq/ Miami/ 63 serum
A/eq/Jammu-Katra/06/08	256	16	32	16
A/eq/Mysore/08	320	<8	16	<8
A/eq/Ahmedabad/09	256	8	16	16

\*indicates testing of virus antigen with homologous serum

Table 2: Haemagglutination inhibition assay titres of serum samples from horses from different regions of the country

Convalescent Equine serum from outbreak	HA antigen raised against								
	A/eq/ Jammu-Katra/6/08	A/eq/ Mysore/ 08	A/eq/ Ahmmedabad/09	A/eq/ Ludhiana/ 87	A/eq/ Miami/ 63	A/eq/ Kentucky/ 81	A/eq/ New Market/ 1/93	A/eq/ New Market/ 2/93	A/eq/ Prague/ 56
Katra (Jammu)	1024 ± 0	846 ± 128	1024 ± 0	192 ± 37	28 ± 4	160 ± 32	896 ± 128	96 ± 18	0
Karnataka	1024 ± 0	1024 ± 0	896 ± 128	96 ± 18	48 ± 9	112 ± 16	896 ± 128	96 ± 18	0
Gujarat	320 ± 64	320 ± 64	256 ± 0	24 ± 5	14 ± 2	28 ± 4	256 ± 0	16	0
Uttarakhand	128 ± 0	112 ± 16	128 ± 0	14 ± 2	10 ± 2	12 ± 2	112 ± 16	16 ± 0	0
West Bengal	112 ± 48	80 ± 16	80 ± 16	8 ± 0	8 ± 0	10 ± 2	104 ± 51	10 ± 2	0
Rajasthan	418 ± 64	512 ± 0	448 ± 64	80 ± 16	40 ± 8	72 ± 20	418 ± 64	80 ± 16	0
Maharashtra	512 ± 0	576 ± 161	512 ± 0	112 ± 16	40 ± 8	80 ± 16	576 ± 161	80 ± 16	0

The titres against the homologous viruses are mentioned in bold letters

### Genetic characterization of EIV isolates

Three genes (HA, NA & NS) each of four different EIV isolates viz. Katra, Mysore, Ahmedabad and Gopeshwar were amplified using self-designed primers. The amplified products were purified and cloned into either pTZ57R/T or pGEM-T Easy vectors. Upon confirmation of the clones, recombinant plasmids were sequenced. The nucleotide sequence data were submitted to NCBI GenBank. The

sequences were aligned and phylogenetic trees were constructed using MEGA 4.0 software.

HA gene: The deduced amino acid sequence of the precursor HA protein was of 567 amino acids. The detailed analysis of the sequences from three isolates revealed single amino acid change in the A/eq/Mysore/08 at position 211 (Lys to Gln) from other two Indian isolates. Comparison of consensus amino acid sequence of predicted matured







HA1 protein with other EI virus (H3N8) isolates depicted more than 99% homology with A/eq/Gansu/7/08, A/eq/Hubei/6/08, A/eq/Inner-Mongolia/8/08, A/eq/Cheshire/3/07, A/eq/Richmond/1/07, A/eq/Solihull/1/07, A/eq/Newmarket/1/07, A/eq/Berkshire/1/07, and A/eq/Essex/3/05. The comparison of amino acid sequence of precursor full-length HA protein showed 98.6% homology of three Indian isolates with A/eq/Newmarket/5/03, A/eq/Bari/05 and 98.23% with A/eq/Kentucky/5/02 isolates (data not shown). Sequence analysis revealed that seven amino acid residues changed at consensus positions (Gly/Asp7Asn; Gln211Lys; Val278Ala; Ala372Thr; Gly379Glu; Thr388Ala; Ile481Leu).

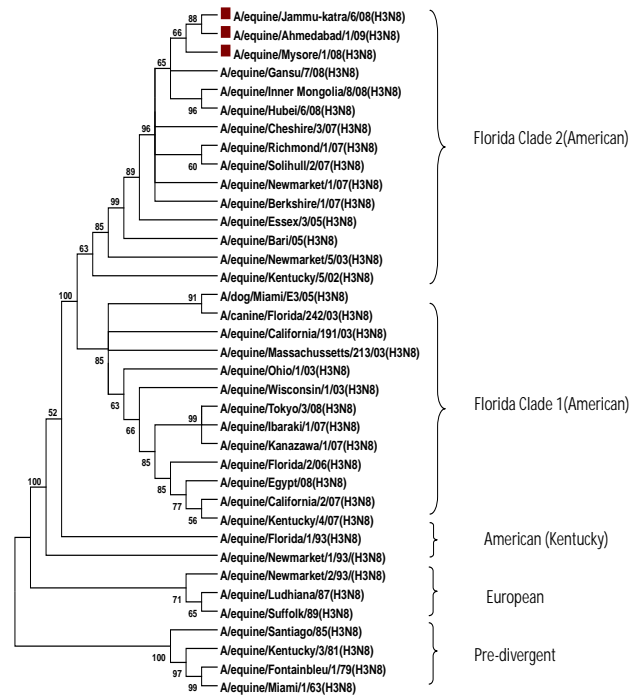
The analysis of amino acid sequence at antigenic sites of HA1 gene revealed changes of two amino acid residues at position 278 (Val to Ala) in the site C and at position 211 (Gln to Lys) in the site D in comparison to all the isolates across various lineages (Table 4). Variation of single amino acid at position 135 (Ile to Arg) in the site A was observed when compared with the strains having closest homology with Indian isolate i.e., A/eq/Inner-Mongolia/8/08 and A/eq/Hubei/6/08 strain.

Phylogenetic analysis of HA gene sequence of A/eq/Jammu-Katra/6/08(H3N8) isolate established broadly the American lineage of the Indian EIV isolates. This virus grouped in Clade 2 of Florida sublineage and depicted closest identity with A/eq/Gansu/7/08, A/eq/Hubei/6/08, A/eq/Inner-Mongolia/8/08, A/eq/Newmarket/1/07 and A/eq/Bari/05. On the other hand, two EIV isolates [A/eq/Ludhiana/87(H3N8) and A/eq/Bhiwani/87 (H3N8)] of 1987 outbreak in India belonged to H3N8 predivergent lineage.

NA gene: The open reading frame (ORF) of the neuraminidase (NA) gene of all the three Indian isolates was 1413 bp length which encodes 470 amino acid residues NA protein. Comparison of the deduced amino acid sequences revealed that Indian isolates shared 92.77% homology among themselves. Mysore/12/08 and Gopeshwar/1/09 isolates showed 99.15% similarity with Chinese isolates at consensus sequences whereas Katra/6/08 isolate shared 96.38% similarity.

NS gene: The nucleotide sequences of NS gene of all the virus isolates comprised of 838 bp encoding predicted two polypeptides of 220 amino acid residues (NS1 protein) and 121 amino acid residues (nuclear export protein, previously called NS2). The predicted amino acid sequences of NS1

gene of the Indian isolates were compared with each other and with other isolates from across the world. Upon comparison, deduced amino acid sequence of NS1 protein of Indian isolates showed 97.26% homology among themselves. Katra/6/08 isolate shared 98.17% similarity with Chinese isolates at the consensus regions while Mysore/12/08 and Gopeshwar/1/09 isolates showed 98.63% and 99.08%, respectively.



Phylogenetic analysis of the HA genes of EIV's

To conclude, the phylogenetic analysis of HA gene sequence of Indian isolates showed that the EIV isolates of current outbreaks were clustered with the Clade 2 of Florida sublineage of the American lineage viruses along with those of China and Mongolia. Surveillance of EI viruses from Europe and North America has revealed that clade 2 and clade 1 viruses of Florida sublineage persisted in Europe and North America, respectively in recent times. The virus responsible for the outbreaks in Japan and Australia was closely related to Clade 1 of Florida sublineage, while, Clade 2 viruses have been predominately responsible for outbreaks in Europe during 2006 and 2007 and Asia during 2007-2009. Phylogram of NA and NS sequences also revealed clustering of Indian isolates of EIV with China and Mongolia isolates along with Clade 2 Florida sublineage isolates.

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





## Resurgence of equine influenza in India

An epizootic of equine influenza (EI) was reported in India in 2008-09 that affected equine population in 11 states viz. J&K, Himachal Pradesh, Delhi, Uttar Pradesh, Haryana, Rajasthan, Gujarat, Maharashtra, Karnataka, West Bengal and Uttarakhand. During 2009, severe outbreaks of EI were reported from the state of Rajasthan, Uttarakhand and Gujarat. Some clinical cases were reported in an annual animal fair held at Tilwara (Barmer) during March 2009. From this place, EI spread to Bikaner, Jodhpur, Pali, Jalore. In April 2009, EI was reported in equines at Ahmadabad, Anand, Kheda, Kutch, Bhavnagar, Patan, Junagadh, Palanpur (Banaskantha),



NRCE team investigating EI outbreak at Gaurikund



EI outbreaks in India (2008-09)

Navsari, Valsad, Amreli, Bharuch, Surat, Bhuj (Kutch) and Kalol (Gandhi Nagar) districts of northern Gujarat. Out of 1670 equines tested, 167 were detected positive for EIV antibodies. The EI outbreak in Uttarakhand started in May 2009 at Gaurikund (Rudrapryag district), an important Hindu pilgrimage site on way to Kedarnath, 14 Km uphill. The animals at Gaurikund exhibited the signs of dry hacking cough, high temperature and watery-to-mucosal nasal discharge. EIV was isolated from nasal swabs of two infected equines from Gaurikund that was typed as H3N8. From there, the disease spread to other districts of the state, viz., Rudraprayag, Uttarkashi, Chamoli, Nainital, Udhamasinghnagar, Champawat, Haridwar, and Dehradun. EIV was also isolated from nasal swab of one infected equine from Uttarkashi. No fresh case of EI has been reported since May 2009.

## Updation of equine influenza vaccine

Epizootic of equine influenza struck India in year 2008 and remained till 2009 covering 11 states of the country. The virus responsible for the outbreak was quite different from the outbreak of 1987 in the country. Accordingly, it was felt necessary to update the existing inactivated aluminium hydroxide adjuvanted vaccine using Ludhiana/87 isolate (a predivergent lineage of the EIV isolate) with the isolates of current outbreak.

For updation of the vaccine, A/eq/Katra (Jammu)/06/08 (H3N8) virus was selected. The virus was grown at passage level 5 in 9-11 days old embryonated chicken eggs and MDCK cells. The virus was purified and inactivated using formalin. The inactivated virus was checked for the sterility and inactivation prior to production of the vaccine. The HA content of the virus was quantified to 20 µg/ml using single radial diffusion method employing standard antiserum obtained from NIBSC, UK. Further the inactivated virus was adjuvanted with aluminium hydroxide gel in equal quantity.

### Potency testing in guinea pigs

The potency of both egg adapted and MDCK-based EIV/Katra vaccines were tested in the three guinea pigs each and results were compared with the previous vaccine (Ludhiana/87). Both cell culture and egg-adapted vaccine generated good immune response in guinea pigs at 4-week post-vaccination (HI titres between 32 and 128).

### Safety and potency testing in experimental equines

Safety and potency testing of the vaccines was also done in the equines, using 6 indigenous horses per vaccine. Each animal was given one ml of vaccine intramuscularly and booster vaccination was done 4 week later. Serum samples from vaccinated animals were collected at weekly interval. Following vaccination, no adverse reaction was observed in vaccinated animals. Virus shedding was also not observed in vaccinated animals till 7-days post-vaccination.





### Kinetics of immune response

Immune response to the vaccine in equine revealed that the egg-adapted Katra virus vaccine showed protective titres by 3<sup>rd</sup> week and MDCK vaccine by 5<sup>th</sup> week post-booster vaccination. On the other hand, Ludhiana/87 vaccine showed rise in titre by 2<sup>nd</sup> week of booster immunization (Fig. 1).

The isotypes of antibody response generated in vaccinated equines were analysed by isotyping ELISA. Both the updated vaccines (egg and MDCK-adapted) generated good IgG<sub>A</sub> and IgG<sub>B</sub> antibodies titres that were higher than those generated by Ludhiana vaccine. On the other hand, egg-adapted Katra vaccine generated higher IgT response as compared to other two vaccines (Fig 2). It is pertinent to mention that IgG<sub>A</sub> and IgG<sub>B</sub> are associated

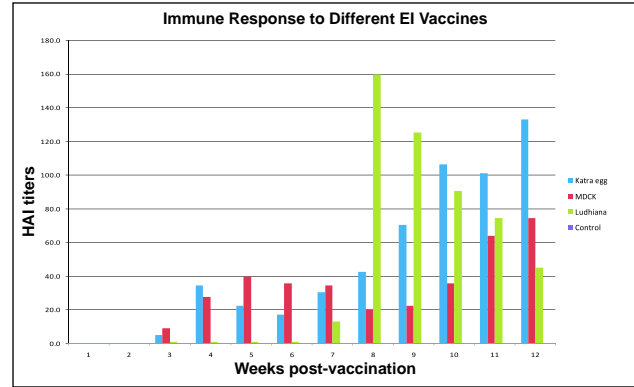


Fig 1: Immune response in horses by HI assay

with protective immune response to vaccine while IgT antibodies represent non-protective immune response to vaccination.

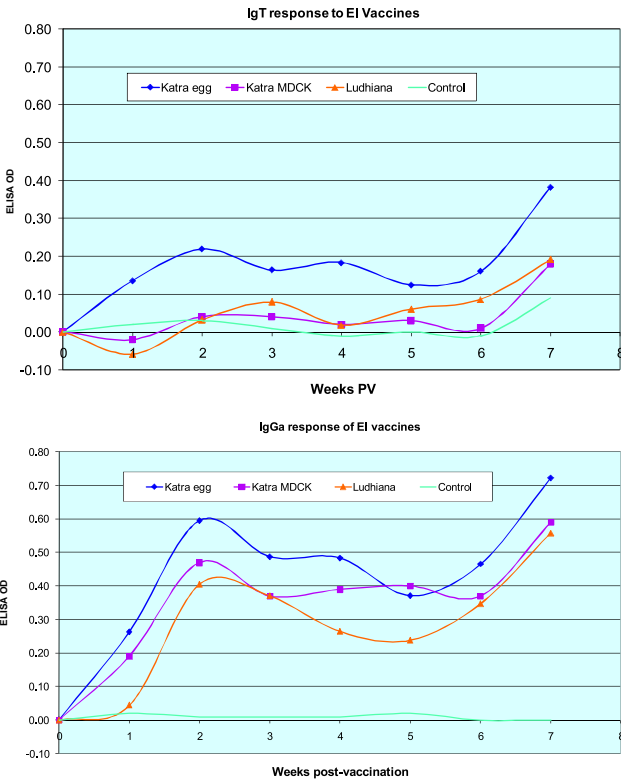
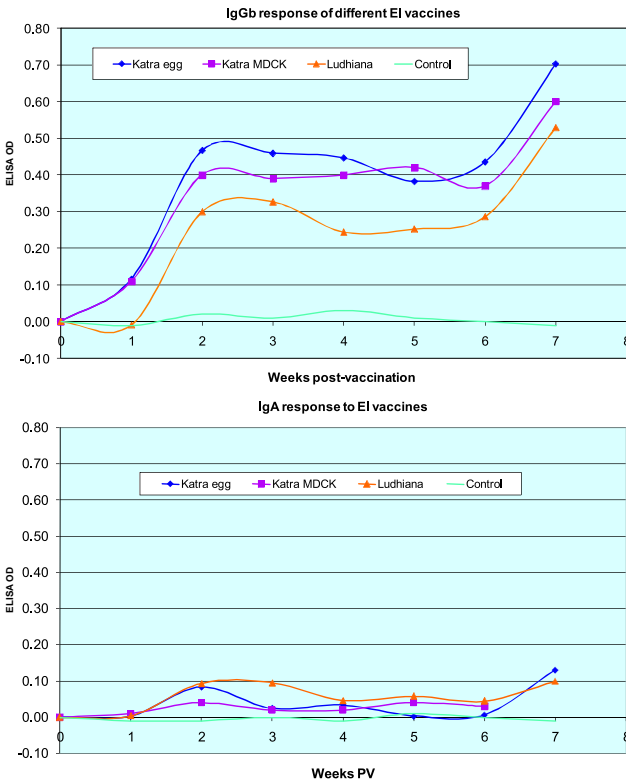


Fig. 2. Isotyping immune response following equine influenza vaccination

### Field trial of equine influenza vaccine in equines

We developed an inactivated equine influenza vaccine using the EIV isolated from the 2008 outbreak of equine influenza (A/eq/Jammu-Katra/6/08/H3N8). The vaccine was evaluated in 150 indigenous horses for the field trials. None of the animals showed any adverse reactions following primary and booster vaccination. Serum samples from 20% (n=30) animals were tested for immune response employing

HI assay. Following vaccination, 28 out of 30 equines showed protective antibody titres four weeks post-booster vaccination (Fig 1). In addition, the vaccine was also tested in six thoroughbred horses in Muktsar (Punjab). None of the vaccinated horses showed any untoward reaction. Mean ( $\pm$ SE) HI antibody titres in horses at 4-week post-primary vaccination was  $83.2 \pm 6.55$  while it was  $153.6 \pm 7.56$  two weeks after booster vaccination.

(RK Singh, Baldev R. Gulati, Nitin Virmani, BK Singh and AK Gupta)





## Characterization of EHV-4 isolates by sequencing

EHV-4 virus isolated previously from two organized farms was characterized genetically based on two genes viz. thymidine kinase (tk) and glycoprotein G (gG). The full-length genes were cloned in pGEM-T Easy vector. The cloned plasmid was RE digested with Not1 and released. The RE digested products of 1137 and 1405 bp (Fig. 1) confirmed for thymidine kinase and Glycoprotein G genes, respectively. Analysis of the sequence of tk gene gave closest homology (99%) with two EHV-4 isolates (AF030027 and D14486). For gG gene, the closest homology (99%) was with NS80567 isolate (AF030027) and 97% with other two isolates (S447961 and M89634). Both

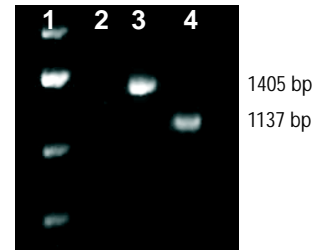


Fig: PCR amplification of glycoprotein G gene (3) and thymidine kinase (4)

the genes from EHV-4 had a homology of 83% with EHV-1 isolates.

