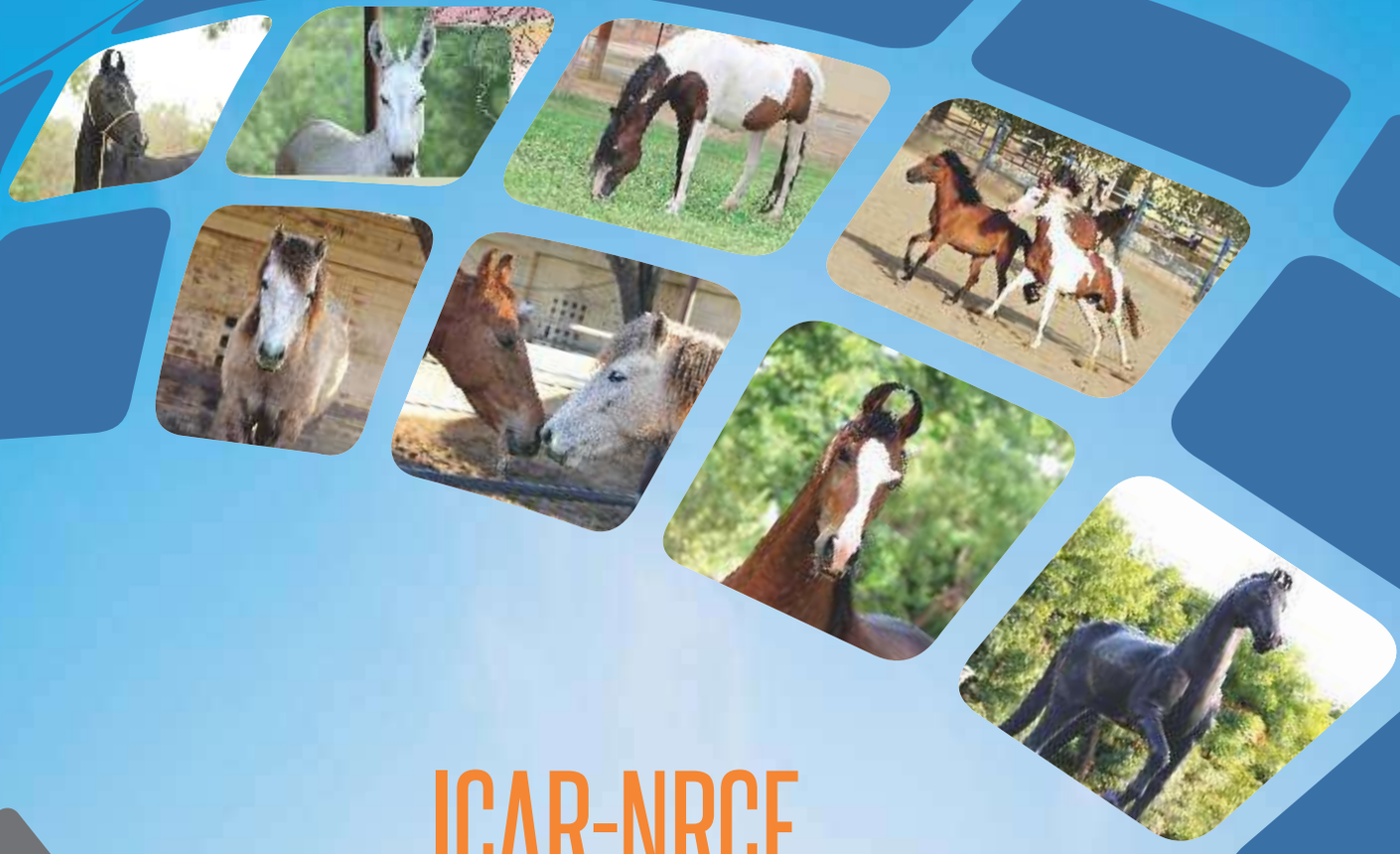
Azadi Ka Amrit Mahotsav



ICAR-NRCE ANNUAL REPORT 2021

भा.कृ.अनु.प. - राष्ट्रीय अश्व अनुसंधान केन्द्र

ICAR-National Research Centre on Equines

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The achievements and activities of the Centre from Jan 2021 to Dec 2021 are presented in this report. Mention of trademark, proprietary product or firm in the report does not constitute an endorsement or rejection of other suitable products or firms.

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Azadi Ka Amrit Mahotsav

ICAR-NRCE ANNUAL REPORT 2021

भाकृअनुप ICAR

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ICAR-National Research Centre on Equines

ICAR-NATIONAL RESEARCH CENTRE ON EQUINES

ICAR-NATIONAL RESEARCH CENTRE ON EQUINES

HISAR 1986

With best compliments from

Dr Yash Pal
Director
ICAR-National Research Centre on Equines
Sirsa Road, Hisar - 125001 (Haryana)

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Director's **Foreword**

Since its inception and humble beginning on November 26, 1985, ICAR-National Research Centre on Equines (ICAR-NRCE) has gained recognition as a premier institution of international stature. The strength that makes ICAR-NRCE truly enduring and unique emanates from our commitment to improve the health and productivity of equines. They are the basis of our growth and inspire us along every path. This is the only premier institute in the country, which is dedicated to work exclusively on equine health and production in the country through research and technological support to the equine stakeholders, breeders, and farmers to enhance their socio-economic status. Considering the immense contribution made by ICAR-NRCE in equine science, the Department of Animal Husbandry, Dairying and Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India has approved it as a National Referral Centre to provide consultancy and testing for health certification and diagnostic services for various equine diseases to stakeholders. The years 2020 and 2021 were unprecedented in terms of challenges and difficulties posed by the COVID-19 pandemic to successfully carry out the mandated research work of the Institute. Despite the challenges, it is heartening to know that our Institute has made remarkable progress in all the research activities. The scientists and all the hard working staff of ICAR-NRCE have accomplished targeted research and performed commendable services for the farmers during this period.

