ICAR-NRCE

Annual Report 2016-17

भाकृअनुप–राष्ट्रीय अश्व अनुसंधान केन्द्र

ICAR-National Research Centre on Equines

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The achievements and activities of the Centre from April 2016 to March 2017 are presented in this report. Mention of trademark, proprietary product or firm in the report does not constitute an endorsement or rejection of other suitable products or firms.

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The American Horse (1999)

A monument to creativity, *The American Horse* was created by famed animal sculptor, Nina Akamu. The work was inspired, in part, by a work created by Renaissance master Leonardo da Vinci for the Duke of Milan in the late 15th century. The project was championed by Frederik Meijer in the late 1990's, resulting in two casts of the 24-feet monument—one for Meijer Gardens & Sculpture Park, Michigan and one for the city of Milan, Italy. In addition, to inspiration from Leonardo, Akamu was also inspired by the history of equine imagery and the study of horses.

Director's Foreword

From the humble beginning on 26 November 1985, National Research Centre on Equines has gained recognition as a premier institution of international stature. The strength that makes NRCE truly enduring and unique emanates from our commitment to improve health and productivity of equines. They are the basis of our growth and inspire us along every path. Our concerted efforts were directed to understand infectious diseases confronting equines to improve the sustainability of equine farming. The Centre is striving hard to make a difference in the lives of the landless and marginal farmers by providing diagnostic, advisory and consultancy services for augmenting equine productivity and utility in agriculture and transport. The research activities at the Centre continue to bridge the gap between basic biology and clinical applications thereby providing cutting edge translational research for improving the health and welfare of the equine population in the country.

The research endeavours of the Centre received major boost during the year through 38 ongoing research projects, including 14 externally funded projects. During the year, scientists published 58 high impact research papers in international and national peer-reviewed journals. In addition, 8 popular articles, 3 book chapters, 18 extension leaflets and 24 research abstracts were also published.

During the year, our efforts for the development of diagnostics, biologicals and therapeutics for major equine diseases continued in right earnest. An updated vaccine for equine influenza was released by DG ICAR and diagnostic kits for rhinopneumonitis and lateral flow assay (LFA) for piroplasmosis were developed. The technologies for vermicomposting of mule dung and semen cryopreservation were also transferred to stakeholders. Some of the newer diagnostic technologies in pipeline include LFA for trypanosomosis, LFA for pregnancy diagnosis, sELISA for equine influenza antigen, synthetic peptide ELISA for EHV1 and r-protein ELISA for glanders.

The Centre maintains nationwide vigilance on infectious equine diseases through an ambitious surveillance and monitoring programme. During the year, 143 new cases of glanders were detected and alert was issued to 9 states for elimination of positive animals. Consultancy services were provided for equine infectious anemia, glanders, equine influenza, EHV1, equine viral arteritis, contagious equine metritis, trypanosomosis, *Salmonella* Abortusequi and African horse sickness, generating revenue of Rs. 48.86 lakh during the year.

The Centre developed capacity for detection of EHV1 latency and neuropathogenicity during the year. In our endeavour to develop refined EHV1 vaccine, three novel adjuvants were evaluated for enhancing protective immunity and we also generated EHV1 bacterial artificial chromosome (BAC) with gE gene deletion for use as an attenuated vaccine. Using reverse genetics approach, a recombinant equine influenza virus having HA and NA genes of H3N8 in the backbone of H1N1 was generated for evaluation as vaccine candidate. Novel drug molecules against *Theileria equi* and trypanosomosis are being developed. Some of these molecules gave promising results in

experimental models and *in vitro* toxicity trials. Our scientists were actively involved in identifying and controlling the microbial agents causing neonatal foal mortality in equines. Mass spectroscopic analysis was done to explore novel proteins of therapeutic value in equine milk, which identified 9 and 8 unique proteins in horse and donkey milk, respectively. The work on assessment of risk factors for colic and development of therapeutic interventions against osteoarthritis using nano-delivery vehicles was initiated. In addition, for emergency preparedness and monitoring of exotic equine diseases, we focused on development of diagnostic competence for Venezuelan equine encephalitis and vesicular stomatitis during the year.

The work on phenotypic and genetic characterization of donkey breeds, Marwari horses and development of DNA typing for parentage testing is in the final stage. The technology for conservation of rarest of the rare horse by embryo cloning is being perfected and we have so far succeeded in production of cloned embryos till 16 to 32 cell stage. In order to improve the height at wither of Marwari animals, semen collected from elite stallions was cryopreserved and used for artificial insemination and these efforts resulted in significant improvement in newborn foals. Monoclonal antibodies were raised against equine chorionic gonadatrophin for development of LFA for pregnancy diagnosis in mares.

Significant progress has been made in the NCVTC culture collection during this year by accessioning 280 new microbes (23 rumen, 26 dairy and 231 veterinary microbes), increasing the total tally to 3219 microbial cultures. The 231 veterinary microbes accessioned during this period include 164 bacteria, 28 viruses, 29 bacteriophages and 10 recombinant clones. New bacterial isolates identified during the year include *Mannheimia caviae* & *Moraxella bovoculi* from sheep, *Lactococcus taiwanensis* from mule dung, *Aeromonas sobria, A. hydrophila, A. veronii*, *Shigella dysenteriae* and *S. sonnei* from poultry intestines. Bacteriophage isolated from *A. hydrophila* made to the cover page of *Journal of Basic Microbiology* during the year. A bacteriophage belonging to family *Myoviridae* isolated from river Ganges exhibited therapeutic value by eliminating infection of *Klebsiella pneumonia* in mouse model. Antiviral activity of SERCA inhibitor was evaluated against *Pestes-des-petits* ruminants virus.

Under lab-to-land programme, scientists interacted with equine owners by participating in three agricultural fairs and conducting 9 equine health camps and provided healthcare to more than 1000 equines. In addition, under *mera gaon mera* gaurav programme, 24 villages were adopted where scientists made 125 visits and conducted 54 interface meetings and 9 trainings, benefitting 1446 rural families. Our initiatives for development of veterinary diagnostic competence in the north-eastern region fructified with transfer of technology for diagnosis of influenza A, Japanese encephalitis and trypanosomosis to the end-users in the region.

I sincerely acknowledge the inspiring guidance, support and encouragement from Dr Triolochan Mohapatra, Secretary DARE and Director General, ICAR; Dr H. Rahaman, Ex-Deputy Director General (Animal Science) and Dr Joykrushna Jena, Deputy Director General (Animal Science). My thanks are also due to the Assistant Director Generals Dr Ashok Kumar (Animal Health), Dr B.S. Prakash (Animal Nutrition & Physiology) and Dr R.S. Gandhi (Animal Production & Breeding) and Principal Scientists (Dr Rajan Gupta, Dr Vineet Bhasin, Dr Jyoti Misri and Dr Neelam Gupta) at ICAR Headquarters for their continuous support.

The publication committee deserves special appreciation for the untiring efforts in compiling, editing and bringing out this afresh looking report in the record time. Many thanks are due to technical, administrative and supporting staff of the institute for their whole-hearted support in carrying out institute activities. I congratulate the devoted team of scientists at NRCE and NCVTC for the strong commitment they have shown to achieve excellence in the research and development. The assiduous efforts put up by the scientists to successfully shouldering various additional responsibilities for smooth functioning of this centre also deserve appreciations. I hope that the Annual Report will be useful for scientists, administrators, entrepreneurs, policy makers and stakeholders working in the field of equine production.

Tripathi

(B.N. Tripathi)

Executive Summary

Horses have been domesticated since prehistoric times and hold a special place in our history & culture. Horses remain preferred mean of transport in hilly and desert terrains of India. To cater to the needs of equine health and augment equine productivity in the country, Indian Council of Agricultural Research established National Research Centre on Equines (NRCE) on November 26, 1985 at Hisar (Haryana). ICAR-NRCE has contributed significantly in the area of diagnosis and control of equine infectious diseases. The Centre has developed diagnostics against various equine diseases like equine herpesvirus, equine rotavirus, equine influenza virus, Japanese encephalitis, equine infectious anemia, glanders, *Theileria equi and Trypanosoma evansi*, etc. In addition, vaccines for EHV1**,** equine influenza, and *Salmonella* Abortusequi have been developed by this centre. The Centre has also established National Centre for Veterinary Type Cultures (NCVTC) for acquisition, authentication, preservation, documentation and conservation of the microbial diversity of animal origin. The Centre has contributed significantly in conservation and characterization of Indian breeds of equines and even established nucleus herds of Marwari, Kathiawari, Zanskari and Manipuri breeds. Recognizing its achievements, ICAR conferred prestigious Sardar Patel Outstanding ICAR Institution Award to NRCE for excellence in research in 2015. During 2016-17, the Centre could make significant research contributions through 38 research projects, including 14 externally funded projects by DBT, DST, ICAR extra mural and OIE. The salient achievements of the Centre during 2016-17 are outlined below.

Nation-wide sero-surveillance of infectious diseases in equines is one of the main activities of NRCE that helps in monitoring the trends of infectious diseases in India. Out of 1588 equines from 8 states tested during 2016-17, 689 (43.38%) were seropositive for *Theileria equi*, 171 (10.76%) for EHV1, 74 (4.65%) for *Trypanosoma evansi,* 73 (4.59%) for Japanese encephalitis and 4 (0.25%) for equine influenza. None of the equines tested was positive for equine infectious anemia, glanders, brucellosis and *Salmonella* Abortusequi. NRCE is recognized as National Referral Centre for diagnosis of important equine infectious diseases by DAHD&F, Government of India for sports, trade and import-export. During the year, a total of 15651 equine samples were tested for various diseases. Out of 13035 equines, 143 were found positive for glanders, while 8 out of 1735 equines were positive for equine influenza. Emergence of glanders outbreak in newer regions is a cause of concern. Whole genome sequencing of two Indian *Burkholderia mallei* isolates revealed that the genome is 5.6 Mb comprising 4713 genes.

Activities were undertaken for surveillance of infectious diseases prevalent in north-eastern region (NER) of India and to augment the diagnostic competence of veterinary diagnostic laboratories in the region for diagnosis of influenza A, Japanese encephalitis and trypanosomosis. Out of 61 pig serum samples, 16 (26.2%) were positive for JEV antibodies. Antibodies to *T. evansi* were detected in 7 (8.97%) cattle and 3 (3.06%) pigs tested from NER. A hands-on training on collection and processing of samples and RT-PCR-based diagnosis of influenza A viruses in swine samples was imparted to laboratory personnel at Assam Agricultural University, Guwahati (AAU). Reagents and SOPs for diagnosis of influenza A viruses, Japanese encephalitis, *T. evansi* were also provided.

The incidence of equine herpesvirus myeloencephalopathy (EHM) has been on the rise world over during last one decade. Most EHV1 isolates causing EHM exhibit a single nucleotide polymorphism (SNP) in the DNA polymerase gene (ORF30) at position 2254 (A2254 to G2254). The A/G SNP analysis by real-time PCR revealed that 118 out of 198 equines (59.6%) were infected with non-neuropathogenic genotype (A2254), while 8 (4%) with the neuropathogenic (G2254) EHV1.

Latency is one phenomenon in equine herpesvirus infection, which leads to lifelong persistence of virus infection. The reactivation of virus from latency leads to

clinical infection. Detection of latency is very tedious process, as virus hides in few cells of trigeminal ganglion or lymphoid tissues. During the year, a real-time RT-PCR was developed to detect latent infection. Out of 113 horses screened, 59 (52.2%) were detected with latent infection.

The inactivated EHV1 vaccine developed by NRCE using indigenous EHV1 isolate has proven to be quite effective in preventing abortions. For improving vaccine, bacterial artificial chromosome (BAC) of EHV1 was generated and characterized by RFLP. In order to develop attenuated vaccine, a gE deletion mutant was developed employing two-step red recombination strategies following *En Passant* mutagenesis process. The gE-deleted BAC construct was confirmed by PCR and RFLP analysis. Further, the expression of recombinant proteins (gB, gD and gM) was optimized in sf9 cells. Recombinant proteins were purified and subjected to western blotting with EHV1 specific serum and all the three proteins reacted specifically.

In order to refine EHV1 vaccine, inactivated EHV1 virus was adjuvanted with three novel adjuvants (advax, montanide and CpG) and evaluated for protective efficacy in BALB/c mice. At 6 days post-challenge, the lesions were of lesser intensity in mice vaccinated with three formulations as compared to non-vaccinated mice. On histological scoring of lung lesions, the lesions were least severe in mice vaccinated with montanide-based vaccine, followed by CpG and advax vaccines. Virus shedding was observed in non-vaccinated and advax vaccinated mice till 6 day post-challenge, whereas in the montanide and CpG groups, the virus was detected till 3 dpc.

To develop refined vaccine for equine influenza, EIV generated by reverse genetics technique was grown by transfection in co-culture of MDCK and T293 cells. The virus was rescued from the culture supernatant, grown in chicken embryos, purified and inactivated for evaluation as vaccine candidate in BALB/c mice.

Development of ready-to-use diagnostics for equine infectious diseases is priority of the Centre. A lateral flow assay for detection of antibodies to trypanosomosis was standardized during the year. On testing 186 serum samples, the sensitivity and specificity of the LFA was found to be 96.31% and 100% vis-à-vis routine ELISA. The shelf life of LFA coated strip stored at 4°C was more than six months. Internal and external validation of the assay was also done during the year. Another LFA for trypanosomosis using recombinant flagellar antigen with nanogold particle is also being standardized.

To develop diagnostics for vesicular stomatitis (VS), a recombinant 393 base construct encoding five antigenic regions of VSV glycoprotein was synthesized and expressed in *E.coli*. The expressed protein reacted with specific antiserum to VS in dot-blot assay. A PCR has been standardized to detect VSV-NJ and VSV-IND using synthetic gene technology. Similarly, for Venezuelan equine encephalitis, a synthetic construct of 921 bases comprising multiple antigenic regions was expressed in *E.coli* and a PCR assay has been standardized to detect multiple serotypes affecting equines.

Neonatal mortality is a significant problem for the equine industry. The etiological investigation in equine farms from different geographic regions resulted in isolation of bacteria comprising *E.coli*, *Klebsiella pneumoniae* and *Salmonella* Enteritidis from neonatal foals. Colic and laminitis are the most common clinical problems of equines. The risk factors associated with colic were assessed and data from 300 horses from Rajasthan and Punjab revealed that sudden feed change and feeding of wheat straw were common causes of colic in this region.

The Centre is pursuing development of novel drugs and nano-formulations for various ailments. For tissue regeneration and osteoarthritis, nano-formulations using minerals and polymers were synthesized. The *in vitro* evaluation and cytotoxicity studies revealed the suitability of developed nanoformulations for evaluation in suitable animal models.

Five drugs affecting metabolic pathways essential for survival of *Trypanosoma evansi* (CPZ, CPA, Indatraline, SC-1and TZD) have been identified. These drugs exhibited considerable *in vitro* growth inhibition of trypanosomes and preliminary *in vitro* toxicity assays revealed that the drugs were not toxic even up to 50 times of effective drug concentration. Similarly, for equine piroplasmosis, novobiocin and harmaline were evaluated for organ toxicity in mice. Some organ dysfunctions were observed when novobiocin was used @ 100 and 200 mg/kg BW, whereas harmaline was found to be quite safe in mice experiments.

In order to develop antiviral therapeutics against *Pestes-des-petits* ruminants virus (PPRV), a library of

host cell kinases and phosphatase inhibitors was screened and sarco/endoplasmic reticulum Ca2+ATPase inhibitor (SERCA) was found to significantly inhibit replication of PPRV. SERCA inhibitor was found to impair late post-entry step of PPRV replication by interfering with the localization (transport) of the viral proteins from cytoplasm to the plasma membrane. The host-targeting agents (such as SERCA inhibitors) are of great clinical importance because they usually do not have a tendency in inducing antiviral drug resistance.

National Centre for Veterinary Type Cultures (NCVTC) activities include isolation, authentication and accessioning of bacteria and viruses of animal and poultry origin. During the year 2016-17, NCVTC accessioned 28 of 41 virus cultures received from various institutions, including duck plague virus, infectious bursal disease viruses and pigeonpox virus. Porcine respiratory viruses (influenza A, PCV2 and PCMV) were detected in pig samples from Chhattisgarh, Maharashtra and Assam by PCR and RT-PCR assays. The porcine circovirus 2 was isolated from positive samples by serial passaging in PK-15 cells.

In order to increase the biodiversity of bacterial cultures in NCVTC, a total of 164 bacteria were accessioned, making cumulative culture collection of 1201 bacteria of veterinary importance. Strains of bacterial isolates were checked by sequencing of 16S rRNA gene and preserved by cryopreservation before accessioning. Many new isolates have been identified during the year like *Mannheimia caviae* & *Moraxella bovoculi* from sheep, *Lactococcus taiwanensis* from mule dung, *Aeromonas sobria, A. hydrophila, A. veronii*, *Shigella dysenteriae* and *S. sonnei* from poultry intestines.

The microbial status of the mule dung samples (41) collected from Katra region was analyzed by isolation, molecular identification and metagenomic analysis. The prominent bacterial pathogen identified in mule dung were *Streptococcus* sp, *Enterococcus* sp, *Staphylococcus aureus* and *S. epidermidis, Enterococcus casseliflavus*, *E. faecium* & *E. mundtii*.

During the year, five bacteriophages from *Shigella* sp were characterized for biological activity, temperature and pH sensitivity. All the five phages were stable at temperature up to 55°C and pH range of 4-10. A bacteriophage belonging to family *Myoviridae* was isolated from *Pseudomonas alcaligenes* in river Ganga, which exhibited thermotolerant and pH tolerant characteristics. This phage was able to eliminate established infection of *Klebsiella pneumonia*, suggesting its therapeutic value. Bacteriophages of *Myoviridae* family were also isolated from equine cadaver affected soil and identified on the basis of 16s rRNA sequence analysis.

To conserve microbial diversity of north-eastern region, seven viruses [fowlpox virus (2), duck plague virus (4), pigeonpox virus (1)]; 14 bacteriophages (against *Salmonella enterica* Paratyphi, *S. enterica* Typhimurium, *Citrobacter freundii*) and 30 bacterial cultures from Guwahati were accessioned in the repository.

Equine Production Campus (EPC) Bikaner is undertaking research on equine production, genetics and breeding, reproduction, physiology and nutrition. Bikaner campus has well maintained herds of Marwari, Kathiawari, Zanskari and Manipuri horses and indigenous & exotic donkeys.

During the year, semen from Marwari, Manipuri and Poitou stallions was cryopreserved for use in artificial insemination (AI). The technology for cryopreservation was demonstrated at Equine Breeding Stud, Hisar. Sperm morphometry from the three indigenous breeds viz. Marwari, Zanskari and Manipuri was studied through computer-assisted semen analyzer (CASA). Survey of field equines in Rajasthan and Punjab revealed abortions (4.94%), repeat breeding (4.94%), cystic ovary (1.23%) and vaginal prolapse (1.23%) as major reproductive problems. In order to improve the growth of foals in Marwari breed, semen collected from elite stallions with good height at wither and true-to-breed phenotypes was used for AI in Marwari mares, which resulted in increase in body weight, body length and height at wither in the progenies.

In order to use embryo cloning for conservation of rarest of the rare horse, we explored the possibility of using the buffalo oocytes and somatic cells from horse for production of cloned embryos and to assess nucleo-cytoplasmic compatibility between these species. Horse skin fibroblasts and buffalo oocytes from slaughterhouse ovaries were fused and cultured to produce embryos. In six fusion experiments, cloned embryos had shown growth till 16 to 32 cell stage.

Monoclonal antibodies were raised against equine chorionic gonadatrophin to develop a lateral flow assay (LFA) for detection of pregnancy in mares. Hyper-immune serum was also raised against eCG for use in sELISA being used for pregnancy diagnosis. The endocrine hormones, FSH and PGF2α were estimated in the blood plasma of Marwari mares and fillies during different reproductive stages to determine underlying causes of fertility, infertility and sub-fertility.

Genetic characterization of donkeys of Rajasthan was studied using 20 microsatellites markers to estimate the genetic variability of Rajasthan donkey population. Marker HTG10 showed the highest number of observed alleles per locus (17), while ASB17 showed the lowest (2) with the 8.158 as mean number of alleles. The results suggest existence of enough genetic variation in the Rajasthan donkey population.

In an attempt to prepare concentrate mixture and total mixed ration from locally available feed ingredients, feeding trials of two concentrate mixtures [(i) 70% oats grain, 20% wheat bran & 10% groundnut cake and (ii) 80% barley grain, 10% wheat bran, 10% mustard cake] were conducted on Marwari stallions. The concentrate mixture containing GNC and oats grain were found to be better wholesomely in terms of digestibility.

Evaluation parameters of stress under draught are very important to derive critical upper limits of load, speed, time and distance of work in equines. Studies with 50 N and 100 N draught loads in donkeys and mules indicated that animals under trotting experienced sufficient physiological and biochemical stress, as exhibited by increased physiological, hematological and biochemical responses. There were significant increases in plasma lactate, urea, creatinine, creatine kinase, lactate dehydrogenase and liver enzymes (AST, ALT, GT, ALP).

Horse and donkey milk is considered to have proteins of therapeutic and cosmetic value. LC-MS/MS spectroscopic analysis identified a total of 212 and 211 proteins, out of which there were 9 and 8 unique proteins in horse and donkey milk, respectively. The identified proteins in each species were grouped based on their molecular functions, biological process, cellular component and protein class. The uncommon proteins identified in horse and donkey milk need to be explored further to identify their biological roles and molecular functions. Horse and mule dung can be converted to vermicompost, which is an excellent, nutrient-rich organic fertilizer for organic farming. The methodology for making vermicompost using dung from mules and horses was optimized and subsequently field tested in Katra region.

During 2016-17, the scientists of the Centre published 58 original research articles in international and national refereed journals. In addition, 8 popular articles, three book chapters, 18 extension leaflets and 24 research abstracts were published by the scientists. The scientists of the Centre presented papers in 31 different national and international conferences, seminars or symposia and also participated in 22 workshops and interactive meets in different parts of the country. Three scientists upgraded their skills by participation in national training programmes while one scientist (Dr Naveen Kumar) participated in three-month international training at University of Edinurgh, UK. Accolades and awards were bestowed to the scientists of the Centre during the year. Dr Anju Manuja was awarded 'Emerging Scientist Award' by Elsevier and Dr Baldev Gulati was conferred prestigious fellowship of Indian Virological Society. Dr Nitin Virmani cleared examination of ICVP and Dr Naveen Kumar received Societal Innovation Award for his work on Johne's disease. Dr Sanjay Ravi received 'Young Scientist Award' for his research presentation in ISSAR conference and Dr Anuradha completed postgraduate diploma in Technology Management in Agriculture with distinction.

Infrastructure development and upgradation was given priority during 2016-17. A new laboratory wing of NCVTC was dedicated to the nation by Dr Trilochan Mohapatra, DG ICAR. In addition, 35 acres of land was reclaimed, making a total of 140 acres under cultivation at Hisar campus. At Bikaner campus, guest house, animal quarantine shed and sick animal shed were inaugurated by Dr H Rahman, DDG (AS) and a stone statuette of Marwari horse was also installed in front of main building at Bikaner.

The centre extended equine welfare activities in different parts of the country by organizing equine health camps and interactive farmer meets to educate equine owners on various aspects of disease control and management. During the year, 9 equine health camps were organized in various parts of Haryana, Rajasthan and Uttarakhand. NRCE adopted 24 villages under 'My Village My Pride' scheme - *Mera Gaon Mera*

Gaurav. The scientists made 125 visits to these villages and conducted 54 interface meetings and 9 trainings, benefitting 1446 rural families. Awareness on different agricultural and animal husbandry practices and cleanliness were created through these visits. Feedback from farmers and equine owners was obtained for further research and development in equine health and production.

The Centre organized various activities under directives from Government of India. Yoga camp (13-21 June) to celebrate International Day of Yoga, Hindi Fortnight (14-26 September) to promote hindi, Sanitation Drive (2-16 October), Vigilance Awareness Week (31 October-5 November), Agriculture Education Day (3 December), National Productivity Week (12-18 February) were celebrated with great fanfare. Foundation Day of the Centre was celebrated on 26 November by organizing Scientists-Veterinarian Interface meeting. Other institutional activities organized were World Veterinary Day (30 April) and National Science Day (28 February).

Technology development, assessment and transfer to end-users are the mainstay activities of the Centre. During the year, rapid diagnostic kit for diagnosis of *Theileria equi* and r-protein-based ELISA kit for differentiation of EHV1/4 infection were developed. An updated vaccine for equine influenza was released by the DG, ICAR. The technologies transferred to end-users include ELISA kit for glanders to state diagnostic laboratories, diagnostics for Japanese encephalitis, trypanosomosis and equine influenza to north-eastern states, technology for semen cryopreservation & artificial insemination to RVC and vermicomposting from mule dung to stakeholders at Katra (J&K). The centre also offers paid consultancy and diagnostic services for important infectious diseases of equines. Under this programme, 8942 equine serum samples were tested for various infectious diseases, including 3806 for equine infectious anemia and 4935 for glanders. The centre generated revenue of Rs 107.81 lakh from its internal sources, including Rs.48.86 lakh from contractual diagnostic services and Rs.7.36 lakh from sale of farm produce.

Introduction

Horses have been domesticated since prehistoric times and hold a special place in our history and culture. Domestication of wild horses played a key role in the rise of larger human settlements and great civilisations. With the advent of modern means of transportation, utility of equines is decreasing resulting in decline in their population. Horses still remain preferred means of transport in hilly and desert terrains for the rural poor, nomadic tribes in the north, north-west and north-eastern parts of India. To cater to the needs of equine health and augment equine productivity in the country, Indian Council of Agricultural Research established National Research Centre on Equines (NRCE) on November 26, 1985 at Hisar (Haryana).

The main campus of NRCE has state-of-the art laboratories and facilities for undertaking research in areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry and biotechnology. The research activities are supported by centralized services such as animal and agriculture farms, experimental animal facility, microbial containment laboratory, AKMU cell, ATIC, library and Info-equine museum. Subsequently, Equine Production Campus (EPC) was established in 1989 at Bikaner to undertake research on equine production, genetics and breeding, reproduction, physiology and nutrition. Bikaner campus has well maintained herd of Marwari, Kathiawari, Zanskari and Manipuri horses and indigenous and exotic donkeys. The National Centre for Veterinary Type Cultures (NCVTC) was established in the year 2005 at NRCE, Hisar, for collection and preservation of microbes of animal origin and veterinary importance. Presently, the Centre is working through 18 network units spread throughout the country. Recognizing its achievements, ICAR-NRCE was conferred Sardar Patel Outstanding ICAR Institution Award by Hon'ble Prime Minister of India Shri Narendra Modi Ji on 87th Foundation Day of ICAR organized at Patna, Bihar on July 25, 2015.

MANDATE OF NRCE

- Basic and strategic research on equine health and production
- To provide advisory and consultancy services and capacity development

"Wherever man has left his footprints in the long ascent from barbarism to civilization, we find the hoof-print of a horse beside it"
– John Trotwood Moore

OBJECTIVES OF NRCE

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

SALIENT ACHIEVEMENTS

During past 31 years, NRCE has contributed significantly in the area of diagnosis and control of equine infectious diseases by providing state-of-the-art diagnostics and biologicals. The Centre is striving hard for conservation and characterization of Indian breeds of equines in the country and even established nucleus herds of representative breeds of equines in its Bikaner campus. Some of the achievements and accolades of the centre are listed below:

Development of diagnostics for equine diseases

The Centre has been recognized as National Referral Centre for diagnosis of important equine infectious diseases by Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, Government of India. The Centre has developed and refined diagnostics against various equine diseases:

- HERP kit for field diagnosis of equine herpesvirus 1 (EHV1) infection.
- COFEB kit for diagnosis of *Theileria equi*.
- A neutralizing monoclonal antibody-based diagnostic kit 'Equiherpes B-ELISA' for EHV1 antibody detection.
- A type-specific ELISA and real-time PCR for differentiation of EHV1 and EHV4 infections.
- Complement fixation and r-protein-based ELISA for diagnosis of glanders.
- A monoclonal antibody-based sandwich ELISA and RT-PCR for detection of equine rotavirus (ERV) from faecal samples.
- RT-PCR and real-time RT-PCR based assays for typing and diagnosis of equine influenza virus.
- A recombinant antigen based-ELISA for detection of antibodies to *Theileria equi*.
- An indirect ELISA using whole cell lysate antigen and PCR for detection of *Trypanosoma evansi*.
- ELISA and RT-PCR for diagnosis of Japanese encephalitis.
- A recombinant protein-based indirect ELISA for serodiagnosis of equine infectious anemia.

Development of vaccines and immuno-biologicals

- Inactivated EHV1 vaccine "Equiherpabort" using indigenous virus for prevention of abortions in mares.
- Inactivated equine influenza vaccine using indigenous isolate (A/equi-2/Ludhiana/87). The vaccine was updated in 2008-09 incorporating recent virus strain {A/eq/Katra-Jammu.06/08 (H3N8)}.
- Bacterin and outer membrane protein-based vaccine for *Salmonella* Abortusequi.
- Monoclonal antibodies against EHV-1, equine rotavirus, equine influenza, Japanese encephalitis and *Trypanpsoma evansi*.

Surveillance and monitoring of equine diseases in India

ICAR-NRCE is involved in nation-wide monitoring and sero-surveillance of important equine infectious diseases with a view to manage, control and eradicate diseases. Some of the salient achievements under seromonitoring include:

- India has gained OIE disease-free status for African horse sickness (AHS) in 2006 based on sero-monitoring data generated by NRCE,.
- Clinical cases of equine infectious anemia (EIA) have not been reported since 1997. Only two sero-positive cases (one mule from Uttarakhand in 2009 and one horse from Haryana in 2011)

were detected and culled. Control of EIA in India was possible due to timely diagnosis and implementing package of practices formulated by NRCE.

- Outbreaks of glanders in equines have been detected since 2006-07 from different states and control measures are being adopted for preventing their further spread.
- Effective control of equine influenza outbreak of 1987 (involving 83000 equines) was done by implementing biosecurity and development of effective vaccine. Similarly, a major outbreak of equine influenza that spread in 13 different states of India during 2008-09 and caused huge mortality and economic losses, was timely diagnosed and controlled in collaboration with state animal husbandry departments.