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

## Seromonitoring of equine diseases

NRCE is undertaking gigantic task of seromonitoring of important equine diseases throughout the length and breadth of the country to know the status of equine diseases with an objective of prevention, control and eradication of equine diseases.

During 2009-10, equine disease sero-survey was conducted in 8 States/ UTs of India, namely Rajasthan, Haryana, Punjab, Uttar Pradesh, Uttarakhand, Madhya Pradesh, Chhattisgarh and Sikkim. During surveillance, samples from 1045 equines were collected. A total of 176 (16.84%) equines had antibodies to equine influenza virus while 28 (2.67 %) were positive for EHV-1 and 107 (10.43%)

were positive for antibodies to Japanese encephalitis virus. Antibodies to *Theileria equi* were detected in 320 (38.09%) of 840 while *T. evansi* antibodies were detected in 49 (5.40%) out of 906 equines tested (Table 1).

None of the 1045 equines tested showed antibodies to Brucellosis and *Salmonella Abortusequi* (H antigen). A total of 13 equines from Chhattisgarh were found positive for glanders during 2009-10. These animals were subsequently euthanized on recommendations of NRCE. Diagnostic services were provided for testing of 4510 equine serum samples for EIA and 3543 samples for glanders from 16 and 8 states/UTs, respectively.

Seroprevalence of various diseases under S&M among indigenous equines

Dis State	EI	<i>T. equi</i>	JE	EHV-1	<i>T. evansi</i>	EIA	Glanders	<i>S. Abortusequi</i>	Brucellosis
U.P.	49(27)	49(7)	49(11)	49	39(2)	49	49	49	49
Punjab	100(36)	68(2)	100(3)	100(2)	32(1)	100	100	100	100
Uttarakhand	288(38)	212(56)	288(32)	288(9)	288(18)	288(1)	288	288	288
Rajasthan	243(26)	243(109)	243(24)	243(4)	231(9)	243	243	243	243
Haryana	199(37)	134(55)	199(29)	199(6)	182(8)	199	199	199	199
Chhattisgarh	63(12)	63(40)	63	63	63(3)	63	63(9)	63	63
Sikkim	32	0	32	32(2)	0	32	32	32	32
M.P.	71	71(51)	71(8)	71(5)	71(8)	71	71	71	71
<b>Total</b>	<b>1045(176)</b>	<b>840(320)</b>	<b>1045(107)</b>	<b>1045(28)</b>	<b>906(49)</b>	<b>1045(1)</b>	<b>1045(9)</b>	<b>1045</b>	<b>1045</b>

Figures in parentheses indicate positive results

(R.K.Singh, S.K.Khurana, B.K.Singh, S.C.Yadav, B.R. Gulati, Praveen Malik, Rajender Kumar, Sanjay Kumar, N.Virmani, Sanjay Barua, R. K. Vaid, A.Arangasamy and Ramesh Dedar)





## Development of recombinant protein-based ELISA for glanders

The clinical and bacteriological diagnosis of glanders is difficult. Diagnosis of glanders is carried out by mallein and serological tests viz. complement fixation test (CFT). The serum becomes positive for CFT within one week of infection. The antigen used in this test was the crude whole cell lysate of *Burkholderia mallei*. Although CFT is a gold-standard test for diagnosis of glanders, the inherent problems associated with this test, like anti-complementary activity in donkey and mule serum warrants for search of newer, sensitive, specific and rapid tests for diagnosis of glanders amongst equines. Considering these facts, newer

assays like recombinant protein based ELISA has been developed. For ELISA, two recombinant antigens developed by Defence Research and Development Establishment (DRDE), Gwalior were evaluated. An indirect ELISA using one of the recombinant antigens (protein B) was developed that has sensitivity and specificity of 100% and 99.3%, respectively in comparison to CFT. The test is safe, rapid, reproducible, specific and sufficiently sensitive for use with field equine samples.

(Praveen Malik, H Singha & R K Singh)

## Glanders reemerged in Chhattisgarh

Glanders is a highly contagious disease of high zoonotic importance and affects primarily the equids. It is notifiable in India and poses a significant human health risk. The disease needs a timely confirmation for applying early mitigation and control strategy.

In our surveillance and monitoring, a total of 5385 equines from different parts of the country were tested for glanders. During November 2009-March 2010, 13 new cases were detected in equines from Raipur city of Chhattisgarh. Two isolates of *Burkholderia mallei* were obtained from nasal swabs of these equines. The adoption of proper sanitary measures after elimination of affected animals in coordination with State Veterinary Services helped in containment of disease in the affected state.



Glanders affected horse showing ulcers in nasal cavity and skin nodules on limbs

## Development of recombinant protein-based Coggin's test and ELISA for serodiagnosis of equine infectious anaemia

Equine infectious anemia (EIA) is characterized by repeated, acute episodes of fever, weight loss, anemia, edema, thrombocytopenia, leukopenia and persistent plasma viremia in equines. Because of its economic impact, EIA falls under regulatory

control program in India.

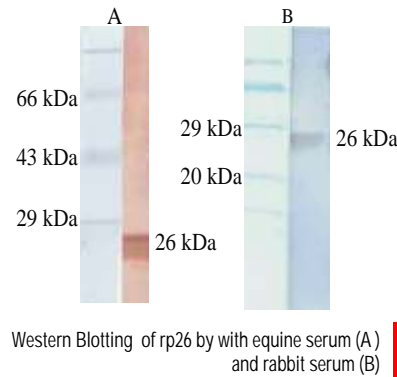
The 'Coggins Test' is used for the detection of specific EIAV antibodies and is recognized by the World Organization for Animal Health (OIE) as the 'gold standard' test for international trade. At NRCE, the test is

being performed using reagents imported from approved commercial sources. The objective of this study was to replace the imported reagents with the indigenously developed recombinant protein to be used in various immunoassays and develop a





field-oriented kit for the diagnosis of EIA. For this, a 675bp fragment of the EIAV gag encoding the protein p26 was commercially synthesized. The synthetic gene was cloned into pQE 30 expression vector, expressed and purified to homogeneity using a nickel charged resin under denaturing conditions. Purified rp26, a 26-kDa protein, reacted in western blot with EIA-positive equine and hyper-immune serum raised in rabbit (Fig.). The rp26-AGID test was carried out using the rp26 and compared with DiaSystems EIA-AGID test kit from



IDEXX Laboratories (Maine, USA) and EIA AGID test kit of VMRD Inc. USA. The recombinant protein so developed was optimized for buffer, agarose concentration and antigen

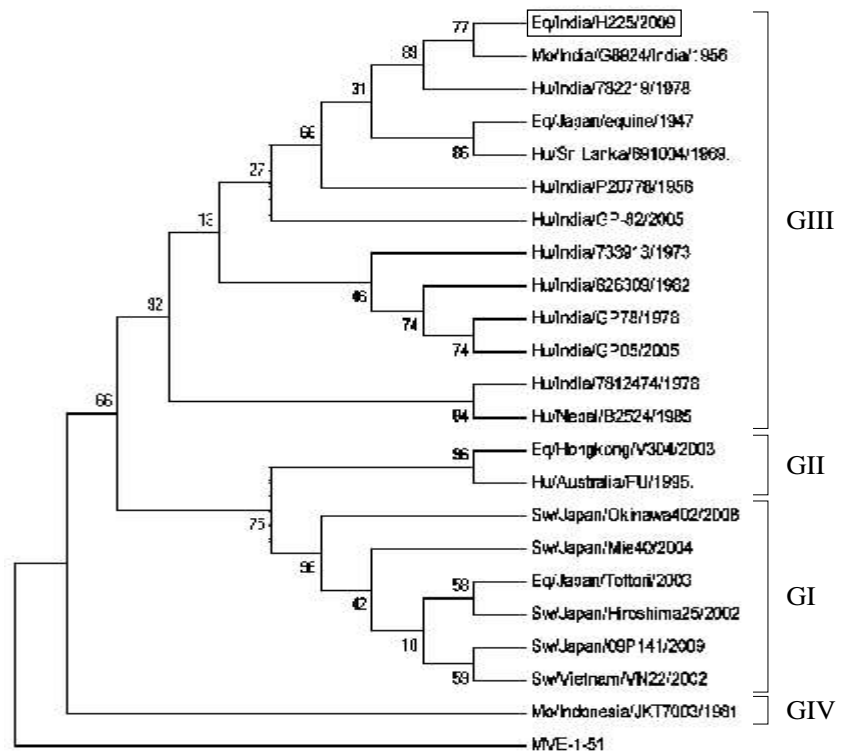
concentration in AGID and indirect ELISA. The repeatability, intermediate precision and reproducibility of tests are being evaluated. On testing with more than 1000 random samples along with 4 known positive samples, the rp26-AGID appears to be sensitive and specific.

Further efforts would be concentrated towards optimization of ELISA, internal/external validation, scaling up of recombinant protein production and transforming the test into a kit.

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

## Isolation and characterization of Japanese encephalitis virus from equines

Japanese Encephalitis (JE) is a mosquito-transmitted disease caused by JE virus causing disease in equines, pigs and humans. It is prevalent in eastern and southern Asia. To know the status of JE among equines in India, a national sero-surveillance indicated that 10% equines (327 of 3286) in India are sero-positive for JE. Antibodies to JEV are widely prevalent in equines of Manipur (91.7%), Gujarat (18.5%), Madhya Pradesh (14.4%) followed by Uttar Pradesh (11.6%). Prevalence of JEV in equine was supported by observation of two clinical cases in Hisar (Haryana) with signs of mild fever, impaired locomotion, recumbency, pedalling of hind legs and death. The serum sample from these equines and a pool of mosquito vector collected from the surrounding field were detected positive for JEV RNA by RT-PCR. Virus was isolated from one of the horse in suckling mice brain and porcine stable cell line. This is the first report of isolation of JEV from a clinical case of JE in equines in India. The envelope gene (1500 bp) of the isolated virus, JE/eq/India/H225/2009 was sequenced. The phylogenetic analysis indicated that the isolate belonged to genogroup



Phylogenetic tree of JEV isolated from an equine

III of JEV and that the equine isolate clustered together with Vellore group of JE isolates from India.

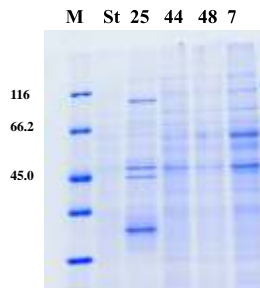
(Baldev R. Gulati, B.K. Singh, Nitin Virmani and H. Singha)



## Development of diagnostics for *Rhodococcus equi*

*Rhodococcus equi* is an important pathogen of equines causing pneumonia in foals resulting in high mortality in endemic farms. Techniques for characterization and diagnosis of the agent were standardized.

During the year, twenty six samples (nasal, faecal, soil) were tested for presence of *R. equi*. These included 12 nasal swabs, 12 faecal samples and 2 soil samples from infected premises. In all, 3 isolates of *R. equi* were recovered from



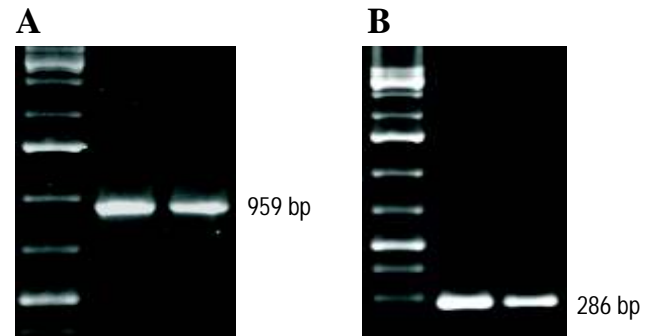
Protein profile of sonicated antigens of *R. equi* isolates

nasal swabs of foals with respiratory problems. These isolates were subjected to *in vitro* antibiotic sensitivity testing to 17 antimicrobial agents and all the isolates were found to be sensitive to chloramphenicol, erythromycin, ciprofloxacin, neomycin and rifampicin. Sonicated *R. equi* antigen from four strains was

prepared and checked by SDS-PAGE. Hyperimmune serum was raised in rabbit using this antigen. Western blotting with standard sonicated *R. equi* antigen revealed immunogenic

proteins with this serum. However, serum sample from clinically positive cases did not react in western blotting with the antigen.

PCRs were standardized with primers targeted against *vapA*, *vapB*, *choE*, *traA* genes of *R. equi* using genomic and



PCR amplification of *choE* gene (A) and *vapB* gene (B) of *R. equi*

plasmid DNA isolated from 20 *R. equi* isolates and one reference strain of *R. equi*. These PCR assays may be useful for early detection and characterization of *R. equi* isolates in equines.

(SK Khurana, Praveen Malik and H Singha)

## Characterization of cysteine proteinases for diagnosis of *Trypanosoma evansi* infection

Several batches of mice/rat adapted, donkey and horse isolate of *T. evansi* were purified by DEAE-cellulose chromatography. The sonicated antigen was prepared using standard protocol. The comparative SDS-PAGE profile of antigens revealed that donkey isolate of *T. evansi* differs from camel isolate antigen, however majority of polypeptides were common in both the isolates (Fig.1). The polypeptides (camel isolate) in the molecular weight range of 55kDa-72kDa were glycoproteinous in nature as detected by glycoprotein detection/staining kit.

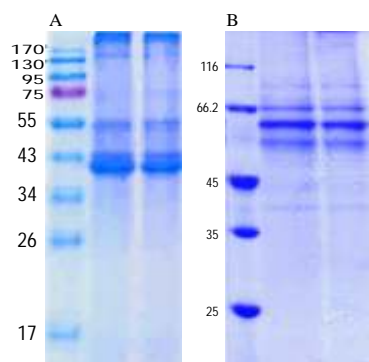


Fig. 1. SDS-PAGE of *T. evansi* antigens of donkey (A); camel isolates (B)

Moreover, few minor bands in molecular wt of 32kDa, 34kDa, 43kDa, and at higher range 72-130 kDa, as well were also glycoproteins as visible along with peroxidase as positive

control (Fig. 2). These glycoproteins are predominant and reactive with experimentally infected serum of rabbits and donkeys as well.

Zymography profile of *T. evansi* of donkey isolates appears to be similar with camel isolate as proteolytic activity observed between 28 and 170k Da regions as evident from zone of clearance by gelatin substrate GS-PAGE in acidic pH. A specific cysteine proteinase inhibitor E-64 – at 10  $\mu$ m concentration – inhibited *T. evansi* cysteine proteinases at mol wt zone of 32kDa, 35kDa, 54 kDa, & 160 kDa. Moreover, zone of clearance at



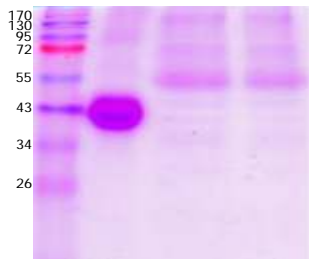


Fig. 2. Glycoproteins in *T. evansi* antigens

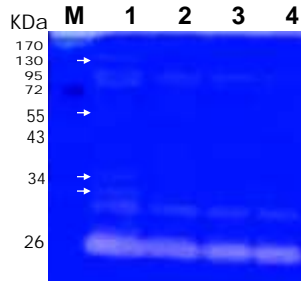


Fig. 3. GS-PAGE of *T. evansi* antigens

Lanes : 1 Antigen without inhibitor;  
 2 10  $\mu$ M E64; 3 20  $\mu$ M E64; 4. 50  $\mu$ M E64

molecular wt 95 kDa also inhibited at 50  $\mu$ M concentration confirm the presence of cysteine proteinase in whole cell lysate *T. evansi* antigen at above zones (Fig.3). Some of these cysteine proteinases were also found reactive in western blot with experimentally-/field-infected serum sample of equines.

An experiment was also carried out to study the kinetics of antibody response in donkeys infected experimentally with *T. evansi*. The serum samples collected sequentially at regular (weekly/fortnightly) interval up to 210 days were subjected to western blot, which identified polypeptides of 33-37, 41-45, 55-57 kDa (Fig. 4). Few bands in the range of 81-95 kDa reacted consistently in chronic infections. Purification of some of these immunoreactive polypeptides

is in progress, in order to characterize and develop specific immunodiagnostic reagent to detect chronic infection.

With a view to identify potential diagnostic markers and drug targets a study has been conducted on clinical proteome of *T. evansi* infection using mass spectrometry. Using shot-gun proteomic approach involving Nano-LC-Quadrupole-Time

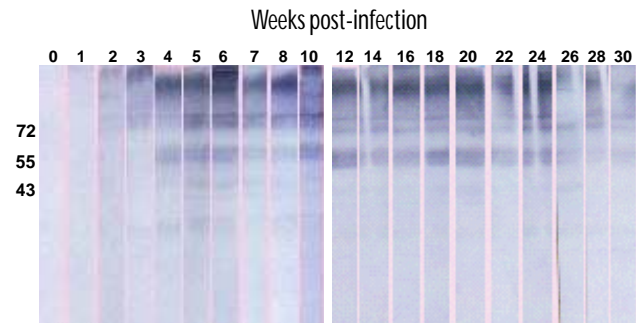


Fig. 4. Western Blot of pooled serum collected (at week/fortnight interval) from donkeys experimentally infected with *T. evansi*

of Flight-Mass Spectrometry, over 160 proteins expressed by *T. evansi* in mice infected with camel isolate were identified. The clinical proteome revealed the presence of known and potential drug targets such as oligopeptidases, kinases, cysteine proteases.

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

## Sero-prevalence of *T. evansi* using antibody -ELISA

The importance of *Trypanosoma evansi* as the etiological agent for surra is often overlooked due to difficulty in accurate diagnosis of the disease. Under field conditions, the disease is generally confused with other chronic wasting diseases, notably helminthosis and malnutrition. In the present study, an antibody-ELISA was developed and used for sero-prevalence study of *T. evansi* in equids of northern region of India.

Whole cell lysate antigen was prepared using purified trypanosome isolated from rat blood infected with *T. evansi*. The optimum dilutions of whole cell lysate antigen, serum samples and conjugate were determined by checker board titration. The diagnostic sensitivity and specificity was found to be 96.22 and 100%, respectively, as compared to western blotting. The ELISA was employed for sero-prevalence study of *T. evansi* infection in equids in the field. Out of 1005 serum samples, 115 (11.44%) samples were positive for

Prevalence of *Trypanosoma evansi* infection in equines

State	Number of samples		
	Tested	Positive	Per cent
Jammu and Kashmir	113	18	15.92
Himachal Pradesh	7	0	0
Haryana	181	5	2.76
Punjab	34	1	2.94
Rajasthan	189	9	4.76
Uttarakhand	325	47	14.46
Uttar Pradesh	156	35	22.43
Total	1005	115	11.44

antibodies against *T. evansi* in northern region of India.