The Centre is working on the projects related to vaccinology, diagnostics, therapeutics, and equine reproduction. The Centre is also concentrating on the emerging areas of research, which include the refinement in production of new generation vaccines; development of rapid diagnostics techniques using nanotechnology; studying the host-pathogen interactions; development of therapeutics by applying ethno-veterinary medicine; creating a repository for microbes and bacteriophages; genetic studies on equine production and augmentation of reproductive efficiencies etc., The research activities at the Centre continue to bridge the gap between basic biology and clinical applications, thereby providing cutting-edge translational research for the amelioration of equine health and welfare in the country. The annual report of the current year displays the various achievements and attempts made to produce viable technologies and efforts for the generation of commercially viable technologies and demand-driven research for the benefit of equine farmers during the year 2021. The research and development activities of ICAR-NRCE are achieved through well-structured research programmes comprising 23 institute and 14 externally funded research projects, which also include collaborative and inter-institutional research projects. ICAR-NRCE has been successful in getting external funding from almost all leading national funding agencies in the field of agricultural and biological sciences.

ICAR-NRCE has initiated research programme at the Centre to estimate the prevalence of bovine and equine CoVs in cattle and equines in different districts of Haryana and Rajasthan states so as to decipher the inter-relatedness of CoVs in different animals, forecast the emergence of pandemics and also assess their zoonotic potential. The detection of SARS-CoV-2 antibodies/virus in different animal species indicates that the virus can also pose a threat to animal health due to reverse zoonosis. ICAR-NRCE has developed a recombinant nucleocapsid protein based indirect ELISA to screen for the presence of SARS-CoV2 antibodies in canines. This ELISA has validated with serum neutralization assay and has been found to have 95.66% sensitivity and 89.76% specificity.

Multiple technologies have been developed for equine welfare. A monoclonal antibody-based ELISA kit for detection of equine influenza (H3N8) antigen was released by the Hon'ble Union Minister of Agriculture and Farmers Welfare, Govt. of India on May 31, 2021. Technology for collection and cryopreservation of equine semen and production of customized artificial vagina was also transferred to equine farmers and breeders. In addition, an encapsulated phage formulation carrying Salmonella phages for therapeutic application in poultry has also been developed and tested during the period under report.

Surveillance and monitoring of equine infectious diseases is one of the priorities of the ICAR-NRCE. During the year 2021, a total of 2000 equine serum samples from 7 states were tested for various equine diseases. Out of total samples tested, the highest sero-prevalence was observed for equine piroplasmosis (28.40%), followed by EHV-1 (7.80%), JE/WNV (7.40%), and Trypanosoma evansi (2.15%). It is important to add here that all the samples tested for the past 11 years were negative for EIA, and this data generated by ICAR-NRCE would be of immense help for initiating procedures to obtain EIA disease free status for the country. Under the glanders surveillance programme, a total of 26257 equine sera obtained from 220 districts in 18 states were tested for glanders. Efforts to develop an immunoassay for detection of specific antibodies for *Streptococcus equi* subspecies *equi* targeting N terminal sequences of SeM and SeQ 2190 genes are also in progress.

Partial genome sequences of two EHV-1 isolates (EHV-1/14 and EHV-1/Meerut) were generated using the NGS platform covering more than 90% of the genome. A refined EHV-1 vaccine was also developed by the researchers of the Centre this year, which is showing better efficacy than the earlier developed vaccine, and at the same time, research for the development of a combined vaccine for both EIV and EHV is ongoing. In order to diagnose JEV infection, a *Taqman*-based real-time PCR was developed for the detection of JEV infection. The assay was specific for JEV and did not cross-react with WNV. For rapid and efficient execution of surveillance activities, glanders ELISA developed by ICAR-NRCE has been provided to 11 state diagnostic laboratories/RDDLs. In this year, 12206 equine samples were screened by ELISA at eight State Labs/RDDLS (Gujarat, Haryana, Himachal Pradesh, Punjab, Rajasthan, Maharashtra, and Jammu & Kashmir).

ICAR-NRCE is continuously working on the development of drugs against various pathogens. A polymerase spiral reaction (PSR) based point of care assay for the rapid detection of *Trypanosoma evansi* has been optimized at the Centre. This assay will help in the monitoring of Surra infection in livestock at field level at a lower cost. Research in the area of nanoencapsulation is a promising field to enhance the therapeutic potential of a drug. In this direction, three naphthoquinone (NTQ) were prepared (encapsulated using gum dammar) and evaluated for their efficacy against *T. evansi* at ICAR-NRCE. The encapsulated NNTQs induced more reactive oxygen species, apoptosis and necrotic effects and thereby had a more inhibitory effect on the growth of *T. evansi* as compared to NTQ by themselves.

ICAR-NRCE also works on genetic diversity analysis of Indigenous horses and equine production related traits. A study on the identification of genomic signatures in Indian equine populations identified a total of 1631 positive selection signatures common among all horse breeds. The high-quality SNP data generated has the potential to be used in genome-wide association studies to explore qualitative trait loci and SNPs related to economically and agronomically important traits in equines. Studies aiming at the improvement of equine production are also going on at the sub-campus of the Centre. ICAR-NRCE continued the work on characterization of Indigenous donkeys (Halari donkey) and donkey milk as these animals play an important role in the livelihood enhancement of their keepers. For optimization of conception rate and fertility, the research work on semen biology and semen extenders was continued. Proteomic analysis of seminal plasma from high and low motile sperm producing stallions identified a total of 1687 proteins, of which 1627 and 1496 proteins were expressed in high- (HM) and low- motile (LM) sperm of stallions, respectively. Purification, molecular characterization, and ligand binding properties of the major donkey seminal plasma protein DSP-1 (Fibronectin type-II family proteins) have also been carried out.