Characterization of equine pathogens

- Nucleic acid sequencing of HA, M, M1 and M2 genes of equine influenza virus (EIV) isolates from 2008 outbreak (A/eq/Jammu-Katra/08, A/eq/Mysore/08 and A/eq/Ahmedabad/09) revealed clustering of Indian and Chinese isolates in a separate cluster designated as "Asian clade" and vaccine updated accordingly.
- Sequencing of VP7 gene of equine rotavirus isolates indicated circulation of G10, G3 and G6 serotypes in India.
- Whole genome sequence analysis of Japanese encephalitis virus isolated from an equine indicated virulent strain of genotype 3 is causing the disease in equine.
- The *in-vitro* cultivation of *T. evansi* and *Theileria equi* was successfully established.
- Experimental mouse models for equine influenza and equine herpesvirus 1 infections developed.

Phenotypic and genotypic characterization of Indian equine breeds

- Six equine breeds namely, Marwari, Kathiawari, Spiti, Zanskari, Bhutia and Manipuri, have been characterized on the basis of their biometric indices and coat colour.
- High genetic diversity observed between Spiti and Thoroughbred, followed by Spiti and Kathiawari while Zanskari and Manipuri are the least differentiated.
- Indian breeds form three distinctive clusters based on Bayesian analysis: (a) Kathiawari; (b) Zanskari, Spiti & Manipuri ponies and (c) Bhutia.

Establishment of nucleus herd

ICAR-NRCE has initiated *in-situ* conservation programme in the form of developing an equine sanctuary at EPC, Bikaner where nucleus herds of different Indian horse breeds are being maintained:

- Marwari horses from Rajasthan; Kathiawari horses from Gujarat; Zanskari ponies from Zanskar valley (Jammu & Kashmir) and Manipuri ponies from Imphal (Manipur) and herds of indigenous and exotic donkeys are being maintained:
- Small grey and large white donkeys for conservation and improvement of donkeys.
- Poitou donkey herd for improvement of indigenous donkeys and for production of superior mules.

Improvement in production potential of equines

- In order to conserve the germplasm of indigenous equine breeds, cryopreservation of semen of Marwari, Zanskari and Manipur stallions and Poitou donkeys has been done.
- Artificial insemination using frozen semen has been perfected for production of superior quality horses, mules and donkeys.
- An eCG based sandwich ELISA has been developed for pregnancy diagnosis between days 30 to 150 of gestation in mares.
- Pregnancy diagnosis between days 14 and 18 post-insemination has been perfected using ultrasonography in donkey and horse mares.


Marwari


Kathiawari


Zanskari

- Donkey fibre has been used to produce carpets by mixing with sheep fibres (40:60).

Utilization of equine energy in agricultural activities

- Single animal drawn matching plough, seed drill (two furrow) and harness have been designed and developed for donkeys and mules for agricultural operations like ploughing and sowing.
- The mules have been used for chaff cutting operation with average output capacity of 660 kg/hour of chopped bajra straw in rotary mode chaff cutter.
- The technique of vermicomposting of equine dung has been optimized for use in agricultural fields.

Patents granted

- A method for preparation of a diagnostic kit useful for forecasting equine herpesvirus-1 disease (Patent No. 55E4-1891278 dated 25.10.2003).
- A method for preparing complement fixation test based (COFEB) kit for diagnosis of *Babesia equi* infection of equines (Patent No. 196690 dated 31.07.2009)

Patents filed

- A highly sensitive kit for detection of antibodies against *Theileria equi* in serum of equids. Application No. 2763/DEL/2012 dated 06.09.2012
- Nano-drug delivery for quinapyramine sulphate. Application No.2560/DEL/2011, dated 06.09.2011.
- Polynucleotide sequence, processes, composition and methods thereof- Application No. 1575/CHE/2010 and PCT/IB 2011/052475.
- A recombinant haemagglutinin domain-containing protein for the detection and diagnosis of glanders and method of preparation thereof. Application No. 1328/DEL/2010 dated 08.06.2010.
- Recombinant *Tss*A protein for detection of antibodies against *Burkoholderia mallei* and uses thereof. Application No. 3610/DEL/ 2015.
- Recombinant *Hcp*1 protein for detection of antibodies against *Burkoholderia mallei* in Equines. Application No. 4120/DEL/ 2015.

Services

ICAR-NRCE provides following services to the farmers and equine breeders:

- Disease diagnostic services for various infectious and non-infectious diseases to equine owners, breeders, state animal husbandry departments, police and army horses.
- Surveillance, monitoring and control of equine infectious diseases in India.
- Health certification for movement of equines within and outside the country to promote export of horses.
- Clinical and diagnostic (including pregnancy diagnosis) services for equine diseases.
- Artificial insemination to augment the production of superior quality horses, mules and donkeys.
- Provision of quality jacks and jennies to various states, breeding societies and farmers, for production of superior quality mules and donkeys.
- Onsite and online consultancy in equine health and production, including toll-free telephonic advisory at Hisar and Bikaner campuses for farmers and stakeholders.
- Trainings and supply of education materials for equine management, production and health.
- Education and awareness of equine farmers by organization of health camps, awareness campaigns and farmers meets in different areas of the country.


Manipuri


Spiti


Bhutia

NATIONAL CENTRE FOR VETERINARY TYPE CULTURES

National Centre for Veterinary Type Cultures (NCVTC) initiated its activities in 2005 for conservation of the microbial diversity of animal origin. The activities comprise acquisition, authentication, preservation, documentation, and repository database management system of animal microbes. A network programme was started in 2010 with its 19 units located in 12 different states viz., Haryana, Rajasthan Uttar Pradesh, Himachal Pradesh, Assam, Jammu & Kashmir, Tamil Nadu, Gujarat, Uttarakhand, Karnataka, Arunachal Pradesh and Nagaland. These network units are contributing in conservation of animal microbial diversity in three specialized areas: veterinary microbes at NRCE Hisar, dairy microbes at NDRI, Karnal and rumen microbes at NIANP, Bengaluru.

At present, NCVTC repository is maintaining a total of 3219 accessioned microbes, including veterinary pathogens (n=2332), rumen microbes (n=354) and dairy microbes (n=533). The year-wise progress in culture collection can be seen in Table 1.

Table 1. Year-wise accessioning of microbial cultures in NCVTC

Type	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	Total
Vet. Microbes									
Bacteria	-	255	185	187	73	227	110	164	1201
Virus	24	68	11	21	11	21	14	28	198
Bacteriophage	-	-	-	-	13	19	44	29	105
Recombinant clone	34	76	81	76	59	140	45	10	521
Phage library	27	-	-	-	-	-		-	27
Genomic DNA	-	-	-	138	38	47	57	-	280
Total	**85**	**399**	**277**	**422**	**194**	**454**	**270**	**231**	**2332**
Rumen microbes									
Anaerobic bacteria	-	-	13	60	28	41	74	23	239
Fungi/Yeast	-	-	76	11	17	3	-	-	107
Meth. Archae	-	-	-	-	8	-	-	-	8
Total	**-**	**-**	**89**	**71**	**53**	**44**	**74**	**23**	**354**
Dairy microbes									
Bacteria	40	78	89	100	125	36	39	26	533
Grand Total	125	477	455	593	372	534	383	280	3219

MANDATE OF NCVTC

- National repository of veterinary, dairy and rumen microorganisms and their identification, characterization and documentation.
- Distribution of microbes for teaching, research and development of new technologies.

Some of the salient achievements of NCVTC are listed below:

Veterinary Microbes

- First laboratory confirmed camelpox virus zoonosis.
- First report on isolation and genetic characterization of swinepox virus from India.
- Accessioning of vaccine strains of viruses *viz.*, Peste des petits ruminants virus, Sheeppox

OBJECTIVES OF NCVTC

- Exploration and collection of microorganisms of animal origin/significance/relevance;
- Central storage of animal microbes from existing culture collection centres, institutions and universities;
- Characterization, documentation and digitization of microbial database of cultures of animal microbes;
- Development of a National Microbial Gene Bank for conserving the biodiversity of animal microbes;
- Conservation (both short-term and long-term) and utilization of microorganisms.

(Srinagar strain), Goatpox virus (Uttarkashi strain), Orf virus (Mukteswar strain), NDV (R2B strain) and NDV (F strain).

- Complete genome sequencing of two isolates of Classical swine fever virus.
- First isolation and characterization of *Bordetella bronchiseptica*, *Actinobacillus equilli*, *Staphylococcus hyicus*, *Trueperella pyogenes.*
- Whole genome sequencing of *Pasteurella multocida* sub sp. *multocida* B:2 serotype.
- First isolation and identification of *Moraxella* (*Branhamella*) *ovis* from ovine keratoconjunctivitis in sheep and methicillin-resistant coagulase negative *Staphylococcus sciuri* from goats.
- Whole genome sequencing of *Trueperella pyogenes, Bordetella bronchiseptica, Pasteurella multocida, Actinobacillus equuli* and *Salmonella* Gallinarum.
- Accessioning of rare strains of bacteria: *Campylobacter* sp., *Bacillus megaterium, Enterococcus casseliflavus, E. cecorum, Barrientosiimonas humi, Corynebacterium amycolatum, Enterococcus devriesei, E. hirae, E. faecium, Nocariopsis alba, Ignatzschineria larvae* and *Escherichia hermanii.*
- Isolation of bacteriophages against a variety of pathogenic bacteria were added to NCVTC repository, including a novel thermotolerant bacteriophage isolated from Ganga river water.

Rumen Microbes:

- Isolation and characterization of seven tannin degrading bacteria-*Streptococcus gallolyticus* from goat, fibre degrading bacteria *Ruminococcus flavefaciens, Prevotella* sp. and *Butyrivibrio* sp. from buffaloes and cattle, and nitrate reducing and cellulose degrading *E. coli* from buffalo.
- Isolation of rumen fungi - *Anaeromyces* sp., *Orpinomyces intercalaris* and *Orpinomyces joyonii* from buffaloes; *Piromyces* sp. and *Neocallimastix* sp. from goats.

Dairy Microbes:

- Preservation of dairy microbes, viz, *Lactobacillus* sp., *Lactococcus* sp., *Lactococcus lactis* ssp. lactis, *Lactococcus lactis*. ssp. Cremoris, *Lactococcus lactis* ssp. lactis bv. Diacetylactis, *Streptococcus thermophilus*, *Leuconostoc* sp., *Bifidobacterium* sp. *Bifidobacterium dentium*, *Bifidobacterium longum, Micrococcus* sp., *Kluyveromyces lactis* and *Saccharomyces bisporus*.
- Combination of *L. lactis* ssp lactis-C12 and *Leuconostoc mesenteroides* ssp. mesenteroides is very suitable for curd and buttermilk preparation.
- Six *Lactobacillus* sp. having phytase degrading potential and strong antifungal activity have been isolated form milk-cereal fermented products (Rabadi samples).
- An amylytic strain of *Pediococcus acidolactici* isolated has potential as starter culture in preparation of milk-cereal fermented products.

Rhodococcus rhodochrous

Serratia marcescens

Bacillus subtilis

LANDMARK ACHIEVEMENTS

Year	Achievement
1985	Foundation of NRCE, Hisar
1987	Detection of first outbreak of equine influenza in northern India
1989	Establishment of Equine Production Campus, Bikaner
1990	Import of Poitou donkeys from France
1995	Cryopreservation of Jack semen for AI
1996	Establishment of a herd of Marwari horses
1996	Crystal structure of mare milk lactoferrin
1996	Production of carpet fabric by blending of donkey and sheep hair
1997	Release of inactivated equine influenza vaccine
2003	Award of Indian patent to HERP kit for diagnosis of EHV1 infection
2005	Development of mAb-based sELISA for detection of rotavirus
2005	Establishment of National Centre for Veterinary Type Cultures (NCVTC)
2006	Collection and cryopreservation of stallion semen at farmers' door
2006	Detection of outbreak of Glanders in equines
2008	Detection of second outbreak of equine influenza
2008	Release of 'Equiherpes B-ELISA' kit for EHV1 diagnosis
2008	Release of 'Pregmare kit' for pregnancy diagnosis in mares
2009	Establishment of a herd of Zanskari ponies
2009	First report of Camelpox zoonosis
2010	Re-emergence of a case of equine infectious anemia
2010	Cryopreservation of Zanskari Stallion semen
2011	First report of Buffalopox virus causing concurrent disease in cow, buffalo and human
2011	Whole genome sequencing of Japanese encephalitis virus isolated from a horse
2011	Whole genome sequencing of *Pasteurella multocida* B:2 strain
2011	Establishment of a herd of small grey & large white indigenous donkeys
2012	Organization of SAARC trainings on equine piroplasmosis under OIE Twinning Programe
2012	Quinapyramine sulfate nanoformulation developed against *Trypanosoma evansi*
2012	Development of r-protein based ELISA for equine infectious anemia
2012	Whole genome sequencing of *Bordetella bronchiseptica*, *Pasteurella multocida*, *Actinobacillus equuli*, *Salmonella* Gallinarum
2012	Technique for vermicomposting using equine dung optimized
2013	Establishment of Microbial Containment Laboratory (BSL-3)
2013	Establishment of ATIC and info-Equine Museum
2014	Development of r-protein based ELISA for diagnosis of *Burkholderia mallei*
2014	Development of r-HSP70 based ELISA for *Trypanosoma evansi* infection
2015	NRCE conferred Sardar Patel Outstanding ICAR Institution Award
2015	Release of 'Equiherpabort vaccine' for prevention of EHV1 abortions in mares
2015	Release of r-protein based *Theileria equi* antibody detection kit
2015	Whole genome sequencing of classical swine fever virus
2016	Organization of SAARC trainings on equine influenza and Glanders under OIE Twinning Programe
2016	Release of updated equine influenza vaccine
2016	Methodolgy for isolation of RNA virus from mixed infection developed
2017	Establishment of a herd of Kathiawari horses

SUMMARY OF EXPENDITURE & REVENUE GENERATION

Rs in Lakh

Summary of Expenditure		
Non-Plan	**2015-16**	**2016-17**
Establishment charges including LSP/PF, wages, OTA	700.46	782.51
Traveling allowances	3.99	4.00
Others charges including equipments & recurring charges	374.65	426.96
Works	0.00	0.00
Total Non -Plan Expenditure	**1079.10**	**1213.47**
Plan	**2015-16**	**2016-17**
Establishment charges including LSP/PF, wages, OTA	0.00	0.00
Traveling allowances & HRD	12.00	9.48
Others charges including equipments & recurring charges	614.70	486.09
Works	54.71	53.92
Total Plan Expenditure	**681.41**	**549.49**
Total Expenditure (Plan, & Non Plan)	**1760.51**	**1762.96**

In Rs

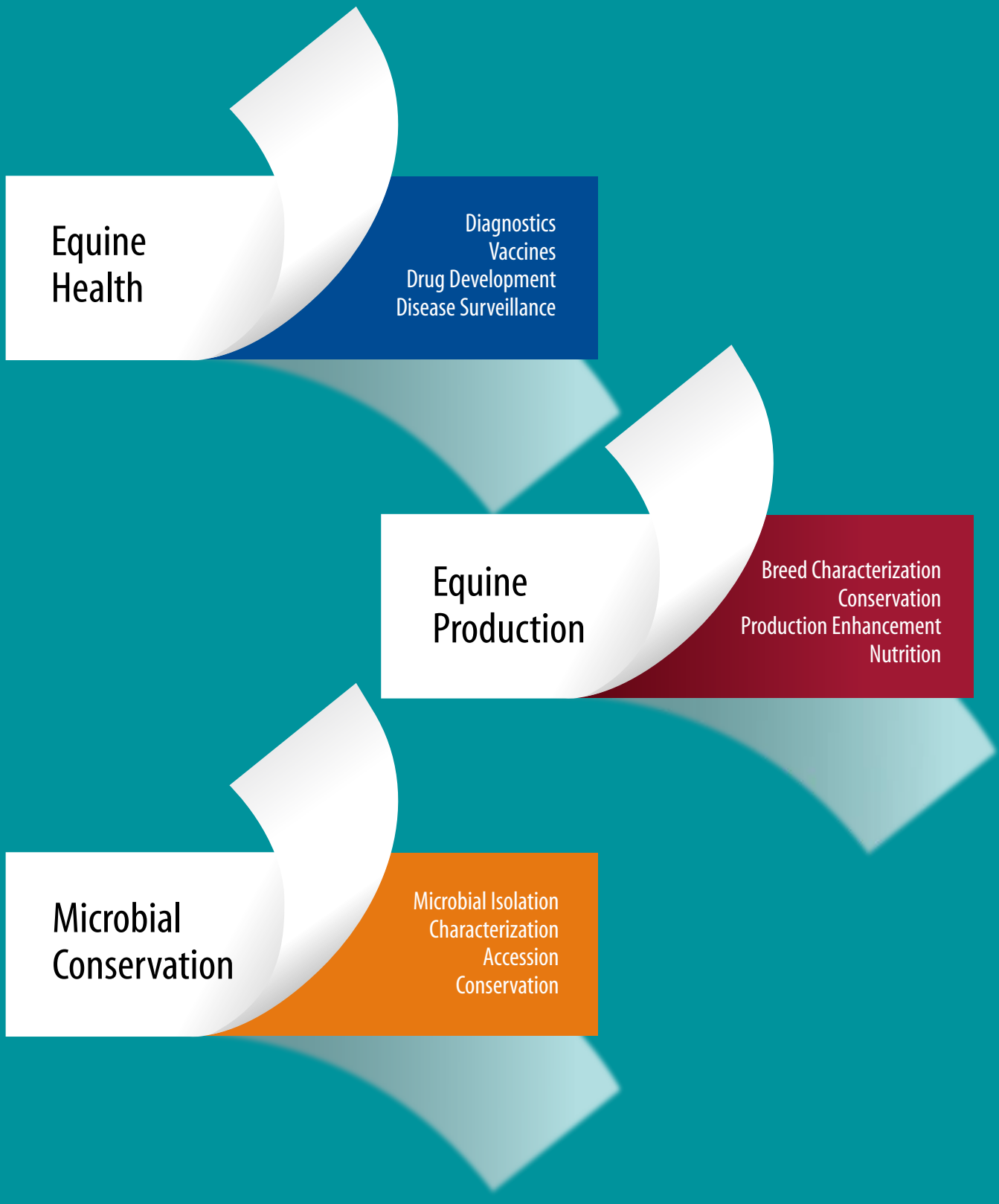
Summary of Revenue Generation	**2015-16**	**2016-17**
Sale of farm produce	495338.00	735840.00
Sale of livestock	977400.00	78000.00
Sale of publications and advertisements	2100.00	500.00
License fee	224020.00	199262.00
Interest on loans and advances	257727.00	212184.00
Interest on short term deposits	1913175.00	2287196.00
Income from internal resource generation	4802276.00	4885843.00
Receipt from services	0.00	367306.00
Other miscellaneous receipts	1728818.00	2015344.00
Total Revenue	**10400854.00**	**10781475.00**

STAFF POSITION AT NRCE AND NCVTC

Name of the Post	NRCE			NCVTC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	1	1	0	-	-	-
Scientific	26	19	7	10	8	2
Technical	24	23	1	1	-	1
Administrative	14	12	2	-	-	-
Supporting	22	20	2	-	-	-

ORGANIZATIONAL SET-UP

Research Achievements



Equine Health

Sero-surveillance of equine infectious diseases in India

Sero-surveillance of infectious diseases in equines is one of the main activities of ICAR/NRCE that helps in monitoring the trends of infectious diseases in India. The centre has National Surveillance and Monitoring Programme as a continuous activity for monitoring of emerging and existing equine diseases. During 2016-17, 1588 equine serum samples from 8 states were tested for various diseases (Table 1). A total of 689 (43.38%) equines were seropositive for *Theileria equi*, 171 (10.76%) for EHV1, 74 (4.65%) for *Trypanosoma evansi,* 73 (4.59%) for JEV and 4 (0.25%) for equine influenza (Table 1). None of the equines sera tested was positive for equine infectious anemia, glanders, brucellosis and *Salmonella* Abortusequi.

Table 1. Seropervalence of important equine diseases of indigenous equines

State	Number tested	Number positive for				
					T.equi	
Jammu & Kashmir	876	1	53	134	250	9
Manipur	10	0	0	0	3	0
Haryana	157	0	2	3	74	43
UP	98	0	2	0	57	6
Punjab	21	0	1	0	12	1
Rajasthan	161	0	2	5	101	7
Uttarakhand	182	2	12	15	145	3
Arunanchal Pradesh	83	1	2	14	47	4
Total Positive (%)	**1588**	**4 (0.25%)**	**74 (4.65%)**	**171** (10.76%)	**689** (43.38%)	**73** (4.59%)

Investigation on field outbreaks of equine infectious diseases in India

ICAR-NRCE is working as National Reference Laboratory for diagnosis of important infectious diseases in equines. The Centre is also involved in testing of all equine samples destined for export/import of equines as per requirement. During 2016-17, 15651 equine samples were tested for various diseases (Table 2). Out of 13035 samples tested, 143 samples from 9 states were positive for glanders. Testing of 1735 samples for equine influenza revealed 8 (0.46%) positive for H3N8 antibodies. None of the serum samples tested were positive for equine infectious anemia and EHV1 infection.

Table 2. Equine samples tested for disease investigation

Disease	Number Tested	Number Positive
Glanders	13035	143
EI	1735	8
EIA	603	0
T. evansi	240	7
EHV1	24	0
T.equi	14	12

Bacteriological analysis on 249 equine clinical samples from different parts of India yielded 56 isolates (Table 3). Investigation of 11 samples received from CMVL, Meerut for Inter laboratory comparison yielded Group D Streptococci (1), *Streptococcus equi* subsp. Equi (1) and *E. coli* (1).

Table 3. Bacteria isolated from clinical samples

Isolate	No.
Streptococcus equi subsp. Equi	16
S.equi subsp. Zooepidemicus	10
Group D Streptococci	1
Group C Streptococci	1
E. coli	6
Streptococcus sp.	14
B. mallei	8
Total	**56**

Post-mortem examination and histopathology of materials received from the field on 9 samples revealed portal cirrhosis and enteritis (1), enteritis (1), bronchopneumonia and hepatitis (1), interstitial pneumonia (1), nephritis and encephalopathy (1) and septicemia (1).

(S.K.Khurana, B.N. Tripathi, S.C.Yadav, B.R. Gulati, Rajender Kumar, Sanjay Kumar, N.Virmani, Sanjay Barua, Rajesh K. Vaid, Ramesh Dedar, H.Singha, Anju Manuja and Balvinder K. Manuja)

Emergence of glanders cases in new territories of India

Glanders is a fatal bacterial disease of equines caused by non-motile gram-negative bacterium *Burkholderia mallei*. The disease in equines is characterized by ulcerating cutaneous nodules, pneumonia, and septicemia (Fig. 1). In general, three clinical forms of the disease namely nasal form, pulmonary form and cutaneous form or 'Farcy' are observed in *B. mallei* infected animals. In India, re-emergence of glanders was reported in 2006, and since then the disease is being reported regularly from Western Uttar Pradesh, Uttarakhand and Himachal Pradesh. The disease has spread to few more states during the year.

A total of 13035 samples were tested under glanders surveillance. The highest number of glanders cases (n=143) were recorded during 2016-17 from 9 states (Table 4). Among these, maximum cases were reported from Gujarat and Uttar Pradesh. All of the infected equines were found serologically positive by CFT (titer 5-160) and in-house ELISA. Surveillance data of last two years indicates detection and reporting of more number of glanders outbreaks in endemic area as well as spread of disease to new territories by movement of infected equines. In addition, 4935 samples were tested for glanders under contractual disease investigation and none was found positive.

Table 4. Glanders cases reported during 2016-17

State	No. of cases
Uttar Pradesh	40
Uttarakhand	3
Jammu & Kashmir	21
Punjab	3
Gujarat	46
Haryana	13
Himachal Pradesh	5
Rajasthan	6
Madhya Pradesh	6
Total	**143**

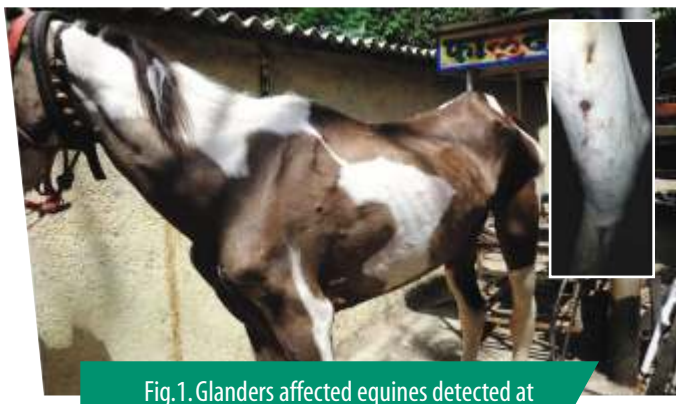


Fig.1. Glanders affected equines detected at different places during 2016-17

(H. Singha, S.K. Khurana and B.N. Tripathi)

Etiology and management of neonatal foal mortality in horses

Neonatal mortality is a significant problem for the equine industry. We initiated a project with an aim to study the incidence of foal mortality and identify the infectious etiological agents. Samples were collected from neonatal foals and adult equines (40 nasal and 69 fecal) from different geographic regions. From these samples, we isolated 69

E.coli, 28 *Klebsiella pneumoniae* and 7 *Salmonella* enteritidis isolates. These isolates were characterized phenotypically and genotypically. All these isolates were confirmed by amplifying their species specific amplicons of 666 bp, 130 bp and 304 bp by PCR, respectively.

All 69 *E.coli* isolates were further characterized for their virulent/pathogenic ability by performing multiplex PCR on eight genes coding for 6 different virulence/pathogenic factors. Three multiplex sets were standardized as follows - 1. *LT*+*dae*E+*hyl*A genes; 2. *pap*+*ast*A genes; 3. *eae*+*stx*1+*stx*2 genes. The *eae* gene (enteropathogenic) was detected in 84.1% of the isolates followed by *pap* (uropathogenic) (81.2%), *ast*A (enteroaggregative) (62.3%), *stx*2 (shigga like toxic) (47.8%) and *LT* (enterotoxigenic) gene (37.7%). The *dae*E and *stx*1 genes were detected only in 13% and 8.7% of the isolates.

All isolates were further subjected to antibiotic sensitivity test using 24 antibiotics. All *E.coli* isolates were found to be 100% sensitive to chloramphenicol followed by gentamicin (98.55%), norfloxacin (97.10%), ofloxacin (97.10%), aztreonam (92.75%), amikacin (92.75%) and meropenem (91.30%). Like-wise, all *K.penumoniae* isolates were found to be 100% sensitive to chloramphenicol, gentamicin and ofloxacin followed by aztreonam and tetracycline (92.90%), amikacin and norfloxacin (78.60%). Among *Salmonella* enteritidis isolates, all were found to be 100% sensitive to chloramphenicol, gentamicin, norfloxacin and ofloxacin followed by ciprofloxacin, aztreonam and ceftriaxone (85.70%), amikacin, kanamycin and tetracycline (71.4%). Overall average multi antibiotic resistance (MAR) value for *E.coli, K.pneumonia* and *S.* enteritidis isolates were 0.22, 0.28 and 0.29, respectively. It can be concluded that all the isolates were found most sensitive to chloramphenicol followed by gentamicin and resistant to penicillin-G and cefotaxime+clavulanic acid followed by ceftazidime+clavulanic acid. Accordingly package-of-practices were devised and communicated to the farmers.

(Sanjay Kumar, Ramesh Dedar, B.R. Gulati, Nitin Virmani and S.K. Khurana)

Assessment of risk factors for laminitis and colic in equines

Colic is recognized as a disease of equines for centuries and a worldwide problem in equines. In Indian equines, colic is one of the most common clinical problems. It is a multi-factorial disease and risk factors vary in different geographical conditions and include quality of drinking water, feed, fodder, management, age, breed, owner, housing, exercise pattern, previous exposure to colic, dental condition etc. Laminitis is another common cause of functional disability in equines in India. A study was initiated to assess the risk factors of colic and laminitis in equines in field conditions.

A total of 300 horses from Hanumangarh, Sri Ganganagar and Bikaner districts of Rajasthan and Mukatsar district of Punjab were included in this study and examined by regular visits. All the horses are being visited regularly and clinically examined each time.

Preliminary data suggests that 10% of horses in the field show repeated incidences of colic. Most of the incidences of colic occurred during the time of feed change. The colic cases were reported more frequently from horses that were fed wheat straw. Gastric ulcers were reported to be another important cause of colic in indigenous horses, which responded by treatment with ranitidine. In addition, cases of chronic laminitis were also observed in 2.5% animals in field.

(R.K. Dedar, P.A. Bala and S.K. Ravi)

Draft genome sequences of *Burkholderia mallei* isolated from glanders cases

Burkholderia mallei is a Gram-negative, non-motile coccobacillus which causes glanders - a fatal disease of equines that may occasionally be transmitted to humans. It is a notifiable disease to the World Organization of Animal Health (OIE). In 2006, a sudden recurrence of the disease was observed in India, which subsequently spread to several

regions of the country in subsequent 10 years, affecting hundreds of equines.

Genome sequence analysis of two *B. mallei* strains, viz, 3076HP_India (mule, 2013) and 3712UP_India (mare, 2015) was done on Illumina HiSeq 2500 using 2125 bp chemistry at Eurofins Genomics (India). The assembled genomes were aligned to the *B. mallei* ATCC 23344 reference genome sequence using MUMmer 3.0 with default parameters and circos plot was generated for graphical representation using Circos v 0.69.1 (Fig. 1). Assembled genome was 5.6 Mb in size with 67.07% G+C content. A total number of 4,713 genes were predicted with average gene size of 987 bp. The genomic data of clinical *B. mallei* isolates will be useful in providing insight into the molecular evolution and epidemiology of the disease.

