Azadi Ka Amrit Mahotsav

भाकृअनुप
ICAR

2022
Annual Report

भा.कृ.अनु.प. - राष्ट्रीय अश्व अनुसंधान केन्द्र
ICAR-National Research Centre on Equines

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ICAR-National Research Centre on Equines

ICAR-NATIONAL RESEARCH CENTRE ON EQUINES


ICAR-NATIONAL RESEARCH CENTRE ON EQUINES

With best compliments from

Dr. TK Bhattacharjya

Director

ICAR-National Research Centre on Equines

Sirsa Road, Hisar - 125001 (Haryana)

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Director's Foreword

In the field of animal sciences, the National Research Centre on Equines (NRCE) is a leading research centre of the Indian Council of Agricultural Research (ICAR), a part of the National Agricultural Research System (NARS). It was founded on November 26, 1985, in Hisar, Haryana, in response to the realisation that equines are crucial to the livelihoods of small and marginal farmers as well as landless labourers. This is the only prestigious institution in the nation that is solely focused on improving the socio-economic situation of the nation's equine stakeholders, breeders, and farmers through technological support and research. Since its establishment, the institute has constantly emphasised its extraordinary accomplishments and pursued basic, applied, and translational research, training, and extension programmes that have identified and characterized Indian equids and improved their health and output over the period. By developing cutting-edge diagnostics and vaccines, the institute has significantly contributed to the eradication and control of some of the most dreaded equine diseases and in improving the health of these animals. The NRCE is tirelessly working to improve horse productivity and quality in agriculture and transportation through R&D, advising, and consultancy services.

Next-generation vaccine development, rapid point-of-care diagnostics, genome editing, host-pathogen interactions, therapeutics like ethno-veterinary medicine, antivirals, bacteriophages, genetic studies on livestock health and production, enhancement of reproductive efficiency, embryo transfer technology, repository of microbes of animal origins, etc. are a few examples of frontier research areas of the institute. By bridging the gap between basic biology and clinical applications, the Centre's research endeavours continue to provide cutting-edge translational research for the improvement of equine health and welfare in the country. The current year's annual report highlights the numerous successes and efforts made to develop workable solutions as well as efforts to build commercially viable innovations and demand-driven research for the benefit of equine rearers in 2022.

The Centre's research endeavours received considerable assistance from 38 ongoing research projects, including 21 projects with outside financing, during the year. In national and international refereed journals during the year, 53 articles were published by the scientists. The researchers also penned through 3 book chapters, several technical bulletins, popular articles, and 48 research abstracts. Besides, research papers were presented by centre researchers at various national and international symposium, conferences, seminars and workshops.

Surveillance and monitoring of equine infectious diseases is one of the major activities of the institute to monitor existing diseases as well as keeps vigilance on exotic diseases. During the year 2022, a total of 2594 equine serum samples from 10 states were tested for various diseases like Equine Infectious Anaemia, Equine Influenza, Trypanosomosis, Equine Herpes Virus-1 and 4, Equine Piroplasmosis, Japanese Encephalitis, *Salmonealla Abortus equi* and Brucellosis. In addition, under contractual diagnostic services, a total 9996 samples were received from organized sectors tested and generated a revenue of Rs. 73.60 lakhs.

In 2022, a total of 27207 equine sera from 226 districts of 18 states were collected and tested for glanders and 106 equids were found positive from 9 states. In zoonotic point of view, 141 sera from occupationally exposed veterinary officers, equine handlers, and laboratory workers were tested and were found negative. The lateral flow assay (LFA) for JEV antibody is being developed and standardized for detection of JEV antibodies in serum samples. Rotaviruses are the most common viral agents associated with foal diarrhoea. In this regard, whole-genome sequencing of four equine rotavirus A (ERV2, ERV3, ERV4 and ERV6) isolates was carried out to determine the genotypic constellations (GCs) of ERVAs and the findings of the study highlighted the terra incognita of the genomic diversity of equine rotaviruses. Equine herpesvirus 1 and 4 has been historically grouped as causal agents of equine rhinopneumonitis and EHV1 have been enlisted as WOAH notifiable terrestrial and aquatic animal diseases with ubiquitous presence. A sequence phylogenetic analysis indicated that EHV-2 and EHV-5 strains from India have shown genetic diversity from existing strains and is the first report of detection of diverse strains of EHV-2 and -5 from India. Versatile POC platform using RT-RPA-CRISPR based LFA assay for detection of SARS-CoV-2 targeting two conserved genes has been developed and could serve as a rapid detection kit of SARS-CoV-2 nucleic acids in humans as well as animals.

Technology development, evaluation, and transfer to end users have been the Centre's major activity throughout the year. During this year, ICAR-NRCE has developed vaccines against Lumpy Skin Disease virus and Coronavirus SARS-CoV-2 for animals. Rapid diagnostic kit for the diagnosis of recombinant nucleoprotein-based indirect ELISA for SARS-CoV-2 antibody detection in canines has been developed. Various technologies such as *Ancovax:* An inactivated vaccine to prevent SARS-CoV2 infection in animals, *Recombinant nucleocapsid protein based indirect ELISA kit:* To detect Anti SARS-CoV2-antibodies in canines (CAN-CoV-2 iELISA kit), *Multi recombinant proteins-based ELISA Kit:* For diagnosis of *Trypanosoma evansi* infection in animals, *Equine parentage testing technology* and *Lumpi-ProVac^{Ind} (Lympy Skin Disease Vaccine)* developed by the scientists of this Institute was released by Hon'ble Minister of Agriculture and Farmers Welfare, Govt. of India and DG, ICAR & Secretary, DARE at Krishi Bhawan, New Delhi. In this year, 6 patents were filed and one was granted, and several MoUs were signed. A total of Rs.186.533 lakhs of revenue was generated through commercialization of various technologies developed at ICAR-NRCE.

To address the rapid decline in the indigenous equine population, the Institute has taken up new initiatives to collect and conserve the embryos from Marwari horses in which two pregnancies were established in mares using fresh semen and frozen semen inseminations derived embryos' transfer. Efforts are being taken up to increase the post-thaw motility in stallion spermatozoa. Addition of *Spirulina platensis* extract significantly increased the cooled and post-thaw semen parameters and antioxidant parameters. The traits like endurance and fertility in indigenous horses are of tremendous importance and hence, the SNP markers associated with them were tested in five indigenous horse breeds. The SNP, BIEC2-1022884 (A>G) was found monomorphic and the rest were polymorphic in indigenous breeds of horses. In an attempt to recognise and characterize the Bhimthadi horse, all the parameters pertaining to a breed have been documented and breed registration is underway. Study aimed to investigate the compositional changes in Halari donkey milk during lactation where the lactose and protein levels showed minor decline from 5.79 – 4.90% and 3.85 – 3.25%, respectively as lactation progressed.

During the year 2022, in the bacterial repository (veterinary microbe component), a total of 138 bacteria were accessioned, making cumulative culture collection to 1721 bacteria of veterinary importance. Similarly, a total

of 32 viruses were accessioned during the year. Lumpy skin disease (LSD) has become the most important animal health problem in India due to high morbidity, mortality, and production losses. A homologous live-attenuated LSD vaccine (Lumpi-ProVac^{Ind}) was developed, released and commercialized from the centre. A novel HRM-based gap-qRT-PCR for identification and quantification of the vaccine and field strain(s) of lumpy skin disease virus was also developed. Efforts are also being made to strengthen the bacteriophage repository and explore their use as therapeutics. Potential bacteriophages against *Aeromonas* species from the fishponds and ESBL strains of *E. coli* and *Salmonella* sp. from poultry origin were isolated and characterized for their antibiotic resistance profile. Some of the significant accessioned cultures are *Moraxella canis, M. ovis, Pasteurella canis, Edwarsiellatarda, Acinetobacter baumanii, Elizabethkingia anopheles, Bordetella parapertussis, B. avium, Streptococcus uberis, St. dysgalactiae* ssp*. dysgalactiae, St. suis, St. gallinaceous, Brevibacillus borstelensis, Coryebacterium faecalis, Coryne. hansanii, C. liphophiloflavum, Pediococcus acidilacti, Lysinibacillus macroides,* and *Gallibacterium anatis*. During this year, several workshops, training programs, equine health camps, MGM activities, stakeholder meetings and community outreach activities were conducted at the institute and fields to improve equine health, conservation and productivity and to create awareness about equine husbandry.

I owe a great deal of gratitude and debt to Dr. Himanshu Pathak, Director General, ICAR and Secretary, DARE, for his unwavering support, direction and guidance in conducting research, education and extension utilizing cutting edge technologies for the benefits of equine and other livestock farmers and stakeholders in the country. I also extend my heartfelt thanks and gratitude to Dr. Trilochan Mohapatra, Former Director General, ICAR and Secretary, DARE for his untiring support to the Institute for carrying out many R&D activities in the advanced areas of Animal Sciences. I am also indebted to Shri Sanjay Garg, Additional Secretary (DARE) & Secretary (ICAR) and Ms. Alka Nangia Arora, Joint secretary (DARE) & Finance Advisor, ICAR for their help to carry out Institute activities very smoothly and to meet out the aspirations of equine and other livestock farmers in the country. I owe a great deal of gratitude to Dr. B.N. Tripathi, Former Deputy Director General (Animal Science), who has consistently been keen to support and mentor our team of scientists and institute in carrying out diversified R&D, education and extension activities with great enthusiasm. I also extend my heartfelt thanks to Dr. J.K. Jena, Deputy Director General (Animal Science & Fishery Science) for his constant support, encouragement and guidance to accomplish Institutional activities with a great success. I also wish to express my gratitude to Dr. Ashok Kumar, ADG (Animal Health), Dr. Amrish Kumar Tyagi, ADG (Animal Nutrition & Physiology), Dr. P.K. Raut, ADG (Animal Production and Breeding), Dr. V.K. Saxena, Former ADG (Animal Production &Breeding), Hon'ble Chairman and Members of RAC, and IMC for their valuable suggestions, support, and guidance in accomplishment of activities of the institute with a great success. I also extend my gratitude to the staff at ICAR Headquarters, particularly Dr. (Mrs.) Jyoti Misri, Pr. Scientist (Animal Health), Dr. Vineet Bhasin, Former Pr. Scientist (AP&B), Dr. Rajan Gupta, Former Pr. Scientist (AN&P), Dr. Rajneesh Rana, Pr. Scientist and others, for their unwavering support and assistance rendered to the Institute to function smoothly.

I also take this opportunity to express my sincere gratitude and appreciation to the Chairman and Members of the Publication Committee for their untiring efforts for compiling and editing the Annual Report thoroughly and nicely showcasing all the Institute activities systematically for easy understanding of the readers. Last but not the least, I would like to express my gratitude to all the scientific, technical, administrative, financial and supporting staff of this Institute for their sincere efforts and commitment for achieving success of different programmes taken up at the Institute during the year and for ultimate benefits of equine stakeholders and farmers in the country.

(TK Bhattacharjya)
Director, ICAR-NRCE

Executive Summary

कार्यकारी सारांश

Horses have been domesticated since prehistoric times and hold a special place in our history & culture. 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 to the area of diagnosis and control of equine infectious diseases. The Centre made significant research contributions through 38 research projects, including 21 externally funded projects by DBT, DST, DRDE, ICAR extramural and NASF during the year. The salient achievements of the Centre during 2022 are outlined below.

Surveillance and monitoring of equine infectious disease are one of the continuous service projects of the institute to monitor existing diseases as well as keep vigilance on exotic diseases. During the year 2022, a total of 2594 equine serum samples from 10 states were tested for various diseases like Equine Infectious Anaemia, Equine Influenza, Trypanosomosis, Equine Herpes Virus-1 and 4, Equine Piroplasmosis, Japanese Encephalitis, *Salmonealla Abortus equi* and Brucellosis. Highest sero-prevalence was observed for *Theileria equi* (38.47%) followed by EHV-1 (9.13 %), *Trypanosoma evansi* (2.66%), JE (0.42%) and EI (0.19%). None of the equines were found to be positive for equine infectious anemia, brucellosis, and *Salmonella Abortus equi*.

Microbiological analysis was carried out on 348 biological and environmental samples originating from Haryana, Uttar Pradesh, Rajasthan, Punjab, and Maharashtra. A total 42 bacterial isolates including *Klebsiella pnuemoniae* (n=18), *E. coli* (*n*=9),

प्रागैतिहासिक काल से ही घोड़ों को पाला जाता रहा है और वे हमारे इतिहास और संस्कृति में एक विशेष स्थान रखते हैं। देश में अश्व स्वास्थ्य की जरूरतों को पूरा करने और अश्व उत्पादकता बढ़ाने के लिए, भारतीय कृषि अनुसंधान परिषद ने 26 नवंबर, 1985 को हिसार (हरियाणा) में राष्ट्रीय अश्व अनुसंधान केंद्र (रा.अ.अनु.के.) की स्थापना की। भा.कृ.अनु.प.– रा.अ.अनु.के. ने अश्व संक्रामक रोगों के निदान और नियंत्रण के क्षेत्र में महत्वपूर्ण योगदान दिया है। केंद्र ने गत वर्ष के दौरान डीबीटी, डीएसटी, डीआरडीई, भा.कृ.अनु.प.–एक्स्ट्रामुरल और डब्ल्यूएएचओ द्वारा 21 बाह्य वित्त पोषित परियोजनाओं सहित 38 अनुसंधान परियोजनाओं के माध्यम से महत्वपूर्ण अनुसंधान योगदान दिया। वर्ष 2022 के दौरान केंद्र की मुख्य उपलब्धियाँ उल्लिखित की गई हैं।

मौजूदा बीमारियों की निगरानी के साथ–साथ विदेशागत रोगों पर निगरानी रखने के लिए अश्व संक्रामक रोगों का सर्वेक्षण और निगरानी, संस्थान की निरंतर सेवा परियोजनाओं में से एक है। वर्ष 2022 के दौरान, कुल 10 राज्यों से 2594 इक्वाइन सीरम नमूनों का परीक्षण विभिन्न बीमारियों जैसे इक्वाइन संक्रामक एनीमिया, इक्वाइन इन्फ्लुएंजा, ट्रिपैनोसोमोसिस, इक्वाइन हर्पीस वायरस –1 (EHV-1) और 4 (EHV-4), इक्वाइन पायरोप्लाज्मोसिस, जापानी एन्सेफलाइटिस, साल्मोनेला एबोर्टस इक्वी और ब्रुसेलोसिस के लिए किया गया था। सबसे अधिक सीरो–व्यापकता थिलेरिया इक्वी (38-47%) की देखी गई। EHV&1 (9-13%), ट्रिपैनोसोमा इवांसाई (2-66%), जेई (0-42%) और ईआई (0-19%) की सीरो–व्यापकता पाई गई। कोई भी घोड़ा संक्रामक एनीमिया, ब्रुसेलोसिस और साल्मोनेला एबोर्टस इक्वी के लिए संक्रमित नहीं पाया गया।

हरियाणा, उत्तर प्रदेश, राजस्थान, पंजाब और महाराष्ट्र से आए 348 जैविक और पर्यावरणीय नमूनों पर सूक्ष्मजीवविज्ञानी विश्लेषण किया गया। इन नमुनों से क्लेबसिएला न्यूमोनिया (सं. = 18), ई. कोलाई (सं. = 9), रोडोकोकस इक्वीई (सं.

Rhodococcus equi (*n*=2), *Streptococcus equi* subsp *zooepedemicus* (*n*=8) and *Burkholderia mallei* (*n*=5) were isolated from these samples.

Under contractual diagnostic services, a total 9996 samples were received from racecourses, turf club, stud farm, riding schools, animal quarantine & certification services (AQCS) and other organized sectors during the year 2022. These samples were tested for various notifiable and exotic diseases. A total of 4968 sera samples for EIA and 3598 samples for glanders were tested. Among exotic diseases, 512 swab samples from for Contagious Equine Metritis (CEM), 275 sera samples for equine viral arteritis (EVA), 258 sera samples for African Horse Sickness (AHS) and 263 sera samples for dourine were received from AQCS, Govt. of India, collected from imported equines. All the samples were found negative for these exotic diseases.

For surveillance, control, and eradication of Glanders in equines from India, National Action Plan on Glanders for control and eradication of glanders was launched by the Department of Animal Husbandry & Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India in 2019. ICAR-NRCE is coordinating the glanders surveillance programme in collaboration with the State Animal Husbandry Department. In 2022, a total of 27207 equine sera from 226 districts of 18 states were collected and tested for glanders. During this year, 106 glanders positive cases were reported in 9 states. Glanders affected states include Uttar Pradesh (n = 55), Uttarakhand (n = 8), Haryana (n = 16), Himachal Pradesh (n = 11), Gujarat (n = 4), Maharashtra (n = 2), Madhya Pradesh (n = 3), Andhra Pradesh (n = 2) and Rajasthan (*n* = 5). It was found that around 50 % of the samples and glanders positive cases originated from Uttar Pradesh. In zoonotic point of view, 141 sera from occupationally exposed humans (Veterinary Officers, equine handlers, and laboratory workers) from Haryana, Uttar Pradesh, Madhya Pradesh, Jammu, and Andhra Pradesh were tested and none of them were found positive.

Three-dimensional bioprinting has emerged as a flexible tool in regenerative medicine. The polymers were integrated to the 3D structure. Encouraging

= 2), स्ट्रेप्टोकोकस इक्वीई सबस्प जूएपेडेमिकस (सं. = 8) और बर्कहोल्डरिया मैलीआई (सं. = 5) सहित कुल 42 जीवाणु पृथक किए गए थे।

संविदात्मक निदान सेवाओं के तहत, वर्ष 2022 के दौरान रेसकोर्स, टर्फ क्लब, स्टड फार्म, राइडिंग स्कूल, पशु संगरोध और प्रमाणन सेवाओं (एक्यूसीएस) और अन्य संगठित क्षेत्रों से कुल 9996 नमूने प्राप्त हुए। इन नमूनों का परीक्षण विभिन्न उल्लेखनीय और विदेशागत बीमारियों के लिए किया गया था। ईआईए के लिए कुल 4968 सीरा नमूने और ग्लैंडर्स के लिए 3598 नमूनों का परीक्षण किया गया। विदेशागत बीमारियों में, संक्रामक इक्वाइन मेट्राइटिस (सीईएम) के लिए 512 स्वैब नमूने, इक्वाइन वायरल आर्टेराइटिस (ईवीए) के लिए 275 सीरा नमूने, अफ्रीकी हॉर्स सिकनेस (एएचएस) के लिए 258 सीरा नमूने और डूरिन के लिए 263 सीरा नमूने एक्यूसीएस, भारत सरकार से प्राप्त हुए थे जो आयातित घोड़ों से एकत्रित किए गए थे। कोई भी नमूना इन विदेशागत बीमारियों से संक्रमित नहीं थे पाया गया।

भारत के घोड़ों में ग्लैंडर्स की निगरानी, नियंत्रण और उन्मूलन के लिए, पशुपालन और डेयरी विभाग, मत्स्य पालन, पशुपालन और डेयरी मंत्रालय, भारत सरकार द्वारा ग्लैंडर्स पर नियंत्रण और उन्मूलन के लिए राष्ट्रीय कार्य योजना 2019 में शुरू की गई थी। भा.कृ.अनु.प.– रा.अ.अनु.के. राज्य पशुपालन विभागों के सहयोग से, ग्लैंडर्स की निगरानी कार्यक्रम का समन्वय कर रहा है। वर्ष 2022 में, 18 राज्यों के 226 जिलों से कुल 27207 इक्वाइन सीरा नमूने एकत्रित किए गए और ग्लैंडर्स के लिए परीक्षण किया गया। इस वर्ष के दौरान, 9 राज्यों में 106 ग्लैंडर्स पॉजिटिव मामले सामने आए। ग्लैंडर्स प्रभावित राज्यों में – उत्तर प्रदेश (सं. = 55), उत्तराखंड (सं. = 8), हरियाणा (सं. = 16), हिमाचल प्रदेश (सं. = 11), गुजरात (सं. = 4), महाराष्ट्र (सं.= 2), मध्य प्रदेश (सं. = 3), आंध्र प्रदेश (सं. = 2) और राजस्थान (सं. = 5) शामिल हैं। यह पाया गया कि लगभग 50% नमूने और ग्लैंडर्स पॉजिटिव मामले उत्तर प्रदेश से सम्बन्धित हुए हैं। जूनोटिक दृष्टिकोण से हरियाणा, उत्तर प्रदेश, मध्य प्रदेश, जम्मू और आंध्र प्रदेश से व्यावसायिक रूप से संपर्क में आने वाले मनुष्यों (पशु चिकित्सा अधिकारी, अश्व संचालक और प्रयोगशाला कर्मचारियों) के 141 सीरा नमूनों का परीक्षण किया गया और उनमें से कोई भी संक्रमित नहीं पाया गया।

त्रि-आयामी बायोप्रिंटिंग, पुनर्योजी चिकित्सा में, एक उपयुक्त साधन के रूप में उभरा है और ऊपर वर्णित कुछ आवश्यकताओं को संबोधित करने के लिए एक मंच प्रदान

results revealed that integration of skin ECM in bioink had improved bioactivity in vitro. Further analysis is underway. The LFA for JEV antibody is being developed and standardized for detection of JEV antibodies in serum samples. The recombinant antigen and BPL-inactivated cell culture grown Japanese encephalitis virus antigen were prepared and preliminary testing results showed that inactivated virus antigen was giving better results in LFA. A spectrum of multispecies sera from equine, guinea pigs and rabbits are being tested on the kits. The internal and external validation of the test is ongoing.

Rotaviruses are the most common viral agents associated with foal diarrhea. Between 2014 and 2017, the annual prevalence of rotavirus in diarrheic foals ranged between 18 and 28 % in Haryana (India). Whole genome sequencing of RVA/Horse-wt/IND/ERV3/2003, RVA/Horse-wt/IND/ERV2/2015, RVA/Horse-wt/IND/ERV4/2017, RVA/Horse-wt/IND/ERV6/2017 was done. Whole-genome sequencing of four equine rotavirus A (ERV2, ERV3, ERV4 and ERV6) isolates was carried out to determine the genotypic constellations (GCs) of ERVAs. The findings of the study highlighted the terra incognita of the genomic diversity of equine rotaviruses and support the need for the surveillance of RVAs in animals and humans with a "one health" approach.

Equine herpesvirus 1 and 4 have been historically grouped as causal agents of equine rhinopneumonitis and EHV1 has been enlisted as WAHO notifiable terrestrial and aquatic animal *disease* with ubiquitous presence. A sequence phylogenetic analysis indicated that Indian EHV-2 and EHV-5 strains from India have marked genetic diversity. The nucleotide sequence identity ranged from 97.30 - 99.32 % for EHV-2 and 98.28 – 100 % for EHV-5 strains. This is the first report of detection of diverse strains of EHV-2 and 5 from India.

The field-deployable point-of-care diagnostic test for rapid detection of SARS-COV-2 is needed for implementation of the control measures. Scientists at this centre report the development of RT-RPA-CRISPR based LFA assay for detection of SARS-CoV-2 targeting two conserved genes. The currently

करता है। पॉलिमर में समाविष्ट पाचित चिकन/पोर्साइन त्वचा से विकसित त्वचा विशिष्ट बायोइंक ने 3डी संरचना प्रदान की। घटक विभिन्न कोशिकाओं को एक अतिरिक्त सतह देते हैं और उपचारात्मक मैक्रोमोलेक्यूल्स जुड़ सकते हैं और अन्त:क्रिया कर सकते हैं। उत्साहवर्धक परिणामों से पता चला कि बायोइंक में त्वचा ईसीएम के एकीकरण से इन विट्रो प्रयोग में बायोएक्टिविटी में सुधार हुआ है।

सीरम नमूनों में जेईवी एंटीबॉडी का पता लगाने के लिए LFA विकसित और मानकीकृत किया जा रहा है। पुन: संयोजक एंटीजन और BPL-निष्क्रिय कोश पालन से विकसित जापानी एन्सेफलाइटिस वायरस एंटीजन तैयार किया गया और प्रारंभिक परीक्षण परिणामों से पता चला कि निष्क्रिय वायरस एंटीजन LFA में बेहतर परिणाम दे रहा था। किटों से घोड़े, गिनी पिग और खरगोशों से प्राप्त बहु-प्रजाति सीरा के एक स्पेक्ट्रम का परीक्षण किया जा रहा है। परीक्षण का आंतरिक और बाहरी सत्यापन जारी है।

रोटावायरस घोड़ों के बच्चों के दस्त से जुड़े सबसे आम विषाणु हैं। वर्ष 2014 से 2017 के बीच, हरियाणा (भारत) में डायरिया से पीड़ित घोड़ों के बच्चों में रोटावायरस का वार्षिक प्रसार 18 से 28% के बीच था। RVA/Horse-wt/IND/ERV3/2003, RVA/Horse-wt/IND/ERV2/2015, RVA/Horse-wt/IND/ERV4/2017, RVA/Horse-wt/IND/ERV6/2017 का संपूर्ण जीनोम अनुक्रमण किया गया था। ईआरवीए के जीनोटाइप (GCS) को निर्धारित करने के लिए चार इक्वाइन रोटावायरस ए (ERV2, ERV3, ERV4 और ERV6) आइसोलेट्स का संपूर्ण-जीनोम अनुक्रमण किया गया था। अध्ययन के निष्कर्षों ने इक्वाइन रोटावायरस की जीनोमिक विविधता की टेरा इनकॉग्निटा पर प्रकाश डाला और "वन हैल्थ" दृष्टिकोण के साथ जानवरों और मनुष्यों में RVA की निगरानी की आवश्यकता का समर्थन किया।

इक्वाइन हर्पीसवायरस 1 और 4 को ऐतिहासिक रूप से इक्वाइन रहाईनोन्यूमोनाइटिस के कारक एजेंटों के रूप में समूहीकृत किया गया है और EHV1 को सर्वव्यापी उपस्थिति के साथ WAHO अधिसूचित स्थलीय और जलीय पशु रोग के रूप में सूचीबद्ध किया गया है। एक अनुक्रम वर्गानुवंशिक विश्लेषण से संकेत मिलता है कि भारत के EHV-2 और EHV-5 प्रभेदों में अधिक विविधता है। न्यूक्लियोटाइड अनुक्रम समानता EHV-2 के लिए 97.30 – 99.32% और EHV-5 प्रभेदों के लिए 98.28 – 100% के बीच थी। यह भारत में EHV-2 और 5 के विविध प्रभेदों का पता लगाने की पहली रिपोर्ट है।

SARS-COV-2 का तेजी से पता लगाने और नियंत्रण उपायों के कार्यान्वयन के लिए फील्ड-परिनियोजन योग्य

developed assay could serve as a versatile POC platform for rapid detection of SARS-CoV-2 nucleic acids in humans as well as animals.

During the pandemic of COVID-19, transmission of SARS-CoV-2 virus was detected in many animals including tigers, lions, minks, canines, felines, and deer. Looking into the threat of transmission of viruses in many species, a recombinant nucleocapsid protein based indirect ELISA kit was developed for detection of SARS-CoV-2 antibody in canines and felines. The developed *i*ELISA is highly specific and doesn't cross-react with other related coronaviruses of canine. The kit has been released by Hon'ble Minister of Agriculture & Farmer Welfare and DG (ICAR) on 9 June 2022. A fixed cell ELISA was also attempted for detection of serum antibodies and identification of asymptomatic carriers of Strangles.

Scientists at the center, developed a multi-host species ELISA for diagnosis of Trypanosomosis in animals, which will be helpful in monitoring and surveillance of infection in wild animals and other warm-blooded vertebrates where host specific secondary antibody conjugates are not available. Theileriosis is one of the very important protozoan diseases of animals. The ICAR-NRCE initiated research on development of vaccine against *Theileria equi* and *Theileria annulata* protozoan parasites. Initially proteomic analysis of Theileria *equi* merozoites and T. *annulata* schizonts was undertaken and merozoites/schizonts were isolated and purified from in-vitro cultures. These samples were submitted for proteomic analysis. The scientists also expressed various genes that will be tested for their vaccine potential.

Dermatophilus congolensis (DC) was isolated and identified by using conventional and molecular methods from the samples obtained from the skin infections of horses. Anti-bacterial activity of some selected plant extracts against this bacterium was observed. The results of this study support that *Eucalyptus camaldulensis*, *Azadirachta indica* and *Aloe vera* have antibacterial activity against *Dermatophilus congolensis* so can be used/ added in topical antibacterial preparations. In another study involving herbal active ingredients like *Eucalyptus*

पॉइंट-ऑफ-केयर (POC) डायग्नोस्टिक परीक्षण की आवश्यकता है। इस केंद्र के वैज्ञानिकों ने दो संरक्षित जीनों को लक्षित करके SARS-CoV-2 का पता लगाने के लिए RT-RPA-CRISPR आधारित LFA परीक्षण को विकसित किया है। वर्तमान में विकसित परीक्षण, मनुष्यों के साथ-साथ जानवरों में SARS-CoV-2 न्यूक्लिक एसिड का तेजी से पता लगाने के लिए एक POC प्लेटफॉर्म के रूप में काम कर सकता है।

COVID-19 महामारी के दौरान, बाघ, शेर, मिंक, कुत्ते, बिल्ली और हिरण सहित कई जानवरों में SARS-CoV-2 वायरस का संचरण पाया गया था। कई प्रजातियों में वायरस के संचरण के खतरे को देखते हुए, शवानों और बिल्लियों में SARS-CoV-2 एंटीबॉडी का पता लगाने के लिए एक पुनः संयोजक न्यूक्लियोकैप्सिड प्रोटीन आधारित अप्रत्यक्ष एलिसा (ELISA) किट विकसित की गई थी। विकसित iELISA अत्यधिक विशिष्ट है और शवानों के अन्य संबंधित कोरोना वायरस के साथ क्रॉस-रिएक्शन नहीं करता है। यह किट 9 जून 2022 को माननीय कृषि एवं किसान कल्याण मंत्री और महानिदेशक (भा.कृ.अनु.प.) द्वारा जारी की गई है। सीरम एंटीबॉडी का पता लगाने और स्ट्रैंगल्स के स्पर्शोन्मुख वाहक की पहचान के लिए एक फिक्स्ड सेल एलिसा का भी प्रयास किया गया था।

केंद्र के वैज्ञानिकों ने निदान के लिए एक मल्टी-होस्ट प्रजाति एलिसा विकसित किया है जो जंगली जानवरों और अन्य गर्म रक्त वाले रीड़दार प्राणियों में संक्रमण की निगरानी और निरीक्षण में सहायक होगा जहां होस्ट विशिष्ट माध्यमिक एंटीबॉडी संयुग्म उपलब्ध नहीं हैं। एक अन्य अध्ययन में थिलेरिया इक्वीई मेरोजोइट्स और टी. एनुलेटा शाईजोन्ट्स को प्रोटिओमिक विश्लेषण के लिए इन-विट्रो कल्चर्स से प्रथम और शुद्ध किया गया था।

घोड़ों की त्वचा के संक्रमण से प्राप्त नमूनों से पारंपरिक और आणविक तरीकों का उपयोग करके डर्मेटोफिलस कॉन्गोलेंसिस (डीसी) को पृथक कर पहचाना गया। इस जीवाणु के प्रतिकूल कुछ चयनित पौधों के अर्क की जीवाणुरोधी गतिविधि देखी गई। इस अध्ययन के नतीजे इस बात का समर्थन करते हैं कि यूकेलिप्टस कैमलडुलेंसिस, अजादिरखता इंडिका और एलोवेरा में डर्मेटोफिलस कश्वन्गोलेंसिस के प्रतिकूल जीवाणुरोधी गतिविधि है, इसलिए इन्हें सामयिक जीवाणुरोधी अनुप्रयोग में इस्तेमाल किया जा सकता है। एक अन्य अध्ययन में यूकेलिप्टस कैमलडुलेंसिस, अजादिरखता इंडिका और एलोवेरा में स्टैफायलोकोकस ऑरियस के प्रतिकूल जीवाणुरोधी

camaldulensis, *Azadirachta indica* and *Aloe vera* proved to have antibacterial activity against *Staphylococcus aureus* and have potential to be used clinically.

To address the rapid decline in the indigenous equine population the center has taken up a new initiative to collect and conserve the embryos from Marwari horses. In this study, the estrus synchronization protocols were optimized and methods of embryo recovery from mares and successful transfer of embryos to the surrogates were optimized. In the Marwari breed for the first time, two pregnancies were established in mares using fresh semen and frozen semen inseminations derived embryos' transfer. Uterine, ovarian, and follicular studies in Marwari mares were also conducted to detect the time of ovulation and variation in some cytokines during the periovulatory period were studied. No significant difference was found in mean values of serum concentration of the cytokines (IL-2, IL-6, IL-1β, TNF-α and VEGF) between different stages of the oestrus cycle in the study. Changes in hematological values during estrus and periovulatory periods indicate a relationship of hematological values with ovulation. Serum concentration of cytokines (IL-2, IL-6, IL-1β, TNF-α and VEGF) and their gene expression do not seem to be related with the ovarian and uterine changes during estrous cycle.

Efforts are being made to increase the post thaw motility in stallion spermatozoa. In this direction a study was conducted to study the effect of supplementation of *Spirulina platensis* extract to semen extender on cooled and post-thaw semen quality in Marwari stallions. Addition of *Spirulina platensis* extract (@50 μg/ml) significantly ($P<0.05$) increased the cooled and post-thaw semen parameters and antioxidant parameters viz., superoxide dismutase, catalase and total antioxidant capacity were significantly ($P<0.05$) increased while lipid peroxidation parameter malondialdehyde was significantly ($P<0.05$) decreased in post-thaw semen by addition of *Spirulina platensis* extract. Another study was also designed to assess if the vital sperm functional parameters of genetically superior stallions producing poor quality semen can be

गतिविधि पाई गई और इन्हें नैदानिक रूप से उपयोग किए जाने की संभावना है।

स्वदेशी घोड़ों की आबादी में तेजी से गिरावट को संबोधित करने के लिए केंद्र ने मारवाड़ी घोड़ों के भ्रूणों को इकट्ठा करने और संरक्षित करने के लिए एक नई पहल की है। इस अध्ययन में, एस्ट्रस सिंक्रोनाइजेशन प्रोटोकॉल को ऑप्टिमाईज किया गया और घोड़ी से भ्रूण की पुनर्प्राप्ति और सरोगेट्स में भ्रूण के सफल स्थानांतरण के तरीकों को ऑप्टिमाईज किया गया। मारवाड़ी नस्ल में पहली बार, ताजा वीर्य और फ्रोजन वीर्य गर्भाधान से प्राप्त भ्रूण के स्थानांतरण का उपयोग करके घोड़ियों में दो गर्भधारण स्थापित किए गए। ओव्यूलेशन के समय का पता लगाने के लिए मारवाड़ी घोड़ियों में गर्भाशय, डिम्बग्रंथि और कूपिक अध्ययन भी किए गए और पेरिओवुलेटरी अवधि के दौरान कुछ साइटोकाईन्स में भिन्नता का अध्ययन किया गया। अध्ययन में मद चक्र के विभिन्न चरणों के बीच साइटोकाईन्स (IL-2, IL-6, IL-1β, TNF-α और VEGF) की सीरम सांद्रता के औसत मूल्यों में कोई महत्वपूर्ण अंतर नहीं पाया गया। एस्ट्रस और पेरीओवुलेटरी अवधि के दौरान हेमेटोलॉजिकल मूल्यों में परिवर्तन ओव्यूलेशन के साथ हेमेटोलॉजिकल मूल्यों के संबंध का संकेत देता है। साइटोकाइन्स (IL-2, IL-6, IL-1β, TNF-α और VEGF) की सीरम सांद्रता और उनकी जीन अभिव्यक्ति एस्ट्रस चक्र के दौरान डिम्बग्रंथि और गर्भाशय में होने वाले परिवर्तनों से संबंधित नहीं दिखता है।

घोड़े के शुक्राणुओं में पिघलाने के बाद की गतिशीलता को बढ़ाने के प्रयास किए जा रहे हैं। इस दिशा में मारवाड़ी घोड़े में ठंडे और पिघलने के बाद वीर्य की गुणवत्ता पर वीर्य विस्तारक में स्पिरुलिना प्लैटेंसिस अर्क के प्रभाव का अध्ययन किया गया। स्पिरुलिना प्लैटेंसिस अर्क (@50 μg/ml) को मिलाने से ठंडे और पिघलने के बाद वीर्य मापदंडों में काफी वृद्धि हुई और एंटीऑक्सीडेंट पैरामीटर जैसे, सुपरऑक्साइड डिसम्यूटेज, कैटालेज और कुल एंटीऑक्सीडेंट क्षमता में काफी वृद्धि हुई ($p<0-05$) जबकि लिपिड पेरोक्सीडेशन पैरामीटर मैलोनडायल्डिहाइड में काफी कमी आई ($p<0-05$)। एक अन्य अध्ययन, यह आकलन करने के लिए भी डिजाइन किया गया था कि क्या खराब गुणवत्ता वाले वीर्य का उत्पादन करने वाले आनुवंशिक रूप से बेहतर स्टैलियन के महत्वपूर्ण शुक्राणु कार्यात्मक मापदंडों को उच्च गुणवत्ता वाले वीर्य का उत्पादन करने वाले स्टैलियन से हेटेरोलॉगस सेमिनल प्लाज्मा (एसपी) (20% और 30%) के पूरक द्वारा बढ़ाया जा सकता है। सामूहिक रूप से, विषमलैंगिक का सेमिनल प्लाज्मा मिलने पर

enhanced by the supplementation of heterologous seminal plasma (SP) (20 % and 30 %) from the stallion producing high quality semen. Collectively, supplementation of heterologous seminal plasma reduced viability and increased RoS production in stallion sperm.

Another study was conducted to find the markers associated with endurance and fertility using SNP Markers. Eight markers associated with endurance were tested in indigenous breeds. Five SNP markers associated with fertility were studied. The SNP BIEC2-1022884 (A>G) was found monomorphic and the rest were polymorphic in indigenous breeds of horse. For identifying the endurance related markers, a total of 425 samples belonging mainly to Sindhi breed were amplified by PCR for respective SNP and sequencing was carried out.

To recognise and characterize the Bhimthadi horse, information regarding its origin, history, development, distribution, population status, traditional breeders, physical characteristics, body weight and reproductive traits have been collected from its breeding tact. The application for registration of the breed was prepared in accordance with the guidelines of ICAR-NBAGR, Karnal and submitted through the Commission Animal Husbandry, Govt. of Maharashtra.

Mathematical functions for the prediction of growth in Marwari horses were derived. The study indicated that the initial growth phase in Marwari and Manipuri continues up to the age of about 6 years and up to the age of 5½ years in Zanskari horses, there after it remains static till about 8-9 years of age; and the cubic function can reliably be used to explain the nature of growth in Marwari, Manipuri and Zanskari breeds of horses. Studies to find the selection signatures through gene annotation and genome wide SNP based genomic diversity and population structure in Indigenous equine breeds are under way.

Since donkey milk has a similar composition to human milk, it has been well demonstrated that it is an excellent alternative. A study was conducted to investigate the compositional changes in Halari donkey milk during lactation. The findings

व्यवहार्यता कम पाई गई और स्टैलियन शुक्राणु में आरओएस उत्पादन बढ़ गया।

एसएनपी मार्करों का उपयोग करके सहनशक्ति और प्रजनन क्षमता से जुड़े मार्करों को खोजने के लिए एक अन्य अध्ययन किया गया। देशी नस्लों में सहनशक्ति से जुड़े आठ मार्करों का परीक्षण किया गया। प्रजनन क्षमता से जुड़े पांच एसएनपी मार्करों का अध्ययन किया गया। SNP BIEC2-1022884 (A>G) को घोड़ों की स्वदेशी नस्लों में मोनोमोर्फिक और बाकी को बहुरूपी पाया गया। सहनशक्ति संबंधी मार्करों की पहचान करने के लिए मुख्य रूप से सिंधी नस्ल से संबंधित कुल 425 नमूनों को संबंधित एसएनपी के लिए पीसीआर द्वारा प्रवर्धित किया गया और अनुक्रमण किया गया।

भीमथाडी घोड़े को पहचानने और उसकी विशेषता जानने के प्रयास में, इसकी प्रजनन पथ से इसकी उत्पत्ति, इतिहास, विकास, वितरण, जनसंख्या स्थिति, पारंपरिक प्रजनकों, शारीरिक विशेषताओं, शरीर के वजन और प्रजनन लक्षणों के बारे में जानकारी एकत्र की गई है। नस्ल के पंजीकरण के लिए आवेदन भा.कृ.अनु.प. – एन.बी.ए.जी.आर., करनाल के दिशानिर्देशों के अनुसार तैयार किया गया और पशुपालन आयोग, महाराष्ट्र सरकार के माध्यम से प्रस्तुत किया गया।

मारवाड़ी घोड़ों की वृद्धि की पूर्व सूचना के लिए गणितीय फलन प्राप्त किए गए। अध्ययन से संकेत मिलता है कि मारवाड़ी और मणिपुरी में प्रारंभिक विकास चरण लगभग 6 साल की उम्र तक और जांस्करी घोड़ों में साढ़े पांच साल की उम्र तक जारी रहता है, इसके बाद यह लगभग 8–9 साल की उम्र तक स्थिर रहता हैय और क्यूबिक फंक्शन का उपयोग मारवाड़ी, मणिपुरी और जांस्करी नस्ल के घोड़ों में वृद्धि की प्रकृति को समझाने के लिए विश्वसनीय रूप से किया जा सकता है। स्वदेशी अश्व नस्लों में जीन एनोटेशन और जीनोम वाइड एसएनपी आधारित जीनोमिक विविधता और जनसंख्या संरचना के माध्यम से चयन हस्ताक्षर खोजने के लिए अध्ययन चल रहा है।

चूँकि गर्दभ के दूध की संरचना मानव दूध के समान होती है, इसलिए यह अच्छी तरह से प्रदर्शित किया गया है कि यह एक उत्कृष्ट विकल्प है। स्तनपान के दौरान हलारी मादा गर्दभ के दूध में होने वाले संरचनात्मक परिवर्तनों की जांच के लिए एक अध्ययन किया गया था। निष्कर्षों से पता चला कि मादा गर्दभ के दूध की संरचना पूरे स्तनपान के दौरान नाटकीय रूप से बदल गई, कोलोस्ट्रम चरण में वसा का उच्चतम स्तर (1.67%) प्रदर्शित हुआ, जो स्तनपान बढ़ने के साथ कम हो गया और अंततः वसा (0.26%) तक पहुंच गया। लैक्टोज और प्रोटीन के

demonstrated that donkey milk's composition changed dramatically throughout lactation, with the colostrum stage exhibiting the highest levels of fat (1.67 %), which decreased as lactation progressed and eventually reached fat (0.26 %). The lactose and protein levels showed minor decline from 5.79 – 4.90 % and 3.85 – 3.25 % respectively. The DNA from the milk somatic cells was isolated and κ-casein, α- Lactalbumin (*LALBA*) genes were characterized in Halari donkey milk. Various transcriptomic and proteomic studies are also ongoing to better characterize and identify nutraceutical properties of donkey milk. Studies on the adaptability of existing goat milking machines for donkey milking showed that the goat milking machine is suitable for donkey milking too and modification of the machine is not needed. New creep feeders were designed and fabricated for optimum growth of the foals.

The activities of NCVTC comprise acquisition, authentication, preservation, documentation, and repository database management system of animal microbes. During the year 22, in the bacterial repository (veterinary microbe component), a total of 138 bacteria were accessioned during the year, making cumulative culture collection to 1721 bacteria of veterinary importance. Similarly, a total of 32 viruses were accessioned during the year.

ROCK1/MLC2/WDR5 cell signaling pathway appears to facilitate BPXV replication by three independent mechanisms; stabilization of viral mRNA, induction of cell contraction that appears to anchor the virus at the replication sites and inhibition of IFN-β induction.

Lumpy skin disease (LSD) has become the most important animal health problem in India due to high morbidity, mortality, and production losses. A homologous live-attenuated LSD vaccine (Lumpi-ProVac^{Ind}) was recently developed by ICAR-NCVTC. Evaluation of the safety, immunogenicity and efficacy of a new live-attenuated lumpy skin disease vaccine was conducted. A minimum Neethling response (0.018 % animals; 5 out of 26940 animals) of the vaccine was observed in the field trials conducted in 26940 animals. There was no

स्तर में क्रमशः 5.79 – 4.90% और 3.85 – 3.25% की मामूली गिरावट देखी गई। दूध की दैहिक कोशिकाओं से डीएनए को अलग किया गया और हलारी मादा गर्दभ के दूध में κ-केसीन, α- लैक्टलबुमिन (LALBA) जीन की विशेषता बताई गई। मादा गर्दभ के दूध के न्यूट्रास्युटिकल गुणों को बेहतर ढंग से चित्रित करने और पहचानने के लिए विभिन्न ट्रांसक्रिप्टोमिक और प्रोटिओमिक अध्ययन भी जारी हैं। मादा गर्दभ का दूध निकालने के लिए मौजूदा बकरी से दूध निकालने वाली मशीनों की अनुकूलनशीलता पर अध्ययन से पता चला कि बकरी का दूध निकालने वाली मशीन मादा गर्दभ का दूध निकालने के लिए भी उपयुक्त है और मशीन में संशोधन की आवश्यकता पर अध्ययन जारी है। गर्दभ के बच्चों के सर्वोत्तम विकास के लिए नए क्रीप फीडरों को डिजाइन और निर्मित किया गया।

एन.सी.वी.टी.सी. की गतिविधियों में पशु रोगाणुओं का अधिग्रहण, प्रमाणीकरण, संरक्षण, दस्तावेजीकरण और भंडार डेटाबेस प्रबंधन प्रणाली शामिल है। वर्ष 2022 के दौरान, जीवाणु भंडार द्धपशु चिकित्सा सूक्ष्म जीव घटकऋ में, कुल 138 जीवाणु शामिल किए गए, जिससे पशु चिकित्सा महत्व के 1721 जीवाणु का संचयित संग्रह हो गया। इसी प्रकार, 2022 वर्ष के दौरान कुल 32 विषाणु संग्रहित हुए।

ROCK1/MLC2/WDR5 सेल सिग्नलिंग मार्ग तीन स्वतंत्र तंत्रों द्वारा ठच्ट रेप्लिकेशन की सुविधा प्रदान करता प्रतीत होता है। वायरल एमआरएनए का स्थिरीकरण, कोशिका संकुचन का प्रेरण जो प्रतिकृति स्थलों पर विषाणु को स्थिर करता प्रतीत होता है और आईएफएन-β प्रेरण का निषेध।

उच्च रुग्णता, मृत्यु दर और उत्पादन हानि के कारण गांठदार त्वचा रोग (एलएसडी) भारत में सबसे महत्वपूर्ण पशु स्वास्थ्य समस्या बन गई है। हाल ही में भा.कृ.अनु.प.- एन.सी.वी.टी.सी द्वारा एक समरूप लाईव-एटेन्यूएटेड LSD वैक्सीन (Lumpi-ProVac*Ind*) विकसित की गई है। एक नए लाईव-एटेन्यूएटेड गांठदार त्वचा रोग टीके की सुरक्षा, प्रतिरक्षाजन्यता और प्रभावकारिता का मूल्यांकन किया गया। कुल 26940 पशुओं पर किए गए क्षेत्रीय परीक्षणों में वैक्सीन की न्यूनतम नीथलिंग प्रतिक्रिया (0.018% जानवरय 26940 पशुओं में से 5) देखी गई। दूध देने वाले पशुओं में दूध की पैदावार में कोई उल्लेखनीय कमी नहीं हुई (सं. = 10108), इसके अलावा गर्भवती पशुओं में गर्भपात या कोई अन्य प्रजनन विकार नहीं था (सं. = 2889)। टीकाकरण के 30 दिन बाद तक क्षेत्र में 85.18% जानवरों में सीरो-रूपांतरण देखा गया। ढेलेदार त्वचा रोग वायरस के टीके और फील्ड स्ट्रेन की पहचान

significant reduction in the milk yield in lactating animals (n = 10108), besides there was no abortion or any other reproductive disorder in the pregnant animals (n = 2889). Sero-conversion was observed in 85.18% animals in the field by day 30 post vaccination. A novel HRM-based gap-qRT-PCR for identification and quantification of the vaccine and field strain(s) of lumpy skin disease virus was also developed. Studies on miRNA profiling of cells infected with LSDV were conducted for the first time at ICAR-NCVTC. This study identified several novel miRNAs and their cellular targets in LSDV-infected cells were identified for the first time in this study. Besides understanding virus replication, virus-host interactions and disease pathogenesis, these miRNAs and their cellular targets may serve as biomarkers and novel targets for therapeutic intervention against LSDV.

For epidemiological studies of coronaviruses in different animals, sampling plans were finalized with the help of NIVEDI, Bengaluru. Out of 1309 bovine samples tested, SARS-CoV-2 was not detected in any sample, but bovine coronavirus was detected in 40 samples. Likewise, out of the 649 equine samples tested, SARS-CoV-2 was not detected in any sample, but equine coronavirus was detected in 2 samples. Omicron variant of SARS-COV-2 from human patients was also isolated and deposited in the repository of NCVTC. The scientists also proved the *in vitro* antiviral activity and therapeutic value of DZNep (3-Deazaneplanocin A; an inhibitor of *S*-adenosylmethionine-dependent methyltransferase) against SARS-CoV-2 replication. A vaccine (ANCOVAX) was also developed to combat the SARS-CoV-2 in animals. The Ancovax was found to be absolutely safe and induced a potent neutralizing antibody - and cell-mediated response against SARS-CoV-2 in laboratory animals (mice and rabbits), dogs, lions/tigers. Studies are also in progress to develop vaccine candidates for SARS-CoV-2 using mRNA-based platform. Scientists also reported the presence of various coronaviruses in bats in northern India.

p38 mitogen-activated protein kinase (p38 MAPK) inhibitor SB239063 and SB203580 were identified as potential hits that resulted in reduced

और मात्रा निर्धारण के लिए एक नया एचआरएम-आधारित गैप-क्यूआरटी-पीसीआर भी विकसित किया गया। गांठदार त्वचा रोग से संक्रमित कोशिकाओं की एमआईआरएनए प्रोफाइलिंग पर अध्ययन पहली बार भा.कृ.अनु.प. - एन.सी.वी.टी.सी में किया गया। इस अध्ययन में कई नवीन miRNAs की पहचान की गई और LSDV-संक्रमित कोशिकाओं में उनके सेलुलर लक्ष्यों की पहचान पहली बार की गई। वायरस रेप्लीकेशन, वायरस- होस्ट इंटरैक्शन और रोगजनन को समझने के अलावा, ये miRNAs और उनके सेलुलर लक्ष्य एलएसडीवी के प्रतिकूल चिकित्सीय हस्तक्षेप के लिए बायोमार्कर और नए लक्ष्य के रूप में काम कर सकते हैं।

विभिन्न जानवरों में कोरोना वायरस के महामारी विज्ञान के अध्ययन के लिए भा.कृ.अनु.प. राष्ट्रीय पशु रोग जानपदिक एवं सूचना विज्ञान संस्थान, बेंगलुरु की मदद से नमूनाकरण योजनाओं को अंतिम रूप दिया गया। परीक्षण किए गए 1309 गोजातीय नमूनों में से किसी भी नमूने में SARS-CoV-2 संक्रमण नहीं पाया गया, लेकिन 40 नमूनों में गोजातीय कोरोना वायरस पाया गया। इसी प्रकार, परीक्षण किए गए 649 घोड़ों के नमूनों में से किसी भी नमूने में SARS-CoV-2 नहीं पाया गया, किंतु 2 नमूनों में घोड़ों में कोरोना वायरस पाया गया। मानव रोगियों से SARS-COV-2 के ओमीक्रॉन वेरिएन्ट को भी अलग किया गया और एन.सी.वी.टी.सी के भंडार में जमा किया गया। वैज्ञानिकों ने SARS-CoV-2 प्रतिकृति के प्रतिकूल DZNep (3-डीजेनप्लानोसिन एय एस-एडेनोसिलमेथिओनिन-निर्भर मिथाइलट्रांसफरेज का अवरोधक) की इन विट्रो एंटीवायरल गतिविधि और चिकित्सीय मूल्य को भी प्रमाणित किया। जानवरों में SARS-CoV-2 का अवरोध करने के लिए एक टीका (ANCOVAX) भी विकसित किया गया था। Ancovax को अति सुरक्षित पाया गया और प्रयोगशाला के जानवरों (चूहे, खरगोश, श्वान, शेर / बाघ) में SARS-CoV-2 के प्रतिकूल एक शक्तिशाली निष्क्रिय एंटीबॉडी और कोशिका-मध्यस्थ प्रतिरक्षा प्रतिक्रिया प्रेरित की गई। एमआरएनए आधारित प्लेटफॉर्म का उपयोग करके SARS-CoV-2 के लिए वैक्सीन उम्मीदवार विकसित करने के लिए भी अध्ययन प्रगति पर है। वैज्ञानिकों ने उत्तर भारत में चमगादड़ों में विभिन्न कोरोना वायरस की उपस्थिति की भी सूचना दी।

p38 माइटोजेन-सक्रिय प्रोटीन काइनेज (p38 एमएपीके) अवरोधक एसबी 239063 और एसबी 203580 को संभावित हिट के रूप में पहचाना गया, जिसके परिणामस्वरूप इन विट्रो में बीपीएक्सवी रेप्लीकेशन कम हो गया, जैसा कि वायरल एमआरएनए / प्रोटीन संश्लेषण, जीनोम

BPXV replication *in vitro,* as evidenced by reduction in viral mRNA / protein synthesis, genome copy numbers and progeny virus particles. p38-α served as a critical cellular factor for the synthesis of BPXV proteins and may serve as a novel target for antiviral drug development against buffalopox. Scientists also developed isothermal "Recombinase Polymerase Amplification" (RPA) based assays for detection of Porcine circovirus 3 (PCV3) in pigs. The developed assay is capable of detecting fg DNA concentration. The specificity of the RPA assay was evaluated against closely related virus-PCV2 and the assay didn't show any cross reactivity.