During the year 2021, a total of 82 bacteria, 31 viruses, and 92 bacteriophages were accessioned, making the total collection of veterinary microbes to 3138. The dairy and rumen microbes' components accessioned 48 and 80 bacteria respectively, thereby making the cumulative culture collection to 4505 microbes in the NCVTC repository. An mRNA vaccine construct targeting the immunogenic protein of SARS-CoV-2 has also been prepared for the development of vaccine candidates against SARS-CoV-2 using an mRNA-based platform. Research work has also been initiated on the generation of immortalized bat cell lines from primary cultures, which will be of use in the isolation of bat viruses and studies on host-virus interactions. Research on the potential use of bacteriophages in biofilm formation has yielded fruitful results. A bacteriophage cocktail to ameliorate *Pseudomonas aeruginosa* infections in biofilms has been formulated, and upon testing, this cocktail efficiently inhibited the bacterial biofilm.

The research endeavors of the Centre received a major boost during the year through 37 ongoing research projects, including 14 externally funded projects. Besides, scientists from the Centre have published 55 research articles in international journals and national peer-reviewed journals in the year 2021. In addition, 19 book chapters/ Technical bulletins/ Popular articles and 13 research abstracts were also published by the scientists in various conferences/ symposia. Scientists from the Centre have also been bestowed with prestigious fellowships and awards from various societies. ICAR-NRCE also imparted training to different stakeholders (veterinarians, medical professionals, students, and equine keepers) and a total of 14 training programmes were organized by the Centre during the period under report. Scientists of the Centre also delivered 35 expert/invited lectures on various workshops and training programmes. Further, for research outreach activities, a Memorandum of Understanding (MOU) has been signed with four institutes/ organisations/ equine breeders in 2021 for co-operation in the areas of research, education, extension, consultancy, capacity building and other areas of national interest. The vision, guidance and technical support provided from time to time by the Hon'ble Chairman and members of QRT, RAC, IMC, and experts of IRC has immensely helped ICAR-NRCE to be in the right direction and be much more focused.

I am extremely grateful and indebted to Dr. Trilochan Mohapatra, Secretary, DARE and Director General, ICAR, for the unstinted support and guidance extended for the development of this institute. I express my sincere gratitude to the Secretary, ICAR and Financial Advisor, ICAR for their immense support. I am highly grateful to Dr. B. N. Tripathi, DDG (AS), who has always been eager to encourage and guide our team of scientists and institute in achieving our goals and meeting our mandates. I am equally thankful to Dr. Ashok Kumar, ADG (AH), Dr. Amrish Tyagi, ADG (ANP) & Dr. V.K. Saxena, ADG (AP&B), for their help, support, and co-operation towards accomplishing various tasks of the institute on time. Hearty thanks are also extended to Dr. Rajan Gupta, Pr. Scientist (AN&P), Dr. Vineet Bhasin, Pr. Scientist (AP&B), Dr. (Mrs.) Jyoti Misri, Pr. Scientist (AH), and other officials of ICAR Headquarters for their constant guidance, help, and support. I also place on record my appreciation for the scientific, technical, administrative and supporting staff of this Centre, who have been sincerely working for the welfare of equine stakeholders and equine farmers. I am sure that with unrelenting cooperation and efforts, we will be able to successfully march ahead to achieve the mandated objectives of this institute. I express heartfelt gratitude to Mr. Manu Sharma for providing scintillating & vivid photographs for ICAR-NRCE Annual Report: 2021.

I congratulate the entire editorial board for doing an excellent exercise meticulously to prepare the ICAR-NRCE Annual Report: 2021 and for the commendable job in bringing out this Annual report in a noteworthy manner, and sincerely wish that this report would best serve the purposes for which ICAR-NRCE was established and would help all concerned with equine husbandry practices in the country.

(Yash Pal)
Director, ICAR-NRCE

Executive **Summary**

कार्यकारी सारांश

The ICAR-National Research Centre on Equines was established on 26th November, 1985 at Hisar (Haryana). The centre is mandated to undertake basic and strategic research on equine health and production and to provide advisory and consultancy services and capacity development. The institute has the state-of-the-art laboratories and facilities for undertaking research in areas of equine science. This institute is striving at its best in making a difference in the lives of the landless and marginal farmers and other stakeholders associated with equine industry. Considering the immense contribution made by ICAR-NRCE in equine science, Department of animal husbandry, dairying and fisheries, Ministry of Fisheries, Animal husbandry and Dairying, Govt. of India has approved it as a National Referral Centre to provide consultancy and testing for health certification and diagnostic services for various equine diseases to stakeholders. In the year 2005, ICAR- NRCE was also entrusted with an additional responsibility of establishing a "National Repository" of animal microbes (National Centre for Veterinary Type Cultures -NCVTC) at its campus for catering to the conservation and preservation of microbial diversity of animal origin. Ever since its establishment, NCVTC has been functioning as an integral but independent part of the Centre.