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







## Surveillance of *Theileria equi* in equines using r-protein ELISA

Equine piroplasmiasis caused by *Theileria equi* is more pathogenic and widespread in equid than that by *Babesia caballi*. We developed a plate ELISA for detecting the *T. equi*-specific antibodies in equine serum samples. Using this assay, we found high sero-prevalence in equine population in different area of the country. For confirmation of findings, we randomly selected 53 samples, representing different geographical regions of the country. The samples were specifically selected to have a representation of sero-

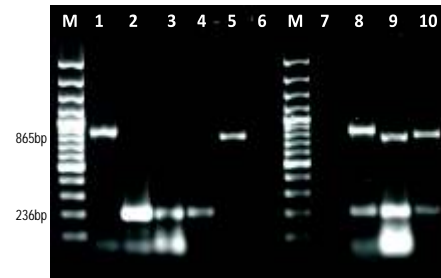
positive and sero-negative (as detected by r-ELISA) equine population. These samples were tested (in duplicate) by OIE-approved CI-ELISA and immunoblotted on recombinant EMA-2 antigen and on *T. equi* native antigen. The results of all the samples are in conformity when tested in r-ELISA, CI ELISA and immunoblot analysis which further validated the results obtained by r-ELISA in sero-prevalence studies.

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

## Multiplex PCR for *Theileria equi* and *Trypanosoma evansi*

There are two important hemoprotozoan diseases of equines in India, caused by *Theileria equi* and *Trypanosoma evansi*. In order to develop sensitive multiplex PCR for simultaneous detection of these pathogens, primers from EMA-1 and 18S rRNA gene of *T. equi* and *T. evansi* were designed, which amplified 236 bp and 865 bp products, respectively. Presently, the suitability of this multiplex PCR in carrier equines with very low parasitaemia is being tested.

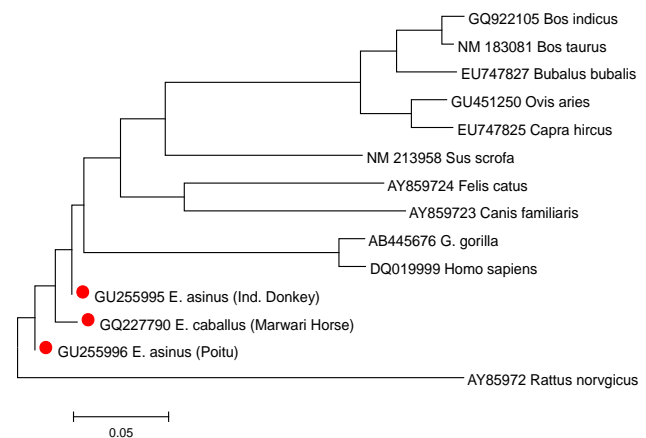
(Sanjay Kumar, Rajender Kumar, A.K. Gupta and S.C. Yadav)



Amplification of *T. equi* and *T. evansi* in PCR (lanes 1-5 and multiplex PCR (lanes 8-10))

## Molecular characterization of toll-like receptor 9 in Marwari horse, Poitu and indigenous donkey

Toll-like receptor 9 (TLR9) has been characterized as a receptor that can recognize unmethylated CpG motif within bacterial DNA and signalling by this receptor triggers pro-inflammatory cytokine response that influences both innate and adaptive immune responses. Although TLR9 is extensively studied in human, mice, swine, canine, bovine, ovine and feline species, there is no report on characterization of TLR9 of Indian breeds of horses, donkeys and Poitu donkey. Expression of TLR9 gene was studied in PBMCs of Marwari horses, Poitu and indigenous donkeys. Partial TLR9 gene amplicons from mRNA were cloned into pGEMT vector and got sequenced. Sequences were submitted to GenBank database. Deduced amino acid sequences of TLR9 proteins were aligned and analysed using MEGA 4. Estimates of evolutionary divergence between the sequences of TLR9 revealed that *Equus caballus* (Marwari breed) and *Equus asinus* differ from



Phylogenetic tree of deduced amino acid sequences of partial TLR9 mRNA of *Equus sp*

human by 19% and 16%, respectively. Phylogenetic tree was constructed by the Neighbour-Joining method (Fig.). The TLR9 proteins from the present study clustered with





*Equus caballus* protein sequences, while human, cattle, dog, sheep, mice, and buffalo formed separate clades. The analysis showed conserved sequences and close association of TLR9 proteins within species and high divergence between species of animals. An understanding of sequence variability of TLR9 amongst different species of

equines would help to better understand the biological responses to CpG DNA. The information can be used to devise new vaccine adjuvants and enhance the immune responses.

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

## Amplification of Mx gene from Marwari horse

The Mx protein confers resistance to Orthomyxovirus infection by modifying cellular functions needed for viral replication. The antiviral properties of Mx proteins are influenced by isoforms and intracellular localization of the individual protein. Mining for variations in Mx genes in resistant and susceptible animals/different breeds of horses may provide insight to the functional diversity within Mx proteins. Blood samples were collected from

clinically normal horses, equine influenza infected and vaccinated animals. RT-PCR was standardized using primers designed for Mx gene. Full length Mx gene was amplified from vaccinated Marwari horse and PCR amplicon has been submitted for sequencing by primer walking.

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

## Phenotypic characterization of Bhutia and Spiti breeds

In an endeavor to evaluate different indigenous breeds of equines found in India, phenotypic characterization of Bhutia and Spiti equids was performed. Bhutia equids originated in the Himalayan region of India, and are now found in Bhutan and India (Sikkim and Darjeeling). Due to their stamina and endurance, these ponies are reared for

decreased drastically from 1682 to 546 during 2003 to 2007 indicating that this breed is on the verge of extinction.

Fifteen different biometrical indices of 35 Bhutia equines in their home tract in Sikkim were recorded. Mean height at withers in Bhutia animals was observed as 126.5 cm, without any significant difference due to sex. In these



Bhutia



Spiti

work, mainly as pack ponies and sometimes for riding. Spiti breed is confined to Lahaul & Spiti, Kinnaur, Kullu, Mandi, Kangra and Shimla districts in Himachal Pradesh. However, the breeding tract is confined to 15 villages of two Panchayats (Kungri and Sagnam) in Pin Valley of Spiti subdivision of Lahaul & Spiti. As per an estimate, total population of Spiti ponies in Himachal Pradesh is approximately 4000. Population of Bhutia ponies has

decreased drastically from 1682 to 546 during 2003 to 2007 indicating that this breed is on the verge of extinction. Fifteen different biometrical indices of 35 Bhutia equines in their home tract in Sikkim were recorded. Mean height at withers in Bhutia animals was observed as 126.5 cm, without any significant difference due to sex. In these ponies, bay was the most common colour (69%), followed by chestnut (23%), grey and other colours (8%). Average body





bones. The legs are thicker and covered with long coarse hairs. The mane is long with hairs of 25 to 40 cm in length. The body is solid, the face is convex, the ears are erect and the eyes are black. The back is straight and the tail is medium in length and straight.

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

## Survey of equine farmers and draught equine population in different regions of India

Surveys of different equine populated regions in India viz. Spiti valley (HP), Hanumangarh, Haldi Ghati, and Jalore (Rajasthan) were conducted to study socio-economic status of equine owners. The horse ownership strength was

found to be maximum in Hanumangarh. In addition, equine management practices like feeding, housing, health care, general management and indigenous technical practices were also studied (Table).

Socio economic status and equine management practices in Rajasthan and Himachal Pradesh

Parameters	Jalore (n=20)	Hanumangarh (n=40)	Haldi Ghati (n=32)	Spiti (n=50)
Literacy (%)	45	86	-	64
Age (Years)	50.1	41.5	-	41.28
Average family size	8.8	7.54	-	6.58
Land holding (in bigha)	46.6	53.64	-	13.04
Landless farmers (%)	25	10	-	0
Horse strength/owner	3.0	4.64	1.3	1.88
Income (Rs)	40000-70000	35000-250000	25000-40000	15000-30000
Vaccination	0	Only tetanus	0	0
Deworming (%)	70	100	100	Occasionally (100)
Foal heat mating	100	100	100	100
Navel care	55%	90%	0	0
Type of dry fodder	Bajra & jowar karbi unchaffed (7.250kg)	Groundnut chara/ jowar karbi/wheat straw (5.54kg)	Klap & hiran grass (ad lib)	barley bhusa, pea bhusa, local grasses
Type of green fodder	Dub grass & lucerne (6.10kg)	Lucerne /dub grass/oat (15.27kg)	Lucerne & bajra (8-10kg)	Grazing (from June onwards for 5-6 months)
Ingredients of concentrate mixture	Bajra (1.95 kg)	Barley/gram/oat (2.39 kg)	Crushed barley (2-2.5kg)	Crushed black pea/barley
Frequency of concentrate feeding	Once daily	Twice daily	Twice daily	Twice daily
Special feeding	Mustard oil in winter	Ghee/milk/ gur in winter/ desi masala	Mustard oil in winter & ghee in summer	Gur in winter
Breed	Marwari	Marwari	Non-descript	Spiti





Utilization of equines in different operations

## Equine management practices in Spiti valley

A survey on the managerial practices followed by horse owners in Spiti valley was conducted and the following observations were made. Horses are stall-fed and looked

indoor during the winter months (November to March) and left for grazing in pastures during the rest of year i.e. from April to October. All the horses of each village are looked



View of Spiti village



Donkeys being used in agricultural operations

after by two people of that village during the grazing in the pasture throughout the day and night on fortnightly rotation basis. Dung is not removed from the sheds throughout the year. By the month of October, the dung lying in the stables decomposes well and then it is used as manure in agricultural fields. Foaling occurs in April-May followed by rebreeding of all the broodmares during foal-heat. In June, all the horses except young stock are shifted to high land pastures till the arrival of winter.

Only selected stallion is used for natural service of mares of two to three villages. The stallion is selected on the basis of body conformation, soundness, co-ordination of legs and against marking on the body. The owners of broodmares are required to pay a nominal fee in cash or kind on account of getting their broodmares served by the selected stallion. Surplus stock of horses is taken to International Lavi fair at



Mule being used as pack animal

Rampur Bushar in Shimla District (November) and Ladarcha Fair at Kaza in Lahaul & Spiti District for sale.

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





## Equine management practices in different parts of Rajasthan

Hanumangarh is situated in the north-west of Rajasthan. Indira Gandhi canal passes through this region. Due to availability of sufficient water, the cropping pattern of Hanumangarh is different from other parts of Rajasthan. Marwari horses are maintained for joy riding, breeding and equestrian events viz., dancing. Most of the equine owners use equine dung as compost in their fields. Majority of equine owners keep the stables neat and clean. Majority of the people keep their horses under pucca roof which are well ventilated. Grooming and hoof care is a regular practice. Shoeing is not in practice in mares while stallions are shod regularly. Only 27% of equine owners spray disinfectants in the stables. Mineral mixture feeding to horses is rare, but common salt is fed regularly. Feeding of ghee, milk and mustard oil particularly to stallions during winter was also reported by some respondents. Haldi Ghati – well known for Maharana Pratap, a great warrior of India – is the part of Rajsamand district of Rajasthan. Male horses are mainly

reared for ceremonial purpose. Horse is offered 1-2 kg gram dal (chickpea) after the completion of ceremonial activity by the party using the animal for ceremony. Grooming, hoof care and shoeing is being done in these animals. The horses are stall-fed and kept in shelter made up of tin or thatch.

Jalore is considered the home tract of Marwari breed of horse in Rajasthan. Majority of equine owners (90%) keep the stables neat and clean. The horses are maintained in intensive (55%) and semi-intensive (45%) manner. Majority of the people maintain their horses under thatch on pillars with no arrangement of water trough in the stables with kuchcha floor. The shoeing is not in practice in mares while stallions that walk on pucca road are shod regularly. Horses are fed jowar/bajra (sorghum/pearl millet) dry fodder without chaffing due to which the wastage of the fodder was observed. They supply bajra as concentrate and tumba (*Citrullus colocynthis*) to prevent colic.

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

## Socio-economic status of donkey and mules owners in Rajasthan and Haryana

A survey of Shergarh, Rattangarh (Rajasthan), Rajli (Haryana) was done to study socio-economic status of donkey and mule owners, management practices and indigenous technical know-how (Table). During surveys, information from 86 respondents of Shergarh village (Jhalawar district), 40 respondents from Rattangarh (Rajasthan) and 40 respondents from Rajli (Haryana) was collected. Majority of donkey owners (83.70%) use donkey

dung for various purposes viz. manure, pot making, brick-kilns etc. The floor of most of the stables was kucha. Majority (95%) of the equine owners groom their animals at least once a day. About half of the respondents (48%) care for the hoof of donkeys. About half of respondents (53.5%) rear other livestock viz. cow, buffalo, goat, camel and mule. The donkeys were offered groundnut bhusa, soya bhusa, wheat bhusa, gram bhusa, dhanian (coriander), jiri and rice bhusi

Socio-economic status of donkey and mules owners in Rajasthan and Haryana

Parameters	Shergarh (n=86)	Rattangarh (n=40)	Rajli (n=40)
Literacy (%)	44	18	47.5
Age (years)	40.38±1.40	38.26±2.38	42.2±2.04
Average family size	6.67±0.32.	4.78±0.43	5.37±0.29
Land holding (bigha/acre)	5.0±0.33 bigha	8.50±4.03 bigha	5.38±0.85 acre
Kuchha housing (%)	82	91	10
Donkey/equine strength/owner	5.15±0.40	1.17±0.08	1.77±0.17
Income/animal (in Rs)	75±1.9	184±11	238±9





Donkey in carting

mainly as dry fodder. Daily mean dry fodder, concentrate and gur supplied to each donkey were  $4.680 \pm 0.049$  kg,  $684 \pm 56$ g and  $112 \pm 28$ g, respectively. In Rattangarh, majority of the owners who use donkeys in carting were having poor harness which is the main reason for gall, wound formation and saddle sores in working donkeys. In Rajli (Haryana), they use animals for transport, ceremonial purposes and breeding. The equines used in carting are shod regularly. Main constituent of dry fodder is wheat straw and jowar, oats and barseem as green fodder. They also provide mix of bajra, barley and gram as concentrate.

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

## Study of donkey and mule production systems in Haryana, Rajasthan and Uttar Pradesh

A total of 368 working donkey and mule owners of Haryana, Rajasthan and UP were selected from the most densely populated area and data were collected through personal interviews. The animals are reared by people involved in occupations like making earthen pots, rearing sheep and goat, working on brick kilns in rural areas and working with donkey carts in semi-urban areas.

Two types of feeding systems are reported in the study area, first complete stall feeding and second stall feeding with some grazing. In case of stall feeding, generally three time feeding in mares is done- morning, noon and evening. Horses are stall-fed in all the states and there is no significant difference among states. Whereas in Haryana, donkeys are in better condition due to stall feeding and significantly different than rest of the two states viz. Rajasthan and U.P. The lack of awareness regarding scientific breeding, feeding practices and lack of financial



Stall Feeding in mules

assistance has been the major constraints faced by 97.55% and 96.74% of the respondents, respectively.

(Niranjan Lal, Ramesh Kumar, Praveen Malik, Rajender Kumar and B. Ganesh Kumar)

## Initiation of vermicomposting

Composting is the process whereby naturally occurring microbes breakdown organic matter. A perfect compost is a soil-like material rich in nutrients and full of roughage which, when added to the garden, improves soil structure and plant health. The critical thing is overcoming the weed problem. Vermicomposting uses specific type of earthworm (*Eisenia fetida*, also known as red worms, tiger worms, red wigglers) that work with other compost organisms to

decompose manure and bedding.

Due to low moisture content in equine dung and poor absorption of water, it does not decompose properly and hence can not be utilized as manure. An attempt was made to convert the dung into vermicompost for its proper use. For this, equine dung was wetted for 15 days before adding earthworms in dung. The water was applied at alternate day to the heap of dung. The heap was turned up two times for





aeration and lowering down the temperature. The trenches for vermicomposting were made inside a shelter made up of cotton green net covered on all sides to maintain optimum temperature and protect from predators. On the bottom, black plastic sheet was laid to prevent earthworms to enter in the soil. Partly decomposed dung was filled in trenches to the height of 1.5 feet. Then, 60 kg culture of earthworm was applied on the top of the dung and then covered with a layer of tree leaves. The water was applied to vermicompost trenches daily to maintain proper humidity. The vermicompost was ready for use in 3 months.

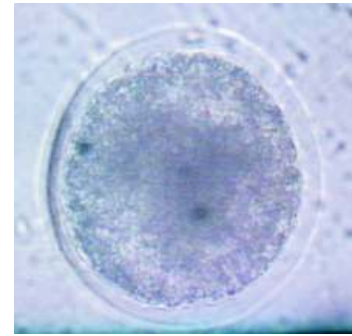


(R.A. Legha)

Application of earth worm culture in equine dung

## Cryopreservation of embryos for conservation of Marwari horses

Synchronization of estrus was carried out in thirteen Marwari mares by prostaglandin F2 alpha. Heat detection was carried out regularly by teasing method with a stallion and monitoring the follicle stage manually as well as ultrasonographically everyday from the 3rd day estrus to ovulation. All the donor mares were artificially inseminated with frozen semen at 24 h interval from follicle size of >35mm to ovulation. The recipient mares were observed for its estrus onset and end of the estrus without insemination. Six embryos were recovered out of twenty one flushings (6-8 days post ovulation) and five were transferred to suitable recipient mares. The pregnancy verification was conducted on day 20 post-ovulation. However, none of the embryo could grow successfully. Work on embryo transfer is being continued.



Seven-days old embryo

(A. Arangasamy and Thirumala Rao Talluri)

## Cryopreservation of equid semen using amides

Stallions show a high degree of individual variation with respect to the cryosurvival of sperm and fertility of frozen-thawed semen. It has been estimated that approximately 30-40 % of stallions produce semen that freezes poorly. Eventhough, glycerol – primary cryoprotectant for other species – has toxic effects on spermatozoa as well as contraceptive effects in the mare. Therefore, we evaluated methyl formamide,

dimethyl formamide and dimethyl sulfoxide as alternative cryoprotectants to glycerol for freezing equine sperm. The sperm motility and livability were studied at pre-freeze and post-thaw stage with various cryoprotectants Methyl formamide did not work as good cryoprotectant.