The phages can be used for phage therapy, as they show a broad biological spectrum and wide stability range for potential bactericidal approaches. Efforts are also being made to strengthen the bacteriophage repository with bacteriophages against pathogenic bacteria and assessment of their synergy with antibiotics. Potential bacteriophages against *Aeromonas* species from the fishponds and ESBL strains of *E. coli* and *Salmonella* sp. from poultry origin were isolated and characterized for their antibiotic resistance profile.

Studies were undertaken to analyse the microbial load of raw donkey milk by enumeration and identification of isolated bacteria. Important genera identified from donkey mare milk were *Staphylococcus* spp, *Mammalicoccus* spp, *Klebsiella* spp, *Acinetobacter* spp, *Agrobacterium* spp, *Bacillus* spp, *Pseudomonas* spp, *Sphingomonas* spp, *Microbacterium* spp, *Enterobacter* spp, *Serratia* spp, *Azospirillum* spp, and *Stutzerimonas* spp Out of 39 isolates, for which molecular identification results were available, maximum 12(30.8 %) isolates were identified as *Mammalicoccus sciuri*, followed by 9 strains of *Staphylococcus* spp. among which 5 strains were of *Staphylococcus saprophyticus*. Microbial identification of milk isolates has revealed presence of some unusual taxa viz., *Agrobacterium fabrum*, *Sphingomonas paucimobilis, Microbacterium zeae, Azospirillum formosense,* and *Stutzerimonas stutzeri.*

Research studies are in progress to investigate the characterization of phages of *Aeromonas* spp.

कॉपी संख्या और प्रोजेनी विषाणु कणों में कमी से प्रमाणित है। p38-α, BPXV प्रोटीन के संश्लेषण के लिए एक महत्वपूर्ण सेलुलर कारक के रूप में कार्य करता है और BPXV के प्रतिकूल एंटीवायरल दवा विकास के लिए एक लक्ष्य के रूप में प्रयोग किया जा सकता है।

वैज्ञानिकों ने सूअरों में पोर्सिन सर्कोवायरस 3 (पीसीवी3) का पता लगाने के लिए आइसोथर्मल "रीकॉम्बिनेज पॉलीमरेज एम्प्लीफिकेशन" (RPA) आधारित परीक्षण भी विकसित किया है। विकसित परख थ्ह डीएनए रेप्लीकेशन का पता लगाने में सक्षम है। त्च्। परख की विशिष्टता का मूल्यांकन निकट से संबंधित वायरस-पीसीवी 2 के लिए किया गया था और परख में कोई क्रॉस रिएक्टिविटी नहीं पाई गई।

जीवाणुभोजी का उपयोग फेज थेरेपी के लिए किया जा सकता है, क्योंकि वे संभावित जीवाणुनाशक दृष्टिकोण के लिए एक व्यापक जैविक स्पेक्ट्रम और व्यापक स्थिरता रेंज प्रदर्शित करते हैं। रोगजनक बैक्टीरिया के खिलाफ बैक्टीरियोफेज भंडार को मजबूत करने और एंटीबायोटिक दवाओं के साथ उनके तालमेल का आंकलन करने के भी प्रयास किए जा रहे हैं। मछली के तालाबों से एरोमोनास प्रजातियों, ई. कोलाई और साल्मोनेला के ESBL उपभेदों के खिलाफ पोल्ट्री मूल से जीवाणुभोजी अलग किए गए और उनकी एंटीबायोटिक प्रतिरोध प्रोफाइल की विशेषता बताई गई।

पृथक बैक्टीरिया की गणना और पहचान द्वारा मादा गर्दभ के कच्चे दूध के माइक्रोबियल लोड का विश्लेषण करने के लिए अध्ययन किए गए। मादा गर्दभ के दूध से पहचानी जाने वाली महत्वपूर्ण प्रजातियाँ ये थी - स्टैफायलोकोकस, मैमैलिकॉकस एसपीपी, क्लेबसिएला एसपीपी, एसिनिटोबैक्टर एसपीपी, एग्रोबैक्टीरियम एसपीपी, बैसिलस एसपीपी, स्यूडोमोनास एसपीपी, स्फिंगोमोनास एसपीपी, माइक्रोबैक्टीरियम एसपीपी, एंटरोबैक्टर एसपीपी, सेराशिया एसपीपी, एजोस्पिरिलम एसपीपी और स्टुटजेरिमोनास एसपीपी। 39 आइसोलेट्स में से, जिनके लिए आणविक पहचान परिणाम उपलब्ध थे, अधिकतम 12 (30.8%) आइसोलेट्स की पहचान मैमैलिकोकस स्किउरी के रूप में की गई, इसके बाद स्टैफायलोकोकस एसपीपी के 9 उपभेदों की पहचान की गई। जिनमें से 5 उपभेद स्टैफायलोकोकस सैप्रोफाइटिकस के थे। दूध के आइसोलेट्स की माइक्रोबियल पहचान से कुछ असामान्य टैक्सा की उपस्थिति का पता चला है, जैसे, एग्रोबैक्टीरियम फैब्रम, स्फिंगोमोनास पश्वसीमोबिलिस, माइक्रोबैक्टीरियम जी, एजोस्पिरिलम फॉर्मोसेंस और स्टुटजेरिमोनास स्टुटजेरी।

The transmission dynamics of AMR *E. coli* from farm to fork. Virulence genes of *Klebsiella pneumoniae* were isolated and characterized by equines. First confirmatory report equine pythiosis in India was reported by isolation and molecular annotation. Isolation and identification of pathogenic bacteria from wetland wild avians was also carried out.

Studies on isolation, characterization, and generation of repositories of *Mycobacterium* species are in progress. *M. kansasii* is a dominant, most virulent species, can cause serious lung infection in immunocompromised humans and the disease resembles tuberculosis. Studying the growth kinematics and mechanisms of biofilms formation by *M. kansasii* is an important area of search for developing new strategies to combat this bacterium and for devising therapeutic agents. Research on assessment of biofilm formation by *Mycobacterium kansasii* can be used to develop new strategies for preventing and treating biofilms related infections. Studies are also underway to decipher the role of MCC genes in survival of *M. kansasii* using CRISPR-Cas9 mediated gene editing in *Mycobacterium kansasii.*

During 2022, the scientists of the Centre published 54 original research articles in international and national refereed journals. In addition, one compendium, eight technical bulletins, three book chapters, 48 chapters in training manuals, besides 54 research abstracts were published by the scientists. The scientists of the Centre presented papers in 12 different national and international conferences, seminars, or symposia, and participated in 22 workshops and interactive meets in different parts of the country.

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

एरोमोनास एसपीपी के जीवाणुभोजियों के लक्षण वर्णन की जांच के लिए अनुसंधान अध्ययन प्रगति पर हैं। एएमआर ई. कोलाई के संचरण की गतिशीलता पर भी अध्ययन चल रहा है। क्लेबसिएला निमोनिया के विषैले जीन्स को घोड़ों में जांचा गया और उनकी विशेषता बताई गई। भारत में पहली पुष्टिकारक रिपोर्ट इक्वाइन पाइथियोसिस अलगाव और आणविक एनोटेशन द्वारा रिपोर्ट की गई थी। आर्द्रभूमि जंगली पक्षियों से रोगजनक जीवाणुओं का अलगाव और पहचान भी की गई।

माइकोबैक्टीरियम प्रजातियों के अलगाव, लक्षण वर्णन और भंडारों के निर्माण पर अध्ययन प्रगति पर है। एम. कंसासी एक प्रमुख, सबसे अधिक विषैली जीवाणु प्रजाति है, जो कमजोर प्रतिरक्षा वाले मनुष्यों में फेफड़ों के गंभीर संक्रमण का कारण बन सकती है और यह रोग तपेदिक जैसा दिखता है। एम. कान्सासी द्वारा बायोफिल्म निर्माण के विकास काइनेमैटिक्स और तंत्र का अध्ययन इस जीवाणु से निपटने के लिए नई रणनीतियों को विकसित करने और चिकित्सीय एजेंटों को तैयार करने के लिए खोज का एक महत्वपूर्ण क्षेत्र है। माइकोबैक्टीरियम कंसासी द्वारा बायोफिल्म निर्माण के मूल्यांकन पर शोध का उपयोग बायोफिल्म से संबंधित संक्रमणों को रोकने और इलाज के लिए नई रणनीति विकसित करने के लिए किया जा सकता है। माइकोबैक्टीरियम कंसासी में CRISPR-Cas9 मध्यस्थ जीन एडिटिंग का उपयोग करके एम. कंसासी के अस्तित्व में एमसीसी जीन की भूमिका को समझने के लिए भी अध्ययन चल रहा है।

वर्ष 2022 के दौरान, केंद्र के वैज्ञानिकों ने 54 अंतरराष्ट्रीय और राष्ट्रीय रेफरीड पत्रिकाओं में मूल शोध लेख प्रकाशित किए। इसके अलावा, एक किताब, आठ तकनीकी समाचार, तीन पुस्तक अध्याय, 48 विस्तार पत्रक और 54 अनुसंधान सार वैज्ञानिकों द्वारा प्रकाशित किए गए थे। केंद्र के वैज्ञानिकों ने 12 विभिन्न राष्ट्रीय और अंतर्राष्ट्रीय सम्मेलनों, सेमिनारों या संगोष्ठियों में भाग लिया। विशेषज्ञ/आमंत्रित व्याख्यानों में शोधपत्र प्रस्तुत किए और देश के विभिन्न हिस्सों में 22 कार्यशालाओं और इंटरैक्टिव बैठकों में भी भाग लिया।

केंद्र ने भारत सरकार के निर्देशों के तहत विभिन्न गतिविधियों का आयोजन किया। अंतर्राष्ट्रीय योग दिवस मनाने के लिए योग शिविर (13-21 जून), हिंदी को बढ़ावा देने के लिए हिंदी पखवाड़ा (14-26 सितंबर), स्वच्छता अभियान (2-16 अक्टूबर), सतर्कता जागरूकता सप्ताह (31 अक्टूबर-5 नवंबर), कृषि शिक्षा दिवस (3 दिसम्बर) और राष्ट्रीय उत्पादकता सप्ताह (12-18 फरवरी) बड़े धूमधाम से

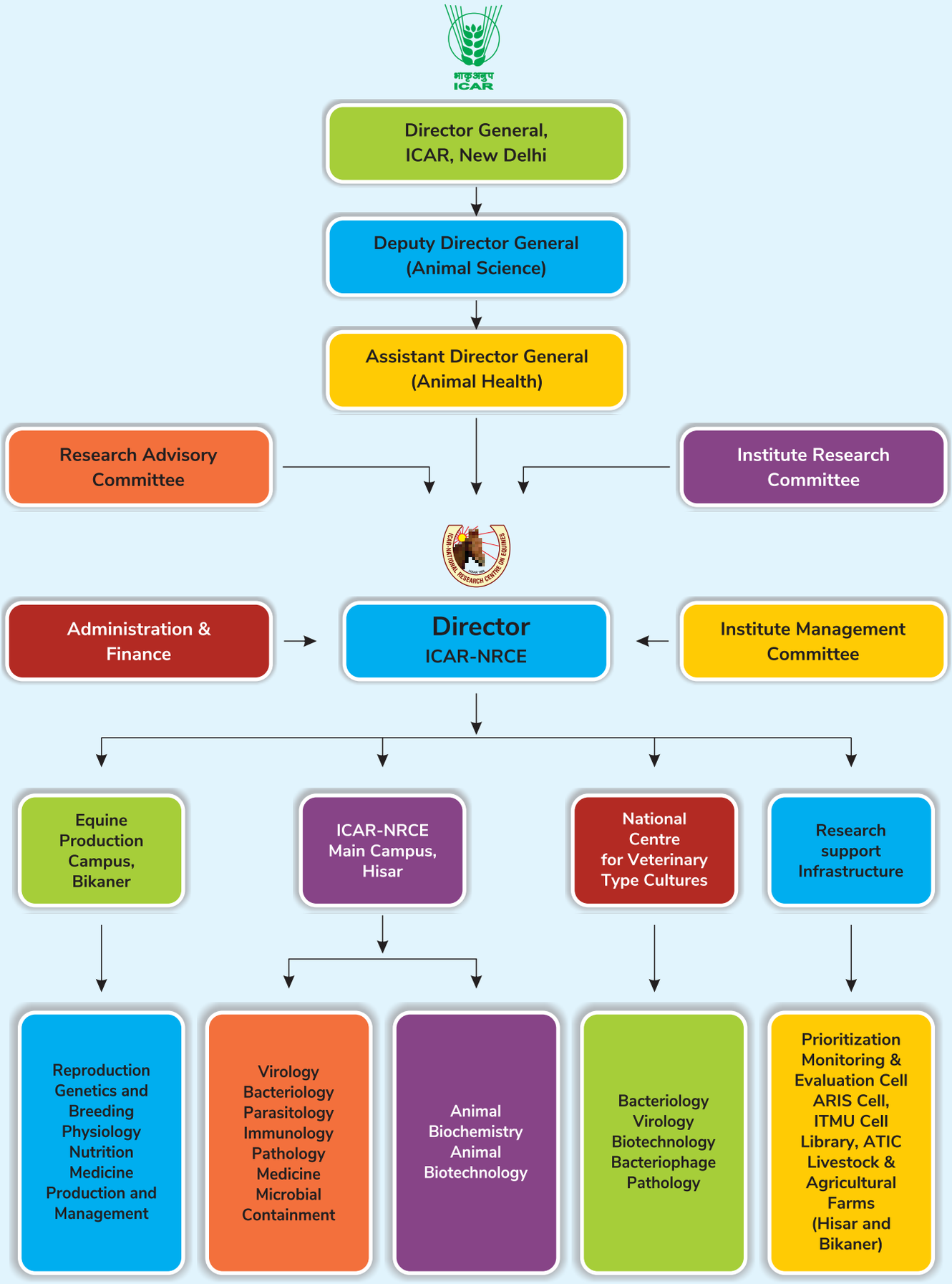
Other institutional activities organized were World Veterinary Day (30 April) and National Science Day (28 February).

The centre also offers paid consultancy and diagnostic services for important infectious diseases of the equines. Under contractual diagnostic services, a total 9996 samples were received from racecourses, turf club, stud farm, riding schools, animal quarantine & certification services (AQCS) and other organized sector during the year 2022. The center generated revenue of Rs 73.60 lakh from contractual diagnostic services.

मनाये गये। 26 नवंबर को वैज्ञानिक-पशुचिकित्सक इंटरफेस बैठक आयोजित करके केंद्र का स्थापना दिवस मनाया गया। आयोजित की गई अन्य संस्थागत गतिविधियों में विश्व पशु चिकित्सा दिवस (30 अप्रैल) और राष्ट्रीय विज्ञान दिवस (28 फरवरी) थीं।

केंद्र घोड़ों के महत्वपूर्ण संक्रामक रोगों के लिए सशुल्क परामर्श और निदान सेवाएं भी प्रदान करता है। इस कार्यक्रम के तहत, 9996 इक्वाइन सीरम नमूनों का परीक्षण विभिन्न संक्रामक रोगों के लिए किया गया, केंद्र ने 73.60 का राजस्व अर्जित किया।

Organizational SET-UP



INTRODUCTION 01

National Research Centre on Equines

Horses hold significant importance in the Indian subcontinent due to their historical, cultural, and economic significance. Horses have deep-rooted cultural value in the subcontinent, often symbolizing power, strength, and nobility. The equestrian tradition is also vibrant in events like horse racing, which has become a popular sport in countries like India, showcasing the continuing appreciation for horses. Furthermore, horses contribute significantly to the economy of the Indian subcontinent. The equine industry, including horse breeding, racing, and tourism generates substantial revenue and employment opportunities. Horse racing events attract a large number of spectators, betting enthusiasts, and tourists around the world and providing a boost to the local economy. Additionally, horse breeding and sales have become a lucrative enterprise, with the demand for well-bred horses rising for recreational purposes such as horseback riding and polo. The economic impact of the equine industry extends to various sectors, including veterinary services, transportation, and hospitality, thus, making it an integral part of the Indian subcontinent's economic landscape.

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 (ICAR-NRCE) on November 26, 1985 at Hisar (Haryana). The state- of-the art laboratories and facilities at the main campus of ICAR-NRCE in Hisar has been undertaking research in areas of equine health, management and production. 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.

ICAR-National Research Centre on Equines, Main Campus, Hisar

Equine Production Campus (EPC) was established in 1989 at Bikaner (Rajasthan) to undertake research on equine production, genetics and breeding, management, reproduction, physiology and nutrition. Bikaner campus has a well maintained herd of Marwari, Kathiawari, Zanskari and Manipuri horses and Halari and exotic donkeys (Poitou). The National Centre for Veterinary Type Cultures (NCVTC) was established in the year 2005 at ICAR-NRCE, Hisar for collection and preservation of microbes of animal origin having veterinary importance. Presently, the Centre is working through 15 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.

ICAR-National Research Centre on Equines, Production Campus, Bikaner (Rajasthan)

MANDATE OF NRCE

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

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 underprivileged under changing environment & socio-economic scenario.

SALIENT ACHIEVEMENTS

During the past 37 years, ICAR-NRCE has contributed significantly in the area of diagnosis and control of equine infectious diseases by developing state-of-the-art diagnostics and biologicals. Some of the major achievements and accolades of the Centre are enlisted below:

Development of diagnostics

The Centre has been recognized as the National Referral Centre for diagnosis of important equine diseases by the 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*.
- 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 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 sero-diagnosis of glanders and equine infectious anemia.
- Lateral flow assay based rapid diagnostic for *Theileria equi* infection.
- Lateral flow assay kit for glanders.
- Lateral flow assay kit for equine infectious anemia.
- Standardization of a nested (gB-nPCR) and real-time PCR (gB-qPCR) targeting gB gene for detection of EHV1 latency.
- Recombinant protein based-Indirect ELISA for detection of JEV specific antibodies in horse and pig.
- Standardization of Multiplex PCR to differentiate *Streptococcus equi* subsp .*equi* and *Streptococcus equi* subsp *zooepidemicus.*
- Lateral flow assay for rapid diagnosis of trypanosomosis using different *T. evansi* antigens.
- ELISA for detection of *T. evansi* antibodies in multiple animal species.
- Monoclonal antibody-based ELISA kit for detection of equine influenza (H3N8) antigen.
- Development of recombinant antigens based-indirect ELISA kit for detection of anti-*Trypanosoma evansi* antibodies in animals.
- Developed of recombinant nucleoprotein based-indirect ELISA for SARS-CoV-2 antibody detection in canines.
- Standardized RT-RPA-CRISPR based-LFA assay for detection of SARS-CoV-2.
- Developed isothermal "Recombinase Polymerase Amplification" (RPA) based assays for detection of Porcine circovirus 3 (PCV3) in pigs.

Development of vaccines and immuno-biologicals

- Inactivated EHV1 vaccine "Equiherpabort" using indigenous virus for prevention of abortions in mares.
- Updated equine influenza vaccine by incorporating recent virus strain {A/eq/Katra-Jammu.06/08 (H3N8)}.
- Bacterin and outer membrane protein-based vaccine for *Salmonella* Abortus equi.
- Monoclonal antibodies against EHV-1, equine rotavirus, equine influenza, Japanese encephalitis and *Trypanosoma evansi*.
- Inactivated EHV1 vaccine using montanide adjuvant (The modified vaccine is currently under trial in horses).
- Encapsulated phage formulation carrying *Salmonella* phages for therapeutic application in poultry.
- Developed of LSD vaccine (Lumpi-ProVac^{Ind}) to prevent Lumpy skin disease (LSD) in animals.
- Developed of SARS-CoV2 vaccines for animals.

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 *Trypanosoma evansi* and *Theileria equi* was successfully established.
- Experimental mouse models for equine influenza and equine herpesvirus-1 infections developed.
- Complete genome sequencing of two EHV1 isolates was carried out using NGS. The primary NGS data obtained covered up to 90% of the genome.
- Sequence comparison of Indian EHV1 isolates with other published isolates revealed that Indian isolates are more closely related to EHV1 isolates (OH03 and VA02) from Japan (97.4- 98.8%).
- Phylogenetic analysis based on US segments classified our isolates into clade 5 along the reference isolates V592.
- Genotypic characterization of *Burkholderia mallei* isolates recovered from glanders outbreaks and currently circulating isolates are differing from the older Indian isolates.
- Whole genome sequencing of RVA/Horse-wt/IND/ERV3/2003, RVA/Horse-wt/IND/ERV2/2015, RVA/Horse-wt/IND/ERV4/2017, RVA/Horse-wt/IND/ERV6/2017 carried out.

Surveillance and monitoring of equine diseases in India

- India has gained WAHO disease-free status for African horse sickness (AHS) in 2014 based on sero-monitoring data generated by ICAR-NRCE.
- Control of EIA in India was possible due to timely diagnosis and implementing a package of practices formulated by ICAR-NRCE.
- Effective control of the equine influenza outbreak of 1987 (involving 83000 equines) was done by implementing bio-security and development of effective vaccines. 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.
- The National Action plan for control and eradication of glanders in India was drafted by ICAR-NRCE and the same has been implemented by the Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India in June 2019.

Establishment of nucleus herd of equines and characterization (phenotypic and genotypic) of Indian equine breeds

- 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 which include Marwari horses from Rajasthan; Kathiawari horses from Gujarat; Zanskari ponies from Zanskar valley (Jammu & Kashmir) and Manipuri ponies from Imphal (Manipur). In addition, Large white (Halari) donkeys for conservation and improvement of donkeys and exotic Poitou donkey herd for production of superior mules are being maintained in the campus.
- Seven equine breeds, namely, Marwari, Kathiawari, Kachchhi-Sindhi, Spiti, Zanskari, Bhutia and Manipuri, have been characterized on the basis of their biometric indices and coat colour.
- Microsatellite marker based genetic diversity analyzed for proposing effectual population breeding and management strategies for future.

Improvement in production potential of equines

- In order to conserve the germplasm of indigenous equine breeds, cryopreservation of semen of Marwari, Kathiawari, Zanskari and Manipur stallions and Halari & Poitou donkeys are being practiced.
- 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 35 to 120 of gestation in mares.

- Pregnancy diagnosis between days 14 and 18 post-insemination has been perfected using ultrasonography in donkeys and mares.
- Donkey fibre has been used to produce carpets by mixing with sheep fibres (40:60).
- Studies on assessment of fertility related genes in stallions have been assessed. Expression of SPATA1, PLCz and CRISP3 fertility genes has been studied and established their correlation with DNA integrity and mitochondrial membrane potential of the stallion spermatozoa.
- Research initiated in the direction of treatment of Fibroblastic sarcoid, proud flesh, Alopecia and Habronemiasis using herbal formulations.
- Developed fatigue cum fitness score card for working equines.
- Customized artificial vagina has been designed for collection and cryopreservation of equine semen and technology also transferred to equine farmers/ breeders.
- Developed donkey milk based products (Bathing soap, Body butter and Lip balm).
- Estrus synchronization protocols were optimized and methods of embryo recovery from mares and successful transfer of embryos to the surrogates were optimized.
- SNP markers associated with fertility in indigenous breeds of horse were studied.
- Compositional changes in Halari donkey milk during lactation were studied.
- New creep feeders were designed and fabricated for optimum growth of the foals.

Utilization of equine energy in agricultural activities

- Single animal drawn matching plough, seed drill (two furrows) and harness have been designed and developed for donkeys and mules for agricultural operations like ploughing and sowing.
- Draught ability studies conducted on adult donkeys using conventional pneumatic two wheel cart.
- The technique of vermin composting of equine dung has been optimized for use in agricultural fields.

Services to 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.
- 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.
- On site and online consultancy in equine health and production, including toll-free telephonic advisory at Hisar and Bikaner campuses for farmers and stakeholders.
- Training and supply of educational materials for equine management, production and health.
- Organization of health camps, awareness campaigns and farmers meets in different areas of the country.
- During the COVID-19 pandemic, ICAR-NRCE served as a COVID-19 testing facility amongst one of the four institutes of ICAR

Patents granted

- Nano-drug delivery for quinapyramine sulphate (Patent No.310429,Application, No.2560/DEL/2011, dated 06.09.2011).
- A method for preparation of a diagnostic kit 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).

- Recombinant *Tss*A protein for detection of antibodies against *Burkoholderia mallei* and uses thereof. Application No.3610/DEL/2015.
- A recombinant protein for diagnosis of glanders (Patent No: 296824, 2018).
- Polymeric metal nanocomposites and methods of synthesis thereof (Patent No. 411620, dated 16.11.2022).

Patents filed

- Polynucleo-desequence, process, composition and methods thereof. Application No. 2560/DEL/2011, dated 06.09.2011.
- Polynucleo-desequence, 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 *Hcp*1 protein for detection of antibodies against *Burkoholderia mallei* in Equines. Application No.4120/DEL/2015.
- *Aerva javanica* extract for the treatment of exuberant granulation tissue and tumors in horses.ApplicationNo.201811048899, dated 24.12.2018.(Provisional).
- Modified vaccine construct for EHV 1 and methods of preparing the same. Application No 202111000312, dated 05.01.2021.
- Monoclonal antibody based immunoassay for detection of equine influenza (H3N8) antigen. Application No 202111004847, dated 04.02.2021.
- Mutated EHV-1(TOH Strain) genome based vaccine construct and method for preparation. Application No.202111057300, dated 09.12.2021.
- Recombinant nucleocapsid protein based indirect ELISA kit for detection of anti SARS-COV-2 antibodies in canines. Application No.202111057358, dated 09.12.2021
- Hydroxychloroquine/chloroquine zinc oxide nanoparticle formulation. Application No.202111057698, dated 11.12.2021
- Hydroxychloroquine/chloroquine zinc oxide nanoparticle formulations. Application No. PCT/IB2022/062019, dated 10.12.2022.
- Recombinant antigens based indirect ELISA kit for detection of anti *Trypanosoma evansi* antibodies in animals. Application No. 202211008619, dated 18.02.2022
- Development of a Novel Modified Attenuated Lumpy Skin Disease Virus (LSDV) For Use as Vaccine Application No.202211013092, dated 10.03.2022
- A novel vaccine formulation (Ancovax) to prevent SARS-CoV-2 infection in animals. Application No. 202211026023, dated 04.05.2022
- A method for encapsulation of bacteriophage cocktail against Salmonella sp for oral delivery in poultry. Application No. 202211050633, dated 05.09.2022
- Development of a Novel test to differentiate the vaccine and field strains of LSDV. Application No. 202211074538, dated 22.12.2022.

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 is in operation with 15 network units located in 9 different states viz., Haryana, Rajasthan Uttar Pradesh, Himachal Pradesh, Assam, Tamil Nadu, Gujarat, Kerala and Karnataka. 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.

National Centre for Veterinary Type Cultures, ICAR-NRCE, Hisar

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.

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.

SALIENT ACHIEVEMENTS

During the past few years, ICAR-NRCE has contributed significantly in the area of conservation of microbial diversity and characterization of microbial pathogens. Some of the major achievements of the Centre are enlisted below.

Veterinary Microbes

- First laboratory confirmed camelpox virus zoonosis.
- First isolation of BoHV-5 from cattle, Swinepox virus from pigs and Lumpy skin disease virus from cattle.
- First confirmatory report equine pythiosis in India.
- First isolation of bacteria such as *Bordetella bronchiseptica* from horse, *Actinobacillus equilli* from foal, *Staphylococcus hyicus* from pig, Methicillin-resistant coagulase negative *Staphylococcus sciuri* from goats, *Trueperella pyogenes, Exiguobacterium* spp. from pigs, *Nocardia otitidiscaviarum* from equine granulomatous pneumonia, *Moraxella* (*Branhamella) ovis* from ovine keratoconjunctivitis in sheep and *Mannheimia varigena* from buffalo.
- Whole genome sequencing of viruses such as SARS-CoV-2, BPXV, LSDV, NDV and Jaagsiekte sheep retrovirus, Avian nephritis virus, Chicken astrovirus and classical swine fever virus.
- Whole genome sequencing of bacteria such as *Pasteurella multocida* sub spp. *multocida* B:2 serotype, *Trueperella pyogenes, Bordetella bronchiseptica, Clostridium botulinum* isolate from horse*, Pasteurella multocida, Actinobacillus equuli* and *Salmonella Gallinarum*.

- Isolation of anaerobic bacterium *viz., Clostridium perfringens*, *Clostridium sordelli* and *Clostridium sporogenes* isolated from disease outbreak in brick-kiln ponies; Isolation of strains of genera *Gemella, Sphingomonas, Ochrobactrum, Rodentibacter, Gallibacterius. Shewanella* and *Aggregatibacter*.
- Isolation of Novel thermo tolerant bacteriophage from Ganga river water against *Klebsiella pneumonia* and Isolation and characterization of bacteriophages against mastitis causing *Staphylococcus aureus.*
- Development of bacteriophage cocktail to ameliorate *Pseudomonas aeruginosa* infections in Biofilms
- Development of phage delivery system for safe oral delivery of bacteriophages in poultry gut.
- Characterization of bacteriophages against ESBL producing bacteria for targeting biofilms in bovines
- Methodologies developed to successfully purify a positive sense-RNA virus (FMDV) from a virus mixture containing a negative sense-RNA virus (PPRV).
- Adopted CRISPR/Cas9-medited gene editing technology to generate knock out cell lines.
- First time demonstrated that MAPK interacting kinase I (MNK1, a host factor) regulates buffalopox virus (BPXV) replication at the level of protein translation initiation.
- Identified the role of heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1, an RNA-binding protein) in regulating early translation to replication switch in SARS-CoV-2 life cycle.
- Evaluated the role of p38 mitogen-activated protein kinase (MAP Kinase) in buffalo pox virus replication
- Generated flexible Gateway ORF library of equine influenza virus to study protein-protein interactions.
- First time demonstrated *in vitro* and *in ovo* broad spectrum antiviral activity of emetine against RNA and DNA viruses (PPRV/NDV/BPX/BHV-1).
- Development of isothermal "Recombinase Polymerase Amplification" (RPA) based assays for detection of Porcine circovirus 2 (PCV2) and 3 (PCV3).
- Developed Lumpy skin disease virus vaccines for animals.
- Developed SARS-CoV2 vaccines for animals.
- Developed recombinant nucleoprotein based-indirect ELISA for SARS-CoV-2 antibody detection in canines

Rumen Microbes

- Isolation and characterization of tannin degrading bacteria such as *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 such as *Anaeromyces* sp., *Orpinomyces intercalaris* and *Orpinomyces joyonii* from buffaloes; *Piromyces* sp. and *Neocallimastix* sp. from goats.

Dairy Microbes

- Preservation of dairy microbes, *viz*, *Lactobacillus* spp; *Lactococcus* spp; *Lactococcus lactis* ssp. lactis; *Lactococcus lactis*. ssp. cremoris; *Lactococcus lactis* ssp. diacetylactis; *Streptococcus thermophiles; Leuconostoc* sp; *Bifidobacterium* sp; *Bifidobacterium dentium; Bifidobacterium longum; Micrococcu* sp; *Kluyveromyces lactis* and *Saccharomyces bisporus*.
- Combination of *L. lactis* ssp Lactis-C12 and *Leuconostoc mesenteroides* subsp *mesenteroides* is very suitable for curd and butter milk 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.

SUMMARY OF EXPENDITURE & REVENUE GENERATION

Summary of Expenditures under Unified budget and NCVTC including SCSP, NEH & TSP

Head-wise Details	2021-2022 (Rs in Lakhs)	2022-2023 (Rs in Lakhs)
Other charges including equipment's and recurring charges	42.94302	742.65240
Establishment charges including LSP/PF, wages, OTA	1201.70000	1267.05000
Travelling allowances and HRD	7.07698	17.52
Works	0	5.82436
Loan and Advances	10.00000	0
Disaster Emergency fund - General	79.99700	75.00000
Disaster Emergency fund - Capital	19.99700	20.00000
New Scheme	0	100.0000
Total	**1361.714**	**2228.04676**

Summary of Revenue Receipts

Head-wise Details	2021-2022 (Rs in Lakhs)	2022-2023 (Rs in Lakhs)
Leave Salary & Pension Contribution	16.57	7.65
Sale of Farm Produce	8.45	2.18
Sale of Livestock.	8.04	20.40
Eco Tourism	1.15	2.83
Rents (Charges & Licence Fee)	1.79	2.58
Contractual Diagnostic Services	75.32	69.54
Sale of cultures / Vaccine	56.49	0.89
Sale of Vaccine	0	3.43
Candidates Tuition Fees, Diploma Charges /Training Fee etc.	1.30	3.51
Interest on Short Term Deposit	4.33	7.77
Interest on short term deposit under DBT/DST Project/Award Money	0	1.95
Interest on Loans & Advances	9.77	4.18
Other Miscellaneous Receipts	30.68	14.92
Total	**213.89**	**141.88**

Staff Position at NRCE & NCVTC

Name of the post	NRCE			NCVTC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	01	01	00	-	-	-
Scientific	23	14	09	10	07	03
Technical	25	19	06	01	-	01
Administrative	19	11	08	-	-	
Supporting	20	14	06	-	-	

LANDMARK ACHIEVEMENTS SINCE INCEPTION

Year	Salient Achievements
1985	Foundation of National Research Centre on Equines, 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
1997	Release of inactivated equine influenza vaccine
2003	Award of Indian patent to HERP kit for diagnosis of EHV1 infection
2005	Establishment of National Centre for Veterinary Type Cultures (NCVTC)
2006	Collection and cryopreservation of stallion semen at farmers' door
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
2010	Re-emergence of a case of Equine Infectious Anaemia (EIA)
2011	First report of Buffalo pox virus causing concurrent disease in cow, buffalo and human
2011	Whole genome sequencing of Japanese Encephalitis (JE) virus isolated from a horse
2011	Establishment of a herd of small grey and large white indigenous donkeys
2012	Organisation of SAARC trainings on equine piroplasmosis under OIE twinning program
2012	Development of r-protein based ELISA for Equine Infectious Anaemia (EIA)
2012	Technique for Vermicomposting using equine dung optimized
2012	Quinapyramine sulfate nanoformulation developed against *Trypanosoma evansi*
2013	Establishment of ATIC and infoequine 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	Organisation of SAARC trainings on equine influenza and glanders under OIE twinning program
2016	Methodology for isolation of RNA virus from mixed infection developed
2017	Establishment of a herd of Kathiawari horses
2018	Ecotourism started at Equine Production Campus, Bikaner
2018	Release of ELISA kits for EHV1/4 and LFA for equine piroplasmosis
2020	Japanese Encephalitis (JE) virus antibody test kit was released
2021	Technology commercialization and transfer on semen collection and cryopreservation in Equines; commercialization of prototype of AV for semen collection from Stallions
2022	Development of Recombinant antigens based indirect ELISA kit for detection of anti-*Trypanosoma evansi* antibodies in animals
2022	Development of LSD vaccine (Lumpi-ProVac^{ind}) to prevent Lumpy skin disease (LSD) in animals
2022	Development of SARS-CoV2 vaccines for animals
2022	Development of recombinant nucleoprotein based indirect ELISA for SARS-CoV-2 antibody detection in canines
2022	Developed isothermal "Recombinase Polymerase Amplification" (RPA) based assays for detection of Porcine circovirus 3 (PCV3) in pigs

EQUINE HEALTH

Sero-surveillance and monitoring of equine infectious diseases in India

Surveillance and monitoring of equine infectious disease are one of the continuous service projects of the institute. The objectives of the project are to keep surveillance of important equine diseases, to investigate disease outbreak and its control. The project plays an important role in monitoring existing diseases as well as keeps vigilance on exotic diseases. During the year 2022, a total of 2594 equine serum samples from 10 states were tested for various diseases like Equine Infectious Anaemia (EIA), Equine Influenza (EI), *Trypanosoma evansi* (Trypanosomosis), Equine Herpes Virus-1 (EHV-1), Equine Piroplasmosis, Japanese Encephalitis (JEV), *Salmonealla Abortus equi* and Brucellosis as indicated in Table. Total number of positive cases and sero-positive percentage are indicated in the table below. Highest sero-prevalence was observed for *Theileria equi* (38.47%) followed by EHV-1 (9.13 %), *Trypanosoma evansi* (2.66%), JE (0.42%) and EI (0.19%). None of the equines were found to be positive for equine infectious anemia, brucellosis, and *Salmonella Abortus equi*.

Sero-prevalence of important equine diseases among indigenous equines (Jan - Dec. 2022)

State/UTs	EIA	EI	Trypano-somosis	EHV-1	Equine Piroplasmosis (*T. equi*)	JEV	*Sal. Ab. equi*	Brucellosis
Uttar Pradesh	1003	1003 (2)	1003 (42)	1003 (92)	1003 (414)	1003 (2)	1003	1003
Haryana	292	292	292 (2)	292 (34)	292 (127)	292(3)	292	292
Madhya Pradesh	413	413	413 (12)	413 (28)	413 (150)	413 (3)	413	413
Rajasthan	152	152	152 (2)	152	152 (105)	152	152	152
Andhra Pradesh	25	25	25(5)	25(7)	25(9)	25	25	25
Chhattisgarh	90	90	90	90(11)	90(18)	90	90	90
Himachal Pradesh	81	81	81(1)	81(13)	81(39)	81	81	81
Manipur	31	31	31	31	31(14)	31	31	31
Uttarakhand	157	157	157	157(21)	157(31)	157	157	157
Jammu	350	350 (3)	350 (5)	350 (31)	350 (91)	350 (3)	350	350
Total	**2594**	**2594 (5)**	**2594 (69)**	**2594 (237)**	**2594 (998)**	**2594 (11)**	**2594**	**2594**
Sero-prevalence (%)	**-**	**0.19**	**2.66**	**9.13**	**38.47**	**0.42**	**-**	**-**

Number in parenthesis indicates sero-positive samples

During the year 2022, a total of 220 samples were tested for Disease Investigation component of the institute. The samples found positive for various diseases viz. Equine Herpes Virus, equine piroplasmosis (*Theileria equi*), Equine Influenza (EI), Japanese Encephalitis/West Nile virus (JE/WNV), *Trypanosoma evansi* (Trypanosomiasis), African Horse Sickness (AHS), *Salmonella Abortus equi* and Brucellosis are shown in Table below.

Number of samples tested under disease investigation (Jan-Dec.2022)

Disease	DI
EHV-1/4	40 (10)
Equine Piroplasmosis *(Theileria equi)*	50 (7)
Equine Influenza (EI)	10 (0)
JE/WNV	20 (5)
T. evansi	40 (4)
African Horse Sickness (AHS)	55 (0)
Sal. Ab. equi & Brucellosis	5 (0)
Total	**220 (26)**

Number in parenthesis indicates sero-positive samples

Microbiological analysis was carried out on 348 biological and environmental samples including nasal swab, tissue, abscess, aborted fetus, semen, water, feed, fecal etc. originating from Haryana, Uttar Pradesh, Rajasthan, Punjab, and Maharashtra. A total 42 bacterial isolates including *Klebsiella pnuemoniae* (n=18), *E. coli* (*n*=9), *Rhodococcus equi* (*n*=2), *Streptococcus equi* subsp *zooepedemicus* (*n*=8) and *Burkholderia mallei* (*n*=5) were isolated from these samples, shown in Table.

Bacteria isolated from biological/environmental samples

Organism	No.	Sample Collection Site	Place of Sample collected
Klebsiella pneumoniae	18	Nasal Swab (9), Lung (2) Liver (1) Feed (2) Water (2) Soil (1) and Frothy Swab (1)	Haryana (8), UP (5), Rajasthan (4), Punjab (1)
E. coli	9	Heart (3), Stomach (2), Large Intestine (1), Liver (1), Kidney (1), and Rectal Swab (1)	Rajasthan (8), Haryana (1)
R. equi	2	Faecal Sample (1), and Nasal Swab (1)	Haryana (1), Rajasthan (1)
Streptococcus equi subsp *zooepidemicus*	8	Kidney (1) Nasal Swab (4) Testicle Swab (1) Water (1) and Frothy Swab (1)	Haryana (5), Rajasthan (3)
Burkholderia mallei	5	Pus (2), Nodular Swab (1) and Testicle Swab (1)	Haryana (4), Maharashtra (1)

Revenue generation through contractual diagnostic services and consultancy

Under contractual diagnostic services, a total 9996 samples were received from racecourses, turf club, stud farm, riding schools, animal quarantine & certification services (AQCS) and other organized sector during the year 2022. These samples were tested for various notifiable and exotic diseases to check ingression of diseases from abroad and monitoring of elite horses in private sectors. A total of 4968 sera samples for EIA and 3598 samples for glanders were tested. Among exotic diseases, 512 swab samples from for Contagious Equine Metritis (CEM), 275 sera samples for equine viral arteritis (EVA), 258 sera samples for African Horse Sickness (AHS) and 263 sera samples for dourine were received from AQCS, Govt. of India, collected from imported equines. All the samples were found negative for these exotic diseases. Revenue of about Rs. 73.60 lakhs were generated through contractual diagnostic service (see details in Table).

Number of samples tested and revenue generation through contractual diagnostic services (Jan-Dec.2022)

Diseases/infection diagnosis	Number of samples tested	Revenue Generated (Rs.)
Equine Infectious Anaemia (EIA)	4968	2732400.00
Glanders	3598	2518600.00
Contagious Equine Metritis (CEM)	512	819200.00
Dourine	263	289300.00
African Horse Sickness (AHS)	258	283800.00
Equine Viral Arteritis (EVA)	275	550000.00
Theileria equi	26	52000.00
Babesia caballi	28	56000.00
Equine influenza	19	10450.00
Equine Herpes Virus-1 (EHV-1)	19	38000.00
Trypanosomosis	4	2200.00
Japanese Encephalitis	1	2000.00
Pregnancy diagnosis	21	4200.00
Salmonella Abortus equi	4	2200.00
Total	**9996**	**73,60,350.00**

(H Singha, Shanmugasundaram K, Baldev R Gulati, Nitin Virmani, Rajender Kumar, Sanjay Kumar, Sanjay Barua, Rajesh K Vaid, Ramesh Dedar, Anju Manuja, Balvinder Manuja, Anubha Pathak, Ana Raj, Yash Pal & TK Bhattacharjya)

Glanders Surveillance Report: 2022

National Action Plan on Glanders for control and eradication of glanders in India was launched by the Department of Animal Husbandry & Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India in 2019. The action plan was undertaken for surveillance, control, and eradication of Glanders in equines from India. This action plan has been framed for surveillance of the entire equine population reared in different management and animal husbandry practices following the conceptual framework of the WOAH Terrestrial Code and the WOAH Terrestrial Manual.

ICAR-NRCE is coordinating the glanders surveillance programme in collaboration with the State Animal Husbandry Department. Two tier diagnostic system (ELISA and CFT) is being followed for the surveillance purposes. In addition, molecular methods (PCR, qPCR) and culture isolation are applied in biological samples collected from glanders outbreaks. In the recent past, NRCE has trained laboratory personnel from different State Lab/RDDLS (Uttar Pradesh, Gujarat, Haryana, Rajasthan, Himachal Pradesh, Madhya Pradesh, Karnataka, Chhattisgarh, Punjab, Maharashtra, Bihar, Jammu, Kashmir) on glanders diagnosis by ELISA developed at NRCE. This was found useful for setting up of network laboratory for rapid and efficient execution of surveillance activities. At present, State laboratories are using commercially available glanders ELISA kit manufactured by Genomix Diagnostic Pvt. Ltd. As per guidelines, net-work laboratories conduct initial screening by ELISA and positive samples are retested and confirmed by complement fixation test (CFT) and molecular methods at NRCE.

In 2022, a total of 27207 equine sera from 226 districts of 18 states were collected and tested for glanders. Out of these, 8889 equine samples were screened by ELISA at 8 State Lab/RDDLS (Gujarat, Haryana, Himachal Pradesh, Madhya Pradesh, Punjab, Maharashtra, Karnataka, and West Bengal). Among these, Himachal Pradesh,

Maharashtra/WRDDL, Karnataka/SRDDL have significantly contributed by testing 3230, 2510 and 1037 equine samples, respectively.

In this year, 106 glanders positive cases were reported in 9 states. Glanders affected states include Uttar Pradesh (n = 55), Uttarakhand (n = 8), Haryana (n = 16), Himachal Pradesh (n = 11), Gujarat (n = 4), Maharashtra (n = 2), Madhya Pradesh (n = 3), Andhra Pradesh (n = 2) and Rajasthan (*n* = 5). It was found that around 50 % of the samples and glanders positive cases originated from Uttar Pradesh. Zone wise surveyed states belong to Northern India (Uttar Pradesh Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand, and Delhi), Western India (Rajasthan, Gujarat, and Maharashtra), Central India (Chhattisgarh and Madhya Pradesh), Southern India (Karnataka, Andhra Pradesh, and Tamil Nadu) and Eastern India (Bihar and West Bengal). State wise glanders surveillance data is shown in Table below.

In zoonotic point of view, 141 sera from occupationally exposed humans (Veterinary Officers, equine handlers, and laboratory workers) from Haryana, Uttar Pradesh, Madhya Pradesh, Jammu, and Andhra Pradesh were tested and none of them were found positive.

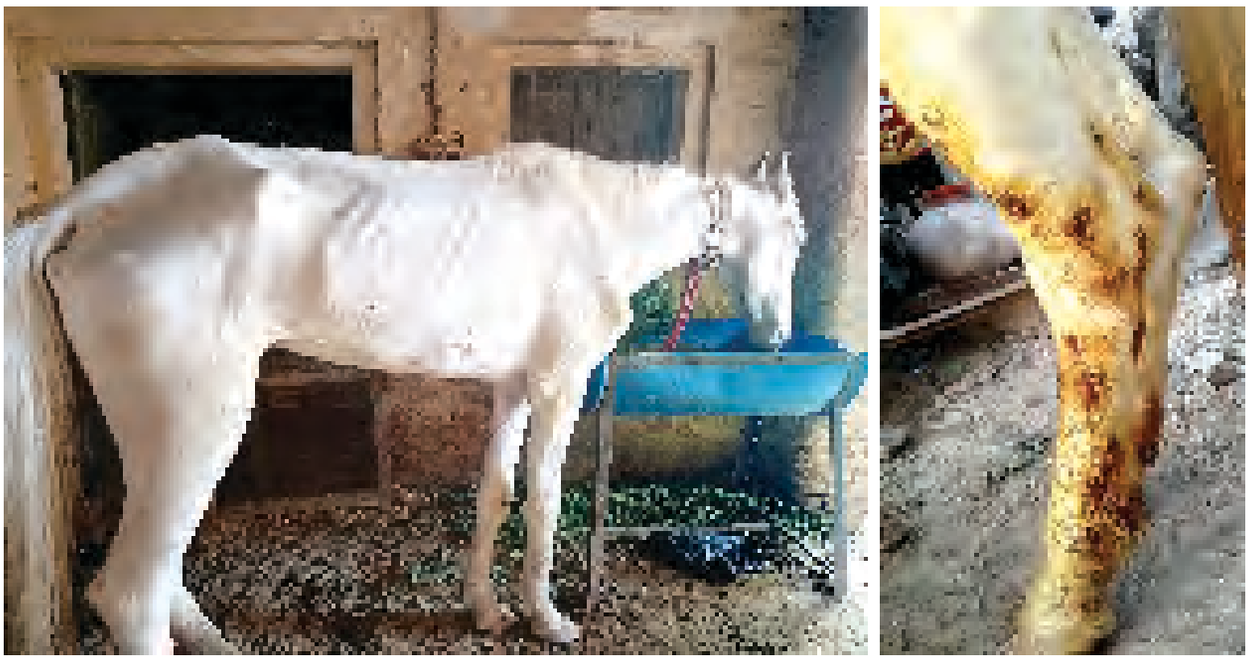
Glanders surveillance data (Jan 2022 - Dec 2022)

Sr. No.	State	No. of Samples Tested	No of samples tested at State Lab/RDDLs	No. of Districts Surveyed	Positive cases
1	Uttar Pradesh	14122	0	75	55
2	Haryana	862	244	10	16
3	Madhya Pradesh	9	557	28	3
4	Himachal Pradesh	1023	3230	9	11
5	Punjab	19	406	20	0
6	Uttarakhand	421	0	5	8
7	Gujarat	21	817	24	4
8	Maharashtra	30	2510	19	2
9	Chhattisgarh	353	0	6	0
10	Tamil Nadu	1	0	1	0
11	Bihar	51	0	3	0
12	Delhi	43	0	4	0
13	Rajasthan	309	0	5	5
14	J & K	877	0	4	0
15	Andhra Pradesh	135	0	5	2
16	Karnataka	21	1037	7	0
17	Manipur	16	0	1	0
18	West Bengal	0	88	2	0
	Total	**18,313**	**8889**	**226**	**106**
	Grand Total	**27207**	**226**	**106**	

Taking account of past five-year surveillance data, it was observed that only 10 to 11 states regularly participated in the glanders surveillance. On the other hand, negligible, irregular or no surveillance was done in North-East and South India. Therefore, pro-active participation of all State Animal Husbandry Department in the glanders surveillance program is necessary to assess state wise sero-prevalence and to devise future strategies for control and eradication of glanders in India.

Glanders affected districts in India (Jan-Dec 2022)

State	Positive case	Distt. / Place
Uttar Pradesh	55	Bahraich, Azampur, Bijnor, Moradabad, Bahraich, Gautam Budh Nagar, Shravasti, Gorakhpur, Sitapur, Varanasi, Meerut, Lakhimpur Kheri, Maharajganj, Mau, Gonda, Agra, Aligarh, Hardoi, Badaun, Rampur, Baghpat, Gorakhpur (22)
Maharashtra	2	Washim, Solapur
Haryana	16	Jind, Sirsa, Jhajjar
Madhya Pradesh	3	Rajgarh, Indore
Himachal Pradesh	11	Shimla, Solan, Kangra
Uttarakhand	8	Dehradun
Gujarat	4	Anand, Ahemdabad, Surat
Andhra Pradesh	2	Guntur
Rajasthan	5	Jhunjhunu, Alwar, Jaipur, Bikaner
Total	**106**	**41-Districts of 9 States**



Representative photographs of glanders affected equines.

(Harisankar Singha, Shanmugasundaram K, Yash Pal & TK Bhattacharya)

Artificial Constructs for Tissue Repair

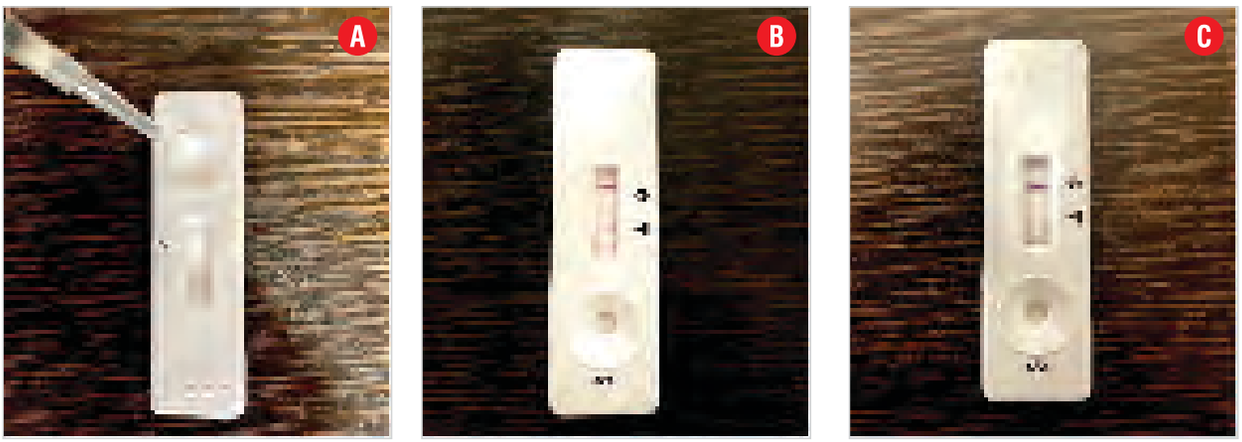
Chronic injuries cause increased pain, suffering, and decreased mobility in affected animals and human beings. Skin is our primary defense against various environmental assaults such as microorganisms, ultraviolet radiation, and toxic or mechanical agents. The injuries may be apt to infection. Three-dimensional bioprinting has emerged as a flexible tool in regenerative medicine and provides a platform for addressing some of the needs described above. The skin specific bioink developed from digested chicken/porcine skin incorporated in polymers provided the 3D structure. The components give an added surface to which various cells and macromolecules

involved in the healing process can attach and interact. DNA was extracted from artificial decellularized skin scaffold. The extracted DNA samples were electrophoresed along with DNA ladder to check the DNA content of both rabbit skin and artificial skin to ensure decellularization of the scaffold. Our data revealed that integration of skin ECM in bioink had improved bioactivity in vitro.

(Anju Manuja, Balvinder Kumar & M Joshi)

Standardization of Lateral Flow Assay for JEV antibodies

The LFA for JEV antibody is being developed and standardized for detection of JEV antibodies in serum samples. The recombinant antigen and BPL-inactivated cell culture grown Japanese encephalitis virus antigen were prepared and preliminary testing results showed that inactivated virus antigen was giving better results in LFA. The standardization of the assay is continued this year. The test has been optimised using equine, guinea pig and rabbit known positive and negative samples. The concentrations of antigen, serum and conjugate were determined by using checkerboard titration, and protocol was developed for obtaining optimal test results. The internal and external validation of the test is ongoing.



The above figures show the test device on a surface. A spectrum of multispecies sera from equine, guinea pigs and rabbits are being tested on the kits.

- In Fig. A, test serum sample is added to the sample pad.
- In Fig. B, the control line (C) and test line (T) develop following incubation indicating positive result.
- In Fig. C, only the control line (C) develops following addition of sample and incubation indicating negative result.