Surveillance and monitoring of equine infectious diseases is one of the priorities of the ICAR-NRCE. During the current year, 2000 equine serum samples from 7 states were tested for various equine diseases. Out of total samples tested the highest sero-prevalence was observed for equine *piroplasmosis* (28.40%) followed by EHV-1 (7.80%), JE/WNV (7.40%), and *Trypanosoma evansi* (2.15%). None of the equines were found positive for equine influenza, equine infectious anemia,

भारतीय कृषि अनुसंधान परिषद् (भा.कृ.अनु.प)– राष्ट्रीय अश्व अनुसंधान केंद्र (रा.अ.अनु.के.) की स्थापना 26 नवंबर, 1985 को हिसार (हरियाणा) में की गई थी। केंद्र अश्वों के स्वास्थ्य और प्रजनन पर बुनियादी और रणनीतिक अनुसंधान करने और सलाह तथा परामर्श सेवाएं एवं क्षमता विकास प्रदान करने के लिए अधिकृत है। संस्थान में अश्व विज्ञान के क्षेत्रों में अनुसंधान करने के लिए अत्याधुनिक प्रयागेशालाएं और सुविधाएं हैं और यह किसानों और अश्व उद्योग से जुड़े अन्य हितधारकों के जीवन में बदलाव लाने के लिए अपना सर्वश्रेष्ठ प्रयास कर रहा है। अश्व विज्ञान में रा.अ.अनु.के. द्वारा किए गए विशिष्ट योगदान को ध्यान में रखते हुए, मत्स्य पालन पशुपालन और डेयरी मंत्रालय, भारत सरकार ने हितधारकों को अश्वों की विभिन्न बीमारियों के लिए स्वास्थ्य प्रमाणन और नैदानिक सेवाओं के लिए परामर्श और परीक्षण प्रदान करने के लिए एक राष्ट्रीय रेफरल केंद्र के रूप में इसे मंजूरी दी है। वर्ष 2005 में, रा.अ.अनु.के. को पशुओं की माइक्रोबियल विविधिता के संरक्षण के लिए अपने परिसर में पशु रोगाणुओं हेतु नेशनल सेंटर फॉर वेटरनरी टाइप कल्चर्स–एनसीवीटीसी का एक ''राष्ट्रीय भंडार'' स्थापित करने की एक अतिरिक्त जिम्मेदारी भी सौंपी गई थी। स्थापना के बाद से एनसीवीटीसी, केंद्र के अभिन्न लेकिन स्वतंत्र हिस्से के रूप में कार्य कर रहा है।

अश्व संक्रामक रोगों की निगरानी भा.कृ.अनु.प.–रा.अ.अनु.के. की प्राथमिकताओं में से एक है। वर्ष 2021 के दौरान, 7 राज्यों से कुल 2000 अश्वों के सीरम के नमूनों का विभिन्न बीमारियों के लिए परीक्षण किया गया। परीक्षण किए गए कुल नमूनों में से उच्चतम सीरो–प्रचलन *इक्वाइन पाइरोप्लाज्मोसिस* (28.40%) के लिए देखा गया, इसके बाद EHV-1 (7.80%), JE/WNV (7.40%), और *ट्रिपैनोसोमा इवांसाई* (2.15%) पाए गए। अश्व इन्फ्लूएंजा, अश्व संक्रामक एनीमिया, ब्रुसेलोसिस और साल्मोनेला एबॉर्टस इक्वाई के लिए कोई भी नमूना सकारात्मक नहीं पाया गया। इसके अलावा, अश्व संक्रामक एनीमिया के निदान के लिए कुल (17 राज्यों से प्राप्त) 14757 सीरम नमूनों पर किए

brucellosis and *Salmonella Abortus equi*. Further, Coggin's and ELISA test conducted on 14757 serum samples (obtained from 17 states) for diagnosis of equine infectious anemia yielded negative results. It is important to add here that all the samples tested for the past 11 years were negative for EIA and this data generated by ICAR-NRCE would be of immense help for initiating procedures on obtaining EIA disease free status to the country. In addition, a total 9937 samples received from race courses, turf club, stud farm, riding schools, animal quarantine & certification services (AQCS) and other organized sectors were also tested for various notifiable and exotic diseases under contractual diagnostic services to check ingression of diseases from abroad and monitoring of elite horses in private sectors. All the samples were found negative for the diseases tested. Under the glanders surveillance programme (Govt of India), 26257 equine sera obtained from 220 districts of 18 states were tested for glanders. Out of these, 148 glanders positive cases were reported from 46 districts of 10 states.

Analysis was also carried out for the presence of other bacterial diseases on 442 clinical samples collected from diseased horses and which resulted in identification and isolation of 133 bacterial isolates. Most prevalent bacteria observed in these samples were *Klebsiella pnuemoniae*, *E.coli*, *Rhodococcus equi*, *Streptococcus zooepidemicus* and *Burkholderia mallei*. Genetic diversity analysis of *K. pneumoniae* isolates obtained from aborted fetus and cervical swabs of mare were also carried out during the period under report. Phylogenetic studies based on 16s rRNA of *K. pneumoniae* (n=15) isolates revealed that *K. pneumoniae* isolates of Indian origin are closely related, but genetically diverse from global *K. pneumoniae* strains. Efforts are also being made to develop an immunoassay for detection of specific antibodies for *Streptococcus equi* subspecies *equi* targeting N terminal sequences of *SeM* and *SeQ 2190* genes.

गए कॉगिन और एलिसा परीक्षण के नकारात्मक परिणाम मिले। यह उल्लेखनीय है कि पिछले 11 वर्षों में परीक्षण किए गए सभी नमूने अश्व संक्रामक एनीमिया के लिए नकारात्मक थे और केंद्र द्वारा उत्पन्न यह डेटा देश को अश्व संक्रामक एनीमिया रोग से मुक्त स्थिति प्राप्त करने की प्रक्रिया शुरू करने में बहुत मददगार होगा। इसके अलावा, रेस कोर्स, टर्फ क्लब, स्टड फार्म, राइडिंग स्कूल, एनिमल क्वारंटाइन एंड सर्टिफिकेशन सर्विसेज (एक्यूसीएस) और अन्य संगठित क्षेत्रों से प्राप्त कुल 9937 नमूनों का परीक्षण भी संविदात्मक निदान सेवाओं के तहत विभिन्न अधिसूचनीय और विदेशी बीमारियों के लिए किया गया। जांच की गई बीमारियों के लिए सभी नमूने नकारात्मक पाए गए। ग्लैंडर्स सर्विलेंस प्रोग्राम (भारत सरकार) के तहत, 18 राज्यों के 220 जिलों से प्राप्त कुल 26257 इक्वाइन सीरम नमूनों का ग्लैंडर्स के लिए परीक्षण किया गया। इनमें से 10 राज्यों के 46 जिलों से 148 ग्लैंडर पॉजिटिव मामले सामने आए।