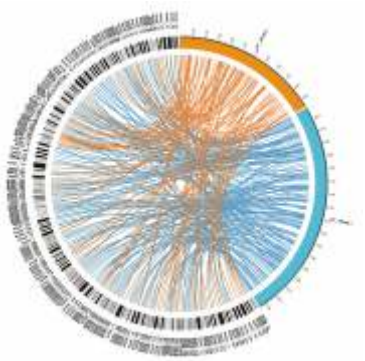


Fig. 1. Circos plot depicts scaffold alignment to two chromosomal sequences NC_006348.1 (Blue) and NC_006349.2 (Orange) of reference genome *Burkholderia mallei* ATCC 23344

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

Characterization of EHV-1 bacterial artificial chromosome (BAC)

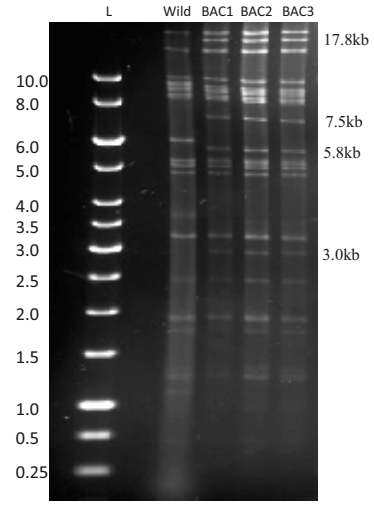


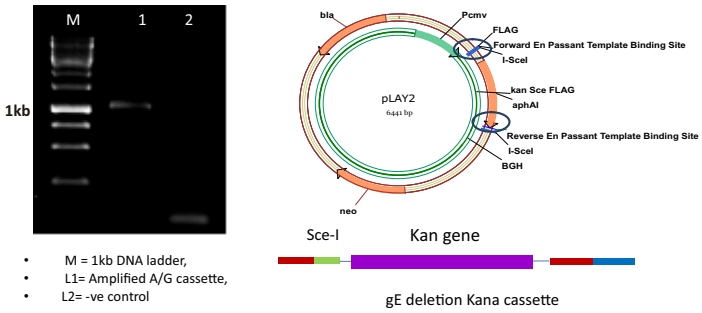
Fig. 1. RE profile of EHV1 BAC digested with *Hind*III showing 4 extra bands

The break-through in cloning the entire genome of viruses as bacterial artificial chromosomes (BAC) has opened up the new vista, which makes it possible to mutate/delete or insert genes in relatively short time into the genome of the virus and make a stable and infectious BAC molecule. The bacterial artificial chromosome (BAC) of EHV1 generated in the previous year was further characterized by isolating it from *E.coli* (DH10) and subjecting to RE analysis by *Hind*III & *Bam*HI and compared with wild virus DNA of EHV1 –Tohana strain. The BAC RFLP profile with *Hind*III showed extra bands at 17.8, 7.5, 5.8 and 3.0 kb position while *Bam*HI profile showed extra bands at 14.1, 4.8 and 3.5 positions (Fig. 1). The findings indicate the insertion of mini F plasmid in place of gp71 gene. Sequencing of plasmid construct of EHV1 confirmed the BAC. *In-silico* analysis of the data by *Hind*III & *Bam*HI profiling revealed presence of extra fragments are due to the insertion of the pHA2 plasmid sequence and circularization of viral DNA in plasmid form.

(Nitin Virmani and B.C.Bera)

Generation of gE deletion mutants of EHV1 employing BAC technology

The infection due to EHV1 is endemic in India and abortion outbreaks lead to huge economic losses. In order to refine existing EHV1 vaccine so as to generate strong cell-mediated immune responses in animals, we initiated studies to develop modified live vaccines. We used EHV1 bacterial artificial chromosome and developed EHV1 deletion mutation. The gE gene encoded by ORF74 has been implicated in reduction of EHV1 virulence, therefore, EHV1 attenuation was done by developing gE deletion mutant as a vaccine candidate. The gE deletion mutant virus of EHV1 was generated employing two-step red recombination strategies following *En Passant* mutagenesis process. For this, PCR products of transfer cassette were electroporated into the *E.coli*-GS1783 strain harboring the BAC construct of EHV1 and mutants were screened against chloramphenicol and kanamycin antibiotics. The



- M = 1kb DNA ladder,
- L1= Amplified A/G cassette,
- L2= -ve control

Fig. 1. PCR amplification from gE mutation cassette

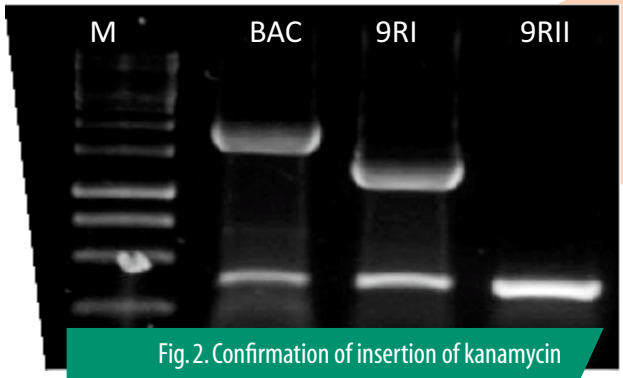


Fig. 2. Confirmation of insertion of kanamycin cassette (1kb) within gE gene by colony PCR

colonies screened against antibiotics were further confirmed by colony PCR targeting the deleted region of the gE gene (Fig. 1), where amplicons of 1.5kb indicates the insertion of kanamycin cassette in the targeted region of gE gene. Subsequently, the inserted kanamycin cassette was removed from the BAC construct employing second red recombination reaction and screening of colonies were carried out by replica plating against kanamycin. The mutant deleted gE-BAC construct was confirmed by PCR (Fig. 2) and RFLP analysis employing *Hind*III *& Bam*HI.

(Nitin Virmani, B.C. Bera and Taruna Anand)

Generation of reverse genetics based equine influenza virus

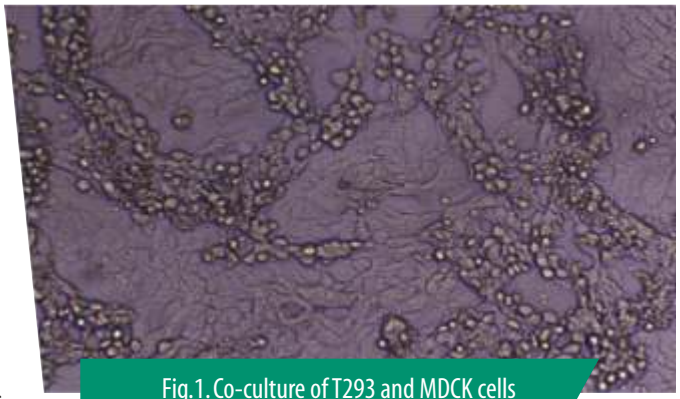


Fig.1. Co-culture of T293 and MDCK cells showing CPE characterstic of EIV

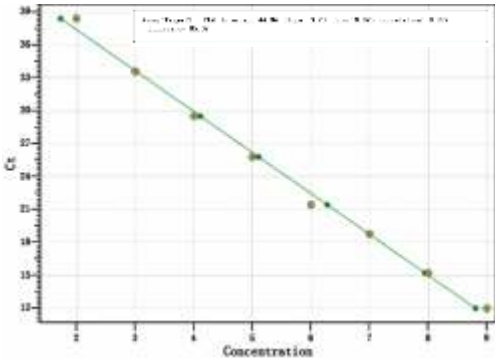
Equine influenza is a global problem and rapid globalization and transport of equines has led to frequent incursions of the disease sans boundaries. The segmented nature of the genome and error prone polymerase enzyme of virus lead to point mutations and re-assortment leading to generation of the escape mutants and rendering vaccines ineffective. Reverse genetics technique has been used as a tool to develop recombinant viruses which can be used as a vaccine candidate. The cloning of HA and NA segments of Indian field isolate of equine influenza virus and rest of the genes from H1N1 in pHW2000 vector was done in previous year. During the year, the recombinant EIV virus was generated through transfection in co-culture of MDCK and T293 cells (Fig. 1) The culture supernatant fluid was collected and inoculated in embryonated chicken eggs.

The recombinant virus was rescued from harvested allantoic fluid and characterized by HA and NA specific RT-PCR and cloning of the product. The virus was serially passaged in chicken embryos. Reciprocal HA titre of the virus was observed as 256 at 6^{th} passage level. The virus was grown in bulk and purified, quantified and inactivated with formalin for immunization in BALB/c mice for the challenge studies.

(Nitin Virmani, Sandeep Bhatia, B.C. Bera, Taruna Anand, Richa Bhatia and Naveen Yadav)

Lytic and latent equine herpesvirus 1 infections in India

Latency is one phenomenon in herpesviruses, which leads to lifelong persistence of virus infection in animals. Once infected, 50-70% horses remain latently infected and reactivation of virus latency may lead to clinical infection. Detection of latency is very tedious process, as virus gets hidden in few cells of trigeminal ganglion or lymphoid tissues. The status of EHV1 latency in India is not known, primarily due to non-availability of tools for detection of latency. We developed assays for detection of latent and lytic EHVI infections and studied their prevelance in India during the year.



Real-time PCR for detection of EHV1 latency

Latent EHV1 Infection: During the year, a real-time RT-PCR targeting sequences related to latency associated transcripts (LATs) corresponding to ORF64 of EHV1 was developed. In addition, RT-PCR and real-time PCR for detection of expression of late structural protein (gB) were developed to detect EHV1 latent infection. Out of a total of 113 equines screened for latency, 41 (36.3%) and 59 (52.2%) were detected latently infected using nested RT-PCR and real-time RT-PCR, respectively. The results prove that majority of equines in India were latently infected with EHV1 virus.

Lytic EHV1 infection: Clinical samples from cases of late-term abortions and respiratory illness were tested for EHV1 infection by a gB-based quantitative real-time PCR. Out of 198 clinical specimens from equines in northern India, 126 (63.6%) were positive for EHV1 infection. Further, the incidence of equine herpesvirus myeloencephalopathy (EHM) has been on the rise world over during last one decade. Most EHV1 isolates causing EHM exhibit a single nucleotide polymorphism (SNP) in the DNA polymerase gene (ORF30) at position 2254 (A2254 to G2254). To know, if there is prevalence of neuropathogenic strains, a real-time PCR was developed to detect single-nucleotide polymorphism (SNP). The A/G SNP analysis at position 2254 of ORF30 by real-time PCR revealed that 118 out of 198 samples (59.6%) were of non-neuropathogenic genotype (A2254) while 8 (4%) had the neuropathogenic marker (G2254). This means that neuropathogenic strains of EHV1 are circulating in India.

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

Optimization of expression and purification of recombinant glycoproteins (gB, gD and gM) of EHV1 in eukaryotic system

The glycoproteins of herpesviruses are known to play an important role in the biology of herpesvirus infection and are also major targets for the host immune system. To explore their potential for immunoprophylaxis, the experession of gB, gD and gM was optimized through variations in temperature and time intervals. The passage 2 recombinant baculoviruses of all the proteins were inoculated into sf9 cells and incubated at 24 & 27°C. Maximum levels of expression of recombinant proteins were observed at 27°C between 48-72 h of incubation. For production of large quantity of recombinant glycoproteins, bulk cultures of confirmed passage 2 recombinant baculoviruses were carried out in suspension culture of sf9 cells for each recombinant protein. Recombinant proteins were purified using Nickel-NTA column by chromatography technique and a high yield homogenous preparation of respective recombinant proteins was obtained (0.8-1mg) from 200 ml suspension culture. Molecular weight of purified fusion recombinant proteins were 45 kDa for gD, 25 kDa for gM and 56 KDa for gB. The proteins were subjected to western blotting with EHV1 specific serum and all the three proteins reacted specifically (Fig. 1). Recombinant proteins of all 3 glycoproteins are to be utilized in mice immunization studies.

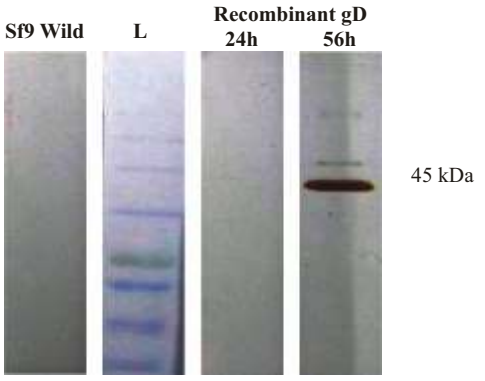


Fig. 1. Western blotting of recombinant glycoprotein D

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

Development of refined vaccine for equine herpesvirus 1 infection

In order to enhance the immunogenicity of existing EHV1 vaccine, the inactivated EHV1 virus was adjvanted with three novel adjuvants (advax, montanide and CpG) and the formulations were tested for protective efficacy in BALB/c mice. For this, BALB/c mice were divided in 5 groups and immunized with inactivated EHV-1 virus vaccine having adjuvants viz. Advax (group 1), Montanide (group 2) and CPG+Montanide (group 3). Group 4 and 5 served as positive and negative controls respectively. The animals were immunized by three doses of each preparation on days

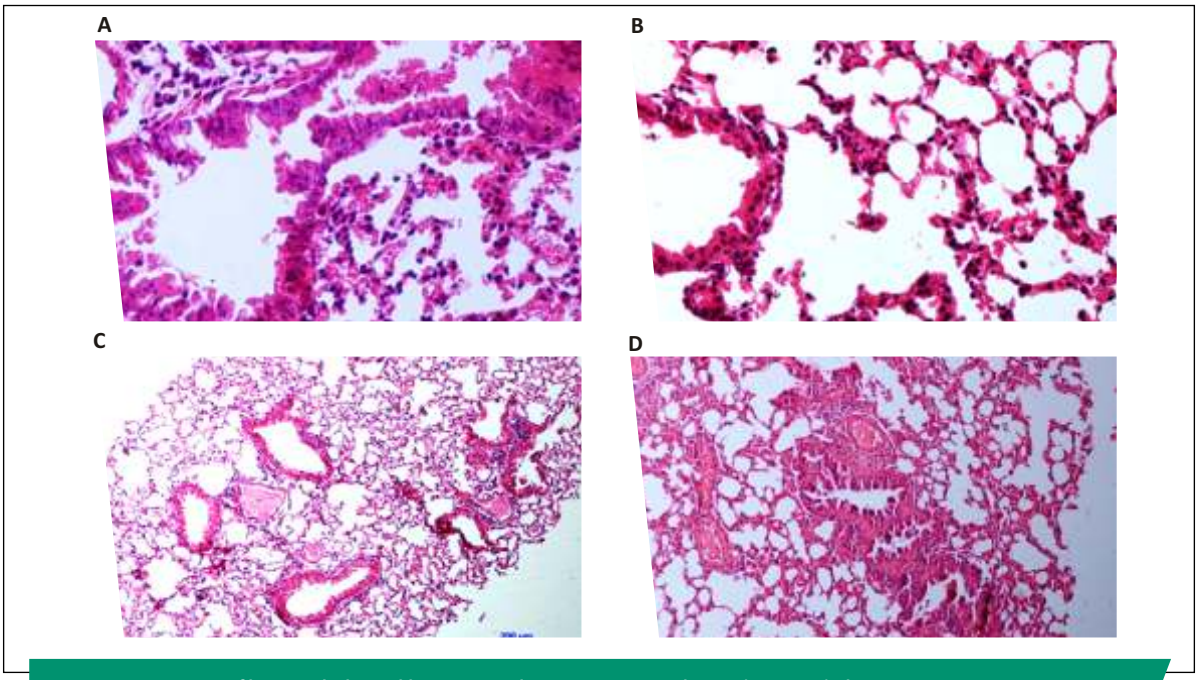


Fig. 1. Comparison of histopathological lesions at 6 dpc non-vaccinated mice (group IV) showing severe necrosis in bronchial epithelium with lymphocytic infiltration (A). Mice with advax vaccine (group I mice) (B) and with montanide (group II) mice (C) showed moderate lymphocytic infiltration in lung while group III mice showed less inflitration, perivascular cuffing and fibroblast proliferation around bronchial epithelium (H.E.X100).

0, 21 and 35 and challenged with purified EHV1 virus intranasally on day 42. After challenge, mice were sacrificed at 1, 3, 6, 13, 21 days post-challenge to monitor for protective efficacy through histopathology, immunohistochemistry, virus isolation, qPCR and immune responses. Mice from all the vaccinated groups showed good humoral immune response as adjudged through indirect ELISA. Non-vaccinated mice on challenge showed signs of EHV1 respiratory infection characterized by reduced feed and water intake, dullness and depression at 1 dpc. However, very mild reduction in feed intake was observed in Advax (3%), CpG (3%) and Montanide (7%) groups. Histopathological studies revealed severe congestion of blood vessels, bronchiolar epithelium hyperplasia, necrosis in lung parenchyma and massive wide spread infiltration of lymphocytes at 3 dpc in animals from positive control. Animals from advax group showed moderate to extensive perivascular and peribronchiolar cuffing with lymphocytes along with bronchiolar hyperplasia, necrosis and lymphocytic infiltration where as animals from montanide group had lesions limited to perivascular and peribronchiolar cuffing with moderate lymphocytic infiltration. Mice from CpG group did not show perivascular and periobronchial cuffing and lymphocytic infiltration was also less as compared to other groups. At 6 dpc in all the vaccinated groups the lesions were of lesser intensity as compared to positive control group, however, syncitia formation and widespread proliferation of macrophages could be seen in positive control infected group mice (Fig. 1). In all three groups with vaccination, the lesions were resolved by 13 dpc, whereas lesions persisted in non-vaccinated mice on challenge. Histological scoring of lung lesions was done on the basis of cellular infiltration, interstitial consolidation, edema, perivascular and peribronchial cuffing and extent of bronchial lesions (Table 1). Maximum lesion score was observed in positive control followed by Advax group, CpG and montanide group in order of decrease of intensity of lesions. Post challenge demonstration of EHV1 virus demonstrated virus isolation from mice from advax and control positive groups till 6 dpc, whereas in montanide and CpG group, the virus was detected till 3 dpc.

Table 1. Histological scoring of the lung lesions

Lung cellular infiltration		Interstitial consolidation and edema (10X)		Perivascular & peribronchial infiltration		Bronchial epithelial cell degeneration and necrosis	
Percent	Grade	Percent area	Grade	Nos of layers	Grade	Percent	Grade
<10	1	<10	1	<5	1	<20	1
10-20	2	10-20	2	5-10	2	20-40	2
20-40	3	20-30	3	10-15	3	40-60	3
40-60	4	30-40	4	15-20	4	60-80	4
≥60	5	≥40	5	≥20	5	≥80	5

(Nitin Virmani, Baldev R. Gulati & B.C. Bera)

Development of diagnostics for vesicular stomatitis and Venezuelan equine encephalitis

The diagnostic facilities for important equine viral diseases of zoonotic importance like vesicular stomatitis (VS) and Venezuelan equine encephalitis (VEE) are not available in India. Nucleic acid based diagnostics and recombinant protein based immunoassays were standardized using synthetic gene technology.

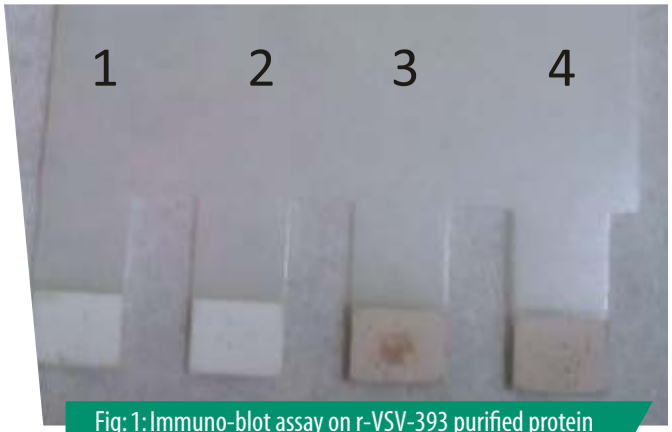


Fig: 1: Immuno-blot assay on r-VSV-393 purified protein against VS Indiana (IND) and VS- New Jersey (NJ) sera (1. PBS; 2. VEEV-420; 3. VS-IND; 4. VS-NJ)

To develop immunoassays, recombinant proteins of diagnostic importance were expressed and purified. A construct of 393 bases coding for five antigenic regions identified by *in silico* analysis from VSV glycoprotein was custom synthesized and expressed in *E.coli*. Recombinant protein has been purified and characterized by Western blotting for use as antigens in immunoassay. The multi-epitope recombinant protein reacted with specific antiserum to VSV-IND in dot-blot assay (Fig. 1). For nucleic acid based diagnostics, a PCR has been standardized to detect VSV-NJ and VSV-IND using synthetic gene technology (Fig. 2a).

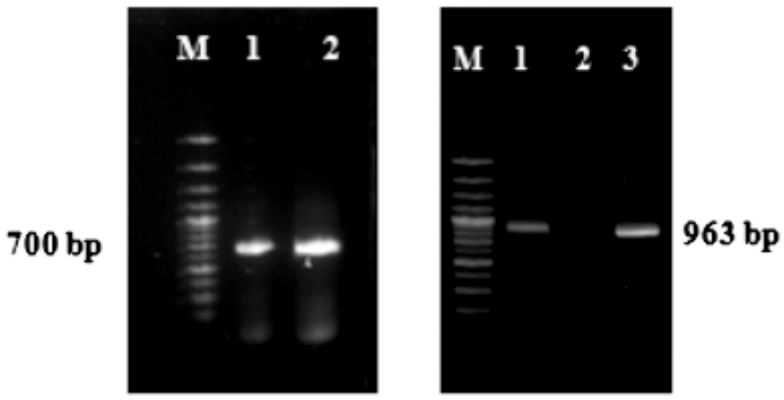


Fig. 2. Gel electrophoresis showing amplified gene products of 700 bp and 963 bp for VSV and VEEV by PCR

For VEEV, twelve immunodominant antigenic regions were identified by *in-silico* analysis from capsid and envelop proteins of VEEV and a synthetic construct of 921 bases comprising multiple antigenic regions was transferred to expression vector and expressed in *E.coli*. Recombinant proteins have been purified and characterized by Western blotting for use as antigens in immunoassays. Nucleic acid based PCR assay has been standardized with the potential to detect multiple serotypes affecting equines using synthetic gene technology (Fig. 2b).

(Balvinder K. Manuja, Anju Manuja, H. Singha and Naveen Kumar)

Lateral flow assay for detection of antibodies to *Trypanosoma evansi*

Routine diagnosis of trypanosomosis is made by the combined use of parasitological, serological (ELISA/ immunoblot) and molecular methods like PCR. However, most of these diagnostic methods require the availability of dedicated laboratory facilities, highly trained laboratory personnel, stable reagents, and multistep sample handling or preparations. There is need for development of a simple, quick diagnostic tool for on-site diagnosis of trypanosomosis field outbreak. Therefore, a lateral flow assay (LFA) was standardized to detect the antibodies in serum samples. The antigen concentration for (test line) and concentration of IgG for (control line) positive controls were standardized using serum samples of ponies experimentally infected (n=6) along with uninfected healthy controls (Fig. 1).

Fig. 1. Lateral flow assay for *T. evansi*

Apart from the experimentally infected samples, a total of 186 field serum samples collected from equines were tested by ELISA and LFA for comparison of LFA vis-à-vis antibody ELISA (Fig. 2). The LFA was 96.31% sensitive and 100% specific (Table 1). Further, we also tested cross reactivity of LFA with serum samples positive for *Theileria equi*, EHV1, equine influenza, and *B.malliei*. None of these positive samples cross reacted with *T.evansi* in LFA.

Table 1. Sensitivity and specificity of LFA vis-à-vis ELISA

ELISA		LFA			
Positive	Negative	Positive	Negative	Sensitivity	Specificity
153	0.00	147	6	96.31%	100%
0	33	0	33		

The shelf life of LFA coated strip stored at 4°C with reference positive and negative serum samples was tested and observed that coated strips were stable up to six months. Internal validation of LFA in three different laboratories at NRCE and external validation from two laboratories was done.

Our results indicate that there is no significant difference in sensitivity and specificity between LFA and ELISA. However, more number of samples need to be tested. LFA has many advantages as compared to ELISA, such as no equipment or trained personnel needed and visually readable results obtained within 10 minutes.

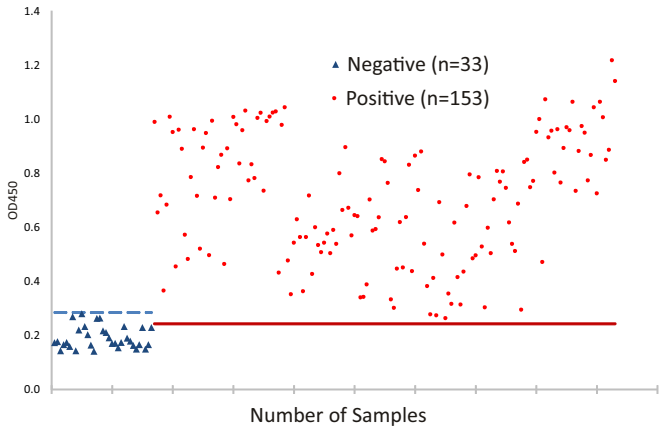


Fig. 2. *T.evansi* antibody levels in field serum samples of equines measured by ELISA

(S.C. Yadav, N. Dilbaghi, Sandeep Kumar and A.K. Gupta)

Development of a lateral flow assay for rapid diagnosis of trypanosomosis using recombinant protein

Presently, whole cell lysate antigen based ELISA/PCR assays are commonly used diagnostic techniques, which can only be performed in the laboratory by skilled technicians. Hence, there is an increasing demand from field veterinarians, farmers and other stakeholders for development of a user friendly, pen-side, rapid and specific diagnostic kit. In the present study, development of a lateral flow assay (LFA) for rapid diagnosis of trypanosomosis was attempted. LFA is a rapid, simple and easy to use (self-performing), cost effective, portable, highly sensitive and specific method in which the diagnosis is completed within 15 min using 1-2 drops of serum sample.

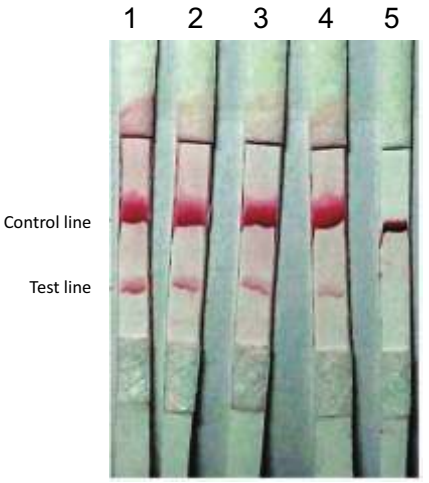


Fig. 1. Lateral flow assay for diagnosis of *T.evansi*

For development of LFA, recombinant flagellar antigen expressed in three batches was purified and pooled. Immuno-reactivity of purified protein was checked by ELISA, dot-ELISA and Western blot. Standardization of LFA using flagellar recombinant antigen and whole cell lysate antigen are in progress (Fig. 1). The preliminary results demonstrated that the 40 nm gold nanoparticles, 1:100 serum dilution are suitable for giving good immunoreactivity and differentiation between known positive and negative serum samples. Different conjugation procedures with nanogold particle/readymade nanogold conjugates are being tried. Preliminary results are encouraging.

(Rajender Kumar)

Development of competence for laboratory diagnosis of infectious animal diseases in North-Eastern Region (NER) of India

Animal population in NER of India has not been investigated for incidence of infectious diseases. There is a constant threat of disease ingression from neighboring countries bordering north-eastern states. In an endeavour to build our capabilities and strengths in the direction of developing latest diagnostics and organize rigorous surveillance of north-eastern states, a surveillance of infectious diseases prevalent in north-eastern states was done during the year.

Development of diagnostic competence of NER laboratories

Activities were undertaken to develop diagnostic facilities for influenza A, Japanese encephalitis and trypanosomosis to augment the diagnostic competence of veterinary diagnostic laboratories in north-eastern states. During the year, a hands-on training on collection and processing of samples and RT-PCR-based diagnosis of influenza A viruses in swine samples was imparted to laboratory personnel at College of Veterinary Sciences, Khanapara, Guwahati. Primer sets, positive RNA/cDNA and SOP for testing of samples for influenza by RT-PCR

NRCE scientist with trainees at Khanapara

were provided. Similarly, reagents and SOPs for RT-PCR and haemagglutination inhibtion for Japanese encephalitis, including primer sets, positive RNA/cDNA, HI antigen, positive controls were provided to AAU, Guwahati. The training and SOPs for antibody ELISA along with reagents (antigen, reference positive and negative serum) were provided for sero surveillance of *T. evansi* infection.

Surveillance of infectious diseases in NER

Influenza A viruses: A total of 84 nasal swabs and 142 serum samples were collected from pigs from various parts of Assam and horses from Arunachal Pradesh. All 84 nasal swabs were subjected to testing for influenza A viruses by universal RT-PCR assay and seven nasal swabs from Assam were positive for amplification of 244 bp of matrix gene for influenza A viruses. None of the horse swab samples were positive for influenza virus. Positive samples were passaged in 7-9 days old embryonated hen's eggs till 4 passages, however no virus could be isolated from any of the samples. Horse serum samples were tested by HI assay for detection of H3N8 viruses and one horse sample showed positive reaction for H3N8 virus infection.

Table 1. Antibodies to *T. evansi* in animals of NER

Species	Number tested	Number (%) Positive
Cattle	78	7 (8.97)
Pig	98	3 (3.06)
Goat	20	0 (0)
Horse	30	0 (0)

Japanese encephalitis virus: Testing of 61 pig serum samples from Guwahati by HI and VNT revealed positivity in 16 (26.2%) samples for JEV antibodies. In addition, 28 duck samples were tested for JEV antibodies and all found negative. Further, recombinant protein based immunoassay employing E protein immunodominant epitope was developed and is being explored for utility in ELISA for JEV seroprevalence.

***Trypanosoma evansi* infection:** Serum samples collected from cattle, pigs, goats and equines were subjected to recombinant protein based ELISA and results are given in Table 1.

(B.N. Tripathi, S.C. Yadav, B.R. Gulati, Rajender Kumar & Nitin Virmani)

Nanoformulations for osteoarthritis and their toxicological evaluation

Osteoarthritis is a common degenerative disease of equines affecting their work performance. Conventionally prescribed drugs like NSAIDs, steroids and local analgesics administered orally, topically or parenterally do not reach therapeutic levels in joint tissue. Intra-articular injections are used for drug delivery but frequent injections aggravate the condition by introducing infections. Therefore, drug delivery using nanoformulations with biocompatible polymers is eyed as potential strategy for its management. It is of significant importance to identify unique natural, non-toxic bioactive materials which are safe, easily available and can help inhibit disease progression. In addition, the tissue regeneration material should have anti-bacterial property to avoid the risk of infection when used.

We synthesized nanoformulations using conventional drugs, minerals and polymers mimicking the natural environment in the cartilage extra-cellular matrix and for bone mineralization. The nanoformulations were characterized for size, morphology, stability and incorporated functional groups. *In vitro* cytotoxicity and cell viability studies were performed by metabolic resazurin assay at different concentrations of the nanoformulations on animal cell lines. Preliminary studies revealed the suitability of developed nanoformulations for evaluation in suitable animal models.

(Anju Manuja, Balvinder K. Manuja and Riyesh T.)