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





# VTCC Research Achievements

## First laboratory confirmed camelpox virus zoonosis

The camelpox virus (CMLV) primarily causes skin eruptions in addition to severe generalized exanthema mostly in camels of 2–3 years of age and outbreaks occur frequently in the north-central regions of the country, the main camel rearing region. However, their zoonotic potential has not been proven conclusively. We report the CMLV outbreaks in camels and its zoonosis on the basis of clinical and epidemiological features along with serological and molecular characterization of the causative agent in three human patients in a Border Security Force (BSF) camp in Barmer district of Rajasthan in 2009 (Fig. 1).

Biological samples (blood, scabs & swabs) were collected from infected animals and in-contact human handlers for identification and characterization of the etiological agent. The disease was confirmed on the basis of isolation of the virus from camels, detection of the CMLV antibodies by

serum neutralization test and amplification of CMLV-specific 243 bp fragment of C18L gene by conventional as well as real-time PCR from camels and humans (Fig 2).

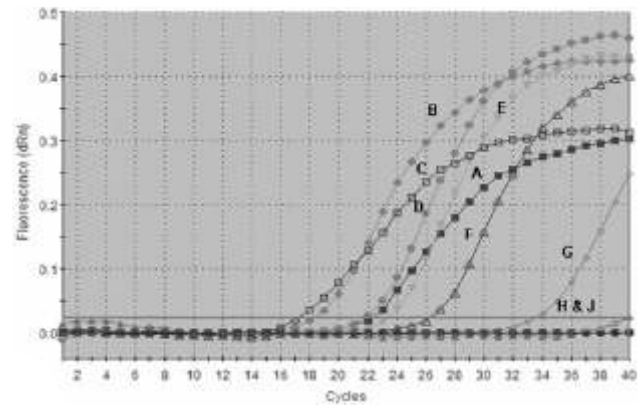


Fig. 2. C18L gene based SYBR Green based real time PCR showing specific amplification from the viral DNA of CMLV-I isolates (A-F).



Fig. 1. Clinical lesions in human patients infected with camelpox virus

Three full-length envelope protein genes (A27L, H3L and D8L) and one partial gene (C18L) of the isolated viruses from camels and clinical sample from humans were cloned and sequenced. Sequence analysis indicated that CMLV isolates shared 97.7–100% sequence identity among themselves at the nucleotide and amino acid level. Furthermore, the phylogenetic analysis based on the amino acid sequences of all the three genes revealed a similar branching pattern with CMLV isolates clustering closely with variola virus (VARV) and vaccinia virus (VACV). There was 100% nucleotide sequence homology in PCR-amplified genes (A27L, H3L and D8L) both from human clinical samples and CMLV (Barmer isolate from camel), further confirming that CMLV (Barmer isolate) was responsible for zoonosis. The close relatedness between the viruses triggers concerns over the possibility of conversion of CMLV which could result in the development of a new human pathogen. Besides, the CMLV zoonoses indicates the changing pattern of host specificity of poxviruses and certainly points towards a declining cohort immunity against Orthopoxviruses in humans, which could be of serious public health concern.

(R.K. Singh, Sanjay Barua, B.C. Bera, K. Shanmugasundaram, R.K. Vaid, P. Malik, B.R. Gulati, G. Nagarajan and K.M.L. Pathak)



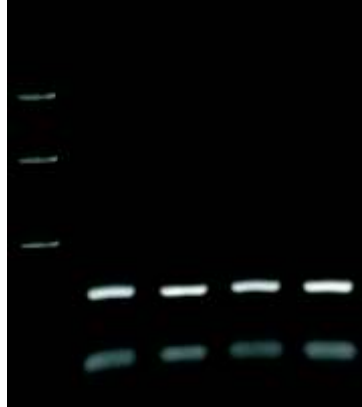




## Isolation and molecular characterization of camelpox virus from outbreaks in Rajasthan and Delhi

We investigated outbreaks of camelpox virus (CMLV) in camels housed in Border Security Force (BSF) camps at Jaisalmer and Barmer districts of Rajasthan and characterized the etiological agent. The first camelpox outbreak was recorded during the second week of December 2008 in Sector Head Quarter (SHQ) of Border Security Force (BSF), Bikaner followed by subsequent outbreaks in Delhi (December 2008), Jaisalmer and Barmer (April-May, 2009). The possible mode of spread of the disease could be attributed to the herds of infected camels in surrounding villages.

Biological samples (blood, scabs & swabs) were collected from infected animals for identification and characterization of the etiological agent. The disease was confirmed on the basis of isolation of the virus in Vero cell line, detection of the CMLV antibodies by serum neutralization test and amplification of CMLV-specific 243 bp fragment of C18L gene by conventional as well as real-time PCR. Three full-



PCR amplification of CMLV specific C18L gene

243 bp

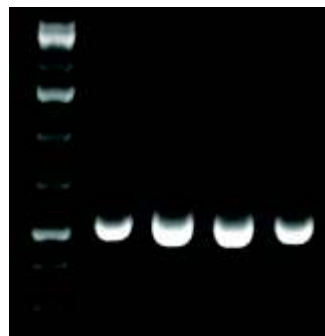
length genes (A27L, H3L and D8L) and one partial gene (C18L) of the isolated viruses were PCR amplified using published primers. The amplicons were cloned and sequenced. The sequences were submitted to GenBank. The ORFs (A27L-333bp, H3L-975bp, and D8L-915bp) were aligned with Orthopoxvirus sequences available in the database using the NCBI BLAST. CMLV isolates shared 97.7–100% sequence identity among themselves at the nucleotide and amino acid level. Furthermore, the

phylogenetic analysis based on the amino acid sequences of all the three genes, followed a similar branching pattern with CMLV isolates clustering closely with variola virus (VARV) and vaccinia virus (VACV). The close relatedness between the viruses triggers concerns over the possibility of conversion of CMLV, which could result in the development of a new human pathogen.

(R.K. Singh, Sanjay Barua, B. C. Bera, K. Shanmugasundaram, R.K. Vaid, B. R. Gulati, N. Kakker, G. Nagarajan and K.M.L. Pathak)

## Isolation and characterization of buffalopox virus from outbreaks in Maharashtra

We investigated outbreaks of Buffalopox in different livestock species (buffaloes, humans, cattle and goats) in Sunasgaon /Gombhi villages in Jalgaon district of Maharashtra in March, 2010. The disease was confirmed on the basis of isolation of the virus in Vero cells from bovine samples, and amplification of BPXV specific 552 bp fragment of A-type



PCR amplification of BPXV specific ATI gene

552 bp

inclusion gene by PCR. Further characterization of the virus is being done to ascertain the significance of changes in host specificity of pox viruses being observed in recent pox outbreaks.

(R.K.Singh, Sanjay Barua, B. C. Bera, Shanmugasundaram, K., R.K.Vaid & B. R. Gulati)





## Field isolation and characterization of bacterial pathogens

Strains of bacteria associated with animal diseases and environment of animals need to be isolated, purified, preserved and studied from taxonomical point of view. In order to achieve this, 162 samples obtained from Delhi, Haryana, Uttarakhand, Uttar Pradesh and Rajasthan from horses, donkeys, pigs and camel were processed for bacterial isolation. The purified isolates were subjected to various staining and media-based phenotypic and biochemical test identification methodology.

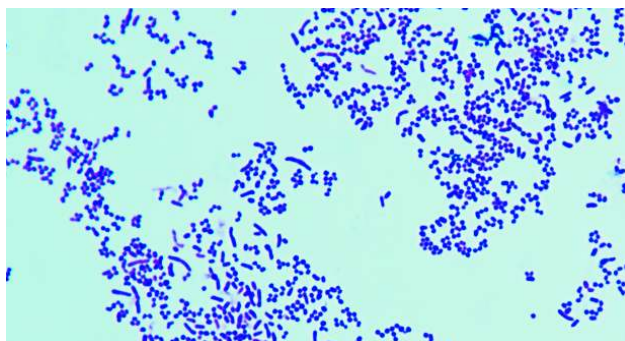


PCR amplification of 16S rRNA genes of different bacterial isolates

Important isolations included *Actinobacillus equuli* and *Serratia marcescens* from vegetative growth on endocardium of horse, and *Pseudomonas aeruginosa* from intestinal contents, *Klebsiella pneumoniae*, *Escherichia coli* and *Edwardsiella* sp from equine abortion cases were made. Other isolates include *Staphylococcus* sp, *Streptococcus* sp, *Enterobacter* sp, *Micrococcus* sp. Sequencing of 16S rRNA of the *Pseudomonas aeruginosa*, *Streptococcus zooepidemicus*, *Rhodococcus equi* and *Bordetella bronchiseptica* has been performed. Under the characterization of 2 isolates of *Pseudomonas aeruginosa* from chronic respiratory tract infection in horse, PCR amplification of outer membrane protein gene (1141bp) was performed. Various isolates have been preserved under -20°C and -70°C conditions.

### *Rhodococcus equi* and *Corynebacterium* sp isolation from feces

In a survey of *Rhodococcus equi* and *Corynebacterium* sp. bacteria in feces of animals, 36 typical *R. equi*, 22 atypical

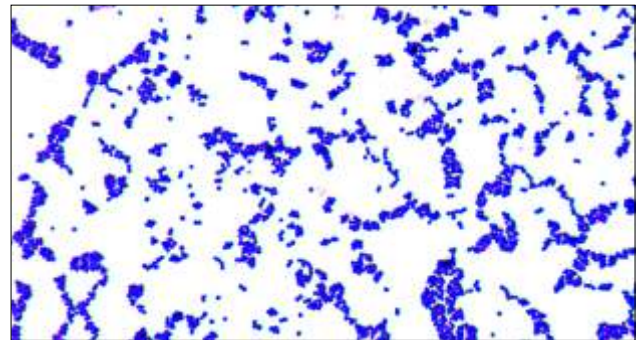


Gram stained atypical *Rhodococcus equi* from camel feces

*rhodococci* and 32 coryneform isolates were obtained. Biochemically, typical *R. equi* were isolated only from horse feces, however, atypical rhodococci were detected from pig, camel and donkey feces. Thirty two *Corynebacterium* isolates were unidentifiable by routine biochemical tests, however, we tentatively diagnosed 3 isolates as *Corynebacterium pseudotuberculosis* and 2 as *C. bovis*.

### Molecular and biochemical characterization of staphylococci from different animals

Molecular characterization of 23 different *Staphylococcus* sp isolates from horse, pig, goats and camel by PCR amplification of genus-specific region (791 bp) confirmed all isolates as *Staphylococcus* sp. The isolates were also characterized by amplification of thermonuclease gene (*nuc*-791 bp) in 15 isolates, clumping factor gene (*clfA* - 638 bp) in 5 isolates, methicillin resistance gene (*mecA* - 533 bp size) of 3 isolates and haemolysin gene (*hly* - 960 bp) in one isolate of *Staphylococcus aureus* has been accomplished. Isolates were also biochemically characterized up to species level.



Gram-stained smear of *Staphylococcus*

Twenty three *Staphylococcus* sp isolates were characterized biochemically and phenotypically up to species level. Various isolates were identified as *S. aureus*, *S. sciuri*, *S. hyicus*, *S. intermedius*, *S. hemolyticus*, *S. hyicus* ssp *chromogenes*, *S. hyicus* ssp *hyicus*, *S. saprophyticus*, *S. warneri*, *S. simulans*. Important finding has been isolation and identification of *Staphylococcus hyicus* from pigs, a pathogen causing greasy pig disease and detection of methicillin-resistant strains of *Staphylococcus sciuri* isolated from goat milk. Literature reports suggest the origin of *mecA* to be possibly from *Staphylococcus sciuri*.

(R.K. Vaid, Mamta Tigga, K. Shanmugasundaram, B.C. Bera, S. Barua and R.K. Singh)



# Technology Development & Assessment

## Field trial of equine herpes virus-1 vaccine

An inactivated EHV-1 vaccine (Equiherpabort) – developed by the Centre – was used for the field trial on 67 pregnant mares. For this, the vaccine in 2 ml dose was inoculated in 58 pregnant mares of 5 month gestation and 9 mares were kept as unvaccinated control. The animals were given booster vaccination on 7<sup>th</sup> month of gestation. There were no post-vaccination untoward effects in vaccinated pregnant mares. All mares before vaccination were negative for EHV-1 antibodies by virus neutralization (VN antibody titre <8). Average serum reciprocal VN antibody titres against EHV-1 in mares at 30 and 60 days post-vaccination were 16. No EHV-1 associated abortion was reported in vaccinated mares.



EHV-1 Vaccine Trial in Mares at Pirkamaria, Hanumangarh

## Validation of immunoassays for differentiation of EHV-1 and EHV-4 infections

Equine herpes virus (EHV-1) causes respiratory disease, abortions, neurological disorders and perinatal foal mortality while EHV-4 is responsible largely for respiratory disease. Due to extensive antigenic and genetic similarity in the two viruses, it becomes difficult to differentiate these two viruses. Serological assays for differentiation of EHV-1 and EHV-4 infections employing recombinant EHV-1 and EHV-4 proteins were developed. Two enzyme immunoassays (single-dilution plate ELISA and immune dipstick) developed using recombinant glycoprotein G antigen from EHV-1 and EHV-4 were validated during the year with field equine serum samples. The plate ELISA was validated using 1600 field serum samples. A total of 136 (8.5%) samples were

positive for EHV-1 while 914 (57.12%) were positive for EHV-4. A total of 48 samples (3.0%) were found positive for both EHV-1 and EHV-4. Immunostick assay was used to screen 250 serum samples to develop a field-based assay for differentiation of EHV-1 and EHV-4 antibodies. The results of the assay were comparable to those obtained by plate ELISA. A total of 148 samples out of 250 have been found positive for EHV-4 while 13 samples were positive for EHV-1. The sensitivity and specificity of the immunostick assay for EHV-4 as compared to plate ELISA was 93.24% and 100%, respectively. The immunostick ELISA was also got validated internally from two laboratories using 30 known samples.

## Validation of the recombinant antigen-based ELISA kit for detection of *Theileria equi* antibodies

Equine piroplasmiasis caused by *Theileria equi* is more pathogenic and widespread in equid than that by *Babesia caballi*. In *T. equi*, two kinds of merozoite surface proteins, equi merozoite antigen (EMA) -1 (34 kDa) and -2 (30 kDa) have been identified as the most immunodominant antigens. *T. equi* merozoites liberally express these surface proteins which are strongly recognized by antibodies

produced in infected animals. We expressed and purified the EMA-2 recombinant protein and developed plate ELISA kit for detecting the *T. equi*-specific antibodies in equine serum samples. Validation of the kit was got done from two internal and three external laboratories during the year. The results of the validation with 48 check samples were identical during internal and external validation.





# Consultancy & Commercialization of Technology

## Consultancy

NRCE – being nodal agency and national referral centre of Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture (Govt. of India) for various equine disease diagnosis – offers consultancy and diagnostic services to various stake-holders for disease investigation and testing for health certification in the country. As part of this programme, on-farm/field visits to different parts of the country are taken by teams of experts from the Centre for attending disease occurrences and outbreaks amongst equines. Besides this, samples submitted by state and regional disease diagnostic labs, regional animal quarantine and certification stations, Indian Polo Association, Equestrian Federation of India, field veterinarians and equine owners are analyzed 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. 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.



NRCE experts attending disease outbreak at Haldwani

During the period under report, a total of 4510 equine serum samples from various sources were examined for equine infectious anaemia (EIA) by Coggins test. All the samples were negative for EIA. The Centre also tested 3543 equine serum samples for glanders using complement fixation test (CFT). In addition, samples from equines were received for testing of contagious equine metritis (CEM), piroplasmiasis, rhinopneumonitis, equine influenza and bacterial infections.

NRCE – together with VTCC – is undertaking work of bacteriological examination of various samples collected mainly by our team of scientists and also received from various equine establishments from different parts of the country. Bacteriological analysis done on 258 samples, originating from Rajasthan, Uttarakhand, Haryana, U.P, Punjab, Gujarat, Chhattisgarh and Delhi, including nasal swabs, vaginal swabs, eye swabs, blood and urine samples, tissues from PM, lesion swabs, faecal samples, rectal swabs and soil samples yielded 54 isolates including *Burkholderia mallei* (2), *Rhodococcus equi* (1), *Streptococcus equi* subsp. *zooepidemicus* (16), *Streptococcus equisimilis* (1), Group C *Streptococci* (14), Group F *Streptococci* (2), *Enterobacter aerogenes* (1), *Staphylococcus* spp. (8), *E. coli* (6), *Enterobacteria* (1). A total of 1684 samples from animal quarantine centres including 1599 vaginal swabs and 85 preputial swabs tested for CEM were negative. Important isolations included *Actinobacillus equuli* and *Serratia marsces* from vegetative growth on endocardium of horse, and *Pseudomonas aeruginosa* from intestinal contents, *Klebsiella pneumoniae*, *Escherichia coli* and *Edwardsiella* from equine abortion cases were made. Other isolates include *Staphylococcus* sp, *Streptococcus* sp, *Enterobacter* sp. and *Micrococcus* sp. Sequencing of 16S rRNA of the *Pseudomonas aeruginosa*, *Streptococcus zooepidemicus*, *Rhodococcus equi* and *Bordetella bronchiseptica* has been performed. Antibiotic sensitivity testing of clinical samples also done and results were conveyed to various concerned quarters. The Centre provides consultancy services to end-users, through extension programmes such as animal health camps, artificial insemination camps, problem solving meets, training-cum-workshops, *Kisan Gosthies*, field visits and on telephone.

### Samples from Animal Quarantine Centres (DAHD & MoA, Gol) tested for contagious equine metritis

Sample	Delhi	Chennai	Total
Vaginal Swab	1483	116	1599
Preputial Swab	69	16	85
Total	1552	132	1684





## Commercialization of Technology

The revenue for the Centre is generated through many activities of which contractual diagnostic services are the major source. During the year, revenue to the tune of Rs.42.88 lakh has been generated through testing of samples for EIA, glanders, CEM, EHV-1, piroplasmiasis, dourine, trypanosomiasis, equine influenza, and *Salmonella* Abortusequi. In addition, improved germplasm

of french jacks and indigenous (Marwari) horses in the form of cryopreserved semen was also provided to the farmers for superior mule production and conservation of Marwari horses. Artificial insemination services using cryopreserved semen were provided in the field in different states of the country along with free pregnancy diagnostic services.