रोगग्रस्त अश्वों से एकत्र किए गए 442 नैदानिक नमूनों पर अन्य जीवाणु रोगों की उपस्थिति के लिए विश्लेषण भी किया गया, जिसके परिणामस्वरूप 133 जीवाणु आइसोलेट्स की पहचान और आइसोलेशन हुआ। इन नमूनों में पाए जाने वाले सबसे प्रचलित बैक्टीरिया *क्लेबसिएला न्यूमोनिया, ई. कोलाई, रोडोकोकस इक्वाई, स्ट्रेप्टोकोकस ज़ूएपेडेमिकस* और *बर्कहोल्डरिया मैलीआई* थे। रिपोर्टाधीन अवधि के दौरान गर्भपात हुए भ्रूण और घोड़ी के ग्रीवा स्वैब से प्राप्त *क्लेबसिएला न्यूमोनिया* आइसोलेट्स का आनुवंशिक विविधता के लिए विश्लेषण भी किया गया। *क्लेबसिएला न्यूमोनिया* (n=15) आइसोलेट्स के 16s rRNA पर आधारित फ़ाइलोजेनेटिक अध्ययनों से पता चला है कि भारतीय मूल के *क्ले. न्यूमोनिया* आइसोलेट्स निकट से संबंधित हैं, लेकिन आनुवंशिक रूप से वैश्विक *क्ले. न्यूमोनिया* स्ट्रेन से भिन्न हैं। SeM और SeQ 2190 जीन के N टर्मिनल अनुक्रमों को लक्षित करने वाले *स्ट्रेप्टोकोकस* समान उप-प्रजातियों के लिए विशिष्ट एंटीबॉडी का पता लगाने के लिए एक इम्युनोऐसे विकसित करने के प्रयास भी जारी हैं।

प्रोटोजोअन रोग जैसे ट्रिपैनोसोमोमिआसिस अश्व के स्वास्थ्य पर गंभीर असर डालते हैं। इस वर्ष अश्व के परजीवी रोगों से संबंधित अनुसंधान पर काफी प्रगति हुई है। *ट्रिपैनोसोमा इवांसाई* का जल्द पता लगाने के लिए पोलीमरेज़ स्पाइरल

The protozoan diseases like trypanosomosis took a very heavy toll on general equine health and well-being. Much progress has been made this year on research related to equine parasitic diseases. A polymerase spiral reaction (PSR) based point of care assay for rapid detection of *Trypanosoma evansi* has been optimized at the Centre. This assay will aid in monitoring of Surra infection in livestock at field level at lower cost. Another study on sero-prevalence of *Trypanosoma evansi* infection in different livestock animals species of Himachal Pradesh state revealed a higher prevalence of *T. evansi* in buffalo (23.57%) followed by cattle (22.52%) and equines (1.82%).

Equine medicine is yet another area in which ICAR-NRCE has made major accomplishments. Research in the area of nanoencapsulation is a promising field to enhance the therapeutic potential of a drug. In this direction, three naphthoquinones (NTQ) were prepared (encapsulated using gum dammar) and evaluated for their efficacy against *T.evansi* at ICAR-NRCE. The encapsulated NNTQs induced more reactive oxygene species, apoptosis and necrotic effects and thereby more inhibitory effect on the growth of *T. evansi* as compared to NTQ by themselves. In another study, ZnONPs encapsulated in alginate/gum-acacia hydrocolloids and further conjugated with iron oxide were tested for their efficacy on intestinal cells (Caco-2) and the results demonstrated that alginate and gum acacia are promising polysaccharides for ZnONPs to protect them against harsh digestive milieu. A non-cytotoxic concentration of the aqueous and ethanol extracts of herbs such as *with A. javanica, Capparis decidua*, *Phoenix dactylifera* and *Ziziphus mauritiana* were tested on equine fibroblasts and the expression of TGF-β1 and TGF-β2 were examined. Extracts prepared from *A. javanica, P. dectylifera* and *Z. mauritiana* have been found to increase the expression of TGF-β1 and/TGF-β2 in equine dermal cells and have the potential to be utilized for clinical use in the treatment of equine

रिएक्शन (पीएसआर) आधारित प्वाईंट ऑफ़ केयर ऐसे को केंद्र में अनुकूलित किया गया है। यह परीक्षण कम लागत पर क्षेत्र स्तर पर पशुधन में सर्रा संक्रमण की निगरानी में मदद करेगा। हिमाचल प्रदेश के विभिन्न जानवरों की प्रजातियों में *ट्रिपैनोसोमा इवांसाई* संक्रमण के सीरो–प्रचलन पर एक अन्य अध्ययन में भैंस (23.57%) में *टी. इवांसाई* के उच्च प्रसार का पता चला है, इसके बाद गाय (22.52%) और अश्वों (1.82%) में भी सीरो प्रचलन पाया गया।