In vitro efficacy of drug molecules against *Theileria equi* parasite and their organ toxicity in mouse model

Equine piroplasmosis, a tick transmitted haemoprotozoan disease caused by *Theileria equi* and/or *Babesia caballi*, poses serious threat and hinders international movement of the infected horses. The latently infected equids develop life-long persistent asymptomatic infections. The currently available drugs are not effective in clearing the parasite completely from the animal.

Theileria parasites alternate their life cycle stages in mammalian host and tick vector. Switches between these stages require calcium-dependent protein kinases (CDPKs). *Teq*CDPK is critical in regulating the motor complex that powers erythrocyte invasion. Quercetin and rutin trihydrate are bumped kinase inhibitors (BKIs). Bumped kinases are required for erythrocytic invasion of the parasite. These drug molecules were tested at various concentrations i.e. rutin trihydrate 0.5-100 µg/ml; quercetin 10-1000 µM. Rutin trihydrate was not at all effective in inhibiting the parasite growth in *in-vitro* culture system, whereas quercetin showed some inhibitory efficacy at higher drug concentration (1000 µM). Altogether these drugs were ineffective in inhibiting *in-vitro* growth of *T.equi* Parasites.

In our previous study, novobiocin, harmaline and barberine were found effective in inhibiting the *T. equi* growth in *in-vitro* culture system. We took these drugs further for studying the organ toxicity trial in mouse model. Novobiocin was tested in different groups of mice at a dosage ranging from 5 to 200 mg/kg b. wt. Drug was injected to the group of mice (n=6) and serum samples were collected on 0, 5, 8 and 14th day of drug administration. Organ specific biochemical biomarkers indicated that drug is safe to use at 5-50 mg/kg body weight (BW). Different organ specific biomarkers were studied – SGOT, SGPT, AP, BUN, Urea, BIT, CK, Glucose and triglycerides. Some organ dysfunctions were observed when novobiocin was used @ 100 and 200 mg/kg BW. Similarly harmaline was injected to group of mice @ 42.0 mg/kg; 17 mg/kg and 6.8 mg/kg and serum samples were collected. This drug molecule was quite safe and no significant alterations in biochemical parameters were observed. Barberine was tested at 12 mg/kg, 6 mg/kg and 3mg/kg BW in a group of mice. This drug molecule was also not found organ toxic at calculated therapeutic dosage.

We also undertook docking studies using novobiocin as a drug Hsp90 as its target. *Theileria equi* specific molecular model of Hsp90 protein was designed (Fig. 1a) and *in silico* interaction of novobiocin was studied. A very high binding energy was found between interacting amino acids and drug molecule (Fig. 1b), indicating specific interaction between and drug and its target.

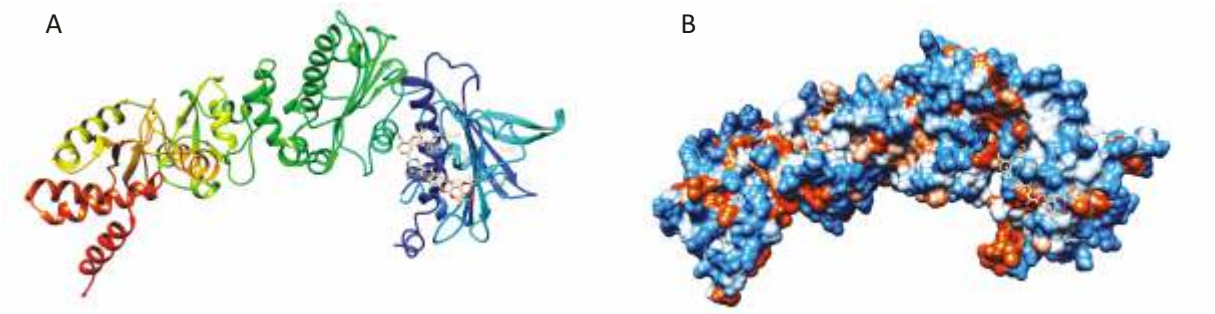


Fig. 1. *Theileria equi* HsP90 target protein (A) and its molecular interaction with novobiocin drug (B)

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

Evaluation of specific novel drug molecules having therapeutic potential against *Trypanosoma evansi* infection

The present constraints in control of trypanosomosis are availability of limited number of effective drugs (diminazene aceturate, quinapyramine and isometamedium) with narrow safety index, non-availability of vaccines due to immune evasion mechanisms developed by the parasite and drug resistance problem. Therefore, there is a need to search for cheaper, more effective, easily available and less toxic alternative chemotherapeutic agents for combating trypanosomosis. In the study, drug targets affecting metabolic pathways essential for survival of parasites have been identified and effects of selected drugs against their targets have been studied (Table 1).

Table 1. Drugs and their targets in *T.evansi* used in the study

Drug Targets	Name of Drugs
Tyrosine kinase inhibitors (PTKIs)	Imatanib, SC-1
Hexokinase-inhibitor	Lonidamine
Trans sialidase inhibitor	Myricetin, Quercitin
Trypanothione reductase	Indatraline
Other target inhibitors	CPZ, CPA, TZD

In total, 9 novel drugs were tested *in vitro* to determine activity against *Trypanosoma evansi* isolate of horse origin (T.ev-India-NRCE-Horse1, Hisar). Quinapyramine sulphate was used as the standard drug in this study. Out of 9, five drugs viz., CPZ, CPA, Indatraline, SC-1and TZD exhibited significant *in vitro* growth inhibition of trypanosomes at different concentrations. Chlorpromazine induced complete growth inhibition at 5 µM concentration. Similarly CPA, Indatraline, SC-1 and TZD showed complete inhibition at 15µM, 2µM, 4µM and 25µM, respectively (Table 2, Fig. 1).

Table 2. Effective concentration of different drugs on *Trypanosma evansi*

Name of Drug	Concentration (mM)	
	Tested	Effective
Imatanib	0.25-15	2-5
Sorafenib (SC-1)	1.5-20	3-4
Myricetin	5-50	25-50
Indatraline	0.05-50	1.5-3.0
CPZ	1-50	5-15
CPA	0.5-15	<5
Thiazolidinedione (TZD)	5-50	10-25
Lonidamine	10-60	–
Quercitin	5-50	–

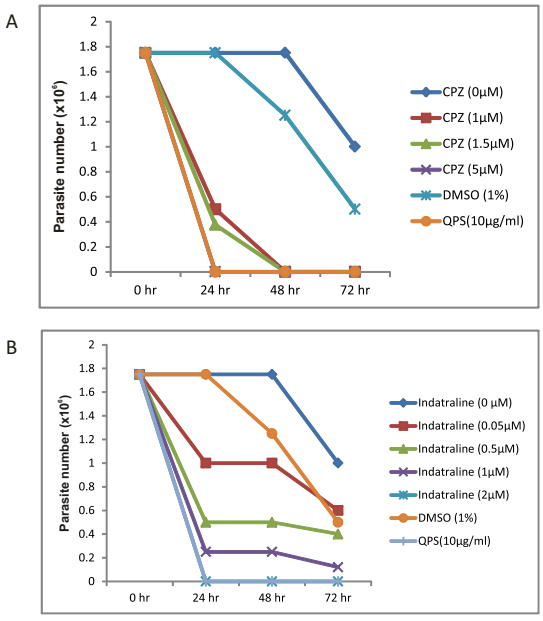


Fig. 1. *In vitro* efficacy of CPZ (A) and indatraline (B) against *Trypanosoma evansi*

In addition, preliminary *in vitro* toxicity assays were performed with these five drugs using PBMCs and Vero cell lines. The results revealed that these drugs were not toxic even up to 50x of effective drug concentration.

(Rajender Kumar)

Equine Production

Optimization of protocols for production of horse *(Equus caballus)* cloned embryos

In India, classical reproduction techniques such as artificial insemination and embryo transfer have been utilized for multiplication of superior animals in cattle/buffalo. Since buffalo oocytes have potential to support donor genome of distinct species, we explored the possibility of using the buffalo oocytes and horse donor genome for the production of cloned embryos and to assess nucleo-cytoplasmic compatibility between these species. This will help us to develop a suitable approach for the cloning of rarest of the rare horse to increase their number. Skin tissues from foals were collected and fibroblast cultures were isolated and characterized for cloning experiments.

Standardization of horse oocyte isolation and maturation: For isolation of the oocytes from ovaries, two protocols were followed; (a) aspiration of follicular fluid from the visible follicles and (b) scrapping of the ovary (Fig.1). After cutting or slicing of the ovary, the visible follicles were aspirated. The ovulatory fossa was identified and it was sliced and washed thoroughly with TCM 199 medium. A combination of slicing, washing and aspiration methods gave the best results for collection of equine oocytes.

Fig.1. Steps involved in isolation of oocytes from the horse ovaries

Production of iSCNT embryos: For production of interspecies horse cloned embryos, buffalo oocytes were isolated from ovaries collected from slaughterhouse. After oocyte maturation, cumulus/zona removal, and manual enucleation, it was fused with horse somatic cells. The fused cells were cultured to produce embryos. Using cell lines isolated from adult mare and a 3 month old foal, six fusion experiments were conducted with buffalo ooplasts. All cloned embryos had shown growth till the 16 to 32 cell stage and further growth was arrested. This study suggests that the buffalo ooplasm can support the reprogramming process for initial stages, favors the embryo genomic activation and can lead to production of iSCNT embryos till 32 cell stage. Experiments are in progress to optimize the culture conditions for production of cloned embryos (Fig.2).

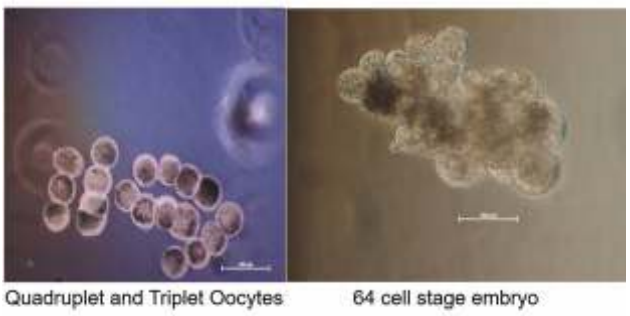


Fig.2. Interspecies embryos produced by using quadruplet and triplet buffalo oocytes

(T.R. Talluri, Naresh Seloker, S.K. Ravi, Taruna Anand, Dharmendra Kumar, P.S. Yadav, S.S. Kashyap and Chandan)

Assessment of reproductive problems in indigenous equines

Indigenous equines are well adapted to different climates in diverse geographical locations. However, information on their reproductive problems is not much reported. Hence, we initiated a survey on reproductive problems in indigenous equines under farm and field conditions.

During the year, pre-breeding examination of external and internal genitalia of farm equids including eight Marwari, five each of Zanskari and Manipuri mares was done. The reproductive problems commonly observed in farm animals were: vaginitis due to poor perineal conformation (Fig.1), anovulation and endometritis (Fig. 2).

Survey of field equines was conducted in Rajasthan (Ganganagar and Hanumangarh) and Punjab (Badal, Bhatinda) states by contacting twenty equine breeders to know the prevailing reproductive disorders in mares. The reproductive disorders reported were abortion (4.94%), repeat breeding (4.94%), cystic ovary (1.23%) and vaginal prolapse (1.23%).

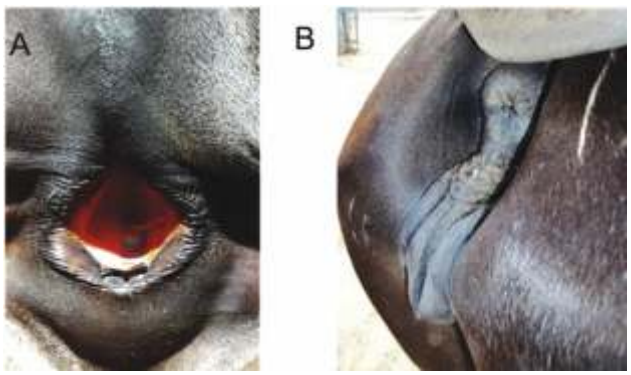


Fig. 1. Vaginitis in a mare and poor perineal confirmation

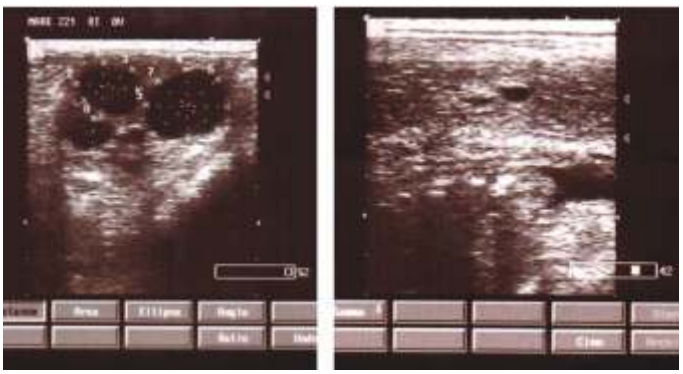


Fig. 2. Multiple follicles on mare ovary (A) & Fluid accumulation in Uterus (B)

(S.K. Ravi, T.R. Talluri, R.K.Vaid, J. Singh and R.A. Legha)

Hormonal profiling of Marwari Mares during different reproductive states

The knowledge of the endocrine status of the mare helps in determining underlying causes of fertility, infertility and sub-fertility. The endocrine hormones, FSH and PGF2α were estimated in the blood plasma of Marwari mares and fillies during estrous cycle and peripartum stages. Growth hormone was studied in plasma of animals of different ages using equine specific kits. It was observed that:

- FSH level was higher in the adult mares and lower in fillies at the 1st day of estrus and in the luteal phase. Cycle-wise, it was higher at the first day of estrus and lower during the luteal phase.
- Fillies exhibited higher FSH during peak estrus stage. This might be because of presence of highly secretive dominant or preovulatory follicles at this stage.
- PGF2α level was higher during the 1st day of estrus and at peak estrus than in the luteal phase. This signifies its role in lysis of corpus luteum resulting in the onset of estrus.
- At one month pre-partum, levels of FSH and PGF2α remained lower, but increased marginally at parturition. At one month post-parturition, animals might have undergone changes in the ovarian cyclicity due to which the levels have changed. A higher PGF2α and lower FSH indicate that the animals had started cycling again after parturition and the higher PGF2α is in relation to corpus luteum lysis.

(Vijay Kumar, Sanjay K. Ravi and R.K. Dedar)

Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines

Valuable germplasm of our indigenous breeds of equines can be cryopreserved *ex situ* for years. Frozen semen can be used in artificial insemination (AI) to multiply germplasm at a faster rate. Frozen semen of large sized Poitou donkeys and the selected indigenous donkeys housed at farm of ICAR-NRCE for production of mules is in high demand.

Semen was collected and cryopreserved from Marwari, Manipuri and Poitou stallions and their seminal parameters were recorded (Table 1). Ten doses of Manipuri horse semen and 75 doses of Marwari & Poitou donkeys were cryopreserved. A total of eighteen mares including Marwari (8), Manipuri (5) and Zanskari (5) were inseminated with frozen semen, out of which 11 became pregnant including two for mule production. Ultrasound guided pregnancy diagnosis was performed at day 15 and 35 post-ovulation in all the inseminated mares. The embryonic loss was observed one each in Marwari and Zanskari mares.

Table 1. Semen characteristics of different breeds of equines

Breed	Total volume (ml)	Gel free volume (ml)	Gel volume (ml)	pH	Sperm conc. (10^6/ml)	Total Motility %	Progressive Motility %	Post thaw Motility %
Marwari	57.28±5.4	44.43±3.92	12.18±2.6	7.65±0.02	210.53±3.66	86.37±1.92	83.37±0.56	36.46±0.86
Manipuri	58.0±4.45	44.15±3.79	13.09±1.40	7.74±0.04	282.12±4.81	92.82±2.73	88.46±0.35	39.65±0.52
Poitou	54.53±7.09	34.93±4.29	19.60±5.6	7.29±0.03	262.33±15.89	80.20±2.82	75±2.84%	39.72±4.80

(S.K. Ravi, T.R. Talluri, J. Singh, R.A. Legha, Yash Pal and A.K. Gupta)

Morphometry studies of indigenous equine spermatozoa

Sperm morphometry is one of the parameters used to determine the fertility of sperms. The biometry of the spermatozoa from the three indigenous breeds viz. Marwari, Zanskari and Manipuri was studied through Computerized Assisted Semen Analyzer (CASA) (Table 1, Fig. 1). Sperm morphology was studied in wet preparations comprising samples fixed in formal-saline under a phase contrast microscope (Olympus, Tokyo, Japan) at a magnification of 100×. A total of 200 sperms in each ejaculate were examined for morphological abnormalities. Five stallions ranging in age from 5 to 7 years were used in this experiment. These stallions were considered to have normal fertility based upon previous breeding history and upon the parameters of sperm number, progressive motility and subjectively assessed spermatozoa morphology. We found variability in the percentage of morphologically normal and abnormal sperm, as well as in sperm head dimensions among native indigenous stallions.

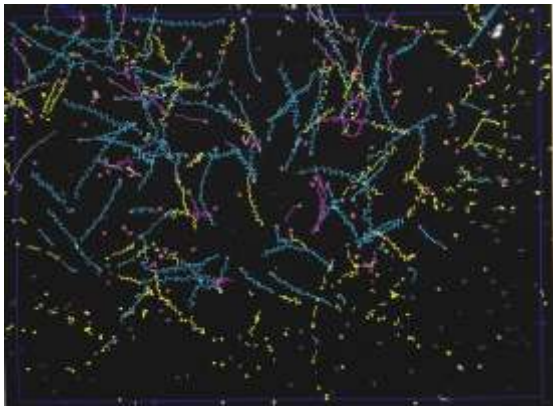
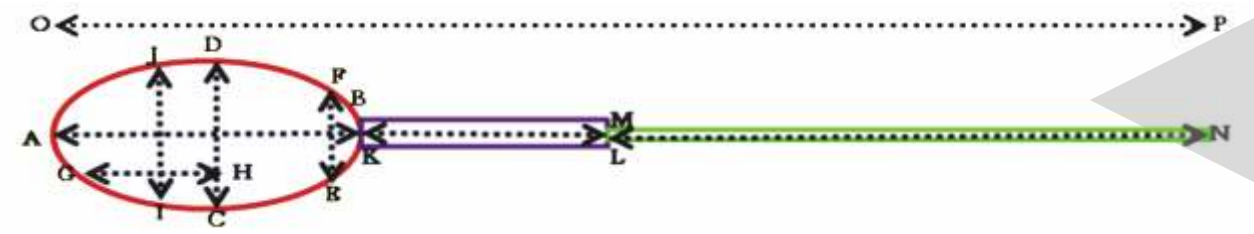


Fig 1.Evaluation of sperm biometry with CASA

Table 1. Biometry parameters of spermatozoa in different breeds

Breed	Biometric parameters*						
	Head length	Head width	Head Elongation	Head Perimeter	Head Area	Tail length	Tail STR
Marwari (6)	4.54±0.11*a	2.69±0.05**	0.62±0.008*	12.53±0.28*	11.47±0.43*a	6.82±0.95*a	46.80±6.07**a
Zanskari (4)	6.06±0.42**b	3.88±0.35*	0.65±0.02	18.26±1.53**	20.95±2.91*b	15.96±0.92**b	64.63±3.11*b
Manipuri (4)	5.32±0.61*c	3.00±0.32*	0.54±0.03	16.35±2.12*	16.24±3.39*c	14.32±2.33**c	57.71±3.75**c

**Means with different superscripts differ significantly (P≤0.05), letters in lowercase denotes significance within breed



*AB= head length, CD= head width, EF= head base, GH= acrosomal cap length, IJ= acrosomal cap width, KL= midpiece length, MN= tail length, OP=total length.

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

Genetic characterization of donkeys of Rajasthan

Microsatellite markers have been used as proven tool for studying genetic diversity. A battery of 20 microsatellites were selected to estimate the genetic variability of Rajasthan donkey population. Basic genetic parameters including observed allele frequencies (Na), effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He), and heterozygote deficit (FIS) in the whole population were calculated by analyzing the genetic data with GenAlEx 6.5 software. Bottleneck events in the population were tested by the mode-shift indicator using Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB).

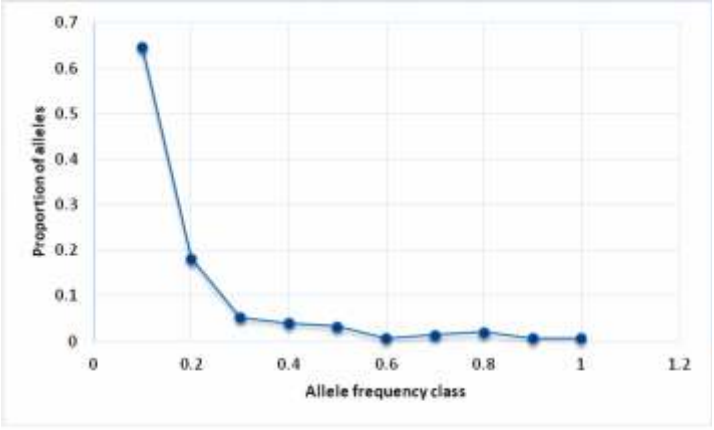


Fig. 1. Proportion of alleles and their distribution in Rajasthan donkey

Reasonable polymorphism in Rajasthan donkey was observed. Marker HTG10 showed the highest number of observed alleles per locus (17) while ASB17 showed the lowest (2) with the 8.158 mean number of alleles. Expected number of alleles varied from 1.043 (ASB17) to 8.785 (AHT05) with the mean of 3.788. The use of microsatellites with a range of polymorphism reduced the risk of overestimating genetic variability, which might occur with microsatellite exhibiting only high polymorphism. Microsatellite preferably should have at least 4 alleles to be useful for the evaluation of genetic diversity, therefore 18 loci were retained for further analysis.

Shannon's information Index (I), a parameter indicative of the informative degree of a marker, ranged from 0.103 (ASB17) to 2.345 (AHT05). Except ASB17, other markers had high I values and thus can potentially be used for diverse genetic applications including linkage mapping, individual identification and parentage testing. Rajasthan donkeys had substantial genetic variation based on gene diversity in addition to the average number of alleles per locus. The observed and expected heterozygosity values ranged from 0.00 (ASB17) to 1 (AHT4) and from 0.042 (ASB17) to 0.886 (AHT05) with an overall mean of 0.627± 0.069 and 0.620±0.054, respectively. Observed heterozygosity was lower than expected showing a departure from Hardy-Weinberg Equilibrium (HWE) and possibility of inbreeding.

It is not clear whether any serious demographic bottlenecks have occurred in this population, hence the mode-shift indicator test was utilized as a method to detect potential bottleneck. The non-bottleneck populations that are near mutation-drift equilibrium are expected to have a large proportion of alleles with low frequency. A graphical representation utilizing allelic class and proportion of alleles showed a normal 'L' shaped distribution (Fig. 1). The L shaped curve indicated the abundance of low frequency (<0.10) alleles. This finding suggested the absence of any detectably large, recent genetic bottleneck (last 40-80 generations) in this population.

(Yash Pal, Rekha Sharma, A.K. Gupta and R.K. Dedar)

Improvement in growth parameters of foals by selective breeding using semen from elite stallions

In order to improve the growth of foals in Marwari breed, elite stallions with good height at wither and true-to-breed phenotypes were selected for collection of semen for use in Marwari mares at our farm. The effect of this selective breeding was evaluated by biometry of Marwari foals from birth (day 0) till 19 months of age (19 M). This helped us to evaluate the effect of selective artificial insemination program in terms of body weight gain, body length and height at wither (Fig 1).

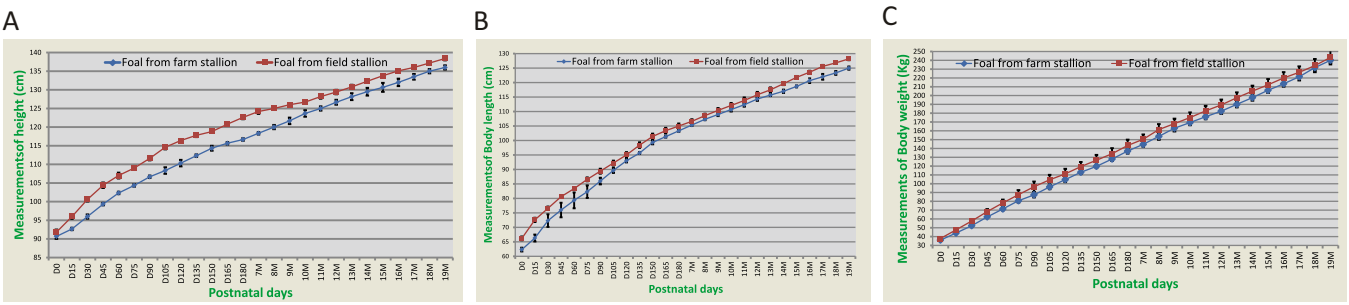


Fig 1. Comparative analysis of height at whither (A), body length (B) and body weight (C) of foals born with semen of farm stallions versus elite field stallions

In addition, reproductive status of 35 indigenous mares from adjoining areas was assessed for determination of estrous cycle stage, AI and pregnancy diagnosis. A total of 12 field mares were presented for AI out of which 9 were inseminated. Pregnancy diagnosis was done in 17 mares and 7 were confirmed pregnant.

(S.K. Ravi, T.R. Talluri, J. Singh, R.A. Legha, Yash Pal and A.K. Gupta)

Assessment of equine breeding, feeding and growth parameters in arid region

The management practices for indigenous equine breeds have not been documented adequately. To record the normal parameters in terms of their growth potential, reproductive performance and nutritional performance in an intensive and arid region, this study was initiated.

In this study, growth pattern of the Marwari foals was studied and biometry was recorded. Through this study, one can analyze the type of feeding to be given to optimize the body growth in foals.

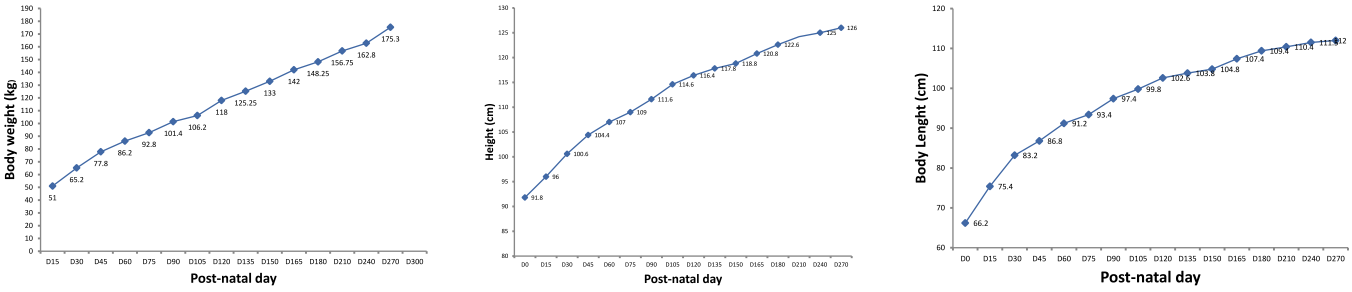


Fig. 1. Biometry of Marwari foals under standard management conditions

Breeding mares in the arid region is biggest challenge to the equine owners as there is little knowledge about the estrus cycle and estrus duration in mares. To generate data for arid zone, the duration of estrus, estrus cycle length

and pre-ovulation follicle size were monitored. The follicular dynamics and reproductive potential were recorded, analysed and tabulated for the three different breeds (Table 2).

Table 2. Reproductive dynamics of equines in arid zone of Rajasthan

Mares	Length of estrus cycle (days)	Estrus duration (days)	Pre-Ovulatory follicle size (mm)	Conception %	No. of AI per conception
Marwari (8)	22.13±0.55	7.62±0.39	44.35±0.99	87.5	1.28
Manipuri (5)	24.25±1.03	8.83±0.40	37.88±1.15	60	1.4
Zanskari (5)	20.86±2.18	8.25±0.25	36.47±0.91	60	1

Feeding balanced diet to mares is very important to achieve fertility and to maintain the pregnancy. The feed formulation (40% oats, 30% gram and 30% wheat bran) was used in mares and feeding trial was conducted on 4 Zanskari and 5 Manipuri mares for 6 months. The animals were also fed *ad libitum* groundnut straw and wheat straw (50:50) as dry forage. The digestibility trials to evaluate efficiency of nutrient utilization indicated that average feed intake and their digestibility was optimal for the mares and hence can be recommended for the mares in arid region.

(R.A. Legha, Vijay Kumar, R.K. Dedar, P.A. Bala, T.R. Talluri, S.K. Ravi and J. Singh)

Development of monoclonal antibodies for rapid pregnancy diagnosis in horse mares

NRCE has previously developed a sandwich ELISA kit for detection of pregnancy in horse mares between 35 to 120 days of gestation. To make it more convenient and farmer friendly, efforts are being made to develop a field based simple, accurate and rapid lateral flow assay (LFA), in which pregnancy hormone namely eCG will be entrapped using monoclonal antibodies.

During this year, monoclonal antibodies were raised against eCG. For this, fresh lot of purified eCG hormone was used for immunization of balb/c mice. In immunized mice, high antibody titre of 1:12800 was observed before fusion with immortal cell line. On fusion, twelve high secretary clones were picked for final cloning using limited dilution. Four monoclones (E1, G11, H3 and B1) with high ELISA OD values were selected after three limited dilutions (Fig.1).

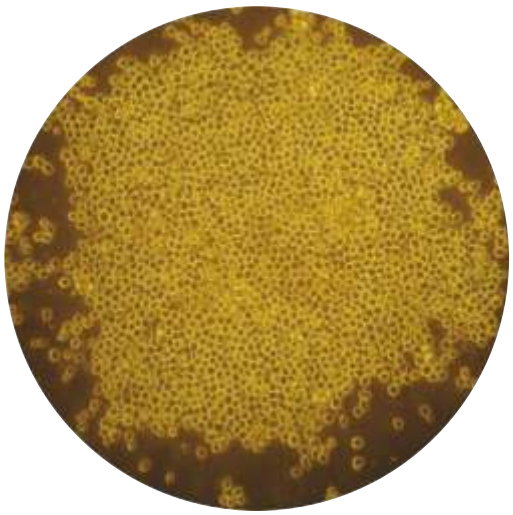


Fig. 1. A monoclone developed against eCG

The monoclonal antibodies were raised as ascites in adult balb/c mice against three individual clones (E1, G11 and H3). All the three monoclonal antibodies were isotyped as IgG1 using mouse monoclonal antibody isotyping kit. Antibody titre (>40,000) was observed to be good enough in sELISA for pregnancy diagnosis. These antibodies will be tagged with nano gold particles for further use in LFA kit.