(Baldev R Gulati & Anubha Pathak)

Whole Genome Sequencing and Phylogenetic Analysis of Equine Rotaviruses

Whole genome sequencing of RVA/Horse-wt/IND/ERV3/2003, RVA/Horse-wt/IND/ERV2/2015, RVA/Horse-wt/IND/ERV4/2017, RVA/Horse-wt/IND/ERV6/2017 was done. For sequencing, barcoded random hexamers were used for reverse transcription and second-strand synthesis. This was followed by amplification with barcoded primers. A Nextera XT library preparation kit (Illumina) was used to construct the sequencing libraries for each of the pooled samples. The standard Illumina MiSeq-based sequencing method was used for genome-wide sequencing of ERVAs.

Sequence analysis : The raw sequence files were analyzed using an in-house bioinformatics pipeline for trimming to remove Illumina adapters using Trimmomatic with a minimum quality score of 20 (v 0.39, https://github.com/usadellab/Trimmomatic). Then, host contamination was removed using bowtie2 and sequences were assembled using SPAdes (v3.15.2, https://github.com/ablab/spades).Extracted contigs were analyzed using BLASTx at NCBI to determine taxonomy. Open reading frames (ORFs) of the assembled contig/genome were predicted using the Vgas tool with default parameters. The genome sequences of all the segments were deposited at NCBI.

Genotyping : The Jillion optimized reimplementation of the RotaC 2.0 annotation pipeline integrated in the Virus Pathogen Database and Analysis Resource (VIPR) along with BLASTn analysis (NCBI)was used for genotyping of the RNA sequence of each segment of ERV2, ERV3, ERV4 and ERV6 to obtain the genotypic constellations.

RVA Strains	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Horse-wt/IND/ERV4/2017/G3P[3]	G3	P[3]	I8	R3	C3	M3	A9	N3	T3	E3	H6
RVA/Horse-wt/IND/ERV6/2017/G3P[3]	G3	P[3]	I8	R3	C3	M3	A9	N3	T3	E3	H6
RVA/Horse-wt/IND/ERV3/2003/G6P[1]	G6	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Horse-wt/IND/ERV2/2015/G6P[1]	G6	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3

Phylogenetic analysis of ERV4 and ERV6 : The sequences of each gene segment in this study were compared with publicly available RVA sequences in GenBank and RCWG to construct data sets of relevant sequences. Multiple sequence alignments of the sequences were done using MUSCLE with default parameters in Molecular Evolutionary Genetics Analysis (MEGA) v11.0.10. The substitution models GTR+G+I for VP1, VP2, VP3, VP4, VP6, VP7, and NSP1, T92+G for NSP3 and NSP4, and T92+G+I for NSP2 and NSP5 were determined as per the corrected Akaike information criterion (AICc). A maximum-likelihood algorithm was utilized to generate phylogenetic trees with relevant substitution models in MEGA11, and the tree topology was confirmed by performing 1,000 bootstrap replicates.

Summary of results: Rotaviruses are the most common viral agents associated with foal diarrhea. Between 2014 and 2017, the annual prevalence of rotavirus in diarrheic foals ranged between 18 and 28% in Haryana (India). Whole-genome sequencing of four equine rotavirus A (ERV2, ERV3, ERV4 and ERV6) isolates was carried out to determine the genotypic constellations (GCs) of ERVAs. A unique bat like ERVA genotypic constellation in ERV4 and ERV6: G3-P[3]-I8-R3-C3-M3-A9-N3-T3-E3-H6, previously reported in the bat strain: RVA/Bat-tc/CHN/MSLH14/2012/G3P[3] was revealed. While a unique bovine like ERVA genotypic constellation in ERV2 and ERV3: G6-P[1-]I2-R2-C2-M2-A3-N2-T6-E2-H3, previously reported in bovine strains and human infant, a case of zoonotic calf to human rotavirus transmission was reported. Both these GCs have been reported in equines for the first time. Particulary the genotypes VP6(I8), VP4(P[1]) and NSP1(A3) have not been reported in equines so far, which makes the Genotypic Constellations unique to equines. The genes NSP3, NSP2 and VP3 of ERV4 and ERV6 clustered with high nucleotide identity (95.82%-99.26%) with bat strains from Africa (Gabon). The genes VP1, VP2, VP7, NSP1, NSP4 of ERV4 and ERV6 clustered with a high nucleotide identity (94.82-97.8%) with bat-like (China and Africa) strains of humans. The phylogenetic analysis and lineage studies of ERV4 and ERV6 were carried out. It was found that VP7 of both isolates clustered in a new lineage (lineage X) of the G3 genotype with bat, human, and alpaca strains. Similarly, VP4 clustered in a distinct P[3] lineage.

Meanwhile, the genes VP1, VP2 VP3, VP4, NSP1, VP7, VP6 of NCDV bovine strains shared 99.85%-100% nucleotide identity with ERV2. Similarly, ERV3 also shared 97.57 %-100 % for all the genes mentioned above except VP1 and VP2. The VP1 gene of ERV3 shared 97.82% nucleotide identity with MUL-13-204 a ressortant human strain isolated in Uganda and VP2 gene shared 94.29% identity with GO34, a caprine strain detected in Bangladesh. A 97.58%-99.73% nucleotide identity was shared with bovine RF strains for the genes NSP3 and NSP4. The VP7 genes of ERV2 and ERV3 shared 99.81% and 91.53% nucleotide identity with previously studied ERV80 and ERV99 equine rotavirus isolates respectively from India which were also found to be bovine-like. While NSP5 genes had 95.38%- 98.49% similarity with bovine strains of Japan and Thailand respectively. These unusual findings highlight the terra incognita of the genomic diversity of equine rotaviruses and support the need for the surveillance of RVAs in animals and humans with a “one health” approach.

(Anubha Pathak & Baldev R Gulati)

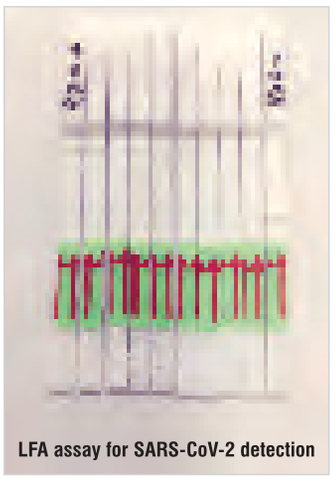
Prevalence of EHV2 and EHV5 infection in equines in India

Equine herpesvirus 1 and 4 have been historically grouped as causal agents of equine rhinopneumonitis and EHV-1 has been enlisted as WOAH notifiable terrestrial and aquatic animal *disease* with ubiquitous presence. However, for other equine herpes viruses not much is known in Indian context. Equine herpesvirus 2 (EHV-2) and equine herpesvirus 5 (EHV-5) are two gamma herpesviruses which have been reported from horses worldwide. EHV-2 is associated with respiratory disorders while EHV-5 causes multinodular pulmonary fibrosis (EMPF). There is no information about the prevalence of EHV-2 and EHV-5 from equines in India. In the present study, a multiplex real-time PCR targeting the glycoprotein (gB) genes of EHV-2 and EHV-5 was developed. The naso-pharyngeal swabs from 238 clinically normal horses below one year of age from equine farms around Hisar (Haryana) were collected during 2019-2021. EHV-2 was detected in 80 (33.61%) while EHV-5 in 86 (36.13 %) equines. Mixed infection was detected in 51(21.42 %) samples. The maximum prevalence of EHV-2 was in 4 to 6 months of age group while EHV-5 in 10-12 month of age group. The partial gB genes of five EHV-2 (444 nt) and five EHV-5 (291 nt) strains were amplified by polymerase chain reaction and sequenced. The sequence phylogenetic analysis indicated that Indian EHV-2 and EHV-5 strains from India have marked genetic diversity. The nucleotide sequence identity ranged from 97.30 - 99.32 % for EHV-2 and 98.28 – 100 % for EHV-5 strains. This is the first report of detection of diverse strains of EHV-2 and 5 from India. Further studies are needed to understand their contributions in respiratory diseases in Indian equines.

EHV -2		EHV-5	
Positive	**Negative**	**Positive**	**Negative**
46	92	50	88
33.33%		36.23%	
n=29 positive for both EHV2 & EHV5			

(Nitin Virmani, Baldev R Gulati, BC Bera & Taruna Anand)

CRISPR-CAS based RPA - lateral flow assay for the detection of SARS-CoV-2 infection



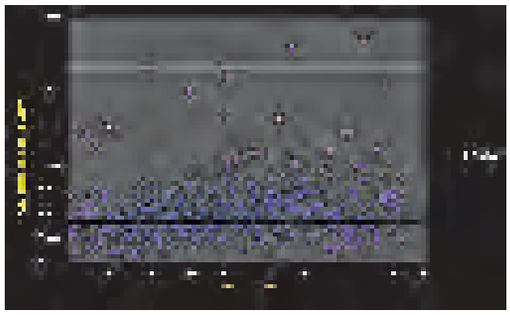
LFA assay for SARS-CoV-2 detection

The field-deployable point-of-care diagnostic test for rapid detection of SARS-COV-2 is needed for implementation of the control measures. In this direction, recently developed CRISPR technology combined with isothermal recombinase polymerase amplification assay is a versatile highly sensitive detection platform for rapid diagnosis of infectious diseases. Here we report the development of RT-RPA-CRISPR based LFA assay for detection of SARS-CoV-2 targeting two conserved genes. Various sets of primers and gRNAs were designed targeting conserved regions of the genes of different lineages of SARS-CoV-2 viruses. The isothermal RT-RPA based amplification reactions were standardized using *in-vitro* transcribed RNAs of the target regions. The optimum amplifications of the targeted regions were confirmed by visualization of the amplicons in agarose gel. Subsequently, CRISPR-CAS reaction was implemented for specific detection of amplicons. Different sets of gRNAs targeting genes were designed and synthesized by *in-vitro* transcription. The CRISP/CAS-gRNA complex and single stranded fluorescence probe were added to the RT-RPA amplicons for cleavage of fluorescence probe in positive reaction. Subsequently, the cleaved probes were detected in pre-coated LFA strips. Upon probe cleavage reaction, the product was mixed with buffer and loaded into LFA strips. In positive reaction, the test line showed a strong band in the test line and light band in control line. The standardized RT-RPA-CRISPR-LFA assay was tested for detection of SARS-CoV-2 using previously isolated RNAs from clinical cases of human SARS-CoV-2 infections. The developed assay successfully detected the positive cases. In conclusion, the developed assay could serve as versatile POC platform for rapid detection of SARS-CoV-2 nucleic acids in humans as well as animals.

(BC Bera, Nitin Virmani & Taruna Anand)

Serological detection of SARs-CoV-2 infection in canines and felines

During the pandemic of COVID19, transmission of SARS-CoV-2 virus was detected in many animals including tigers, lions, minks, canines, felines and deer's. Looking into the threat of transmission of viruses in many species, a recombinant nucleocapsid protein based indirect ELISA kit was developed for detection of SARS-CoV-2 antibody in canines and felines. The developed iELISA is highly specific and doesn't cross-react with other related coronaviruses of canine. The sensitivity and specificity of the assay was evaluated using serum samples collected from canines during earlier preserved pre-COVID19 period and COVID19 period samples as well as canine corona virus vaccinated animals. The iELISA assay showed 95.66% sensitivity and 94.06% specificity for detection of anti-SARS-CoV-2 N IgG antibodies in canines. More than 2500 canine serum samples were tested by developed iELISA and result showed 44.53% positivity for SARS-CoV-2 antibody. The field samples (nos-54) collected from feline tested by using the developed iELISA assay showed ~28% positivity for COVID-19 infection. The Indian patent has been filed for IPR protection of developed iELISA kit. The kit has been released by Hon'ble Minister of Agriculture & Farmer Welfare and DG (ICAR) on 9 June 2022.



Canine samples tested by iELISA

(Nitin Virmani, BC Bera & Taruna Anand)

Development of diagnostics for strangles in equines

Strangles, caused by *Streptococcus equi* subsp. *equi*, is the most frequently diagnosed infectious disease of horses worldwide. The diagnosis of *S. equi* infection has traditionally relied on cumbersome tests like isolation and biochemical characterization of the organism. *S. equi* persists in chondroids, or possibly as a biofilm on mucosal surfaces, and can intermittently shed from carrier animals into the environment allowing transmission to naive individuals. The lack of clinical signs in persistently infected carriers emphasizes the need for effective testing procedures for identification of persistently infected carriers. *S. equi* synthesizes an outer membrane protein of 58 kDa encoded by the *SeM* gene that is particularly important as diagnostic antigen for strangles. *S. equi* M (SeM) protein-based ELISA has been exploited for the identification of horses with high antibody titres. *S. equi* SeM protein has a homologue, SzM, in *S. zooepidemicus* that shares near identity with SeM across the C-terminal two-thirds of this protein, which compromises the specificity of the assay. The PCR-based tests developed for *S. equi* targeted the 5' region of the SeM gene. However, this region is highly variable and some strains of *S. equi* isolated from persistently infected carriers lack the target region. Specific and sensitive tests are needed to detect serum antibodies against *S. equi* to identify the carrier animals and differentiate them from *S. zooepidemicus.* At the genomic level the two subspecies, *S. equi* and *S. zooepidemicus*, are very closely related sharing a large portion of the genome, therefore only a few areas are suitable to be chosen for assays enabling differentiation between them. Scanty research in India despite the high occurrence of strangles necessitates the development of specific immunological and molecular diagnostic assays.

To develop serological assay for detection of serum antibodies and identification of asymptomatic carriers a fixed cell ELISA has been attempted. The inactivated bacterial cells were fixed in the wells of ELISA plates. The surface antigens of these coated cells were reacted with the serial dilutions of the test and control serum samples. The binding of specific antibodies was further detected by addition of specific conjugate and enzymes. The initial experiments have indicated the usefulness of the assay for detection of antibodies. The assay is being further standardized to give specific results with high sensitivity.

To develop a Point-of-Care (POC) diagnostic kit for rapid detection of *Streptococcus equi*, *in silico* analysis of the reference genomic sequences of *S. equi* and *S. zooepidemicus* initiated to identify unique sequences of diagnostic importance.

(Balvinder Kumar, RK Vaid, Anju Manuja, Shanmugasundarm K & Harisankar Singha)

Development of multi-host species ELISA for diagnosis of Surra

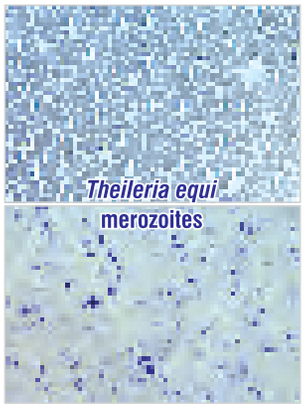
Surra is an infectious disease caused by the protozoan parasite, *Trypanosoma evansi* in domestic, wild and companion animals and the disease is widespread in Africa, the Middle East, Asia, South and Central America. There is no species barrier and infection can be transmitted from one infected animal to any healthy animal by the biting of tabanid flies. This disease is responsible for heavy production losses in Indian livestock which is estimated at the tune of US $ 671.1 million. There is no serological tool available to date which can detect the *T. evansi* infection in multiple host species. The aim of the study was to explore the use of non-species-specific enzyme conjugated proteins *viz.*, protein A, protein G and chimeric protein A/G in place of species-specific enzyme conjugated secondary antibodies in enzyme linked immunosorbant assay (ELISA) for detection of antibodies against *T. evansi* infection in wide range of animal host. Relative avidity index (Table) was determined by using urea as a chaotropic agent, which revealed the binding affinity of serum IgG of six different animal species with non-species-specific enzyme conjugated proteins (protein A, protein G and chimeric protein A/G). The binding affinity of these proteins was compared with anti-species secondary antibody conjugates. Indirect ELISA was performed using whole cell lysate *T. evansi* antigen and non-species-specific conjugates for six animal species viz. equine, cattle, buffalo, camel, pig, and dog. The data depicts that chimeric A/G protein conjugate showed comparable results with that of anti-species secondary antibody conjugates. The diagnostic sensitivity and specificity for chimeric A/G protein conjugate varied in range from 59.94-100% and 79.20-100 % at 95 % confidence interval, respectively for different livestock species. From this study, we concluded that sero-diagnosis of *T. evansi*, a multi-host species parasite, can be carried out by using non-species-specific A/G protein conjugate, which overcomes the limitation of requirement of host species specific conjugated secondary antibody. The study will be helpful in monitoring and surveillance of infection in wild animals and other warm-blooded vertebrates where host specific secondary antibody conjugates are not available.

Relative avidity index (RAI) in multiple hosts using different conjugates at 2M urea

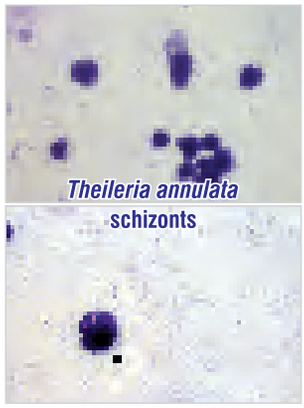
Sr No	Animal Species Samples	Anti-species HRP conjugate(%)	Protein A HRP conjugate (%)	Protein G HRP conjugate(%)	Chimeric protein A/G HRP conjugate (%)
1	Equine	95.3	75.2	98.1	87.0
2	Cattle	86.4	59.8	74.0	78.1
3	Buffalo	91.3	55.2	89.0	64.0
4	Dog	95.5	97.2	70.2	94.4
5	Pig	74.8	75.3	66.3	71.0
6	Camel	-	64.0	51.2	58.6

Isolation and purification of *Theileria equi* merozoites and *T. annulata* schizonts from in-vitro cultures for proteomic analysis

Theileria equi (Hisar Strain) parasites were cultured in horse red blood cells (RBCs) through continuous microaerophilic stationary-phase (MASP) culture system. *Theileria equi* infected RBCs (*i*RBCs) were collected at peak parasitaemia (8-10 %) from in-vitro MASP culture system. These *i*RBCs were treated on ice flakes for ½ h and suspended in PBS/M-199 medium. This suspension was overlaid onto Percoll/phosphate-buffered saline and centrifuges. The supernatant was collected and centrifuged at 800 X *g* for 5 min at 4 °C. Supernatant discarded and *T. equi* merozoite pellet was suspended in PBS (Fig 1A). *Theileria equi* free merozoites were counted in haemocytometer under microscope. *Theileria annulata* (Hisar Strain) infected bovine cell line was cultured in RPMI 1640 complete medium supplemented with heat-inactivated foetal calf serum, L-glutamine and antibiotic solution. The cell line was incubated at 37°C in a humidified atmosphere with 5% CO_2, 3% O_2, and 95% N. *Theileria annulata* schizonts were isolated by collecting the infected culture. Cells were counted haemocytometer and adjusted to $4.0x10^7$/µl. Cells were pelleted by centrifugation at 8000x *g* for 20 min. Pellet was washed with PBS and finally pellet was suspended in 100 µl (Fig B). These purified Theileria infected cells were further subjected to proteomic analysis.



Initiation of *in-vitro* culture of *T. equi* and *T. annulata* and purification of merozoties and schizonts



(Sanjay Kumar, Rajender Kumar, Shanmugasundaram K, Geetanjali & Simran)

Antibacterial activity of some desert herbs against *Dermatophilus congolensis*

Dermatophilus congolensis (DC) was isolated and identified by using conventional and molecular methods from the sample obtained from the skin infections of horses. Anti-bacterial activity of some selected plant extracts against this bacterium by using agar well diffusion method and broth micro-dilution method was also observed. The peculiar "*Tram track*" appearance of coccoid forms in stained smears prepared from culture revealed the presence of *Dermatophilus congolensis*. Isolated colonies from scab materials of horses on horse blood agar plate were rough, adherent, haemolytic and whitish gray in color. Isolated colony were fermented dextrose, catalase positive and indole negative. 16S rDNA PCR yielded 500 bp amplicon specific for *Dermatophilus congolensis* and the result of gene sequence further confirms the presence of this bacteria. Sequence analysis of a sheep DC isolate showed 96 % sequence homology with other DC isolates of Tamil Nadu. Antibacterial screening revealed that both organic and inorganic extract of *Eucalyptus Camaldulensis* showed highest antibacterial activity against *Dermatophilus congolensis* with zone of inhibition in range of 13 mm to 21 mm and MIC in range of 1.562 to 3.125 mg/ml. *Azadirachta indica* (Chloroform, methanolic and ethanolic extract) and *Aloe vera* (methanolic extract) also showed antibacterial activity against this pathogen with zone of inhibition 15 mm to 21 mm (MIC - 3.125 to 6.25 mg/ml) and 11 mm (MIC - 25 mg/ml), respectively. The results of this study support that *Eucalyptus camaldulensis*, *Azadirachta indica* and *Aloe vera* have antibacterial activity against *Dermatophilus congolensis* so can be used/ added in topical antibacterial preparations.

(RK Dedar, Naveen Kumar, T Rao, RA Legha, Yash Pal, P Karela, Mukuni Kumari & TK Bhatacharjya)

Antibacterial activity of desert herbs against *Staphylococcus aureus*

Isolation and molecular characterization of *Staphylococcus aureus* from skin lesions of horses and evaluation of *in-vitro* antibacterial activity of aqueous, methanolic, ethanolic, chloroform and petroleum ether extract of leaves *Capparis decidua*, *Calotropis gigentean*, *Leptadenia* pyrotechnica, *Aerva javanica*, *Azadirachta indica*, *Aloe vera* and *Eucalyptus camladulensis*. *Methods :* Swabs were collected from skin lesion of horses and inoculated in nutrient broth and later in nutrient agar. Bacteria was identified by morphological characters of the colony, biochemical test, and PCR. Agar well diffusion and broth dilution methods were used to determine the antibacterial activity of seven locally available plants against *Staphylococcus aureus*. *Results :* The bunches of grapes like cocci in stained smears prepared from culture and results of various biochemical test and result of PCR confirm the presence of *Staphylococcus aureus*. Antibacterial screening revealed that both organic and inorganic extract of *Eucalyptus Camaldulensis* showed highest antibacterial activity against *Staphylococcus aureus* with zone of inhibition in range of 15 mm to 21 mm and MIC in range of 1.562 to 3.125 mg/ml. *Azadirachta indica* (Chloroform, methanolic and ethanolic extract) and *Aloe vera* (methanolic and ethanolic extract) also showed antibacterial activity against this pathogen with zone of inhibition 15mm to 17.33mm (MIC- 3.125 to 25 mg/ml) and 9mm to 12mm (MIC- 12.5 to 25mg/ml), respectively. Conclusion: The results of this study support that *Eucalyptus camaldulensis*, *Azadirachta indica* and *Aloe vera* have antibacterial activity against *Staphylococcus aureus* and have potential to be used clinically.

(RK Dedar, Naveen Kumar, T Rao, RA Legha, Yash Pal, P Karela, Mukuni Kumari & TK Bhatacharjya)

First molecular evidences of *Anaplasma phagocytophilum* in horses from India

Anaplasma phagocytophilum is a Gram-negative intracellular bacterium, known for causing fever, depression, ataxia, jaundice and edema of limbs in horses. This report describe the first report of clinical cases of *Anaplasma phagocytophilum* infection in horses (n=23) in India. Disease was afebrile in most of the horses. Hindleg weakness and ataxia were the most common clinical signs. Recumbency, oedema and respiratory distress were the other clinical sigs present in some horses. Blood of the 15 horses was presented for laboratory examination. On microscopic examination of blood smear basophilic morulae were observed in neutrophils of the 9 horses. On PCR examination of 15 horses, 9 horses showed amplification of *Anaplasma phagocytophilum specific gene* segments. All the horses showing clinical signs of hind leg weakness and ataxia (n=23) including PCR positive horses (n=9) were treated with oxytetracycline @ 11 mg per kg body weight once daily intravenously for 8 days after diluting in normal saline 1000 ml. Twenty horses out of 23 horses including 7 of the 9 PCR positive horses showed complete clinical recovery from the disease with oxytetracycline treatment. We also found amplification of *Anaplasma phagocytophilum* specific gene segment in a tick sample collected from a horse stable.

(RK Dedar, P Karela, Ram Kumar, Naveen Kumar, TR Talluri, Sanjay Kumar, Rajender Kumar, J Singh. RA Legha, Yash Pal, & TK Bhattacharya)

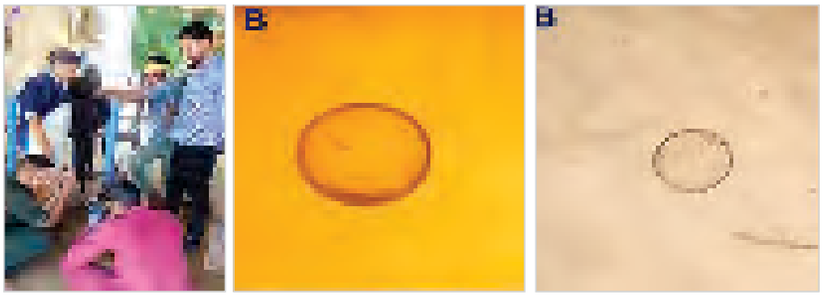
EQUINE PRODUCTION

Optimisation of procedures for non-surgical recovery and bio-banking of equine embryos

Standardization of estrus synchronization protocols in Marwari mares: For standardization of estrus synchronization, both the protocols of extending the luteal phase using CIDR and shortening the luteal phase with PGF2α were tried. CIDR, was inserted in 20 mares for 10 days. After removal of the CIDR, all the 20 mares (100 %) expressed the estrus symptoms with an average time interval of 2.25 ± 0.39 days. Under PGF2α protocol 38 treatments were given under which 29 mares responded and exhibited estrus within 3.44 ± 0.18 days.

CIDR regime in mares for estrus synchronization. B. Application of CIDR in mares

Standardization of flushing of the mares for recovery of embryos: The mares which exhibited estrus on teasing were subjected for examination twice daily to find out the best possible time of ovulation. The mares were artificially inseminated with either fresh semen or frozen semen at near time of ovulation for getting good conception rates. The mares were flushed at different time points after ovulation and the effect of day of flushing on embryo recovery rate was studied. A total of 26 flushings were carried out on day 6.5, 7.5, 8.5 and 9.5. Maximum embryo recovery rate (75 %) was observed on day 8.5 after ovulation. The recovered embryos were successfully transferred to surrogates and two surrogate mares became pregnant through embryo transfer in Marwari mares (Embryos out of fresh semen and frozen semen inseminations).



Flushing of Mare for recovery of embryo in process. B. Recovered Embryos of early blastocyst of 7.5 day.

Summary of the flushing experiments for the recovery of embryos

Day of flushing	Total flushings	Embryos recovered	Recovery rate (%)
Flushing on 6.5-day post - AI	5	2	40.0
Flushing on 7.5-day post - AI	8	3	37.5
Flushing on 8.5-day post - AI	8	6	75.0
Flushing on 9.0-day post -AI	5	3	60.0
	26	**14**	**53.84**

(TR Talluri, Yash Pal, RA Legha, RK Dedar & Sajjan Kumar)

Studies on ultrasonographic, haematological and immunological markers changes during oestrus cycle in mares

A study was designed to observe structural changes in ovaries and uterus by transuterine ultrasonography (TRUS) during oestrus cycle and study haematological and immunological marker (IL-2, IL-6, IL-1β, TNF-α and VEGF) changes during oestrus cycle in Marwari mares. Only single major follicular wave pattern was observed by TRUS in all the mares. The details of the follicular dynamics were listed in Table. The shape of the pre-ovulatory follicle changed from spherical to pear shaped structure in 6 out of 7 mares. Endometrial folds of the uterus were observed edematous in the estrus phase. The edema reduced or almost disappeared 12 - 24 hours prior to the ovulation. Significant changes in the TRBC, Hb, PCV, MCV, MCH, MCHC and TLC were observed during the estrus cycle by hematological examination.

No significant difference was found in mean values of serum concentration of the cytokines (IL-2, IL-6, IL-1β, TNF-α and VEGF) between different stages of oestrus cycle in the study. RT-qPCR revealed that all the 5 genes (IL-2, IL-6, IL-1β, TNF-α and VEGF) expressed in each mare at different stages of oestrus cycle. No significant difference ($P<0.05$) was observed between mean Ct-value of IL-2, IL-6, TNF-α and VEGF genes at different stages of oestrus cycle. Changes in haematological values during estrus and periovulatory period indicate a relationship of haematological values with ovulation. Serum concentration of cytokines (IL-2, IL-6, IL-1β, TNF-α and VEGF) and their gene expression do not seem to be related with the ovarian and uterine changes during oestrus cycle.

Follicular dynamics in Marwari mares

S. No.	Follicular characteristics	Mean ± SE	Range (days)
1	Number of follicles emerged	10.42±0.37	8.0 - 15.0
2	Maximum diameter of pre-ovulatory follicle (mm)	43.21±0.61	40.5 - 48.9
3	Growth of the dominant follicle per day (mm)	2.86±0.26	2.0 - 3.9
4	Inter-ovulatory interval (days)	21.5±0.78	19.0 – 25.0
5	Duration of estrous phase (days)	8.85±0.51	7.0 – 10.0
6	Duration of diestrous phase (days)	12.71±0.61	10.0 – 15.0
7	Interval between onset of estrous to ovulation (days)	7.57±0.53	6.0 – 10.0
8	Interval between ovulation to end of estrous (days)	1.28±0.42	0 – 3.0

(Saurabh Daria, TR Talluri, Ashok Kumar & Sajjan Kumar)

Addition of *Spirulina platensis* extract to stallion semen extender improves the post – thaw semen quality

A study was conducted to study the effect of addition of *Spirulina platensis* extract to semen extender on cooled and post-thaw semen quality in Marwari stallions. Gel free semen was diluted with primary extender in equal volume (1:1) and after then with secondary semen extender alone (control, C) and supplemented with different concentrations of *Spirulina platensis* extract i.e. 10 μg/ml (T1), 20 μg/ml (T2) and 50 μg/ml (T3). Then extended semen of each group (i.e. C, T1, T2, T3) was kept at 4°C for 2 hours for equilibration and then plunged into

liquid nitrogen for cryopreservation. Addition of *Spirulina platensis* extract significantly ($P<0.05$) increased the cooled and post-thaw parameters of progressive sperm motility, sperm viability, sperm plasma membrane integrity (HOST), acrosome integrity, DNA integrity Antioxidant parameters superoxide dismutase, catalase and total antioxidant capacity were significantly ($P<0.05$) increased while lipid peroxidation parameter malondialdehyde was significantly ($P<0.05$) decreased in post-thaw semen by addition of Spirulina platensis extract. Maximum beneficial effect of addition *Spirulina platensis* extract was observed at concentration of 50 μg/ml. Hence, addition of *Spirulina platensis* extract to semen extender significantly increased the freezability of semen and cryo-survival rate of spermatozoa.

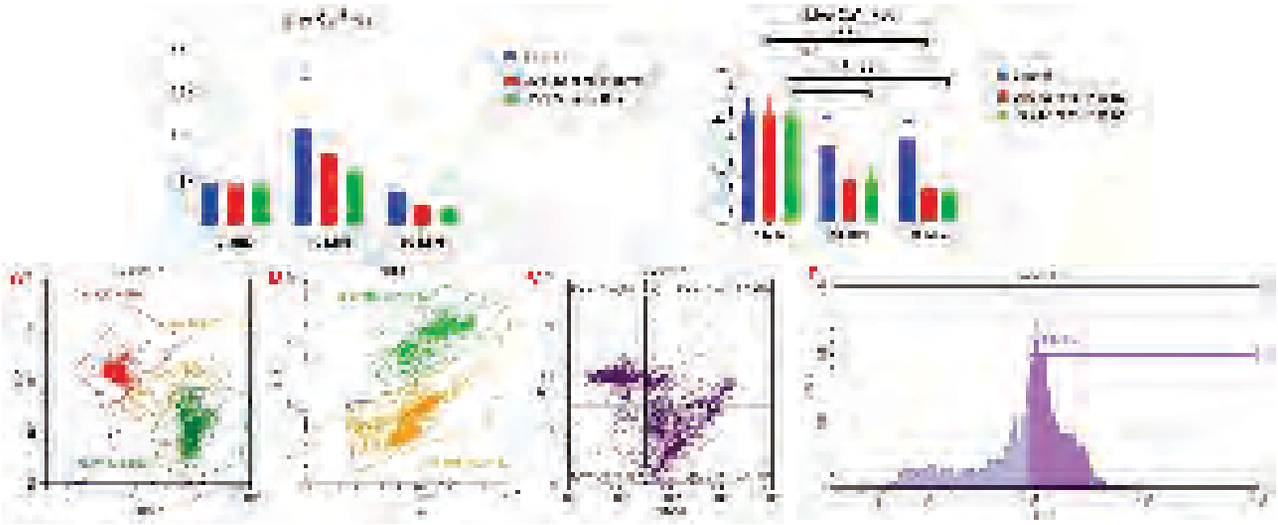
Anti-oxidant effects of *Spirulina platensis* extract to semen extender

Groups	SOD (ng/ml)	CAT (Catalase) (ng/ml)	T-AOC (Total Antioxidant capacity) (ng/ml)	MDA (Malondialdehyde) (nmol/ml)
Control	2.65 ± 0.21^{a}	26.81 ± 0.48^{a}	1.79 ± 0.07^{a}	2.90 ± 0.011^{c}
T1 (10 μg/ml)	4.08 ± 0.30^{b}	27.05 ± 0.49^{a}	2.19 ± 0.09^{b}	2.70 ± 0.012^{b}
T2 (20 μg/ml)	5.81 ± 0.27^{c}	31.35 ± 0.39^{b}	3.06 ± 0.12^{c}	2.68 ± 0.012^{b}
T3(50 μg/ml)	10.63 ± 0.30^{d}	37.68 ± 0.48^{c}	5.51 ± 0.16^{d}	1.26 ± 0.024^{a}

(Vishal Yadav, TR Talluri, Sandeep Dholpuria & Sajjan Kumar)

Heterologous seminal plasma reduces the intra-cellular calcium & sperm viability of cryopreserved stallion spermatozoa

A study was designed to assess the vital sperm functional parameters of genetically superior stallions producing poor quality semen can be enhanced by the supplementation of heterologous seminal plasma (SP) from the stallions producing high quality semen. Spermatozoa from poor quality semen producing stallions were divided into three aliquots; two aliquots were supplemented with SP obtained from good quality semen producing stallions at the rate of 20 % and 30 %, while the third aliquot as control (0 % SP) and incubated at 37 °C for 30 min. Using Flow Cytometry, sperm membrane integrity, mitochondrial membrane potential (MMP), mitochondrial superoxide (mtROS) generation and intracellular calcium status were assessed at different time intervals during incubation. It was observed that the dead sperm population increased during incubation in both 20% and 30% SP supplemented groups. On the other hand, no significant changes in were observed in MMP in both the control and the treatment groups at different time intervals. Interestingly, it was found that sperm mtROS production increased ($p<0.01$) during incubation in SP supplemented groups as compared to the control group. The proportion of live spermatozoa with high intracellular calcium was reduced during incubation in SP incubated groups. Supplementation of heterologous seminal plasma reduced viability and increased ROS production in stallion sperm.



(TR Talluri, Nilendu Paul & A Kumaresan)

Endurance and fertility analysis in indigenous horses using Single Nucleotide Polymorphisms (SNP) markers

The traits like endurance and fertility in indigenous horses are of tremendous importance and hence the SNP markers associated with them were tested in five indigenous breeds viz. Marwari, Kathiawari, Sindhi, Manipuri and Zanskari for polymorphism. Eight markers associated with endurance were tested in indigenous breeds. In all 503 samples belonging to indigenous breeds were amplified by PCR for respective SNPs and sequencing was carried out. The SNP BIEC2-1022884 (A>G) was found monomorphic and rest were polymorphic in indigenous breeds of horse. The genotype and allele frequency were calculated in each breed, the pooled over information has been summarized and presented in Table.

Genotype- and allele-frequency of SNP markers associated with endurance in indigenous breeds (pooled over Marwari, Kathiawari, K-Sindhi, Manipuri and Zanskari) of horse.

SNP Marker	N	Genotype Frequency					Allele Frequency		
BIEC2-755603	54	AA	AC			CC	CC		C
		0.26	0.43			0.31	0.31		0.53
BIEC2-755604	60	CC	CT			TT	TT		T
		0.27	0.48			0.25	0.25		0.49
BIEC2-977605	58	AA	CC	CG	AG	GG	A	C	G
		0.00	0.00	0.05	0.07	0.88	0.03	0.03	0.94
BIEC2-363958	45	GG	GT			GG	G		T
		0.60	0.31			0.09	0.76		0.24
BIEC2-11782	68	CC	CT			TT	C		T
		0.85	0.10			0.05	0.90		0.10
BIEC2-620109	62	CC	CT			TT	C		T
		0.65	0.26			0.09	0.77		0.23
MSTN	105	CC	CT			TT	C		T
		0.02	0.25			0.73	0.14		0.86

Five SNP markers associated with fertility were studied. In all 277 samples belonging to indigenous breeds were amplified by PCR for respective SNP and sequencing was carried out. The genotype and allele frequency were calculated in each breed, the pooled over information has been summarized and presented in Table.

Genotype- and allele-frequency of SNP markers associated with fertility in indigenous breeds (pooled over Marwari, Kathiawari, K-Sindhi, Manipuri and Zanskari) of horse.

SNP Marker	N	Genotype Frequency			Allele Frequency	
BIEC2_952439	48	CC	CT	TT	C	T
		0.04	0.08	0.88	0.08	0.92
PLCZ1_2	66	GG	GT	TT	G	T
		0.65	0.26	0.09	0.78	0.22
PLCZ1_3	65	AA	AG	GG	A	G
		0.82	0.18	0.00	0.91	0.09
FKBP6_1	29	AA	AG	GG	A	G
		0.52	0.17	0.31	0.60	0.40
FKBP6_2	69	AA	AC	CC	A	C
		0.32	0.43	0.25	0.54	0.46

The endurance racing (*Rewal Chaal*) events held at different places were covered and in all 425 samples belonging mainly to Sindhi breed were amplified by PCR for respective SNP and sequencing was carried out. The genotype and allele frequency were calculated the information has been presented in Table.

Genotype-and allele-frequency of SNP markers associated with endurance in K-Sindhi horses covered during various racing events.

SNP Marker	N	Genotype Frequency			Allele Frequency		
BIEC2-755603	59	AA	AC	CC	A		C
		0.35	0.41	0.24	0.56		0.44
BIEC2-755604	63	CC	CT	TT	C		T
		0.33	0.43	0.24	0.55		0.45
BIEC2-977605	54	AG	CG	GG	A	C	G
		0.04	0.07	0.89	0.02	0.04	0.94
BIEC2-363958	65	GG	GT	GG	G		T
		0.65	0.32	0.03	0.81		0.19
BIEC2-11782	64	CC	CT	TT	C		T
		0.86	0.14	0.00	0.93		0.07
BIEC2_620109	65	CC	CT	TT	C		T
		0.43	0.54	0.03	0.70		0.30
MSTN	55	CC	CT	TT	C		T
		0.05	0.25	0.70	0.18		0.82

(SC Mehta & TR Talluri)

Characterisation and recognition of Bhimthadi horse

The breeding tract of the Bhimthadi horse was visited and the information regarding its origin, history, development, distribution, population status, traditional breeders, physical charateristics, body weight and reproductive traits have been collected. The breeding tract extends in North from 15.7469ºN to 19.9897ºN and in East 76.414ºE to 73.3249ºE encompassing the Pune, Ahmednagar, Satara, Solapur, Sangli and Kolhapur. The application for registration of the breed was prepared in accordance with the guidelines of ICAR-NBAGR, Karnal and submitted through the Commission Animal Husbandry, Govt. of Maharashtra. The adult male and female animals weighed on an average 260 kg (180 - 320 kg) and 245 kg (175 - 273 kg), respectively. The averages of important body measurements along with range and number of observations of adult Bhimthadi horses has been presented in Table.

Body measurements of Bhimthadi horses.

Parameter	Stallion			Mare		
	Average (cm)	Range (cm)	N	Average (cm)	Range (cm)	N
Height at withers	130.3	117-140	237	128.5	114-135	78
Height at croup	129.3	117-138	237	127.5	110-135	78
Body length	132.5	111-142	237	130.6	107-136	78
Girth	142.0	126-160	237	138.5	118-156	78
Face length	52.0	43-58	237	50.5	42-55	78
Face width	19.0	14-22	237	19.0	14-22	78
Ear length	15.0	10-18	237	15.0	10-18	78
Ear width	10.0	7-15	237	10.0	7-15	78

Parameter	Stallion			Mare		
	Average (cm)	Range (cm)	N	Average (cm)	Range (cm)	N
Space between Eyes	17.0	11-21	237	17.0	11-21	78
Length of fore arm	91.0	79-98	237	87.0	79-95	78
Height at knee	38.0	30-48	237	37.0	29-45	78
Height at hock	45.0	35-55	237	44.0	33-55	78
Distance between fetlock to coronet	9.0	7-11	237	8.5	7-11	78
Chest width	27.0	24-30	237	26.0	24-30	78
Shank (Cir)	17.5	13-19	237	16.5	15-18	78
Throat Latch	67.0	44-72	237	63.0	44-70	78
Poll to withers length	70.0	61-77	237	69.5	60-76	78
Distance between Withers and croup	66.5	58-76	237	66.0	58-75	78
Distance between Croup and head of tail	30.0	26-35	237	28.0	24-32	78
Tail Length	72.0	39-100	237	70	35-98	78

(SC Mehta & SD Sorate)

Analysis of quantitative traits for genetic improvement of indigenous equines

Growth Curve Analysis : Mathematical functions for the prediction of growth in Marwari horses were derived. The Logarithmic, Power, S and Cubic functions were derived with respective R^2 values of 0.955, 0.833, 0.897 and 0.980 for average body weights to explain the age-weight relationship in Marwari horses from birth to 11½ years of age. Looking at the distribution of observed data along the course of predicted curve and goodness of fit on average body weight the Cubic equation can reliably (R^2=0.984) be utilized for prediction of body weight with respect to age. Though, the effect of sex was non-significant (P>0.05) but the male animals were heavier than the female animals from birth to 3 years of age, separate prediction equations were derived for the two sexes. Similarly, the cubic equations were derived for Manipuri and Zanskari horses. The study indicated that the initial growth phase in Marwari and Manipuri continues up to the age of about 6 years and up to the age of 5½ years in Zanskari horses, there after it remains static till about 8- 9 years of age; and the cubic function can reliably be used to explain the nature of growth in Marwari, Manipuri and Zanskari breeds of horses.

(SC Mehta & Jitender Singh)

Analysis of runs of homozygosity and its distribution for selection signatures in Indigenous Equine breeds

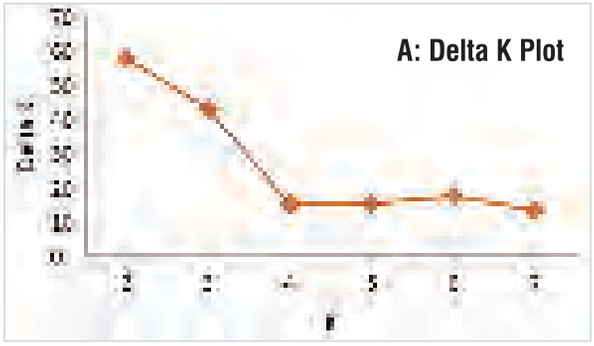
The blood samples were collected from 07 horse breeds, namely Manipuri (17), Zanskari (20), Bhutia (20), Spiti (16), Kathiawari (10), Marwari (10) and Thoroughbred (3) covering 96 genotypes. Samples were genotyped with an average recall rate of 92.6%for each sample. A total of 5444 homozygous segments were identified. The mean number of runs of homozygosity (ROH) per animal was highest in Spiti, *i.e.,*67.69± 42.06 (with number ranging of 26 to 154 and length ranging from 1 Mb to 124 Mb) while the lowest was in Bhutia, *i.e.,*48.05 ± 27.21 (with number ranging of 20 to 116 and length ranging from 1 Mb to 126 Mb). After computing the average length of ROHs for all breeds, it was observed that Marwari breed had lowest average length of 4.6 Mb and Thoroughbred had highest average length of 5.7 Mb. Significant number of ROHs were observed on chromosome 1, *i.e.,*373 (6.85%) and the chromosome 30 had least number of ROHs, *i.e.,*66 (1.01%) while on chromosome 32 had no ROH. Details of the ROHs for each chromosome is given in Figure. It is also showing the percentage variation of the ROHs of each chromosome for all breeds.

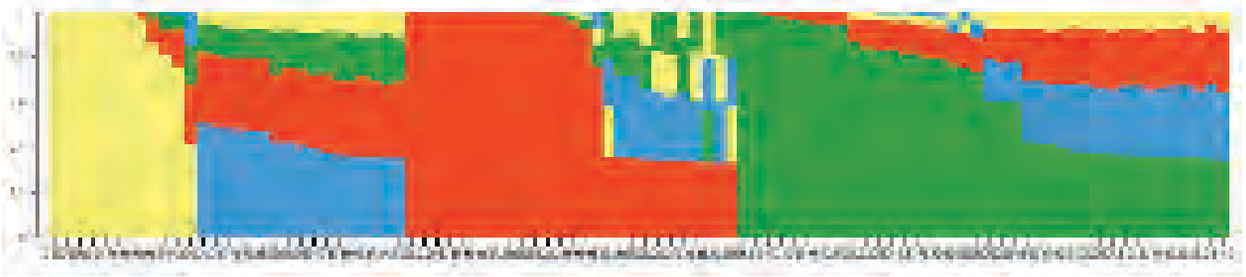
Chromosome-wise ROH variation for all breeds

(Anuradha Bhardwaj, Sarika, Jay Kumar, MA Iqbal, Yash Pal, Varij Nayan, Sonali, Ram Avatar Legha, TR Talluri, TK Bhattacharjya & BN Tripathi)

Genome-wide SNP based genomic diversity and population structure in Indian horses

SNP detection in the 7 indigenous breeds via whole genome sequencing approach was attempted in this study. In our study, total 96 samples categorised under seven breeds and 620721 SNPs have been considered for ascertaining the ROH patterns amongst all the seven breeds. Over 5444 ROH islands were mined and maximum number of ROHs were present in Zanskari while Thorough was confined least number of ROHs. Gene enrichment of these ROH islands, produced 6757 functional genes with AGPAT1, CLEC4 and CFAP20 as important gene family. However, QTL annotation revealed that maximum QTLs were associated with Wither's height trait ontology that falls under Growth trait in all the seven breeds.



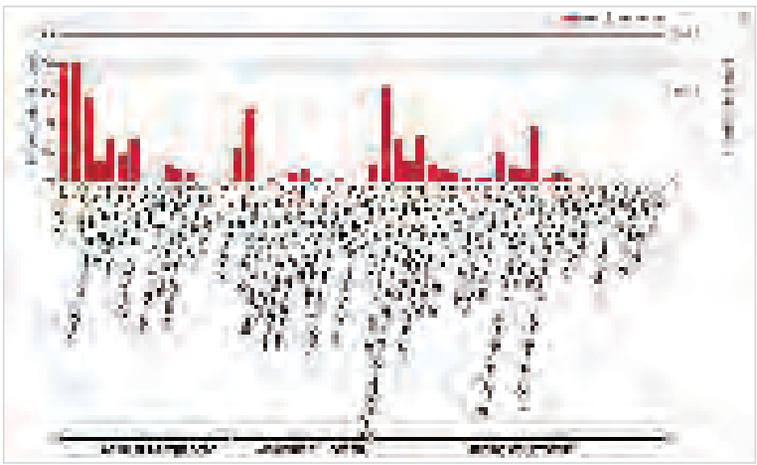


Population structure analysis of 96 samples of Equus caballus based on SNPs. A: Population estimation using mean of estimated LnP(D) with K ranging from 1 to 7. B: Clustering of 96 individuals presented by different colors i.e. 1. Red, 2. Green, 3. Blue, and 4. Yellow inferred by Structure analysis. cEach bar representative an individual, where different colors in that individual representing the estimated admixture using the Q statistic.

(Anuradha Bhardwaj, Sarika, MA Iqbal, Yash Pal, Varij Nayan, Sonali, Ram Avatar Legha, TR Talluri, TK Bhattacharjya & BN Tripathi)

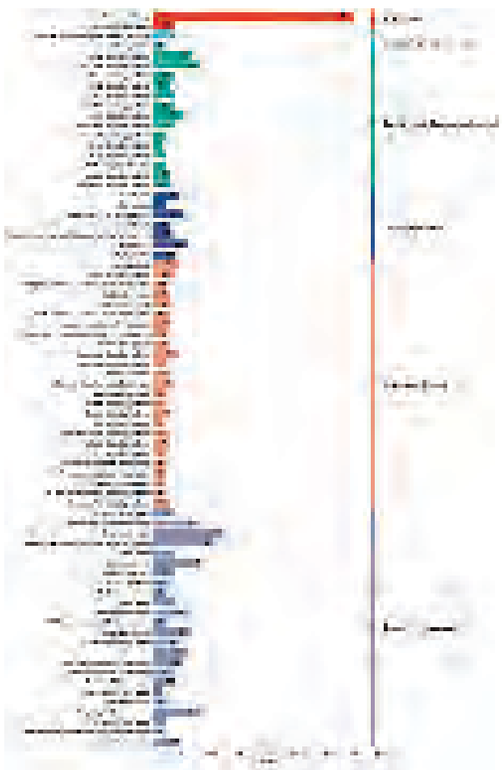
Gene annotation and enrichment analysis based on SNPs analysis through axiom equine array

SNP annotation by SnpEff produced total 18,395 candidate genes within ROH islands. In all the breeds Gene Ontology terms and pathways associated with these genes were verified for evidence of functional enrichment. This produced 6757 genes, which were further categorized into Biological Processes, Molecular Functions and Cellular Components. The details of their GO IDs have been analysed. Detailed gene ontology segregation according to the category has been provided in the figure.



Gene function with their counts according to the category in which they fall.

Pathway analysis revealed that most of the genes were involved metabolic pathways, followed by pathways related to cancer. Most of the genes covered under ROH islands were related to various diseases in horse directly or indirectly. Top genes based on gene count and P-value were chosen and plotted and is given the figure below.



Gene Ontology and pathways analysis associated with candidate genes for functional enrichment

(Anuradha Bhardwaj, Yash Pal, Sarika, Jay Kumar, MA Iqbal, Varij Nayan, Ram Avatar Legha, TR Talluri, TK Bhattacharjya & BN Tripathi)

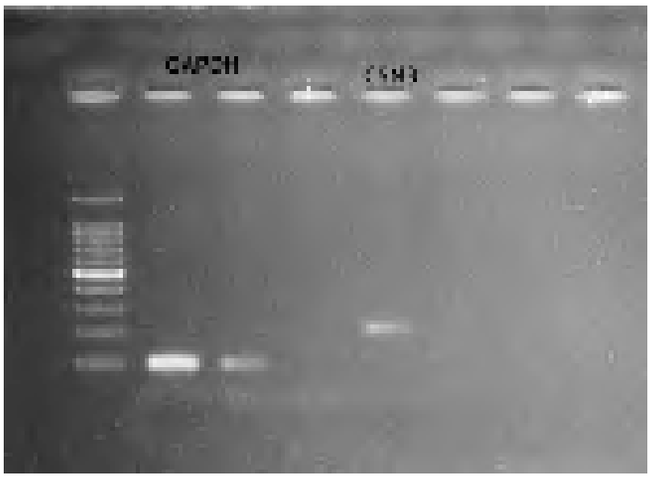
Compositional changes in Halari donkey milk during lactation

Since donkey milk has a similar composition to human milk, it has been well demonstrated that it is an excellent alternative. The content of donkey milk changes during lactation, nevertheless, has not been thoroughly researched. This study aimed to investigate the compositional changes in Halari donkey milk during lactation. Donkey milk samples were collected at different stages of lactation, including the colostrum, transitional, and mature milk stages. Milk samples were collected from healthy halari jennies, reared at donkey farm in ICAR-NRCE, Hisar, Haryana, India. A total 270 raw samples of Halari donkey milk form nine different animals were collected weekly for seven months of lactation. The milk samples were analysed for various components including fat, solids not fat, protein, and lactose. The findings demonstrated that donkeys' milk composition changed dramatically throughout lactation, with the colostrum stage exhibiting the highest levels of fat (1.67 %), which decreased as lactation progressed and eventually reached fat (0.26 %). The lactose and protein levels showed minor decline from lactose (5.79 – 4.90 %) and protein (3.85 – 3.25 %). Overall, this study provides important insights into the compositional changes that occur in donkey milk during lactation, which may have implications for the nutritional value and health benefits of this unique milk.

(Anuradha Bhardwaj, Renu Garhwal, Karnam Sangwan, Varij Nayan, TR Talluri, Ram Avatar Legha & Yash Pal)

Milk somatic cell DNA isolation and characterization of κ-casein gene in Halari donkey milk

Halari donkey breed is one of the indigenous breeds of India and its population is rapidly decreasing. Its milk has shown significant antimicrobial properties and other health benefits especially among infants suffering from cow milk protein allergy. κ-casein, a major milk protein has been studied in other animal species majorly and in donkeys. The key source of DNA in those studies was blood. In this study, we extracted DNA from the milk somatic cells which may be regarded as a non-invasive source for DNA. PCR was performed specifically to amplify donkey κ-casein gene (*CSN3)*. A total of 235 bp of the κ-casein gene (*CSN3*) was amplified using the given primers- F: 5'-GATGACAACTCTATTTCCCCCT-3' and R: 5'-CCAGGGTCAGGTCTTGCT-3. The genomic DNA extracted from the milk somatic cells were used as a template in the PCR reaction set up. A total volume of 25 μL or reaction mixture contained 12.5 μL of PCR master mix (thermo scientific), 1μL of each forward and reverse primer,and 2 μL of DNA sample. It was found that the DNA extracted from it amplified the κ-casein gene specific primers showing that the milk somatic cells can be used as a source for the extraction of DNA for molecular studies.