अश्व चिकित्सा विज्ञान के क्षेत्र में भी केंद्र ने प्रमुख उपलब्धियां हासिल की हैं। एक दवा की चिकित्सीय क्षमता को बढ़ाने के लिए नैनो एनकैप्सूलेशन के क्षेत्र में अनुसंधान एक आशाजनक संकेत है। इस दिशा में, तीन नेफ्थोक्विनोन (एनटीक्यू) तैयार किए गए (गम डैमर का उपयोग करके एनकैप्सूलेटेड) और *टी. इवांसाई* के खिलाफ उनकी प्रभावकारिता के लिए मूल्यांकन किया गया। एनकेप्सूलेटेड एनएनटीक्यू ने अधिक प्रतिक्रियाशील ऑक्सीजन प्रजातियों, एपोप्टोसिस और नेक्रोटिक प्रभावों को प्रेरित किया और इस तरह *टी. इवांसाई* के विकास पर एनटीक्यू की तुलना में अधिक निरोधात्मक प्रभाव दर्शाया। एक अन्य अध्ययन में, एल्गिनेट/गम–बबूल हाइड्रो कोलोइड्स में निहित और आयरन ऑक्साइड के साथ संयुग्मित जनोपस का आंतों की कोशिकाओं (Caco-2) पर प्रभावकारिता के लिए परीक्षण किया गया। परिणामों ने प्रदर्शित किया कि एल्गिनेट और गम बबूल ZnONPs बेहतरीन पॉलीसेकेराइड हैं जो ZnONPs की कठोर पाचन वातावरण में रक्षा कर सकते हैं। जड़ी–बूटियों के जलीय और इथेनॉल के अर्क की एक गैर–साइटोटॉक्सिक सांद्रता जैसे कि *ऐ जावनिका, कैपारिस डेसीडुआ, फीनिक्स डेक्टाइलिफेरा* और *ज़िज़िफस मॉरिटियाना* का परीक्षण टीजीएफ 1 और टीजीएफ 2 की अभिव्यक्ति पर इक्वाइन फाइब्रोब्लास्ट कोशिकाओं पर किया गया। *ऐ जावनिका, फीनिक्स डेक्टाइलिफेरा और ज़िज़िफस मॉरिटियाना* से तैयार किए गए अर्क को अश्व की त्वचीय कोशिकाओं में TGF 1 और / TGF 2 की अभिव्यक्ति को बढ़ाने के सक्षम पाया गया है और इनमें अश्व त्वचा रोगों के उपचार में उपयोग किए जाने की क्षमता है। हैस्पेरिडीन (HS), एक फ्लेवोनोइड ($C_{28}H_{34}O_{15}$) जिनमें हाइपोग्लाइसेमिक और हाइपोलिपिडेमिक गुण हैं, का मूल्यांकन गर्भ में हेमेटोलॉजिकल और

skin diseases. Hesperidin (HS), a flavanoid ($C_{28}H_{34}O_{15}$) reported for its hypoglycemic and hypolipidemic properties has been evaluated on haematological and serum-biochemical profile in donkeys to assess whether these flavonoids may have potential to be used for the treatment of hyperlipidemic donkeys. The study revealed that hesperidin can be supplemented to the donkeys at lower dose to reduce serum triglyceride and cholesterol levels in donkeys.

ICAR-NRCE also works on genetic diversity analysis of Indigenous horses and equine production related traits. Study on identification of genomic signatures in Indian equine populations identified 1631 positive selection signatures common among all horse breeds. The high-quality SNP data generated has the potential to be used in genome-wide association studies to explore qualitative trait loci and SNPs related to economically and agronomically important traits in equines. In order to initiate molecular selection and to identify the animals at an early age for endurance potential and fertility, the associated SNP markers were tested in Indigenous breeds of horses *viz.* Marwari, Kathiawari, Sindhi, Manipuri and Zanskari for polymorphism. Out of 13 SNP markers, BIEC2-1022884 (A>G) was found to be monomorphic and the rest 12 SNPs were found polymorphic. An association study is being carried out at the centre to further expand the scope of these findings. In another study, genetic diversity analysis in Indigenous horses and ponies with use of genome-wide SNPs was carried out using a panel of 24 polymorphic microsatellites. The study revealed a high number of alleles and heterozygosity in horses. 21197 unique SNPs were identified for differentiation among Marwari, Kathiawari, Kachchhi-Sindhi and Thoroughbred horses along with Zanskari and Manipuri ponies. Sequencing and phylogenetic analysis studies on Myostatin (MSTN) gene, a member of the Tβ superfamily that regulates both the number and growth of muscle fibers to reduce skeletal muscle

सीरम–बायोकेमिकल प्रोफाइल पर किया गया है ताकि यह आकलन किया जा सके कि फ्लेवोनोइड्स में हापरलिपिडेमिक गर्दभों के उपचार में उपयोगी हैं या नहीं। अध्ययन से पता चला है कि गर्दभों में सीरम ट्राइग्लिसराइड और कोलेस्ट्रॉल के स्तर को कम करने के लिए कम खुराक में हैस्पेरिडीन प्रदान कर उनमें सीरम ट्राइग्लिसराइड और कोलेस्ट्रॉल का स्तर कम किया जा सकता है।