### Technologies Developed by NRCE



- ❑ Herpkit for diagnosis of EHV-1 infection in equines.
- ❑ Monoclonal antibody-based blocking ELISA (Equiherpes B-ELISA) for diagnosis of EHV-1 infection.
- ❑ Monoclonal antibody-based sandwich ELISA for diagnosis of equine rotavirus infection.
- ❑ Pregmare kit for pregnancy diagnosis in mares.
- ❑ Recombinant antigen-based ELISA kit for diagnosis of *Babesia equi* infection in equines.
- ❑ Semen freezing technology and artificial insemination for superior mule production and *in situ* conservation of true- to-breed horses.
- ❑ Inactivated equine influenza vaccine.
- ❑ COFEB Kit for diagnosis of piroplasmiasis in equines.
- ❑ Updated equine influenza vaccine incorporating 2008 EIV isolate.





## Education & Training

### A Short Course on Equine Influenza Organized

In view of re-emergence of equine influenza in India during 2008-2009, a need was felt to update the knowledge and skills of field veterinarians in equine influenza diagnosis, control and emergency preparedness. Therefore, NRCE organized a short course on 'Equine Influenza Diagnosis and Control' during August 31-September 5, 2009. In this course, 23 veterinarians from 10 different states participated. Dr. B.K. Singh, Principal Scientist was the Course Director. During the course, 5 practicals were conducted and 17 expert lectures were delivered by NRCE scientists on the topics including equine influenza virology, epidemiology, molecular biology,



The Chief Guest releasing a compendium of lectures

pathology, immune response, recent developments in diagnostics & prophylaxis, disease control and biosecurity. Addressing the participants during the valedictory function, Dr P.K. Uppal, Technical Director (RWITC, Pune) shared experiences gained during equine influenza outbreak of 1987 and elaborated the biosecurity measures to be taken to deal with such outbreaks.

### A Short Course on Equine Health and Management for Paramilitary and Field Veterinarians Organized

Equines hold special importance in hilly terrain for military and paramilitary forces. In addition, some states are laying emphasis on equine use. There has been great demand from some states and para-military forces for imparting training to their veterinarians on salient aspects of equine health and production. Accordingly, NRCE organized a short course on "Equine Health & Production Management" during January 20 to February 2, 2010. In this course, 20 equine veterinarians from North-East and Gujarat participated. This 14-day training course comprised theory lectures and practical demonstrations on various health and production



Dr S.C. Yadav, Course Director briefing participants about the Course

management aspects. The Course was organized for one week each at Hisar and Bikaner campus. Dr. S.N. Tandon, and Dr. S. C. Yadav were the Course Directors. A compendium was also published and released by Director, NRCE, Dr R.K Singh. Compendium contained more than 25 expert lectures on various aspects of equine health and management.





## Expert Lectures Organized at NRCE, Hisar

- ❑ Mr. Guy Delhomme, France, delivered lecture on 'Equine Reproduction and Embryo Transfer,' on April 16, 2009, sponsored by IMV (India) Pvt Ltd.
- ❑ Dr Utpal Tatu, Professor of Biochemistry, Indian Institute of Sciences, Bangalore delivered a talk on 'Clinical Proteomics of Trypanosomal Infections in Animals,' on May 14, 2009.
- ❑ Dr M.M. Parida, Deputy Director, DRDE, Gwalior delivered a lecture on 'Resurgence of Explosive Unprecedented Chikungunya Epidemic; Virologist's Insight: Complete Characterization to Complete Solution' on June 12, 2009.



Dr D.N. Tripathy (right) with scientists at NRCE

- ❑ Dr D.N. Tripathy, Professor, University of Illinois, USA delivered a talk on 'Strategies for Development of Animal Vaccines' on December 15, 2009 on the occasion of Silver Jubilee Celebrations of NRCE.
- ❑ Dr Rajneesh Sharma, Patent Attorney, New Delhi delivered a talk on 'IPR Issues in Biotechnology and Patenting' on July 21, 2009.
- ❑ Dr. Colin G. Rousseaux, Adjunct Professor, Department of Pathology and Laboratory Medicine, University of Ottawa, Canada visited this Centre on March 9, 2010. Dr. Rousseaux is the world-renowned toxicopathologist and is the editor of 'Handbook of Toxicologic Pathology'. During his visit, he delivered a lecture on "Spontaneous lesions of the gastrointestinal system". The lecture was attended by the scientists from NRCE, CIRB and CCS HAU, Hisar.



Scientists attending the lecture by Dr Colin at NRCE



## Lectures by NRCE Scientists published in Compendia

1. Arangasamy, A. and Talluri, T.R. 2010. Artificial Insemination and factors affecting Conception rate in Equines. In: Compendium on Equine Health and Production Management (eds. Gulati, B.R., Khurana, S.K., Pal, Y., Kumar, R. and Vaid, R.K.), NRCE Publications, Hisar, pp.85-87.
2. Arangasamy, A. and Talluri, T.R. 2010. Cryopreservation and Semen Evaluation Methods in Equines. In: Compendium on Equine Health and Production Management (eds. Gulati, B.R., Khurana, S.K., Pal, Y., Kumar, R. and Vaid, R.K.), NRCE Publications, Hisar, pp.68-72.
3. Bansal, R.S. and Singh, J. 2010. Technique of Artificial Insemination and Per-rectal method of Pregnancy Diagnosis





- in Equines. In: Compendium on Equine Health and Production Management (eds. Gulati, B.R., Khurana, S.K., Pal, Y., Kumar, R. and Vaid, R.K.), NRCE Publications, Hisar, pp.83-84.
4. Barua, S. Virmani, N. and Bera, B.C. 2009. Diagnosis of equine influenza. In: Compendium on Equine Influenza Diagnosis and Control (eds. Gulati, B.R., Kumar, R., Malik, P. and Vaid, R.K.), NRCE Publications, Hisar, pp.17-22.
  5. Barua, S. Bera, B.C. and Yadav, S.C. 2010. Collection and Dispatch of Biological Samples for Diagnosis of Infectious Diseases of equines. In: Compendium on Equine Health and Production Management (eds. Gulati, B.R., Khurana, S.K., Pal, Y., Kumar, R. and Vaid, R.K.), NRCE Publications, Hisar, pp. 17-21.
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# QRT, RAC, IRC & IMC Meetings

## Quinquennial Review Team Meeting

Quinquennial Review Team (QRT) for NRCE - under the chairmanship of Dr. J.M. Nigam - met three times between October 12, 2008 and July 14, 2009 to review the progress done by the Centre during 2003-2008. The QRT Chairman presented his report before IMC on July 14, 2009. The Chairman was of the view that number of animals available at Equine Production Campus, Bikaner should be increased for tangible research and sustainable equine production. Scaling up of frozen semen and artificial insemination for public use was also suggested. QRT emphasized that the time has come for the NRCE to fructify its research products and go beyond diagnostic services to draw up national programmes for long-term disease prevention, surveillance and disease management strategies. QRT was of the opinion that Veterinary Type Culture Centre has immense opportunities to collect and preserve nationally important



Director NRCE briefing NRCE achievements to QRT members

veterinary pathogens and vaccine strains. The QRT also emphasized the need to orient training programmes with defined objectives and course modules covering both equine health and production improvement.

## Actions taken on QRT recommendations

The Centre has initiated following actions on the recommendations of the QRT:

- a. For conservation of indigenous equine germplasm, a nuclear herd of 12 Zanskari ponies has been established at our Bikaner Campus. These animals were procured from their native herd at Ladakh in J & K during October 2009.
- b. NRCE has scaled up frozen semen and artificial insemination facility for farmers at its Hisar and Bikaner Campus for production of true-to-breed Marwari horses and superior quality mule production. More than 4000 frozen semen doses are being maintained for regular use.
- c. The Centre has initiated nation-wide programmes for surveillance and monitoring of diseases. This has helped in control of equine influenza in India during 2008-09 and containment of glanders outbreak of 2009 in Raipur (Chattisgarh). NRCE has also updated equine influenza vaccine incorporating recent EIV isolate (EIV/2008/Katra/H3N8).
- d. VTCC has been geared up for establishing repository of veterinary pathogens. A network project has been initiated to make collaborative repository of animal, dairy and veterinary microbes. A new laboratory building for VTCC is nearing completion.
- e. Based on the QRT recommendations, NRCE organized a short course for field veterinarians on 'Equine Influenza Diagnosis and Control' during August 31-September 5, 2009. In addition, a model training was conducted for equine stake holders at NRCE during March 23-30, 2009. More such training programmes will be taken up in future.
- f. The Centre has taken initiatives to gather information on equine husbandry practices. Equines in Rajasthan, Haryana, Uttar Pradesh, Himachal Pradesh and Jammu & Kashmir have been surveyed for attaining information on equine demography, husbandry practices and disease profile.
- g. Three scientists of the Centre have been nominated for advance international training in the area of bioinformatics (Dr Rajesh Kumar Vaid & Dr Sanjay Kumar) and biosecurity (Dr Sanjay Barua) under NAIP.





## XII Research Advisory Committee Meeting

The XII RAC meeting was held under the chairmanship of Dr. A.T. Sherikar, Former Vice-Chancellor, Maharashtra Animal and Fishery Sciences University, Maharashtra on May 26, 2009 to discuss various scientific, administrative and policy matters of NRCE related to research. Other members of RAC who attended the meeting included Col. (Dr.) B. Raut, Consultant to DRDO, Dr. S.N. Maurya, Ex. OSD & VC, (UPDDUPCVVAS), Dr. D.V. Rangnekar, Ex. Vice President, BAIF, Dr. R.K. Singh, Director, NRCE, Dr. Lal Krishna, ADG (AH), Sh. Shivlal R. Daga, Dr. Baldev R. Gulati, Principal Scientist.

The RAC reviewed the ongoing research projects in the area of equine production, health, extension and Veterinary Type Culture Centre and also approved 7 new research projects to be taken up by the Centre. The RAC desired that in view of

equine influenza outbreak in various parts of the country, the Centre should provide training on management of equine influenza for various stake-holders, including veterinary practitioners.

Dr. R.K. Singh, Director NRCE informed that this Centre is included as one of the sub-centre of 'AICRP on utilization of animal energy with enhanced system efficiency' to undertake research on use of equines in rotary mode systems and in agricultural operations. He also emphasized that more research proposals should be submitted for funding from external agencies. The Chairman RAC congratulated the Director and applauded the scientists of NRCE for their sincere contribution in the ongoing research programmes and other developmental activities of the Centre. He emphasized the need of extending the research benefits of the Centre to the society and also for commercialization of technologies being developed at the Centre. He also desired that basic data on equine nutrition and equine farming system need to be generated.

### Research Advisory Committee

- ❑ Dr A.T. Sherikar, Ex-VC, MAFSU, Mumbai
- ❑ Dr R.K. Singh, Director, NRCE, Hisar
- ❑ Col (Dr) B. Raut, Consultant DRDO, FRL, Chandigarh
- ❑ Dr R.C. Katoch, Ex-Dean, COVS, CSKHPKV, Palampur
- ❑ Dr S.N. Maurya, Ex-OSD & VC, (UPDDUPCVVAS), Mathura
- ❑ Dr D.V. Rangnekar, Ex-Vice President, BAIF, Pune
- ❑ Dr Lal Krishna, ADG (AH), ICAR, New Delhi
- ❑ Sh Shivlal R. Daga, Mumbai
- ❑ Sh Zavaray S. Poonawalla, Pune
- ❑ Dr B.R. Gulati, PS & I/c PME Cell, NRCE, Hisar

### New Research Projects Approved

- ❑ Molecular epidemiology and development of vaccine & diagnostics for equine influenza
- ❑ Studies on donkey lactoferrin and its therapeutic application
- ❑ Studies on four class II genes of major histocompatibility complex in donkeys
- ❑ Development of Enzyme Immunoassays for hormones for optimization of equine reproduction
- ❑ Draughtability studies and utilization of equine (mule and donkey) energy in agricultural operations including transport

## 30<sup>th</sup> Institute Management Committee Meeting

The 30<sup>th</sup> Institute Management Committee meeting of NRCE was held on 14<sup>th</sup> July 2009 under the chairmanship of Dr. R.K. Singh, Director. The Committee was apprised of QRT report by QRT Chairman, Dr J.M. Nigam. The IMC agreed to the recommendations of the QRT. The IMC also recommended for replacement of old research equipments and vehicles of the Centre. In addition, IMC agreed to the proposal of purchase of some need-based research equipments for the Centre.





## Institute Research Committee Meeting

The annual meeting of Institute Research Committee was held under the chairmanship of Dr. R.K. Singh, Director, NRCE, Hisar on August 17-18, 2009 to review the research achievements of the ongoing projects for the year 2008-09 and to consider new research project proposals. In the IRC, Dr. M.P. Yadav, Dr. G. Butchaiah, Dr. A.K. Gahlot, Dr. S.K. Aggarwal, Col B. Raut and Dr. Aves Khan were invited as external experts. The Chairman emphasized that time-line in the ongoing and new projects should be strictly adhered to and the results of each research project should be presented quantitatively. The technologies ready for transfer should be popularized through website, press, media and public-private interface. The Chairman also emphasized on sharing of resources for optimal use.



IRC meeting in progress

## 31<sup>st</sup> Institute Management Committee Meeting

The 31<sup>st</sup> Institute Management Committee meeting of NRCE was held on 18<sup>th</sup> January 2010 under the chairmanship of Dr. R.K. Singh, Director at NASC, New Delhi. The Committee approved the price for testing of various equine diseases and pregnancy diagnosis. The IMC also approved the construction for BSL-III facility for VTCC by seeking consultancy from HSCC, Noida. The IMC also recommended replacement of old research equipments and agreed to the proposal of engaging need-based contractual technical assistants in various research projects.



31st IMC meeting at NASC, New Delhi

### Institute Management Committee

- Dr R.K. Singh, Director, NRCE, Hisar
- Dr Lal Krishna, ADG (AH), ICAR, New Delhi
- The Director General, Deptt. of Animal Husbandary & Dairying, Govt. of Haryana, Panchkula
- The Director, Deptt. of Animal Husbandry, Govt. of Punjab, Chandigarh
- The Dean, COVS, Bikaner, Rajasthan
- Sh Shiv Lal Daga, Mumbai
- Sh Zavary S. Poonawalla, Pune
- Dr S.N. Singh Technical Manager, BIOVET, Bangalore
- The Finance & Accounts Officer, IASRI, Pusa Campus, New Delhi
- Dr B.K. Singh, Pr. Scientist, NRCE, Hisar
- Dr. S.K. Khurana, Sr. Scientist, NRCE, Hisar
- Dr. R.C. Sharma, Sr. Scientist, EPC, NRCE, Bikaner
- Dr. Rajender Kumar, Sr. Scientist, NRCE, Hisar
- Asstt. Admn. Officer, NRCE, Hisar





# Workshop, Seminar & Institutional Activities

## Interactive meet of Network Units of VTCC

The Interactive meet of "Network Units of Veterinary Type Culture Centre" was held at NASC Complex, New Delhi on January 8, 2010 under the chairmanship of Dr. Lal Krishna, ADG (AH). The meeting was attended by 22 delegates from 17 network units from all over the country. Addressing the delegates, Dr. Lal Krishna emphasized the need to develop animal microbial repository in our country for future use. He proposed to conduct quarterly group wise meetings and half-yearly Network Meeting.

Dr. R. K. Singh, Director, NRCE briefed the delegates about the salient aspects of Veterinary Type Culture Centre. He desired that that molecular characterization of isolates is important and we should characterize and fully sequence at least 3 genes of a microbe. It was also emphasized to develop protocols/SOPs for preservation procedures for different groups. It was decided in the meet that the followings will be technical coordinator for smooth functioning of the network centres:

- ❑ Dr. R. K. Singh, Director, NRCE, Hisar for Veterinary Pathogens
- ❑ Dr. D.N. Kamra, Principal Scientist, Animal Nutrition, IVRI, Izatnagar for Rumen Microbes
- ❑ Dr. Rameshwar Singh, Principal Scientist, Dairy Microbiology, NDRI, Karnal for Dairy Microbes.



Delegates attending the Network Meet

## Round Table Conference on Equine Influenza at Raipur

During Silver Jubilee Year celebrations, NRCE organized a Round Table Conference on "Current Equine Influenza Outbreaks" at Indira Gandhi Krishi Vishvavidhyalaya, Raipur (Chhattisgarh) on January 27, 2010. This was organized on the sidelines of XXIV Annual Convention of Indian Association of Veterinary Microbiologists and Immunologists

(IAVMI) and specialists in infectious diseases held during January 27-29, 2010. The Round Table was chaired by Dr. M.P. Yadav, Former Vice Chancellor, SVBPUAT, Meerut, Prof. P.K. Uppal (Technical Director, RWITC, Pune) and Prof. M.C. Sharma (Director, IVRI, Izatnagar) contributed as resource scientists. The conference was attended by scientists from various institutes,

officials from Remount Veterinary Corps and representatives of various Turf Clubs. Dr R.K. Singh, Director, NRCE made a presentation on "Equine Influenza and Vaccination Strategies: An Overview". He briefed the delegates about the epizootic of equine influenza in the country during 2008-09 and various biosecurity measures adopted to control the ailment.



Director NRCE addressing delegates in Round Table Conference





## Equine Health Camps

The Centre organized eleven Equine Health Camps, four in Haryana (Rajli on 6th August, 2009, Bharu Keda on 4th September, 2009, Beri on 26th September, 2009, and Julana 22nd February, 2010), six in Rajasthan (Rattangarh on 30th August, 2009, Shergarh on 1st September, 2009, Pushkar on 27-28th October, 2009, Jhalarpattan on 29-30th October, 2009, Hanumangarh on 17-19th February, 2010 and Tilwara on 7-9th March, 2010) and one in Punjab

(Mukatsar on 6-12th January, 2010). These health camps were organized in collaboration with state animal husbandry departments. The animals were clinically examined for their soundness and were treated on-the-spot for various ailments. The common clinical conditions observed were lameness, wound, colic, parasitic infestations, respiratory problem, retention of urine, etc. Anthelmintic drugs were administered for deworming of equids.