Phylogenetic relationship of the CSN3 peptide sequence of Halari donkey (*Equus asinus*) with other mammals. Molecular phylogenetic analysis was performed from the data provided by the multiple sequence alignment

patterns of amino acids. The sources of all the sequence data are represented in the phylogenetic tree. The phylogeny is an un-rooted tree recovered using the maximum likelihood (ML) method based on 1,000 bootstrap replications and Jones–Taylor–Thornton (JTT) matrix-based model by the MEGA 11.0 program. Only bootstrap value greater than [0 % is shown on the branch].

(Anuradha Bhardwaj, Prashant Singh, Varij Nayan, TR Talluri, Ram Avatar Legha, Yash Pal & BN Tripathi)

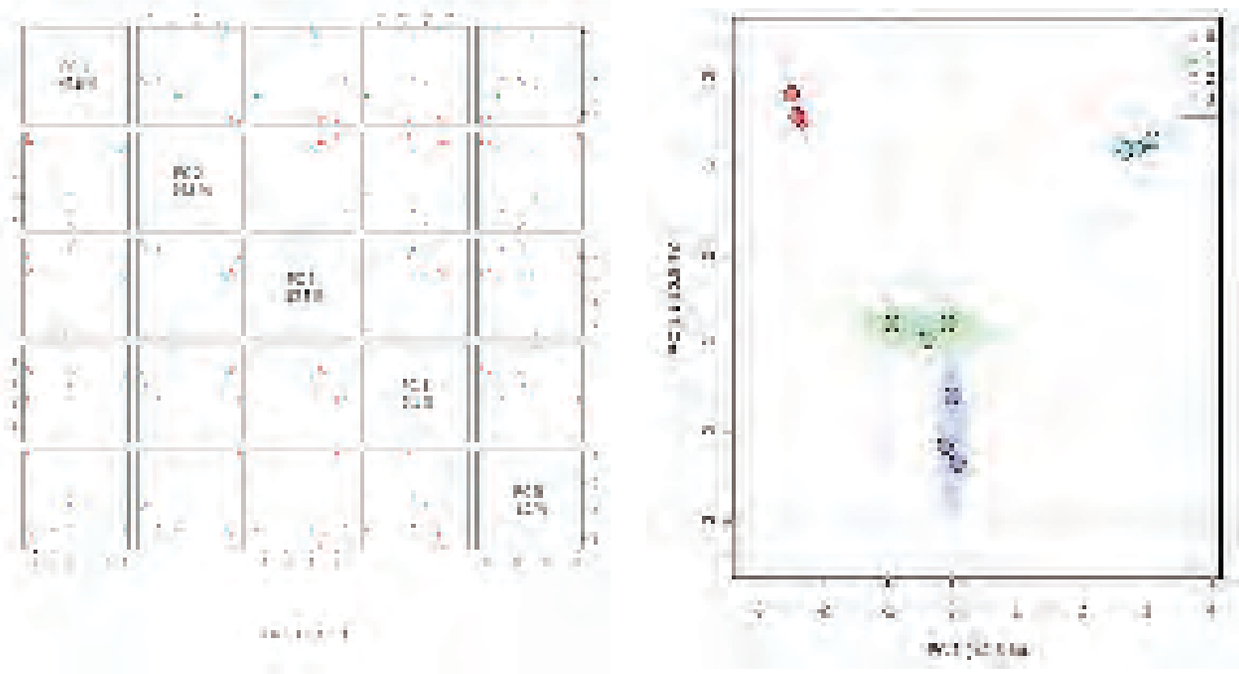
Molecular amplification and identification of the Halari donkey α- lactalbumin (*LALBA*) gene.

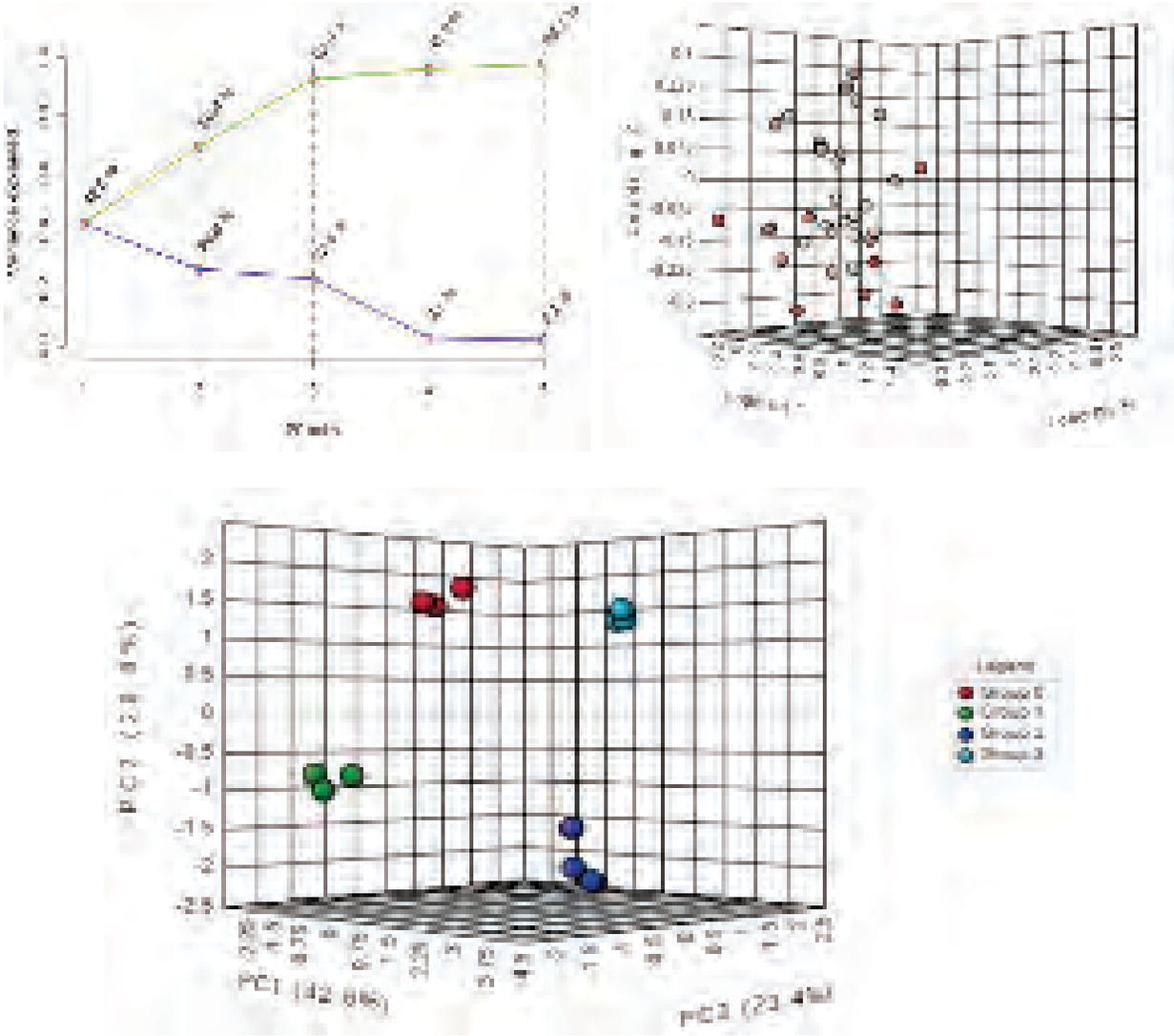
In India, among the three registered breeds of donkeys, Halari donkey, a native breed of the Saurashtra region of Gujarat, is considered as an asset for farmers. They must be characterized morphologically and genetically to conserve them. It is reported that in donkey milk, *LALBA* concentration is around 1.8 g/l which is very close to human milk. The *LALBA*, as a part of lactose synthase complex, plays a central role in the lactose formation in milk. Also, it has been reported that it possesses antiviral, anti-tumor and anti-stress properties. So, the aim of this study is to isolate the somatic cells and DNA from donkey milk for amplification of the *LALBA* gene for further characterization in the indigenous donkey population of India. A total of 151 bp of the α-lactalbumin gene (*LALBA*) was amplified using the given primers- F: 5'-GCCCCCATTTCAGGTTCTTG-3' and R: 5'-ATTCAGGCAAAGTGACGCCT-3'. For future research applications, further down-streaming applications of PCR based amplification of the α-lactalbumin gene will shed more light on its milk properties in Halari donkeys.

(Anuradha Bhardwaj, Prashant Singh, TR Talluri, Varij Nayan, Ram Avatar Legha, Yash Pal & TK Bhattacharjya)

Chemometric analysis of donkey colostrum and donkey milk during different lactation stages

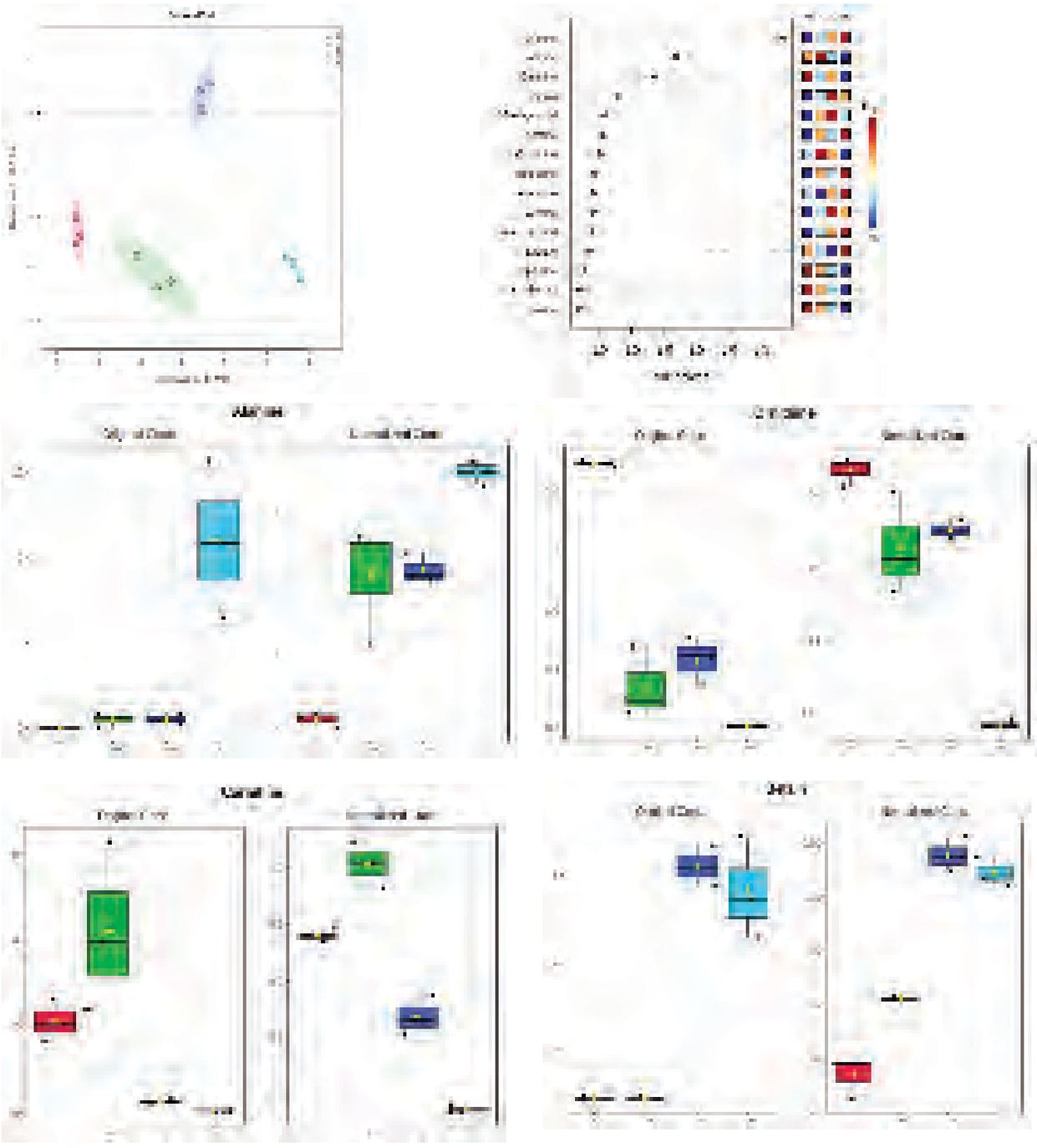
Similarity and differences in the sample of different lactation stages were also detected by performing different chemometric analysis as Principal Component Analysis (PCA). As shown in figure A, PCA score plot revealed five principal components namely, PC1 which represented 42.6 % variation among the groups, PC2 representing 26.8 % variation, PC3 representing 23.4 % variation, PC4 and PC5 representing 3.1 % and 2.1 % variation respectively.





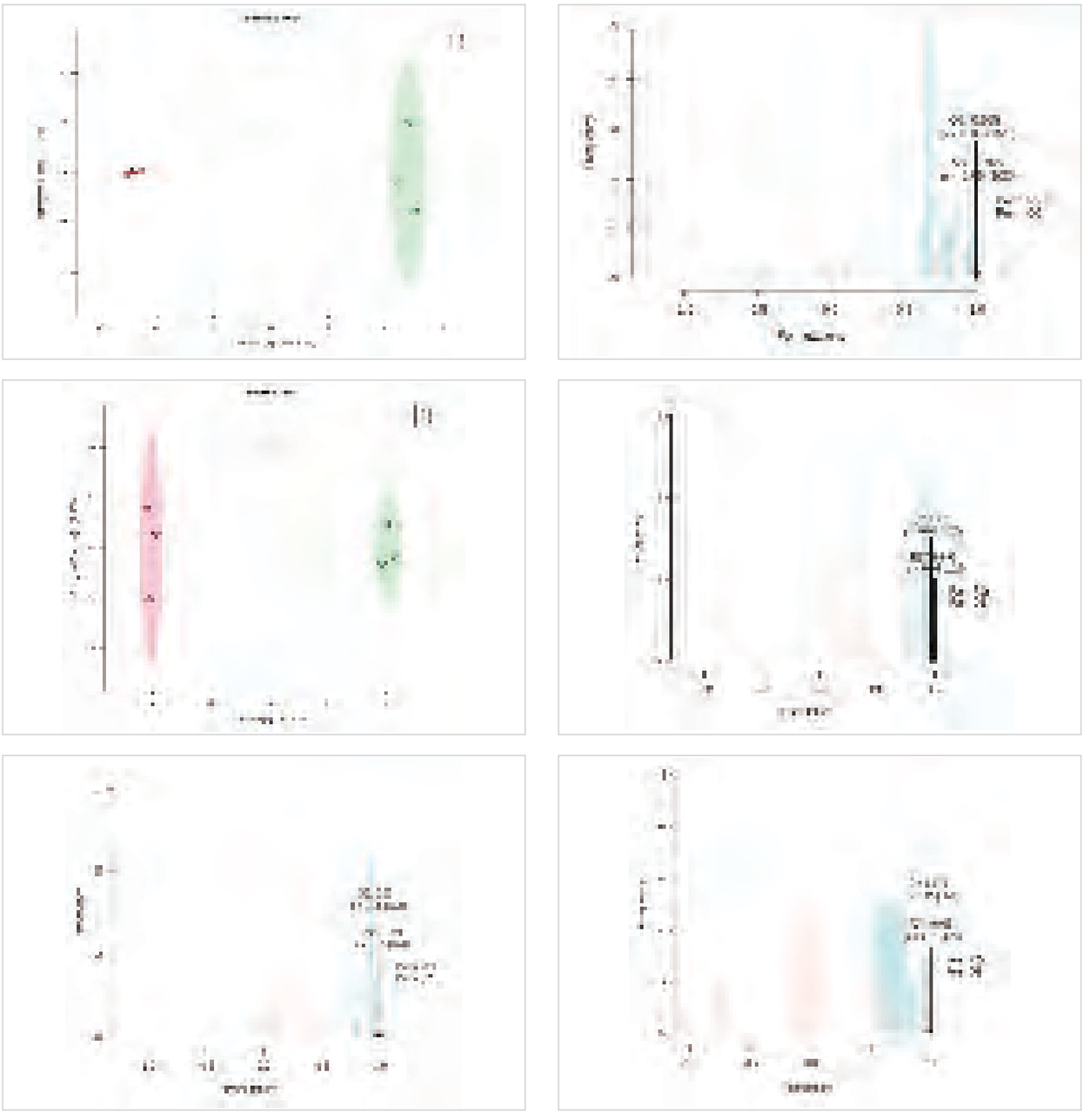
Principal component analysis (PCA) of identified metabolites at different lactation stages in donkey colostrum and milk. (A) Pairwise score plots between the selected PCs (principal components), group 0= colostrum, group 1= early lactation, group 2 = mid lactation, group 3 = late lactation; (B) Score plots between the selected PCs showing explained variances in brackets; (C) Scree plot showing the variance explained by PCs. The green line on top shows the accumulated variance explained and the blue line underneath shows the variance explained by individual PC; (D) Loading plot for the selected PCs; (E) 3D score plot between the selected PCs showing explained variances in brackets.

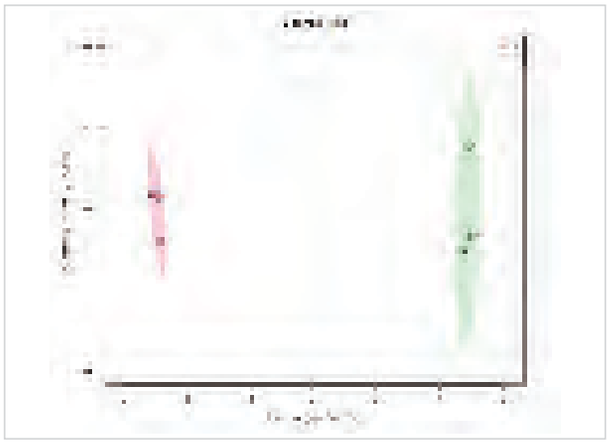
Differential analysis: Partial Least Squares – Discriminant Analysis (PLS-DA) and Orthogonal Partial Least Squares–Discriminant Analysis (OPLS-DA) were also performed to study the variation of metabolites among different lactation stages in detail. The supervised PLS-DA was used to improve the separation between the four groups (lactation stages). The groups were well distinguished using PLS-DA models, demonstrating strong reliability within each group. All four groups could be distinguished well by PLS-DA, as shown in Fig. B, since the groups were segregated on the left and right sides of the origin point (x-axis). Between each of the four separate groups, two main components (components 1 and 2) were indicated. These components provided an explanation for the percentage of variance between all four groups of different lactation stages, for example, (A) depicts that component 1 accounts for the 42.2 % of the variation among the different lactation stages, component 2 accounts for the 25.1 % among all four lactation stages. X axis in the plot displays the score of components 1 and Y axis displays the scores of components 2 respectively.

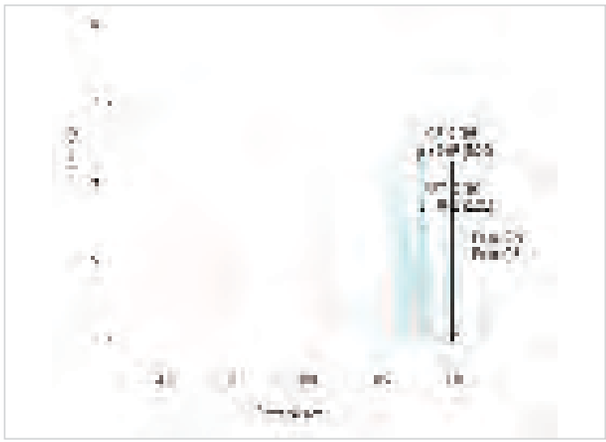


(A) Scores plot between the selected PCs. Component 1 accounts for the 42.2 % variability and component 2 accounts for 25.1 % variability. (B) Important features identified by PLS-DA according to their VIP scores. The coloured boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study. (C) Box whisker plots of metabolites with highest VIP scores (a-e). The concentrations of a chosen feature from all samples are shown by black dots on each figure. The notch shows the 95% confidence interval around each group's median. Differences between groups are indicated by notches that do not overlap, and each box's yellow diamond displays the mean concentration of each group.

Orthogonal Partial Least Squares : Discriminant Analysis (OPLS-DA) demonstrated the distinction between different lactation stages of donkey colostrum and milk samples namely, 0 - Colostrum (CL) and 1 - early lactation (EL) (Fig C (A1)), 1 – early lactation (EL) and 2 – mid lactation (ML) (Fig 3 (B1)), 2 – mid lactation (ML) and 3 – late lactation (LL) (fig 4 (C1)) and 0 – colostrum (CL) and 3 – late lactation (LL) (Fig 3 (D1)). OPLS-DA models also displayed distinct separation among the four lactation stages. Score plots clearly indicate the differences as the different groups in each score plot were present on the left and right side of the x-axis (origin point). Component 1 and component 2 for each stage were identified with their percent variation between two lactation stages. To verify the models and determine if the separation was statistically significant, permutation tests were used. Fig 3 (A2, B2, C2, D2) represented the results of premutation tests for the respective OPLS-DA models of different lactation stages. Values of the permutation tests for goat milk yoghurt with various storage times include R^2 (measure of model fit to original data) and Q^2 (measure of consistency between original and cross-validation predicted data). Lactation stage 0-1 ($R^2 = 0.998$ and $Q^2 = 0.992$), lactation stage 1-2 ($R^2 = 0.999$ and $Q^2 = 0.970$), lactation stage 2-3 ($R^2 = 0.997$ and $Q^2 = 0.979$) and lactation stage 0-4 ($R^2 = 0.999$ and $Q^2 = 0.995$) were close to 1. This demonstrated that the metabolite cluster separation seen by OPLS-DA models between the various lactation stages was statistically significant.







The score scatter plot as shown by Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) (A1, B1, C1, D1) and permutation tests for OPLS-DA models (A2, B2, C2, D2) for samples of donkey colostrum and milk at different lactation stages: (A1 and A2) colostrum versus early lactation; (B1 and B2) early lactation versus mid lactation; (C1 and C2) mid lactation versus late lactation; (D1 and D2) colostrum and late lactation. (OPLS-DA displays distinct separation between two groups of donkey colostrum and milk samples during different lactation stages; R2 denotes the model's fit to the initial data, and Q2 assesses the congruence between the initial and cross-validation projected data.

(Anuradha Bhardwaj, Renu Garhwal, Karnam Sangwan, Rahul Mehra, Varij Nayan, Manish Tiwari, Sarika, Mir Asif Iqbal, Man Mohan Singh Chauhan, Ram Avatar Legha, Harish Kumar, & Yash Pal)

Studies on suitability of goat milking machine for milking the mares.

Nowadays, equids milk, especially donkey milk is getting popularize among the educated people and many new entrepreneurs are adopting donkey farming and establishing donkey dairies. Presently, the milking of equines is mainly done by strip method, and it is unhygienic and time consuming. Keeping in view the need of the donkey dairies and efficient milking, the project was initiated to test the adaptability of existing goat milking machines for donkey milking. We purchased the parts of milking machine used for goat milking and assembled it at our center. First, we trained two Poitou and one Halari donkeys for milking with machine and these were trained within 10 -15 days period. We tried milking of donkeys with this machine, and it is working well, and no modification of machine is required except under scaling the milk cane as it is of 20 Kg capacity which is not required for equine milking as the milk yield is very less in comparison of cattle and buffaloes. At present we are having only 2-3 female donkeys in milking, and we are milking these animals with machine. In the next foaling season of equines, we will conduct full study on machine parameters as well as animal parameters and compare the hand milking and machine milking. In next season we will also train the mares also for machine milking.

Milking machine and donkey mare during milking

(RA Legha & Yash Pal)

Designing, fabrication and use of creep feeders for foal optimum growth rate in indigenous equids

Creep feeder is used to provide a balanced, high-quality concentrate to young nursing foals prior to weaning. The practice of creep feeding serves to supply nutrients beyond what a foal receives from the milk. Also, broodmare feeds contain between 10 % and 14 % crude protein, which is inadequate for growing foals. Keeping in view the importance of creep feeding in foal management we designed and fabricated the creep feeder for foal feeding. We fabricated the creep feeder of ply board. The dimension of top cover was 9x9 inches and depth was 6 inches. The oval shape whole was made in top cover (3" width and 5" length) which allows foal to consume from feeder while mare could not reach to feed inside the creep feeder. The cost of making this type of creep feeder is about Rs. 600/- only. The price of available foal creep feeder online is about Rs 5000/- per piece. It takes about 7 days to train the foal to eat from creep feeder. Till now we have fabricated only one feeder and only one foal is trained for feeding. We will fabricate some more feeders with wooden material, so that it may not be damaged with moisture content of feed put inside the feeder. In the next foaling season, we will study the effect of creep feeding on growth performance of foals.

(RA Legha & Yash Pal)

NATIONAL CENTRE FOR VETERINARY TYPE CULTURES

Authentication and accessioning of viruses of animal origin :

NCVTC virus repository is being strengthened with the addition of viruses from different geographical locations of the country through the deposition/collection of isolates and clinical samples from different animals and poultry. The samples were processed for isolation of different viruses. The details of virus authentication / isolation are as follows.

i. **Acquisition / Receipt of viral isolates:** A total of 42 viral isolates were received as deposits from NCVTC Network units. The deposits included 26 bluetongue viruses from ICAR-NIVEDI, seven bovine coronaviruses from ICAR-NRCE, seven lumpy skin disease viruses from NCVTC and ICAR-NIVEDI, one Avian Infectious Bronchitis Virus from Assam Agricultural University and one Rous sarcoma virus (RSV-1) from IVRI (Table).

Acquisition/Receipt of viral isolates during 2022-23

Depositor	Virus isolates	Number
NCVTC, ICAR-NRCE (Hisar)	Lumpy skin disease virus	2
ICAR-NIVEDI (Bengaluru)	Bluetongue virus	26
	Lumpy skin disease virus	5
ICAR-NRCE (Hisar)	Bovine coronavirus	7
AAU (Guwahati)	Avian Infectious Bronchitis Virus	1
ICAR-IVRI (Izatnagar)	Rous sarcoma virus (RSV-1)	1
	Total	**42**

i. **Authentication of isolates /samples:** All the viruses received at the repository were processed for authentication and authentication details are as follows.

a. **Isolation and authentication of Lumpy skin disease virus (LSDV):** Two viruses isolated at NCVTC, Hisar and five LSDV isolates submitted by ICAR-NIVEDI, Bengaluru were processed for authentication. The viruses were passaged in Lamb Testicle and Kidney cells for assessing its viability and later authenticated by PCR amplification of RPO30 (606 bp) gene. Five isolates were confirmed and accessioned in the repository.

b. **Authentication and accessioning of bovine corona virus:** A total of seven bovine corona viruses submitted from ICAR-NRCE were processed for authentication at NCVTC repository. The genomes were amplified by PCR amplification of spike gene and RNA polymerase gene. The viability of the viruses was assessed by passing into NLBK cells. Six of these isolates could be confirmed and accessioned in the repository.

c. **Authentication and accessioning of Bluetongue virus:** 26 BTV isolates received from ICAR-NIVEDI were processed for isolation and confirmation of the virus. The viruses were authenticated by PCR amplification of NS1 gene (273 bp) and passage into BHK21 cells. 21 of these viruses were finally confirmed and accessioned in the repository.

d. **Authentication of Rous sarcoma virus:** One virus submitted by ICAR-IVRI was authenticated by PCR amplification of group specific antigen (326bp). The virus is being passage into embryonated eggs for assessing the viability.

e. **Authentication of Infectious bronchitis virus:** One virus submitted by AAU; Guwahati was authenticated by PCR amplification of spike gene (326 bp). The virus is being passage into embryonated eggs for assessing the viability.

ii. **Accession of viral isolates:** All the 32 viruses authenticated at the repository during the period under report were accessioned (VTCC AVA 349- 380), which include bluetongue viruses (n = 21), bovine coronaviruses (n=6) and lumpy skin disease viruses (n = 5) (Table).

List of viruses accessioned during the year 2022-23

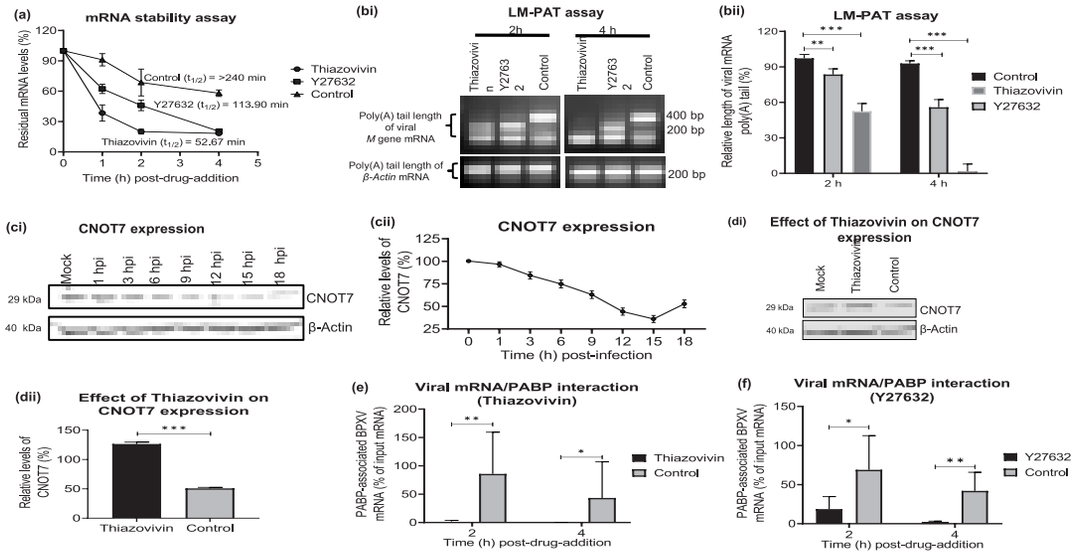
Depositor	Virus isolates	Number
NCVTC (Hisar)	Lumpy skin disease virus	2
ICAR-NIVEDI (Bengaluru)	Bluetongue virus	21
	Lumpy skin disease virus	3
ICAR-NRCE	Bovine coronavirus	6
	Total	**32**

iii. **Revival of preserved isolates:** A total of 30 previously preserved viruses including NDV (12), SWPV (1), FMDV (1), BTV (12), and IBDV (2) and IBV (2) were revived and checked for assessing their viability and these viruses were found viable.

(Sanjay Barua, Naveen Kumar & Riyesh T)

Inhibition of ROCK1/MLC cell signaling pathway induces deadenylation of buffalopox mRNA in the infected cells

Rho-associated coiled-coil kinase 1 (ROCK1) intracellular cell signaling pathway regulates cell morphology, polarity, and cytoskeletal remodeling**.** We observed the activation of ROCK1/myosin light chain (MLC)/WD repeat domain 5 (WDR5) signaling pathway in buffalopox virus (BPXV) infected Vero cells. Disruption of ROCK1 signaling pathway resulted in reduced BPXV replication in vitro (evidenced by reductions in viral mRNA/protein synthesis, genome copy numbers and progeny virus particles), besides providing protection to embryonated chicken eggs against lethal BPXV infection. It was demonstrated for the first time that inhibition of ROCK1 signaling by specific small molecule chemical inhibitor Thiazovivin induces deadenylation of viral mRNA (mRNA decay), a phenomenon which is mediated via displacement of Poly(A)-binding protein (PABP) and recruitment of CCR4-NOT [a multi-protein complex that has both a poly (A) 32-52 exonuclease and a ubiquitin ligase activity] (Fig.). Besides, it was also observed that BPXV infected cells undergo cell shrinking in ROCK1/MLC-dependent manner which eventually led to the cytoplasmic localization of WDR5. WDR5 interacts with the viral proteins and appears to anchor the virus at the replication sites in the infected cells. In addition, cytoplasmic localization of WDR5 also prevented the transactivation of the IFN-β gene which resulted in an increase in virus replication. Finally, it was demonstrated that the long-term sequential passage (P = 50) of BPXV in the presence of Thiazovivin does not select for any drug-resistant virus variants, suggesting that antiviral drug resistance is unlikely to occur against ROCK1 inhibitors. In conclusions, ROCK1/MLC2/WDR5 cell signaling pathway appears to facilitate BPXV replication by three independent mechanisms; stabilization of viral mRNA, induction of cell contraction that appears to anchor the virus at the replication sites and, inhibition of IFN-β induction.

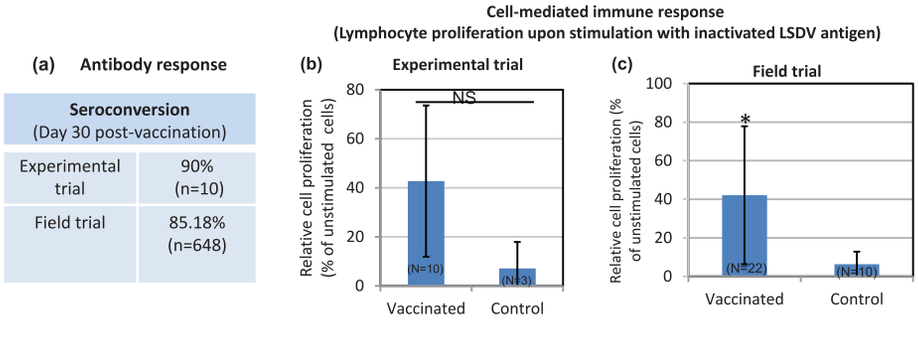


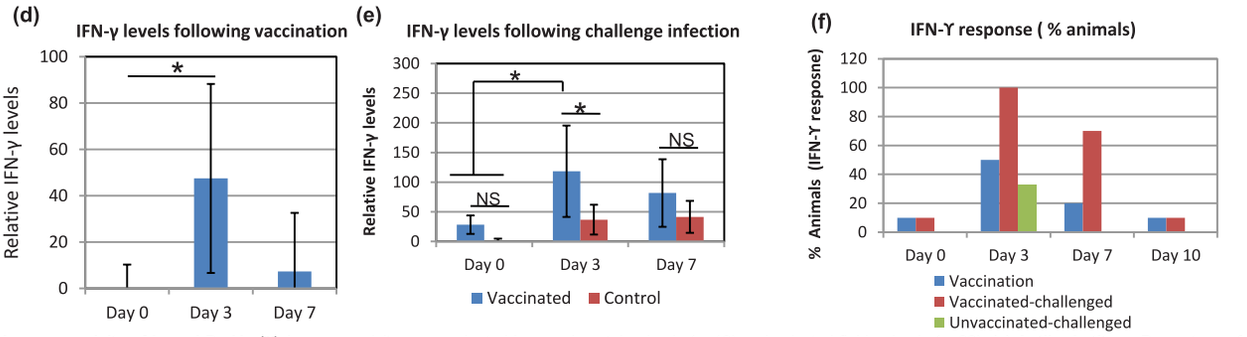
Thiazovivin induces viral mRNA decay. ***(a) Measurement of mRNA stability:*** Vero cells, in triplicates, were infected with BPXV at MOI of 5. At 9 hpi, cells were treated with Thiazovivin (1 µg/ml), Y27632 (1.5 µg/ml) or equivalent volumes of DMSO in the presence of Actinomycin D (5 µg/ml). Cells were subjected to RNA isolation at indicated time points (post-drug treatment) and subjected to cDNA synthesis and quantified for BPXV *M* gene by qRT–PCR. The relative levels of viral mRNA at 0 h, 1h, 2h and 4 h in Thiazovivin or Y27632- and control-treated cells are shown. n= 3 independent experiments. ***(b) LM-PAT assay.*** Vero cells were infected with BPXV. At 9 hpi, the cells were treated with the inhibitors (Thiazovivin or Y27632) or DMSO in the presence of Actinomycin D. At indicated times post-drug-addition, cells were scrapped to isolate the RNA. The RNA was allowed to react with an adaptor oligo(dT) primer in the presence of T4 DNA ligase and subjected to cDNA synthesis. The length of poly(A) tail of viral (*M*) and cellular (β-actin) mRNA were determined by PCR by using an anchor primer and a gene-specific primer (bi). The length of the PCR product (BPXV *M* gene) in inhibitor-treated and control-treated cells was measured (ImageJ) and the relative length of the viral mRNA poly(A) tail (% of control) at different times treatment was calculated (bii). Data are presented as mean with SD. n= 3 independent experiments. ***(c) Kinetics of CNOT7 expression.*** Vero cells were either mock-infected or infected with BPXV at MOI of 5. Cell lysate were prepared at indicated time points and subjected for detection of CNOT7 levels in Western blot analysis. The levels of CNOT7 (upper panel) and β-actin (house-keeping control protein, lower panel) are shown (**ci**). The line graph (**cii**) shows the band intensity of the protein at different times post-infection. The blots were quantified by densitometry (ImageJ) and the data are presented as mean with SD. n= 3 independent experiments. ***(d) Effect of Thiazovivin on CNOT7 expression.*** Vero cells were either mock-infected or infected with BPXV at MOI of 5. Thiazovivin or vehicle controls were added at 6 hpi. Cell lysates were prepared at 9 hpi and subjected for detection of CNOT7 levels in Western blot analysis (**di**). The histogram (**dii**) shows the band intensity of the protein. The blots were quantified by densitometry (ImageJ) and the data are presented as mean with SD. n=3 independent experiments. **(e and f)** ***ROCK1 inhibition blocks interaction of BPXV mRNA with PABP (CHIP assay).*** Vero cells, in triplicates, were infected with BPXV at MOI of 5. At 9 hpi, the cells were treated with ROCK1 inhibitors (Thiazovivin and Y27632) or vehicle control. At 2h and 4 h post-drug addition, the cell lysates were prepared as per the procedure described for CHIP assay (materials and method section). The clarified cell lysates were incubated with α-PABP or equivalent volume of the IP buffer (Beads control), followed by incubation with Protein A Sepharose® slurry. The beads were then washed five times in the IP buffer. To reverse the cross-linking, the complexes were then incubated with Proteinase K. Finally, the reaction mixtures were centrifuged and the supernatant was subjected to cDNA preparation and quantitation of BPXV RNA (M gene) by qRT-PCR. The percentage of the input BPXV mRNA bound to PABP in Thiazovivin-treated (e) and Y27632-treated cells (f) is shown. n=3 independent experiments. Error bars indicate SD. Pair-wise statistical comparisons were performed using Student's t-test (*** = P<0.001, ** = P<0.01, * = P<0.05).

(Naveen Kumar & Sanjay Barua)

Evaluation of the safety, immunogenicity and efficacy of a new live-attenuated lumpy skin disease vaccine in India

Lumpy skin disease (LSD) was reported for the first time in India in 2019 and since then, it has become endemic. Since a homologous (LSD-virus based) vaccine was not available in the country, goatpox virus (GPV)-based heterologous vaccine was authorized for mass immunization to induce protection against LSD in cattle. This study describes the evaluation of safety, immunogenicity and efficacy of a new live-attenuated LSD vaccine developed by using an Indian field strain, isolated in 2019 from cattle. The virus was attenuated by continuous passage (P = 50) in Vero cells. The vaccine (50th LSDV passage in Vero cells, named as Lumpi-ProVac^{Ind}) did not induce any local or systemic reaction upon its experimental inoculation in calves (n = 10). At day 30 post-vaccination (pv), the vaccinated animals were shown to develop antibody- and cell-mediated immune responses (Fig.) and exhibited complete protection upon virulent LSDV challenge. A minimum Neethling response (0.018 % animals; 5 out of 26940 animals) of the vaccine was observed in the field trials conducted in 26940 animals. There was no significant reduction in the milk yield in lactating animals (n = 10108), besides there was no abortion or any other reproductive disorder in the pregnant animals (n = 2889). Sero-conversion was observed in 85.18% animals in the field by day 30 pv.



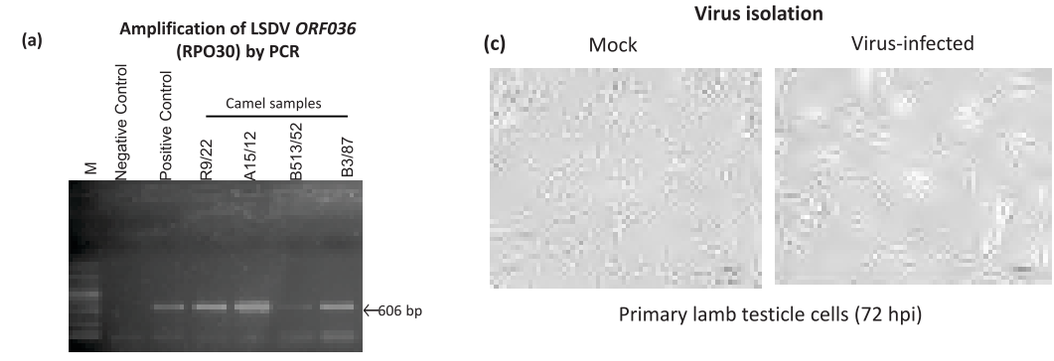


Immunogenicity of Lumpi-ProVac^{Ind}. Immunogenicity was evaluated in experimental animals (n=10) and selected field animals (n=22). **(a) Antibody titers.** Percentage of animals (experimental and field trials) that revealed detectable anti-LSDV antibodies in serum by virus neutralization assay is shown. **(b) Lymphocyte proliferation assay (experimental trial).** PBMCs were separated from the blood collected from vaccinated (n=10) or unvaccinated (n=3) calves at day 30 pv. PBMCs were cultured in RPMI and stimulated with UV-inactivated LSDV. Relative proliferation of lymphocyte from vaccinated as compared to unvaccinated animals is shown. **(c) Lymphocyte proliferation assay (field trial).** PBMCs were separated from the blood collected from vaccinated (n=22) or unvaccinated (n=10) animals (all age groups) at day 30 pv and stimulated with UV-inactivated LSDV. Relative proliferation of lymphocyte from vaccinated as compared to the unvaccinated animals is shown. **(d) IFN-ϒ levels following vaccination.** Sera from experimental calves, separated at indicated times post-vaccination were subjected for determination of IFN-ϒ by using Bovine IFN-ϒ-ELISA kit. **(e) IFN-ϒ levels following challenge infection.** Sera from vaccinated, vaccinated-challenged and unvaccinated-challenged were examined for determination of IFN-ϒ. **(f) Percentage of animals that developed IFN-ϒ response.** Percentage of animals that exhibited IFN-ϒ response at indicated a times following LSDV exposure is shown is shown. *=P<0.05, NS=non-significant difference.

(Naveen Kumar, Sanjay Barua & Riyesh T)

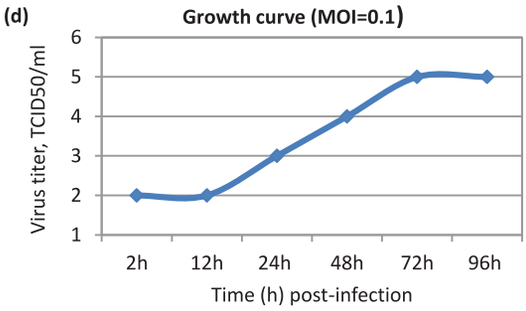
Isolation of LSDV from Camel, Rajasthan

LSD is primarily a disease of cattle. Buffaloes may sometimes develop mild illness; however, other domestic animals are considered resistant to LSD. We confirmed the LSDV infection in camels (Bikaner/Rajasthan) as evidenced by skin nodules on the body surface of the affected camels, isolation of LSD virus (LSDV) and amplification of LSDV-specific gene segments from the skin nodules (PCR) (Fig.), nucleotide sequencing of the viral genome and demonstration of anti-LSDV antibodies in serum. Phylogenetic analysis based on nucleotide sequencing of ORF011, ORF012 and ORF036 revealed that the virus (LSDV/Camel/India/2022/Bikaner) is related to the historical NI-2490/Kenya/KSGP-like field strains which are predominantly circulating in the Indian subcontinent. This is the first report wherein LSDV has been shown to infect camels.



(b) Anti-LSDV antibody titer

Camel ID	Antibody titer
R9/22	<4
A15/12	16
B513/52	16
B3/87	8

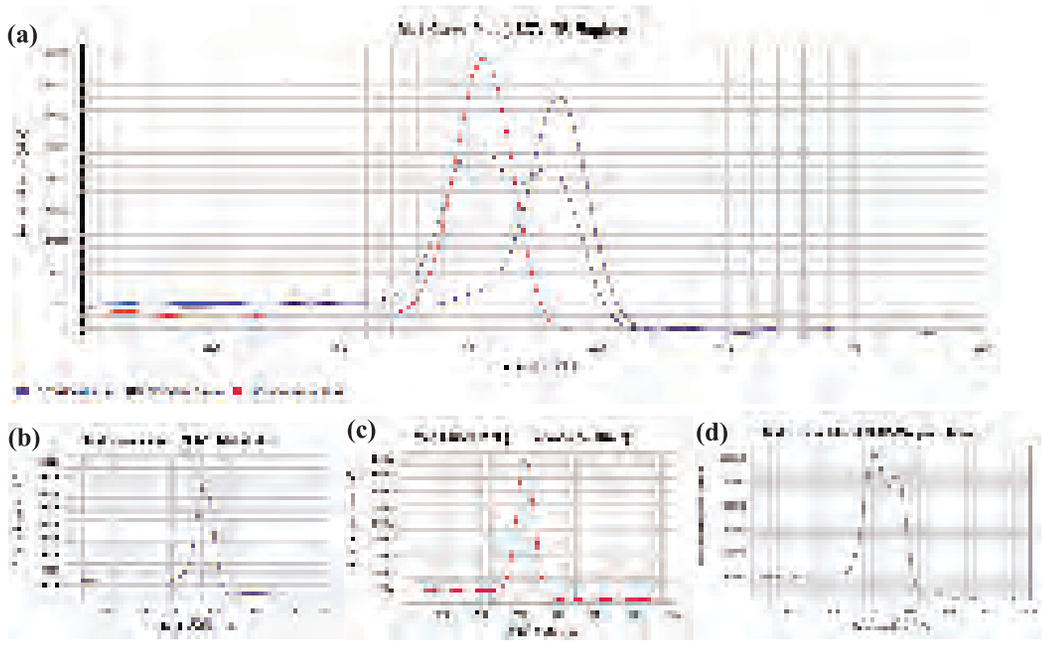


Isolation and identification of Lumpy skin disease virus from camel. . ***(a) Detection of LSDV genome in skin nodules.*** Pieces of the skin nodules were collected in 3 ml DMEM and subjected to DNA extraction. The LSD-specific gene ORF012 (Ank) segment was amplified by PCR. ***(b) Detection of anti-LSDV antibodies in serum.*** Serum samples were collected 3-6 days after the appearance of skin nodules. Samples were initially heated at 56°C for 30 min to inactivate the complements. Two-fold serum dilutions were mixed with 10^3 $TCID_{50}$ of LSDV/Ranchi/P50 and incubated for 90 min at 37°C. Thereafter, virus-antibody mixture was used to infect Vero cells. At 72 hpi, the cells were observed for CPE to determine the antibody titres. ***(d) Virus isolation.*** An aliquot of the virus (800 µl filtrate) was used to infect confluent monolayer of primary lamb testicle cells for 2 h followed by addition of fresh growth medium. The cells were observed daily for the appearance of CPE. CPE observed at the second blind passage in one of the samples (skin nodule) derived from camel is shown ***(b) Growth curve.*** Primary lamb testicle cells, in triplicates, were infected with virus infected cell culture supernatant (obtained in blind passage 2) at MOI of 0.1 for 2 h, followed by washing with phosphate buffered saline (PBS) and addition of fresh medium. The virus released in the infected cell culture supernatant at indicated time points was quantified by determination of TCID50.

(Naveen Kumar & Sanjay Barua)

A novel HRM-based gap-qRT-PCR for identification and quantitation of the vaccine and field strain(s) of lumpy skin disease virus

Lumpy skin disease (LSD) has become the most important animal health problem in India due to high morbidity, mortality, and production losses. A homologous live-attenuated LSD vaccine (Lumpi-ProVac^{Ind}) was recently developed by using a local LSD virus (LSDV) strain (LSDV/2019/India/Ranchi) in India which is likely to replace the existing practice of vaccinating cattle with goatpox vaccine in the country. It is essential to differentiate the vaccine and field strains, if a live-attenuated vaccine has been used for control and eradication of the disease, particularly in endemic areas. As compared to the prevailing vaccine and field/virulent strains, the Indian vaccine strain (Lumpi-ProVac^{Ind}) has a unique deletion of 801 nucleotides in its inverted terminal repeat (ITR) region. We exploited this unique feature in the Indian vaccine strain and developed a novel high-resolution melting-based gap quantitative real-time PCR (HRM-gap-qRT-PCR) (Fig.) for rapid identification and quantitation of the vaccine and field strain(s) of LSDV.



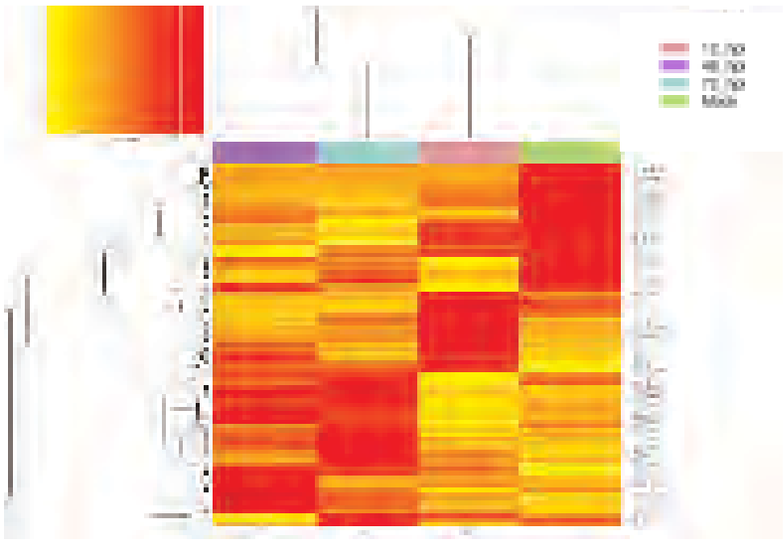
High resolution melt curve analysis of ITR region of LSDV: The ITR region of LSDV vaccine and field strains were amplified as described in material and methods. The Tm of PCR-amplicons were examined by change in their relative fluorescence intensity over a temperature range of 60–95°C in 0.2°C increments by using QuantStudio™ Design and Analysis Software v1.4.2. **(a and b)** Melting curve of LSDV field strain (Blue line; Tm=78.5°C).(**a and c**) Melting curve of LSDV vaccine strain (Red line; Tm=75.62°C). (**a and d)** The mixed (vaccine plus field strain) population of LSDV produces two amplicons with Tm of75.29°C and 78.12°C.

(Naveen Kumar & Sanjay Barua)

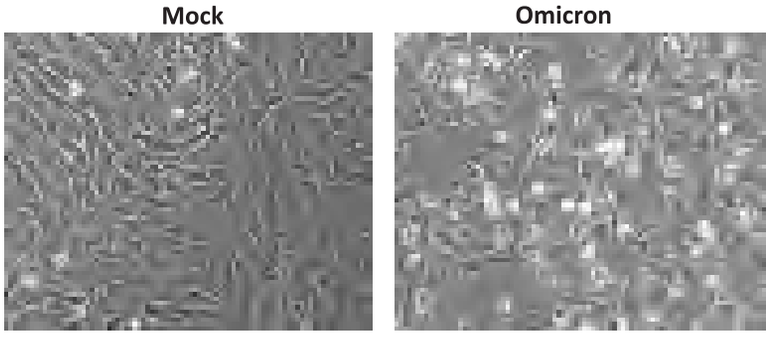
Studies on the miRNA response to LSD virus infection

Lumpy skin disease (LSD) was restricted to African continent for many decades. During the last few years, transcontinental spread of LSD has been observed in some European and several Asian countries. India experienced its first outbreak in 2019. Due to heavy economic losses, LSD has become the most important animal disease in India. LSD virus (LSDV) is poorly studied. The precise information on all the viral encoded factors and their interactions with cellular counterparts is lacking. miRNAs are 22-nucleotide long non-coding RNAs that can influence gene expression and hence regulate various aspects of host and pathogen biology. However, information on the miRNA response to LSDV infection is completely missing in the literature. In this study, miRNA profiling of cells infected with LSDV was conducted for the first time. As compared to the mock-infected cells, LSDV-infected primary lamb testicle (LT) cells revealed dysregulation of 64, 85, and 85 miRNAs at 12 hours post-infection (hpi), 48 hpi and 72 hpi, respectively, several of which were novel in nature (Fig.). While some of these miRNAs were found to be specifically dysregulated at a particular time point following LSDV infection, others were commonly dysregulated across all three time points. Furthermore, analysis of the differentially expressed miRNA–mRNA interaction

networks, Gene ontology analysis of the predicted targets and KEGG analysis of the highly enriched pathways revealed several cellular factors/pathways that may potentially regulate LSDV infection. Three of the commonly dysregulated miRNAs viz; miR-3959-3p and miR-432, and miR376e- 5p identified in miRNA profiling studies were further validated by quantitative real-time PCR (qRT-PCR) in LSDV infected Vero cells. In conclusion, several novel miRNAs and their cellular targets in LSDV-infected cells were identified for the first time in this study. Besides understanding virus replication, virus-host interactions and disease pathogenesis, these miRNAs and their cellular targets may serve as biomarkers and novel targets for therapeutic intervention against LSDV.



miRNA profiling of primary lamb testicle cells infected with LSD virus. Heatmap showing hierarchical cluster analysis of top dsyregulated miRNAs in mock-infected and LSDV-infected primary lamb testicle cells at various times post-infection. The color code on the heat nap is linear red as upregulated and yellow as downregulated miRNA expression. Each row represents a miRNA and each column represents a sample. Heat map plot was generated by R package based on log2FC.