भा.कृ.अनु.प.–रा.अ.अनु.के. देशी अश्वों के आनुवंशिक विविधता विश्लेषण और अश्वों के उत्पादन से संबंधित लक्षणों पर भी काम करता है। भारतीय अश्वों की आबादी में जीनोमिक हस्ताक्षर की पहचान पर अध्ययन ने सभी अश्वों की नस्लों के बीच आम तौर पर 1631 सकारात्मक चयन हस्ताक्षरों की पहचान की। उच्च गुणवत्ता वाले एसएनपी डेटा का उपयोग जीनोम–बाइड एसोसिएशन अध्ययनों में गुणात्मक विशेषता लोसाई और एसएनपी का पता लगाने के लिए किया जा सकता है। जो आर्थिक और कृषि संबंधी महत्वपूर्ण लक्षणों से संबंधित है। आणविक चयन शुरू करने एवं कम उम्र में जानवरों में अच्छी एन्ड्योरेंस क्षमता और प्रजनन क्षमता वाले अश्वों का पता लगाने के लिए संबंधित एसएनपी मार्कर्रों का परीक्षण बहुरूपता के लिए मारवाड़ी, काठियावाड़ी, सिंधी, मणिपुरी और जांस्करी घोड़ों की स्वदेशी नस्लों में किया गया। 13 एसएनपी मार्कर्रों में से, बीआईईसी 2-1022884 (ए>जी) मोनोमोर्फिक पाए गए और शेष 12 एसएनपी पॉलीमॉर्फिक पाए गए। इन निष्कर्षों के दायरे को और विस्तारित करने के लिए केन्द्र में एक एसोसिएशन अध्ययन किया जा रहा है। एक अन्य अध्ययन में, जीनोम–बाइड एसएनपी के उपयोग के साथ स्वदेशी अश्वों और टट्टूओं में आनुवंशिक विविधता विश्लेषण 24 पॉलीमॉर्फिक माइक्रोसेटेलाइट्स के एक पैनल का उपयोग करके किया गया। अध्ययन ने घोड़ों में अत्याधिक संख्या में एलील और हेटेरोज़यगोसिटी का खुलासा किया। जांस्करी और मणिपुरी टट्टू के साथ मारवाड़ी, काठियावाड़ी, कच्छी, सिंधी और थोरोब्रेड घोड़ों के बीच भेदभाव के लिए 21197 अद्वितीय एसएनपी की पहचान की गई थी। मयोस्टैटिन (MSTN) जीन पर अनुक्रमण और फ़्लायोजेनेटिक विश्लेषण अध्ययन, T सुपरफैमिली का एक सदस्य जो अश्वों में कंकाल की मांसपेशी द्रव्यमान को कम करने के लिए मांसपेशी फाइबर की संख्या और वृद्धि दोनों को नियंत्रित

mass in equines revealed that there exists haplotype diversity and MSTN gene has been equally distributed among the different breeds of equines.

ICAR-NRCE continued the work on characterization of Indigenous donkeys (Halari donkey) and donkey milk as these animals play an important role in livelihood enhancement of its keepers. Gene sequencing and phylogenetic anlaysis of the mitochondrial D-loop region of Halari donkeys identified 8 haplotypes with haplotype diversity of 0.8152 and nucleotide diversity of 0.12811 indicating that the population has high genetic diversity. The physico-biochemical properties of Halari donkey milk are also being evaluated at the centre.

Analysis of quantitative traits for genetic improvement of indigenous equines was also carried out during this period. The breeding value for the height at withers (150.45 cm), body length (151.97 cm), heart girth (169.73 cm) and body weight (366.3 kg) for Marwari horses was estimated and the heritability of height at withers, body length, heart girth and body weight has been estimated to be 0.396±0.586, 0.370±0.777, 0.507±1.95 and 0.597±0.612, respectively. Efforts are also being made to characterize Bheemthadi horse breed. Significant efforts are also going to develop a fatigue cum fitness score card for working equines.

For optimization of conception rate and fertility the research work on semen biology and semen extenders were continued. Proteomic analysis on seminal plasma of high- and low-motile sperm producing stallions identified 1687 proteins, of which 1627 and 1496 proteins were expressed in high- (HM) and low-motile (LM) sperm of stallions, respectively. High abundance of protein related to dysregulated oxidative metabolism found in low- motile (LM) sperm of stallions has been proposed as the underlying etiology for poor sperm motility in LM group stallions. Purification, molecular characterization and ligand binding properties (with erythrocytes (a model cell

करता है, से पता चला है कि हैप्लोटाइप विविधता मौजूद है और MSTN जीन को समान रूप से वितरित किया गया है।

केंद्र ने देशी गर्दभों (हलारी गर्दभ) और गर्दभ के दूध के लक्षण वर्णन पर काम जारी रखा क्योंकि ये पालकों की आजीविका बढ़ाने में महत्वपूर्ण भूमिका निभाते हैं। हलारी गर्दभों के माइटोकॉन्ड्रियल डी-लूप क्षेत्र के जीन अनुक्रमण और फ़ाइलोजेनेटिक एनालिसिस ने 0.8152 की हैप्लोटाइप विविधता और 0.12811 की न्यूक्लियोटाइड विविधता के साथ 8 हैप्लोटाइप की पहचान की, जो दर्शाता है कि जनसंख्या में उच्च आनुवंशिक विविधता है। केंद्र में हलारी गर्दभ के दूध के भौतिक-जैव रासायनिक गुणों का भी मूल्यांकन किया जा रहा है।

इस अवधि के दौरान स्वदेशी अश्वों के अनुवांशिक सुधार के लिए मात्रात्मक लक्षणों का विश्लेषण भी किया गया। मारवाड़ी अश्वों में विदर तक ऊँचाई (150.45 से.मी.), शरीर की लंबाई (151.97 से.मी.), हार्ट गर्थ (169.73 से.मी.) और शरीर के वजन (366.3 किलोग्राम) का प्रजनन मूल्य का अनुमानित किया गया तथा विदर तक ऊंचाई, शरीर की लम्बाई, हार्ट गर्थ और शरीर के वजन की अनुवांशिकता को क्रमशः 0.396 ± 0.586, 0.370 ± 0.777, 0.507 ± 1.95 और 0.597 ± 0.612 पाया गया। भीमथाड़ी घोड़े की नस्ल को चिन्हित करने का भी प्रयास किया जा रहा है। काम करने वाले अश्वों के लिए एक थकान सह फिटनेस स्कोर कार्ड विकसित करने के लिए भी महत्वपूर्ण प्रयास किए जा रहे है।