Equine Health Camps at Rajli (Haryana) and Tilwara (Rajasthan)

## Farmer Goshthies

During 2009-10, fifteen farmer goshthies, farmer interactive meets and equine husbandry awareness programmes were organized in Haryana, Rajasthan, Punjab and Uttar Pradesh states. In these activities, interactive question answer sessions were conducted to understand the problems of equine owners and to provide expert advice in the areas of equine health, feeding and management. NRCE also participated in animal fairs organized at various places of Haryana, Rajasthan and Punjab, and displayed technologies developed by the Centre. The equine owners were educated and literature related to equine husbandry was distributed among farmers during goshthis.



Farmer Goshthy at Sawai Madhopur

## Participation in Melas and Exhibitions

During the period under report, the Centre participated in animal fairs and exhibitions at Hanumangarh, Muktsar, Tilwara, Meerut, CIRB Hisar and NDRI Karnal. The farmers were informed about the latest developments in the area of equine husbandry. Technologies generated by NRCE were exhibited in these fairs. Feedback from the equine owners about the problems faced by them in rearing of equines was received and farmers were provided package of practices for equine management.



Dignitaries visting NRCE stall at CIRB, Hisar





## स्वतंत्रता दिवस समारोह उत्साह से मनाया

राष्ट्रीय अश्व अनुसन्धान केन्द्र ने 15 अगस्त 2009 को धूमधाम से स्वतंत्रता दिवस मनाया। इस अवसर पर डा. राजकुमार सिंह, निदेशक ने ध्वजारोहण कर स्वतंत्रता दिवस समारोह का शुभारम्भ किया। उन्होंने अधिकारियों एवं कर्मचारियों को स्वतंत्रता दिवस की शुभकामनाएँ देते हुए केन्द्र की गतिविधियों पर प्रकाश डाला और केन्द्र के विकास के लिए राष्ट्रनिर्माताओं एवं महापुरुषों द्वारा दिखाये गये मार्ग का अनुसरण कर पूरी तरह समर्पित होने का आह्वान किया। उन्होंने बताया कि इस वर्ष केन्द्र ने देश में अश्व फ्लू के निदान एवं रोकथाम में सराहनीय योगदान किया है तथा बीकानेर परिसर में विभिन्न स्वदेशी अश्वों की संख्या में वृद्धि की जा रही है।

इस अवसर पर केन्द्र में देशभक्ति से परिपूर्ण कविता पाठ एवं भाषण प्रतियोगिता का कार्यक्रम आयोजित किया गया।



स्वतंत्रता दिवस पर ध्वजारोहण करते हुए

## हिन्दी सप्ताह का आयोजन

केन्द्र में राजभाषा कार्यान्वयन समिति के तत्वाधान में 14-19 सितम्बर 2009 को हिन्दी सप्ताह का आयोजन 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 की अन्य संस्थाओं के कर्मचारियों ने बढ़-चढ़ कर भाग लिया। केन्द्र के निदेशक डा. राजकुमार सिंह ने अपने अध्यक्षीय भाषण में हिन्दी दिवस की बधाई देते हुए सभी अधिकारियों एवम् कर्मचारियों से अधिकाधिक कार्य हिन्दी में करने का आह्वान किया। सप्ताह के दौरान सुलेख, श्रुतलेख, प्रश्नोत्तरी, शब्दावली आदि प्रतियोगिता में केन्द्र के सभी वर्गों के कर्मचारियों ने भाग लिया। इसी श्रृंखला में आयोजित काव्य गोष्ठी में बतौर अध्यक्ष, हरियाणा के राज्यकवि उद्यभानु हंस की पंक्तियों ने समस्त श्रोतागणों का मन मोह कर भावविभोर कर दिया।

ज्यूँ कृष्ण का अनुराग है कालिंदी से,  
ज्यूँ नारी का सिंगार है सुलभ बिन्दी से,  
गंगा का हिमालय से है जो नाता अटूट,  
संबंध-वही है हिन्द का हिन्दी से।

काव्य गोष्ठी में हिसार के गणमान्य कवि गीतकार गज़लकार, श्री राधेश्याम शुक्ल जी, श्री रघुबीर अनाम, श्री सतीश कौशिक, श्री महेन्द्र जैन, श्री सुरेश भारती नादान, श्री नरेश शर्मा,

श्री ओम प्रकाश दिलबर ने अपनी रचनाओं को निराले अंदाज में प्रस्तुत कर श्रोताओं के दिल को छू लिया।

समारोह के समापन पर मुख्य अतिथि राज्यकवि उद्यभानु हंस जी ने विभिन्न प्रतियोगिताओं के विजेताओं को पुरस्कृत किया। कविता पाठ में जीवन बीमा निगम, हिसार के श्री नरेश शर्मा ने प्रथम, केन्द्रीय भैंस अनुसंधान संस्थान की श्रीमती शम्मी त्यागी और श्री संदीप कुमार ने क्रमशः द्वितीय तथा तृतीय स्थान प्राप्त किया। ट्रेक्टर ट्रेनिंग सेंटर के श्री नरेश दत्त शर्मा, केन्द्रीय भैंस अनुसंधान संस्थान के डा. सुधीर खन्ना तथा भारतीय संचार निगम के श्री केशा राम को सान्त्वना पुरस्कार से सम्मानित किया गया।



हिन्दी दिवस पर आयोजित काव्य गोष्ठी





## स्थापना दिवस समारोह

राष्ट्रीय अश्व अनुसंधान केन्द्र के स्थापना दिवस एवं रजत जयंती शुभारंभ समारोह के अवसर पर केन्द्र परिसर में 26 नवंबर 2009 को एक अश्व प्रदर्शनी एवं अश्व क्रीड़ा प्रतियोगिताओं का आयोजन किया गया। इस अवसर पर डॉ राजकुमार सिंह, निदेशक ने संस्थापक निदेशक प्रेम कुमार उप्पल, मुख्य अतिथि एवं सरदार वल्लभ भाई पटेल कृषि विश्वविद्यालय मेरठ के पूर्व कुलपति प्रो महेन्द्र पाल यादव, विशिष्ट अतिथि का स्वागत किया तथा केन्द्र की उपलब्धियों के बारे में जानकारी दी। समारोह को सम्बोधित करते हुए डॉ उप्पल ने कहा कि आज के बदलते पर्यावरण के परिवेश में अश्व रोग एवं उत्पादन सम्बंधित समस्याओं के समाधान के लिए नई



स्थापना दिवस पर आयोजित अश्व पालक संगोष्ठी



मुख्य अतिथि द्वारा अश्व प्रदर्शनी का अवलोकन

नीतियां निर्धारण करने की आवश्यकता है। डॉ यादव ने अश्व संरक्षण के क्षेत्र में केन्द्र के सराहनीय योगदान के बारे में अश्व पालकों को जानकारी दी। इन कार्यक्रमों में हरियाणा एवं राजस्थान के अश्व पालकों ने भाग लिया तथा संस्थान के कार्यों व अश्वों के पालन के लिए महत्वपूर्ण जानकारी प्राप्त की। आस-पास के गांवों के अश्वों एवं अश्वपालक बन्धुओं के कल्याण हेतु एक अश्व पालक संगोष्ठी का आयोजन भी किया गया। इस अवसर पर केन्द्र के अधिकारियों एवं कर्मचारियों ने पशुशाला, कृषि फार्म एवं रिहायशी क्षेत्र में पर्यावरण सुधार हेतु पौधारोपण भी किया।

## गणतंत्र दिवस पर रंगारंग कार्यक्रम

संस्थान के निदेशक डा. राजकुमार सिंह ने तिरंगा फहराकर गणतंत्र दिवस समारोह का शुभारंभ किया। उन्होंने वैज्ञानिकों एवं कर्मचारियों को गणतंत्र दिवस की शुभकामनाएं देते हुए अपने संबोधन में केन्द्र की उपलब्धियों का विवरण दिया। देशभक्ति एवं मानवीय मूल्यों पर जोर देते हुए निदेशक महोदय ने राष्ट्रीय उन्नति को सर्वोपरि मानते हुए अग्रसर रहने की प्रेरणा दी। इस अवसर पर केन्द्र के सभागार में बच्चों द्वारा देशभक्ति से परिपूर्ण रंगारंग कार्यक्रम का आयोजन भी किया गया।



गणतंत्र दिवस पर ध्वजारोहण कार्यक्रम







## Visit of Dignitaries

### Dr Sherikar visits NRCE

Dr. A.T. Sherikar, Former Vice Chancellor, Maharashtra Animal & Fishery Sciences University, visited the Centre on May 26, 2009 to Chair the XII RAC of the Centre. He reviewed the progress made by the Centre in R&D and expressed his satisfaction with the excellent work being done by the Centre in the area of equine disease diagnosis, control and in conservation of indigenous breeds of equines.



### Delegates from Sri Lanka visits NRCE

A 4-member delegation from Council for Agricultural Research Policy (CARP), Colombo, Sri Lanka visited Centre on October 1, 2009 under "Work Plan in the field of Livestock Production R&D". The delegates were apprised about the activities of the Centre in the area of equine production and health and role played by the Centre for improvement in equine husbandry.

### Dr Modayil visits Bikaner Campus

Prof. (Dr.) M.J. Modayil, Member, Agricultural Scientists' Recruitment Board, New Delhi visited Equine Production Campus, Bikaner, on March 12, 2010 and interacted with the Scientists and staff members. He delivered a lecture on "Personnel Management in ARS" which was attended by the scientist of all the ICAR Institutes viz, NRCE, NRCC, CSWRI, CAZRI and CIAH. He was impressed to see the true-to-breed germplasm of Marwari and Zanskari animals at the farm.



### Dr Pradhan reviews BSL-III construction

A meeting for pre-validation evaluation of the BSL-III laboratory was held at NRCE, Hisar on 5th December 2009. Dr. H.K. Pradhan, WHO (India) Consultant was invited by NRCE. Dr Pradhan reviewed the construction of the facility and he was satisfied with the quality of construction of BSL-III facility. He provided valuable inputs for completion of process of internal and external validation of the laboratory.





# Infrastructure & Developmental Activities

## Development of repository of Veterinary Type Cultures

Infrastructure for Veterinary Type Culture Centre is being developed at the Centre. The construction of first phase of laboratory building is nearing completion. A BSL-III facility for handling of hazardous animal microbes is to be established at VTCC for which, a provision of Rs 8.0 crore has been earmarked in the XI Plan.

The activities of VTCC entail reposition of veterinary pathogens from different sources, their characterization and preservation. Under this activity, different viral isolates including pox, herpes and rotaviruses have already been acquired from ICAR institutes/Veterinary colleges and deposited in the VTCC repository. Five buffalopox virus isolates and three camelpox virus (CMLV) isolates deposited in the VTCC repository have been authenticated. Furthermore three bovine herpes and four rotaviruses (bovine & human) are in the process of authentication. Twenty seven recombinant clones of phage display library of

single-domain antibodies of Indian desert camel have been deposited in the VTCC repository. Multiple copies of different virus isolates have been preserved in freeze dried form as well as at  $-70^{\circ}\text{C}$  for future use. The acquisition, authentication and preservation of different viruses along with the recombinant clones would be useful in establishing a national repository of the characterized viral isolates for future conservation and distribution.



VTCC laboratory under construction

## Land reclamation and development

NRCE campus at Hisar has the problem of salinity and water logging, making the land unfit for cultivation of fodder crops. During the year, thirty acres of saline land was reclaimed for cultivation of crops. The area was reclaimed to reduce the salinity by using gypsum, manure & refilling of sandy loam soil. Sheep manure was mixed in field and saline resistant varieties of oat, berseem & lucerne were sown in this area for production of feed and fodder.

About fifty acre of barren land was weeded out, harrowed & developed for future crop cultivation. Different species of plants were planted in this area to adopt agri-silvi-pasture system of agro-forestry. The unlined sewerage drain passing through the campus was covered with



Micro sprinkler system for irrigation in newly developed lawn.

RCC. A lawn was developed around it to solve the problem of water logging & seepage in the premises. About 1500 plants of different species were planted along the roadside and in farm area.

At Equine Production Campus Bikaner, a lawn in about 3.5 acres was developed and 600 saplings were planted for greening of the campus and to protect animals from harsh climatic conditions.





## Agricultural Farm Production

Crop production at Agriculture Farm, Hisar (2009-10)

Crop	Quantity (Qt.)
Green fodder	
Sorghum sudan grass	395
Maize+cowpea+pearl millet	187
Sorghum sudan grass+cowpea	156
Berseem	179
Lucerne	75
Oat	286
Total feed and green fodder	1278
Other produce	
Oat grain	127
Oat straw	205

During the period, a total of 1278 quintal of green fodder, 205 Qt. dry fodder and 125 Qt. of grains of different fodder crops were produced and supplied to animals at Hisar (Table). The efforts put in this activity, not only resulted in self-sufficiency of the centre in terms of fodder requirement, but surplus is being sold for revenue generation. At our Equine Production Campus, Bikaner, 1084 Qt. of green fodder was produced in about 10 acres of land.

Crop production at Agriculture Farm, Bikaner (2009-10)

Crop	Quantity (Qt.)
Lucerne	607
Oat	315
Bajra	162
Total green fodder	1084

## Livestock

The Centre has got a nucleus herd of Marwari, Kathiawari horses, Zanskari ponies and exotic donkeys at Hisar and Bikaner campuses (Table). The mules and ponies produced at the farm are also maintained by the Centre. The stallions at

Bikaner campus are used for collection and cryopreservation of semen for use in the artificial insemination. The frozen semen is used for superior mule production and propagation of indigenous germplasm in the field.

Herd strength at Equine Production Campus, Bikaner for the year 2009-10

Category	Sex	Horses		Ponies		Exotic donkeys	Mules	Total
		Marwari	Kathiawari	Zanskari	Others			
Stock as on 01.4.2009	M	11	01	-	-	11	03	82
	F	36	-	-	03	15	02	
Births during the year	M	06	-	-	-	03	-	12
	F	02	-	-	-	01	-	
Purchased during the year	M	-	-	04	-	-	-	12
	F	-	-	08	-	-	-	
Deaths during the year	M	01	-	-	-	01	-	05
	F	02	-	01	-	-	-	
Balance as on 31.3.2010		52	01	11	03	29	05	101

Herd strength at Main Campus, Hisar for the year 2009-10

Category	Horses		Non-descript ponies	Mules	Poitou Donkeys	Total
	Marwari	Others				
Adult Male	0	2	0	1	2	05
Adult Female	10	1	2	0	3	16
0-3 years	11	0	2	1	1	15
Total	21	3	4	2	6	36





## Personnel Milestones

### OUR NEW COLLEAGUES

- ❑ Dr. Taruna Anand joined as Scientist (Biotechnology) at Veterinary Type Culture Centre, on September 4, 2009.
- ❑ Dr. Ajay Kumar Raut joined as Scientist (Veterinary Extension Education) at this Centre on March 15, 2010.

### PROMOTIONS

- ❑ Sh. Subhash Chander, UDC, has been promoted as Assistant w.e.f. May 16, 2009.

### SELECTION/APPOINTMENT

- ❑ Dr Baldev R. Gulati, Senior Scientist selected as Principal Scientist (Veterinary Microbiology) at NRCE, Hisar on April 30, 2009.
- ❑ Dr R.S. Bansal, Farm Manager (Livestock) relieved from NRCE on September 2, 2009, subsequent to his selection as Senior Scientist (LPM), at Project Directorate on Cattle, Meerut Cantt. (UP).
- ❑ Sh Gopal Nath, SSS, selected as T-1(Driver) w.e.f. November 11, 2009.
- ❑ Smt Soma Devi joined as SSS on May 5, 2009.

### TRANSFERS

- ❑ Dr Yashpal, Senior Scientist from NRCE, Hisar to EPC, Bikaner on March 6, 2010.
- ❑ Sh Gopal Nath, T-1 from NRCE, Hisar to EPC, Bikaner on March 6, 2010.
- ❑ Sh Raghbir Singh, T-1 from EPC, Bikaner to NRCE, Hisar, March 6, 2010.

### STUDY LEAVE

- ❑ Dr Mamta Tigga, Scientist has been granted study leave for 3 years to pursue her Ph.D at College of Veterinary Sciences, Durg w.e.f. January 7, 2010.

- ❑ Dr Ramesh Dedar, Scientist has been granted study leave for 3 years to pursue his Ph.D at RAU, Bikaner w.e.f. April 6, 2009.
- ❑ Dr Prokasananda Bala, Scientist has been granted study leave for 3 years to pursue his Ph.D at NDRI, Karnal w.e.f. August 1, 2009.

### AWARDS AND RECOGNITIONS

- ❑ Dr Anju Manuja, Senior Scientist, was selected for Bharat Jyoti award by Indian International Friendship Society, New Delhi.
- ❑ Best oral presentation award on the article entitled "Recombinant antigen based ELISA for detection of antibodies against *Theileria equi* protozoa" authored by Kumar S., Kumar R., Gupta A. K. and Yadav S. C. presented in XX National Congress of Veterinary Parasitology organized by Dept. of Vet. Parasitology, CCShAU, Hisar w.e.f. Feb. 18-20, 2010.
- ❑ Dr. A. Arangasamy, Scientist has been selected for Boyscast Fellowship 2009-2010 by Department of Science and Technology, Ministry of Science and Technology, Government of India for undergoing 12-month specialized advanced training programme on Reproduction technology at Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, USA.
- ❑ Dr. Balvinder Kumar, Senior Scientist has been awarded Dr. V.D. Kashyap and Ms. Manju Utreja Gold medals for Ph.D research by CCS Haryana Agricultural University, Hisar.
- ❑ Drs. Sanjay Kumar, S. Barua and R. K. Vaid, Senior Scientists were awarded 3 month International training grant by National Agricultural Innovation Project (NAIP), ICAR, New Delhi.