In addition, hyper immune serum was also raised against eCG in a lab animal for use in sELISA (Pregmare kit) with a titre of 1: 6400. Using pregmare kit, 150 pregnant mare serum samples were also tested successfully.

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

Physiological and biochemical indices of stress under draught load in equines

Physiological and biochemical indices of stress under draught are very important to derive critical upper limits of load, speed, time and distance of work in equines. The stress evaluation in working equines would also help in devising suitable working protocols for equine welfare. Preliminary studies were conducted with 50N and 100N draught loads in donkeys and mules to study their performance and stress levels. In another trial, draught load was carried over 10 KM distance for seven continuous days so as to study the effect in a simulated situation of owner's equines under draught.

Overall results showed that animals under trotting experienced sufficient physiological and biochemical stress, as exhibited by increased physiological, hematological and biochemical responses. There was significant increase in plasma lactate, urea, creatinine, creatine kinase, lactate dehydrogenase and liver enzymes (AST, ALT, GT, ALP). Most of these responses returned to below critical limits after 20 min to 4 hour of rest, indicating that the stress induced were within the physiological adaptation of the equines.

Non-recovery of heart rates under 64 beats/min after 20 min of rest be used as a critical indicator of excessive stress, which would require the animal owner to reduce either load, speed, or time of work in the equines.

(R.A. Legha, Vijay Kumar and Yash Pal)

Development of a lifting device for sick and recumbent equines

A large animal lifting device has been developed which can be used to lift recumbent animals for treatment and rescue operations in difficult situations. The animal lifting device and slings have been designed and fabricated in collaboration with the Central Institute of Agricultural Engineering Bhopal. A device capable of lifting up to 2000 kg weight was developed based on body dimension size, weight data for different animals, safe areas on animal body for using slings. The device and slings were tested for lifting and transport of bullock of 537 kg and Jenny of 234 kg (Fig. 1).

Fig. 1. Evaluation of lifting device on Poitou jenny (234 Kg)

(R.A. Legha, Vijay Kumar and Yash Pal)

Vermicomposting of mule and horse dung

Utilization of mules for transport in hilly regions leads to deposition of mule dung on hilly tracks. There is a continuous problem of piles of mule dung on hilly tracks. Horse and mule dung can be converted to vermincompost, which is an excellent, nutrient-rich organic fertilizer for sustainable organic farming. Vermicomposting with cattle dung is routine and well established practice, however, composting using equine dung is not well established. In the present study, we standardized methodology for making vermicompost using dung from mules and horses at our farm. Subsequently, the technology was field tested in Katra region.

At our farm, dung of mules was collected daily and 10 quintals mule dung was filled in HDPE vermibed and applied one crateful (14 kg) vermiculture having about 3-4 kg earthworms on the top layer of the dung and mixed in upper layer. A fine layer of neem leaves (*Azadirachta indica*) was also applied on the top to protect the earthworms from predators. Small quantity of water was regularly sprinkled at 2-3 day interval on the bed to maintain moisture. Similalry, one bed of horse dung was also filled on the same day for comparison. In the same manner, beds with dung

from mules and horses were prepared for simple composting (without worms). The mule and horse vermicompost were analyzed for pH, temperature and chemical composition (Table 1).

During 16 week period, the pH, temperature and dry matter of vermicompost ranged between 7.68 & 8.07, 30°C & 14°C and 19.36 & 62.53%, respectively. On the other hand, in case of simple composting, pH, temperature and dry matter ranged from 7.7 to 8.25, 48°C to 28°C and 24.32 to 65.86%, respectively. It took 3-3.5 months for conversion of dung to vermi-compost production. There was no significant difference in duration of vermicompost conversion between mule and horse dung.

A team from ICAR-NRCE visited the Jammu and Katra areas and collected the mule dung for the preparation of vermicompost and chemical composition of the vermicompost was compared with that of mule dung (Table.1).

Table 1. Chemical composition of vermicompost from mule dung prepared at Katra

Item	Moisture %	Total Nitrogen %	Total Phosphorus %	Total Potassium %	Electrical conductivity
Fresh mule dung from Katra	6.41	0.11	0.43	0.30	3.0
Prepared vermi-compost from lower layer	6.17	0.11	0.15	0.10	1.42
Prepared vermi-compost from upper layer	5.22	0.11	0.14	0.09	1.02
Biogas sludge	6.07	0.08	0.35	0.14	2.60

(B.N. Tripathi, R.K. Vaid and R.A. Legha)

Evaluation of total mixed rations for maintenance horses

The main concern of indigenous equine owners is maintenance of animal in healthy condition by feeding concentrate mixture prepared from locally prepared ingredients. Information pertaining to the requirement of the maintenance of Marwari horses is meager.

In an attempt to prepare concentrate mixture and total mixed ration (TMR), 24 feed samples were collected from Bikaner region. Based on Proximate principles and Van Soest's fibre analysis by in vitro analysis for evaluating digestibility, two concentrate mixtures were selected (i) 70% oats grain, 20% wheat bran & 10% groundnut cake and (ii) 80% barley grain, 10% wheat bran, 10% mustard cake.

The feeding trials using these two concentrate mixtures were conducted on five Marwari stallions (Average body wt. 338.4 kg). The animals were fed roughages ad libitum (wheat straw and groundnut haulm 50:50). The results were compared with standard concentrate mixture (30% gram, 40% oat grains and 27% wheat bran).

The body condition and body weight were regularly monitored. It took time for the animals to adjust with the mustard cake but digestibility was higher (60.96%), compared to standard (55.87%) and groundnut cake fed group (56.04%). However, glycemic index (GI) was better in the standard group (66.4) as compared to the treatments (80.7 and 87.3). Thus, the concentrate mixture containing GNC and Oats grain were found to be better wholesomely in term of digestibility.

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

Comparative proteomics analysis of horse, donkey and cow milk

Donkey milk is considered to have some characteristic proteins, which have therapeutic and cosmetic values. Therefore, the proteomic analysis of donkey milk vis-à-vis horse and cow milk was carried out. Milk samples from donkey and horse mares (n=4 each) were collected and processed after removing fat and casein. These samples were further subjected to protein quantification and protein profiling on SDS-PAGE (Fig. 1).

Species-wise samples were pooled and further processed for analysis by LC-MS/MS spectroscopy. Obtained proteomic spectrum was annotated with respect to UniPort (http://www.uniprot.org/) bovine and equine

database. Each protein listed in horse and donkey mare's milk was BLAST searched in NCBI database. A total of 212 and 211 proteins in horse and donkey mares were identified, respectively. Venn diagram analysis showed that out of these listed proteins, 203 proteins (92.3%) were common between horse and donkey (Fig. 2). There were 9 and 8 unique proteins in horse and donkey milk, respectively.

The identified proteins in each species were grouped based on their molecular functions, biological process, cellular component and protein class (according to PANTHER geneontology). Catalytic activity (43%) and binding proteins (35%) appeared to be dominant functional group in milk proteins in horse and donkey mares. Transporter activity proteins were only 8%. Milk of these species contains lot of proteins which are involved in various biological processes. Proteins involved in cellular (83) and metabolic processes (70) are represented maximum in the milk.

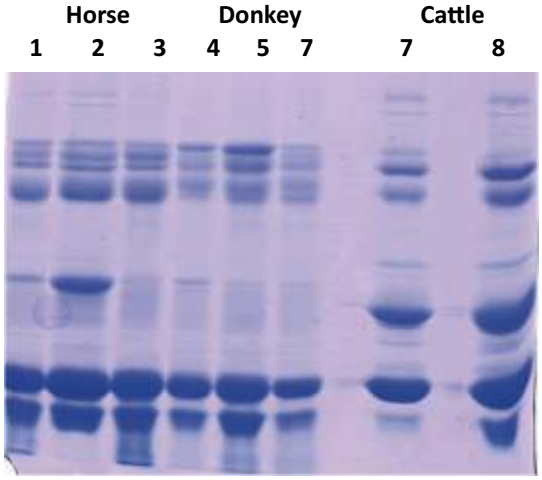


Fig. 1. SDS-PAGE protein profiling of horse, donkey and cattle milk

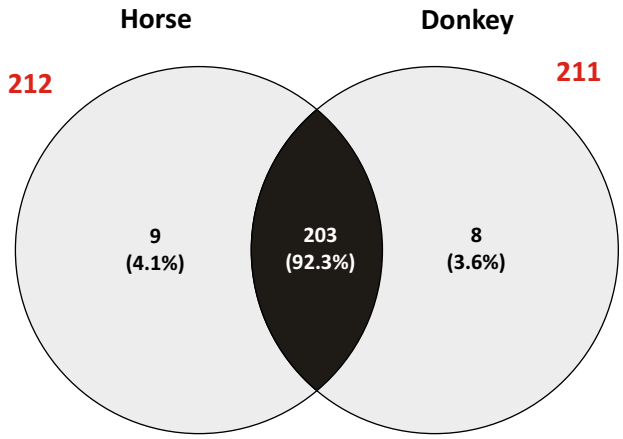


Fig. 2. Venn diagram analysis showing relationship between total proteome obtained from horse and donkey mare milk

Proteins involved in biological regulation, stimuli response and cellular component organization are >20. A total of 116 proteins were identified representing cellular component group of proteins. Cellular and organelle component protein groups were highly represented. Macromolecular complex, membrane and extracellular region proteins are also vividly represented. This milk proteomic data represented 22 protein classes involving 178 proteins. Nucleic acid binding, transferase, hydrolase, cytoskeletal protein, cell adhesion molecule, enzyme modulator, etc are the major protein classes represented in the horse and donkey mare milk.

A few uncommon proteins identified in horse and donkey milk need to be explored further to identify their biological role and molecular functions. Quantifications and expression behaviour would further help in assigning biological role to these proteins. This proteome has been defined as per geneontology database and has generated the basic data for further use.

(Yash Pal, Sanjay Kumar, Anuradha Bhardwaj, R.A. Legha and A.K. Mohanty)

National Centre for Veterinary Type Cultures

National Centre for Veterinary Type Cultures (NCVTC) activities include isolation, authentication and accessioning of microbes of animal and poultry origin. In addition, NCVTC also maintains microbial cultures, including various cell lines of animal, human and poultry origin in its repository. NCVTC receives microbial cultures from various network units located in different parts of the country. These network units submit their cultures to the NCVTC repository for authentication, accessioning and long term preservation in the repository. Upon receipt, these cultures are examined for their viability and identity. Once authentication process is completed these cultures are finally accessioned in the repository.

Isolation, authentication and accessioning of viruses

The Virology activities include maintenance and preservation of cell lines, isolation, passaging, revival, viability checking and preservation of virus isolates. The viruses deposited in the repository are passaged in appropriate cell lines, for ascertaining the viability, bulk cultivated and cryo preserved.

At present, NCVTC has 19 network units depositing microbial cultures in repository, of which 8 network units are exclusively dedicated to the reposition of microbes of veterinary importance. During the year 2016-17, NCVTC processed a total of 41 virus cultures received from various institutions (Table 1) including 8 virus cultures isolated from field samples during disease investigation.

Among these, fowlpox virus, Infectious bronchitis virus, duck plague virus, infectious bursal disease viruses, pigeonpox viruses were new additions to the NCVTC. The newly added poultry virus isolates exhibited classical lesions in embryonated chicken eggs upon passaging. The authentication and accessioning of pigeonpox virus and IBV was done by passaging in chicken embryo and by PCR (Figs 1-2).

Table 1. List of cultures processed during the year 2016-17

Source and Virus name	Submitted	Accessioned
College of Veterinary Sciences, Khanapara, Guwahati		
Fowlpox virus	4	2
Duck plague virus	4	4
Duckpox virus	1	0
Pigeonpox virus	1	1
Tamilnadu Veterinary and Animal Sciences University, Chennai		
Newcastle disease virus	2	2
Classical swine fever virus	1	1
Infectious bronchitis virus	1	1
Infectious laryngotracheitis virus	2	0
Fowl adeno virus	2	0
Chicken infectious anemia virus	1	0
Infectious bursal disease virus	2	0
ICAR-Indian Veterinary Research Institute, Izatnagar		
Bovine herpes virus-1	5	5
Canine parvovirus	3	0
NCVTC, National Research Centre on Equines, Hisar		
Classical swine fever virus	2	2
Newcastle disease virus	1	1
Sheep poxvirus	2	2
Infectious bursal disease virus	3	3
Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar		
Newcastle disease virus	4	4
Total	**41**	**28**

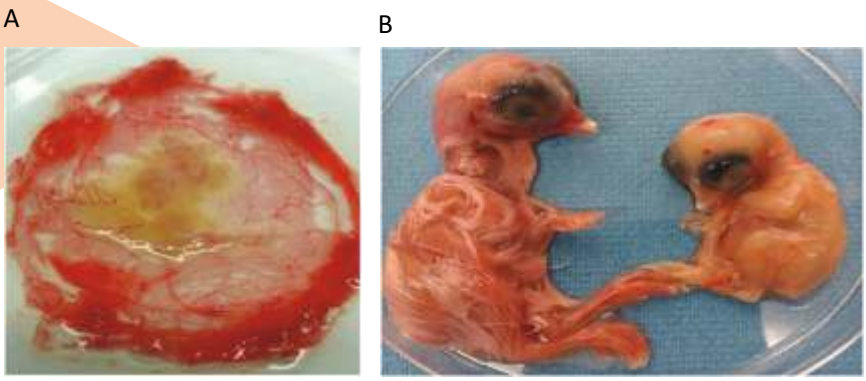


Fig1. Passaging of viruses in chicken embryos. Typical pox lesion of pigeon poxvirus on CAM on 5 dpi (A) and curling and dwarfing of embryo on 4 dpi by IBV (B)

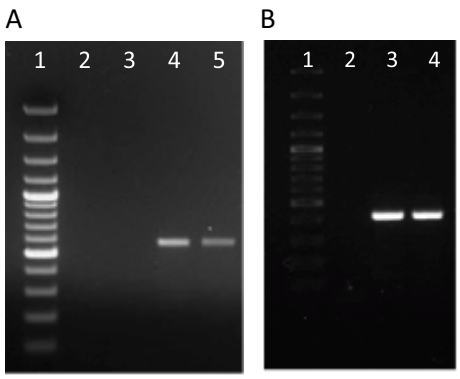


Fig. 2. PCR identification of accessioned viruses. Pigeonpox virus DNA polymerase gene amplicon of 550 bp (A) and IBV spike gene based amplicon of 386 bp (B)

(Naveen Kumar, Riyesh T, B.C. Bera, Taruna Anand and Sanjay Barua)

Investigation of an outbreak of sheeppox virus infection at Dobhi village, Hisar

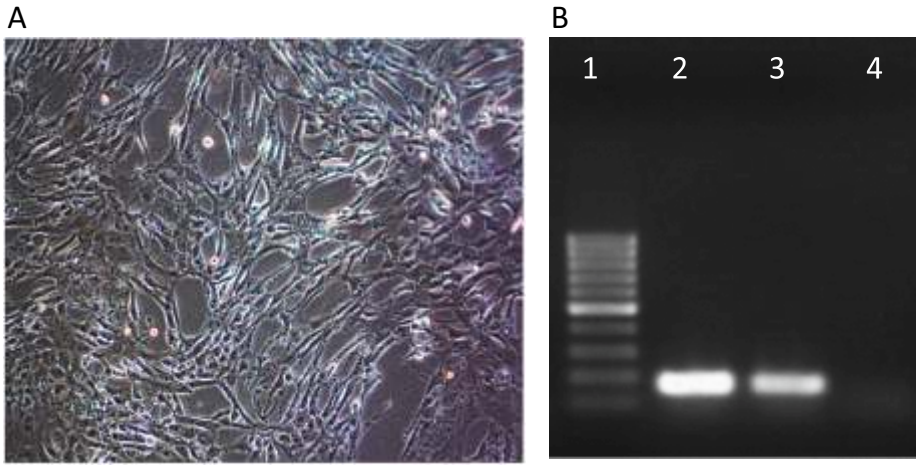


Fig. 1. Isolation and confirmation of sheeppox virus. CPE in lamb testicle cells on 4 dpi (A) and PCR amplification of P32 amplicon of 200 bp (B)

Sheep and Goat Pox (SGP) are highly contagious viral diseases of sheep and goats. Due to high morbidity and mortality associated with disease in susceptible animals, these viruses have major impact on small ruminant production. Sheep pox virus (SPV) and goat pox virus (GPV) were once believed to be strains of the same virus, but genetic sequencing has now demonstrated them to be separate viruses. The genus Capripox comprises sheeppox, goatpox and lumpy skin disease viruses as the etiological agents of sheeppox, goatpox and lumpy skin disease in cattle, respectively. Capripoxviruses are double-stranded DNA viruses belonging to the family *Poxviridae* subfamily *Chordopoxvirinae*. SGPV and LSDV are very closely related genetically, sharing at least 96% nucleotide identity.

An outbreak of sheeppox was reported in Dobhi, Hisar. Affected animals exhibited fever and the presence of pox-like lesions on skin. The macules enlarge to form papules which progress into scabs that eventually leave scars. Specimens (scab from pock lesions) were subjected for virus isolation in primary lamb testicle cells and detection of capropoxvirs-specific genome. At passage 3, cytopathic effects were observed in primary lamb testicle cells. Capripoxvirus was detected in cell culture adapted virus at P3 by PCR targeting P32 gene (Fig. 1). Two representative isolates, one from lamb and one from adult sheep were accessioned and preserved at NCVTC repository.

(Naveen Kumar, Riyesh T., Balvinder K. Manuja, S.C. Yadav and Sanjay Barua)

Prevalence of porcine respiratory viruses and development of their repository

Porcine respiratory diseases are major problems to the swine industry worldwide. To investigate the prevalence of respiratory viruses circulating among pigs, a total of 321 biological samples (nasal swabs, serum, and tissue) were collected from infected and apparently healthy pigs from various farms in Chhattisgarh (Nardaha, Manacamp, Kashiram, Somani & Durg), Maharashtra (Nagpur) and Guwahati (Hekera, Turukpara and AAU, Khanapara) (Fig.1).

Fig.1. Collection of samples from pigs

The nasal swab samples were processed for detection of various respiratory viruses by PCR and RT-PCR assays. Studies revealed positive results in samples for influenza A viruses (n= 28), PCV2 (n= 32) and PCMV (n= 11). Interestingly, one sample collected from Guwahati was positive for both PCV2 and PCMV (Fig. 2).

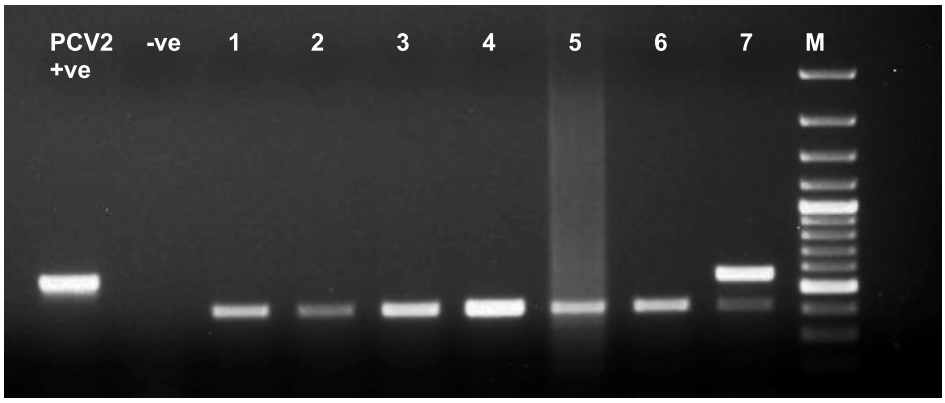


Fig. 2. Multiplex PCR based detection of PCV2 (565 bp) & PCMV (413 bp) viruses in clinical samples

The presence of viruses in samples was further confirmed by sequencing of PCR products. The PCR amplicons of various virus-specific regions i.e. 244 bp of M gene of influenza virus; 490 bp of ORF2 of PCV2 and 413 bp of gB gene of PCMV were amplified from the clinical samples, purified and sequenced. The sequence data subjected to BLAST, NCBI homology analysis and revealed 90-99.9% identity to the respective viruses. The porcine circovirus 2 was isolated from positive samples by serial passaging in PK-15 cells and isolates were confirmed by PCR and sequencing of partial ORF2 region of the virus. The study showed that three viruses namely influenza A virus, PCV2 and PCMV are circulating among pigs.

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

Sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) regulates PPR virus replication

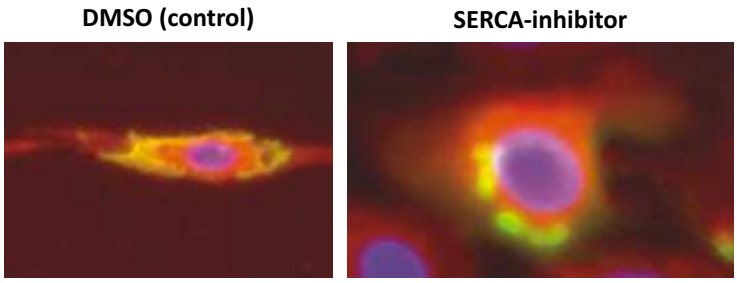


Fig. 1 Immunofluorescence assay showing localization of PPRV and SERCA proteins in SERCA inhibitor-treated and -untreated cells

Despite the availability of suitable vaccines, peste des petits ruminants (PPR) continues to be leading cause of livestock morbidity and mortality. The antiviral therapeutics assume enormous importance in providing instantaneous protection. However, antiviral therapeutics against PPR and other viral diseases of animals are unavailable.

In order to develop antiviral therapeutics against PPR, we screened a library of host cell's kinase and phosphatase inhibitors, where the yields of infectious virions were measured in the presence of the inhibitors or vehicle-control. At a noncytotoxic concentration, one of the inhibitor [sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) inhibitor] was found to significantly inhibit replication of PPRV, suggesting SERCA (a host protein) is critically required for PPRV replication. With the help of a time-course assay, it was demonstrated that SERCA inhibitor impairs late post-entry step of PPRV replication. SERCA inhibitor was not found to affect PPRV attachment to host cells. Likewise, no effect of SERCA inhibitor was seen on viral entry, RNA synthesis and budding. With the help of immunofluorescence assay, it was demonstrated that SERCA inhibitor interferes with the localization (transport) of the viral proteins from cytoplasm to the plasma membrane (Fig. 1). To demonstrate the interaction between SERCA and PPRV, a co-immunoprecipitation assay is currently underway. Besides, sequential passage of PPRV was performed in presence/absence of SERCA inhibitor.

We have defined a host target (SERCA) for development of antiviral therapeutics against PPRV. As compared to the compounds that directly act on viral proteins, host-targeting agents (such as SERCA) are of great clinical importance because they usually do not have a tendency in inducing antiviral drug resistance.

(Naveen Kumar and Sanjay Barua)

Accessioning of bacterial cultures

The importance of obtaining culture and preserving them *ex situ* has been increasing day by day. The pathogenic strains of bacteria isolated from animals need to be conserved for their future use. In order to increase the biodiversity of bacterial cultures in NCVTC, a total of 164 bacteria were accessioned during the year, making cumulative culture collection of 1201 bacteria of veterinary importance. Cultures were obtained from CIRG, Makhdoom; CMVL, Meerut; DUVASU, Mathura; CSWRI, Avikanagar; TANUVAS, Chennai; IVRI, Izatnagar; College of Veterinary Sciences, Khanapara; CSKHPKV, Palampur; NIVEDI, Bengaluru; NRCC, Bikaner; SKUAST, Jammu and NCVTC, Hisar (Table 1 and Fig. 1).

Table 1. Bacteria isolated and accessioned

Mannheimia caviae	*Plesiomonas shigelloides*	*Moraxella bovoculi*
Aeromonas trota	*Aeromonas veronii*	*Aeromonas. Sobria*
Streptococcus equines	*Streptococcus parasanguinis*	*Aerococcus urinaequi*
Truperella abortisuis	*Raultella terrigena*	*Bacillus licheniformis*
Kocuria marina	*Kocuria rhizophila*	*Pseudomonas flouroscens*
Lactococcus plantarum	*Lactococcus taiwanensis*	*Lactococcus fermentans*
Corynebacterium kutscheri	*Acinetobacter variabilis*	*Corynebacterium pseudotuberculosis*
Enterococcus kobei	*Enterococcus mundtii*	*Enterococcus casseliflavus*
Staphylococcus gallinarum	*Staphylococcus muscae*	*Staphylococcus chromogenes*
Acinetobacter hemolyticus	*Acinetobacter indicus*	*Acinetobacter baumanii*

Strains of other bacterial isolates like *Pasteurella multocida* ssp. multocida, *Aeromonas hydrophila, A. veronii, Salmonella Gallinarum, Proteus mirabilis, Pseudomonas aeruginosa, Rhodococcus equi, Staphylococcus aureus, Bacillus licheniformis, Bacillus cereus, Brucella melitensis, Listeria monocytogenes,* many serogroups of *E. coli, Salmonella* Typhimurium, *Acinetobacter baumanii, Enterococcus* sp, and many other including Clostridium sp. were also accessioned. These cultures were checked by sequencing of 16S rRNA gene and housekeeping genes and preserved by cryopreservation before accessioning.

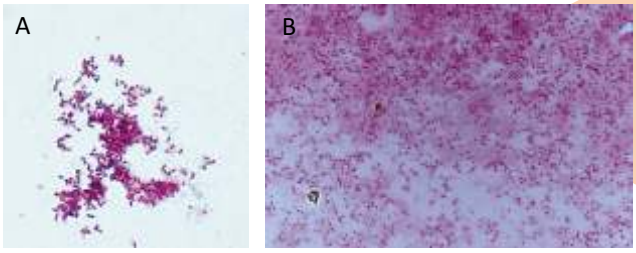


Fig. 1. Bacterial isolates accessioned. *Streptococcus parasanguinis* (A) and *Mannheimia caviae* (B)

Out of 103 pathological and environmental samples [equines (22), vermicomposting (38), buffalo (30), sheep (11), monitor lizard (1), pigs (1)], 319 isolations were made. Many new isolates have been identified like *Mannheimia caviae* and *Moraxella bovoculi* from sheep, *Lactococcus taiwanensis* (3 strains) from mule dung, *Aeromonas sobria, A. hydrophila, A. veronii, Shigella dysenteriae, Shigella sonnei* from poultry intestines; *Corynebacterium lactis.*

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

Pathogenic microorganisms in mule dung in hilly regions in India

Utilization of mules for transport in hilly regions leads to deposition of mule dung on hill tracks. This is one of the unique problems found in the hill tracts of tourism and religious importance in India. Mule dung contains microorganisms and pathogens such as *Clostridium, Rhodococcus*, etc. A study was conducted to evaluate the microbial status of the mule dung collected from Katra region (n=45). The dung samples were subjected to metagenomic analysis to understand the taxa level diversity of bacterial microbes in mule dung. Further, isolation, molecular identification by 16S rRNA based sequence data and, enumeration of different bacterial classes on selective media were done to quantify the amount of bacteria on a cfu/gram basis.

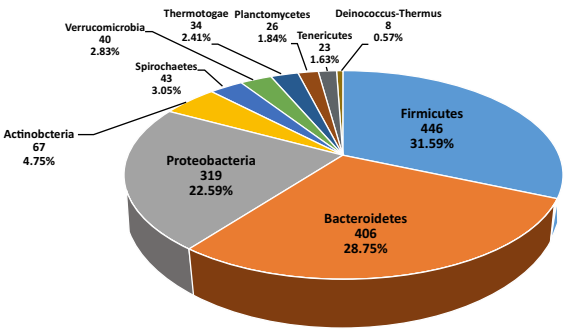


Fig. 1. Phylum level classification results of mule dung metagenomic analysis

In metagenomic studies, the raw paired end reads data were obtained with mean length of 298 bp, 17,46,583 reads per sample, and a total of 623178 merged reads. The results revealed that, Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, were the main phyla (83% of all OTU) in the mule dung sampled, and rest of the minor phyla identified were of Actinobacteria, Spirochaetes, Verrucomicrobia, Thermotogae, Plantomycetes etc. (Fig. 1).

From the selective media, 362 bacterial isolates were picked up from plates and cryopreserved, and few of the unique or haemolytic isolates were targeted for molecular identification. The significant finding has been identification of *Enterococcus casseliflavus* as the most dominant enterococci followed by *Enterococcus faecium & Enterococcus mundtii* (Table 1). The fecal enterococci *Enterococcus casseliflavus* was the most predominant in mule faeces followed by detection of one each isolate of *Enterococcus faecium* and *Enterococcus mundtii*.

Table 1. Some of the isolates identified from mule dung representing different taxa

Prolinoborus fasciculus	*Escherichia hermanii*	*Enterobacter kobei,*
Serratia marcescens	*Enterococcus casseliflavus* (9 hemolytic isolates)	*Enterococcus faecium,* and *Enterococcus mundtii*
Streptococcus equines	*Lactococcus taiwanensis* (4 isolates)	*Acinetobacter hemolyticus*

Four *Lactococcus taiwanensis* strains (Mu21A, Mu23A, Mu30A, and Mu36A) were phylogentically analysed with other *Lactococcus* sp. type strains using 16S rRNA sequences, three of them clustered as outgroup indicating probability of novel taxa, whereas one strain (Mu21A) clustered close to Type strain (Fig. 2).