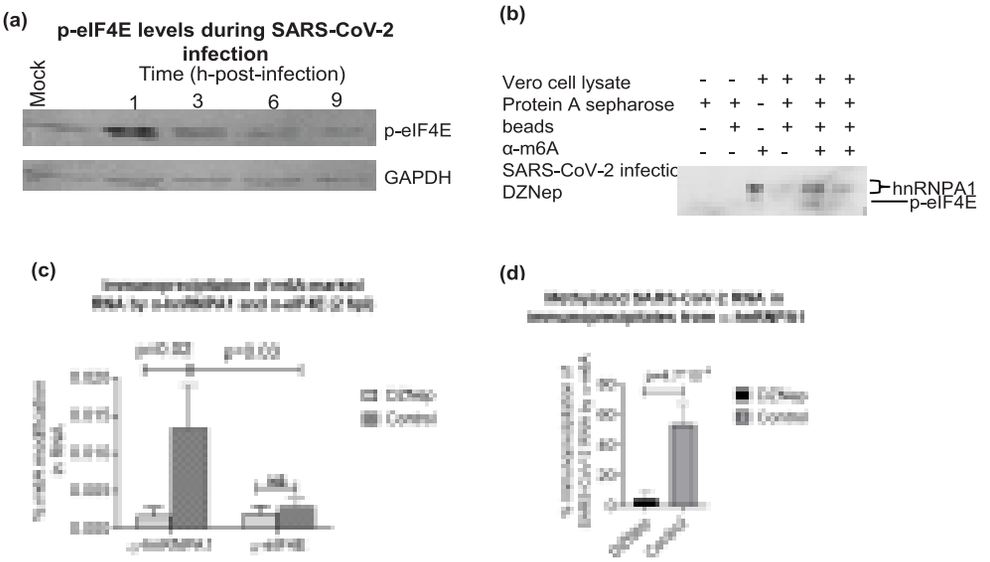
Isolation of SARS-CoV-2 Omicron from human patients. Samples were received from Maharaja Agarsen Medical College Agroha, Hisar, Haryana. Virus was isolated in Vero cells. Two isolates (VTCCAVA 336 and VTCCAVA337) deposited to the national repository of animal microbes (National Center for Veterinary Type Cultures, Hisar)

(Naveen Kumar & Baldev R Gulati)

Epitranscriptomic regulation of SARS-CoV-2 replication

We report the *in vitro* antiviral activity of DZNep (3-Deazaneplanocin A; an inhibitor of *S*-adenosylmethionine-dependent methyltransferase) against SARS-CoV-2, besides demonstrating its protective efficacy against lethal infection of infectious bronchitis virus (IBV, a member of the *Coronaviridae* family). DZNep treatment resulted in reduced synthesis of SARS-CoV-2 RNA and proteins without affecting other steps of viral life cycle. We demonstrated that deposition of N6-methyl adenosine (m6A) in SARS-CoV-2 RNA in the infected cells recruits heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), an RNA binding protein which serves as a m6A

reader. DZNep inhibited the recruitment of hnRNPA1 at m6A-modified SARS-CoV-2 RNA which eventually suppressed the synthesis of the viral genome (Fig.). In addition, m6A-marked RNA and hRNPA1 interaction was also shown to regulate early translation to replication switch of SARS-CoV-2 genome. Furthermore, abrogation of methylation by DZNep also resulted in defective synthesis of the 5' cap of viral RNA, thereby resulting in its failure to interact with eIF4E (a cap-binding protein), leading to a decreased synthesis of viral proteins. Most importantly, DZNep-resistant mutants could not be observed upon long-term sequential passage of SARS-CoV-2 in cell culture. In summary, we report the novel role of methylation in the life cycle of SARS-CoV-2 and propose that targeting the methylome using DZNep could be of significant therapeutic value against SARS-CoV-2 infection.



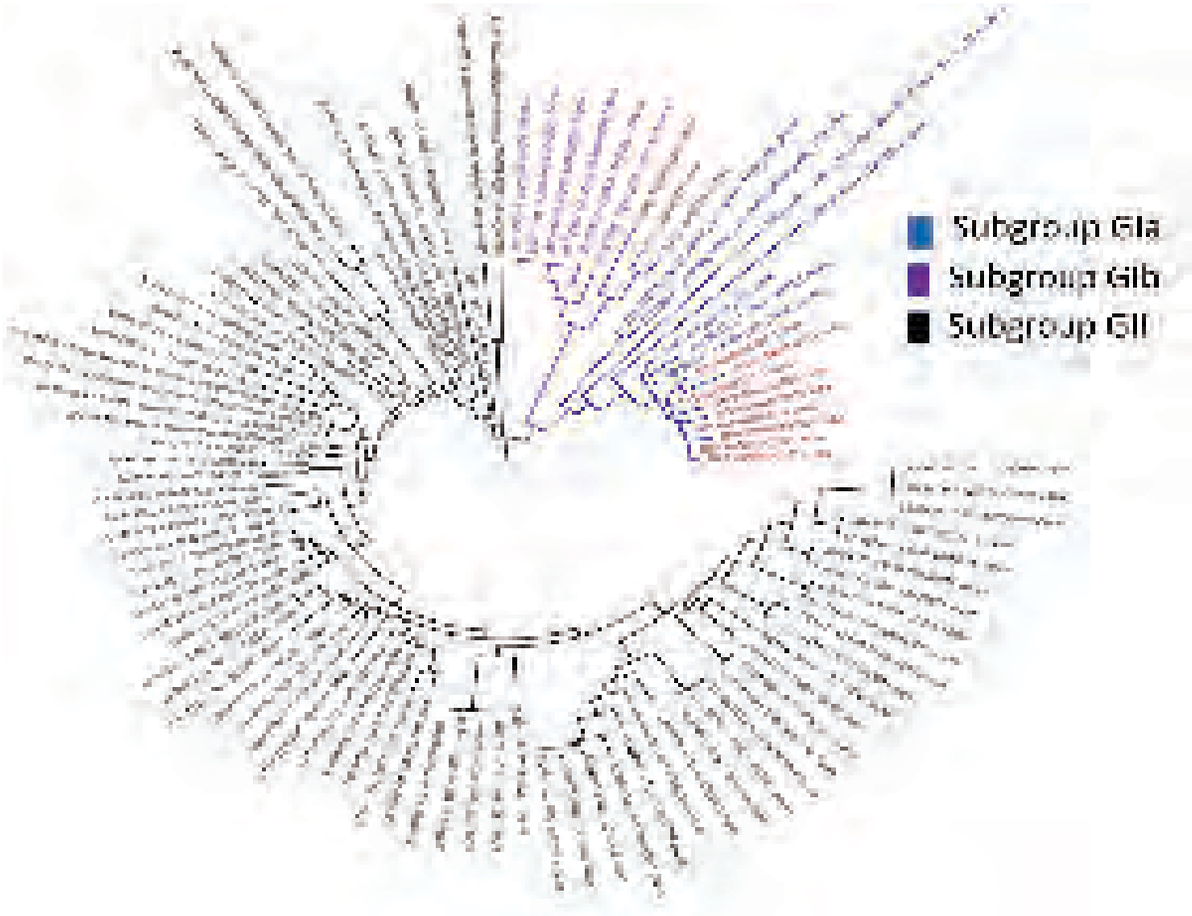
m6A modifications of viral RNA act as a molecular switch from translation to replication of SARS-CoV-2 RNA. ***(a) Kinetics of eIF4E activation in SARS-CoV-2-infected cells.*** Vero cells were infected with SARS-CoV-2 at MOI of 5 and the cell lysates were subjected to determination of the levels of p-eIF4E and GAPDH in a Western blot analysis. ***(b) Levels of α-hnRNPA1 and α-eIF4E in the cell lysate immunoprecipitated by α-m6A.*** Confluent monolayers of Vero cells were infected with SARS-CoV-2 at MOI of 5 for 1 h, followed by washing with PBS and the addition of fresh MEM having DZNep (1 µg/ml) or equivalent volume of DMSO. At 2 hpi cells were subjected to covalently cross-link proteins and nucleic acid for 10 min. The cells lysates and cytosolic fractions were prepared as described in materials and methods under CLIP assay. The cytosolic fraction was subjected to immunoprecipitation by α-m6A. Proteins (hnRNPA1 and eIF4E) interacting with m6A-marked-RNA were probed from the immunoprecipiate (protein-RNA complex) by Western blot analysis. ***(c) Levels of m6A-modifed RNA in the cell lysates (at 2 hpi) immunoprecipitated with α-hnRNPA1 and α-eIF4E.*** Confluent monolayers of Vero cells were infected with SARS-CoV-2 at MOI of 5 for 1h in the presence of DZNep (1 µg/ml) or equivalent volume of DMSO, followed by washing with PBS and the addition of fresh MEM having either DZNep or vehicle control. At 2 hpi, cells were subjected to covalent cross-linking. The cells lysates were then incubated with α-hnRNPA1 or α-eIF4E and the immunoprecipitates were subjected to determination of m6A methylome by EpiQuikTM m6A RNA Methylation Quantification Kit (Colorimetric). (d) ***Levels of m6A-modifed SARS-CoV-2 RNA.*** DZNep-treated or vehicle control-treated cells (at 2 hpi) were first immunprecipitated by α-hnRNPA1. The hnRNPA1-bound RNA (immunoprecipitate) was purified (Triazol) and again subjected to immunoprecipitation using α-m6A. The relative levels of m6A-modified SARS-CoV-2 RNA in the immunoprecipitate were quantified by qRT-PCR and expressed as % of input (RNA immunoprecipitated by α-hnRNPA1) SARS-CoV-2 RNA. Values are means ± SD and representative of the result of at least 3 independent experiments.

(Naveen Kumar, Sanjay Barua & Baldev R Gulati)

Studies on prevalence of coronaviruses in bovine and equine populations

For epidemiological studies of coronaviruses in different animals, sampling plans were finalized with the help of NIVEDI, Bengaluru. The sampling plan was based on Cluster sensitivity of 0.9, Design level prevalence of 0.3, Cluster Level prevalence of 0.03. The assays for detection of animal CoVs were standardized using different published primers and probes, with modifications. Whole genomes of 12 Bovine coronaviruses and 2 SARS-CoV-2 were conducted.

Out of 1309 bovine samples tested, SARS-CoV-2 was not detected in any sample, but bovine coronavirus was detected in 40 samples. Likewise, out of the 649 equine samples tested, SARS-CoV-2 was not detected in any sample, but equine coronavirus was detected in 2 samples. Bovine coronavirus was isolated (12 isolates) in NLBK cells and deposited to NLBK cells. Whole genome sequencing of 12 BCoVs was conducted in-house on Nanopore MinION. Nine viruses clustered with the classical Bovine corona viruses in global subgroup Gla (China, USA, Germany) whereas 3 viruses clustered with the subgroup Glb (France in 2017) (Fig.). This indicates co-circulation of two subgroups and also might be a recent independent introduction after 2017.



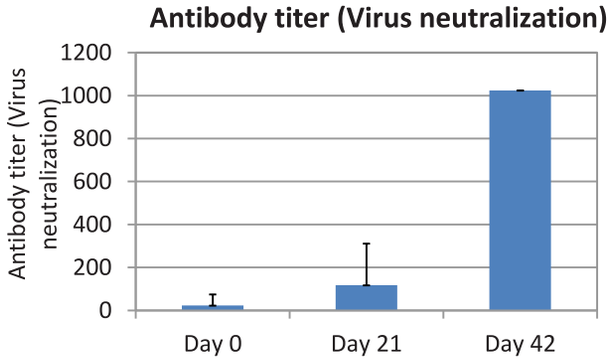
Phylogeny of Bovine coronaviruses. Nasal/fecal samples were collected from buffaloes and cows. WGS of 12 BCoVs was conducted in-house on Nanopore MinION. Nine viruses clustered with the classical Bovine corona viruses in global subgroup GIa (China, USA, Germany) whereas 3 viruses clustered with the subgroup GIb (France in 2017). This indicates co-circulation of two subgroups and also might be a recent independent introduction after 2017.

(Baldev R Gulati, Naveen Kumar, Riyesh T & Shanmugasundaram K)

Evaluation of the safety and immunogenicity of an inactivated SARS-CoV-2 vaccine in animals

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19 in humans has been shown to readily infect and occasionally cause death in cat, dog, deer, lions/tigers/leopards and minks. In India, SARS-CoV-2 Delta (identical to human Delta SARS-CoV-2 strains) infection has been reported in lions (); other pet animals (dog, cat) are also at high risk due to their close contact with human population. Jumping of SARS-CoV-2 from humans to animals might accelerate its evolution and hence affect surveillance and control strategies of COVID-19 in humans. Therefore, implementing effective risk management measures to prevent the transmission of SARS-CoV-2 from humans to animals and then back to humans is a major task of veterinary services.

We isolated the Delta variant of SARS-CoV-2 from a COVID-19 confirmed patient, followed by its genetic (whole genome sequencing) and antigenic characterization. The virus was sequentially passaged (n=10) until it produced sufficient viral titer in the Vero cells. The P10 SARS-CoV-2 (Delta) was used to prepare an inactivated (*β-Propiolactone* inactivated and aluminium hydroxide gel adjuvanted) vaccine termed Ancovax. The Ancovax was found to be absolutely safe and induced a potent neutralizing antibody- and cell-mediated immune response against SARS-CoV-2 in laboratory animals (mice and rabbits), dogs (Fig.), lions/tigers.



Immunogenicity of Ancovax in dogs. Six BSF dogs were immunized with Ancovax. Booster dose was injected at day 21 post-primary vaccination. Blood was collected at day 21 and day 42 post-primary immunization and antibody titers were determined by virus neutralization assay.

(Naveen Kumar, Sanjay Barua, Baldev R Gulati & Yash Pal)

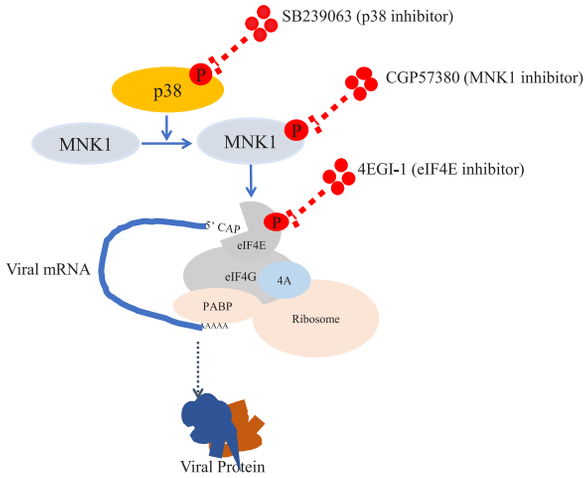
Role of p38 Map Kinase in Buffalopox virus replication

Buffalopox is an emerging viral zoonosis. The prophylactic and therapeutic agents that can be used to control buffalopox are lacking. Some directly acting poxvirus inhibitors have been described but these are prone to induce the generation of drug-resistant mutants, besides their potential carcinogenic effects. By screening a library of small molecule chemical inhibitors, the p38 mitogen-activated protein kinase (p38 MAPK) inhibitor SB239063 and SB203580 were identified as potential hits that resulted in reduced BPXV replication *in vitro,* as evidenced by reduction in viral mRNA / protein synthesis, genome copy numbers and progeny virus particles. The p38 is a serine / threonine protein kinase which transduces signals from inflammation stimuli. BPXV infection was shown to induce biphasic activation of p38 at 2-4 hpi and 20-24 hpi, and inhibition of p38-MAPK suppresses buffalopox virus (BPXV) protein synthesis by targeting p38-MNK1-eIF4E signaling.

To provide insights into the evolution of drug resistance, the resistant mutants by long-term sequential passages (P; n=60) in the presence of p38 inhibitor (SB239063) were selected. The P60-SB239063 virus exhibited significant resistance to SB239063 as compared to the P60-Control virus. To provide mechanistic insights on the acquisition of resistance by BPXV-P60-SB239063, the p38-α and p38-ϒ (isoforms of p38) knockout Vero cells were generated by CRISPR/Cas9-mediated genome editing. It was demonstrated that unlike the WT virus which is dependent on p38-α isoform, the resistant virus (BPXV-P60-SB239063) switches over to use p38-ϒ to efficiently replicate in the target cells. This is rare evidence wherein a virus was shown to bypass the dependency on a critical cellular factor under selective pressure of a drug.

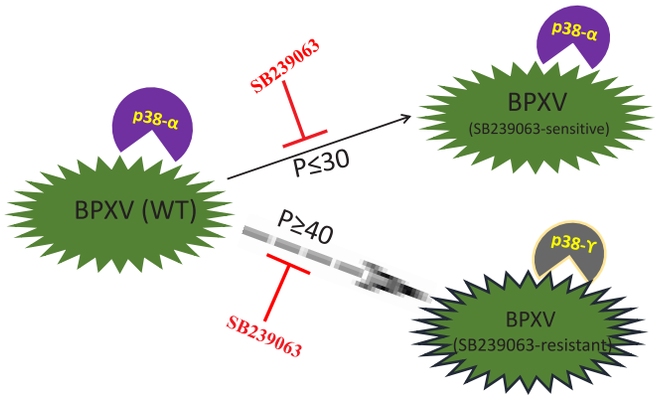
To conclude, p38-α serves as a critical cellular factor for the synthesis of BPXV proteins and may serve as a novel target for antiviral drug development against buffalopox. BPXV does not easily select SB239063-resistant mutant. However, long-term selective pressure of SB239063 allows the virus to switch over to use p38-ϒ, so as to become resistant against the p38-α inhibitor.

p38-MNK1-eIF4E signaling axis regulates translation of BPXV proteins.



Disruption of p38/MNK1/eIF4*E* signaling by chemical inhibitors results in reduced synthesis of viral proteins, and thereby may serve as a target for antiviral drug development.

BPXV switches to use p38-ϒ under long-term selective pressure of p38-α inhibitor



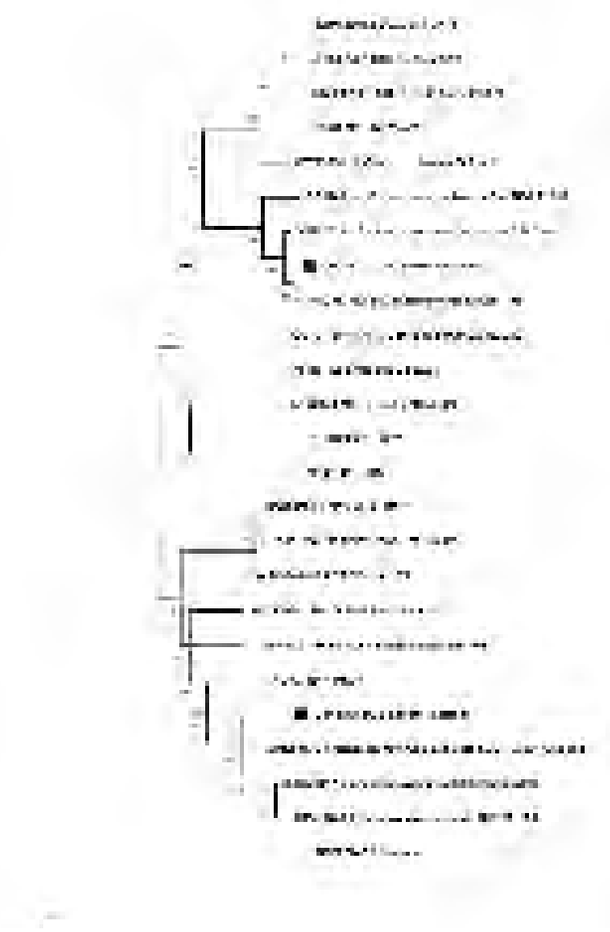
Long-term sequential passage ($P \geq 40$) of BPXV in Vero cells in the presence of p38-α inhibitor (SB239063) induces generation of viral mutants that preferentially utilize p38-ϒ instead of p38-α, thereby becoming resistant against the targeting agent (SB239063).

(Sanjay Barua & Naveen Kumar)

Detection of alpha coronavirus and beta coronavirus from bat guano samples

Bats are the second most diverse mammals on earth after rodents, comprising approximately 22% of all named mammal species. India has a diverse population of bats with recorded 117 species of bats belonging to 39 genera under eight families. Except few studies on *Pteropus* and *Rousettus* bats, no systematic studies have been carried out to assess the viral diversity in bat population in India. Bats' nocturnal activity and their powered flight make it difficult to study bats and their infectious agents under field conditions. Hence, the present study was undertaken to explore the virus diversity in the guano samples of bats from Rajasthan and Haryana. In this regard, during the year we collected a total of 219 guano samples from different bat roosts located at various places in Haryana and Rajasthan states. Overnight fecal/urine samples were collected by a non-invasive method by spreading polythene sheets near bat roosts. Both insectivorous and frugivorous bats were targeted in sample collection (samples were processed separately). Out of 219 samples, 162 were from fruit bats and 57 samples were from insectivorous bats. All these samples (a pool of 5 samples were made) were tested for Coronavirus, Rotavirus, Reovirus, Paramyxovirus and Adenoviruses. Five samples collected from insectivorous bats from

Bikaner, Rajasthan and two pooled samples collected from frugivorous bats from Panchkula, Haryana were PCR positive for coronavirus by spike gene-based PCR using pancorona primers (The samples were negative for all the other four viruses tested). The amplified PCR products were sequenced commercially to identify the virus. Sequence and phylogentic analysis revealed that the samples from insectivorous bats from Bikaner, Rajasthan shared the highest similarity with alphacoronavirus and is closely related to a batvirus (Bat180/Eswatini/2014) from the USA, whereas samples from frugivorous bats from Panchkula, Haryana shared highest similarity with betacoronavirus of bat origin and was closely related to a batvirus from Thailand (BtCoV/B55440/Pte_lyl/CB1-THA) (Fig.). This study provides insights into the presence of various coronaviruses in bats in northern India.



Phylogenetic analysis of coronaviruses from bats based on partial spike gene (S) sequences: This analysis involved 25 nucleotide sequences. The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary distances were computed using the Maximum Composite Likelihood method (in the units of the number of base substitutions per site). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

(Riyesh T, Shanmugasundaram K, Tirumala Rao Talluri, Rajesh K Vaid, Naveen Kumar, Sanjay Barua, Yash Pal & TK Bhattacharjya)

Development of repository of viruses of livestock

The respiratory diseases in livestock species cause significant health problem leading to huge economic losses. The spectrum of respiratory pathogens, especially viral agents circulating in different livestock species has not been thoroughly explored. Further, repository of respiratory viruses of livestock with long term storage of authenticated viruses will be a ready-made asset for the scientific community to explore these biological resources for future development of diagnostics, immunobiologicals, as reference material, comparative biological studies, etc. In this direction, the project was formulated to develop repository of respiratory viruses of different livestock species. The biological samples were collected from sheep and goats for identification and isolation of respiratory

viruses. The biological samples (Nasal swabs: 111 nos) were collected from goats (77 nos) and sheep (34 nos) from villages like Panihari (48 nos), Dandoor (24 nos) and local meat shops in Hisar (39 nos). The nasal swab samples were collected in viral transport media and transported to laboratory for testing for molecular detection of respiratory viruses. The viral nucleic acids (DNA and RNA) were isolated from nasal swabs using commercial kits. The nucleic acids were subjected to identification of targeted viruses viz., BPIV3 and BRSV by RT-PCR. None of the tested samples were positive for respiratory viruses.

(BC Bera, Taruna Anand, Nitin Virmani & Baldev R Gulati)

Development of isothermal "Recombinase Polymerase Amplification" (RPA) based assays for detection of Porcine circovirus 3 (PCV3) in pigs

The Porcine circovirus 3 (PCV3) is a recently identified emerging virus causing reproductive failure in pigs. The reproductive failure and piglet mortality are being reported in pig which incurs huge economic losses in swine industry. To overcome such important disease conditions, there is need to implement measures for identification, prevention and control to improve the economic status and sustainability of pig owners. This warrants the development of new generation diagnostics for early, rapid, and specific diagnosis of the disease. In this direction, the isothermal recombinase polymerase amplification (RPA) assays are being standardized for development point-of-care diagnostics for detection of PCV3. The RPA assays are more specific, highly sensitive and require less laboratory equipments to be used at field setup. Different sets of primers were designed targeting the conserved regions of PCV3 genome and standardized the RPA assay using plasmids of cloned genome of PCV3. The RPA assay was standardized using specific reagents from TwistDX, UK. The RPA amplicons were detected in gel electrophoresis. The results indicated that the designed primer sets were successfully amplified in the targeted regions of PCV3 virus. For estimation of detection limit of the assay, the cloned plasmid was serial diluted and subjected to RPA assay. The developed assay is capable of detecting fg DNA concentration. The specificity of the RPA assay was evaluated against closely related virus-PCV2 and the assay didn't show any cross reactivity.

(BC Bera, Taruna Anand & Nitin Virmani)

Development of vaccine candidates for SARS-CoV-2 using mRNA based platform

The aim of the project is to develop self-replicating mRNA vaccine candidates targeting different antigens of SARS-CoV-2. Different mRNA vaccine constructs were designed, synthesized codon optimized construct, cloned into self-replicating machinery and produced in-vitro transcribed RNA of the constructs. The good quality IVT-mRNA was synthesized and confirmed in RNA gel electrophoresis. Subsequently, the mRNA construct of one antigen was formulated as liposome by encapsulation of the IVT mRNA in lipid mixture. Different lipids in various molar ratios were prepared and mixed with mRNA. Then the lipid-mRNA mix was passed through extruder to prepare nanosize liposome. The prepared LNPs were dialyzed and the size of the LNPs was estimated using Zetasizer. The result indicated formation of nano size particle of the liposome preparation. Subsequently, the encapsulation efficiency was evaluated using Ribogreen assay and observed higher encapsulation efficiency. The morphology of the liposomes of mRNA construct was analyzed by transmission electron microscopy (TEM). The TEM image showed liposomes are globular and well separated nanoparticles.

(BC Bera, Taruna Anand & Nitin Virmani)

Strengthening of bacteriophage repository with bacteriophages against pathogenic bacteria and assessment of their synergy with antibiotics

In the current investigation, the isolation and characterization of lytic bacteriophages against ESKAPE group of pathogens was carried out and the assessment of their synergistic effects with antibiotics was also performed. Prior to the isolation of bacteriophages, various bacterial strains of ESKAPE pathogens were isolated, identified and characterized for their antimicrobial susceptibility profiles and these strains were used as hosts to enrich the bacteriophages from diverse environmental or clinical samples. A total of 15 (15.15 %) out of 99 strains of ESKAPE pathogens used in this study were found to be extensively drug resistant (XDR) and 72 (72.7 %) were classified as multiple drug resistant (MDR). Out of the total 75 samples screened for bacteriophage isolation, only

15% yielded bacteriophages against ESKAPE pathogens. These mainly included samples from hospital and community sewage. There was a considerable difference in the bacteriophage distribution frequency with phages of *P. aeruginosa* being the most abundant (36.1 %) and those of *E. faecium* being the least abundant (2.7 %). Molecular, proteomic, and lytic profiles of bacteriophages were also screened to assess their lytic potential by spot assay & efficiency of plating (EOP) assay.

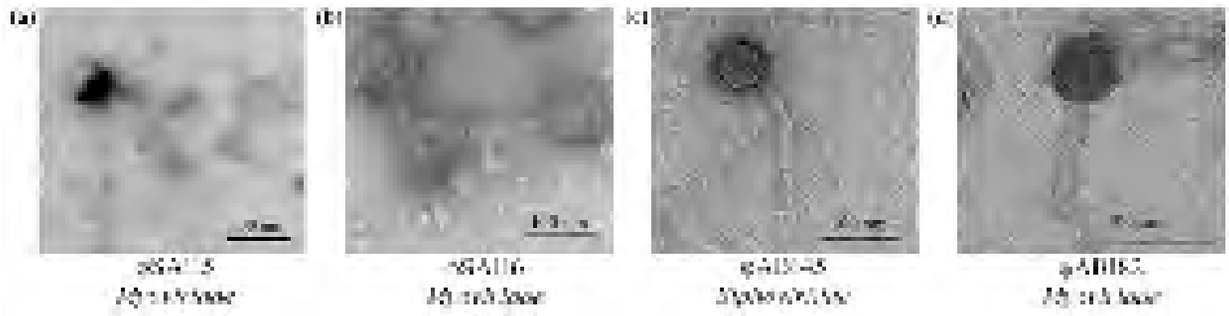
A total of 36 different bacteriophages were isolated and characterized *in vitro* for their structural properties. *A. baumannii* phage ϕAB181 and *K. pneumoniae* phage ϕKP207 were highly thermotolerant and were stable up to 80°C. *A. baumannii* phage ϕAB181 was also found to be stable at extreme acidic pH2. *E. faecium* phage ϕEF283 had the smallest burst size of 62 progeny virions per infected cell, and *S. aureus* phage ϕSA114 & *A. baumannii* phage ϕAB182 had the largest burst sizes of 290 and 287, respectively. *A. baumannii* phage ϕAB182 had the shortest eclipse and latent period of 9 min and *A.baumannii* phage ϕAB181, *K. pneumoniae* phage ϕKP205, and *P. aeruginosa* phage ϕPA180 had the longest latent periods of ~35 min each. Most of the phages isolated against ESKAPE pathogens belonged to the family *Myoviridae* (50 %), followed by *Podoviridae* (33.3 %) and *Siphoviridae* (16.6 %) Fig. *A. baumannii* phage ϕAB181 formed plaques with a prominent halo zone representing bacterial exopolysaccharide degradation which kept on increasing up to 50 days of observation upon incubation at 37°C. Based on diversity analysis by amplification of major capsid protein gene (*g23*), a total 8 out of 36 bacteriophages were classified as T4-like myophages. Phylogenetic analysis of *g23* gene amplicon of *A. baumannii* phage ϕAB182 with other related phages revealed it to be closely related with another *A. baumannii* lytic phage Acj9. Based on spot assay *E. aerogenes* phages ϕEF196, ϕEF198, ϕEF199 and *A. baumannii* phage AB181 were found to have narrow spectrum of lysis and *P. aeruginosa* phages ϕPA170, ϕPA172, ϕPA180, ϕPA176 and *A. baumannii* phage ϕAB182 had broad spectrum of lysis. EOP assay was found to present a better analysis of the lytic profiles of bacteriophages than spot assay. *K. pneumoniae* phages ϕKP194, ϕKP195 and *P. aeruginosa* phage ϕPA180 consistently established a highly productive lysis (EOP > 0.5) in most cases of successful infections.

Assessment of bacteriophages synergy with antibiotics : After the most suitable bacteriophages were selected and further tested for their synergistic effects with routinely used antibiotics. Eight bacteriophages against four different bacterial species of ESKAPE group were tested with an array of antibiotics using disc embedded double agar overlay method, time kill curve assays after determining respective minimal inhibitory concentrations and optimal multiplicities of infection. Synergy between phages and antibiotics was also tested for suppression of resistance development using mutant frequency assay.

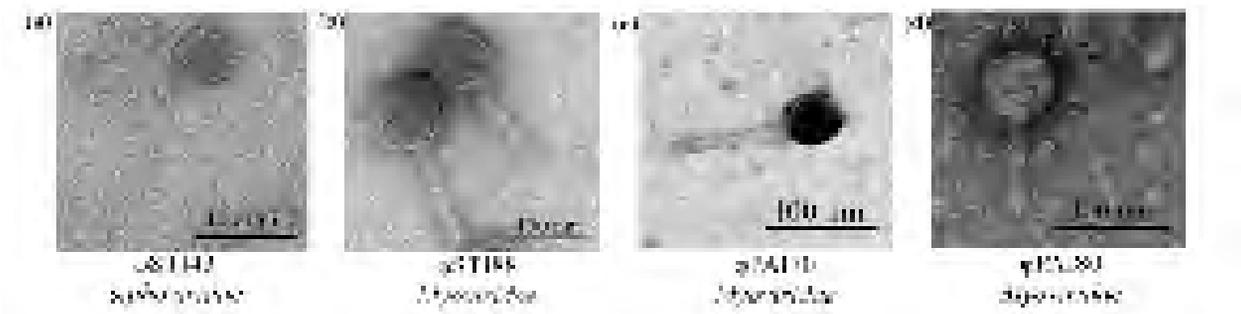
S. aureus phages ϕSA115 ($p<0.0001$) and ϕSA116 ($p<0.0001$) showed highest synergy with β-lactam antibiotic cephalothin at 1/2 of MIC value. *K. pneumoniae* phage ϕKP202 demonstrated highest synergy with cefotaxime ($p<0.001$) and ceftazidime ($p<0.001$); and phage ϕKP205 demonstrated highest synergy with aztreonam ($p<0.0001$), followed by ceftazidime ($p<0.0001$) with 1/4 of MIC values. *A. baumannii* phage ϕAB182 demonstrated highest synergy with colistin ($p<0.0001$), followed by ceftazidime ($p<0.0001$), polymyxin ($p<0.001$), and cefotaxime ($p<0.001$) at 1/4 of respective MIC values in time kill assays. *P. aeruginosa* phage ϕPA176 demonstrated highest synergy with aztreonam ($p<0.0001$) and piperacillin ($p<0.0001$); ϕPA180 also demonstrated highest synergy with aztreonam ($p<0.0001$) and piperacillin ($p<0.0001$) at 1/4 of respective MIC values. Bacteriophages ϕAB182, ϕPA176 and ϕPA180 re-sensitized the antibiotics ceftazidime, aztreonam and piperacillin by lowering their MICs (2 - 4-fold) from resistant limits to susceptible limits. PAS was observed mainly with the bacterial cell wall or membrane targeting antibiotics whereas antibiotics that act on bacterial ribosomal machinery showed antagonism with bacteriophages. Combined application of phages with antibiotics was able to suppress the emergence of phage or antibiotic resistant mutants. *P. aeruginosa* phage ϕPA180 demonstrated strongest suppression of resistance evolution.

Further few selected bacteriophages were tested individually and along with antibiotics for removal of mono- or muti-species biofilms employing qualitative assessment with tube method and quantitatively with microtitre plate method and scanning electron microscopy. Some bacteria were strong biofilm producers such as *P. aeruginosa, K. pneumoniae* and *A. baumannii* strains. As visualized by FE-SEM analysis, bacteriophages ϕKP205, ϕPA180, ϕAB181 and AB182 eradicated/degraded the 48 hr old biofilms respectively of *K. pneumoniae*, *P.*

aeruginosa, and *A. baumannii* within only 12 hr of treatment duration. A strong halo producing phage ϕAB182 successfully eradicated the resilient multi-layered biofilms of hypermucoid *A. baumannii* (Fo-53-5BP lab strain). The combined application of bacteriophage cocktail with colistin successfully eradicated the 48 hr old mixed species (*A. baumannii*, *P. aeruginosa* and *K. pneumoniae*) biofilm more efficiently as compared to the individual treatments and *A. baumannii* phage ϕAB182 significantly eradicated 48 hr old biofilm of MDR *A. baumannii* VTCCBAA1084 in combined application with colistin, polymyxin B, ceftazidime and cefotaxime. Based on the inferences of the present investigation, it can be concluded that preliminary characterization of bacteriophages is crucial for selection of the best candidates for successful therapeutic outcome.

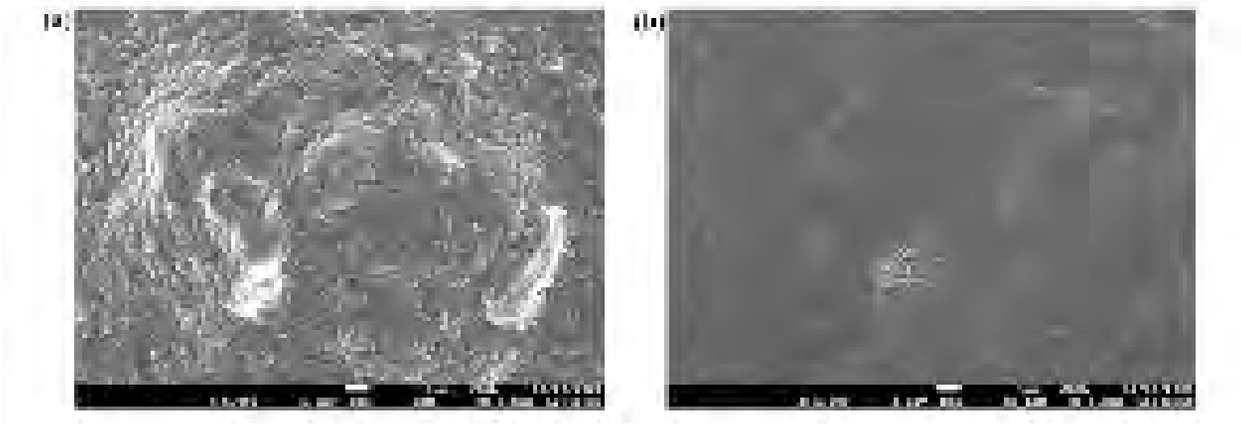


Transmission electron micrographs of (a) ϕSA115 (b) SA116, (c) ϕAB145 and (d) ϕAB182. Phages were negatively stained with 2% uranyl acetate and visualized using an accelerating voltage of 80 kV.

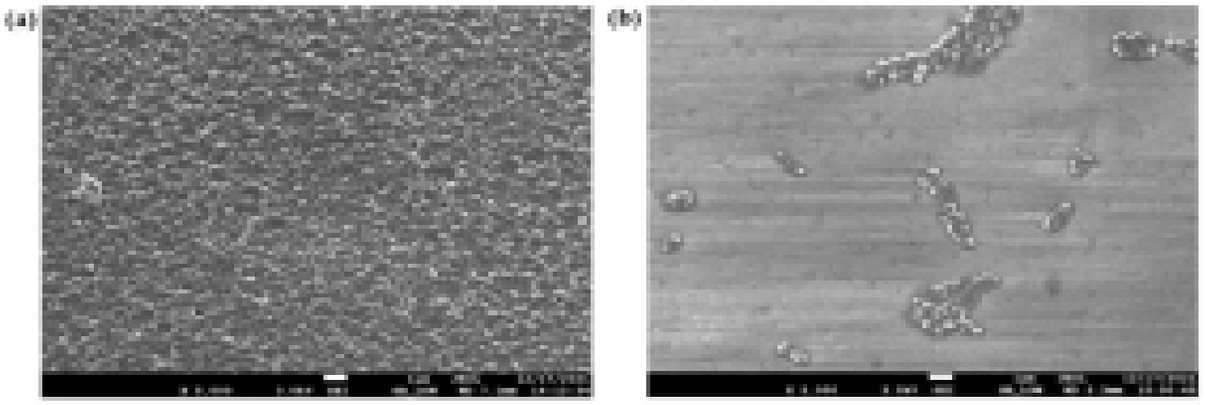


Transmission electron micrographs of (a) ϕST143 (b) ST188, (c) ϕPA170 and (d) ϕPA176. Phages were negatively staind with 2% uranyl acetate and visualized using an accelerating voltage of 80 kV.

A cocktail of four phages of *P. aeruginosa* (ϕPA170 + ϕPA172 + ϕPA177 + ϕPA180) was formulated and it was able to significantly eradicate 4-day old biofilm of XDR *P. aeruginosa* strain VTCCBAA1047 (Fig.).



Eradication of XDR *P. aeruginosa* biofilm on urinary catheter as visualized using Field Emission Scanning Electron Microscopy (FE-SEM). (a) 4 day old biofilm of XDR *P. aeruginosa* strain VTCCBAA1047 on inner walls of urinary catheter with multi-layered protrusions of biofilm cells (5000 X), and (b) eradicated biofilm of *P. aeruginosa* by cocktail of phages ϕPA170 + ϕPA172 + ϕPA177 + ϕPA180 (5000 X)



Eradication of XDR *P. aeruginosa* biofilm on glass cover slips as visualized using FE-SEM. (a) 4 days old biofilm of XDR *P. aeruginosa* strain VTCCBAA1047 on borosilicate glass coverslips. Tightly packed cells of *P. aeruginosa* in monolayer can be seen (5000 X); and (b) eradicated biofilm of *P. aeruginosa* by cocktail of phages ϕPA170 + ϕPA172 + ϕPA177 + ϕPA180 (5000 X).

Isolation and characterization of bacteriophages against *Aeromonas* species : Potential bacteriophages were isolated against *Aeromonas* species from the fishponds. These phages were targeted against *Aeromonas* sp., *Stenotrophomonas maltophilia* and *Pseudomonas* sp. Phages against aeromonads comprised of *A. veronii, A. hydrophila, A. caviae, A. jandaei* and other *Aeromonas* spp. The SDS-PAGE based protein profile of whole phage particles, Restriction endonuclease digestion pattern and RAPD amplification pattern of phage nucleic acids revealed that all phages were distinct. Transmission electron micrographs (TEM) showed that 12 Aeromonas phages belong to *Myoviridae* family, and 1 phage (ϕAq12) belongs to *Podoviridae* family. All the phages were characterized by the following parameters: Temperature: activity range 4-60°C ; pH: activity range 3-10; Stability assays using chloroform conc.:5-30% and bile salts:1% and 2%; One-step growth curve analysis and host range. The biological characterization and experimental findings on the isolated phages showed their specificity for several bacterial isolates. This will be helpful in the logical selection of bacteriophages for use in phage therapy in aquaculture. The phages can be used for phage therapy, as they show a broad biological spectrum and wide stability range for potential bactericidal approaches.

Isolation and characterization of bacteriophages against ESBL strains of *E. coli* and *Salmonella* spp. : ESBL strains of *E. coli* and *Salmonella* sp. from poultry origin were isolated and characterized for their antibiotic resistance profile. Using these strains as hosts, a total of 17 bacteriophages against *Salmonella* sp. (BPA215, BPA216, BPA217, BPA218, BPA219, BPA229, BPA274, BPA277, BPA278, BPA280, BPA281, BPA282, BPA284, BPA285, BPA318, BPA319, BPA320) and 29 bacteriophages against *E. coli* (BPA211, BPA212, BPA213, BPA214, BPA221, BPA271, BPA272, BPA273, BPA275, BPA276, BPA279, BPA308, BPA309, BPA310, BPA311, BPA312, BPA314, BPA315, BPA316, BPA317, BPA321, BPA322, BPA323, BPA324, BPA325, BPA326, BPA327, BPA328, and BPA329) were isolated and characterized using temperature profiling, pH stability. These phages were also tested for their lytic spectrum and lytic productivity using spot assay and EOP assay. Potential candidates of bacteriophages were selected based on the above-described assays and were characterized genomically. Whole genome sequencing was performed, and sequences were assembled using hybrid assembly. Phylogenetic analysis of phage BPA310 infecting *E. coli* demonstrated it to be a novel phage and phage BPA219 of *Salmonella* was found to be like Salmonella phage NINP13076 which belongs to family Caudoviricetes; Vequintavirinae; Seunavirus. Phage selection is an essential criterion for application in therapy. The current research was focused on genomic characterization of potential pahge candidates to select appropriate ones for further application in therapy on poultry farms.

(Taruna Anand, Nitin Virmani, Rajesh K Vaid & BC Bera)

Authentication and accessioning of bacteria

During the year of 2022, nearly 200 cultures were processed, out of which 138 cultures were accessioned in the bacterial repository which has led to total strength of bacterial culture collection to 1721 veterinary bacteria. Cultures were mainly submitted from IVRI, Izatnagar; College of Veterinary Sciences, Palampur; CSWRI,

Avikanagar; College of Veterinary Sciences, Durg, and NRCC, Bikaner, apart from NCVTC Bacteriology laboratory, however most accessions during this period were from IVRI, Izatnagar. Besides this, filed sample lots were received comprising of pathological/other samples submitted/collected at NCVTC bacteriology laboratory from different species of animals viz., equines–nasal swab, PM samples, organ tissues, stomach contents, foal lung sample, uterus swab, fecal sample): 31 isolates; rabbit disease outbreak samples : 4 isolates; Pig nasal swabs, tissue samples from Rajasthan: 9 isolates; donkey/mare milk (61 isolates), and bat samples were processed for bacterial isolation and a total of 218 bacteria were isolated and cryo-preserved in the repository under the general category of preservation.

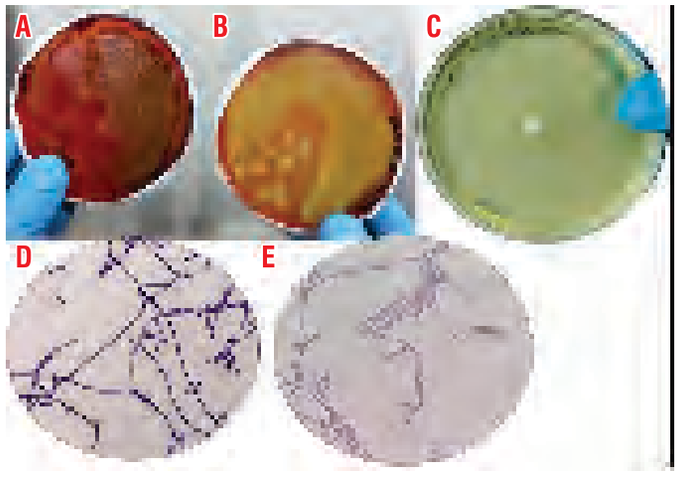
Some of the significant accessioned cultures are *Moraxella canis, M. ovis, Pasteurella canis, Edwarsiella tarda, Acinetobacter baumanii, Elizabethkingia anopheles, Bordetella parapertussis, B. avium, Streptococcus uberis, St. dysgalactiae* ssp*. dysgalactiae, St. suis, St. gallinaceous, Brevibacillus borstelensis, Coryebacterium faecalis, Coryne. hansanii, C. liphophiloflavum, Pediococcus acidilacti, Lysinibacillus macroides,* and *Gallibacterium anatis*. The Anaerobic culture of *Dermatophilus congolensis* was also accessioned. A total of 20 cultures of *Pasteurella multocida* belonging to Serotype F3, A3 and B, isolated from cattle; sheep, buffalo, quail, and chicken have also been accessioned.

The total of 138 bacterial accessions were distributed among the 23 taxa belonging to following genera: *Moraxella* spp., *Enterococcus* spp., *Serratia* spp., *Edwardsiella* spp., *Acinetobacter* spp., *Bacillus* spp., *Elizabethkingia* spp., *Klebsiella* spp., *Bordetella* spp., *Brevibacillus* spp., *Streptococcus* spp., *Proteus* spp., *Staphylococcus* spp., *Corynebacterium* spp., *Pediococcus* spp., *Escherichia* spp., *Pasteurella* spp., *Lysinibacillus* spp. , *Pseudomonas* spp. , *Gallibacterium* spp. and *Dermatophilus* spp., have been cryopreserved, which is a significant addition to NCVTC in 2022.

(RK Vaid, Taruna Anand, BC Bera, Riyesh T & Shanmugasundaram K)

DBT Network Programme on Anthrax Diagnosis and Control in India

Anthrax is an important zoonotic disease endemic in Southeastern States of India, which causes losses in livestock, wild animals, and occasional outbreak of cutaneous and intestinal anthrax in humans due to handling or consumption of tainted animal products in remote areas. The work on collection, and phenotypic and genotypic characterization of cultures started in BSL III laboratory after due regulatory approval from IBSC, followed by DBT and RGGM/IBKP application approval received (IBKP/TAI No C100511). The main aim of project is establishment of Culture Collection of *Bacillus anthracis* cultures for posterity for which culture receipt and processing, primary protocol has been established. Since these are category 'A' infectious agents, the UN2814 certified biosafety containers are used for transportation. A biosafety drill was performed on *Bacillus cereus* cultures to test and approve the standard operating procedure including receipt of cultures, their examination, storage, registration, temporary storage, culturing on media, study of phenotypic characters like staining procedure, DNA extraction, followed by proper discard and decontamination of media plates/broth.



Bacillus cereus **cultures phenotypic characterization under biosafety drill (A, B)** ***B. cereus*** **colonies on sheep blood agar, (C) Penicillin susceptibility test, (D) Gram-staining, (E) Schaeffer and Fulton Spore Staining.**

In the biosafety drill, *Bacillus cereus* cultures, which are clubbed with *B. anthracis* as common genomospecies, were used. The cultures were streaked for their properties on Sheep Blood Agar (SBA), and staining was performed by various methods to study cell morphology. Penicillin sensitivity was also ascertained. The focus of the drill was to examine procedures in order to avoid spillage and splashing of culture media and ease of performing various tests.

Interestingly, while the *B. cereus* were expectedly ß- hemolytic on SBA (VTCCBAA 182, 443, 567, 737) one strain (VTCCBAA692) was weakly-hemolytic on SBA (Fig.). Moreover, it formed typical morphology of *B. anthracis* on observation after staining, in which it forms Gram-positive rods as tram car arrangement. As part of project, *Bacillus* spp. cultures [*Bacillus paramycoides* (VTCCBAA1525); *Bacillus thuringiensis* (BAA1527); *Bacillus cereus* (BAA1538); *Bacillus licheniformis* (BAA1055); *Bacillus subtilis* (BAA1060; BAA183); *Bacillus mycoides* (BAA479) and *Bacillus paralicheniformis* (BAA5)] were distributed to project component unit ICAR-National Research Centre on Meat, Hyderabad on the behalf of this project for use as negative controls.

(RK Vaid, BC Bera & Shanmugasundaram K)

Isolation and identification of pathogenic bacteria from wetland wild avians

Migratory and resident wild wetland birds are important reservoirs and spreaders of zoonotic and antimicrobial-resistant (AMR) bacteria. The migratory wild birds constitute heavy traffic, as nearly 5 billion individuals fly across continents biannually. This large migration is a potential source of the global transfer of several pathogens. Fecal samples of various wild avifauna were obtained from area of Basai wetlands in Gurgaon district (Haryana) (Fig.). Out of these samples, after subjecting them to isolation protocol, 75 cultures were isolated. Apart from phenotypic characterization, these were subjected to 16S rRNA PCR product sequencing. Isolates with complete sequences (72) were subjected to BLASTn homology search. As a result, 26 distinct species belonging to 15 different genera were identified as following: *Acinetobacter venetianus* (1), *Aeromonas caviae* (1), *Aeromonas enteropelogenes* (1), *Aeromonas rivipollensis* (2), *Aeromonas veronii* (12), Atypical *Eschrichia* sp. close to *Eschrichia ruysiae* (6), Atypical *Escherichia* sp. close to *Shigella flexnerii* (1), Atypical *Pseudomonas* spp. close to *Pseudomonas peli* (1), *Eschrichia albertii* (1), *Eschrichia fergusonii* (10), *Enterobacter hormaechei* (1), *Enterococcus columbae* (5), *Enterococcus faecalis* (3), *Enterococcus hirae* (4), *Exiguobacterium indicum* (7), *Klebsiella aerogenes* (1), *Klebsiella pneumoniae* (1), *Klebsiella quasipneumoniae* (1), *Lactococcus lactis* (1), *Macrococcus canis* (1), *Plesiomonas shigelloide*s (1), *Priestia aryabhattai* (1), *Pseudomonas juntendi* (1), *Pseudomonas oleovorans* (1), *Shigella flexnerii* (6) and *Staphylococcus gallinarum* (1). Highest bacterial diversity was observed in samples of Common Coot (*Fulica atra*) harbouring 10 distinct species which included *Acinetobacter venetianus*, *Aeromonas enteropelogenes, Aeromonas veronii,* Atypical *Eschrichia* sp. close to *Eschrichia ruysiae, Enterobacter hormaechei, Enterococcus hirae, Klebsiella aerogenes, Klebsiella pneumonia, Klebsiella quasipneumoniae* and *Staphylococcus gallinarum.* Nine distinct species were obtained from samples of Common Moorhen (*Gallinula chloropus*). In samples of purple moorhen (*Porphyrio porphyrio*), seven distinct species of genera *Escherichia* sp. *Enterococcus, Lactococcus lactis* and *Priestia aryabhattai* were identified. Five distinct species from samples of Red wattled lapwing (*Vanellus indicus*) and White breasted waterhen (*Amaurornis phoenicurus*) were individually obtained. Red wattled lapwing was found harbouring bacteria of genera *Aeromonas* and *Pseudomonas*. Genera of bacteria *Escherichia* spp. *Shigella , Enterococcus,* and *Exiguobacterium* were present in White Breasted Waterhen samples.