# Staff at NRCE

**DIRECTOR : DR. R. K. SINGH**

## Scientists at NRCE, Hisar

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

## Scientists at EPC, Bikaner

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

## Scientists at VTCC, Hisar

1.	Dr Sanjay Barua	Senior Scientist
2.	Dr R. K. Vaid	Senior Scientist
3.	Dr Mamta Tigga	Scientist
4.	Dr B.C. Bera	Scientist
5.	Dr K. Shanmugasundaram	Scientist
6.	Dr Sarita Yadav	Scientist
7.	Dr Taruna Anand	Scientist

## Technical Staff at EPC, Bikaner

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

## Technical Staff at VTCC, Hisar

1.	Sh Manoj Kumar, T-3	Lab. Technician
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## Technical Staff at NRCE, Hisar

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

## Administrative Staff at NRCE, Hisar

1.	Sh R.A. Parashar	AFAO
2.	Sh Hawa Singh	AAO
3.	Sh Ram Pal	Assistant
4.	Sh S.P. Kaushik	Assistant
5.	Sh Subhash Chander	Assistant
6.	Sh Ashok Arora	Stenographer, Gr-III
7.	Sh Pratap Singh	LDC
8.	Sh D.D. Sharma	LDC
9.	Sh Om Prakash	LDC

## Administrative Staff at EPC, Bikaner

1.	Sh Mahender Singh	LDC
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## 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 Deepak Kumar
11.	Sh Satbir Singh	12.	Sh Hanuman Singh
13.	Sh Subhash Chander	14.	Sh Ishwar Singh
15.	Sh Ram Singh	16.	Sm. Ram Kali
17.	Smt Santra	18.	Sh Sant Ram
19.	Smt Soma Devi		

## Supporting Staff at EPC, Bikaner

1.	Sh Raju Ram
2.	Sh Mahabir Prasad





## List of Ongoing Research Projects

### Equine Health

	Title	Team	Date of Start	Date of Completion
1.	Development of vaccine(s) against equine herpes virus-1 infection.	B.K. Singh*, B.R. Gulati & N. Virmani	June, 2003	March, 2010
2.	Development of diagnostic tests for equine trypanosomosis (Surra).	Rajender Kumar*, Sanjay Kumar & S.C. Yadav	June, 2003	Dec, 2009
3.	Development of sensitive and specific diagnostic tests for detection of equine piroplasmosis	Sanjay Kumar*, Rajender Kr., A.K. Gupta & S.C. Yadav	May, 2004	Dec, 2009
4.	Studies on the improvement of the diagnostics for differentiation between EHV-1 & 4 infections employing molecular techniques	Nitin Virmani*, B. K. Singh & Baldev R. Gulati	May, 2004	March, 2010
5.	Development of diagnostics for <i>Rhodococcus equi</i> infection in foals	S.K. Khurana*, Praveen Malik	May, 2004	March, 2010
6.	Development of sensitive and specific diagnostics for Japanese encephalitis in equines	Baldev R. Gulati*, B.K. Singh, N. Virmani & H.S. Singha	Oct, 2006	March, 2010
7.	Surveillance, monitoring and control of emerging and existing diseases of equines	R.K. Singh*, B.K. Singh, S.K. Khurana, S.C. Yadav, Baldev R. Gulati, Rajender Kr, P. Malik, Sanjay Kumar, Nitin Virmani, Sanjay Barua, R. K. Vaid, A. Arangasamy & Ramesh Dedar	April, 1995	Continuous Service Project
8.	Cysteine proteinase, a defined antigen of <i>T. evansi</i> for control of trypanosomosis	S.C. Yadav*, Rajender Kumar, Sanjay Kumar & A.K. Gupta	Sept, 2008	Aug, 2010
9.	Role of TLR-9 for CpG immunomodulation in buffalo calves	Anju Manuja*	July, 2009	Jan, 2010
10.	Genetic and antigenic differentiation of equine influenza viruses	B.K. Singh*, Nitin Virmani, B. C. Bera, B. R. Gulati, K. Shanmugasundaram & R. K. Singh	Sept, 2009	Aug, 2012
11.	Development of diagnostics for equine influenza	Nitin Virmani*, Baldev. R. Gulati, Bidhan. C. Bera, B.K. Singh & R.K. Singh	Sept, 2009	Aug, 2012
12.	Evaluation and Updation of the inactivated equine influenza virus vaccine	R.K. Singh*, Baldev R. Gulati, Nitin Virmani, A.K. Gupta & B.K. Singh	Oct, 2009	Sep, 2012
13.	Diversity of Mx gene and association of polymorphic markers with susceptibility vis-à-vis resistance against Equine Influenza	Balvinder Kumar*, R.C. Sharma, Anju Manuja & R.K. Singh	Sept, 2009	Aug, 2012
14.	Studies on donkey lactoferrin and its therapeutics application	Sanjay Gupta*, A.K. Gupta, T.P. Singh, R. Kumar, R.K. Vaid, Anju Manuja & R.K. Singh	Sept, 2009	Aug, 2010





## Equine Production

Title	Team	Date of Start	Date of Completion
1. Molecular characterization of indigenous breeds of horse for genetic diversity within and between different breeds.	A.K. Gupta*, S.C. Gupta, S.N. Tandon, Mamta & Neelam Gupta	Oct, 2006	March, 2010
2. Cryo-preservation of embryo for conservation of Marwari Horse	A. Arangasamy*, T. Rao Talluri & R.K. Chaturvedi	April, 2008	March, 2010
3. Studies on class I and II genes of Major Histocompatibility Complex in donkeys	R. C. Sharma*, Balvinder Kumar & A.K. Gupta	April, 2010	March, 2013
4. Cryopreservation of equid semen using amides	A. Arangasamy*, T. Rao Talluri, Yash Pal, & Jitendar Singh	Sept, 2009	Aug, 2011
5. Draughtability studies and utilization of equine (mule and donkey) energy in agricultural operations including transport	Yash Pal*, R A Legha, P. A. Bala, N. Lal & A.K. Gupta	April, 2009	March, 2011

## Extension

Title	Team	Date of Start	Date of Completion
1. Studies on existing management system and utilization of donkeys and mules for sustainable livelihood	Niranjan Lal*, Yash Pal, R.A. Legha & R.K. Singh	Sept, 2009	Agu, 2011

## VTCC

Title	Team	Date of Start	Date of Completion
1. Isolation, maintenance and characterization of bacterial pathogens and their molecular identification	Rajesh Kumar Vaid*, Sanjay Barua, Mamta Tigga, K. Shanmugasundaram, & B.C. Bera	June, 2007	May, 2010
2. Isolation, molecular characterization and reposition of viruses of animal origin	R.K. Singh*, Sanjay Barua, B.C. Bera, R. K. Vaid, K. Shanmugasundaran, & Baldev R. Gulati	Sept, 2009	Aug, 2012





# Participation in Trainings, Conferences & Symposia

## Participation in Trainings

1. Dr S.K. Khurana, Sr. Scientist, participated in a two-day training programme on "Project Management" organized by Consultancy Development Centre, Core-IV-B, 2nd Floor, India Habitat Centre, Lodhi Road, New Delhi during May 27-28, 2009.
2. Dr Mamta Tigga, Scientist, Hisar participated in a short course on "Advances in molecular diagnosis of important bacterial diseases of animals" organized by Division of Bacteriology, IVRI, Izatnagar during September 15-24, 2009.
3. Dr A. Arangasamy and Dr T.R. Talluri, Scientists participated in 21-day training programme on "Genomic and Proteomic Tools for Animal Reproduction Research" organized by National Institute of Animal Nutrition and Physiology, Hosur Main Road, Adugodi-P.O., Bangalore (Karnataka) during November 10-30, 2009.
4. Dr Baldev Gulati, Dr. Nitin Virmani, Dr. Anju Manuja, Dr. Balvinder Kumar, Dr. Sanjay Barua, Dr. B.C. Bera, and Dr. Shanmugasundaram, K., participated in training on "Bioinformatics and its applications for Animal Sciences" at Bioinformatics Centre, College of Basic Sciences, CCS Haryana Agricultural University, Hisar, from August 1-7, 2009.

## Participation in Symposia and Conferences

1. Dr R.A. Legha, Sr. Scientist, participated in XI coordination committee Meeting held at MPUAT, Udaipur from June 15-16, 2009.
2. Dr R.K. Singh, Director, participated in National Conference of Indian Society for Study of Animal Reproduction (ISSAR), held at Central Institute for Research on Buffaloes, Hisar on June 27, 2009.
3. Dr Yash Pal, Dr R.A. Legha, and Dr T.R. Talluri, participated in "Interactive Meet on Buffalo Reproduction", organized by Central Institute for Research on Buffaloes, Hisar (Haryana) on June 27, 2009.
4. Dr R.K. Singh, Director participated and presented an expert lecture in "Indo-China Symposium on Buffalo Production", organized by Indian Society for Buffalo Development (ISBD) at Central Institute for Research on Buffaloes (CIRB), Hisar during October, 19-20, 2009.
5. Dr Baldev R. Gulati, Dr Praveen Malik, Dr R.K. Vaid, Dr Harisankar Singha, participated in "Indo-China Symposium on Buffalo Production", organized by Indian Society for Buffalo Development (ISBD) at Centre Institute for Research on Buffaloes (CIRB), Hisar during October 19-20, 2009.
6. Dr Nitin Virmani, Sr Scientist, participated and presented a paper entitled "Epizootic of Equine Influenza in India in 2008-09" in XXVI Conference of Indian Association of Veterinary Pathologists, organized by Department of Veterinary of Pathology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana during October 28-30, 2009.
7. Dr R.K. Vaid, Sr Scientist, participated and presented a paper entitled "Isolation Biochemical Characterization and Identification of *Rhodococcus equi* and *Corynebacterium* spp from Equines and Porcine Faeces" in VIII Conference of Indian Association of Veterinary Public Health Specialists, organized by ICAR Research Complex for NEH Region Sikkim Centre, Tadong, Gangtok (Sikkim) during November 6-7, 2009.
8. Dr R.K. Singh, Director, delivered a lecture entitled 'Present Status of Equines in India and Breeds Needing Conservation' in Model Training Course on "Conservation of Threatened Breeds of Livestock in India" held at NBAGR, Karnal, during November 16-23, 2009.
9. Dr Baldev R Gulati and Dr Sanjay Barua, participated in a WHO-sponsored Workshop on "Laboratory Biosafety and Biosecurity" at HSADL, Bhopal during November 17-19, 2009.
10. Sh K.S. Meena, Farm Manager and Sh. Manoj Kumar, T-3 participated in eminar on 'Energy Conservation in







- Agriculture and Domestic Sector' organized by Dakshin Haryana Bijli Vitran Nigam, Hisar during December 13-14, 2009.
11. Dr Rajender Kumar, Sr. Scientist, participated in one day National Conference organized by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi during January 15, 2010.
  12. Dr A.K. Gupta, Pr. Scientist, participated in XXIV Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases and International Conference on Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products, organized by College of Veterinary Science & Animal Husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during January 27-29, 2010.
  13. Dr B. K. Singh, Pr. Scientist, participated and presented a paper entitled "Immune response of equine herpes virus-1 vaccine in animals of an organized farm at Hisar (Haryana)" in XXIV Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases and International Conference on "Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products" organized by College of Veterinary Science & Animal Husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during January 27-29, 2010.
  14. Dr Baldev R. Gulati, Pr. Scientist, participated and presented a paper entitled "Japanese Encephalitis among Equines in India: Sero-prevalence, Virus Isolation, and Phylogenetic Analysis of Envelope gene" in XXIV Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases and International Conference on "Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products" organized by College of Veterinary Science & Animal Husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during January 27-29, 2010.
  15. Dr Praveen Malik, Sr. Scientist, participated and presented a paper entitled "Detection of Glanders in Chhattisgarh during-2009" in XXIV Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases and International Conference on "Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products" organized by College of Veterinary Science & Animal Husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during January 27-29, 2010.
  16. Dr Nitin Virmani Sr. Scientist, NRCE, Hisar participated and presented a paper entitled "Phylogenetic analysis of haemagglutinin gene of equine influenza viruses isolated during 2008-09 epizootic in India" in XXIV Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases and International Conference on "Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products" organized by College of Veterinary Science & Animal Husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during January 27-29, 2010.
  17. Dr A.K. Gupta, Pr. Scientist, participated in International Buffalo Conference on "Optimizing buffalo Productivity through Conventional and Novel technologies" organized by Central Institute for Research on Buffaloes, at New Delhi during February 01-04, 2010.
  18. Dr B.K. Singh, Dr S.C. Yadav, Dr Praveen Malik, Dr. Balvinder Kumar, Dr R.A. Legha,, participated in International Buffalo Conference on "Optimizing buffalo productivity through conventional and novel technologies" organized by Central Institute for Research on Buffaloes, at New Delhi during February, 1-4, 2010.
  19. Dr Anju Manuja, Sr. Scientist, participated and presented a paper entitled "Toll like receptor 9 gene expression in fibroblast cells derived from dermal tissue of buffalo (*Bubalus bubalis*)" in International Buffalo Conference on "Optimizing buffalo Productivity through Conventional and Novel technologies" organized by Central Institute for Research on Buffaloes, at New Delhi during February 01-04, 2010.
  20. Dr A.K. Gupta, Pr. Scientist, participated and presented a paper entitled "Phenotypic characterization of Bhutia and Zanskari equine breeds-Need for their





- conservation" in National Symposium on "Challenges to domestic animal biodiversity and action plan for its management and utilization" organized by Department of Animal Genetics and Breeding, College of Veterinary Sciences and Animal Husbandry, Anand Agricultural University, Anand (Gujarat) during February 10-11, 2010.
21. Dr Yash Pal, Sr. Scientist, participated in National Symposium on "Challenges to domestic animal biodiversity and action plan for its management and utilization" organized by Department of Animal Genetics and Breeding, College of Veterinary Sciences and Animal Husbandry, Anand Agricultural University, Anand (Gujarat) during February 10-11, 2010.
  22. Dr S.C. Yadav, Pr. Scientist, participated and presented a paper entitled "Identification of immunodominant *T. evansi* antigen by Western Blot Assay in chronic experimental infections" in XX National Congress of Veterinary Parasitology focal theme "Parasitology Today-Ecology to Molecular Biology" organized by Department of Veterinary Parasitology, CCS, HAU, Hisar during February 18-20, 2010.
  23. Dr Rajender Kumar, Sr. Scientist, participated and presented a paper entitled "Sero-prevalence of *Trypanosoma evansi* in equids of northern region of India using antibody-ELISA" in XX National Congress of Veterinary Parasitology focal theme "Parasitology Today-Ecology to Molecular Biology" organized by Department of Veterinary Parasitology, CCS, HAU, Hisar during February 18-20, 2010.
  24. Dr Sanjay Kumar, Sr. Scientist, participated and presented a paper entitled Recombinant antigen based ELISA for detection of antibodies against *Theileria equi* protozoa in XX National Congress of Veterinary Parasitology focal theme "Parasitology Today-Ecology to Molecular Biology" organized by Department of Veterinary Parasitology, CCS, HAU, Hisar during February 18-20, 2010.
  25. Dr Sanjay Kumar, Sr. Scientist, participated and presented a paper entitled Cloning and sequencing of 18s RNA gene of *Trypanosoma evansi* in XX National Congress of Veterinary Parasitology focal theme "Parasitology Today-Ecology to Molecular Biology" organized by Department of Veterinary Parasitology, CCS, HAU, Hisar during February 18-20, 2010.
  26. Dr Anju Manuja, Sr. Scientist, participated and presented a paper entitled "Indian isolates of *Theileria annulata* differ at molecular as well as antigenic level but show conserved protein profile" in XX National Congress of Veterinary Parasitology focal theme "Parasitology Today-Ecology to Molecular Biology" organized by Department of Veterinary Parasitology, CCS, HAU, Hisar during February 18-20, 2010.
  27. Sh Raj Kumar Dayal, T-3 (Lab Technician) NRCE, Hisar participated in XX National Congress of Veterinary Parasitology focal theme "Parasitology Today- Ecology to Molecular Biology" being organized by Department of Veterinary Parasitology, CCS, HAU, Hisar during February 18-20, 2010.
  28. Dr Sanjay Barua, Sr. Scientist, participated and presented a paper entitled "Molecular characterization of camelpox virus from outbreaks in Rajasthan in 2008-09" in XIX National Conference on "Recent trends in viral disease problems and management" organized by Department of virology, Sri Venkateswara University, Tirupati (A.P.) during March 18-20, 2010.
  29. Dr A.K. Gupta and Dr Rajender Kumar attended "ICAR Zonal Technology-Management and Business Planning and Development Meeting-cum-Workshop (North Zone-II), held at Indian Veterinary Research Institute, Izatnagar from March 26-27, 2010.





## List of Publications

### List of published papers

1. Arangasamy, A. 2010. Effect of stallion seminal plasma proteins on in vitro capacitation of equine spermatozoa. *Indian Journal of Animal Sciences* 80: 107-109.
2. Arangasamy, A., Bansal, R.S. and Singh, J. 2009. Quality assessment of frozen Poitu jack semen by Hos test and conception rate studies. *Indian Veterinary Journal* 86: 1138-1140.
3. Arangasamy, A., Singh, J., Bansal, R.S. and Tandon, S.N. 2009. Endometritis in a Marwari mare- a case study. *Indian Journal of Animal Reproduction* 30: 84.
4. Bansal R.S., Yash Pal, Pareek, P.K. and Gupta, A.K. 2009. Progesterone profile during various physiological stages in mares. *Indian Veterinary Journal* 86: 481-483.
5. Dey, S., Dwivedi, S.K., Malik, P., Panisup, A.S., Tandon, S.N. and Singh, B.K. 2010. Mortality associated with heat stress in donkeys in India. *Veterinary Record* 166: 143-144.
6. Khurana, K.L., Banerjee, D.P. and Gupta, A.K. 2009. Experimental immunization of rabbits with whole tick extract antigens of *Hyalomma anatolicum anatolicum*: Cross resistance against *H. dromedarii*. *Journal of Veterinary Parasitology* 23: 57-59.
7. Khurana, S.K. and Malik, P. 2009. Status of *Mycoplasma equigenitalium* among indigenous equines. *Indian Journal of Veterinary Research* 18: 17-19.
8. Khurana, S.K., Malik, P., Virmani, N. and Singh, B.R. 2009. Prevalence of *Rhodococcus equi* infection in foals. *Indian Journal of Veterinary Research* 18: 20-22.
9. Malik, P. and Kalra, S.K. 2009. Prevalence of Group C streptococci amongst equines in India. *Indian Journal of Animal Sciences* 79: 459-465.
10. Malik, P. and Kalra, S. K. 2009. Variability and protective efficacy of M protein of streptococci of equine origin. *Indian Journal of Animal Sciences* 79: 466-469.
11. Malik, P., Khurana, S.K., Singh, B.K. and Dwivedi, S.K. 2009. Recent outbreak of Glanders in India. *Indian Journal of Animal Sciences* 79: 1015-1017.
12. Roy, N., Nageshan, R. K., Pallavi, R., Chakravarthy, H., Chndran, S., Kumar, R., Gupta, A.K., Singh, R.K., Yadav, S.C. and Tatu, U. 2010. Proteomics of *Trypanosoma evansi* infection in rodents. *PLoS ONE* 5(3): e9796.
13. Sharma, R.C., Mehta S.C., Bansal R.S. and Pathak, K.M.L. 2009. PCR-RFLP profile of MHC-DRB3 class II genes in Marwari horses. *Indian Journal of Animal Sciences* 79: 1036-39.
14. Singh, B.R., Chauhan, M., Sindhu, R.K., Gulati, B.R., Khurana, S.K., Singh, B., Singh, H.S. and Yadav, R.P. 2010. Diseases prevalent in equids in India: a survey of veterinary practitioners. *Asian Journal of Animal and Veterinary Advances* 5: 1-10.
15. Virmani, N., Bera, B.C., Singh B.K., Shanmugasundaram, K., Gulati, B.R., Barua, S., Vaid, R.K., Gupta, A.K. and Singh, R.K. 2010. Equine influenza outbreak in India (2008-09): Virus isolation, sero-epidemiology and phylogenetic analysis of HA gene. *Veterinary Microbiology* 143: 224-237.
16. Yash Pal and R. A. Legha 2009. Seminal characteristics of Marwari stallions. *Indian Veterinary Journal* 86: 918-920.
17. Yash Pal, Legha, R.A. and Tandon, S.N. 2009. Comparative assessment of seminal characteristics of horse and donkey stallions. *Indian Journal of Animal Sciences* 79: 1028-2.