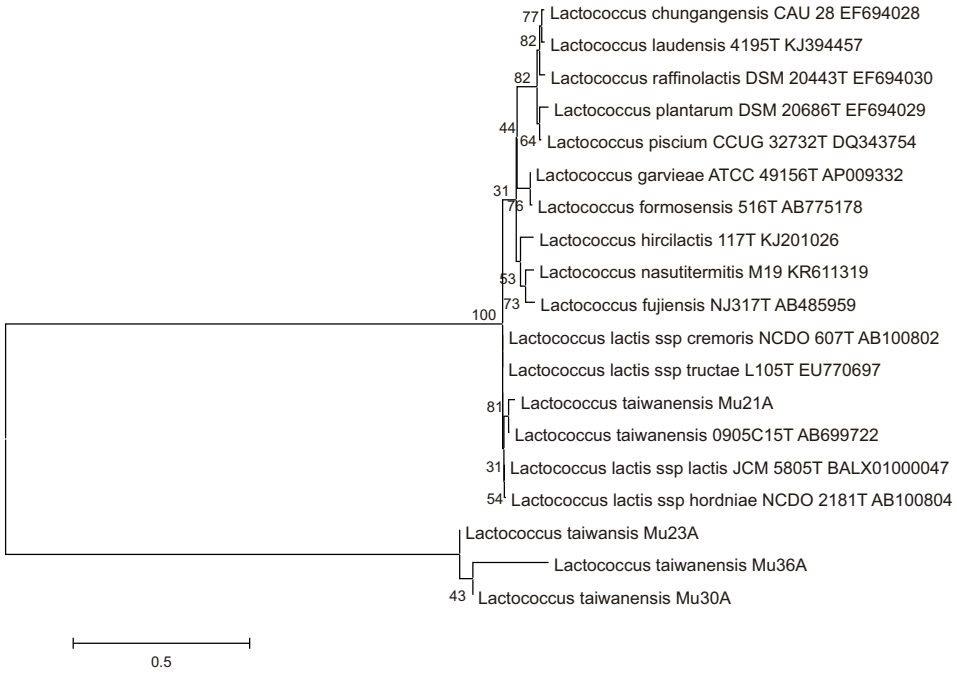


Fig. 2. Phylogenetic analysis of *Lactococcus taiwanensis* isolated from mule dung

(R.K. Vaid, Taruna Anand, R.A. Legha and B.N. Tripathi)

Characterization of bacteriophages isolated from *Shigella* sp.

The emergence of multi-drug resistance in *Shigella*, the causative agent of bacillary dysentery is becoming a serious concern and calls for the rational use of antibiotics. There is need to evaluate alternative treatment measures, incuding bacteriophage therapy. During the year, five *Shigella* sp. bacteriophages were characterized by assessment of biological activity, temperature and pH sensitivity.

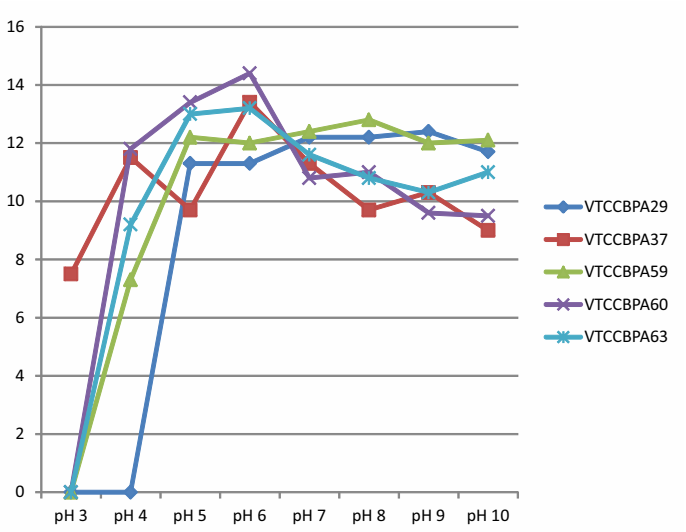


Fig.1: The pH sensitivity of bacteriophages against *Shigella* sp.

For this, five *Shigella* sp bacteriophages - VTCCBPA29 (isolated from poultry litter), VTCCBPA37 (isolated from village sewage), VTCCBPA59, 60 and 63 (isolated from animal dung) were used. All five phages were stable up to temperature of 55°C. Upon assessment of pH sensitivity, BPA37 was found to be stable in pH range of 3-10 whereas rest of the phages were stable at pH 4-10 (Fig. 1). The plaque characteristics ranged from pin-point to 2-3 mm in size. The protein profiles were generated in 10% SDS-PAGE gels. The phages were characterized by biological activity using spot test. Further characterization of the *Shigella* phages by PCR is underway.

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

Therapeutic trial of a novel thermotolerant bacteriophage against *Klebsiella pneumonia* in mouse model

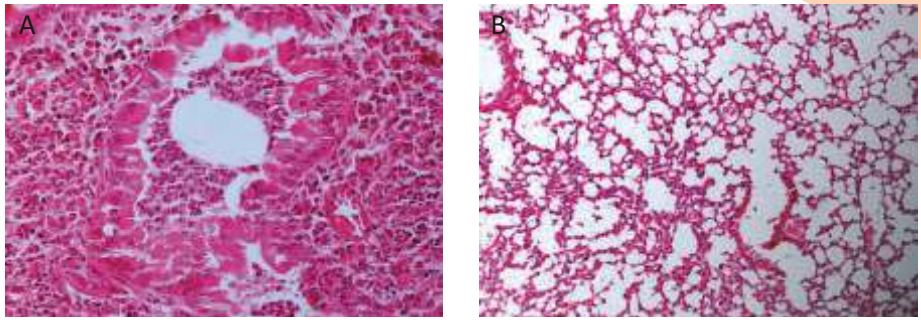


Fig. 1. Lung sections showing frank necrosis in alveolar parenchyma and bronchiolar epithelium with neutrophilic infiltration (A) and open alveolar spaces with mild neutrophilic infiltration (B) at 3 dpi in a mouse infected with *Klebsiella pneumoniae* infection (H&E 400X)

Bacteriophages influence biogeochemical and ecological processes by controlling bacterial diversity and density. In this study, a novel bacteriophage was isolated from river Ganga and characterized. The phage was found to exhibit thermotolerant and pH tolerant characteristics. The corresponding host bacteria was identified as *Pseudomonas alcaligenes* by biochemical characterization and 16s rRNA sequencing. Upon visualization by transmission electron microscopy, the phage BPA43 was found to belong to family *Myoviridae*. The protein profile of phage BPA43 showed major protein bands of 32 kDa and 20 kDa. The phage was used for therapeutic trial against *Klebsiella pneumoniae* (MTCC109). The phage was able to completely eliminate established infection in mouse model (Fig. 1) within a period of 10 days.

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

Equine cadaver soil as a source of bacteriophages

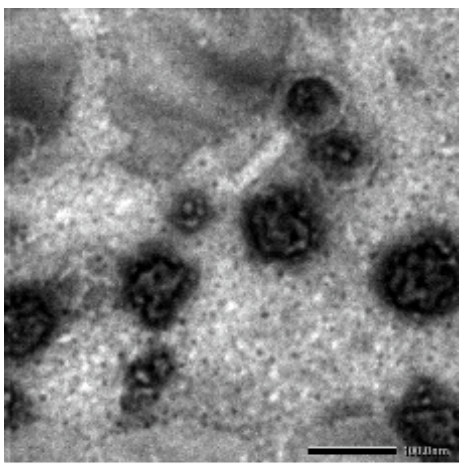


Fig. 1. *Myoviridae* isolated from equine cadaver affected soil

Bacteriophages are abundantly present in environment and they evolve rapidly with the host evolution. Their presence in soils of disposal sites including cadaver affected sites is important to assess the biodegradation patterns and to gain an insight into the issue of animal-soil transmission of microbes and to depict the role of phages in biological dynamics of manure thus formed. Bacterial isolates, *viz*., *Arthrobacter creatinolyticus*, *Bacillus pichinotyi*, *Bacillus cereus* group (Bcg) member and *Caryophanon* spp were isolated from the equine cadaver affected soil and identified on the basis of 16s rRNA sequence analysis. Using these host bacteria, enrichment of bacteriophages was successfully carried out from cadaver affected soil. The plaque characteristics and the phage titre (PFU/ml) of purified and polyethylene (PEG) concentrated bacteriophages were noted. The phage isolated against the BCG host morphologically resembles *Myoviridae* (Fig. 1) and has an isometric head of 69 nm diameter and a tail of 131 nm with visible base plate. The one-step growth experiment revealed that the average burst size of BPA38 was about 200 PFU/cell, and the latent period was 30 minutes. The bacteriophage BPA38 was biologically active within the temperature range of 4-45°C however it lost complete biological activity beyond 55°C temperature. The bacteriophage showed biological activity ranging from slight (+), moderate (++) to significant against 6/19 (31.6%) of a total of 19 *Bacillus* spp. isolates tested including *B. cereus* isolated from goat mastitic milk.

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

Conservation of animal microbial diversity in north-eastern region of India

National Centre for Veterinary Type Cultures is engaged in identification, characterization, and reposition of microbial cultures/isolates collected from NER and in establishing regional replicate repository at College of Veterinary Sciences, Khanapara, Guwahati. During the year, eight viruses were accessioned from NER including fowlpox virus (2), duck plague virus (4), pigeonpox virus (1) and Newcastle disease virus (1).

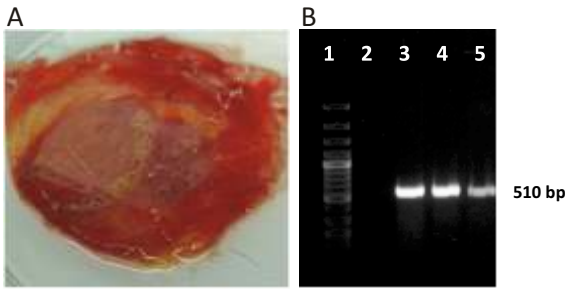


Fig. 1. Isolation of fowlpox virus in chicken embryo CAM (A) and confirmation by PCR (B)

Authentication and accessioning of fowlpox virus: Two fowlpox isolates (RR/2016/690 and RR/2016/328) deposited by COVSc, Khanapara, Guwahati were processed for virus identification by PCR targeting DNA polymerase gene and propagated in embryonated chicken eggs for assessing viability (Fig. 1). Both these isolates were subsequently accessioned in the repository.

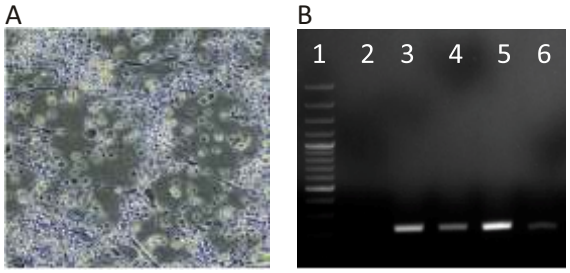


Fig. 2. Duck plague virus isolation in chicken embryo fibroblast (A) and confirmation by PCR (B)

Authentication of duck plague virus: Four duck plague virus isolates (RR/2016/686, RR/2016/687, RR/2016/688, RR/2016/689) deposited by COVSc, Khanapara, Guwahati were propagated in embryonated chicken eggs and identified by PCR targeting DNA polymerase gene (Fig. 2). The viruses produced cytopathic effect consisting of cell rounding and desquamation in chicken embryo fibroblast culture and subsequently accessioned in the repository.

Authentication of pigeonpox virus: One pigeonpox virus isolate (RR/2016/692) deposited by COVSc, Khanapara, Guwahati was also processed for virus identification by PCR targeting DNA polymerase gene and propagated in embryonated eggs for assessing viability. The virus produced typical pock lesion on CAM of chicken embryo.

Accessioning of bacteriophages: Sewage, soil and fecal materials were collected from poultry and piggery farms from NER for isolation of bacteriophages. Fourteen bacteriophages were isolated and accessioned against *Salmonella enterica* Paratyphi, *S. enterica* Typhimurium, *Citrobacter freundii*, etc (Fig. 3).

Fig. 3. Bacteriophage against *Salmonella enterica* isolated from sewage of pig farm

Accessioning of bacterial cultures: A total of 61 bacterial cultures were received from COVSc, Khanapara, Guwahati and ICAR Research Complex for N.E.H (Meghalaya) and 30 cultures were authenticated for accessioning in the repository.

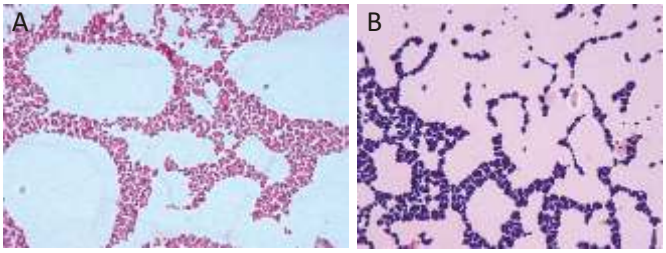


Fig. 4. Bacterial isolation from NER. VTCCBAA1145 *E.coli* (A) and VTCCBAA1139 *Staphylococcus aureus* (B)

The accessioned bacterial cultures include 14 *Escherichia coli* isolates from fecal swabs of duck, piglet, deer and elephant (Fig. 4a), two *Staphylococcus aureus* isolates from cattle blood and lung of swine (Fig. 4b), one isolate each of *Streptococcus* sp (duck), *Pseudomanas* sp. *and Bacillus* from different animals. Three cultures obtained from Yak were of *Escherichia marmotae, E.fergusonii and E.coli*. The accessioned cultures have been preserved by cryopreservation and accession numbers communicated.

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

Technology Development, Transfer and Commercialization

ICAR-National Research Centre on Equines is continuously striving for the upliftment of equine sector in the country since its inception. The institute's major efforts are focussed on development of technologies for improvement in equine health, production and utilization. Many diagnostic kits, vaccines and biologicals have been developed by the scientists of ICAR-NRCE for technology transfer and commercialization.

Technologies for commercialization

- Updated equine influenza vaccine
- Inactivated equine herpesvirus-1 vaccine (Equiherpabort)
- Equiherpes B-ELISA kit for diagnosis of EHV1 infection
- Monoclonal antibody-based ELISA kit for diagnosis of rotavirus infection
- Recombinant antigen ELISA kit for *Theileria equi* diagnosis
- Recombinant protein based ELISA for diagnosis of glanders
- Recombinant protein based ELISA for diagnosis of EIA
- Pregmare kit for pregnancy diagnosis in mares
- Cryopreservation of equine semen

Technologies being developed

- Recombinant gG-based type-specific ELISA for differentiation of EHV1 & 4 infection
- Recombinant protein based ELISA for diagnosis of trypanosomosis
- Lateral flow assay for diagnosis of trypanosomosis/equine piroplasmosis
- Lateral flow assay for pregnancy diagnosis in mares
- Monoclonal antibody-based sandwich ELISA for equine influenza virus

TECHNOLOGY DEVELOPMENT & ASSESSMENT

Updated equine influenza vaccine released by ICAR-NRCE

An outbreak of equine influenza occurred during 2008-09 that was caused by equine influenza virus of subtype H3N8 belonging to Clade 2 of Florida sub lineage. The disease resulted in heavy morbidity and led to huge economic loss. Subsequently, the previously developed vaccine was updated using A/eq/Katra (Jammu)/06/08 (H3N8) virus with HA content of 20 µg/dose. The vaccine virus was grown in embryonated chicken eggs, purified by ultracentrifugation and inactivated by formalin. Field trials in 150 horses resulted in development of protective antibody titres, without any adverse reactions or clinical signs, following booster vaccination after four weeks.

DG, ICAR releasing equine influenza vaccine

It will be very useful for vaccinating the animals, which are under continuous movement inside and outside the country. The technology was released by Secretary DARE & Director General ICAR on August 22, 2016 at ICAR-NRCE, Hisar.

Field validation of rapid diagnostic kit for *Theileria equi*

Field validation of the LFA kit

Equine piroplasmosis is an acute, sub-acute or chronic tick-borne disease of equines, caused by an intra-erythrocytic haemo-protozoa *Theileria equi* or *Babesia caballi*. Significant segment of the Indian equine population (~35%) is latently infected and diagnosis of these animals is of more relevance to prevent spread of the parasitic infection to naïve animals. In an effort to provide a farmer friendly field test kit, the Centre has successfully developed lateral flow assay (LFA) for diagnosis of *T. equi* infection. The kit is based on a recombinant *T. equi* merozoite surface antigen (EMA-2) conjugated with gold-nano particles. Optimum conditions for LFA w.r.t. conjugation, membrane type and pore size, sample dilution, etc., have been standardized. During the year, field validation of LFA kit was done and results were compared with ELISA, cELISA (VMRD, USA), MASP *in vitro* culture and PCR.

A total 97 bio-samples collected from Haryana, Rajasthan and Gujarat were tested in LFA and results were compared. Out of total 97 serum samples tested, 73 were found positive in ELISA's and 69 in LFA. *Theileria equi* specific DNA could be demonstrated in PCR in 63 samples, while we were successful in demonstrating live *T. equi* parasites through MASP culture system in 62 blood samples. The diagnostic sensitivity (Dsn) and specificity (Dsp) of LFA vis-à-vis ELISA were 0.945 and 0.916, respectively, indicating its applicability on the field samples.

Validation of r-protein ELISA kit for differentiation of EHV1/4 infection

EHV1 and EHV4 together are responsible for equine rhinopneumonitis, an OIE listed disease of equines. EHV1 is also the foremost cause of abortions and neurological disorders. The differential diagnosis of EHV1 and EHV4 viruses is often complicated due to antigenic cross-reactivity between the two viruses. We developed a recombinant protein based ELISA kit for differential diagnosis of EHV1 & EHV4 infections.

This type-specific ELISA has been validated using field serum samples in single dilution format. The ELISA was employed on 1600 field equine serum samples and a total of 136 (8.5%) samples were found positive for EHV1, while 914 samples (57.12%) were found positive for EHV4. A total of 48 samples (3.0%) were found positive for both EHV1 and EHV4.

The ELISA was transformed into a kit format and 659 more serum samples were screened by for detection of EHV1/4 antibodies. A total of 42 serum samples (6.37%) were found positive for EHV-1 while 425 samples (64.49%) were positive for EHV-4. The shelf life of the kit was found to be more than 4 months.

TRANSFER OF TECHNOLOGIES

ELISA for screening of glanders provided to State Diagnostic Laboratories

On the backdrop of emergence of glanders outbreak in India since last one decade, NRCE developed a recombinant protein-based ELISA. This indirect ELISA is based on recombinant *B.mallei* protein and has been validated at NRCE. The reproducibility and repeatability of the assays has been tested in six laboratories using blind serum panel and is currently under international validation process in OIE Referral Laboratory on glanders, FLI Germany.

In order to develop diagnostic competence of state diagnostic laboratories, training was imparted to veterinary officers from Haryana, Himachal Pradesh and Gujarat. After training, ELISA reagents were provided to start testing at Ahmedabad, Sonepat and Shimla for screening of equine glanders. A total of 5335 samples were tested at Ahmedabad and 1564 at Sonepat laboratory.

Field demonstration of vermicomposting of mule dung

Utilization of mules for transport in hilly regions leads to deposition of mule dung on hilly tracks. There is a continuous problem of piles of mule dung on hilly tracks. Shri Mata Vaishno Devi Shrine Board requested NRCE to provide solution for mule dung disposal on pilgrimage route. Accordingly, the technology for vermicomposting of mule dung was standardized at our farm. Subsequently, the technology was field tested in Katra region. The kits for vermicomposting of mule dung were supplied to Katra region and quality of the vermicompost so generated was analyzed.

Vermicompost prepared from mule dung

Demonstration of semen cryopreservation technology to equine breeding stud

The Centre has perfected the technology for semen cryopreservation of stallions in field for use in artificial insemination. Equine Breeding Stud Hisar is currently using fresh semen of the stallions for artificial insemination. EBS showed keen interest in AI using cryopreserved semen. Accordingly, a team of scientists from EPC, Bikaner demonstrated the technique of AI using frozen semen to the EBS personnel. Semen was collected from seven exotic stallions and evaluated for seminal characteristics and freezability at EBS, Hisar. The semen from exotic stallions was cryopreserved using conventional vapor freezing technique.

Demonstration of semen collection and cryopreservation

COMMERCIALIZATION

Revenue generation through diagnostic services and consultancy

During 2016-17, diagnostic services were provided to stakeholders from various states viz., Maharashtra, Rajasthan, Delhi, Haryana, Punjab, Tamil Nadu, Uttar Pradesh, Madya Pradesh, Karnataka, Arunchal Pradesh, Andhra Pradesh, Uttarakhand, Gujarat, Jammu & Kashmir and West Bengal.

Under contractual services, 3806 equine samples were tested for equine infectious anemia and 4935 for glanders. Among other diseases, 173 vaginal swab samples for contagious equine metritis, 19 samples for equine viral arteritis and 25 samples for African horse sickness were tested. Through these services, revenue of about Rs 52.21 lakhs was generated during the year, including Rs. 2854900 by Glanders testing and 1753533 by EIA testing (Table 1).

Table. 1: Revenue generated by disease testing services

Disease	Revenue (in Rs)
Glanders	2854900
EIA	1753533
CEM	227000
Piroplasmosis	141500
EHV1	16000
EI	52000
EVA	40000
AHS	30500
Dourine	29000
JE/WNV	24000
T. evansi	38000
Rotavirus	10500
Bacterial analysis	5000
Total	**5221933**

Education and Trainings

Farmers' trainings on integrated farming in arid zone

The Centre in collaboration with Society for Agriculture and Arid Ecology Research organized four 2-days farmers' trainings on "शुष्क क्षेत्र मे समन्वित फसल प्रणाली" on 9-10 February, 11-12 February, 20-21 February and 26-27 February 2017. In these training programmes, scientists from ICAR-NRCE, ICAR-CIAH and ICAR-CAZRI trained farmers on knowhow's in modern agriculture practices in farming and agriculture. In these trainings, 129 farmers from various villages of Bikaner district learned about livestock rearing, vermicompositing process, innovative methods of agriculture and horticulture in the arid region of Rajasthan. This programme was sponsored by ATMA, Bikaner.

Participants in training on integrated farming

Hands on training on pregnancy diagnosis to ITBP officers

ITBP officers with the Director NRCE

A two-day hands-on-training on 'Pregnancy diagnosis by ultrasonography' was organized for the Officers from the Indo-Tibetan Border Police on 25-26 October 2016. In this training, seven participating veterinarians were delivered lectures and imparted practical training on pregnancy diagnosis by rectal examination and ultrasonography.

Biosafety training for NRCE staff and students

Basic biosafety training was organized on 15 October 2016 at NRCE, Hisar. In this, 36 scientists, technicians, students and other project staff were imparted training on laboratory biosafety and a lecture-cum-practical demonstration on Biosafety in research laboratories was conducted.

Expert lectures by NRCE scientists in training courses

1. Dr. Baldev R. Gulati delivered an invited lecture on "Equine herpesvirus diagnosis" in training course on "Diagnosis of livestock diseases: a molecular approach", Department of Animal Biotechnology, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana on 02 March 2017.
2. Dr. Baldev R. Gulati delivered an invited lecture on "Risk analysis and biosafety in research laboratories" in training course on "Basic biotechnology and bioinformatics tools", Department of Animal Biotechnology, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana on 03 June 2016.

3. Dr. Baldev R. Gulati delivered a lecture on "Biosafety in research laboratories" at ICAR-NRCE, Hisar, Haryana on 15 October 2016.
4. Dr. Baldev R. Gulati delivered a lecture on "Characterization of monoclonal antibodies by immunoblotting" in 29th CAFT training on "Modern technologies for production and applications of antibodies for animal health improvement", Department of veterinary microbiology, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana on 06 March 2017.
5. Dr. Baldev R. Gulati delivered a lecture on "Isotype determination of monoclonal antibodies by ELISA" in 29th CAFT training on "Modern technologies for production and applications of antibodies for animal health improvement", Department of veterinary microbiology, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana on 07 March 2017.
6. Dr. Baldev R.Gulati delivered a lecture on "Trends in diagnosis and vaccinology for equine herpesvirus infections in India" in International Winter School on "Role of molecular biology in disease diagnosis and development of new generation vaccines", School of animal biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab on 19 November 2016.
7. Dr. Naveen Kumar delivered an invited lecture on "Isolation, identification and purification of multiple viruses from mixed infection" in training on "Diagnosis of livestock diseases: a molecular approach", Department of animal biotechnology, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana from 14 February-06 March 2017.
8. Dr. Rajesh K. Vaid delivered an invited lecture on "Advanced techniques in microbial identification'' in International Winter School on "Role of Molecular Biology in Disease Diagnosis and Development of New Generation Vaccines", School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab on 19 November 2016.
9. Dr. Talluri Rao, Dr. P.A. Bala and Dr. S.K. Ravi delivered lectures for the trainees of Service Selection Board (Vet Wing) at NRCE, Hisar, Haryana on 15 June, 2016.
10. Dr. Yash Pal delivered an expert lecture on "Application of ultrasound in equines" in training course on "Ultrasonography and fertility management in large animals" at Central Institute for Research on Buffaloes, Hisar, Haryana on 29 November 2016.
11. Dr. Yash Pal delivered an expert lecture on "Use of ultrasonography in equine reproduction" in training on "Ultrasonography and fertility management in large animals" at Central Institute for Research on Buffaloes, Hisar, Haryana on 26 July, 2016.

Participation of Scientists and Staff in Trainings

1. Dr. Naveen Kumar participated in training course on "Genome-wide siRNA screens for foot-and-mouth disease virus (FMDV) and herpes simplex virus (HSV)" at Division of Infection and Pathway Medicine, University of Edinburgh, UK under Commonwealth professional fellowship, UK, from 01st March -31st May, 2017.
2. Dr. Sanjay K. Ravi participated in a training course on "Hands on training on *in vitro* fertilization technology" at Animal Biotechnology Centre, NDRI, Karnal, Haryana from 15-24 November 2016.
3. Dr. Sanjay Kumar participated in the training on "Development of signalling pathway networks and analysis of transcriptomics and proteomics data" at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala from 28-30 November 2016.
4. Dr. Taruna Anand participated in "HRD nodal officers training" at NAARM Hyderabad, Telengana from 16-18 February 2017.
5. Sh. Ram Pal, Assistant Administrative Officer, Sh. Subhash Chander, Assistant and Sh. Sunil Sharma, Assistant participated in the training on "e-Procurement solution through CPP Portal" at ICAR-NDRI, Karnal, Haryana from 21-22 July 2016.
6. Shri Sunil Sharma, Assistant participated in the training course on "Purchase Management in Government" at Institute of Secretariat Training & Management (ISTM), New Delhi from 30 November to 1 December 2016.

Post-graduate students guided at NRCE

Sr. No.	Student	Guide	Topic
PhD			
1.	Narender Singh Rathore, RAJUVAS	T.R. Talluri	Isolation, culture and characterization of stem cells derived from extra embryonic tissues of indigenous equines
2.	Ashok Choudhary, RAJUVAS	T.R. Talluri	Evaluation of various parameters affecting semen quality in Marwari stallion
3.	Sheetal Saini, CDLU	H.S. Singha	Expression of recombinant equine cytokines and analysis of their biological activities
4.	V. Balena, IVRI	B.N. Tripathi	Generation of recombinant equine herpesviruses 1 through BAC mediated deletion mutagenesis and their comparative pathogenicity and immunogenicity in murine model
5.	Ramesh Kumar, LUVAS	N. Virmani	Pathological investigation and protective immunity of recombinant vaccine candidates of equine influenza virus in BALB/C mice
6.	Deepak Kumar Sharma, RAJUVAS	Naveen Kumar	Dynamics of inflammasome activation following exposure to PPR virus
MVSc			
7.	Tarachand Nayak, RAJUVAS	R.K. Dedar	Studies on prevalence and risk associated factors of enteric salmonella (inv A gene) in Horses in Bikaner
8.	Preeti S., RAJUVAS	R.A. Legha	Study on the effect of North and south faced housing system, on the performance of growing foals in semi arid conditions of Rajasthan
9.	Manish Songara, RAJUVAS	R.A. Legha	Effect of dietary inclusion of azolla on nutrient utilization and semen quality of Marwari stallions
10.	Sourabh Kant, IVRI	Yash Pal	Effect of caffeine as an additive in semen extender to improve frozen thawed semen quality of Marwari horses and exotic donkeys
11.	Manu K Mathew, IVRI	N. Virmani	Immunological and pathological evaluation of protective efficacy of inactivated recombinant EIV vaccine candidate with different adjuvant formulations in murine model
12.	Ameya Gupte, LUVAS	B.R. Gulati	Development of peptide ELISA for serodiagnosis of equine *herpesvirus* 1
13.	Amit Chotia, RAJUVAS	Vijay Kumar	Effect of draught loads on hematobiochemical indices during carting in donkeys.
14.	Pradeep Kumar Godwal, RAJUVAS	R.A. Legha	Haematobiochemical indices of heat tolerance in exotic donkeys (*Martina franca*)
15.	Rabina Kumar, RAJUVAS	Sanjay Kumar Ravi	Studies on the post thaw semen quality of Marwari horses and Poitou donkeys with addition of Alpha-tocopherol, Pentoxyphylline and Tuarine in semen extender
16.	Ankur Verma, RAJUVAS	R.K. Dedar	Therapeutic efficacy of aqueous extract of *Ocimum sanctum* (Tulsi) on oxidative stress in horses
17.	Prerna Yadav, RAJUVAS	R.K. Dedar	Therapeutic efficacy of aqueous extract of *Withania somnifera (Ashwa Gandha)* on oxidative stress in horses
18.	Prashant Kumar, RAJUVAS	T.R. Talluri	Effect of ascorbic acid and glutathione on prefreeze and post thaw quality of equine semen

Workshop, Seminar and Institutional Activities

World Veterinary Day celebrated with equine farmers at Umra

The Centre organized an equine health camp-cum-interactive meet at village Umra, Hisar (Haryana) on the occasion of "World Veterinary Day" on 30 April 2016 on the theme 'promoting use of science for the welfare of farmers'. Scientists educated equine owners about latest developments in equine husbandry and provided solutions to problems faced by equine owners in housing, hoof care, shoeing and grooming of animals. On this occasion, sick equines were examined by a multidisciplinary team of veterinarians and free of cost treatment was provided for parasitic infestations, wound infections and lameness. The clinical samples were collected for laboratory disease investigation.

Animal health camp at Umra, Hisar

Yoga camp on International Yoga Day

A nine day Mass Yoga Performance Camp was organized at NRCE Hisar from 13-21 June 2016. In this Camp, all employees of NRCE and their family members practised Yoga and Pranayama as per Common Yoga Protocol developed by Ministry of Ayush, GoI. On International Day of Yoga on 21 June, expert lectures on "Yoga and Health" and "*Yoga for Peace and Harmony*" were also organized.