Water Birds in and around Basai Wetlands (Gurgaon)

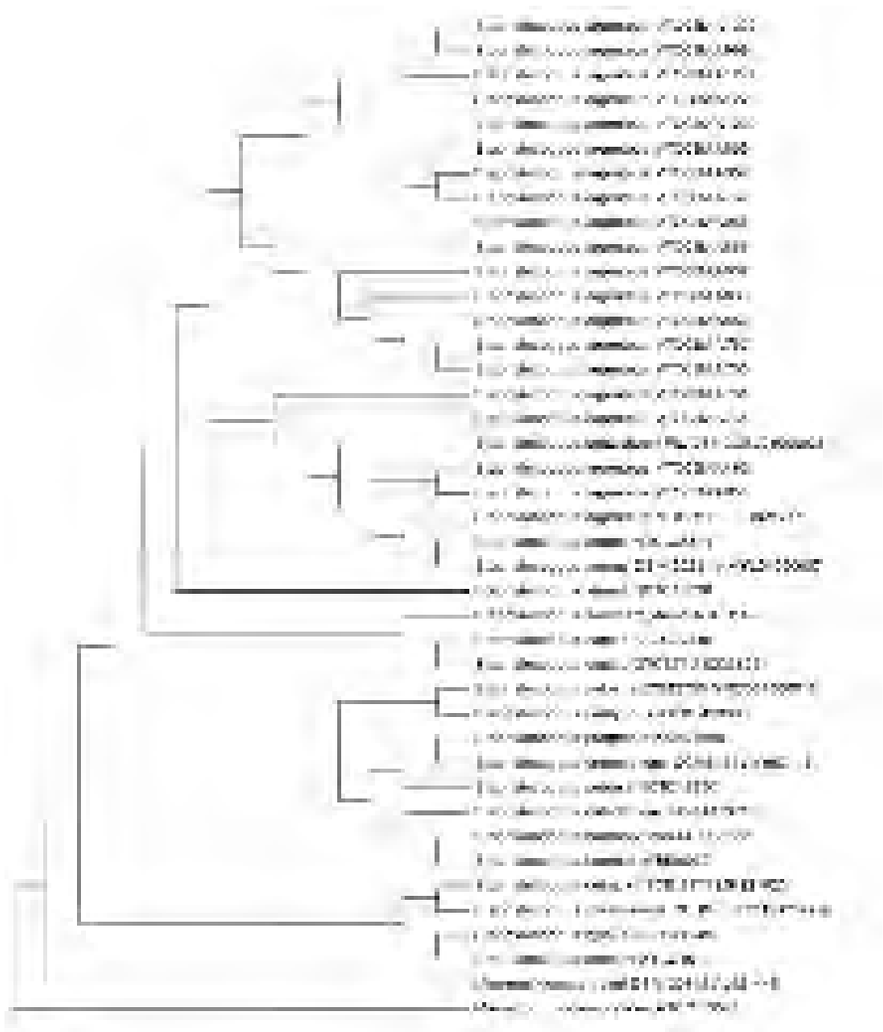
Purple swamp-hen | White breasted water hen | Red wattled lapwing | Common coot

Various avifauna which were sampled in Basai wetlands for bacterial species

(Priya & RK Vaid)

Identification and differentiation of *Staphylococcus aureus* cultures into *S. aureus* and *Staphylococcus argenteus* strains

Members of genera *Staphylococcus* spp. are one of the most important veterinary bacterial pathogens, as they can cause a variety of infections and diseases in multiple host species, including humans and animals. *Staphylococcus argenteus* is a novel staphylococcal species closely related to *Staphylococcus aureus* and is considered as a part of the S. *aureus* complex which also includes *Staphylococcus schweitzeri*. *S. argenteus* was first reported in northern Australia as a *Staphylococcus aureus* clone complex (CC) (grouped as CC75). There is a phenotypic similarity between *S. argenteus* and *S. aureus* and routine biochemical tests used in clinical laboratories fail to distinguish the 2 taxa. In the NCVTC Bacterial repository, 73 historical cultures purported to be *Staphylococcus* spp. were subjected to molecular identification by 16S rRNA sequencing. A total of 49 cultures were identified as belonging to *Staphylococcus* spp., 8 as *Mammalicoccus* spp. and 16 remained unidentified. Out of 49 *Staphylococcus* spp. cultures, the maximum number of isolates i.e., a total of 19 (39%) cultures were identified as *S. argenteus*. These cultures were isolated from the horse (1), dog (2), buffalo (3), and cattle (13). The nineteen isolates of *S. argenteus* formed a large clade, which was further subdivided into 3 different clades consisting of 8, 7, and 4 strains respectively. The smallest subclade consisting of VTCCBAA761, BAA766, BAA492, and BAA853 were placed together with *S. argenteus* MSHR1132 FR821777, strain *S. schweitzeri* FSA084 CCEL01000025, and 2 strains of *S. aureus*. The near complete sequence of 16S rRNA genes was utilized to infer phylogeny (Fig.). *S. argenteus* is also coagulase-positive and is important etiological agent of bovine mastitis and may be involved in community associated methicillin infections, its identification is important.



Phylogenetic analysis of *Staphylococcus argenteus* historical isolates

(Ankush & RK Vaid)

Bacteriological quality of donkey mare milk and identification of bacteria

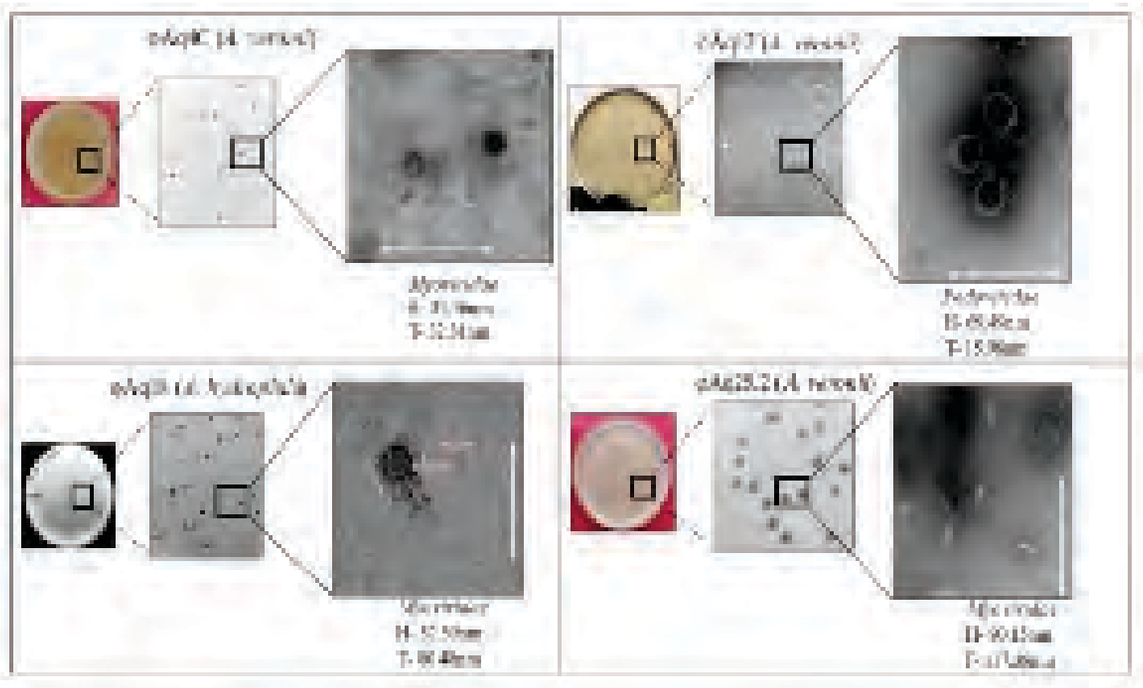
The production and consumption of donkey (*Equus asinus*) milk is almost negligent, however, given its economic importance as source of this scarce product and its exotic importance, it can be exploited for development of novel products and for niche consumption. However, given the fact that it is a mammalian secretion with rich nutritive composition of protein, sugar, fats, and vitamins, it eventually gets contaminated either during milking or some bacteria may gain entry into teat orifices and thereby get secreted into the milk. Since hygienic milk production is important for use as human consumption, milk needs to be characterised by an acceptable microbial load. Bacterial flora in raw milk may lead to spoilage or may constitute a public health risk. Therefore, we have undertaken to analyse the microbial load of raw donkey milk by enumeration and identification of isolated bacteria. The bacterial colonies growing on media were characterized by standard phenotypic and molecular level up to species level. The quantitative idea of bacterial load in milk and identification of bacteria may give a good idea of the type of microbial hazard raw donkey milk may represent. A total of 95 samples of donkey milk were received in the bacteriology laboratory. The samples were collected in 50 ml. sterile test tubes and brought to the laboratory and were processed immediately. Enumeration was done by plating appropriate decimal dilution on 5% SBA and incubation at 37°C for 24 hours. Colonies were counted and expressed in cfu. ml^{-1}. Results indicated that aerobic plate count (APC) of bacteria ranged between minimum 1×10^{1} to 3.2×10^{9} cfu/ml. The internationally acceptable values for raw cattle milk meant for human consumption as raw milk is $>3x\ 10^{4}$ cfu/ml). A total of 175 bacterial colonies were marked and isolated based on morphology, abundance, and hemolysis. Out of this a majority, 109 (62.3%) were Gram-positive and remaining 66(37.7%) were Gram-negative. Important genera identified from donkey mare milk were *Staphylococcus* spp, *Mammalicoccus* spp, *Klebsiella* spp, *Acinetobacter* spp, *Agrobacterium* spp., *Bacillus* spp, *Pseudomonas* spp., *Sphingomonas* spp. , *Microbacterium* spp, *Enterobacter* spp, *Serratia* spp., *Azospirillum* spp., and *Stutzerimonas* spp. Out of 39 isolates, for which molecular identification results were available, maximum 12(30.8%) isolates were identified as *Mammalicoccus sciuri*., followed by 9 strains of *Staphylococcus* spp. among which 5 strains were of *Staphylococcus saprophyticus*. The presence of coliforms (*Enterobacter* spp and *Klebsiella* spp.) in raw milk is not acceptable. Among Gram-negative isolates identified, maximum (4) 10.3% belonged to *Enterobacter* spp. Microbial identification of milk isolates has revealed presence of some unusual taxa viz., *Agrobacterium fabrum*, *Sphingomonas paucimobilis, Microbacterium zeae, Azospirillum formosense,* and *Stutzerimonas stutzeri. Staphylococci* and *Mammaliicocci* are opportunistic pathogens, however, have ability to cause a wide range of severe infections. *Mammalicoccus sciuri* has been reportedly isolated from many cases of bovine mastitis, and in other animals such as goats, piglets, and dogs.

(RK Vaid, Anuradha Bharadwaj & Taruna Anand)

Characterization of Phages of *Aeromonas* spp. isolated from fishery pond water

A total of 66 villages in Hisar and Fatehabad district were surveyed, and fisheries pond water was sampled for isolation, identification, and characterization of *Aeromonas* species. Molecular identification of 97 isolates by sequencing of *gyr*B gene showed the prevalence as- *Aeromonas veronii, A. hydrophila*, *A. jandaei* , *A. caviae* , *A. punctata* , *A. sobria* and *A. culicicola*. On average, enolase (*eno*, 98%) was the most frequently detected virulence gene in *Aeromonas* species isolated from all ponds, followed by hemolysin (*asa*1, 86%) and cytotoxic enterotoxin (*act*, 88%). From the pond water samples, using isolated aeromonads as host, a total of 39 bacteriophages (34-*Aeromonas* species; 3-*Stenotrophomonas maltophilia*; 2-*Psuedomonas* species) were isolated from fish culture pond water. Phages against aeromonads comprised of 13-*A. veronii*; 11-*A. hydrophila*; 6-*A. caviae*; 1-*A. jandaei*; 4-*Aeromonas* spp. SDS-PAGE protein profile, RE digestion pattern and RAPD amplification pattern revealed that all phages were distinct. TEM observation revealed that the phages had tails and thus belonged to the order Caudovirales. Transmission electron micrographs revealed that 12 *Aeromonas* phages belong to *Myoviridae* family, and 1 phage (ϕAq12) belonged to *Podoviridae* family (Fig.). Different plaques appeared on the appropriate host lawn after 20-24 h incubation at 37°C, and plaques of all the thirty-four phages were morphologically dissimilar with diameters ranging from 1 mm to 5 mm, with a clear/transparent zone in the middle. The host range of phages was evaluated by including a taxonomically diverse panel of 103 motile aeromonads from different pond waters. All phages could infect more than one strain (other than the indicator host), showing broad infectivity against

phylogenetically distant species in *Aeromonadaceae* family. Phage ΦAq16.1 (*A. veronii*) infected more strains but showed only clear plaques on a few, whereas phage ΦAq96 (*A. hydrophila*) was able to lyse approx. only 25% of strain tested, but more efficiently. The one-step growth experiment performed to determine the latent time and phage burst size revealed considerable heterogeneity in the propagation among different phages in their appropriate host cells. Phage stability assays at different pH and temperatures, chloroform and bile salts were also performed to ascertain utility of phages. The collection of phages in this study can be used in control and prevention of aeromonads in aquaculture.

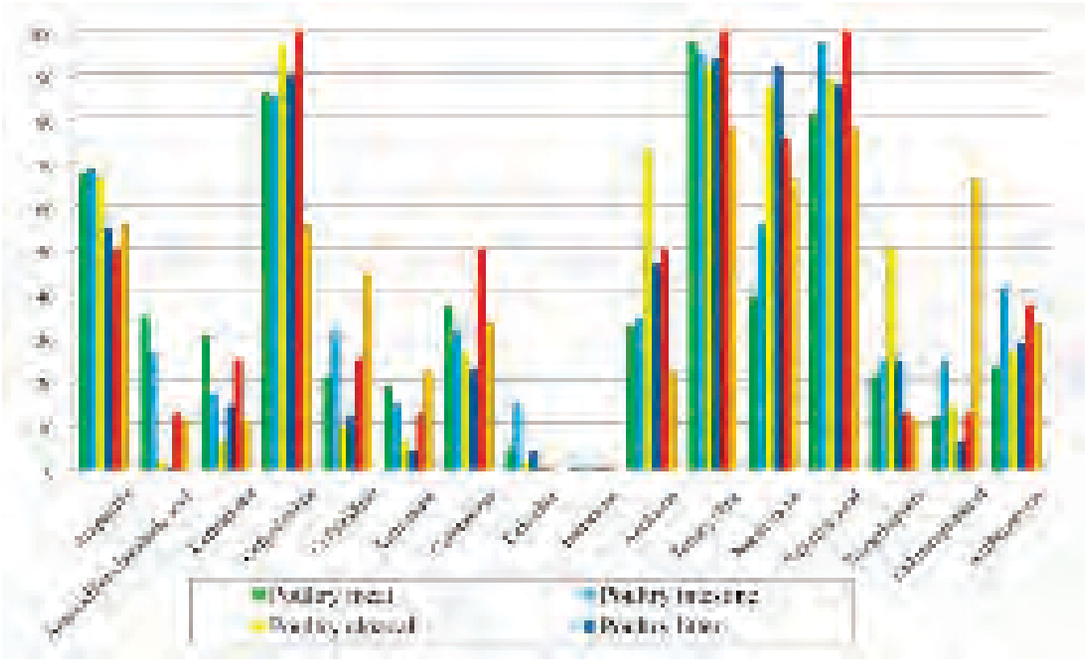


Plaque morphology analysis of *Aeromonas* phages by Transmission Electron Microscopy.

(Alka Nokhwal, Taruna Anand & RK Vaid)

Extended Spectrum Beta Lactamase *Escherichia coli* detected in poultry samples in Hisar district

The use of antibiotics in intensive poultry farming industry causes the emergence of antibiotic-resistant bacteria in poultry, which may be transmitted to humans through poultry products, and the farm environment, which is a serious emerging public health concern. For studying AMR *Escherichia coli* in different settings of poultry farming system, we obtained and processed 253 samples including poultry intestine samples, and raw meat samples, from retail meat shops: cloacal swabs, and litter material, from farm environment. Human hands swabs and house fly (*Musca domestica*) pools were obtained from both retail shops and poultry farms. A total of 213 *E. coli* isolates were obtained identified by phenotypic, biochemical, and molecular methods. The bacterial isolates from poultry meat, poultry intestine, poultry cloacal swabs, poultry litter isolates, human hands, and *M. domestica* were used for AMR testing (Fig.). The antibiogram studies of 213 isolates by disk diffusion assay revealed that 22 *E. coli* (10.3 %) isolates were extended-spectrum beta-lactamase (ESBL) producing isolates. Two *E. coli* isolates were confirmed phenotypically as AmpC-beta-lactamase produced by combined disc method. The ESBL *E. coli* isolates comprise of 9 meat isolates (40.9 %), 4 poultry intestine isolates (18.2 %), and 3 cloacal and poultry litter isolates each, respectively (13.7 %). ESBLs were also confirmed among one human hand swabs isolate (4.5 %) and 2 *M. domestica* isolates (9.1%). Among ESBL *E. coli* isolates, all were positive for AmpC gene, followed by *bla*CTX-M Gp-1 (11, 50 %), *bla*TEM 1 & 2 (8, 36.4 %), *bla*SHV-1 (1, 4.3%), ACC (1, 4.5 %) and CIT gene (1, 4.5 %) positive, respectively. The ESBL, *E. coli* were detected not only in the farm environment at production stages but also at retail shops. The human handlers and flies may be an important source of spread of AMR resistant *E. coli* in the environment. There is a need to further study the transmission dynamics of AMR *E. coli* from farm to fork.



Resistance of the *Escherichia coli* isolates from different poultry sources

(Upender, Taruna Anand & RK Vaid)

Detection of virulence genes in *Klebsiella pneumoniae* isolated from equines

Klebsiella pneumoniae is an opportunistic pathogen and causes urinary, respiratory tract, digestive, reproductive tract and septic infections in humans and a wide variety of animals. In horses, *K. pneumoniae* causes pneumonia, epidemic metritis, cervicitis, abortions, and septicaemia. Little information is available on the impact of *K. pneumoniae* infections in horses, disease epidemiology, molecular epidemiology, and antimicrobial resistance profiles. Therefore, this study was undertaken to characterize the virulence factors (*fimH-1, entB, mdtK, kfu* and *arb*) in clinical strains of *K. pneumoniae* isolated from biological samples (heart blood, cervical and vaginal swabs, foetal stomach contents and kidney) of equine origin heart blood, cervical and vaginal swabs, foetal stomach contents and kidney. A total of 15 *K. pneumoniae* were used for this study. All 15 isolates were found to be positive for the presence of *fimH, mdtK, kfu, entB*, and negative for *arb*. Thus, it can be concluded that *K. pneumonia* might be considered a major emerging pathogenic bacterium with clinical significance and needs further detailed studies.

(Shanmugasundaram K, Singha H, Indu Rani & Yash Pal)

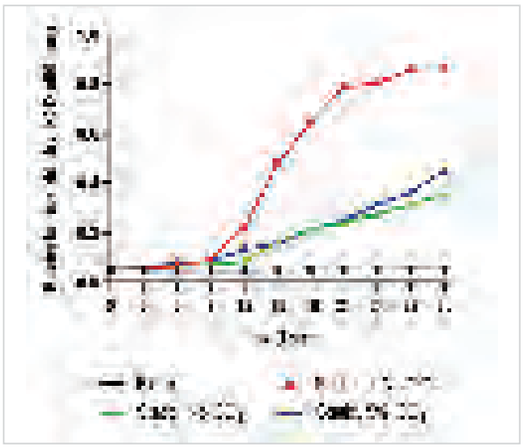
Detection of equine pythiosis in a stallion

Pythiosis is an exudative, tumor-like, granulomatous, invasive, and itchy lesion of cutaneous and subcutaneous tissues in humans and other animals. The disease is caused by the fungus like pathogen *Pythium insidiosum,* which belongs to the class *Oomycetes,* Order *Pythiales*, family *Pythiaceae* and the genus *Pythium.* In India, pythiosis in humans has been reported but not in equines. We have presumably confirmed the first case of pythiosis from Kathiyawari horse in India from clinical samples (Kunkers and skin) received from veterinary surgeon (Tamil Nadu). Kunkers and skin tissue samples were sliced with scalpel blades and the genomic DNA was extracted with DNeasy Blood and tissue kit (Qiagen) as per the manufacturer's protocols. For isolation, slices of kunkers and skin samples for paled on Sabouraud dextrose agar and incubated at 37°C to monitor the fungal growth. Fungal colonies were observed in overnight incubation. Genomic DNA was extracted by using DNeasy Blood and tissue kit (Qiagen). For PCR amplification, universal primers targeting ITS regions were used and amplified products were purified and sequenced. The sequences were matched with *Pythium insidiosum* sequences available in the GenBank and as per our knowledge this is the first report of equine pythiosis in India.

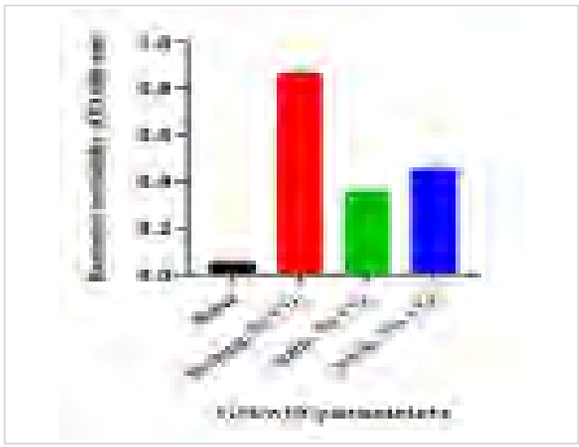
(Shanmugasundaram K, Indu Rani, Singha H, Saranya & Yash Pal)

Evaluation of *Mycobacterium kansasii* growth kinetics under different *in-vitro* conditions

Mycobacterium kansasii is the second most frequently isolated non-tuberculosis mycobacteria (NTM) species in Asian countries. This opportunistic pathogen is responsible for chronic lung infections resembling pulmonary tuberculosis, especially in immune compromised individuals. Studying bacterial growth curve is essential in predictive microbiology and is indispensable in diverse fields of biotechnology, drug design, genetics, ecology, etc. Poorly defined growth kinetics due to its prolonged generation time, severely restrict the understanding of disease progression. Thus, this study was aimed to determine the growth curve of *M. kansasii* under different *in vitro* culture conditions. *M. kansasii* (ATCC12478) was grown at 37°C in Middlebrook 7H9 broth supplemented with 10% OADC at three different conditions: (i) shaking, without CO_2 (ii) static, without CO_2 and (iii) static, with 5% CO_2. Growth curve was plotted with the $OD_{600\ nm}$ values measured at an interval of 3 days successively up to 30 days. Results indicated that the *M. kansasii* from group 1 achieved log phase after 9th day of incubation and in other 2 groups, it was observed after 12th day of incubation (Fig.) . However, the cells from group 1 were able to grow 1.9 and 2.4 times more robustly than group 2 and group 3, respectively (Fig.). Cells grown under agitated conditions were able to show rapid multiplication than static conditions irrespective of the presence or absence of CO_2. Devising optimal growth parameters for multiplication of *M. kansasii* under *in vitro* conditions will have wide applications in genetic manipulations and devising of therapeutic strategies for *M. kansasii* infections.



Growth curve



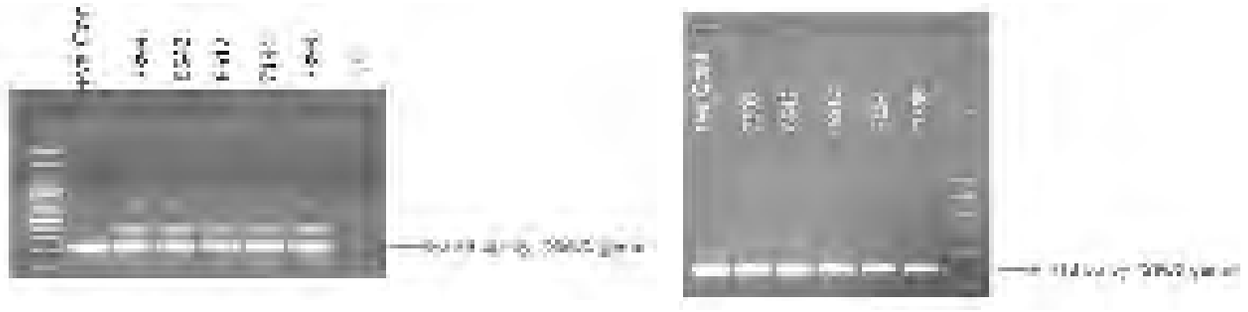
Bacterial growth at 30th day of incubation

(Indu Rani, Shanmugasundaram K, Rakesh Kumar & RK Vaid)

Isolation, characterization and generation of repository of *Mycobacterium* species

A total of biological samples (n=104) samples was collected for isolation of mycobacterial species. It includes cattle (n=18) and buffaloes (n=2) fecal samples, bat guano samples (n=56) human sputum (n=15), cattle blood (n=7), nasal swabs from cattle (n=10), human blood (n=1) and water samples (n=5) were collected. Acid fast staining of cattle fecal (n=10) and nasal (n=10) samples were performed, and all samples found to be negative for the presence of acid-fast bacilli. Genomic DNA extraction from bat guano samples were optimized with spiking of *M. smegmatis*. For this purpose, two commercial kits were used, namely QIAamp Fast DNA Stool and Quick fungal/Bacterial DNA (Zymo Research). It has been noticed that QIAamp Fast DNA Stool yields better results. Genomic DNA was extracted from Human sputum (n=15) samples and has been tested for the identification of MTBC complexes with published MTBC species-specific primers and this work is under progress. This will be useful for the detection of *M. bovis* infection in humans. Genomic DNA extraction from cattle fecal samples and PCR amplification: Genomic DAN was extracted from 10 fecal samples and subjected to PCR amplification to detect the Mycobacterial species. One fecal sample was found to be positive for afb gene and another fecal sample was found positive for *hspx* gene. Recently, *Mycobacterium* subsp *paratuberculosis* (n=4) species has been isolated from sheep intestinal samples. Isolated colonies were screened for acid-fastness by Ziehl-Neelsen stain and found positive for the presence of acid-fast bacilli. Furthermore, for the species identification, two set of primers, namely,

IS900F 5′-CCGCTAATTGAGAGATGCGATTGG-3′; IS900R5′- AATCAACTCCAGCAGCAGCGCGGCCTCG-3′, and MAP IS900 F- GAAGGGTGT TCGGGGCCGTCGCTTAGG-3 and MAP IS900R - GGCGTTGAGGTCGATCGC CCA CGTGAC-3 were used and these primers were amplified expected size of 229 bp and 413bp respectively (Fig.). All four isolates were subcultured in Middlebrook 7H11 agar and Middlebrook 7H9 broth. Further molecular characterization will be upon successes full sub-culturing.

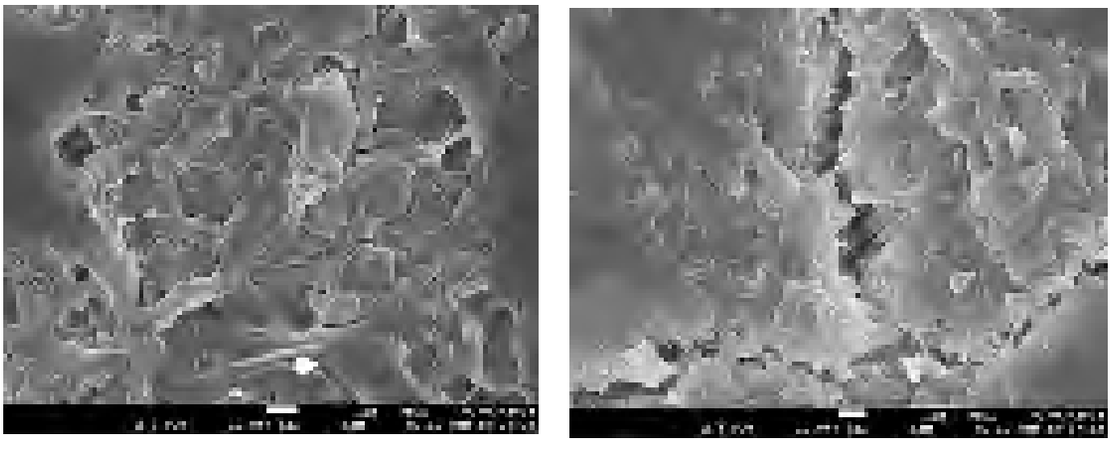


PCR amplification of MAP isolates

(Shanmugasundaram K, Indu Rani, RK Vaid, Riyesh T & BC Bera)

Assessment of Biofilm formation by *Mycobacterium kansasii*

Mycobacterium kansasii is ubiquitous acid-fast, non-motile, photochromogenic and slow-growing non-tuberculosis mycobacterial species. *M. kansasii* is a dominant, most virulent species, can cause serious lung infection in immunocompromised humans and the disease resembles tuberculosis. One of the major pathogenic factors of mycobacteria is the formation of biofilms. Mycobacterial biofilms are complex communities of cells that are embedded in an extracellular matrix of polysaccharides, proteins, and lipids. These biofilms can be difficult to treat with antibiotics, as the bacteria within the biofilms are often less susceptible to antimicrobial agents. Therefore, understanding the mechanisms of biofilms formation by *M. kansasii* is an important area of search for developing new strategies to combat this bacterium. Hence, the present study was aimed to assess the biofilms forming ability of *M. kansasii*. Biofilms of *M. kansasii* (ATCC12478) strain were developed on borosilicate glass cover slips as per the standard protocols and four weeks old biofilms were visualized using Field-Emission Scanning Electron Microscopy (FE-SEM) in a JSM-7610F Plus Scanning electron microscope (Jeol, Akishima, Japan). We observed the tightly packed monolayers of *M. kansasii* on cover slip (Fig.).These SEM imaging of biofilms is a useful tool for studying the structure and function of complex bacterial communities and further characterization is under progress. This information can be used to develop new strategies for preventing and treating biofilms related infections.



Field- Emission Scanning electron micrographs of *Mycobacterium kansasii* biofilms.

(Indu Rani, Rakesh Kumar, Shanmugasundarm K & RK Vaid)

CRISPR- Cas9 mediated gene editing in *Mycobacterium kansasii*

Mycobacterium kansasii is an acid-fast and causes cause range of infections in immunocompromised individuals, including pulmonary tuberculosis. Prevalence of *M. kansasii* infection in India is underreported due to misdiagnosis of NTM infections and treated as tuberculosis. There is a lack of functional genomics studies to understand the role of several genes in bacterial survival in hosts, environments, host-pathogen interactions, and virulence. Several studies have validated the function of MCC genes in survival of many *Mycobacterium* spp. Therefore, in this study we were using CRISPR-Cas9 approach to decipher the role of MCC genes in survival of *M. kansasii*. We designed sgRNA for MCC genes using CHOPCHOP tools and custom PAM sites were used to identify the target sites, designed primers using NCBI primer designing tool. Plasmid pCRISPRx-Sth1Cas9-L5 was digested using BsmBI enzyme, dephosphorylated and purified by gel purification kit. Phosphorylated and annealed oligos containing BsmBI overhangs were annealed into the plasmid. After ligation transformed colonies were observed on the LBA plates containing 50μg/ml Kanamycin. Upon transformation, there were, and this may be due to some off target effects or CRISPR –Cas9 and hence further troubleshooting is under progress.

(Indu Rani, Shanmugasundarm K, Rakesh Kumar, Singha H & RK Vaid)

Total microbial accessions in the NCVTC repository

In the current year, a total of 351 microbes were accessioned in the NCVTC repository thereby leading to a cumulative strength of 4815 microbes (till December, 2022). The bacterial repository increased its strength to 1723 bacterial isolates with addition of 120 cultures. Some of the significant accessioned cultures are *Moraxella canis, M. ovis, Pasteurella canis, Edwarsiella tarda, Acinetobacter baumanii, Elizabethkingia anopheles, Bordetella parapertussis, B. avium, Streptococcus uberis, St. suis, St. gallinaceous, Brevibacillus borstelensis, Coryebacterium faecalis, Coryne. hansanii, C. liphophiloflavum, Lysinibacillus macroides,* and *Gallibacterium anatis*. A total of 42 virus cultures were processed of which 32 virus isolates were accessioned in the virus repository, thereby increasing the strength of the virus repository to 384 virus isolates. The important virus isolates accessioned include, bluetongue viruses, bovine coronaviruses and lumpy skin disease viruses. Furthermore, 44 bacteriophages were also accessioned during the year to have a total collection of 329 bacteriophages in the NCVTC repository. The rumen microbial repository at NIANP Bengaluru, added 96 rumen bacteria during the year and making the total strength to 703. Similarly, the dairy microbe's repository at NDRI increased its strength to 780 with the accession of 70 bacteria (Table).

Year-wise microbial accession in the NCVTC repository

Year	2009-17	2017-18	2018-19	2019-20	2020-21	2021-22	2022*	Total
Veterinary Microbes								
Bacteria	1201	70	123	95	50	63	120	1723
Virus	198	27	31	44	24	31	47	384
Bacteriophage	105	24	8	8	48	92	44	329
Recombinant clone	521	36	16	8	0	0	0	581
Phage library	27		0	0	0	0	0	27
Genomic DNA	280	8	0	0	0	0	0	288
Total	**2332**	**165**	**178**	**155**	**122**	**186**	**185**	**3332**
Rumen microbes								
Anaerobic bacteria	253	37	49	46	62	80	96	588
Fungi/Yeast	107	0	0	0	0	0	0	107
Meth. Archae	8	0	0	0	0	0	0	8
Total	**368**	**37**	**49**	**46**	**62**	**80**	**96**	**703**
Dairy microbes								
Bacteria	547	30	36	44	20	48	70	780
Total	**547**	**30**	**36**	**44**	**20**	**48**	**70**	**780**
Grand total	**3247**	**232**	**263**	**245**	**204**	**314**	**351**	**4815**

***Data from Jan.-Dec. 2022**

Distribution of microbial isolates to Stakeholders

During the year 2022, The following bacterial cultures (n=24) such as *Pasteurella multocida, Staphylococcus aureus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus fecalis, Salmonella* Gallinarum (n=5), *Escherichia fergusonii* (n=3), *Escherichia hermanii, Pasteurella multocida* (n=2), *Salmonella Typhimuriuma and Shigella spp* were distributed for research and teaching purposes. In addition, Bacteriophage against *E.coli, Bacillus*, and *Staphylococcus* sp and 23 viruses comprising Buffalopox virus, Bovine herpes virus-1, Bovine rotavirus, Camelpox virus, Equine herpes virus-1, Canine adenovirus, Sheeppox virus, Goatpox virus, Orf virus, *Peste des petits ruminants virus* (Small ruminant morbilli virus), Fowl adeno virus, Duck plague virus, Fowlpox virus, Infectious bronchitis virus, Infectious bursal disease virus, Chicken astro virus, Avian nephritis virus, Avian rotavirus, Avian reo virus, Pigeonpox virus, Newcastle disease virus and Porcine circo virus were also distributed to different stakeholders. Cell lines *viz.,* PK-15, Lamb Testicle cells, HeLa cells, RK-13, BHK-21, MDCK, Vero Cells and MDBK were distributed to different universities and research organization for teaching and research purpose.

(RK Vaid, Sanjay Barua, Naveen Kumar, Shanmugasundaram K, Riyesh T, BC Bera & Taruna Anand)

TECHNOLOGY DEVELOPMENT, TRANSFER AND COMMERCIALISATION

03

Since its inception, ICAR-National Research Centre on Equines is continuously striving for the upliftment of equine sector in the country, oriented its research, and focused on the development of farmer friendly technologies for improvement in equine health, production, and utilization in the country. Many diagnostic kits, vaccines and biologicals developed by the scientists of ICAR-NRCE are routinely used in the field. Many of the novel and innovative technologies are under development, transfer, and commercialization.

I. List of Technologies Developed by ICAR-NRCE, Hisar

A. Vaccines for Equines and other Livestock species

i. Equiherpabort vaccine.
ii. Updated Equine Influenza Vaccine.
iii. A modified vaccine construct for EHV1 and methods of preparing the same
iv. Corona virus SARS-CoV-2 vaccine for animals
v. Lumpy Skin Disease Vaccine

B. Disease Diagnostics for Equines and other Livestock species

i. Equiherpes B-ELISA Diagnostic Kit.
ii. ELISA for differentiation of EHV1 & 4 Infections.
iii. Equine Rotavirus Diagnostic kit.
iv. Equine Infectious Anaemia (EIA) ELISA Diagnostic kit.
v. Equine Japanese Encephalitis Virus Antibody Test Kit, iELISA.
vi. Monoclonal antibody based immunoasssay for detection of equine influenza (H3N8) antigen.
vii. Canine SARS-CoV-2 Antibody detection ELISA Kit.
viii. Glanders ELISA Kit.
ix. *Theileria equi* ELISA Diagnostic kit.
x. Lateral flow assay for diagnosis of *Theileria equi* infection.
xi. Surra (*Trypanosoma evansi*) ELISA Diagnostic Kit.

C. Drug Development and delivery Technologies

i. Polymeric metal nanocomposites and methods of synthesis there of.
ii. *Aerva javanica* extract for the treatment of exuberant granulation tissue and tumors in horses.
iii. *Aerva javanica* extract for the treatment of seasonal dermatitis in horses.

D. Equine Reproduction Technologies

i. A pregnancy diagnostic kit for equine based on detection of eCG by ELISA (Pregmare Kit).
ii. Customised AV (artificial vagina) for semen collection from Stallions
iii. Semen collection and cryopreservation in Indigenous horses
iv. Equine DNA Parentage Testing Kit

E. Equine Products Technologies

i. Processes for formulation of donkey-milk based cosmetic products – bathing soap, body butter, lip balm.

III. Technology Development & Assessment

a. Recombinant antigens based indirect ELISA kit for detection of anti-*Trypanosoma evansi* antibodies in animals

Recombinant antigens based indirect ELISA kit for detection of anti- *Trypanosoma evansi* antibodies in animals involve preparation of pool/mixture of four recombinant antigens *viz.*, Calflagin proteins (Clf), Invariable surface glycoproteins (ISG-65), Tandem repeat proteins (TR) and Variable surface glycoproteins (VSG) in specified ratio and their use in development of a kit/device for detection of antibodies in serum of animals, more specifically against *Trypanosoma evansi*. The specific recombinant antigens which strongly reacts with antibodies against *T. evansi*, but which do not cross-react with antibodies against other protozoan infections viz., *Theileia* spp, *Babesia* spp, etc. The diagnostic sensitivity and specificity of iELISA using mixture of antigens was found to be 94.54% (88.72%-98.36%) and 97.87% (92.96%-99.76%), respectively. This ELISA kit able to detect antibodies against *T. evansi* from 10th day on ward post-infection. This kit provides an alternative of whole cell lysate antigen of *Trypanosoma evansi*, which required use of no laboratory animals, and is more uniform, more sensitive, more specific, less complicated, and is applicable on different animal species/ wide host range, readily usable in field laboratory conditions and can be transformed in to point-of-care device.

Inventors : Rajender Kumar, Sanjay Kumar, BC Bera & BN Tripathi

b. Development of Ancovax vaccine

Development of Ancovax, a vaccine to prevent SARS-CoV-2 infection in animals. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19 in humans, has been shown to readily infect and occasionally cause death in cats, dogs, deer, lions/tigers/leopards, and minks. In India, SARS-CoV-2 Delta (identical to human SARS-CoV-2 Delta strains) infection has been reported in lions; other pet animals (dogs, cats) are also at high risk due to their close contact with human population. Jumping of SARS-CoV-2 from humans to animals might accelerate its evolution and hence affects surveillance and control strategies of COVID-19 in humans. SARS-CoV-2 vaccination in animals is not only essential to prevent animal-to-animal transmission of SARS-CoV-2 but also essential to prevent zoonotic transmission back to humans.

Product (Ancovax) description *:* Ancovax contains inactivated SARS-CoV-2 (Delta) antigen with Alhydrogel as an adjuvant. Ancovax is safe in dogs/ lions/leopards/mice/rabbits. It induces a potent neutralizing antibody- and cell-mediated immune response against SARS-CoV-2 in animals. The antibodies elicited by Ancovax can neutralize both Delta and Omicron variants of SARS-CoV-2.

Inventors : Naveen Kumar, Sanjay Barua & BN Tripathi

c. Development of India's first LSD vaccine (Lumpi-ProVac^{Ind}) to prevent Lumpy skin disease (LSD) in animals

LSD was reported first time in India from Odisha state in 2019, has spread across the country. The current outbreaks of the disease are occurring with high morbidity and mortality in the cattle population of the country. The emerging evidence suggest that the LSD virus (LSDV) can also cause mild infection in buffaloes, horses and camel. The disease in cattle is characterized by development of skin nodules which is associated with fever, enlargement of lymph nodes and depression, eventually resulting in reduced milk yield, abortion in pregnant animals and sterility in bulls. *Capripoxvirus* which includes LSDV, sheepox virus (SPV) and goat pox virus (GPV) are genetically quite similar and cannot be distinguished serologically. Therefore, SPV/GPV-based vaccine (heterologous vaccine) is usually authorized to induce cross protection against LSD in cattle, where homologous LSD vaccine is not available. The Government of India also authorized the use of goatpox vaccine to control LSD in cattle. However, heterologous vaccine provides partial protection and is not as efficacious as homologous vaccine.

ICAR-National Research Centre on Equines (ICAR-NRCE), Hisar (Haryana), in collaboration with ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar (UP) developed a homologous live-attenuated LSD vaccine, named Lumpi-ProVac^{Ind}. Lumpi-ProVac^{Ind} is safe in cattle/buffaloes and induces LSDV-specific antibody- and cell-mediated immune response, besides providing a complete protection against lethal LSDV infection.

Product description: The virus used for developing Lumpi-ProVac^{Ind} was isolated at ICAR-NRCE Hisar from skin scab collected from a naturally LSDV infected cattle at Ranchi (Jharkhand) in 2019 (LSDV/2019/India/Ranchi). The virus was attenuated in Vero cells and the 50th passage of the virus was used to prepare the vaccine. A single dose of the vaccine (Lumpi-ProVac^{Ind}) contains $10^{3.5}TCID_{50}$ of live-attenuated LSD virus (Ranchi strain).

Lumpi-ProVac^{Ind} is used for the prophylactic immunization of cattle/buffaloes. Calves are vaccinated after 3 months of age. The immunity induced by homologous live-attenuated LSD vaccines usually persists for a minimum period of 1 year.

Inventors: Naveen Kumar, Sanjay Barua & BN Tripathi

d. Recombinant nucleoprotein based indirect ELISA for SARS-CoV-2 antibody detection in canines

During the pandemic of COVID19, transmission of SARS-CoV-2 virus was detected in many animals including tigers, lion, minks, canines, felines and deers. Looking into the threat of transmission of virus in many species, a recombinant nucleocapsid protein based indirect ELISA kit was developed for detection of SARS-CoV-2 antibody in canines and felines. The developed iELISA is highly specific and doesn't cross-react with other related coronaviruses of canine. The sensitivity and specificity of the assay was evaluated using serum samples collected from canines during earlier preserved pre-COVID19 period and COVID19 period samples as well as canine corona virus vaccinated animals. The iELISA assay showed 95.66 % sensitivity and 94.06 % specificity for detection of anti-SARS-CoV-2 N IgG antibodies in canines. More than 2500 canine serum samples were tested by developed iELISA and result showed 44.53 % positivity for SARS-CoV-2 antibody. The field samples (nos-54) collected from feline tested by using the developed iELISA assay showed ~28 % positivity for COVID-19 infection. The assay has been into a kit which contains ready to use recombinant nucelocapsid protein coated plates which are ready to use and can be stored at 40C. The kit has capacity to test 45 samples in duplicate.

Inventors: Nitin Virmani, BC Bera & Taruna Anand

III. Release of Technologies

a. Release of Ancovax vaccine

Ancovax vaccine development technology and the kit were released on June 10, 2022 by Honb'le Union Minister of Agriculture and Farmers Welfare Shri Narender Singh Tomar jee.

Release of Ancovax- SARS-CoV-2 vaccine for animals

b. Release of India's first LSD vaccine (Lumpi-ProVac^{Ind})

ICAR-National Research Centre on Equines (ICAR-NRCE), Hisar (Haryana), in collaboration with ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar (UP) developed a homologous live-attenuated LSD vaccine, named Lumpi-ProVac^{Ind}. Lumpi-ProVac^{Ind}. This technology and the vaccine was released by Honb'le Union Minister of Agriculture and Farmers Welfare Shri Narender Singh Tomar Ji on August 10, 2022.

Release of the LSD vaccine technology (Lumpi-ProVac^{Ind}) by Honb'le Union Minister of Agriculture, Shri Narender Tomar Ji

c. Release of Recombinant antigens based indirect ELISA kit for detection of anti -*Trypanosoma evansi* antibodies in animals

A kit developed by ICAR-NRCE scientists for detection of anti-*Trypanosoma evansi* antibodies in animals. This kit was released by Hon'ble Minister of Agriculture and Farmers Welfare on 9th June 2022 at Krishi Bhawan, New Delhi.

Anti-*Trypanosoma evansi* antibodies detection ELISA kit developed by ICAR-NRCE team

IV. List of Technologies developed and released

a. Technology entitled "*Ancovax: An inactivated vaccine to prevent SARS-CoV2 infection in animals*" was released by Hon'ble Minister of Agriculture and Farmers Welfare on 9th June 2022 at Krishi Bhawan, New Delhi.

b. Technology entitled "*Recombinant nucleocapsid protein based indirect ELISA kit for detection of Anti SARS-CoV- antibodies in canines (CAN-CoV-2 iELISA kit)*" was released by Hon'ble Minister of Agriculture and Farmers Welfare on 9th June 2022 at Krishi Bhawan, New Delhi.

c. Technology entitled "*Multi recombinant proteins-based ELISA Kit for diagnosis of Trypanosoma evansi infection in animals*" was released by Hon'ble Minister of Agriculture and Farmers Welfare on 9th June 2022 at Krishi Bhawan, New Delhi.

d. Technology entitled "*Equine parentage testing technology*" was released by Hon'ble Minister of Agriculture and Farmers Welfare on 9th June 2022 at Krishi Bhawan, New Delhi.

e. Technology entitled "*Lumpi-ProVac^{Ind} (Lympy Skin Disease Vaccine)*" was released by Hon'ble Minister of Agriculture and Farmers Welfare on 10th August 2022 at Krishi Bhawan, New Delhi.

V. Patents Granted/Filed

a. Patent Granted

Polymeric metal nanocomposites and methods of synthesis thereof (Patent No. 411620, dated 16.11.2022, Application No.201911009696, Dated 13.03.2019).

Inventors: Anju Manuja, Balvinder Kumar, Riyesh T & BN Tripathi

b. Patents Filed

S. No.	Title of Patent	Name of the Inventors	Filed	Institutes
1.	Recombinant antigens based indirect ELISA kit for detection of anti *Trypanosoma evansi* antibodies in animals.	Rajender Kumar, B.C. Bera, Sanjay Kumar, Khushboo Sethi, Kanisht Batra, Saroj Kumar, Bhupendra N. Tripathi.	Application No. 202211008619, dated 18.02.2022	NRCE, Hisar
2.	Development of a Novel Modified Attenuated Lumpy Skin Disease Virus (LSDV) For Use as Vaccine	Naveen Kumar, Sanjay Barua and Bhupendra N. Tripathi.	Application No.202211013092, dated 10.03.2022	NRCE, Hisar
3.	A novel vaccine formulation (Ancovax) to prevent SARS-CoV-2 infection in animals.	Naveen Kumar, Sanjay Barua, Bhupendra N. Tripathi, Yash Pal, Baldev R. Gulati, Nitin Khandelwal, Ram Kumar and Yogesh Chander	Application No. 202211026023, dated 04.05.2022	NRCE, Hisar
4.	A method for encapsulation of bacteriophage cocktail against Salmonella sp for oral delivery in poultry.	Taruna Anand, Nitin Virmani, B.C. Bera, Rajesh Kumar Vaid, Manju Bernela, R.K. Singh, B.N. Tripathi	Application No. 202211050633, dated 05.09.2022	NRCE, Hisar
5	Development of a Novel test to differentiate the vaccine and field strains of LSDV.	Naveen Kumar, Ram Kumar, Sanjay Barua, Bhupendra N. Tripathi	Application No. 202211074538, dated 22.12.2022	NRCE, Hisar
6	Recombinant nucleoprotein based indirect ELISA for SARS-CoV-2 antibody detection in canines	Nitin Virmani, B.C. Bera & Taruna Anand	Application No. 20211157358, dated 9.12.2021	NRCE, Hisar
7	Hydroxychloroquine/ chloroquine zinc oxide nanoparticle formulations	Anju Manuja, Balvinder Kumar, Rajender Kumar, Yash Pal and Minakshi Prasad	Application No. PCT/IB2022/062019, dated 10.12.2022	NRCE, Hisar

VI. MoU for Cooperation in Research and Education

- An MoU/MoA was made with Dr Jeya Bhaskaran, Pune, Maharashtra on two technologies of Semen collection and cryopreservation in Equines and Artificial Vagina for semen collection in Stallions. These technologies were transferred to Mr Manusharma during 12th -15th, Feb 2022

Team: TR Talluri, Yash Pal, SK Ravi & RA Legha

- An MoU/MoA was made with Mr Manu Sharma, Patiala, Punjab on two technologies of Semen collection and cryopreservation in Equines and Artificial Vagina for semen collection in Stallions. These technologies were transferred to Mr Manu Sharma during 12th -15th, April 2022

Team: TR Talluri, Yash Pal, SK Ravi & RA Legha

- An MoU/MoA was made with Mr Ananth Singh Rathore, Kelwa, Udaipur on the technology of Semen collection and cryopreservation in Equines. This technology was demonstrated at his place and was transferred to Mr Ananth Singh Rathore during 25th -28th, June, 2022

Team: TR Talluri, Yash Pal, SK Ravi & RA Legha

- An MoU/MoA was made with Dr Jignesh Wala, Junagarh, Gujarat on the technology of Artificial Vagina for semen collection in Stallions. This technology was transferred to Dr Jignesh Wala, Junagarh, Gujarat during 12th -15th, Nov 2022

Team: TR Talluri, Yash Pal, SK Ravi & RA Legha

VII. Commercialization of NRCE Technologies

Commercialisation and licensing LSD vaccine technology to Biovet Pvt Ltd, Malur, Karnataka.

a. **Commercialisation of LSD vaccine technology:** Three firms namely M/s Biovet Pvt Ltd, Malur, Karnataka, M/s Indian Immunologicals Ltd, Hyderabad and M/s Institute of Veterinary Biological Products, Pune purchased the technology, each costing Rs. 75.00 Lakhs.

Inventors: Naveen Kumar, Sanjay Barua & BN Tripathi

b. **Recombinant Hcp1 antigen-based ELISA for diagnosis of *Burkholderia mallei* infection (Glanders):** The technology has been transferred to Genomix Molecular Diagnostic Pvt Ltd, Hyderabad through Agrinnovate India Ltd. (AgIn). The agreement was signed on 5th April, 2022 for a period of 5 years. The license fee of the technology was Rs. 4 Lakh and 5% royalty on commercial sales of the kit.

Inventors: Harisankar Singha, Praveen Malik & BN Tripathi

VIII. Commercialisation and Revenue Generation from the Sale and Service of NRCE Technologies

S. No.	Period	Name of the Technology Transferred	Name of the Licensee/party	Revenue Generated	Date of Licensing
1.	2021-22	Semen collection and cryopreservation in Indigenous horses	Dr. S. Jeya Bharath, 5th Sense Animal Health Care, Shop No.4, E Wing, DSK Vidyanagari Phase II, Sus Road, Pune	Rs. 0.5 lakh towards technology fee.	04.02.2022
2.	2021-22	Customised AV for semen collection from stallions	Dr. S. Jeya Bharath, 5th Sense Animal Health Care, Shop No.4, E Wing, DSK Vidyanagari Phase II, Sus Road, Pune	Rs. 0.15 lakh towards technology fee.	04.02.2022
3.	2021-22	Updated Equine Influenza Vaccine	Biovet Private Limited, KIADB Industrial Area, Malur, Karnataka	Rs. 15.00 lakh	10.03.2022
4.	2021-22	A modified vaccine for EHV-1 construct and method for preparation	Biovet Private Limited, KIADB Industrial Area, Malur, Karnataka	Rs. 15.00 lakh	10.03.2022
5.	2022-23	Recombinant antigen-based ELISA for diagnosis of *Burkholderia mallei* infection (GLANDERS)-HCP1	M/s Genomix Molecular Diagnostic Pvt Ltd. & Genomix CARL Pvt Ltd., Pulivendula, Kadapa Distt (AP)	Rs. 4.00 lakh	05.04.2022
6.	2022-23	Semen collection and cryopreservation in Indigenous horses	Shri Anant Singh Rathore, Fateh Stud Farm, Kelwa C/o Hotel Rampratap Palace, 5-B Alkapuri, Near Fateh Sagar Lake, Udaipur, Rajasathan	Rs. 0.5 lakh towards technology fee.	29.06.2022
7.	2022-23	Customised AV for semen collection from stallions	Dr Balender Kumar, V.P.O. Malladkhera, Distt. Hanumangarh, Rajasthan	Rs. 0.15 lakh towards technology fee.	09.07.2022
8.	2022-23	Semen collection and cryopreservation in Indigenous horses	Mr. Munish Dev, Long Riders ranch, Village Fatehpur, Sangrur Road, Patiala, Punjab	Rs. 0.5 lakh towards technology fee.	09.07.2022
9.	2022-23	Customised AV for semen collection from stallions	Mr. Munish Dev, Long Riders ranch, Village Fatehpur, Sangrur Road, Patiala, Punjab	Rs. 0.15 lakh towards technology fee.	09.07.2022
10.	2022-23	Lumpy Skin Disease Vaccine	Biovet Private Limited, KIADB Industrial Area, Malur, Karnataka	Rs. 75.0 lakh	16.09.2022

S. No.	Period	Name of the Technology Transferred	Name of the Licensee/party	Revenue Generated	Date of Licensing
11.	2022-23	Customised AV for semen collection from stallions	Dr Jignesh V Vadalia, Block No.102, Haveli Apartment, Silver Park, B/H Golden Heights, Zanzarda Chokdi, Junagarh-362001, Gujarat	Rs. 0.15 lakh towards technology fee.	12.10.2022
12.	2022-23	Lumpy Skin Disease Vaccine	Indian Immunological Ltd, Hyderabad	Rs. 75.0 lakh	13.10.2022
13.	2022-23	Lumpy Skin Disease Vaccine	Institute of Veterinary Biological Products, Govt. of Maharashtra, Pune	Rs. 75.0 lakh	29.12.2022
			Total Revenue Generated	**Rs. 261.1 lakhs**	

EDUCATION AND TRAININGS 04

1. Training Organized

A. Training Programme on "*Approaches for Diagnosis of Zoonotic Bacterial Infections*"

Three days training programme on "Approaches for Diagnosis of Zoonotic Bacterial Infections was organized at ICAR-NRCE from March 03 to 05, 2022. A total of 40 participants from human health sectors and animal husbandry departments and wildlife sectors of Haryana and Punjab states attended the training. During this training program, expert lectures on zoonotic diseases such as glanders, brucellosis, mycobacterial infections and paratuberculosis were delivered. Hands on training were also imparted on laboratory diagnosis of glanders, tuberculosis, paratuberculosis and brucellosis.

Participants of the Three days Training Program

Release of Compendium of the Training Program by Dr Dolly Gambhir, Incharge State IDSP

B. Training of Field Veterinary Officers :

Mass awareness of veterinary officers and equine stake holders on glanders disease is one of the instrumental factors for effective implementation of physical, clinical, and serological surveillance, control, and containment of glanders outbreak. In 2022, a three-day training programme was organized at NRCE, Hisar on "*Diagnosis and control of Equine Glanders*" from 6-8 September 2022. A total of 5 Veterinary Officers from Uttarakhand state participated in the training programs. A glanders testing laboratory was established at Srinagar, Pauri-Garhwal under the technical supervision of NRCE and trained personnel. This net-work laboratory will conduct glanders surveillance for Uttarakhand state.