### Accepted research papers

1. Arangasamy, A., Singh, J., Sharma, R.C., Singh, R.K., Bansal, R.S., and Tandon, S.N. 2010. Ultrasonographic detection of foetal resorption and persistent corpus luteum in a Poitu jenny. *Indian Veterinary Journal*.
2. Arangasamy, A., and Bhure, S.K. 2010. SDS-Polacrylamide gel electrophoresis of Equid seminal plasma Proteins. *Indian Journal of Animal Reproduction*.
3. Bansal, R.S., Yash Pal and Pareek, P.K. 2009. Ultrasonographic imaging for early pregnancy diagnosis in mares. *Indian Journal of Animal Reproduction*.





4. Chugh, M., Gulati, B.R., and Gakhar, S.K. 2010. Monoclonal antibodies AC-43 and AC-29 disrupt the Plasmodium vivax development in Indian malaria vector *Anopheles culicifacies* (Diptera: culicidae). Journal of Biosciences.
5. Singh, B.R., Gulati, B.R., Virmani, N., and Chauhan, M. 2010. Outbreak of abortions and infertility in thoroughbred mares associated with waterborne *Aeromonas hydrophila*. Indian Journal of Microbiology.
6. Talluri, T.R., Arangasamy, A., Singh, J., Singh, R.K., and Tandon, S.N. 2010. Scrotal biometry in Marwari stallions. Indian Veterinary Journal.
7. Vaid, R.K. 2010. Prokaryotic Wealth. Current Science.

#### Research papers presented in conferences

1. Arangasamy, A., Talluri, T.R., Chaturvedi, R.K., and Singh, R.K. 2009. Synchronization of Marwari mares for induction of estrus using Prostaglandin F2 alpha. In: Proceedings of XXV Annual Convention & International Symposium on "Expanding the horizons of reproductive technologies for augmenting fertility in farm and pet animals in the global scenario. December 10-12th, Namakkal, Tamil Nadu.
2. Barua, S., Bera, B.C., Shanmugasundaram, K., Gulati, B.R., Malik, P., Nagarajan, G., Gnanavel, V., Gadhvi, S., Yadav, V., Bhanuprakash, V., Kakker, N.K., Singh, R.V., Sardarilal, Pathak, K.M.L., and Singh, R.K. 2010. Molecular characterization of Camelpox virus from outbreaks in Rajasthan in 2008-09. In: Proceedings of XIX National conference on "Recent Trends in Virological Disease Problems and management. Department of Virology Sri Venkateswara University, Tirupati from March 18-20, pp 21
3. Bera, B.C., Gnanavel, V., Barua, S., Shanmugasundaram, K., Gadhvi, S., Yadav, V., Nagarajan, G., Bhanuprakash, V., Gulati, B.R., Kakker, N.K., Malik, P., Singh, R.V. Sardarilal, Pathak, K.M.L. and Singh, R.K. 2010. Zoonotic Camelpox virus infection in India. In: Proceedings of International conference on Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products and XXIV Annual Convention of Indian Association of Veterinary Microbiologist, Immunologist and Specialist in infectious diseases, College of Veterinary and Animal Husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur from January 27 to 29, p 197.
4. Gulati B.R., Singha H., Singh B.K., Virmani N., and Singh R.K. 2010. Japanese Encephalitis among equines in India: Sero-prevalence, virus isolation, and phylogenetic analysis of envelope gene. In: Proceedings of International conference on Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products and XXIV Annual Convention of Indian Association of Veterinary Microbiologist, Immunologist and Specialist in infectious diseases, College of Veterinary and Animal Husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur from January 27 to 29, p88.
5. Gupta, A.K., Yash Pal, Tandon, S.N, Gupta, S.C., and Gupta, N. 2010. Phenotypic characterization of Bhutia and Zanskari breed of equines- need for their conservation. In: Proceedings of National Symposium on 'Challenges to domestic animal biodiversity and action plan for its management and utilization' held at Anand Agricultural University, Anand from February 10-11, p161.
6. Kumar R., Yadav, S.C., Kumar, S., and Khurana, S.K. 2010. Sero-prevalence of *Trypanosoma evansi* in equids of northern region of India using antibody-ELISA. In: Proceedings of XX National Congress of Veterinary Parasitology on "Parasitology today-ecology to molecular biology" organized by Dept. of Vet. Parasitology, CCSHAU, Hisar w.e.f. February 18-20, p. 19-20.
7. Kumar, S., Jangra, K., Kumar, R., and Manuja, A. 2010. Cloning and sequencing of 18S RNA gene of *Trypanosoma evansi*. In: Proceedings of XX National congress of Vety. Parasitology and Symposium on Parasitology Today-Ecology to Molecular Biology February 18-20, CCSHAU, Hisar, p.37.
8. Kumar, S., Kumar, R., Gupta, A. K., and Yadav, S. C. 2010. Recombinant antigen based ELISA for detection of antibodies against *Theileria equi* protozoa. In: Proceedings of XX National Congress of Veterinary Parasitology on "Parasitology today-ecology to





- molecular biology" organized by Dept. of Vet. Parasitology, CCSHAU, Hisar w.e.f. February 18-20, p. 37-38.
9. Malik, P., Singha, H., Khurana, S.K., Jain, S., Avasthi, V.S., and Singh, R.K. 2010. Detection of glanders in Chattisgarh during 2009. In: Proceedings of International conference on Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products and XXIV Annual Convention of Indian Association of Veterinary Microbiologist, Immunologist and Specialist in infectious diseases, College of Veterinary and Animal Husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur from January 27 to 29, p58
  10. Manuja, A., Kumar, B., Khanna, S., and Sethi, R.K. 2010. Quantitative expression of Toll Like Receptor 9 gene in PBMCs of buffalo calf following stimulation with CpG-ODN. In: Proceedings of International Buffalo Conference on 'Optimizing Buffalo Productivity Through Conventional and Novel Technologies.' February, 1-4, New Delhi, India, Vol II, pp 12-13
  11. Manuja, A., Singh, S.R., Malhotra, D.V., Sangwan, A.K., Kadian, S.K., Nichani, A.K. 2010. Indian isolates of *Theileria annulata* differ at molecular as well as antigenic level but show conserved protein profile. In: Proceedings of XX National congress of Vety. Parasitology and Symposium on Parasitology Today- Ecology to molecular Biology February 18-20, CCS HAU, Hisar. p.43.
  12. Manuja, A., Kumar, B., Yadav, P.S., and Sethi, R.K. 2010. Toll Like Receptor 9 gene expression in fibroblast cells derived from dermal tissue of buffalo (*Bubalus bubalis*). In: Proceedings of International Buffalo Conference on 'Optimizing Buffalo Productivity Through Conventional and Novel Technologies.' February, 1-4, New Delhi, India, Vol II, p 13.
  13. Rana, N., Raut, A.A., Khurana, S.K., Manuja, A. and Saini, A. 2010. Isolation and Characterization of *Escherichia coli* and *Salmonella* associated with healthy and diarrhoeic neonatal calves. In: Proceedings of International Buffalo Conference on 'Optimizing Buffalo Productivity Through Conventional and Novel Technologies.' February, 1-4, New Delhi, India.
  14. Shanmugasundaram, K., Abraham, M.J., and Lalitha, C.K. 2009. A case report-Tuberculosis in camel (*Camelus dromedarius*). 2009. In: Proceedings of XXVI Annual Conference of Indian Association of Veterinary Pathologist CL Davis Satellite Seminar on Advanced Descriptive Techniques- Ultrastructure, Cytology and Immunohistochemistry and international Symposium on "Philosophy of Disease Diagnosis through Morphological to Biomolecular Approaches and Core Theme Diagnostic Pathology" GADVASU, Ludhiana, Punjab, from October, 28-30 p186.
  15. Singh, B.K., Virmani, N., Gulati, B.R., and Singh, R.K. 2010. Immune response of equine herpes virus-1 vaccine in animals of an organized farm at Hisar (Haryana). In: Proceedings of International conference on Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products and XXIV Annual Convention of Indian Association of Veterinary Microbiologist, Immunologist and Specialist in infectious diseases, College of Veterinary and Animal Husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur from January 27 to 29 p 49.
  16. Singh, R.K., Manuja, B. and Gulati, B.R. 2010. Modern Trends in New Generation Vaccine Biotechnology. In: Proceedings of International Buffalo Conference on 'Optimizing Buffalo Productivity Through Conventional and Novel Technologies,' New Delhi. February 1-4, Vol I, p 76-84.
  17. Singh, R.K., Kumar, S., Kumar, R., and Malik, P. 2010. Climate change and its effect on animal health. In: Proceedings of International Buffalo Conference on 'Optimizing Buffalo Productivity Through Conventional and Novel Technologies', New Delhi. February 1-4, Vol-I, p 258-271.
  18. Talluri, T.R., Mutha Rao, M., Babu Rao, K., and Venu Gopal Naidu, K. 2009. "Studies on ejaculate characteristics and fertility in Ongole bulls" In: Silver Jubilee Annual Convention of The Indian Society for the Study of Animal Reproduction and International Symposium on "Expanding The Horizons of Reproductive Technologies for Augmenting Fertility In Farm and pet Animals In the Global Scenario" held at





- Veterinary College and Research Institute, Namakkal, Tamilnadu, India.
19. Talluri, T.R., Chandra Prasad, B. and Mallikharjuna Rao, CH "Dystocia due to a Cyclopa (cebocephalus) monster and its management in a buffalo – a case report" In: Silver Jubilee Annual Convention of The Indian Society For the Study Of Animal Reproduction And International Symposium on "Expanding The Horizons of Reproductive Technologies for Augmenting Fertility In Farm And pet Animals In the Global Scenario" held at Veterinary College and Research institute, Namakkal, Tamilnadu, India.
  20. Vaid, R.K., Tigga, M., Kumar, A., Shanmugasundaram, K., Bera, B.C. Virmani, N., and Barua, S. 2009. Isolation, biochemical characterization and identification of *Rhodococcus equi* and *Corynebacterium* spp. from equine and porcine faeces. In: Proceedings of National Symposium and VII Conference of Indian Association of Veterinary Public Health Specialist on "Trans-boundary zoonotic diseases: Challenges and strategies", ICAR Complex for NEH region, Sikkim Centre, Tadong, Gangtok, Sikkim from November 6 to 7, p 35
  21. Virmani, N., Singh, B.K., Bera, B.C., Gulati, B.R., Shanmugasundaram, K., Gupta, A. K. and Singh, R.K. 2009. Epizootic of equine influenza in India in 2008-09. In: Proceedings of XXVI Annual Conference of Indian Association of Veterinary Pathologist CL Davis Satellite Seminar on advanced Descriptive Techniques-Ultrastructure, Cytology and Immunohistochemistry and international Symposium on "Phylosophy of Disease Diagnosis through Morphological to Biomolecular Approaches and Core Theme Diagnostic Pathology", GADVASU, Ludhiana, Punjab from October, 28-30, p 174
  22. Virmani, N., Bera., B.C., Singh., B.K., Shanmugasundaram, K., Gulati., B.R., Gupta, A. K., and Singh, R.K. 2010. Phylogenetic analysis of Haemagglutinin gene of equine influenza viruses isolated 2008-09 epizootic in India. In: Proceedings of International conference on Protecting Animal Health: Facilitating Trade in Livestock and Livestock Products and XXIV annual convention of Indian association of veterinary microbiologist, immunologist and specialist in infectious diseases, college of veterinary and animal husbandry, Indira Gandhi Krishi Vishwavidyalaya, Raipur from January 27 to 29, p 89.
  23. Yadav S.C., Kumar, R., Kumar, S. and Gupta, A.K. 2010. Identification of immunodominant *Trypanosoma evansi* antigens by western blot analysis in chronic experimental infections. In: Proceedings XX National Congress of Veterinary Parasitology on "Parasitology today-ecology to molecular biology" organized by Dept. of Vet. Parasitology, CCSHAU, Hisar w.e.f. February 18-20, p 42-43.

#### COMPENDIA PUBLISHED

1. Compendium on Equine Health and Production Management (eds. Gulati, B.R., Khurana, S.K., Yashpal, Kumar, R. and Vaid, R.K.), NRCE Publications, Hisar, 2010.
2. Compendium on Equine Influenza Diagnosis and Control (eds. Gulati, B.R., Kumar, R., Malik, P. and Vaid, R.K.), NRCE Publications, Hisar, 2009.



प्रशिक्षण

हिसार के राष्ट्रीय अश्व अनुसंधान केंद्र में चल रहा है शोध कार्य

अब गधे-घोड़े जोतेंगे खेत



हिसार। अब खेती में ऊंट व बैल की जगह गधे-घोड़े हल खींचते नजर आएंगे। अक्सर बोझा ढोने के काम में इस्तेमाल किए जाने वाले गधे व खच्चरों को अब हल चलाने का प्रशिक्षण दिया जा रहा है। वैज्ञानिकों का मत है कि ये पशु खेती के कार्य में सफलता हासिल कर सकते हैं। खेती में अश्व प्रजाति के उपयोग पर हिसार स्थित राष्ट्रीय अश्व अनुसंधान संस्थान में रिसर्च किया जा रहा है। यहां देखा जा रहा है कि गधे, घोड़े व खच्चर किस प्रकार के हल व अन्य खेती के उपकरण चलाने में

सहायता सिंह

सक्षम रहेंगे तथा वे किस प्रकार की भूमि पर अधिक कार्य कर सकेंगे। पन्नासरी सिंह ने इस शोध को कर रहे वैज्ञानिक डॉ. यशपाल ने बताया कि भारत में अभी तक कृषि क्षेत्र में गधे व घोड़ों का प्रयोग नहीं किया जाता है। जबकि केन्या, नामीबिया व आर्जीका आदि देशों में गधों व घोड़ों का इस्तेमाल खेती के कार्यों में किया जाता है। डॉ. यशपाल ने बताया कि रिसर्च के लिए भोपाल स्थित केंद्रीय कृषि अभियांत्रिकी संस्थान से वॉन जैसे हल व गाड़ियां आदि आ रहे हैं। वहीं वरिष्ठ वैज्ञानिक डॉ. प्रवीण मलिक ने बताया कि शोध की शुरुआत के पीछे यह उद्देश्य रहा कि जो छोटे किसान ट्रैक्टर का ऊंट-बैल नहीं खरीद सकते उनके लिए

स्वास्थ्य प्रशिक्षण शिविर में हुआ अश्वों का उपचार



डॉ. आरके सिंह (दोसरे से दाएं) अश्वों का स्वास्थ्य जांच कर रहे हैं। डॉ. अश्वनी सिंह (बाएं) अश्वों का उपचार कर रहे हैं। डॉ. अश्वनी सिंह (दोसरे से दाएं) अश्वों का उपचार कर रहे हैं।

केन्या, नामीबिया व आर्जीका में होता है इनका खेती में उपयोग

खच्चर में विकसित की जाएगी प्रजनन क्षमता

अनुसंधान

अश्व अनुसंधान केंद्र में चल रहा शोध

अनुसंधान

अश्व अनुसंधान केंद्र में चल रहा शोध



बवंर फटाफट

गंधीयत की रोक के लिए निःशुल्क टीकाकरण

अनुसंधान

अश्व अनुसंधान केंद्र में चल रहा शोध

अश्व अनुसंधान का निरीक्षण

अनुसंधान

अश्व अनुसंधान केंद्र में चल रहा शोध

अश्व पालकों को जागरूक करने को अश्व अनुसंधान केंद्र गंभीर : डा. अंजू

कुल्लू, अश्व पालकों को जागरूक करने के लिए अश्व अनुसंधान केंद्र द्वारा एक कार्यक्रम का आयोजन किया गया। डॉ. अंजू ने कहा कि अश्व पालकों को जागरूक करने के लिए अश्व अनुसंधान केंद्र गंभीर है। डॉ. अंजू ने कहा कि अश्व पालकों को जागरूक करने के लिए अश्व अनुसंधान केंद्र गंभीर है।



अश्व रोग जांच व स्वास्थ्य परीक्षण कैम्प

हनुमानगढ़ (अनिल खंडू)। हनुमानगढ़ में अश्व रोग जांच व स्वास्थ्य परीक्षण कैम्प का आयोजन किया गया। डॉ. अश्वनी सिंह ने कहा कि अश्व पालकों को जागरूक करने के लिए अश्व अनुसंधान केंद्र गंभीर है। डॉ. अश्वनी सिंह ने कहा कि अश्व पालकों को जागरूक करने के लिए अश्व अनुसंधान केंद्र गंभीर है।

अश्व अनुसंधान केंद्र में चल रहा शोध

अश्वों को नहीं मनुष्य को भी खतरा

अश्वों को नहीं मनुष्य को भी खतरा

अश्वों को नहीं मनुष्य को भी खतरा

अश्वों को नहीं मनुष्य को भी खतरा

राष्ट्रीय अश्व अनुसंधान केंद्र

राजस्थान के परिवेश में गर्दनों पर शोध की योजना : सिंह

राजस्थान के परिवेश में गर्दनों पर शोध की योजना : सिंह



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