Celebrating International Day of Yoga at NRCE

हिन्दी पखवाड़े में विभिन्न प्रतियोगिताओं का आयोजन

भारत सरकार की राजभाषा विकास नीति के अंतर्गत इस वर्ष राजभाषा कार्यन्वयन समिति के तत्वावधान में हिन्दी के प्रयोग को अधिकाधिक प्रोत्साहित करने के लिए केन्द्र में हिन्दी पखवाड़े का आयोजन 14 से 28 सितम्बर, 2016 तक किया गया। इस दौरान विभिन्न ज्ञानवर्धक एवं रूचिपूर्ण हिन्दी प्रतियोगिताओं क्रमश: निबंध प्रतियोगिता, हिन्दी परिच्छेद अनुवाद, हिन्दी श्रुतलेख, हिन्दी आशुभाषण, हिन्दी प्रश्नोत्तरी, हिन्दी कविता पाठ एवं सुलेख, टंकण आदि का आयोजन किया गया। उद्घाटन सत्र में एक हिन्दी कार्यशाला का आयोजन भी किया गया जिसमें ठाकुरदास भार्गव सीनियर सैकेण्डरी स्कूल के डॉ. शमशेर सिंह तथा साहित्यकार एवं गजलकार श्री महेन्द्र जैन विशिष्ट अतिथि के रूप में उपस्थित थे। डॉ. शमशेर सिंह ने हिन्दी को एक वृहद भाषा के रूप में परिभाषित करते हुए इसे एक सहज,

मेजर जनरल डॉ. रंजीत सिंह हिन्दी पखवाड़े में सम्बोधित करते हुए

कविता-पाठ प्रतियोगिता में भाग लेते हुए प्रतिभागी

सरल व आमजन की भाषा बताया। विशिष्ट अतिथि श्री महेन्द्र जैन ने अपनी मधुर अवाज में गजल प्रस्तुत कर श्रोताओं को मंत्रमुग्ध कर दिया।

हिन्दी पखवाड़े का समापन समारोह में मेज़र जनरल डॉ. रंजीत सिंह, कुलपति चौधरी रणबीर सिंह विश्वविद्यालय, जीन्द मुख्य अतिथि एवं श्री महेन्द्र पाल कुलश्रेष्ठ, निदेशक, राष्ट्रीय सूचना केन्द्र, हिसार एवं श्री पी.के. पाण्डेय, निदेशक, उत्तरी क्षेत्र कृषि मशीनरी प्रशिक्षण एवं परीक्षण संस्थान, ट्रैक्टर नगर, सिरसा रोड, हिसार विशिष्ट अतिथियों के रूप में मौजूद थे। समापन समारोह के दौरान सरकारी कर्मचारियों के लिए लिए कविता पाठ एवं हिन्दी शब्दानुवाद प्रतियोगिता का आयोजन एवं पुरस्कार वितरण किया गया। कार्यक्रम के अध्यक्ष डॉ. भूपेन्द्र नाथ त्रिपाठी ने अपने संबोधन में हिन्दी को एक परिष्कृत व प्रभावशाली भाषा बताया। निदेशक महोदय एवं मुख्य अतिथि ने हिन्दी पखवाड़ा में बच्चों की प्रतियोगिता की सराहना की और उन्हें पुरस्कार देकर प्रोत्साहित किया।

Farmers meet at EPC, Bikaner on Foundation Day

Equine Production Campus, Bikaner organized an equine owners-famers-scientists interactive meet on 28 September 2016 to celebrate its Foundation Day. Addressing equine owners, Chief Guest Brig S.S Kashyap, Commandant, Equine Breeding Stud, Hisar emphasized the need for conservation of indigenous breeds of equines. Dr B.N. Tripathi, Director NRCE explained the role of Bikaner campus in conservation and characterization of indigenous equines and asked farmers to use services of artificial insemination for superior equine production.


Brig. Kashyap addressing farmers

Vigilance awareness week celebrated for promoting integrity


Staff taking pledge for eradicating corruption

The Vigilance Awareness Week was celebrated from 31 Oct to 5 Nov. 2016 on the theme "Public participation in promoting integrity and eradicating corruption". On this occasion, all employees were administered the pledge for promoting integrity and eradicating corruption. Addressing staff members, Dr. B.N. Tripathi, Director, ICAR-NRCE emphasized on changing our system to prevent corruption and asked to orient towards education of customers, clients and the users of the services provided by the Centre.

Sanitation drive launched to clean NRCE campus

Under *Swachh Bharat Mission* of Government of India, employees of the Centre participated in 21 weekly cleaning activities during 2016-17. In addition, campaign for promoting cleanliness was also spread to rural areas by educating the farmers about cleanliness under *Mera Gaon Mera Gaurav* program of the centre. NRCE also organized cleanliness drive from 2-16 October 2016 with taking a pledge for cleanliness. During the fortnight drive, daily activities were taken for cleaning the campus both at Hisar and Bikaner.

NRCE employees cleaning campus at Hisar & Bikaner

Scientists-Veterinarian Interface meeting on NRCE Foundation Day

The 32nd Foundation Day of NRCE was celebrated on 26 November 2016. On this occasion, a Scientist-Veterinarian interface meeting was organized to create awareness on glanders, influenza and other infectious diseases. In the inaugural function, Maj Gen (Dr) Shri Kant, Vice-Chancellor, LUVAS, Hisar, Chief Guest appreciated that NRCE has made its mark in diagnosis and control of equine diseases at international level. Dr S.K. Dwivedi, Former Director NRCE and Brigadier S.S. Kashyap, Commandant, EBS, Hisar graced the occasion as Guests of Honor. Dr BN Tripathi, Director NRCE apprised about the steps taken by NRCE in timely diagnosis and control of outbreaks of glanders and equine influenza. In the interface meeting chaired by Dr S.K. Dwivedi, 16 veterinarians interacted with NRCE scientists on management of emerging outbreaks of infectious equine diseases.

Experts interacting in Scientist-Veterinarian Interface meeting

Career opportunities in agriculture discussed on Agriculture Education Day

Scientists addressing the students at village Rawalwas

The Centre celebrated "Agriculture Education Day" on 3 December 2016 in villages Siswala and Rawalwas, Hisar. NRCE scientists delivered lectures to the school students and their guardians about career opportunities in agriculture. The students were encouraged to take agriculture and animal husbandry as an optional subject in senior secondary school. The students were provided guidance on procedure for obtaining admission in professional courses in agriculture and veterinary sciences.

Profitable use of farm waste promoted during National Productivity Week

National Productivity Week was celebrated from 12 - 18 February 2017 at ICAR-NRCE, Hisar. To increase the income of farmers and equine owners, training on 'Use of farm waste and equine dung for vermicomposting' was given to the farmers and women belonging to a social group *Anubhuti.* An interactive campaign on "From waste to profit through reduce, recycle & reuse" was taken up in rural areas promoting profitable utilization of farm waste and animal dung, especially equine dung.

Farmers during training on vermicomposting

NRCE celebrated National Science Day with specially abled children

An interactive meet of specially abled childrens, their parents, school teachers was organized on 28th February, 2017 at Village Neoli Khurd (Hisar) on the occasion of National Science day. A team of NRCE scientists and child psychologist Dr Bharti Arora interacted with specially abled children and their parents to identify the problems being faced by them. Solutions were provided after discussion with school principal and village panchayat. On this occasion, games and competitions were organized for physically challenged/specially abled children.

NRCE scientists interacting with specially abled children

NRCE outreaches to farmers in farmer's fairs in different institutes

The Centre participated in three farmer fairs organized by different ICAR institutes to showcase the activities to farmers.

In three-day regional agriculture fair "*Krishi Kumbh*-2016" organized by ICAR-Indian Institute of Farming Systems Research during 28-30 November 2016 at Muzaffarnagar (Uttar Pradesh), NRCE scientists interacted with farmers and demonstrated technologies generated by NRCE to the farmers and stakeholders, who showed keen interest in horse rearing.

Participating in Krishi Kumbh-2016 at Muzafarnagar

NRCE participated in a three-day farmers' fair, *Krishi Unnati Mela*, organized by Indian Agricultural Research Institute, New Delhi from 15-17 March 2017. In the fair, NRCE scientists showcased equines and technologies developed for the benefit of equine owners, with an objective of development, empowerment and progress of the farmers.

NRCE also participated in Buffalo Fair organized by Central Institute For Research on Buffaloes, Hisar on 4 February 2017 and displayed NRCE activities to participating animal owners.

Dignitaries visiting NRCE stall at IARI and CIRB

Scientists of Bikaner campus participated in Western Regional Agriculture Fair-2017 at Swami Keshwanand Rajasthan Agricultural University, Bikaner from 18-20 February 2017, disseminated the knowledge to the equine farmers about equine rearing and its prospects.

Participating in Agriculture fair at RAU, Bikaner

Mera Gaon Mera Gaurav programme helps in 'lab to land' dream of NRCE

My Village My Pride scheme - *Mera Gaon Mera Gaurav* - was launched by the Hon'ble Prime Minister on 25 July 2015 with an aim to promote the direct interface of scientists with the farmers to hasten the lab to land process and to provide farmers with required information, knowledge and advisories on regular basis by adopting villages. NRCE has adopted 24 villages through six teams of scientists. Between April 2016 and March 2017, scientists made 125 visits to various villages, where 54 interface meetings and 9 trainings were conducted benefitting 1446 rural families (Table 1). Linkages were established with different State departments including local authorities for tackling problems related to farmers. Awareness on different Agricultural and Animal husbandry practices and hygiene were created. Dr Ashok Kumar Gupta was the nodal officer of the MGMG program.

Table 1. Summary of activities organized under MGMG

Activity	Number	No. of farmers benefitted
Visit to villages by teams	125	1446
Interface meeting/ *Goshthies*	54	586
Trainings conducted	9	261
Mobile based advisories	16	395
Literature support provided	68	919
Awareness created	53	1556
Linkages developed with other agencies	46	996

NRCE Scientists interacting with farmers in villages around Hisar and Bikaner

IRC, RAC and Review Meetings

Institute Research Committee reviews research projects

Annual IRC meeting of ICAR-NRCE was held during May 2-3, 2016 under the chairmanship of Dr. B.N Tripathi, Director to review and discuss achievements in various research projects at the Centre. There are 31 on-going research projects, including 11 in equine health, 10 in equine production and 10 in NCVTC. The scientists presented the progress made in these projects and provided inputs for further improvement. Five new research proposals were also discussed and approved, including development of area-specific mineral mixtures for equines, risk assessment for colic & laminitis, and management of equine reproductive problems. Addressing IRC meeting, Dr Tripathi advised scientists to publish research findings in high impact journals. He emphasized that scientists should devote more time on bench work and encouraged to submit projects for grant from national and international agencies. The need to formulate hypothesis/technology-based research projects was also emphasized in the meeting.

Annual Review of Network Units of NCVTC at Karnal

NCVTC annual review meeting in progress

The seventh annual review meet of Network Units of NCVTC was organized at ICAR-NDRI-Karnal on January 09, 2017. The meeting was chaired by Dr H. Rahman, DDG (Animal Science) in presence of Dr A.K. Srivastava, Director, ICAR-NDRI, Karnal and Dr Ashok Kumar, ADG (Animal Health). Dr B.N. Tripathi, Project Coordinator, NCVTC and Director, ICAR-NRCE, Hisar apprised the members of the progress made by the repository over the years. Dr Rahman emphasised the need to focus on conservation of only the most important microbial cultures in view of financial constraints. Dr. Srivastava highlighted the public health significance of zoonotic microbes and their importance for human health. He stressed on testing the probiotic efficacy of dairy microbes as they are highly relevant to therapeutic use in Indian context. Dr B.N. Tripathi, Project Coordinator, NCVTC presented an overview of NCVTC and apprised the members that NCVTC repository has now collection of more than 3000 microbes and distribution of cultures to stakeholders has been initiated from this year. Investigators from 19 network units presented the progress during the year on rumen, dairy and veterinary microbes. The committee recommended for identification and conservation of vaccine & challenge strains for important veterinary pathogens from buffalo, cattle & pigs and to organize a workshop for training for adoption of uniform culture deposition and accessioning procedures.

RAC reviews the research activities of the Centre

The XIX meeting of Research Advisory Committee of the Centre was organized on March 25, 2017 to review the research priorities of the Centre and to advise on new initiatives. Welcoming RAC members, Dr Tripathi acknowledged the guidance of the members during last three years for achieving the goal of NRCE. He presented action taken report on RAC recommendations and research achievement of the Centre during 2016-17. The progress made in each Unit of NRCE was presented by in-charges of each section. Dr Gowda, Chairman RAC emphasized that scientists at Equine production campus, Bikaner should focus on applied research to address the issues faced by

equine keepers in breeding, reproduction, nutrition and shelter management for sustainable equine production. The committee recommended undertaking research for development of new generation vaccines, penside diagnostics and herbal drugs for control and treatment of equine diseases. The RAC also applauded the initiatives of the Centre for development of area-specific mineral mixtures, parentage testing in equines and bacteriophage therapy. The RAC encouraged to adopt multidisciplinary approach for better utilization of country's infrastructural resources and quality research output and to develop R&D linkages with other institutes and stakeholders.

RAC members discussing research priorities

Members of RAC	
Dr. R.N.S. Gowda	Former Vice Chancellor, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar (Chairman RAC)
Dr. S. K. Srivastava	Former Head, Veterinary Bacteriology and Mycology, IVRI, Izatnagar
Dr. V.D. Sharma	Dean, Sai Institute of Paramedical Allied Sciences, Dehradun
Dr. Ashok Kumar	ADG (AH), ICAR, New Delhi
Dr. J.R. Rao	Emeritus Scientist, NAARM, Hyderabad
Dr. K.C. Varshney	Prof. & Head, Department of Veterinary Pathology, Rajiv Gandhi College of Veterinary and Animal Sciences, Puduchery
Sh. Gajendra Pal Singh	Progressive equine breeder, Jodhpur
Shri Ranjeet Pawar	AT & PO, Pune
Dr. B.N. Tripathi	Director, ICAR-NRCE, Hisar
Dr. Yash Pal	Pr. Scientist, ICAR-NRCE, Hisar (Member Secretary)

Visit of Dignitaries

Dr Mohapatra appreciates R&D activities at NRCE Campus

DG-ICAR addressing staff of NRCE and CIRB

Dr Trilochan Mohapatra, Secretary DARE and Director General ICAR, New Delhi visited NRCE on 22 August 2016. During his visit, Dr B.N. Tripathi, Director NRCE briefed him about ongoing research activities at the Centre. Dr Mohapatra visited all the laboratories and had personal interaction with the scientists. Appreciating the research activities and infrastructure at the Centre, he emphasized the need to publish papers in high impact journals and commercialization of technologies. He congratulated NRCE staff for excellent maintenance and upkeep of the campus. Dr Mohapatra desired that the technologies developed by institute should be transferred to end users and wished that scientists should deliver more and better than before.

Dr Srivastava motivates scientists for team work

Dr Srivastava addressing scientists at NRCE

Dr A.K. Srivastava, Member Agricultural Scientists Recruitment Board and Dr Rameshwer Singh, Ex-Director, Directorate of Knowledge Management in Agriculture visited NRCE on 4 February 2017. The dignitaries were briefed about the research activities of NRCE-Hisar by the Dr B.N. Tripathi, Director, NRCE. Delivering motivational address, Dr Srivastava asked the scientists to believe in self and work in a collaborative mode to achieve the higher goals.

Director General RVS applauds contributions of NRCE

DG, RVS interacting with scientists of NRCE

Lt Gen A.J. Singh, Director General, Remount Veterinary Services visited the centre on 11 November 2016. Welcoming DG RVS, Dr B.N. Tripathi, Director NRCE highlighted the role and contributions of NRCE in management of infectious equine diseases, especially in control of glanders, equine influenza and equine herpesvirus outbreaks. Lt Gen Singh complimented the scientists of NRCE for the yeomen service to the nation in the field of equine research, investigation and control of diseases and for improvement in the management and breeding practices. He emphasized the need for more collaborative efforts between RVC and NRCE for addressing reproductive problems in equines.

Dr Rahman visits EPC, Bikaner

Dr H. Rahman, Deputy Director General (Animal Science), ICAR, New Delhi visited Equine Production Campus, Bikaner on 16 May 2016. Dr Rahman inaugurated the newly constructed animal quarantine shed, sick animal shed and rest house facilities. He was accompanied by Prof A.K. Gahlot, Vice Chancellor, Rajasthan University of Veterinary & Animal Sciences, Bikaner and Dr B.N. Tripathi, Director NRCE. Dr Rahman appreciated the equine sanctuary being maintained at the Campus and asked scientists to work with greater vigour for conservation of different equine breeds.

Dr Rahman interacting with the scientists

Dr Gurbachan Singh emphasizes on research with global imprint

Dr Gurbachan Singh, Chairman, Agricultural Scientists Recruitment Board visited Bikaner campus on 21 January 2017. Welcoming Dr Singh, Dr B.N. Tripathi, Director, NRCE apprised about the activities of NRCE and efforts being made for conservation of indigenous equine breeds by NRCE. Dr Singh encouraged the scientists to reap benefits of early promotions through direct selection. He congratulated the scientists for their efforts in conservation and propagation of indigenous equine breeds and encouraged them to carry out cutting edge research in equine management to make a global imprint.

Chairman ASRB during visit to Bikaner Campus

Dr Prasad appreciates research activities of the Centre

Dr Prasad interacting with scientific staff of NRCE

Dr C.S. Prasad, former Director NIANP, Bangalore and Dr S.V.N. Rao, Professor & Head, Veterinary & Animal Husbandry Extension Education, Rajiv Gandhi College of Veterinary and Animal Sciences, Puducherry visited NRCE on 26 October 2016. Dr Prasad appreciated excellent infrastructure facilities and good research being done at the Centre.

Dr M P Yadav appreciates research on equine diseases

Dr M.P. Yadav, Former Director ICAR-NRCE, Former Director ICAR-IVRI, Former Vice-chancellor, DUVASU, Mathura visited the Centre on 29 October 2016. During the visit he interacted with scientists of the institute and applauded the role of Centre in the development of diagnostics and vaccines for management of infectious equine diseases. He also appreciated efforts of the Centre in conservation of microbial diversity at NCVTC.

Dr Yadav interacting with scientists of NRCE

Visitors from Abroad

Visitors assessing NRCE for international meeting

- Dr. Shaikh Mohammad Bokhtiar, Director and Md. Nure Alam Siddiky, Senior Program Officer (Livestock) from SAARC Agricultural Centre, Dhaka, Bangladesh visited the Centre on 23 August, 2016. During the visit, they were briefed about the activities and achievements of the Centre. They were highly impressed by the laboratory facilities at NRCE and expressed the need to explore collaborations in the area of equine infectious diseases.
- Dr Oleksandr Tashyrev and Dr Vera Govorukha from Zabolotny Institute of Microbiology and Virology, National Academy of Science, Ukraine visited the Centre on 16 September 2016. During the visit, they interacted with scientists and visited different laboratories.
- Professor Stacey Shultz-Cherry, St Jude Graduate School of Biomedical Sciences, Memphis, USA, Professor Ann Cullinane, Irish Equine Centre, Ireland and Professor Thomas Chambers, Gluck Equine Research Centre, Kentucky, USA visited NRCE on 17-18 October 2016 as a part of the team from International Society for Influenza and Respiratory Virus Diseases (ISIRV) for assessing the facilities at NRCE and ICAR Delhi for hosting 4th/5th Neglected Flu meeting in India. They also met Director General, ICAR and DDG (AS) at head quarters prior to visiting ICAR-NRCE. They were impressed by the research being carried out at NRCE and showed keen interest in the activities.

Infrastructure and Developmental Activities

New laboratory wing of NCVTC inaugurated

The second phase of NCVTC building was inaugurated by Dr Trilochan Mohapatra, Hon'ble Director General, ICAR on 22nd August, 2016 in the presence of Dr. H. Rahaman, DDG (AS), ICAR and Dr B.N.Tripathi, Director NRCE. The new wing has state-of-the-art laboratories for poultry virology, anaerobic bacteriology, mycology, bacteriophage, central instrumentation and bioinformatics. New equipments including ultra centrifuge, deep freezers, ELISA plate reader, water purification system, PCR machine, shaker incubators, refrigerators and autoclave were procured for strengthening the repository related activities.

DG, ICAR inaugurating new laboratory wing

New infrastructure developed at EPC Bikaner

During the year, the Centre focussed on infrastructure improvement at EPC Bikaner. A guest house was opened, which has two well furnished rooms, a kitchen and one dining hall. In addition, new sick animal shed, quarantine animal shed and semen laboratory were constructed during this year. These facilities were inaugurated on 16th May 2016 by Dr. H. Rahman, DDG (Animal Sciences) in the presence of Prof A.K. Gahlot, Vice-chancellor, RAJUVAS and Dr. B.N. Tripathi, Director NRCE.

A statuette of horse constructed with single rock was unveiled at the campus during the foundation day celebrations of the campus by Brig. S S Kashyap, Commandant Equine Breeding Stud, Hisar in the presence of Dr. B.N. Tripathi, Director NRCE. A pool was built for swimming of horses and to treat the horses with musculoskeletal disorders by hydrotherapy. In addition, a new tube well, generator with 100 KVA capacity, weigh bridge and chaff cutter were also installed.

Dr. Rahman inaugurating quarantine animal shed

Brig. Kashyap unveiling the horse statuette

Agricultural Production: During 2016-17, 220 acres of land was brought under cultivation, 140 acre at Hisar and 80 acres at Bikaner. The land was rotationally used for cultivating green fodder, dry fodder and grains for feeding equines kept at our farms. During the year, total farm production was 3659.67 quintals, including 2488.39 quintal of green fodder, 590.52 quintal of dry fodder and 580.69 quintal of grains (Table 1). By sale of surplus farm produce, a total income of Rs10,09,603/- was generated during the year, Rs.5,77,555/- from Hisar and Rs.4,32,048/- at Bikaner Campus.

Table 1. Agricultural production at NRCE

Type of crop	Production (in Quintal)	
	Hisar	Bikaner
GREEN FODDER		
Oats	209.0	377.60
Berseem	120.0	-
Lucerne	31.0	579.60
Sorghum, sudan grass+Cowpea	240.0	-
Sorghum sudan grass	195.0	633.30
Cowpea	42.0	-
Sewan grass	-	24.40
Napier grass	-	34.60
Azola	-	1.84
Total	**837.0**	**1651.39**

Type of crop	Production (in Quintal)	
	Hisar	Bikaner
DRY FODDER		
Oats, bajra, wheat straw, etc	165.62	424.90
GRAINS		
Oats	89.75	29.70
Mustard	30.45	-
Wheat	284.55	-
Bajra	22.74	-
Guar	-	40.50
Barley	-	47.30
Moth	-	35.70
Total	**427.49**	**153.2**

Livestock Strength

During the year, Kathiawari mares (2) and two true-to-breed Marwari stallions were purchased for our Bikaner farm. At present, 136 equines of various breeds are being maintained at Hisar (Table 2) and Bikaner (Table 3), including 53 horses, 31 ponies, 46 donkeys and 6 mules. At Bikaner campus, there are 112 equines, including Marwari (37) & Kathiawari (2) horses; Zanskari (15) & Manipuri (14) ponies; Poitou (27) & indigenous (12) donkeys and mules (6).

Marwari stallion and Kathiawari mares procured

Table 2. Equine herd strength at Hisar campus

Category	Horses		Ponies		Donkey		Mules		Total
	M	F	M	F	M	F	M	F	
Stock as on 01.04.2016	5	16	2	0	2	3	0	0	28
Births	0	0	0	0	2	0	1	0	3
Deaths	0	0	0	0	0	0	0	0	0
Euthanized	0	0	0	0	0	0	0	0	0
New Receipts	0	0	0	0	0	0	0	0	0
Auctioned	1	6	0	0	0	0	0	0	7
Balance as on 31.03.2017	**4**	**10**	**2**	**0**	**4**	**3**	**1**	**0**	**24**

Table 3. Equine herd strength at EPC, Bikaner

Category	Horses				Ponies				Donkey				Mules		Total
	Marwari		Kathiawari		Zanskari		Manipuri		Poitou		Indigenous				
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Stock as on 01.04.2016	08	18	0	0	05	07	05	06	09	14	05	06	03	01	87
Births	01	04	0	0	01	02	01	02	03	02	0	01	01	0	18
Deaths	0	01	0	0	0	0	0	0	0	0	0	0	0	0	01
Euthanized	0	0	0	0	0	0	0	0	01	0	0	0	0	0	01
New Receipts	02	05	0	02	0	0	0	0	0	0	0	0	0	0	09
Auctioned	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balance as on 31.03.2017	**11**	**26**	**0**	**02**	**06**	**09**	**06**	**08**	**11**	**16**	**05**	**07**	**04**	**01**	**112**

Awards, Recognitions and Personal Milestones

Emerging investigator award to Dr Anju Manuja

Dr Anju Manuja, Principal Scientist was awarded "Emerging Investigator Award" by Elsevier Journal Nanostructures and Nano Objects (NANOSO) at International Conference on Nanostructures Materials and Nanocomposites (ICNM 2017), Mahatma Gandhi University, Kottayam, Kerala during 10-12 February 2017. The award was conferred in recognition of her excellent research and high impact publications in the area of nanotechnology.

Dr Baldev R Gulati conferred IVS Fellowship

Dr Baldev R. Gulati, Principal Scientist has been elected Fellow of Indian Virological Society (FIVS-2016) for his outstanding contribution to the field of veterinary virology. This award was conferred on him during International Conference of Indian Virological Society (IVS) on "Global Perspectives in Virus Disease Management" at Indian Horticulture Research Institute, Bengaluru, Karnataka during 8-10 December 2016.

Diplomate of ICVP award to Dr Nitin Virmani

Dr Nitin Virmani successfully cleared examination of "Indian College of Veterinary Pathologists" and awarded "Diplomate of ICVP" during XXXIII Annual Meeting of Indian Association of Veterinary Pathologists held at Department of Veterinary Pathology, College of Veterinary Science & Animal Husbandry, Anjora, Chhattisgarh on 09-11 November 2016.

Societal Innovation Award to Dr Naveen Kumar

Societal Innovation Award for the development of indigenous vaccine against Johne's Disease in Domestic Livestock was given to Dr Naveen Kumar, Senior Scientist along with scientists from CIRG, Makhdoom (Singh SV, Gupta S, Chaubey KK, Bhattacharya) in 43rd Meritorious Invention Awards Ceremony & Conference on "Leveraging innovation ecosystem for accelerating startups" at Indian National Science Academy, New Delhi during 24-25 March 2017.

Young Scientist Award to Dr Sanjay Kumar Ravi

Dr Sanjay K. Ravi, Scientist received "Young Scientist Award" for poster presentation entitled "Effect of fish oil supplementation on certain biochemical parameters in mares" in "XXXII Annual Convention of the Indian Society for the Study of Animal Reproduction at College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati during 6-8 December, 2016.

Dr Anuradha completes PG diploma

Dr Anuradha Bhardwaj, Scientist completed one year post graduate diploma in Technology Management in Agriculture (PGD-TMA) with distinction from University of Hyderabad and ICAR-NAARM, Hyderabad, Telengana.

New joining and transfers

1. Shri Ashok Kumar, SSS joined at EPC- Bikaner on 23 July 2016.
2. Dr Sandeep K. Khurana, Principal Scientist has been transferred to ICAR- CIRB, Hisar on 31 March 2017.
3. Dr Vijay Kumar, Scientist has been transferred to ICAR-CSWRI, Avikanagar on 31 March 2017.

Promotions

1. Dr Balvinder K. Manuja has been promoted to the post of Principal Scientist with effect from 13 August 2014.
2. Dr Anju Manuja has been promoted to the post of Principal Scientist with effect from 8 August 2014.
3. Dr Ramesh K. Dedar has been promoted to the post of Scientist (Sr. Scale) with effect from 8 January 2012.
4. Dr Prokasananda Bala has been promoted to the post of Scientist (Sr. Scale) with effect from 8 January 2012.
5. Dr Tirumala R. Talluri has been promoted to the post of Scientist (Sr. Scale) with effect from 7 January 2013.
6. Dr Riyesh Thachamvally has been promoted to the post of Scientist (Sr. Scale) with effect from 28 August 2014.
7. Dr Sanjay K. Ravi has been promoted to the post of Scientist (Sr. Scale) with effect from 29 August 2014.
8. Shri Sajjan Kumar, Sr. Technical Assistant has been promoted to the post of Technical Officer with effect from 29 June 2016.
9. Shri Suresh Kumar, Sr. Technical Assistant has been promoted to the post of Technical Officer with effect from 29 June 2016.