Participants of the Training Program

C. Training programme on "*Entrepreneurship Development Programme on Donkey Farming*"

As mechanization advances, the use of donkeys has dwindled at a faster pace. As per the 20th Livestock census, the indigenous donkey population registered a drastic decline of approximately 62%. This necessitates adopting necessary conservation strategies to save this endangered species. However, an increase in the demand for donkey milk and its by-products has been observed together with that of meat products that are commercialized in the European markets and as specific dietary products. Even in our country, many upcoming farmers are adopting donkey milk as a business venture due to its unique rheological properties. ICAR-NRCE is also working in this direction of developing a donkey dairy unit and aiming at studying the special properties of the donkey milk. In this endeavour to appraise about the various products of donkey milk and how to make donkey farming as an entrepreneurship, a three-day training programme entitled "Entrepreneurship Development Programme on Donkey Farming" was inaugurated by Dr Yash Pal, Director, ICAR-NRCE on 23.08.2022. To participate in this training programme, 13 entrepreneurs from seven different states (Rajasthan, Delhi, Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, and Telangana) have arrived at Equine Production Campus, ICAR-NRC on Equines, Bikaner.

Participants interacting with the Director, ICAR-NRCE and Experts

2 Post Graduate Students' Research and Guidance

Sr No	Name of the student	Name of the Guide	Project/Dissertation Title	Completed/ Ongoing
Post-Doctoral Student				
1.	Ruma Rani	Dr Rajender Kumar	Development of recombinant antibody-based nano-diagnostic lateral flow assay for rapid detection of *Trypanosoma evansi* infection at Point-of-Care	Ongoing
Ph.D Students				
1.	Kapil Kumar Gupta (ICAR-IVRI, Izatnagar)	Dr Sanjay Kumar	Evaluation of in-vitro growth inhibitory activity of Artemisia scoparia extract and its lead molecules against Theileria equi and their associated cyto-/organ-toxicity on equine cell line and mice model	Completed
2.	Mamta (CBLU, Bhiwani)	Dr Sanjay Kumar	Anti-theilerial and anti-plasmodial activities of herbal based selected lead drug molecules and elucidation of their targets	Ongoing
3.	Dharvi (LPU, Phagwara)	Dr Balvinder Kumar	Epidemiological and Molecular Investigation of Strangles in Equine Population of Northern India	Ongoing
4.	Diksha Sharma (LUVAS, Hisar)	Dr Rajender Kumar	In vitro and in vivo evaluation of chemotherapeutic potential of alkaloids against *Trypanosoma evansi.*	Ongoing
5.	Snehil Gupta (LUVAS, Hisar)	Dr Rajender Kumar	Screening, identification and evaluation of some novel target specific therapeutic compounds against *Trypanosoma evansi*	Completed
6.	Aashwina Madhwal (ICAR-IVRI, Izatnagar)	Dr Nitin Virmani	Development of modified live EHV1 vectored bivalent vaccine candidate against equine influenza virus & equine herpesvirus 1: Protective efficacy studies in mouse challenge model	Ongoing
7.	Vaishali (LUVAS, Hisar)	Dr Sanjay Barua	Epidemiological Studies on porcine astrovirus in piggery units in Haryana	Completed
8.	Yogesh Chander (GJU & ST, Hisar)	Dr Sanjay Barua	Role of p38 Map Kinase in Buffalopox virus replication	Ongoing
9.	Swati Rani (GJU & ST, Hisar)	Dr Anju Manuja	Synthesis, characterization and biological activity of chloroquine derivatives, their complexes and nanocomposites	Ongoing

Sr No	Name of the student	Name of the Guide	Project/Dissertation Title	Completed/ Ongoing
10.	Indu Rani (CCSHAU, Hisar)	Dr Shanmugasundaram K	Targeted genome editing using CRISPR-Cas9 approach to decipher the functional role of predicted genes in survival of *Mycobacterium kansasii*	Ongoing
11.	Medhavi Vashisth (CCSHAU, Hisar)	Dr Taruna Anand	Characterization of bacteriophages against ESKAPE	Completed
12.	Anubala Jaglan (CCSHAU, Hisar)	Dr Taruna Anand	Exploring Bacteriophage derived endolysins for targeted delivery into biofilm forming Bacteria	Ongoing
13.	Sonali (Maharaja Agarsen University, Baddi, Solan)	Dr Anuradha Bhardwaj	Comparative Genomic Studies on Horse and Donkey Performance Genes	Ongoing
14.	Renu Garhwal (Amity University, Jaipur)	Dr Anuradha Bhardwaj	Characterization of physicochemical qualities of donkey milk and its utilization in value added dairy products	Ongoing
15.	Karnam Sangwan (Amity University, Jaipur)	Dr Yash Pal	Preparation and optimization of watermelon rind powder based double emulsion and its incorporation in donkey milk derived dairy product	Ongoing
16.	Ajmer Singh (Singhania University, Pacheri Bari, Jhunjhunu)	Dr Yash Pal	A sociological study of socio-economic aspects of equine farmers in Haryana (India)	Ongoing
MVSc/MSc Students				
1.	Naintara Thapa (GJU & ST, Hisar)	Dr Harisankar Singha	To determine IgG antibody titre against recombinant *B. mallei* Hcp1, TssA and TssB in glanders positive equines	Completed
2.	Mukuni Kumari (RAJUVAS, Bikaner)	Dr Ramesh K Dedar	Evaluation of in vitro antibacterial activity of some plant extracts against *Staphylococcus aeurus* and *Dermatophilus cpngolensis* isolated from skin lesion of horses	Completed
3.	Priyanka Karela (RAJUVAS, Bikaner)	Dr Ramesh K Dedar	Effect of various desert herbs (*A. javanica, C. decidua, P. dectylefera, Z. mauritiana*) on expression of TGF beta in skin fibroblast of horse	Completed
4.	Sakshi Pandita (LUVAS, Hisar)	Dr Naveen Kumar	Studies on the miRNA response to LSD virus infection	Ongoing

Sr No	Name of the student	Name of the Guide	Project/Dissertation Title	Completed/ Ongoing
5.	Lokender Singh (LUVAS, Hisar)	Dr Naveen Kumar	m6A modification of SARS-CoV-2 RNA: Exploring the epitranscriptomic regulation of proinflammatory cytokine production	Ongoing
6.	Ram Kumar (RAJUVAS, Bikaner)	Dr Naveen Kumar	Role of ROCK-1 signaling in buffalpox virus replication	Completed
7.	Anmol Dhiman (IVRI, Izatnagar)	Dr Sanjay Kumar	In-vitro inhibitory efficacy of equine merozoite surface antigen-2 antisera during *Theileria equi* invasion into erythrocytes	Ongoing
8.	Shaurabh Daria (RAJUVAS,Bikaner)	Dr TR Talluri	Studies on Ultrasonographic, Haematological and Immunological Markers Changes during Oestrus Cycle in Mares	Ongoing
9.	Vishal Yadav (RAJUVAS, Bikaner)	Dr TR Talluri	Effect of Addition of Spirulina platensis Extract to Semen Extender on Cooled and Post-Thaw Semen Quality of Marwari Stallion	Ongoing
10.	Naina Paswan (BASU, Patna)	Dr TR Talluri	Effect of melatonin supplementation to the semen extender on cryopreserved stallion semen parameters	Ongoing
11.	Supriya (IVRI, Izatnagar)	Dr Nitin Virmani	Attenuation of Recombinant EHV1 Through Deletion of Glycoprotein I and its Pathological and Immunological Study in Murine Model for Selection of an Improved Vaccine Candidate	Ongoing
12.	Priya (GJU & ST, Hisar)	Dr Nitin Virmani	Diagnosis of Equine Herpesvirus 4 and its differentiation from Equine Herpesvirus 1 infection	Ongoing
13.	Ruchika (GJU & ST, Hisar)	Dr Nitin Virmani	Diagnosis of EHVI employing molecular techniques and serum neutralization assay	Ongoing

3 Trainings/ Worshops/Webinars/Meetings (National and International) attended by the Scientists

Sr No	Name of Staff	Name of Training	Organizing Institute	Period	Duration
1.	Dr SC Mehta	Webinar on "Awareness and Implementation Webinar on Indian Standards on Cattle Feed and Feed Ingredients".	Bureau of Indian Standards and CLFMA	16th March, 2022	1 day

Sr No	Name of Staff	Name of Training	Organizing Institute	Period	Duration
2.	Dr SC Mehta	23rd meeting of Animal Husbandry, Feeds and Equipment Sectional Committee, FAD 5.	Bureau of Indian Standards	22nd March, 2022	1 day
3.	Dr Sanjay Barua	International satellite seminar on Inadequacies of veterinary vaccines against emerging infectious diseases in animals including poultry	Rajasthan University of Veterinary & Animal Sciences, Bikaner and Indian Association for the Advancement of Veterinary Research at Udaipur, Rajasthan	9th April, 2022	1 day
4.	Dr RK Vaid	Use of validated protocol for estimation of antimicrobial usages (AMU) at farm level	ICAR-Directorate of Poultry Research, Hyderabad and National Institute of Plant Health Management, Hyderabad	25th - 26th April 2022.	2 day
5.	Dr Harishankar Singha, Dr Shanmugasundaram K, Dr Riyesh T, Dr Anubha Pathak, & Sh. Ajmer Singh	Workshop on NGS Pipeline and Computer Aided Drug Designing	ICAR-NRCE, Hisar in association with Altem Technologies Pvt Ltd., Bengaluru	25th -27th April, 2022	3 day
6.	Dr SC Mehta	Webinar on “Building a shared future for all life” by Dr. B.P. Mishra, Director, NBAGR, Karnal. Organised on International Biodiversity Day.	National Bureau of Animal Genetic Resources, Karnal	22nd May, 2022	1 day
7.	Dr SC Mehta	National Symposium on Indian Agriculture after Independence. (Virtual)	DDG (Agril Engineering), ICAR, New Delhi	24th May, 2022	1 day
8.	Dr SC Mehta	FAD 5 /P-4: Standards for transportation of animals	FAD 5 / P-4 Bureau of Indian Standards	16th June, 2022	1 day
9.	Dr SC Mehta	International Yoga Day	Kendriya Yoga evm Prakrit Chikitsa Parishad, Ayush Mantralaya, Government of India	21st June, 2022	1 day

Sr No	Name of Staff	Name of Training	Organizing Institute	Period	Duration
10.	Ms Ana Raj J	Orientation Workshop for Nodal Officers of Disaster Management of Ministries/ Departments of Government of India	National Institute of Disaster Management (NIDM), New Delhi	27th – 28th June, 2022	2 days
11.	Dr SC Mehta	Webinar on "Assessing Animal Science Technology Contributions to Livestock and Poultry Sector Growth - Need of the Hour" by Dr. K M Bujarbaruah.	DDG (Animal Science), ICAR, New Delhi	28th June, 2022	1 day
12.	Dr RK Vaid, Dr Rajender Kumar & Dr Nitin Virmani	India Animal Health Summit 2022, NASC Complex, New Delhi	Department of Animal Husbandry and Dairying, *New Delhi*	6th - 7th July 2022	2 days
13.	Dr SC Mehta	Azadi Ka Amrit Mahotsav, Lecture by Dr. K. K. Sharma (Virtual),	ICAR Hq, New Delhi	14th July, 2022	1 day
14.	Dr SC Mehta	Executive Committee Meeting	ISAGB, Karnal	15th July, 2022	1 day
15.	Dr SC Mehta	Stakeholders meeting to discuss Standard National Action Plan 2022 for Agriculture Sector.	Bureau of Indian Standards	18th Aug., 2022	1 day
16.	Dr RK Vaid & Dr Shanmugasu-ndaram K	Workshop on Biosafety and biosecurity issues in relation to Anthrax	Department of Vet Public Health, College of Veterinary Sciences, LUVAS, Hisar	25th -26h Aug., 2022	2 days
17.	Dr Anuradha Bhardwaj	ILRI-ICAR Sponsored five days Hands-on-Training Advanced Biotechnological Approaches to Augment Productivity in Poultry for Ensuing Food and Nutritional Security	ICAR- Directorate on Poultry Research, Hyderabad	20th -24th Sept., 2022	5 days

Sr No	Name of Staff	Name of Training	Organizing Institute	Period	Duration
18.	Sh. Ajmer Singh	National Conference on Business, Economics, Social Science and Humanities (NCBESSH-22)	ARDA Conference, Chennai, organized at Patna	2nd Oct., 2022	1 day
19.	Dr Thirumala Rao Talluri	National Dialogue on Semen Biology for enhancing the fertility	DUVASU, Mathura	14th Oct., 2022	1 day
20.	Sh. Ajmer Singh	National Conference on Business, Economics, Social Science and Humanities (NCBESSH-22)	ARDA Conference, Chennai, organized at Udaipur	6th Nov., 2022	1 day
21.	Dr Naveen Kumar	National Workshop and Brainstorming on "Strategy on control and Eradication of Formidable Transboundary Viral diseases of Livestock"	GADVASU, Ludhiana	14th -15th Nov., 2022	2 days
22.	Dr Yash Pal, Dr RA Legha & Dr Thirumala Rao Talluri	Stakeholder meeting on "Improvement of Donkey & Non-Bovine Milk"	ICAR-NRC on Camel, Bikaner in collaboration with International Livestock Research Institute (ILRI)	13th Dec., 2022	1 day

4. Lead Papers/Invited Papers/Invited Expert Lectures

1. Dr Anju Manuja delivered an Invited lecture on "Nano-based drug delivery systems for therapeutic applications in animals" in XXXIV Annual Conference of IAVMI on the theme "Current Trends in Immunodiagnostics and Vaccinology for Health of Livestock & Poultry" from May 27-28, 2022.
2. Dr Anuradha chaired a session at National Conference on "Natural Sciences - Exploration through Innovations (NSEI)" at School of Basic and Applied Sciences, Maharaja Agrasen University, Baddi, during MAY 27-28, 2022.
3. Dr Harisankar Singha delivered six expert lectures on "Diagnosis of glanders: serological and molecular methods", "Glanders surveillance status and National Action plan" and "Zoonotic importance of glanders" in 3 days Training Programmes (2 programmes) organized at NRCE, Hisar on 03-05 March 2022 and 6-8 September, 2022 and two Workshops organized at Rohtak on 15 March, 2022 and at NRCE, Hisar on 6 July, 2022 for Medical /Veterinary Officers and livestock farmers on Glanders.
4. Dr Naveen Kumar delivered an invited lecture on "Development of SARS-CoV-2 vaccine for animals: Indian perspectives" in 22nd Veterinary Congress, Indian Association for Advancement of Veterinary Research (IAAVR) on "Advancement in Veterinary Medical Research contributing to "One health" for betterment of animal and public health and their welfare, held at RAJUVAS, Udaipur form April 8-9, 2022.

5. Dr Naveen Kumar Invited to deliver a lecture on “Lumpy skin disease: Indian Perspectives” Invited by Rajasthan University of Veterinary and Animal Sciences (RAJUVAS), Bikaner on December 28, 2022.

6. Dr Naveen Kumar Invited to deliver a lecture on “Management of Lumpy skin disease in India” in a symposium organized by West Bengal University of Veterinary and Animal Sciences and West Bengal Veterinary Council on December 22, 2022.

7. Dr Naveen Kumar invited to deliver and expert lecture on “Host-directed antiviral therapy” in Indian Virological Society, National conference VIROCON 2021 on “Emerging and reemerging viral diseases – climate change impacts and mitigation” by virtual mode On March 27, 2022.

8. Dr Naveen Kumar invited to deliver expert views for NAVS-GADVASU National Workshop and Brainstorming on “Strategy on control and Eradication of Formidable Transboundary Viral diseases of Livestock” organized by GADVASU Ludhiana from November 14-15, 2022.

9. Dr Nitin Virmani delivered an invited lecture on “Current Trends in Immunodiagnostics and Vaccinology for Health of Livestock & Poultry" during XXXIV Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in infectious diseases and National Conference on “Current Trends in Immunodiagnostics & Vaccinology for Health of Livestock & Poultry” at LUVAS, Hisar from May 27 to May 28, 2022

10. Dr Nitin Virmani delivered an invited talk on “Spatio-Temporal Analysis of Important Equine Viral Respiratory Pathogens and Vaccine Development Strategies for their Control” during International Conference (IAVP) on “Global Challenge in Rapid Diagnosis and Management of Animal and Poultry Diseases for Improved Health and Productivity” held at College of Veterinary Science, Telangana Veterinary University, Hyderabad on November 17-19, 2022.

11. Dr Riyesh T. delivered an invited lecture on “Avian influenza” in the one day “Public Health Awareness Workshop on Zoonotic Diseases” held at Maharishi Dayanand University, Rohtak. The event was organized by ICAR- NRCE, Hisar in collaboration with department of Animal Husbandry, Haryana on 15 March 2022.

12. Dr Sanajay Barua Invited as a speaker in the international satellite seminar on Inadequacies of veterinary vaccines against emerging infectious diseases in animals including poultry on 9th April 2022 on the topic entitled “Development of a homologous lumpy skin disease virus vaccine in India” organized by Rajasthan University of Veterinary & Animal Sciences, Bikaner and Indian Association for the Advancement of Veterinary Research at Udaipur, Rajasthan

13. Dr Sanjay Barua delivered an invited lecture on “Development of a homologous lumpy skin disease virus vaccine in India” in XXIX Annual conference of IAAVR, organized by Rajasthan University of Veterinary & Animal Sciences, Bikaner, and Indian Association for the Advancement of Veterinary Research [IAAVR] on 8-9 April 2022. on “Advancement in Veterinary Medical Research contributing to “One health” for betterment of animal and public health and their welfare, held at Udaipur from April 8-9, 2022.

14. Dr Sanjay Kumar delivered a invited lecture on “Homology Modeling and Drug Design” during National Workshop on Novel strategies in Genome based therapeutics organized by Department of Genetics, Maharishi Dayanand University, Rohtak on 11th October, 2022.

15. Dr SC Mehta acted as Panel speaker at Brain Storming Session -Breed Watch List: Status & Way Forward during National Symposium on “Contemporary Technology for Animal Genetic Resource (AnGR) Management” at ICAR- NBAGR, Karnal during September 21-22, 2022.

16. Dr SC Mehta delivered a lead Paper on “Improvement of efficiency and profitability from hill and pack animals”. In: XVI Annual Conference of Indian Society of Animal Genetics & Breeding (ISAGB) organized by ICAR-DPR, Hyderabad during 2nd & 3rd December 2022.

17. Dr SC Mehta delivered a lecture on “Status and Conservation of Donkey Genetic Resources” in three days training programme on Entrepreneurship development Programme in Donkey Farming (on 17.10.2022) organized by ICAR-NRCE, EPC, Bikaner during October 17-19, 2022.

18. Dr SC Mehta delivered a lecture on "Status and Conservation of Donkey Genetic Resources" in three days training programme on Entrepreneurship development Programme in Donkey Farming (on 2.9.2022) organized by ICAR-NRCE, EPC, Bikaner during September 2-4, 2022.

19. Dr SC Mehta delivered a lecture on "Status and Conservation of Donkey Genetic Resources" in three days training programme on Entrepreneurship development Programme in Donkey Farming (on 22.8.2022) organized by ICAR-NRCE, EPC, Bikaner during August 22-25, 2022.

20. Dr SC Mehta delivered a lecture entitled "Application of Molecular Markers for Improvement of Plant Production" in ICAR Winter School on "Advances in Irrigation Technology and Nutrient Management in Arid Horticultural Crops" on 26.3.2022. Organised by Swami Keshwanand Rajasthan Agricultural University, Bikaner during March 8-28, 2022.

21. Dr SC Mehta delivered an expert lecture on "Prospects and constraints in equine production vis- a-vis management of zoonotic diseases" in DBT sponsored workshop on "Recent Advances in Diagnosis and Management of Zoonotic Diseases" on 22.4.2022. Organised by ICAR-NRC on Camel, Bikaner during April 19-25, 2022.

22. Dr SC Mehta delivered expert lecture on "Indigenous Horse Breeds of India" and conducted the session that was organised by Indigenous Sports Horse Equestrian League (ISHEL) on August 27, 2022.

23. Dr Shanmugasundaram K delivered an expert lecture on "Bovine Tuberculosis" in the public Awareness Workshop on Zoonotic Diseases. Maharishi Dayan and University, Rohtak, and the event was organized by ICAR- NRCE, Hisar in collaboration with department of Animal Husbandry, Haryana on 15 March 2022.

24. Dr Shanmugasundaram K delivered an expert lecture on "Diagnosis of glanders: serological and molecular methods" during training programme for Field Veterinary Officers' on Hands on Training Programme on Diagnosis and Control of Equine Glanders. This training program organised at ICAR-NRCE, Hisar, 06-09 September 2022.

25. Dr Shanmugasundaram K delivered an expert lecture on "Sample collection, processing and dispatch for glanders diagnosis" during training programme for Field Veterinary Officers' Hands on Training Programme on Diagnosis and Control of Equine Glanders. This training program was organised at ICAR-NRCE, Hisar, 06-09 September 2022.

26. Dr Shanmugasundaram K delivered an expert lecture on "Zoonotic Mycobacterial infections of animals, their diagnosis and control" in the training Programme on Approaches for Diagnosis of Zoonotic Bacterial Infections. This training program organised by ICAR NRCE on 03-05 March 2022.

27. Dr Taruna Anand delivered a special address at the round table session under "One Health Approach to AMR & its challenges in Animal Origin Food Value Chain" on Nov 2, 2022 "in the brainstorming session, at Bihar Animal Science University, Patna, Bihar on 25, March 2022.

28. Dr Taruna Anand delivered an invited lecture on "Novel therapeutic strategy of Phage Therapy to Tackle Emerging Antimicrobial Resistance – an exclusive way to treat biofilm forming and food borne pathogens" during XXXIV Annual Convention of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases organized from May 27-28, 2022, at Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana.

29. Dr Taruna Anand delivered an invited talk entitled "Efficacy Studies on Bacteriophages at Two Fronts: Single Phage in Combination with Antibiotics Assessed in vitro and Phage Cocktails Applied in vivo Lab Animal Models" during Society for Bacteriophage Research and Therapy during IIIrd International Conference on Bacteriophage Research and AMR, 26h-27th November 2022, Karnatak University, Dharwad, Karnataka.

30. Dr TR Talluri delivered a talk on "Emerging techniques for assessing the fertility in male livestock" at DST-SERB sponsored workshop conducted on Genome analysis methods for molecular genetic studies and disease diagnosis from 9th-10th March, 2022 at ICAR-NRC on Camel, Bikaner, Rajasthan.

31. Dr TR Talluri delivered an expert lecture on "Cell culture techniques for diagnosis of viral diseases" at Karyashala organized by SERB-DST (High end workshop) on "Recent Advances in Diagnosis and Management of Zoonotic Diseases "from 19th to 25th April 2022 at ICAR-NRC Camel, Bikaner, Rajasthan.

32. Dr TR Talluri delivered an invited talk on "Exceptions in equine reproduction" at College of Veterinary Sciences, GADVASU, Ludhiana, Punjab on 28th March 2022 under NAHEP plan of Institutional developmental plan.

33. Dr TR Talluri delivered an invited talk on "Reproductive disorders in equines" at College of Veterinary Sciences, GADVASU, Ludhiana, Punjab on 30th March 2022 under NAHEP plan of Institutional developmental plan.

34. Dr TR Talluri delivered an invited talk on "Status and applications of ART technologies in equine reproduction in India" on at College of Veterinary Sciences, GADVASU, Ludhiana, Punjab on 29th March 2022 under NAHEP plan of Institutional developmental plan.

35. Dr TR Talluri presented an invited lead paper on "Application of ART in equine reproduction to conserve the indigenous equine germplasm" at XXXVII Annual Convention of ISSAR conducted on "Optimizing animal reproduction through recent techniques of biotechnology, nutraceuticals and alternative medicine" from 16-18 November 2022 at College of Veterinary Science and Animal Husbandry, Jabalpur.

36. Dr TR Talluri presented an oral presentation on "Multi-omics analyses for bull fertility prediction" at International conference on reproductive healthcare and 32nd Meeting of the ISSRF held at New Delhi from 11-13th Feb 2022.

37. Dr TR Talluri delivered an expert lecture on "Emerging techniques for assessing the fertility in male livestock"at DST-SERB sponsored workshop conducted on Genome analysis methods for molecular genetic studies and disease diagnosis.9th-10th March, 2022.Pg no 77-87.

38. Dr TR Talluri delivered an expert lecture on "Reproduction in donkeys" during the training programme on Entrepreneurship development programme on Donkey farming. Sept 02-04, 2022.

39. Dr TR Talluri delivered an expert lecture on "Reproduction in donkeys" during in three days training programme conducted on "Entrepreneurship development Programme in Donkey Farming" organized by ICAR-NRCE, EPC, Bikaner during August 22-25, 2022.

40. Dr TR Talluri delivered an expert lecture on "Collection and cryopreservation of jack semen" during in three days training programme conducted on "Entrepreneurship development Programme in Donkey Farming organized by ICAR-NRCE, EPC, Bikaner during Sept 02-04, 2022.

41. Dr TR Talluri delivered an expert lecture on "Collection and cryopreservation of jack semen" In three days training programme organized on Entrepreneurship Development Programme in Donkey Farming organized by ICAR-NRCE, EPC, Bikaner during August 22-25, 2022.

42. Dr Yash Pal delivered an invited talk on "Pregnancy diagnosis in mares" on 28th March 2022 under NAHEP plan of Institutional developmental plan at College of Veterinary Sciences, GADVASU Ludhiana, Punjab.

43. Dr Yash Pal presented an invited lead paper on "Nutraceutical Properties of Equine Milk". In Compendium of "IVth International Conference in Hybrid Mode on Innovative and Current Advances in Agriculture & Allied Sciences" (ICAAAS-2022) during 12-14 June 2022 at Himachal Pradesh University, Summer Hill, Shimla (H.P.) India pp 50-54.

WORKSHOPS, SEMINARS AND INSTITUTIONAL ACTIVITIES 05

A. Workshops Organized

1. Workshop on NGS Pipeline and Computer Aided Drug Designing conducted at ICAR-NRCE

A Workshop on "Next Generation Sequence Pipeline and Computer Aided Drug Designing" was organized at ICAR-NRC on Equines, Hisar from 25th to 27th April 2022. This workshop was organized in collaboration with Altem Technologies Pvt. Ltd, Bengaluru. In this workshop, a total of 22 participants representing different ICAR institutes (ICAR-IVRI, ICAR-CIRB, ICAR-NRCE, ICAR-NDRI); States Universities (LUVAS, Hisar; CBLU, Bhiwani; GJU & ST, Hisar; GCW, Hisar) have participated. A hands-on-Practice session were delivered on the Fundamentals Pilot Script and Protein Modelling, structure-based drug designing, simulation, and virtual screening. Ms Dhivya Shanmugarajan, Senior Application Scientist, Altem Technologies was the resource person for this workshop. The Bioinformatics Facilities operational at ICAR-NRCE was utilized in this workshop. Dr Sanjay Kumar and Dr Rajesh Kumar Vaid were the Organising and Co-organising Secretaries for this workshop.

Participants of the three days Bioinformatics Workshop at ICAR-NRCE, Hisar

2. Public Health Awareness Workshop

One day "Public Health Awareness Workshop on Zoonotic Diseases" was held at Maharishi Dayanand University, Rohtak, on 15 March, 2022 and the event was organized by ICAR- NRCE, in collaboration with the Department of Animal Husbandry, Haryana. In this workshop, 60 participants from the Animal Husbandry department and farmers of Rohtak and adjacent districts took part. The goal of this workshop was to inform the Animal Husbandry workers and farmers about common zoonotic diseases that can be spread from the livestock. Lectures and discussions on various zoonotic diseases such as Glanders, Bovine Tuberculosis, Brucellosis, Leptospirosis, Scrub Typhus, Rabies, Bird flu and Japanese Encephalitis were delivered and followed by a discussion with the participants.

3. Workshop on "Zoonotic Diseases: Prevention and Control"

One day Workshop on "Zoonotic Diseases: Prevention and Control" was organised at ICAR-NRCE on 06

July, 2022. The objective of the programme was to commemorate the World Zoonoses Day and also to apprise the participants about importance of zoonotic diseases. A total of 101 participants from different fields such as medical and veterinary professionals, researchers, laboratory technicians, students/RA/SRF were attended the workshop. Dr Ratna Bharti, Chief Medical officer, Hisar and Brig. SS Balaje, Commandant EBS, Hisar graced the occasion as Chief Guest and Guest of Honor respectively. Emphasize was given on better collaboration and real time sharing of data (on zoonosis) between medical, veterinary and wildlife professional for the control of zoonotic diseases in the region.

Participants during workshop session Zoonotic Diseases: Prevention and Control

4. A Workshop for Forest Officers on Awareness of Wildlife Protection Act and Zoonotic Diseases from Wildlife at ICAR-NRCE

A Workshop was conducted at ICAR-NRCE, Hisar on 26th June, 2022 for Forest Officers on Awareness of Wildlife Protection Act and Zoonotic Diseases from Wildlife. Officers from Wildlife department of Hisar, Fateahbad, Sirsa, Jind, Bhiwani, Charkhi-dadri took part in the workshop. DFO, Mr. Ved Prakash Singh and Inspector Mr. Jaivinder Singh delivered talks about the Wildlife Protection Act and Dr. Baldev Raj Gulati, Principal Scientist of the centre delivered lecture on Zoonoses and Wildlife: One Health approach.

5. Rabies awareness workshop

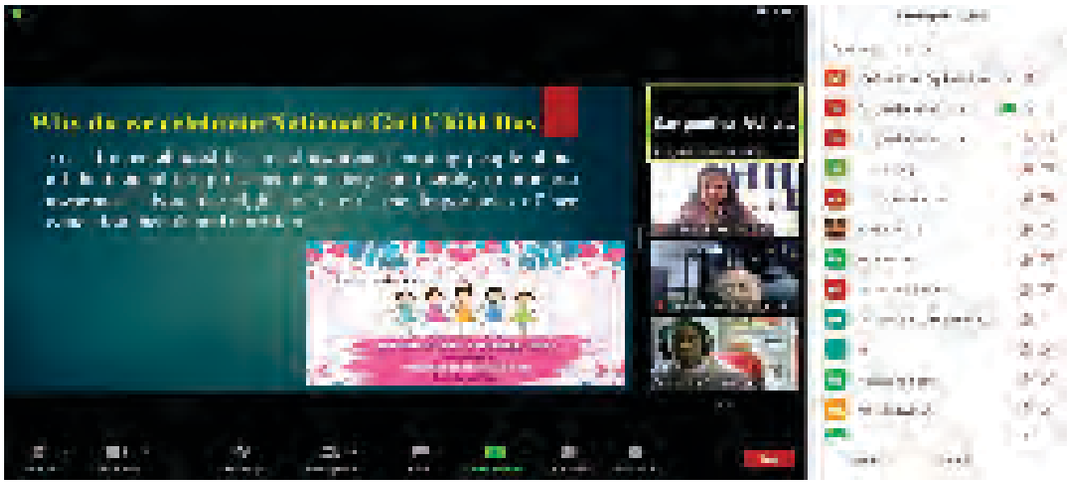
A workshop on “World Rabies Day" was organised by ICAR-NRCE on 27 September 2022 at Shree Krishna Pranami Public School Hisar Haryana on the eve of World Rabies Day. A total of 200 students participated in this awareness workshop. A lecture describing the spread of the disease and its symptoms in humans and dogs and measures to be taken for prevention and control of rabies was delivered. The role of young adults in fulfilling the mission of eradication of Rabies by 2030 was elaborated to the students. The program was successfully conducted under the patronship of Dr Yash Pal, Director, ICAR-NRCE and the chairmanship of Dr Baldev Raj Gulati, Principal Scientist, ICAR-NRCE.

School Students and NRCE Experts during workshop on “World Rabies Day”

B. Seminars and Webinars Organized

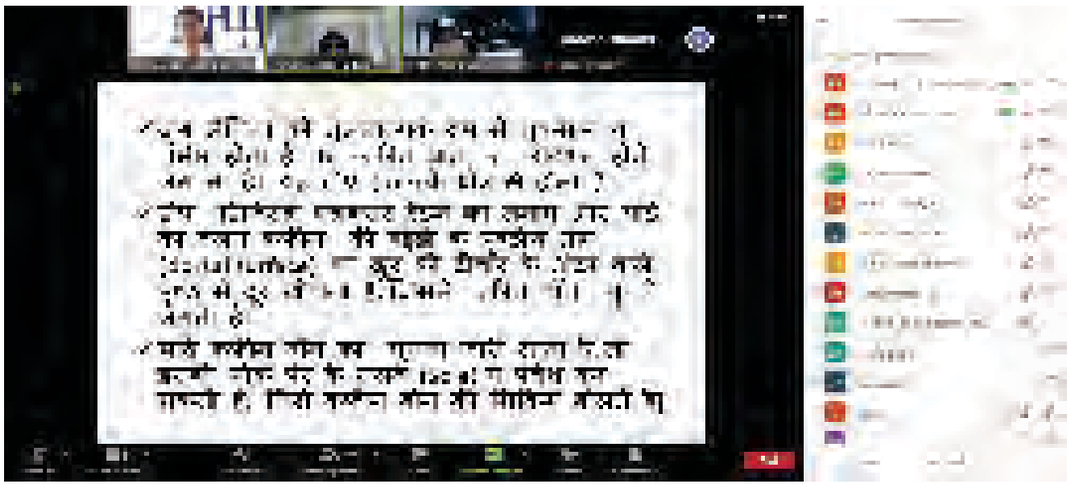
1. *Azadi ka Amrit Mahotsav* webinar lecture series

a) Strategies for nutritional empowerment of girl children: A webinar on 'Strategies for nutritional empowerment of girl children' was organised through zoom platform on 28th January, 2022 at 3 PM. A total of 28 participants attended the lecture and got awareness about the status of girl children in the state of Haryana. Director, ICAR-NRCE stressed on the importance of empowerment of girl children and women in his opening remarks. Dr Sangeetha V, Senior Scientist, ICAR-IARI delivered a lecture on the nutritional options to improve women's health in India. She also addressed the problems faced by girl children and the significance of "National Girl Child Day" observed on January 24 every year.

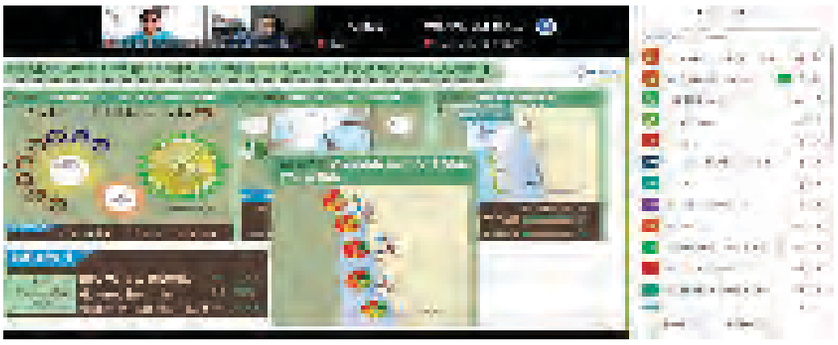


b) Virtual mode workshop on "*Karma* and Spirituality": A workshop was organised in the zoom platform in collaboration with Science of Spirituality (SOS), New Delhi on 11/02/2022 (Friday) at 3.30 PM. A total of 38 participants including scientific, technical and administrative staff of the centre attended the workshop and were benefitted.

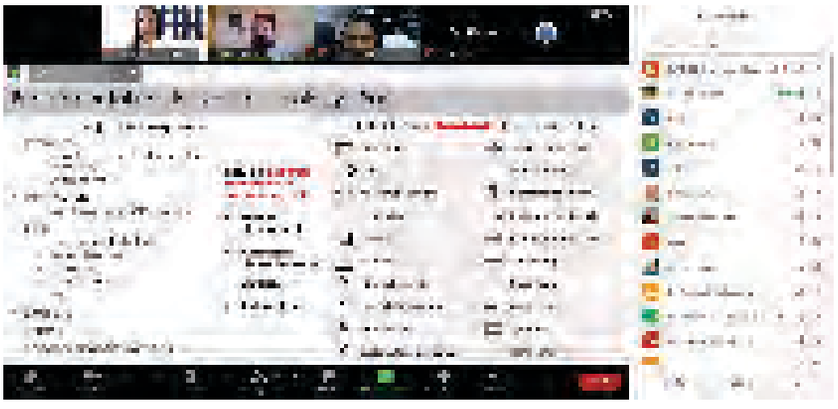
c) Laminitis: Treatment and Management: A webinar on "Laminitis: Treatment and management" was organised on February 23, 2022 at 3.30 PM for all equine stakeholders including farmers, veterinarians and scientists. A total of 34 participants attended the lecture and were benefitted. The session was chaired by Dr Yash Pal, Director, ICAR-NRCE.



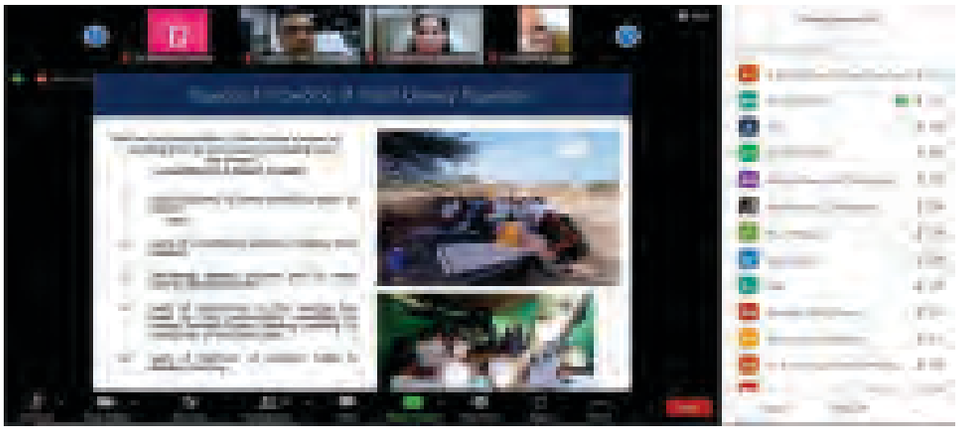
d) National Science Day: A national webinar was organised on National science day 28 February, 2022. Dr P Krishnan, Director, BOBP-IGO delivered a lecture on "Strengthening science-policy interface for a sustainable future". A total of 37 participants attended the online lecture conducted on the zoom platform. Dr Yash Pal, Director, ICAR – NRCE chaired the session.



e) Frontiers in synthetic biology: A webinar on "Frontiers in synthetic biology" was organised at ICAR-NRCE, Hisar on 14/03/2022. Dr Chetan Aditya, Institute Pasteur, Paris delivered the lecture and explained different research avenues in synthetic biology. Total of 39 participants attended the lecture including scientists, students and technicians.



f) Conservation and management of Halari donkey: ICAR-NRCE, Hisar celebrated the World Donkey day 2022 by organizing a webinar on "Conservation and management of Halari donkey" on May 7, 2022. Dr Manoj Mishra, Executive Director (Sahjeevan) and Director (Livelihoods) for Centre for Pastoralism delivered the expert lecture which was participated by 56 equine stakeholders like scientists, veterinarians, development professionals, students, NGOs, etc. The talk was focused on the prominence of donkeys in rural livelihoods and the alarming rate of donkey population decline.



C. Participation in Melas and Exhibitions

1. State level Animal Fair 2022, Bhiwani

A three-day animal fair was organised by the Department of Animal Husbandry and Dairying, Government of Haryana from 25 - 27 February 2022. ICAR-NRCE, Hisar has reached out to more than 1000 visitors/farmers through its stall in the *mela*. The visitors were made aware about different equine breeds and diseases. Technologies developed at NRCE are also displayed for the benefit of different stakeholders.

2. *Pashudhan Mela*, Tilwara, Rajasthan

A three-day animal fair was organised by ICAR at Tilwara, Rajasthan from 1 to 3 April, 2022. ICAR-NRCE, Hisar has reached out to more than 1200 visitors through its stall in the mela. The visitors were made aware about different equine breeds, equine diseases, and technologies of ICAR-NRCE.

3. ICAR-NRCE Participation in Exhibition at Directorate of Poultry Research

Exhibition at National conference on "Innovations in animal genetics and breeding for sustainable productivity of livestock and poultry" organized by ICAR – Directorate of Poultry Research (DPR), Hyderabad during December 2 to 3, 2022. The scientists from the centre took part and showcased the various technologies developed by the ICAR-NRCE.

Participation in Exhibition at Directorate Poultry Research

D. Equine Health Camps/ *Kisan Ghoshthi* Organized

***Mera Gaon Mera Gaurav* - Kisan Ghoshthi :** Scientists and Staff members from EPC, ICAR-NRCE, Bikaner visited the adopted village Bhojoosar, Bikaner under MGMG activity. Importance of deworming and the role of macro and micro minerals in health, vigour and fertility were discussed. Control of ectoparasites was also explained. Entrepreneurship in the livestock sector was explained with local success stories. The medicines for deworming, mineral mixture and ecto-parasiticidal were distributed to 21 farmers possessing 156 cattle, 1 camel and 1 buffalo.

Staff members from EPC with villagers at Bhojoosar, Bikaner

Equine Health Camp

a. Kherampur: As a part of the 75th year of Indian independence (*Azadi ka Amrut Mahotsav*), an equine health camp was organized at Kherampur village, Hisar on March 8, 2022. In the health camp, 14 horses were examined by a multidisciplinary team of scientists and technical officers of the centre and addressed the treatment for various ailments. The equine bio-samples were also collected from the animals for different epidemiological studies. After the camp, a farmer – scientist interaction meeting (*Kisan gosthi*) was organized in the veterinary dispensary premises. The scientists responded to the queries of equine owners on deworming, vaccination, pregnancy etc. and encouraged them to undertake income generation activities with horses. The equine owners also shared their difficulties in equine management, which they are experiencing daily. Finally, they were appraised about the extension services provided by the centre.


Equine Health Camp at Kherampur

b) Equine health camp at Rodha, Bhiwani : An equine health camp was organized at Rodha village, Bhiwani on April 12, 2022. In the health camp, total 9 horses were examined by a multidisciplinary team of scientists and technical officers for ailments, if any. Based on examination, prescriptions were given to the equine keepers and the details of their equines were registered. Pregnancy diagnosis was also performed and injured horses were treated.


Equine Health Camp at Rodha

c) Equine Health Camp and Kishan Gosthi, Julana, Jind (Haryana) : The health camp was organised in June 23, 2022. In this camp 10 equines were brought and 25 equine farmers took part in Kisan Gosthi.

d) Equine Health Camp and Kishan Gosthi, Pabra, Hisar (Haryana) : The health camp was organised in July 27, 2022. In this camp 12 equines were examined for illness by multidisciplinary team of scientists and technical officer. Pregnancy diagnosis was done in 4 mares by rectal palpation. Biological sample were collected from all available equines and all were provided anthelmintic bolus and mineral mixture. Kisan Gosthi was organized and 50 farmers took part and were benefitted.

E. Institutional Activities

1. Swachhta Pakhwada

Swachhta Pakhwada activities were organised from 16/12/2022 to 31/12/ 2022, NRCE, Hisar and EPC Bikaner including '*Kisan Diwas*' on 23rd December 2022 at ICAR-NRCE. Various activities have been carried out

such as taking *Swachhta* pledge, stock taking on digitization of office records/ e-office implementation, sanitation and SWM cleanliness and sanitation drive in programmes in the villages adopted under the *Mera Gaon Mera Gaurav* village community. *Swachhta* activities including implementation SAP, awareness on recycling of wastewater, water harvesting for kitchen gardens in residential colonies, Lecture on "Biological Waste Disposal" by Dr Sanjay Barua, Pr. Scientist, NCVTC, ICAR-NCRE, celebration of Special Day – *Kisan Diwas*, cleaning of public places, community market places and/or nearby tourist/selected spots, organising competition in the campus, etc were conducted in the NRCE, Hisar and EPC Bikaner.

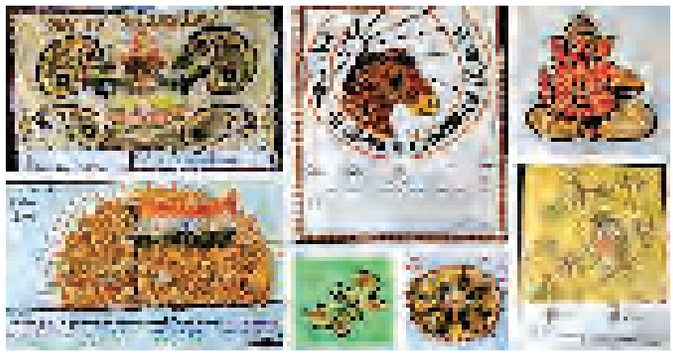
Taking Swachhta Pledge at NRCE Main Campus

Village Community Activity by EPC, Bikaner

2. Organizing SEEDARIUM competition (*Rangoli* with pulses only)

A SEEDARIUM Competition was organized by ICAR-NRCE, Hisar to mark the celebration of 4th. "World Pulses Day" on February 10, 2022. The staff of ICAR-NRCE, Hisar along with the students and kids participated in the competition with great zeal. The *rangoli* with pulses only was selected to spread the awareness about the importance of pulses in foods and to illustrate their nutritional value. A glimpse of the competition has been provided below. Prizes were also decided for the winners.

Participants on Rangoli Competition



SEEDARIUM (Rangoli with pulses only)

3. Republic Day celebrations at Centre

Republic Day celebrations at the main campus of ICAR-NRCE and EPC Bikaner celebrated with great enthusiasm and pride on January 26, 2022. The Director at ICAR-NRCE Hisar and Officer I/C at EPC, Bikaner hoisted the National Flag and inspired the staff and families by commemorating a series of incidents in the history of India.

Republic day celebrations at ICAR-NRCE, Hisar & EPC, Bikaner

4. Independence day celebrations ICAR-NRCE

Independence Day programme was celebrated on August 15, 2022, in the campus premises. After hoisting of the National Flag, Dr Yash Pal, Director inspired the staff and families by commemorating the martyrs' struggle for freedom from British rule. While recalling the achievements of the institute for the year, the Director congratulated the staff and inspired the staff to accomplish the new horizons in their scientific endeavour.

Independence day celebrations ICAR-NRCE, Hisar

5. Tiranga Yatra

Tiranga Yatra was organized on 12 August, 2022 under "*Azadi Ka Amrit Mahotsav* " and the National Flags were distributed for "*Har Ghar Tiranga*". The *Tiranga* Yatra was led by honourable Vice-chancellor of Bikaner Technical University Prof. Ambrish S Vidyarthi, and the programme was sponsored and participated by Bharat Vikas Parishad, Samta Seva Kendra, Engineers' Association (retired) and staff members.

Staff and other personnel participating in the *Tiranga Yatra*

6. International *Yoga Day* celebrated at the Centre

International Yoga Day was celebrated at ICAR-NRCE, Hisar at the Centre on 21 June 2022. The Staff members of the Centre collectively and actively participated in *Yoga* Day celebrations. *Yoga* activities were conducted by Sh.Ajmer Singh. He explained the benefits of each *yoga* for health. Congratulating staff members on the occasion of International *Yoga* Day, Dr. Yash Pal, Director, NRCE emphasized the importance of *Yoga* for improving health and productivity of the employees. He encouraged staff members to regularly organize such activities at the NRCE Campus.

Yoga Day Celebration at ICAR-NRCE, Hisar

7. Celebration of 38th Foundation day at ICAR-NRCE Hisar

The 38th foundation day of ICAR-NRCE was celebrated on November 26, 2021 with great fan favour. On this occasion a tree plantation drive was organized in the animal shed area of the campus. Institute staff members participated in the event under the chairmanship of Dr Yash Pal, Director, ICAR-NRCE.

Tree Plantation on Foundation Day of ICAR-NRCE, Hisar

8. Celebration of Foundation day at EPC, ICAR-NRCE Bikaner

The 34th Foundation Day was celebrated with great pride and enthusiasm at Equine Production Campus, ICAR-NRCE, Bikaner on 28 September,2022. On this occasion an interactive meet with the equine farmers and breeders was organized. A Horse show was also conducted, and the winners were awarded. A quiz programme was also conducted for the farmers on the basic upkeep of the equines. On this occasion a technical bulletin entitled "Stallion Semen Evaluation Techniques' ' was released by the Chief Guest, Dr A Sahoo, Director, ICAR-NRC on Camel, Bikaner, Rajasthan. Dr Yash Pal, Director, ICAR-NRCE presided over the function and appraised the dignitaries about the research activities and accomplishment of the Centre in equine health and production. Director. During this occasion, outstanding scientific team award at NRCE for the year was also conferred on Dr TR Talluri, Dr RA Legha and Dr Yash Pal. These awards were distributed by the Chief Guest. On this occasion Dr Nirmala Saini, Incharge, ARC, ICAR-CSWRI, Bikaner and Dr S K Yadava, Incharge, Regional station, ICAR-CAZRI, Bikaner also present as Guests of honour.


Celebration of Foundation day and facilitating the equine farmers at EPC Bikaner


Release of technical bulletin and award distribution at Foundation day EPC, Bikaner

9. Fit India Freedom Run 3.0 activity

From 2nd October to 31st October 2022 under *Azadi Ka Amrit Mahotsav* (AKAM) initiative, fit India Freedom Run 3.0 activity was organized in the ICAR-NRCE, Hisar and EPC Bikaner. All staff members participated with great zeal and ran around campus in the event.


10. आयोजित तिमाही हिन्दी बैठकें/कार्यशालायें/गतिविधियां

भा०कृ०अनु०परि०–राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार में दिनांक 14.09.2022 से 20.09.2022 की अवधि के दौरान हिन्दी सप्ताह का आयोजन किया गया। हिन्दी सप्ताह के दौरान हिन्दी कार्यशाला, हिन्दी टंकण, हिन्दी शब्दावली, वर्ग पहेली, हिन्दी प्रश्नोत्तरी, हिन्दी की प्रसिद्ध रचनाएं व उनके रचनाकार और आशुभाषण प्रतियोगिता में प्रथम, द्वितीय व तृतीय स्थान प्राप्त करने वाले सभी विजेताओं को नकद पुरस्कार एवं प्रशस्ति प. प्रदान कर सम्मानित किया गया। मूल्यांकन समिति द्वारा चुने गए हिन्दी में सर्वाधिक कार्य करने वाले कर्मचारियों को भी पुरुस्कृत किया गया। हिन्दी कार्यशाला के दौरान डॉ० राजपाल सहायक प्राध्यापक (हिन्दी शाखा), राजकीय स्नातकोत्तर महाविद्यालय, हिसार ने अपने कथन में हिन्दी का उच्चारण

व उनके शब्दों की गरीमा के बारे में बताया। समापन समारोह के मुख्य अतिथि श्री मुकेश जैन, निदेशक उत्तरी क्षेत्र फार्म मशीनरी प्रशिक्षण और परीक्षण संस्थान (टी०टी०सी०), हिसार ने इस बात पर जोर दिया कि जिन देशों ने उन्नति की है उनकी अपने एक भाषा के रूप में भी पहचान बनी है, वे अपनी भाषा में बात करने में गर्व महसूस करते है। तिमाही के दौरान हिन्दी कार्यान्वयन हिन्दी सलाहकार समिति एवं हिन्दी में किये गए सर्वाधिक कार्य के मूल्यांकन के लिए गठित समितियों की बैठक आयोजित की गई।

वर्ष 2022 के दौरान राष्ट्रीय अश्व अनुसंधान केन्द्र, अश्व उत्पादन परिसर, बीकानेर में :

दिनांक	गतिविधियां
25.01.2022	**मुख्य हिन्दी वक्ता** : डॉ० वत्सला पाण्डेय, वरिष्ठ साहित्यकार एवं लेखक, बीकानेर **व्याख्यान विषय** : ''राष्ट्र का स्वाभिमान और स्वमान हिन्दी'' विषय पर कार्यशाला **मुख्य अतिथि** : श्री राजेन्द्र जोशी, साहित्यकार, कवि एवं शिक्षाविद, बीकानेर
30.06.2022	**मुख्य हिन्दी वक्ता** : श्री ताराचन्द रेपस्वाल, प्रधानाध्यापक, राजकीय प्रवेशिका संस्कृत विद्यालय, बीकानेर **व्याख्यान विषय** : ''हिन्दी भाषा में अनुस्वार का उपयोग एवं शुद्ध उच्चारण'' विषय पर कार्यशाला **मुख्य अतिथि** : प्रो. सतीश कुमार गर्ग, माननीय कुलपति, राजस्थान पशु चिकित्सा एवं पशु विज्ञान, विश्वविद्यालय, बीकानेर
14.09.2022 21.09.2022	**मुख्य अतिथि** : प्रो. एव.सी. गोस्वामी, Dean Student's Wlfare (DSW), राजस्थान पशु चिकित्सा एवं पशु विज्ञान विश्वविद्यालय, बीकानेर **कार्यकारी अध्यक्ष** : डॉ. रामेश कुमार देदर, कार्यकारी अध्यक्ष राजभाषा एवं प्रभारी, अश्व उत्पादन परिसर, बीकानेर

अश्व उत्पादन परिसर में हिन्दी सप्ताह का आयोजन

IRC, RAC AND IMC MEETINGS 06

1. 25th Research Advisory Committee (RAC) meeting of ICAR- NRCE

The 25th RAC meeting of ICAR- National Research Centre on Equines was held under the Chairmanship of Dr. M C Sharma (Former Director, IVRI) on 06th June 2022 to review the research for the year 2021-22. Dr. Yash Pal, Director, ICAR-NRCE gave a brief presentation on the overall achievement of the Centre for the year 2021-22 which was followed by presentations on equine health, equine production and NCVTC. A total of 37 research projects were discussed in the meeting. Director ICAR-NRCE presented an overview of the achievements, activities of the institute and action taken reports on the recommendations of previous RAC meetings. He also apprised the technologies developed and released by the institute. Action taken report was thoroughly discussed and the Chairman expressed his satisfaction on the initiatives taken by ICAR- NRCE based on the RAC recommendations. The chairman appreciated the contribution of the scientists for achievements of the Centre in the field of Research and Development. The Chairman emphasized the need to transfer the technologies to equine farmers and also initiate extension activities in equine populated areas of Himachal Pradesh, Jammu and Kashmir, Uttrakhand etc. In his concluding remarks the Chairman urged the scientists to work hard for the upliftment of the equine sector in the country.

25th Research Advisory Committee meeting being chaired by Dr. M C Sharma (Former Director, IVRI)

2. Annual Institute Research Committee (IRC) Meeting (2021-22)

The annual IRC meeting for the year 2021-22 of ICAR-NRCE was held on 30 June, 21 July, and 22 August 2022 under the chairmanship of Dr. Yash Pal, Director, ICAR-NRCE. A total of 32 research projects and 2 new concept notes were discussed in the meeting. Dr Yash Pal, Director, ICAR-NRCE appreciated the research activities and output from the scientists. He motivated the scientists to do research relevant to the stakeholders' needs. He also motivated the scientists to apply for external funding. He also guided the scientists to work in collaborative mode and to share resources for judicious utilization. He requested to focus on commercialization of technologies and based on farmers' demand.

Annual Institute Research Committee Meeting chaired by Dr Yash Pal, Director ICAR-NRCE

3. Half Yearly Institute Research Committee (IRC) Meeting (2022-23)

The Half Yearly IRC meeting for the year 2022-23 of ICAR-NRCE was held on 22 December 2022 under the chairmanship of Dr. TK Bhattacharjya, Director, ICAR-NRCE. In this meeting, 35 research projects including 16 externally funded projects were discussed and two concept notes were presented and discussed. The Chairman congratulated and complimented the institute scientists for running 16 externally funded projects. He urged all the scientists to apply for at least 2-3 externally funded projects every year so that the institute can be self-sufficient in terms of funds. The Chairman also asked the scientists to aim for development of at least one product per year (in the form of vaccine/diagnostic kit) and also requested to identify the major issues in the field and the research should be focused on addressing the same. He also indicated that there is a need to develop cost effective vaccines and point of care diagnostics. He opined that, scientists working in equine production area should concentrate on quantitative traits of economic importance, evaluation of donkey milk for its commercial use, draftability/load carrying capacity of donkeys and mules, germplasm conservation of animals located in southern and north-eastern part of the country. And whole genome sequencing of all established and non-characterized equine breeds to estimate genetic distance among them. He further stressed that NCVTC scientists should go for whole genome sequencing of all important microbial isolates available with them. He also instructed scientists to develop a road map for the commercialization of existing technologies. In his concluding remarks, the Chairman congratulated all the scientists for the good research work they have done during the period under report.

Half Yearly Institute Research Committee Meeting chaired by Dr TK Bhattacharjya, Director ICAR-NRCE

4. Institute Management Committee Meeting (IMC):

The 41st Institute Management Committee meeting of ICAR- NRCE was held on 6th August 2022 under the chairmanship of Dr Yash Pal, Director ICAR-NRCE, Hisar. The IMC meeting confirmed the Proceedings of 40th meeting of IMC held on 15.07.2020. The action taken report of the 40th IMC meeting was presented and discussed. The following agendas were approved in this meeting:

1. Constitution of Grievance Committee at NRCE, Hisar.
2. Write-off losses of animals at Equine Production Campus, ICAR-NRCE, Bikaner and Main Campus, NRCE, Hisar.
3. Extension of Terms of Part Time Doctor (AMAs) at NRCE, Hisar.
4. Financial status of NRCE, Hisar: Smt. Shammi Tyagi, Sr. Finance & Accounts Officer briefed the IMC about the financial status of the Centre. She informed that during the financial year 2021-22, there was 100 % utilization of funds under NRCE including SCSP, NEH & TSP as well as NCVTC.

5. Memorandum of Understanding (MoU)

The ICAR-NRCE, Hisar linked the Memorandum of Understanding with the Guru Angad Dev Veterinary & Animal Sciences University (GADVASU), Ludhiana and Swami Keshwanand Rajasthan Agricultural University, Bikaner for the cooperation in the areas of Research and Education. Dr. Yash Pal (Acting Director) NRCE, Hisar and Vice Chancellors of the Universities signed the Memoranda. With the MoU, the NRCE and Universities have agreed for collaborative programmes in the fields of research, education, training and capacity building, extension consultancy and other areas of national interest. Both the partners have also agreed for mutually recognizing the faculty of both the Institutes for the research and teaching purposes, wherein, the students and faculties can carry out the specific, research and outreach activities at the laboratories of these institutions.

VISIT OF DIGNITARIES 07

1. Dr BN Tripathi, DDG (AS) visited Equine production Campus, ICAR-NRCE, Bikaner, Rajasthan

Dr BN Tripathi, Deputy Director General (Animal Science), ICAR visited the Equine production Campus, ICAR-NRCE, Bikaner, Rajasthan on 27th May 2022. During his visit to the Centre Dr BN Tripathi inaugurated the *Kisan Seva Kendra* cum Equine Museum, Post-Mortem facility and a feed unit. He also interacted with the scientists of the Campus and urged them to reorient the research priorities for bringing out innovations that promote novel technologies for the benefit of equine breeders and farmers. Dr Tripathi also interacted with the equine farmers and farmers belonging to the SC&ST community and distributed mineral mixtures and certificates for progressive farmers. Dr. Yash Pal, Director, ICAR-NRC on Equines and Dr. Artabandhu Sahoo, Director, ICAR-NRC on Camel also present in the interactive meeting.

Dr BN Tripathi, DDG (AS), inaugurating the *Kisan Seva Kendra* cum Equine Museum at EPC, ICAR-NRC on Equines, Bikaner, Rajasthan

DDG (AS) inaugurating the Equine feed unit at EPC, ICAR-NRC on Equines, Bikaner, Rajasthan

2. Padma Shri Dr Omesh Kumar Bharti visited ICAR-NRCE

Dr Omesh Kumar Bharti, Padma Shri awardee and a leading expert in rabies visited the campus on 6th July, 2022. He visited various laboratories of the institute and delivered an expert lecture on the topic "Intersectoral co-ordination saves lives of animals from impending rabies- A One Health Approach" on "World Zoonoses Day".

Dr Omesh Kumar Bharti addressing the audience on world zoonoses day

3. Col. K. Lakshmi Narayan appreciates NRCE research activities

Col. K. Lakshmi Narayan, RVC Centre, Meerut visited ICAR-NRCE, Hisar on Aug 11, 2022 along with seven Army officers. He interacted with scientists of the Centre on the research activities being undertaken on equine health and production. He thanked Director, NRCE for whole hearted support in managing health issues of equines at Meerut Centre.

4. Sh. G.P Sharma, Director Finance, ICAR visits ICAR-NRCE

Sh. G.P Sharma, Director Finance visited ICAR-NRCE on Apr 30, 2022. During his visit the water logging problems at the farm areas of ICAR-NRCE were discussed and he assured all support to ICAR-NRCE.

5. Dr. PK Uppal, applauds contributions of NRCE

Dr PK Uppal, Former Director, ICAR-NRCE visited the institute on May 28, 2022. Dr Uppal applauded the research work being undertaken by the scientists and was impressed with the technologies being generated by the NRCE team.

6. Sh. RK Singh, appreciates R&D activities at NRCE

Sh. RK Singh Secretary DADF, New Delhi visited the Centre Oct 29, 2022 along with other staffs of DADF. He congratulated NRCE staff for the brilliant research work being carried out at the Centre and motivated scientists to work in team mode to achieve more in coming years

Sh. RK Singh visits large animal shed, ICAR-NRCE, Hisar

7. Prof Jitender Prasad, Chairman Dept. of Sociology, MDU Rohtak visits ICAR-NRCE

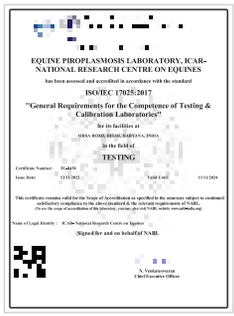
Prof Jitender Prasad, Chairman Dept. of Sociology, MDU Rohtak visited ICAR-NRCE on 25 August, 2022. Prof. Prasad visited different laboratories of the campus and was very much impressed by activities undertaken at the Centre

INFRASTRUCTURE, DEVELOPMENTAL ACTIVITIES AND HERD STRENGTH 08

1. e-Governance

At NRCE e-governance has already been made functional. FMS/MIS has been functioning for a long time, while e-office has also been functioning since July 2020. Files are being moved through e-office which has enabled expeditious disposal of matters. FMS/MIS is being used effectively for various matters including pay roll module and leave applications and sanctioning. e-HRMS is also likely to be implemented shortly. Annual Performance Appraisal Reports (APARs) of all the staff (except Scientific) are being written in Smart Performance Appraisal Report Recording Online Window (SPARROW) from the year 2021-22. SPARROW is also being implemented for writing APARs of Scientists from the year 2022-23.

2. Accreditation of Equine Piroplasmosis Laboratory as per ISO-17025:2017


EQUINE PIROPLASMOSIS LABORATORY, ICAR-NATIONAL RESEARCH CENTRE ON EQUINES

has been assessed and accredited in accordance with the standard

ISO/IEC 17025:2017

"General Requirements for the Competence of Testing & Calibration Laboratories"

for its facilities at

SIRSA ROAD, HISAR, HARYANA, INDIA

in the field of

TESTING

Certificate Number: TC-8438

Issue Date: 12/11/2022

Valid Until: 11/11/2024

This certificate remains valid for the Scope of Accreditation as specified in the annexure subject to continued satisfactory compliance with the requirements of the Accreditation Standard & the rules of NABL. (To see the scope of accreditation of the laboratory, please visit NABL website www.nabl.qci.org)

Name of Legal Identity :- ICAR-National Research Centre on Equines

Signed for and on behalf of NABL

Dr. S. Venkateswaran
Chief Executive Officer

Equine Piroplasmosis Laboratory at ICAR-NRCE was accredited as per ISO-17025:2017 NABL guidelines. The accreditation will be for a period of one year (11th November 2024) as per certificate number TC-8438. NABL accreditation was issued in compliance with General Requirements for the Competence of Testing & Calibration Laboratories. The scope of the laboratory accreditation includes – diagnosis of the bio-samples using ELISA, cELISA and IFAT for *Theileria equi* and *Babesia caballi* infection.

3. Inaguaration of *Kisan Seva Kendra* cum Equine Museum at EPC, Bikaner

Dr BN Tripathi, Deputy Director General (Animal Science), ICAR visited the Equine production Campus, ICAR - National Research Centre on Equines, Bikaner, Rajasthan on 27th May 2022. During his visit to the centre Dr. B N Tripathi, DDG (AS), ICAR, has inaugurated the *Kisan Seva Kendra* cum Equine Museum. The museum displays of know-hows ad facts about various breeds of equines and importance of equines.

Dr BN Tripathi, DDG (AS), inaugurating the *Kisan Seva Kendra* cum Equine Museum at EPC, ICAR-NRC on Equines, Bikaner, Rajasthan

4. Inaguaration of Feed unit and postmortem facilities at EPC Bikaner

Dr B N Tripathi DDG (AS), during his visit to Equine Production campus, ICAR-NRC on Equines, Bikaner has also inaugurated a Post-Mortem facility. This facilitates the systematic postmortem examination of the equines. During his visit, he also inaugurated other facilities like feed unit, which will serve the purpose of making feed pellets.

DDG (AS) inaugurating the Equine feed unit & Post-Mortem Facility

In his visit, DDG (AS) has interacted with the scientists of the campus, he urged scientists for focusing on the outreach & effective communication of the scientific research to the stakeholders and reorienting the research priorities for bringing out innovations that promote novel technologies for the benefit of equine breeders and farmers. DDG (AS) has also interacted with the equine farmers and farmers belonging to the SC&ST community and distributed mineramixture and certificates for progressive farmers.

Earlier, welcoming the dignitaries, Dr Yash Pal, Director, ICAR-NRC on Equines, underlined the Institute's achievements and its mandates. A total of 40 stakeholders participated in the Interface Meeting. Dr Artabandhu Sahoo, Director, ICAR-NRC on Camel, also participated in the interaction meeting of Hon'ble DDG with equine farmers.

Equine herd strength at EPC, Bikaner during the period 01/01/2022 – 31/12/2022

	Marwari		Kathiawari		Zanskari		Manipuri		Poitou		Halari		Mule		Nukra		Total
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Stock as on 01.01.2022		**32**	**02**	**05**	**03**	**06**	**03**	**02**	**11**	**23**	**03**	**06**	**02**	**01**	**02**	**00**	**123**
Birth		3	0	0	0	0	0	0	3	3	0	1	0	0	0	0	16
Purchased		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Death		0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	4
Auctioned /Sold		8	1	1	0	0	0	0	6	9	0	1	1	1	0	0	37
Balance as on 31.12.2022		**27**	**01**	**04**	**03**	**06**	**03**	**01**	**06**	**17**	**03**	**06**	**01**	**--**	**02**	**--**	**98**
Total	**45**		**05**		**09**		**04**		**23**		**09**		**01**		**02**		**98**

Equine herd strength at ICAR-NRCE, Hisar during the period 01/01/2022 – 31/12/2022

	Horses		Ponies		Donkeys		Total
	M	F	M	F	M	F	
Stock as on 01.01.2022	**02**	**07**	**--**	**05**	**07**	**13**	**34**
Birth	01	--	--	--	06	04	11
Purchased	--	--	--	--	--	--	--
Death	01	02	--	--	01	--	4
Auctioned /Sold	--	--	--	--	--	--	--
Balance as on 31.12.2022	**02**	**05**	**--**	**05**	**12**	**17**	**41**
Total	**07**		**05**		**29**		**41**

AWARDS, RECOGNITIONS, PERSONAL MILESTONE, NEW JOINING AND PROMOTIONS

09

1. Awards & Recognitions

a. **Dr Taruna Anand** received Outstanding Scientist Award, 2022 from Society for Bacteriophage Research and Therapy during IIIrd International Conference on Bacteriophage Research and AMR, 26h-27th November 2022, Karnatak University, Dharwad, Karnataka.

b. **Dr Taruna Anand** got selected to serve as core committee member of Society for Bacteriophage Research and Therapy.

c. **Dr Riyesh T** received best oral presentation award (second prize) for the oral presentation delivered on the topic entitled "A calcimimetic compound AC265347 impairs multiple steps of Newcastle disease virus replication" in the National conference on Current trends in immunodiagnostics & vaccinology for health of livestock & poultry organized by Dept of Veterinary microbiology, College of Veterinary Sciences, LUVAS, Hisar from May 27-28, 2022.

d. **Dr BC Bera** received "ISVIB-IVRI Mukteswar Albert Linghard Memorial Award-2021" by Indian Society for Veterinary Immunology and Biotechnology (ISVIB) in 2022.

e. **Dr BC Bera** received first prize for best oral presentation on "Recombinant neurovirulent Equine herpesvirus-1 generated from non-neurogenic strain by inserting point mutation (A2254G) in DNA polymerase gene" in National Conference of Virology (VIROCON-2021) at All Indian Institute of Medical Sciences, Hyderabad, Telengana on March 26-28, 2022.

f. **Dr Sanjay Barua** was nominated as a member of DPC under CAS for conducting DPC for considering cases of promotion of Scientists of ICAR- DFMD, ICFMD, Arugul, Bhubaneswar, and Odisha on 19.07.2022 & 20.07.2022.

g. **Dr Sanjay Barua** was nominated by the Council (ICAR) as a Member of Institute Management Committee (IMC) of ICAR- DFMD, ICFMD, Arugul, Bhubaneswar, Odisha for a period of 3 years w.e.f. 18.10.2022.

h. **Dr TR Talluri, Dr Yash Pal and Dr RA Legha** were awarded with Certificate of Appreciation from ICAR-NRCE in recognition of outstanding team contributions in the scientific category on 15th Aug 2022.

i. **Dr S C Metha** has been nominated as the member of the committee constituted for deciding "Standards on Cattle Housing", FAD-5, Bureau of Indian Standards.

j. **Dr Anuradha Bhardwaj** has been nominated as Resource Person for ILRI's Stakeholders meetings at Delhi, Lucknow, Dehradoon and Bikaner for expertise on donkey milk and milk products.

k. **Dr Anuradha Bhardwaj** has been nominated as Resource Person for Round Table Conference on "Harnessing Full Nutraceutical Potential of Non-Bovine Milk" organized by National Research Centre on Camel at Bikaner on 28th April, 2022.

l. **Dr TR Talluri** was selected for best PDF research story of AWSAR award instituted by DST.

m. **Dr TR Talluri** was awarded with First prize in the oral presentation category during the 5th Global Meet on Science and Technology for minimizing innovation conducted by the Hi-Tech Horticultural society at Meerut on 08-9th Dec 2022.

n. **Dr TR Talluri** and his team were awarded with Certificate of Appreciation from ICAR-NRCE in recognition of outstanding team contributions in the scientific category on 15th Aug 2022.

o. **Dr Anju Manuja** was selected as Fellow of National Academy of Agricultural Sciences, India.

p. **Dr Anju Manuja** was selected felicitated by OM Sterling University for the exemplary efforts in the health field on International Women's Day.

q. **Drs Gulati BR , Pathak A*, Maan S , Mor S , Kumar D, Soman R , Punia S , Chaudhary D, Khurana** awared with Best oral presentation award on the topic "Whole Genome Sequencing Reveals Equine Rotavirus A of Bat Origin from India" at XXXIV Annual convention of Indian association of veterinary microbiologists, immunologists and specialists in infectious diseases and national conference on "Current Trends in Immunodiagnostics & Vaccinology for Health of Livestock & Poultry" held from May 27-28, 2022 at (ICAR-Centre for Advanced Faculty Training) College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.

2. Personal Milestones

a. **Dr Rajesh Kumar Vaid** took up as In-Charge National Centre for Veterinary Type Cultures, NRCE, Hisar from 8th September 2022 onwards.

b. **Dr Taruna Anand** (I/c Bacteriophage Laboratory at NCVTC) got selected as ICAR-National Fellow with effect from 16th November, 2022 and was granted a project for Rs.1.44cr (5 years) on bacteriophage application.

3. New Joining

a. **Shri Raj Kumar** joined as Sr. Administrative Officer at this Centre on 23rd May, 2022 on transfer from ICAR-IIMR, Ludhiana. He has about 36 years of experience in administration.

b. **Dr Tarun Kumar Bhattacharjya** joined on 15.11.2022 as Director of ICAR-NRCE.

Dr. Yash Pal Welcoming Dr. T. K. Bhattacharjya

4. Transfer

a. **Dr. Praveen Malik** Principal Scientist transferred on 19.09.20221 to enable him to join his duties at ICAR Hqrs, New Delhi.

b. **Sh. K.S. Meena**, ACTO transferred to ICAR-IISWC, RC, Kota, Rajasthan on 16.03.2022.

c. **Dr. B.R. Gulati**, Principal Scientist transferred on 13.11.2022 to ICAR- NIVEDI, Bengaluru, Karnataka for the post of Director.

5. Superannuation

Sh. Mardan, SSS, retired on 31.01.2022.

Sh. S.P. Kaushik, AAO retired on 31.03.2022.

RESEARCH PUBLICATIONS 10

A. Research publications of ICAR-NRCE scientists during the year 2022

1. Anand T, Vashisth M, Jaglan A, Nokhwal A, Virmani N, Bera BC, Vaid RK, Singh RK, Tripathi BN. Phage therapy in tackling AMR: Potential and prospects. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases. 2022;43(1):50-7.
2. Balena V, Pradhan SS, Bera BC, Anand T, Sansanwal R, Khetmalis R, Madhwal A, Bernela M, Supriya K, Pavulraj S, Tripathi BN. Double and quadruple deletion mutant of EHV-1 is highly attenuated and induces optimal immune response. Vaccine. 2022 (Accepted).
3. Bera BC, Anand T, Pavulraj S, Balena V, Pradhan S, Singh RK, Tripathi BN, Virmani N. Attenuation of equine herpesvirus 1 through deletion of gE gene and its pathological evaluation in murine model. Acta Virologica. 2022 (Accepted).
4. Bhutia WD, Gupta S, Rani R, Batra K, Sethi K, Kumar S, Kumar R. In vitro and in vivo evaluation of kinase and protease inhibitors against *Trypanosoma evansi*. Veterinary Research Communications. 2022 Jun 25:1-3.
5. Brangsch H, Singha H, Laroucau K, Elschner M. Sequence-based detection and typing procedures for *Burkholderia mallei*: Assessment and prospects. Frontiers in Veterinary Science. 2022;9.
6. Chander Y, Kumar R, Verma A, Khandelwal N, Nagori H, Singh N, Sharma S, Pal Y, Puvar A, Pandit R, Shukla N. Resistance evolution against host-directed antiviral agents: Buffalopox virus switches to use p38-ϒ under long-term selective pressure of an inhibitor targeting p38-α. Molecular Biology and Evolution. 2022 Sep;39(9):msac177.
7. Deepak D, Jhamb D, Nirwan S, Juneja R, Singh J, Gaur M, Talluri T. Comprehensive study on dynamics of early embryonic development in Marwari mares. The Indian Journal of Animal Sciences. 2022;92(3).
8. Ebenezer Samuel King JP, Kumaresan A, Talluri TR, Sinha MK, Raval K, Nag P, Karuthadurai T, Aranganathan V. Genom-wide analysis identifies single nucleotide polymorphism variations and altered pathways associated with poor semen quality in breeding bulls. Reproduction in Domestic Animals. 2022 Oct;57(10):1143-55.
9. Ebenezer Samuel King JP, Sinha MK, Kumaresan A, Nag P, Das Gupta M, Arul Prakash M, Talluri TR, Datta TK. Cryopreservation process alters the expression of genes involved in pathways associated with the fertility of bull spermatozoa. Frontiers in Genetics. 2022 Oct 25;13:1025004.
10. Elango K, Kumaresan A, Talluri TR, Raval K, Paul N, John ES, Peter MK, Patil S, Verma A. Impact of sperm protamine on semen quality and fertility. Journal of Reproductive Healthcare and Medicine. 2022;3(5):1.
11. Garhwal R, Sangwan K, Mehra R, Kumar N, Bhardwaj A, Pal Y, Buttar HS, Kumar H. A Systematic Review of the Bioactive Components, Nutritional Qualities and Potential Therapeutic Applications of Donkey Milk. Journal of equine veterinary science. 2022 May 6:104006.
12. Giri SK, Nayan V, Legha RA, Pal Y, Bhardwaj A. Characterization of Partial Sequence of Myostatin Gene Exon 2 along with SNP detection in Indian Horse Breeds (*Equus caballus*). Journal of Equine Veterinary Science. 2022 Sep 1;116:104047.

13. Gupta S, Vohra S, Sethi K, Gupta S, Bera BC, Kumar S, Kumar R. In vitro anti-trypanosomal effect of ivermectin on *Trypanosoma evansi* by targeting multiple metabolic pathways. Tropical Animal Health and Production. 2022 Aug;54(4):240.

14. Gupta S, Vohra S, Sethi K, Gupta S, Kumar S, Kumar R. Gene expression study to elucidate the anti-trypanosomal activity of quinapyramine methyl sulphate (QPS). Parasitology International. 2022 Dec 1;91:102632.

15. Jaglan AB, Anand T, Verma R, Vashisth M, Virmani N, Bera BC, Vaid RK, Tripathi BN. Tracking the phage trends: A comprehensive review of applications in therapy and food production. Frontiers in Microbiology. 2022 Nov 24;13:993990.

16. Jhamb, D., Sharma, S., Talluri, T.R., Nirwan, S.S., Juneja, R., Pargi, K., Tanwar, A., Gaur, M., Gautam, L.K. and Kumar, P. (2022). Effect of trehalose addition in semen extender on sperm abnormalities of cryopreserved stallion semen. Haryana Vet. 60(2): 166-169.

17. Jhamb D, Tallurib T, Junejaa R, Sharmaa S, Nirwana S, Mehtab S, Kumarb R, Kumara P, Pargia K, Gaura M. Cryopreservation has detrimental effect on mitochondrial membrane potential, DNA integrity and oxidative parameters of equine spermatozoa. Cryobiology. 2022 Dec 1;109:55.

18. Kues WA, Kumar D, Selokar NL, Talluri TR. Applications of genome editing tools in stem cells towards regenerative medicine: An update. Current Stem Cell Research & Therapy. 2022 Apr 1;17(3):267-79.

19. Kumar A, Bhalothia SK, Talluri TR, Dangi SS, Tomar AK, Patel AK, et al. Melatonin and Canthaxanthin Ameliorate Oxidative Stress and Improve Semen Quality: A Special Reference to Ram. Andrology.2022 Aug 10;11:266.

20. Kumar N, Barua S, Kumar R, Khandelwal N, Kumar A, Verma A, Singh L, Godara B, Chander Y, Kumar G, Riyesh T, Sharma DK, Pathak A, Kumar S, Dedar RK, Mehta V, Gaur M, Bhardwaj B, Vyas V, Chaudhary S, Yadav V, Bhati A, Kaul R, Bashir A, Andrabi A, Yousuf RW, Koul A, Kachhawaha S, Gurav A, Gautam S, Tiwari HA, Munjal VK, Gupta MK, Kumar R, Gulati BR, Misri J, Kumar A, Mohanty AK, Nandi S, Singh KP, Pal Y, Dutt T, Tripathi BN. Evaluation of the safety, immunogenicity and efficacy of a new live-attenuated lumpy skin disease vaccine in India. Virulence. 2023 Dec;14(1):2190647.

21. Kumar N, Tripathi BN. A serious skin virus epidemic sweeping through the Indian subcontinent is a threat to the livelihood of farmers. Virulence. 2022 Dec 31;13(1):1943-4.

22. Kumar R, Bhardwaj P, Soni M, Choudhary S, Singh R, Kumar A, Asrani RK, Virmani N, Gupta VK, Tripathi BN. Cytopathological, ultrasonographic and hematological studies on the ameliorative effects of *Saussurea costus* (Falc.) Lipsch root extract against DMBA induced mammary tumors in female Sprague Dawley rats. Indian Journal of Veterinary Pathology, 2022 Jan to Mar; 46 (1): 59-69.

23. Kumar R, Chander Y, Khandelwal N, Verma A, Rawat KD, Shringi BN, Pal Y, Tripathi BN, Barua S, Kumar N. ROCK1/MLC2 inhibition induces decay of viral mRNA in BPXV infected cells. Scientific Reports. 2022 Oct 24;12(1):17811.

24. Kumar R, Duhan JS, Manuja A, Kaur P, Kumar B, Sadh PK. Toxicity assessment and control of early blight and stem rot of Solanum tuberosum L. by mancozeb-loaded chitosan–gum acacia nanocomposites. Journal of Xenobiotics. 2022 Apr 14;12(2):74-90.

25. Kumar R, Gupta S, Bhutia WD, Vaid RK, Kumar S. Atypical human trypanosomosis: Potentially emerging disease with lack of understanding. Zoonoses and Public Health. 2022 Jun;69(4):259-76.

26. Kumar R, Khandelwal N, Chander Y, Nagori H, Verma A, Barua A, Godara B, Pal Y, Gulati BR, Tripathi BN, Barua S. S-adenosylmethionine-dependent methyltransferase inhibitor DZNep blocks transcription and translation of SARS-CoV-2 genome with a low tendency to select for drug-resistant viral variants. Antiviral Research. 2022 Jan 1;197:105232.

27. Kumar R, Sethi K, Gaur DK, Goyal SK, Kumar S, Jain S, Kumar S. Use of recombinant calflagin protein as a potential candidate for diagnosis of *Trypanosoma evansi* infection. Veterinary Parasitology. 2022 Oct 1;310:109776.

28. Kumar R, Sethi K, Jindal N, Kumar S, Tripathi BN. Immunosorbent assay for detection of *Trypanosoma evansi* infection in multiple host species using chimeric protein A/G conjugate. Research in Veterinary Science. 2022 Dec 20;152:604-9.

29. Kumar S, Ashraf M, Pannu U, Mehta SC. Single Nucleotide Polymorphism in Interleukin-6 and Interleukin-8 Genes of Equines. Journal of Equine Veterinary Science. 2022 Oct 1;117:104058.

30. Kumari A, Sharma P, Bhardwaj A. Exploration and Insights of Potential Probiotics of Donkey Milk from a Rural Indian Village. Biology and Life Sciences Forum. 2022; 15(1):35.

31. Madhwal K, Supriya, Rhushikesh S. Khetmalis, Stephanie S. Pradhan, Venkataramireddy Balena, Bidhan Chandra Bera, Taruna Anand, B.N. Tripathi and Virmani Nitin. Comparative pathology of equine herpesvirus 1 and equine influenza infection in BALB/c mouse model". 2022; Indian J Veterinary Pathology.

32. Manuja A, Kumar B, Athira S, Sarkar P, Riyesh T, Kumar N, Tripathi BN, Mann B. Zinc oxide nanoparticles encapsulated in polysaccharides alginate/gum acacia and iron oxide nanomatrices show enhanced biocompatibility and permeability to intestinal barrier. Food Hydrocolloids for Health. 2022 Dec 1;2:100050.

33. Manuja A, Kumar B, Chhabra D. Chloroquine Chaos and COVID-19: Smart delivery perspectives through pH sensitive polymers/micelles and ZnO nanoparticles. Arabian Journal of Chemistry. 2022 Nov 30:104468.

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35. Nayan V, Singh K, Iquebal MA, Jaiswal S, Bhardwaj A, Singh C, Bhatia T, Kumar S, Singh R, Swaroop MN, Kumar R. Genome-Wide DNA Methylation and Its Effect on Gene Expression During Subclinical Mastitis in Water Buffalo. Frontiers in Genetics. 2022 Mar 15;13:828292.

36. Nokhwal A, Anand T, Vaid RK. Bacteriophage therapy: an emerging paradigm in fish disease management. Aquaculture International. 2023 Apr;31(2):777-805.

37. Pal V.K., Singh A., Kumar S. and Kumar R. 2022. Chemotherapeutic effect of trypanocidal drugs in naturally occurring equine trypanosomosis. Haryana Veterinarian, 61: 38-41.

38. Pathak A, Gulati BR, Maan S, Mor S, Kumar D, Soman R, Punia S, Chaudhary D, Khurana SK. Complete Genome Sequencing Reveals Unusual Equine Rotavirus A of Bat Origin from India. Journal of Virology. 2022 Oct 26;96(20):e01408-22.

39. Rani R, Narsiman B, Varma RS, Kumar R. Gum-based nanocapsules comprising naphthoquinones enhance the apoptotic and trypanocidal activity against *Trypanosoma evansi*. European Journal of Pharmaceutical Sciences. 2022 Apr 1;171:106118.

40. Rani R, Sethi K, Gupta S, Varma RS, Kumar R. Mechanism of Action and Implication of Naphthoquinone as Potent Anti-trypanosomal Drugs. Current Topics in Medicinal Chemistry. 2022 Oct 1;22(25):2087-105.

41. Rani R, Sethi K, Kumar S, Varma RS, Kumar R. Natural naphthoquinones and their derivatives as potential drug molecules against trypanosome parasites. Chemical Biology & Drug Design. 2022 Dec;100(6):786-817.

42. Raval, K., Paul, N., Nag, P., K, E., Pal, Y., R A, L., Talluri, T. R., & Kumaresan, A. Asthenozoospermic stallions tend to have high acrosome reacted spermatozoa as evidenced by dual fluorescent staining assay. The Indian Journal of Animal Sciences, 2022 92(8), 946–949.

43. Sharma P, Kumari A, Bhardwaj A, Poonia A. Blue green algal farming revolution: An eco-friendly top-up step for Agribusiness advancements. InIOP Conference Series: Earth and Environmental Science 2022 Aug 1 (Vol. 1057, No. 1, p. 012014). IOP Publishing.

44. Sharma P, Bhardwaj A, Kumari A, Pal Y. Prebiotics and Probiotics: It's Impact on Host as Internal Healers. Journal of General medicine and Clinical Practice. 2022.5(2); DOI: 10.31579/2639-4162/064

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47. Sinha MK, Kumaresan A, Rao Talluri T, Ebenezer Samuel King JP, Prakash MA, Nag P, Paul N, Raval K, Kamaraj E, V A. Single nucleotide polymorphisms cumulating to genetic variation for fertility in crossbred (Bos taurus × Bos indicus) bull spermatozoa. Animal Biotechnology. 2022 Sep 20:1-2.

48. Suthar A, Gopalakrishnan A, Maji C, Dahiya RK, Kumar R, Kumar S. Evaluation of the inhibitory efficacy of quaternary ammonium compounds on in vitro growth of *Theileria equi* parasite in MASP culture. International Journal for Parasitology: Drugs and Drug Resistance. 2022 Dec 1;20:11-6.

49. Talluri TR, Kumaresan A, Paul N, Sinha MK, Ebenezer Samuel King JP, Elango K, Sharma A, Raval K, Legha RA, Pal Y. High throughput deep proteomic analysis of seminal plasma from stallions with contrasting semen quality. Systems Biology in Reproductive Medicine. 2022 Jul 4;68(4):272-85.

50. Talluri TR, Kumaresan A, Sinha MK, Paul N, Ebenezer Samuel King JP, Datta TK. Integrated multi-omics analyses reveals molecules governing sperm metabolism potentially influence bull fertility. Scientific Reports. 2022 Jun 23;12(1):10692.

51. Thukral H, Shanmugasundaram K, Riyesh T, Kumar N, Singha H, Gambhir D, Laura A, Tiwari S, Gulati BR. Multisectoral Prioritization of Zoonotic Diseases in Haryana (India) Using One Health Approach. Preventive Veterinary Medicine. 2022 (Accepted)

52. Unnati, Bhardwaj, A., Sonali, Nayan, V., Goutam, U., Legha, R.A., Pal, Y., Kumar, J., Giri, S.K. and Tripathi, B.N. Mitochondrial DNA Variation and Genetic Relationships in Indian Halari Donkey Breed using D-Loop Region. Indian Journal of Animal Research. 2022;DOI: 10.18805/IJAR.B-4912.

53. Vashisth M, Yashveer S, Anand T, Virmani N, Bera BC, Vaid RK. Antibiotics targeting bacterial protein synthesis reduce the lytic activity of bacteriophages. Virus Research. 2022 Nov 1;321:198909.

54. Vashisth M, Yashveer S, Jaglan AB, Virmani N, Bera BC, Vaid RK, Anand T. Synergy of a virulent phage (ϕAB182) with antibiotics leading to successful elimination of biofilms formed by MDR *Acinetobacter baumannii*. Canadian Journal of Microbiology. 2022 Sep 29;68(12):731-46.

B. Research Abstracts Published in Conferences/ Symposia

1. Ajmer Singh and Yash Pal. "A Cross Sectional Study to Improve Equine Husbandry in Haryana" in National Conference on Business, Economics, Social Science and Humanities (NCBESSH-22) held at Udaipur, India on 06 November, 2022.

2. Ajmer Singh and Yash Pal. "Identification of Causes and Constraints of Equine Farmers in Haryana" in National Conference on Business, Economics, Social Science and Humanities (NCBESSH-22) held at Patna, India on 02 October, 2022.

3. Alka Nokhwal, Anu Bala, Medhavi Vashisth and Taruna Anand "Isolation and characterization of broad-spectrum bacteriophages virulent to *Aeromonas veronii* isolates of environmental origin" in 3rd International conference on Bacteriophage Research and Antimicrobial Resistance organized by Karnatak University, Dharwad and Society for Bacteriophage Research and Therapy, India during 26-27, November 2022.

4. Alka Nokhwal, R. K. Vaid, Ravikant, Taruna Anand & Rachna Gulati. "Occurrence, molecular characterization, and antibiogram of *Aeromonas* spp. isolated from low salinity fish culture ponds in Hisar district" in XXXIV Annual convention of Indian association of veterinary microbiologists, immunologists and specialists in infectious diseases and national conference on Current Trends in Immunodiagnostics & Vaccinology for Health of Livestock & Poultry organized at College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar held from 27-28 May, 2022.
5. Alka Nokhwal, R. K. Vaid, Ravikant, Taruna Anand and Rachna Gulati. "Occurrence, molecular characterization, and antibiogram of *Aeromonas* spp. isolated from low salinity fish culture ponds in Hisar district", in XXXIV Annual convention of Indian association of veterinary microbiologists, immunologists and specialists in infectious diseases and national conference on Current Trends in Immunodiagnostics & Vaccinology for Health of Livestock & Poultry organized at College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar held from 27-28 May, 2022.
6. Alka Nokhwal, Ravikant, Taruna Anand and R.K. Vaid. "Isolation and characterization of lytic bacteriophages consortia against ESBL and AmpC producing aquatic strains of *Aeromonas hydrophila*" in National conference on virology, organised by All India Institute of Medical Sciences, Hyderabad, Telangana during 26-28 March, 2022.
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C. Books

अश्व पालन व अश्व चिकित्सा लेखक डॉ. रमेश कुमार देदड़, डॉ यश पाल, रामावतार लेघा, डॉ. तिरुमला राव ताल्लुरी ISBN 978-81-959087-0-7

D. Books Chapters

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2. Paul, N., Talluri, T.R., Nag, P., Raval, K., and Kumaresan, A. (2022). Nano Purification of Semen: A Novel Technique for Enrichment of Superior Quality Spermatozoa. In: Kumaresan, A., Srivastava, A.K. (eds) Frontier Technologies in Bovine Reproduction. Springer, Singapore. http://doi.org/10.1007/978-981-19-3072-0_6

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E. Compendium Published

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G. Technical /Popular Articles

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3. RK Dedar, T R Talluri, RA Legha, SC Mehta and Yash Pal. 2022. Prevention management and treatment of laminitis in horses. Souvenir of All India Marwari Horse Society. Pg. No. 90-91.
4. Shanmugasundaram K, Singha H, Indu Bisla, R. K. Vaid and Yash Pal. 2022. Mycobacterial infection in equines. National workshop on Manipuri pony conservation and sustainable management: Emerging issues and challenges. Pp56-59. 06 October 2022. Organised by Directorate of research Central Agricultural University, Imphal under the aegis of ICAR-NRCE, Hisar
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H. Training Manual Chapters

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6. Jitendar Singh, T. R. Talluri, R. K. Dedar, R. A. Legha, S. C. Mehta, A. Bhardwaj and Yash Pal. Pregnancy diagnosis using ultrasound technique method. Training programme on Entrepreneurship development programme on Donkey farming. Sept 02-04, 2022; Pg No.120-126.
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44. TR Talluri, Yash Pal, RA Legha, SC Mehta,A Bhardwaj, RK Dedar and J Singh. Collection and cryopreservation of jack semen. Training programme on Entrepreneurship development programme on Donkey farming. Sept 02-04, 2022; Pg No. 89-95.

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46. Yash Pal and TR Talluri. Application of artificial insemination technology for speedy improvement of performance of desirable traits in horses and ponies. National workshop organised on “Manipuri pony conservation and sustainable management: emerging issues and challenges” on 6th October 2022 at CAU, Manipal. Pg No. 13-22.

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PARTICIPATION, PRESENTATION IN SEMINARS, CONFERENCES AND SYMPOSIA

11

A. Conferences/Symposia (National and International) attended by the Scientists

Sr. No.	Name of Staff	Name of Training	Organizing Institute	Period	Duration
1.	Dr BC Bera	International Conference of Indian Society for Veterinary Immunology and Biotechnology (ISVIB) & National Conference on "Transforming Livestock Economy through Innovations in Immunology and Biotechnology"	GADVASU, Ludhiana, Punjab on	4th -5th February 2022.	2 Days
2.	Dr Thirumala Rao Talluri	International conference on reproductive healthcare and 32nd Meeting of the ISSRF	All India Institute of Medical Sciences, New Delhi	11th-13th February 2022	3 Days
3.	Dr Thirumala Rao Talluri	DST-SERB sponsored workshop conducted on Genome analysis methods for molecular genetic studies and disease diagnosis.	ICAR-NRC on Camel, Bikaner, Rajasthan	9th-10th March 2022	2 Days
4.	Dr BC Bera & Dr Shanmugasundaram K	National Conference of Virology (VIROCON-2021) on "Emerging and Re-emerging viral diseases –Climate change impacts and mitigation"	All Indian Institute of Medical Sciences, Hyderabad, Telengana on.	26th -28th March, 2022	3 Days
5.	Dr Naveen Kumar	National conference VIROCON 2021 on "Emerging and reemerging viral diseases – climate change impacts and mitigation"	Virtual mode	27th March, 2022	1 Day
6.	Dr Naveen Kumar	22nd Veterinary Congress, Indian Association for Advancement of Veterinary Research (IAAVR) on "Advancement in Veterinary Medical Research contributing to "One health" for betterment of animal and public health and their welfare	RAJUVAS, Udaipur	8th - 9th April, 2022	2 Days

7.	Dr Harishankar Singha Dr Shanmugasundaram K Dr Riyesh T & Dr Anubha Pathak	XXXIV Annual convention of Indian association of veterinary microbiologists, immunologists and specialists in infectious diseases and national conference on "Current Trends in Immunodiagnostics & Vaccinology for Health of Livestock & Poultry"	ICAR-Centre for Advanced Faculty Training) College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar	27th -28th May, 2022	2 Days
8.	Dr Harishankar Singha & Dr Shanmugasundaram K	28th Annual Scientific Conference of Bangladesh Society for Veterinary Education and Research (BSVER AS Con XXVIII).	Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh (Virtual mode)	28th -29th May, 2022	2 Days
9.	Dr SC Mehta	National Symposium on "Contemporary Technology for Animal Genetic Resource (AnGR) Management".	SOCDAB and National Bureau of Animal Genetic Resources, Karnal	21st -22nd Sept., 2022	2 Days
10.	Dr Thirumala Rao Talluri	XXXVII Annual Convention of ISSAR conducted on "Optimizing animal reproduction through recent techniques of biotechnology, nutraceuticals and alternative medicine	College of Veterinary Science and Animal Husbandry, Jabalpur.	16th -18th Nov., 2022	3 Days
11.	Dr BC Bera	International Conference of Indian Association of Veterinary Pathologists on "Global Challenge in Rapid Diagnosis and Management of Animal and Poultry Diseases for Improved Health and Productivity	College of Veterinary Science, Telangana Veterinary University, Hyderabad	17th -19th Nov., 2022	3 Days
12.	Dr SC Mehta	XVI Annual Conference of Indian Society of Animal Genetics & Breeding (ISAGB)	ISAGB and ICAR-DPR, Hyderabad	2nd-3rd Dec., 2022	2 Days

ON-GOING RESEARCH PROJECTS 12

A. Equine Health

Sr. No.	Title	Team	From	To	PIMS Code/Page
1.	Surveillance, Monitoring and Control of Emerging and Existing Diseases of Equines	H. Singha*, B.R. Gulati (upto 12.11.22), R. Kumar, S. Kumar, N. Virmani, S. Barua, R.K. Vaid, R. Dedar, A. Manuja, Balvinder Kumar, K. Shanmugasundaram, Anubha Pathak, Ana Raj and Yash Pal	April, 1997	Continuous Service Project	IXX00257
2.	Biomacromolecules based nanoscaffolds for wound healing using 3D printing	Anju Manuja*, Balvinder Kumar and Riyesh T.	Oct., 2020	Sept., 2023	IXX15412
3.	Development of improved serological diagnostic assays for Surra using Trypanosoma evansi recombinant protein	Rajender Kumar*, Sanjay Kumar & BC Bera	July, 2021	June, 2022	IXX15796

B. Equine Production

Sr. No.	Title	Team	Date of Start	Date of Completion	PIMS Code
1.	Explicating genomic insights of Indigenous equines breed population through "*Computational Genomics* " and "*Artificial Intelligence*" based approaches	Anuradha Bhardwaj*, Sarika, Yash Pal, MA Iqbal and Dinesh Kumar	Dec., 2019	Nov., 2022	IXX15401
2.	Elucidation of physico-chemical, metabolomic and functional attributes of indigenous donkey milk	Anuradha Bhardwaj*, Yash Pal, RA Legha, Varij Nayan, AK Singh, PN Raju, Rajan Sharma and RK Vaid	July, 2020	June, 2023	IXX15412
3.	Characterization and recognition of Bhimthadi horse	SC Mehta* and Sachin D. Sorate	July, 2021	June, 2023	IXX15797
4.	Analysis of quantitative traits for genetic improvement of indigenous equines	SC Mehta*, RA Legha and J. Singh	April, 2021	March, 2026	IXX15798

* Principal Investigator

C. Extension Project

Sr. No.	Title	Team	From	To	PIMS Code
1.	Impact of social networking in equine extension and advisory services	Ana. J. Raj*, Ramesh Kumar Dedar, GuruRaj Makarabbi	Jan., 2021	Jan., 2023	IXX15419

* Principal Investigator

D. National Centre for Veterinary Type Culture

Sr. No.	Title	Team	Duration	To	PIMS Code
1.	Authentication and accessioning of viruses of animal origin (Service Project)	Sanjay Barua*, Naveen Kumar, B.C. Bera, Riyesh T. and Taruna Anand	May, 2015	Service Project	IXX11882
2.	Phenotypic and genotypic authentication and preservation of network bacterial isolates	R.K. Vaid*, Taruna Anand, B.C. Bera, Riyesh T. and K. Shanmugasundaram	June, 2015	Service Project	IXX11884
3.	Isolation, characterization and generation of repository of Mycobacterium species	Shanmugasundaram K.*, R.K. Vaid, B.C. Bera and B.N. Tripathi	Oct., 2017	March., 2022	IXX13994
4.	Development of respository of respiratory viruses of livestock and isothermal based diagnostics for rapid identification.	B.C. Bera*, Nitin Virmani, Taruna Anand, B.R. Gulati (upto 12.11.22) and Riyesh T	Aug., 2020	July, 2023	IXX15338
5.	Indian network for fisheries and animal antimicrobial resistance (INFAAR)	R.K. Vaid*, Taruna Anand, H.S. Singha and Anubha Pathak	June, 2018	March, 2025	IXX15418
6.	Isolation and characterization of bacteriophages against important biofilm forming bacteria	Taruna Anand*, Nitin Virmani, B.C. Bera, and RK Vaid	April, 2021	March, 2024	IXX15795
7.	A study on bat virome for unravelling the viral diversity in India	Riyesh T*, Naveen Kumar, Shanmugasundaram K, RK Vaid and Sanjay Barua	April, 2021	March, 2024	IXX16007
8.	Adaptation of Lumpy skin disease virus in Vero cells	Naveen Kumar*, Riyesh T and Sanjay Barua	Jan., 2021	Jan., 2024	IXX16675
9.	Evaluating immunogenicity of the attenuated SARS-CoV2 in mice	Naveen Kumar*, B.R. Gulati (upto 12.11.22), Sanjay Barua and Riyesh T	Jan., 2021	Jan., 2022	IXX16831

* Principal Investigator

E. External Funded Projects

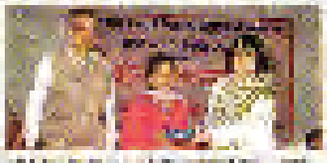
Sr. No.	Title	Team	From	To	PIMS Code
1.	All India Coordinated Research Project on Utilization of Animal Energy with enhanced system efficiency (AICRP on UAE)	R A Legha* and Yash Pal	July, 2009	March, 2022	OXX00486
2.	Investigating mechanism underlying acquisition of antiviral drug resistance against host targeting agents.	Naveen Kumar* and Sanjay Barua	March, 2019	March, 2022	OXX04469
3.	National One Health Program on Prevention and Control of Zoonotic Diseases (NOHPPCZ) Project: Regional Coordination center under program for Inter-Sectoral Coordination for prevention and control of Zoonotic Diseases	**Bacterial Diseases:** Harisankar Singha, Shanmugasundaram K, Anubha **Viral Diseases:** B. R Gulati (upto12.11.22) Dr Naveen Kumar, Dr Riyesh	June, 2019	March, 2023	OXX04686
4.	Role of p38 MAP kinase in buffalopox virus replication	Sanjay Barua* and Naveen Kumar	Jan, 2020	Jan 2023	OXX04792
5.	Epidemiological studies and development of antiviral therapeutics against coronaviruses	BR Gulati* (upto 12.11.22) Naveen Kumar, Riyesh T and Shanmugasundaram K	June, 2021	May, 2024	OXX4935
6.	Validation and translation of the vaccines as well as diagnostic technologies developed in Phase-I of ADMaC".	B.R. Gulati* (upto 12.11.22) Anubha Pathak & Riyesh T.	April, 2021	March, 2024	OXX4940
7.	Development of ML and ANN-based breed and individual identification system for equine population differentiation	Anuradha Bhardwaj*, Yash Pal and R.A. Legha	July, 2020	June, 2025	OXX5012
8.	DBT Network Programme on Anthrax Diagnosis and Control in India	R.K. Vaid*, B.C. Bera, K. Shanmugasundaram	Sept., 2021	Sept., 2024	OXX5383
9.	Development of Diagnostics for Coronavirus infections	Nitin Virmani* and Naveen Kumar	June, 2021	May, 2023	OXX5111
10.	Studies on host pathogen interaction and development of vaccine against zoonotic coronaviruses	B.C. Bera*, Nitin Virmani & Taruna Anand	June, 2021	May, 2024	OXX5382
11.	Survelliance of Rotavirus a Genotypes in bovine and Equines of India for Identification of Potential Vvaccine a candidates	B.R. Gulati* (upto 12.11.22) Anubha Pathak	April, 2022	March, 2025	OXX5368

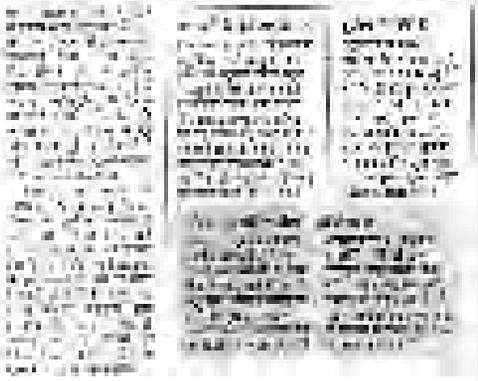
Sr. No.	Title	Team	From	To	PIMS Code
12.	Optimisation of procedures for non-surgical recovery and bio-banking of Marwari breed horse embryos	T.R. Talluri*, Yash Pal, RA Legha & RK Dedar	April, 2022	March, 2025	OXX5369
13.	Utilization of desert plants for the treatment of skin diseases of Horses	R.K. Dedar*, RA Legha Yash Pal, TR Talluri & Naveen Kumar	April, 2022	March, 2025	OXX5370
14.	Translation of nano based quinapyramine sulphate formulation into product and its evaluation against Trypanosoma evansi in animals	Anju Manuja*, Rajender Kumar and Balvinder Kumar	April, 2022	March., 2025	OXX5388
15.	Development of vaccine against animal's haemoprotozoan parasites for mitigating biotic stress	Sanjay Kumar*, Rajender Kumar & K. Shanmugasundaram	Oct., 2022	May, 2025	OXX5445
16.	Isolation, identification and characterization of SARS-CoV-2 from sewage and domestic wastewater and COVID-19 patients from Hisar (Haryana)	Naveen Kumar* & Riyesh	June, 2022	June 2023	--
17.	Development and evaluation of genetically engineered vaccine candidates for African swine fever, Equine Herpes virus-1 and Equine Influenza	Nitin Virmani* Sanjay Barua, Naveen Kumar, BC Bera & Taruna Anand	June, 2022	May, 2025	--
18.	CRP on Vaccine and Diagnostics: “Development and validation of multiplex assays for laboratory diagnosis of emerging equine herpesviruses (EHV2 & EHV5)”	Nitin Virmani*, BC Bera & Taruna Anand	Jan., 2021	Dec., 2024	--
19.	CRP on Vaccine and Diagnostics: “Development of antigen detection point-of-care diagnostics for haemoprotozoan diseases of equine”	Sanjay Kumar* & Rajender Kumar	Jan., 2021	Dec., 2024	--
20.	CRP on Vaccine and Diagnostics: “Development of point of care diagnostics for strangles in equines “	Balvinder Kumar*, RK Vaid, Anju Manuja, K Shanmugasundaram & Harisankar Singha	Jan., 2021	Dec., 2024	--
21.	CRP on Vaccine and Diagnostics: “Development of RPA-LFA based point-of-care diagnostic assay for rapid detection and differentiation of equine herpes viruses 1&4”	B.C. Bera*, Nitin Virmani & B.C. Bera	Jan., 2021	Dec., 2024	--

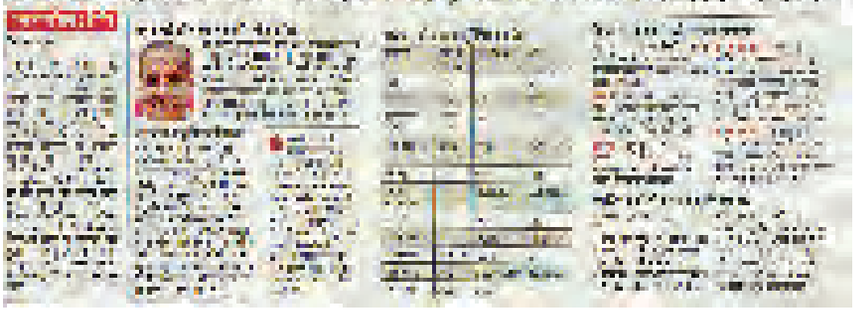
* Principal Investigator

समाचार पत्रों में एनआरसीई

महिला पशुपालकों को गधी व घोड़ी के दूध की विशेषताएं बताई







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