Director : Dr B. N. Tripathi

SCIENTIFIC STAFF

Main campus, Hisar

1. Dr Ashok Kumar Gupta, Principal Scientist
2. Dr Suresh Chander Yadav, Principal Scientist
3. Dr Yash Pal, Principal Scientist
4. Dr Baldev Raj Gulati, Principal Scientist
5. Dr Rajender Kumar, Principal Scientist & National Fellow
6. Dr Sandip Kumar Khurana, Principal Scientist
7. Dr Nitin Virmani, Principal Scientist
8. Dr Anju Manuja, Principal Scientist
9. Dr Balvinder Kumar, Principal Scientist
10. Dr Sanjay Kumar, Principal Scientist
11. Dr Mamta Chauhan, Sr Scientist
12. Dr Anuradha Bhardwaj, Scientist
13. Dr Harishankar Singha, Scientist

Equine Production Campus, Bikaner

1. Dr Ram Avatar Legha, Principal Scientist
2. Dr Vijay Kumar, Scientist
3. Dr Ramesh.Kumar Dedar, Scientist
4. Dr Prokasananda Bala, Scientist
5. Dr Thirumala Rao Talluri, Scientist
6. Dr Sanjay Kumar Ravi, Scientist

NCVTC, Hisar

1. Dr Praveen Malik, Principal Scientist (on deputation)
2. Dr Sanjay Barua, Principal Scientist
3. Dr Rajesh Kumar Vaid, Principal Scientist
4. Dr Naveen Kumar, Sr. Scientist
5. Dr Taruna Anand, Scientist
6. Dr Bidhan Chandra Bera, Scientist
7. Dr Shanmugasundaram Karuppusamy, Scientist
8. Dr Riyesh Thachamvally, Scientist

ADMINISTRATIVE STAFF

Main campus, Hisar

1. Sh. A.G. Barapatre, Administrative Officer
2. Smt. Shammi Tyagi, Assistant Finance & Accounts Officer
3. Sh. Ram Pal, Assistant Administrative Officer
4. Sh. Surender Pal Kaushik, Assistant Administrative Officer
5. Sh. Ashok Kumar, Personal Assistant
6. Sh. Subhash Chander, Assistant
7. Sh. Pratap Singh, Assistant
8. Sh. Sunil Sharma, Assistant
9. Sh. Dinesh Datt Sharma, Upper Division Clerk
10. Sh. Om Parkash, Upper Division Clerk
11. Sh. Deepak Kumar, Lower Division Clerk

Equine Production Campus, Bikaner

1. Sh. Mahender Singh, Lower Division Clerk

TECHNICAL STAFF

Main campus, Hisar

1. Sh. Krishan Kumar Gupta, Chief Technical Officer
2. Sh. Kirpa Shankar Meena, Senior Technical Officer
3. Sh. Partha Pritam Chaudhary, Senior Technical Officer
4. Sh. Diger Dev Pandey, Senior Technical Officer
5. Sh. Sita Ram, Senior Technical Officer
6. Sh. Ajmer Singh, Technical Officer
7. Sh. Sanjeev Kumar, Technical Officer
8. Sh. Sajjan Kumar, Technical Officer
9. Sh. Suresh Kumar, Technical Officer
10. Sh. Joginder Singh, Senior Technical Assistant
11. Sh. Mukesh Chand, Senior Technical Assistant
12. Sh. Raj Kumar Dayal, Senior Technical Assistant.
13. Sh. Arun Chand, Senior Technician
14. Sh. Raghbir Singh, Senior Technician

Equine Production Campus, Bikaner

1. Dr. Jitender Singh, Senior Technical Officer
2. Sh. Kamal Kumar Singh, Senior Technical Officer
3. Sh. Brij Lal, Technical Officer
4. Sh. Narender Chauhan, Technical Officer
5. Sh. R.A. Pachori, Technical Officer
6. Sh. Om Parkash, Senior Technical Assistant
7. Sh. S.N. Paswan, Technical Assistant
8. Sh. Rajender Singh, Technical Assistant
9. Sh. Gopal Nath, Technician

SKILLED SUPPORTING STAFF

Main campus, Hisar

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

Equine Production Campus, Bikaner

1. Sh. Raju Ram
2. Sh. M.P. Meena
3. Sh. Ashok Kumar

Publications

Research Articles

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5. Kumar N, Barua S, Riyesh T, Tripathi BN. 2016. "Isolation and purification of multiple viruses from mixed infection". In: 1st International Agrobiodiversity Congress, science, technology, policy and partnership, organized by Indian Society of Plant Genetic Resources Biodiversity International, New Delhi, India, November 6-9.
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3. Bhardwaj A, Panghal S, Chauhan M, Pal Y. 2016. "Genetic markers for selection of animal genetic resources". Compendium of ICAR sponsored short course on "Recent models and methods for analysis of farm animal data for devising suitable breeding and management strategies", ICAR-CSWRI, Avikanagar, Rajasthan, July 11-21.
4. Dedar RK. 2016. "Some important aspects of equine colic" - http://www.punjabpashudhan.in/some-important-aspects-of-equine-colic/
5. Gulati BR, Sarika, Arora D. 2017. "Characterization of monoclonal antibodies by immunoblotting". CAFT training in Veterinary Microbiology on Modern technologies for production and applications of antibodies for animal health improvement". Department of Veterinary Microbiology, LUVAS, Hisar, Haryana, March 06.
6. Gulati BR, Sarika, Arora D. 2017. "Isotype determination of monoclonal antibodies by ELISA". CAFT training in Veterinary Microbiology on Modern technologies for production and applications of antibodies for animal health improvement". Department of Veterinary Microbiology, LUVAS, Hisar, Haryana, March 07.
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4. Manuja A. 2016. Therapeutic interventions against trypanosomiasis. Curr Top Med Chem. Bentham Science Publishers, USA.
5. Talluri TR, Ravi SK, Singh J, Gupta AK, RA Legha, Pal Y and Tripathi BN. 2016. Pregnancy diagnosis in equines. pp No 32-38. In Souvenir All India Marwari Horse Society (2016-17).

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2. यश पाल, आर ए लेघा, पार्वती शर्मा एवं अनुराधा भारद्वाज 2016, उत्तम नस्ल के खच्चर उत्पादन में भाकृअनुप–राष्ट्रीय अश्व अनुसंधान केंद्र का योगदान । पशुधन प्रकाश (सप्तम अंक–2016) पृष्ठ 37–39.
3. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, कोलिक के सामान्य कारण व बचाव, अश्व उत्पादन परिसर, रा. अ. अनु. के.,बीकानेर, पत्रक सं –2

4. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों में लेमीनाइटिस की बीमारी के कारण व बचाव, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर , पत्रक सं –3

5. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों के बछेरों में गोल कृमि संक्रमण के लक्षण एवं बचाव, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर, पत्रक सं –4

6. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों के बछेरों में रोड़ोकोकक्स नुमोनिया संक्रमण, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर, पत्रक सं –5

7. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों के नवजात बछेरों में दस्त व बुखार, अश्व उत्पादन परिसर, रा.अ.अनु.के., बीकानेर, प=क सं –6

8. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों में सर्रा रोग, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर, पत्रक सं –7

9. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों में स्ट्रेंगल्स रोग, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर, पत्रक सं –8

10. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों में ग्लैंडर्स रोग, अश्व उत्पादन परिसर,, रा. अ. अनु. के., बीकानेर, पत्रक सं –9

11. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों में टेटनस रोग, रा. अश्व उत्पादन परिसर,, रा. अ. अनु. के., बीकानेर, पत्रक सं –10

12. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों में इक्वाइन पाईरोप्लास्मोसिस रोग, अश्व उत्पादन परिसर,, रा. अ. अनु. के., बीकानेर, पत्रक सं –11

13. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों में हर्पीस वाइरस संक्रमण, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर, पत्रक सं –12

14. रमेश देदड़, विजय कुमार, पी ए बाला, एस के रवि, टी राव, आर ए लेघा, 2017, अश्वों में कवक विषाक्तता (माइकोटोक्सिन टोक्सिसिटी), अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर, पत्रक सं –13

15. विजय कुमार ,रमेश कुमार देदड़, प्रकाश आनन्द बाला, तल्लुरी राव, संजय कुमार रवि, राम अवतार लेघा, यशपाल, 2017, अश्वों में उच्च ताप–तनाव के लक्षण और बचाव, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर, पत्रक सं–17

16. विजय कुमार, रमेश देदड और प्रकाश आनंद बाला, ताल्लुरी राव, संजय कुमार रवि, राम अवतार लेघा, यश पाल, 2017, भार–ढोने और कार्य करने वाले अश्वों में थकान के लक्षण कैसे पहचानें, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर, पत्रक सं. 19

17. प्रकाश आनंद बाला, विजय कुमार, रमेश देदड और तल्लुरी राव, संजय कुमार रवि, राम अवतार लेघा, 2017, घोड़ों के लिए खनिज मिश्रण, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर, पत्रक सं. 20

18. प्रकाश आनंद बाला, विजय कुमार, रमेश देदड और तल्लुरी राव, संजय कुमार रवि, राम अवतार लेघा, 2017, घोड़ों के लिए पोषण प्रबंधन के नुस्खे, अश्व उत्पादन परिसर, रा. अ. अनु. के., बीकानेर , पत्रक सं. 21

19. संजय कुमार रवि, तिरुमला राव ताल्लुरी और राम अवतार लेघा, 2017, अश्वों में प्रजनन प्रबंधन, टीएसपी मैनुअल ''जनजातीय क्षेत्रों में पशुपालन (जनजातीय उपयोजना) में भा.कृ.अनु.प.–रा.उष्ट्र अनुसंधान केन्द्र प्रकाशन, पृष्ठ सं. 62–65

20. अनुराधा भारद्वाज एवं बी. एन. त्रिपाठी, 2016, अश्व विज्ञान की अभिनव उपलब्धियाँ, राजभाषा आलोक वार्षिक संकलन, पृष्ठ सं. 13–15

Participation, Presentation in Seminars, Conferences & Symposia

1. Dr Anju Manuja delivered an invited lecture on "Nanotechnology: Challenges and applications in veterinary sciences" in Fourth International Conference on "Nanostructured Materials and Nanocomposites (ICNM 2017)" at Mahatma Gandhi University, Kottayam, Kerala from 10-12 February 2017.
2. Dr Baldev R. Gulati delivered a lecture on "Molecular epidemiology of equine rotavirus in India: Evidence of inter-species transmission" in National Seminar on "Diarrhoeal disease burden and management: special reference to north eastern India", Department of molecular biology and biotechnology, Tezpur University, Assam from 10-11 March 2017.
3. Dr Baldev R. Gulati delivered an invited a lecture on "One Health: Role of veterinarian in containment of viral zoonotic diseases", World Veterinary Day celebration, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana on 28 April 2016.
4. Dr Baldev R.Gulati delivered an invited lecture on "Traditional and Contemporary Approaches for Diagnosis of Equine Herpesvirus 1 Infection" in International Conference on "Global perspectives in virus disease management (VIROCON 2016)", ICAR- Indian Institute of Horticultural Research, Bengaluru, Karnataka from 7-10 December 2016.
5. Dr Balvinder K. Manuja delivered an invited lecture on "Toxicological evaluation of nanoformulations for drug delivery" in Fourth International Conference on "Nanostructured Materials and Nanocomposites (ICNM-2017)" at Mahatma Gandhi University, Kottayam, Kerala from 10-12 February 2017.
6. Dr Bhupendra N. Tripathi delivered an invited lecture on "Horse rearing: An upcoming enterprise for the farmers" in a Training Course on " Extension strategies for sustainable entrepreneurship in livestock sector' at Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab from 01-08 August 2016.
7. Dr Bhupendra N. Tripathi attended "XXXIII Annual Meeting of IAVP and National Symposium on "Innovative approaches for diagnosis and control of emerging and re-emerging diseases of livestock, poultry and fish" at Department of Veterinary Pathology, College of Veterinary Science & Animal Husbandry, Kamdhenu Vishwavidyalaya, Anjora, Chhattisgarh from 09-11 November 2016.
8. Dr Bhupendra N. Tripathi delivered an invited lecture on "Control strategies of emerging and re-emerging disease of livestock & birds" at Sardarkrushinagar Dantiwada Agriculture University, SK Nagar, Gujarat on 28 January 2017.
9. Dr Bhupendra N. Tripathi delivered a lecture on "Better equine health, better perforamce" at 9th Livestock Championship and Agri. & Livestock Expo-2016 at Sri Muktsar Sahib, Punjab on 3 December 2016.
10. Dr Bhupendra N. Tripathi delivered a lecture on "Control and prevention of equine glanders in Uttar Pradesh" in a One Day Workshop on "Surveillance of glanders" organized by Animal husbandry department, Lucknow, Uttar Pradesh on 13 January 2017.
11. Dr Bhupendra N. Tripathi delivered a lecture on "An overview of equine glanders and equine influenza" in "2nd Asian African Congress of Mycobacteriologist" at Razi vaccine & serum research institute, Karaj, Iran on 01 March 2017.
12. Dr Harishankar Singha participated in international conference on "Technological advancement for sustainable agriculture and rural development (TASARD-India-2017", Society for Plant Research and African Asian Rural Development Organization, NASC Complex, New Delhi from 20-22 February 2017.

13. Dr Harishankar Singha participated in XXX Annual convention of "Indian Association of Veterinary Microbiology & Immunology and National Symposium" on "Challenges in animal health for higher productivity and income to farmers IAVMI-2017" at Nagpur Veterinary College, Seminary Hills Nagpur, Maharashtra from 10-12 February 2017.
14. Dr Nitin Virmani delivered an invited lecture on "Important viral diseases of equines and their status in India" in XXXIII Annual Meeting of IAVP and National Symposium on "Innovative approaches for diagnosis and control of emerging and re-emerging diseases of livestock, poultry and fish" at Department of Veterinary Pathology, College of Veterinary Science & Animal Husbandry, Kamdhenu Vishwavidyalaya, Anjora, Chhattisgarh from 9-11 November 2016.
15. Dr Prokasananda Bala delivered a lecture on "Feeding strategies for equids in Rajasthan" at "Global Rajasthan Agricultural Meet- (GRAM)", Jaipur, Rajasthan on 11 November 2016.
16. Dr Rajesh K. Vaid participated in International Symposium on "Microbial ecology and systematic", at MCC-NCCS, Pune, Maharashtra from 16-17 September 2016.
17. Dr Rajesh K. Vaid presented a paper on "Role of Veterinary Type Culture Collection in capturing equine microbial biodiversity" in 1st International Agrobiodiversity Congress on "Science, Technology, Policy and Partnership" organized by Indian Society of Plant Genetic Resources Biodiversity International at New Delhi from 06-09 November 2016.
18. Dr Rajesh K. Vaid presented a report on "NCVTC Microbial Repository'' in Conference on "Microbial resource centers and conservation of microbial diversity" and 13th meeting of the "Asian consortium for the conservation and sustainable use of microbial resources (ACM)" at Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology (CSIR-IMTECH), Chandigarh, Haryana from 08-10 November 2016.
19. Dr Riyesh T participated in the iinternational conference on "Global perspectives in virus disease management", at ICAR- Indian Institute of Horticultural Research Bengaluru, Karnataka from 08-10 December, 2016.
20. Dr Sandeep K. Khurana delivered an expert lecture on "Glanders: An Overview" in Foundation Day at ICAR-NRCE, Hisar, Hayrana on 26 November 2016.
21. Dr Taruna Anand participated in National Conference on "Trends in Nanobiotechnology (NCTN -2016)" organized by Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana from 29-30 November 2016.

Participation in Workshop

1. Dr Anju Manuja and Dr Yash pal participated in international workshop on "Milk: naturally nanostructured food" at NDRI, Karnal, Haryana on 30 November 2016.
2. Dr Anuradha Bhardwaj participated in the BTIS-NET workshop on "Bioinformatics for genomics and proteomics analysis" at Animal Biotechnology Centre, NDRI, Karnal, Haryana on 17 March 2017.
3. Dr Bhupendra N. Tripathi participated in joint international workshop organized by ICAR on "Production welfare research" with University of Edinburg, UK, at NASC Complex, New Delhi on 01 December 2016.
4. Dr Rajender Kumar attended workshop on "Procedures for filing patents" at NDRI, Karnal, Haryana on 16 December 2016.
5. Dr Rajender Kumar participated in national workshop on "Drug discovery technology- A molecular modelling, simulation & dynamics approach" at Department of Bio and Nano Technology, Guru Jambheshwar University of Science & Technology, Hisar, Haryana from 20-22 March 2017.
6. Dr Rajesh K. Vaid participated in workshop on "16S rRNA Sequence based Bacterial Identification" jointly organized by CDC, Atlanta, USA and Thermo Fisher at Gurgaon, Haryana from 14-16 February 2017.
7. Dr Rajesh K. Vaid participated in workshop on "Human genome and transcriptome analysis", at Institute of Bioinformatics, Bengaluru, Karnataka from 25-28 July 2016.
8. Dr Ramesh K. Dedar attended International workshop on "Metabolomics for plant, human and animal health -2016" organised by The

Energy and Resources Institute (TERI) at New Delhi from 17-18 November, 2016.

9. Dr Ramesh K. Dedar and Dr Vijay Kumar attended one day workshop on "Extraction, Purification, Identification and quantitation'' organized by Waters India Pvt. Ltd, at New Delhi on 19 April 2016.
10. Dr Riyesh T attended a workshop on "Scientific writing, e-books and publication process" organized by Directorate of Knowledge Management in Agriculture- ICAR at Central Institute for Research on Buffaloes, Hisar on 16 November, 2017.
11. Dr Sanjay Barua attended workshop on scientific writing, e-books and publication process" organized by Directorate of Knowledge Management in Agriculture- ICAR at Central Institute for Research on Buffaloes, Hisar on 16 November 2017.

Participation in Interactive meet/review meet/other meet

1. Dr Balvinder Kumar participated in Krishi Unnati Mela-2017 at ICAR-IARI, New Delhi from 15-17 March 2017.
2. Dr Bhupendra N. Tripathi , Dr Balvinder Kumar, Dr Anju Manuja participated in Northern Regional Agriculture Fair at GIC ground Muzaffarnagar, Uttar Pradesh from 28-30 November 2017
3. Dr Bhupendra N. Tripathi, Dr Balvinder Kumar, Dr Naveen Kumar, Dr Anuradha Bhardwaj participated in one day Wellcome trust-DBT meeting at IVRI, Izatnagar, Uttarpradesh on 27 April 2016.
4. Dr Bhupendra N. Tripathi participated in Industry – Interface meet organized by the Training & Education Centre, IVSRI, Pune Campus, Maharashtra on 10 February 2017.
5. Dr Bhupendra N. Tripathi participated in interactive meet on "Camel and human medicine" organized by NRCC, Bikaner, Rajasthan on 16 May 2016.
6. Dr Bhupendra N. Tripathi visited as a member of the Expert Committee constituted by GoI, DADF, Krishi Bhavan, New Delhi to review the status of equine disease diagnosis carried out by the approved laboratories i.e. RWITC & WRRL, Pune, Maharashtra on 09 February 2017.
7. Dr Bhupendra N. Tripathi and Dr Rajesh K. Vaid attended the 1st PRMC meeting of the project "Feasibility studies on biogas and compost production from mule dung in hilly regions in India'' at NEERI, Nagpur, Maharashtra on 21 October 2016.
8. Dr R. A. Lega, Dr Talluri T. Rao and Dr P.A. Bala participated in a field day on "Genetic improvement of Magra Sheep in Farmers" flocks under ICAR network project being conducted at Village Kotada, Rajasthan on 04 November 2016.
9. Dr R.K. Dedar, Dr P.A. Bala, Dr Talluri T. Rao and Dr S.K. Ravi participated in India's Western Regional Agriculture Fair -2017 conducted at RAU, Bikaner, Rajasthan from 18-20 February 2017.
10. Dr Vijay Kumar, Dr R.K. Dedar, Dr P.A. Bala, Dr Talluri T. Rao and Dr S.K. Ravi participated in scientist and equine owners interactive meet conducted on the occasion of foundation day of Equine Production Campus, Bikaner, Rajasthan on 28 September 2016.
11. Dr Vijay Kumar, Dr R.K. Dedar and Dr Talluri T. Rao participated in scientists and progressive farmers interactive meet at RAJUVAS, Rajasthan from 09-10 March 2017.

On-going Research Projects

Sr.No.	Title	Team
1.	Surveillance, monitoring and control of emerging and existing diseases of equines (continuous service project since 1995)	S.K. Khurana*, S.C. Yadav, Baldev R. Gulati, Rajender Kumar, Sanjay Kumar, N. Virmani, Sanjay Barua, Rajesh Vaid, Ramesh Dedar, H. Singha, Anju Manuja, Balvinder Kumar and B.N. Tripathi
2.	Evaluation of *in vitro* growth inhibitory efficacy of some novel synthetic drug molecules against *Theileria (Babesia) equi* haemoprotozoa (Nov 2013- March 2017)	Sanjay Kumar*, Rajender Kumar and A.K. Gupta
3.	Investigations on neuropathogenic and non–neuropathogenic variants of equine herpesvirus-1 and associated latency among equines in India (Sep 2013- March 2017)	Baldev R. Gulati,* Nitin Virmani and Riyesh T.
4.	Pathology of EHV-1 infection in BALB/c mice post- immunization with glycoprotein (gB, gD & gM) and bacterial artificial chromosome construct of EHV-1(Oct 2013-March 2017)	Nitin Virmani*, Baldev R. Gulati and B.C. Bera
5.	Generation of reverse genetics based equine influenza virus and explore its potential as vaccine candidate through challenge studies in mice model	Nitin Virmani, B.C. Bera and Taruna Anand
6.	Development of diagnostics for emergency preparedness and monitoring of emerging equine viral diseases (April 2014- March 2017)	Balvinder Kumar.* H.S. Singha, Naveen Kumar and Anju Manuja
7.	Nanobased therapeutic interventions against osteoarthritis(April 2016-March 2019)	Anju Manuja*, Balvinder Kumar and Riyesh T.
8.	Endocrine, biochemical and gene expression profiling of reproductive states in Marwari Mares (Oct 2012-Sept 2016)	Vijay Kumar*, Sanjay K. Ravi and R.K. Dedar
9.	Evaluation of total mixed rations for maintenance horses (March 2014-Feb 2017)	P.A. Bala*, R.K. Dedar and N.V. Patil
10.	Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines- (continuous service project since April 2015)	S.K. Ravi*, T.R. Talluri, J. Singh, R.A. Legha, Yash Pal and A.K. Gupta
11.	Characterization of donkey milk with emphasis on important milk proteins (Oct 2012-Sept 2016)	Yash Pal*, Sanjay Kumar, R.A. Legha, Anuradha Bhardwaj and A.K. Mohanty
12.	Development of rapid diagnostic test for pregnancy diagnosis in horse mares (Jan 2015-June 2017)	A.K. Gupta*, Yash Pal, Sanjay Kumar and Sanjay K. Ravi
13.	Genetic characterization of Marwari horses for selection of true to breed animals (July 2015-June 2018)	Anuradha Bhardwaj*, A.K.Gupta, Yash Pal, Mamta Chauhan and Vijay Kumar
14.	Development of DNA typing facility for parentage testing in horses (Oct 2015-March 2017)	Mamta Chauhan* Anuradha Bhardwaj, Yash Pal, B.N. Tripathi and A.K. Gupta
15.	Assessment of risk factors of equine laminitis and colic (Sep 2016- Aug 2019)	Ramesh Kumar Dedar*, P.A. Bala and Sakar Palecha
16.	Approaches to the diagnosis and management of reproductive failure in equines (May 2016- March 2019)	S.K. Ravi*, T.R. Talluri, R.K. Vaid, J. Singh and R.A. Legha
17.	Assessment and optimization of equine management in an intensive system (continuous service project since June 2016)	R.A. Legha*, Yash Pal, Vijay Kumar, R.K. Dedar, P.A. Bala, T.R. Talluri, S.K. Ravi and J. Singh.
18.	Area specific mineral mixture for equine of Rajasthan (June 2016- May 2018)	P.A. Bala*, R. K. Dedar and R. Nehra
19.	Optimization of inter/intra species somatic cell nuclear transfer technique for production of horse (*Equus caballus*) cloned embryos (Nov 2015-Feb 2018)	T.R. Talluri*, Sanjay Kumar Ravi, Taruna Anand, Naresh Seloker, Dharmendra Kumar and P.S. Yadav

Sr.No.	Title	Team
20.	Development of bacteriophage repository (Oct 2013- March 2017)	Taruna Anand*, R.K. Vaid, Sanjay Barua and B.C. Bera
21.	Authentication and accessioning of viruses of animal origin (continuous service project since 2015)	Sanjay Barua*, Naveen Kumar, B.C. Bera, Riyesh T. and Taruna Anand
22.	Isolation, characterization and development of repository of poxviruses of caprine, ovine and bovine origin (May 2015- April 2018)	Sanjay Barua,* Naveen Kumar, B.C. Bera, Riyesh T. and Taruna Anand
23.	Phenotypic and genotypic authentication and preservation of network bacterial isolates (June 2015- May 2018)	R.K. Vaid*, Taruna Anand, B.C. Bera and Riyesh T.
24.	Prevalence studies for porcine respiratory viruses and development of their repository (Jan 2016-Dec 2018	B.C. Bera,*, Sanjay Barua, Taruna Anand and Nitin Virmani
Externally funded projects at ICAR-NRCE/NCVTC		
25.	Development of sensitive and specific diagnostic assays for detection of *Trypanosoma evansi* infection in animals using modern molecular tools (National Fellow Scheme) **-** (April 2011-April 2019)	B.N. Tripathi*, Sanjay Barua, Nitin Virmani, S.C. Yadav, Baldev R. Gulati, Rajender Kumar, R.K. Vaid, B.C. Bera, Taruna Anand and Riyesh T.
26.	Advanced animal diagnostic and management consortium (ADMaC (Sep 2013-April 2019)	Sanjay Kumar*, Ramesh Dedar, Baldev R. Gulati, Nitin Virmani and S.K. Khurana
27.	All India network programme on neonatal mortality in farm animals (Jan 2015- March 2017)	Sanjay Kumar*, Ramesh Dedar, Baldev R. Gulati, Nitin Virmani and S.K. Khurana
28.	Consortia research project on vaccines and diagnostics (May 2015- March 2017)	Component-I (Baldev R. Gulati and Nitin Virmani) Component-II (Nitin Virmani, Baldev R. Gulati and B.C. Bera) Component-III (Sanjay Kumar and Rajender Kumar)
29.	Pathogenecity and immunogenicity of recombinant neurogenic and non-neurogenic mutant equine herpesvirus-1 (in tissue explants and murine model) and their potential as vaccine candidate(s) (Jan 2016- March 2017)	Nitin Virmani*, B.C. Bera and Taruna Anand
30.	Validation study of a western blot (WB) technique and ELISAs for serological diagnosis of glanders in equids for the purpose of certifying freedom from infection in individual animals for trade or movement (Dec 2015- Nov 2017)	H.S. Singha* and B.N. Tripathi
31.	Development of nano gold based immunochromatography/immune dot blot assay for detection of *Trypnosoma evansi* (April 2014-May 2016)	Neeraj Dilbaghi*, S.C. Yadav, Sandeep Kumar and A.K. Gupta
32.	Molecular epidemiology of Japanese encephalitis virus in pigs and mosquitoes in Assam (Jan 2017- Dec. 2019)	Baldev R. Gulati
33.	All India coordinated research project on utilization of animal energy with enhanced system efficiency (July 2009- March 2017)	R.A. Legha*, Vijay Kumar and Yash Pal
34.	Network project on characterization of donkeys of Rajasthan (April 2014-Sep 2016)	Yash Pal*, A.K. Gupta and R.K. Dedar
35.	CRP on Agrobiodiversity (August 2015- July 2017)	Sanjay Barua*, R.K. Vaid, Naveen Kumar, Taruna Anand, B.C. Bera, Riyesh T.
36.	Targetting a host cell protein kinase for development of antiviral therapeutics against PPR virus (Aug 2015- March 2018)	Naveen Kumar* and Sanjay Barua
37.	Generation of induced pluripotent stem (iPS) cells from buffalo fetal fibroblasts through non-viral approaches (Feb 2016- Aug 2017	Dharmendra Kumar*, Naresh L. Selokar, P.S. Yadav and Taruna Anand
38.	Feasibility studies on biogas and compost production from mule dung in hilly regions in India (Nov 2015-Oct 2017)	B.N. Tripathi*, R.K. Vaid and R.A. Legha

*Principal Investigator

NRCE in News

यहां भी दे गुजराती गधे, कुछ बाहर भेजे, बाकी पर बेरोजगारी की मार

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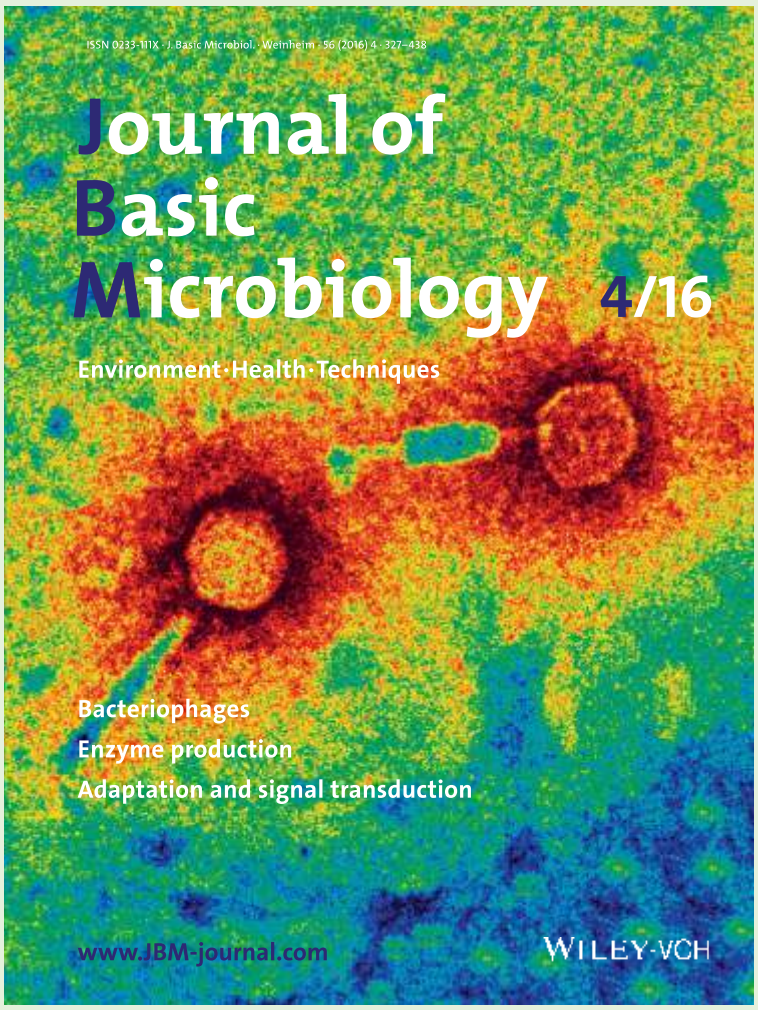
ग्लैंडर्स की चपेट में आए पांच राज्य

किस राज्य में कब कितने मिले ग्लैंडर्स केस



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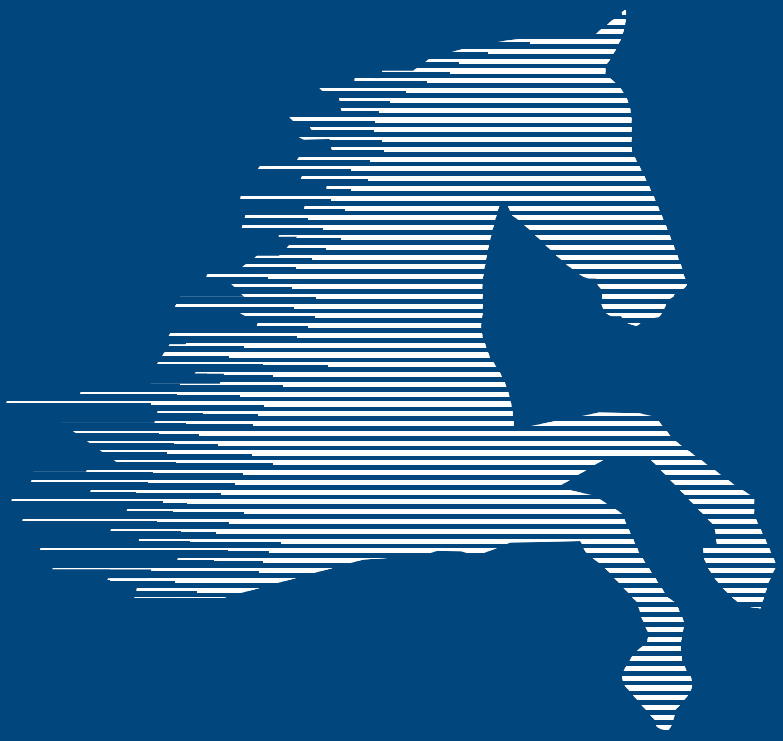

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