गर्भाधान दर और प्रजनन क्षमता के अनुकूलन के लिए वीर्य जीव विज्ञान और वीर्य विस्तारण पर शोध कार्य जारी रखा गया था। उच्च और निम्न गतिशील शुक्राणु उत्पादक स्टैलियन के वीर्य प्लाज्मा पर प्रोटीन विश्लेषण ने कुल 1687 प्रोटीनों की पहचान की, जिनमें से 1627 और 1496 प्रोटीन क्रमशः स्टैलियन के उच्च (एचएम) और निम्न-गतिशील (एलएम) शुक्राणु में व्यक्ति किए गए। स्टैलियन्स के निम्न-गतिशील स्पर्म में पाए जाने वाले डिसरेगुलेटेड ऑक्सीडेटिव मेटाबॉलिज्म से संबंधित प्रोटीन की उच्च प्रचुरता को निम्न-गतिशील स्पर्म ग्रुप स्टैलियन्स में खराब स्पर्म मोटिलिटी के लिए अंतर्निहित कारक तत्व के रूप में प्रस्तावित किया गया है। केन्द्र में प्रमुख गर्दभ सेमिनल प्लाज्मा प्रोटीन डीएसपी-1 (फाइब्रोनेक्टिन टाइप-II फैमिली

membrane) of the major donkey seminal plasma protein DSP-1 (Fibronectin type-II family proteins) has also been carried out at the Centre. The study concluded that DSP-1 binding is mediated by a specific interaction with choline phospholipids and results in membrane perturbation. Hence, the binding of this protein to sperm plasma membrane could be of physiological significance.

Effect of addition of lyophilized heterologous seminal plasma and colostrum to semen extenders on cooled and post-thaw stallion semen quality was evaluated. The study suggested that supplementation of seminal plasma in semen extender (2 mg/ml) may have detrimental effects on cooled and post thaw semen quality, however, colostrum supplementation at the same dose may have beneficial effects. Similarly, effect of zinc and gold nanoparticles on cooled and post-thaw quality of stallion semen was also evaluated and it was found that addition of ZnNp (2mg/ml) to the freezing extender improves stallion post-thaw semen quality but not in the cooled semen. Addition of AuNp (2mg/ml) to the freezing extender does not alter stallion cooled and post-thaw semen quality. Adding to this data, study on supplementation of 50 mM trehalose to the semen extender resulted in better sperm quality after cooling and freezing and improved the fertility rates.

The National Centre for Veterinary Type Cultures (NCVTC) established at ICAR-NRCE has a mandated aim of authentication, preservation and distribution of microbial cultures of veterinary importance. During the year 2021 a total of 82 bacteria, 31 viruses and 92 bacteriophages were accessioned making the total collection of veterinary microbes to 3138. The dairy microbe and rumen microbes component accessioned 48 dairy and 80 rumen bacteria respectively there by making the cumulative culture collection to 4505 microbes in the NCVTC repository. The major viruses accessioned this year include Lumpy skin

प्रोटीन) का शुद्धिकरण, आणविक लक्षण वर्णन और एरिथ्रोसाइट्स के साथ लिगैंड बाइंडिंग गुण (एक मॉडल सेल झिल्ली) के साथ का विश्लेषण भी किया गया है। अध्ययन ने निष्कर्ष निकाला कि डीएसपी-1 बंधन को कोलीन फॉस्फोलिपिड के साथ एक विशिष्ट अंत: क्रिया द्वारा मध्यस्थ किया जाता है और परिणामस्वरूप झिल्ली में गड़बड़ी होती है। इसलिए, शुक्राणु प्लाज्मा झिल्ली के लिए इस प्रोटीन का बंधन फैसिओलॅजिकल महत्व का हो सकता है।

कूल्ड और पोस्ट थॉ स्टैलियन वीर्य गुणवत्ता पर लियोफिलाइज्ड हेटेरोलॉगस सेमिनल प्लाज्मा और कोलोस्ट्रम को वीर्य विस्तारकों में मिलाने के प्रभाव का मूल्यांकन किया गया। अध्ययन से यह जानकारी प्राप्त हुई कि वीर्य विस्तारक (2 मिलीग्राम/ एमएल) में सेमिनल प्लाज्मा मिलाने से कूल्ड और पोस्ट थॉ स्टैलियन वीर्य की गुणवत्ता पर हानिकारक प्रभाव पड़ सकता है, हालांकि इस ही खुराक में कोलोस्ट्रम मिलाने के लाभकारी प्रभाव हो सकते हैं। इसी तरह, स्टैलियन वीर्य की कूल्ड और पोस्ट थॉ गुणवत्ता पर जस्ता और सोने के नैनोकणों के प्रभाव का भी मूल्यांकन किया गया और यह पाया गया कि फ्रीजिंग एक्सटेंडर ZnNp (2 mg/ml) मिलाने से स्टैलियन पोस्ट–थॉ वीर्य की गुणवत्ता में सुधार होता है लेकिन कूल्ड वीर्य में नहीं। फ्रीजिंग एक्स्टेंडर में AuNp (2 mg/ml) मिलाने से स्टैलियन कूल्ड और पोस्ट–थॉ वीर्य की गुणवत्ता में कोई बदलाव नहीं आता है। इस डेटा को जोड़ते हुए, वीर्य विस्तारक में 50 एमएम ट्रेहलोस मिलाने से शीतलन के बाद बेहतर शुक्राणु गुणवत्ता पाई गयी और प्रजनन दर में भी सुधार हुआ।

नेशनल सेंटर फॉर वेटरनरी टाइप कल्चर (एनसीवीटीसी) पशु चिकित्सा की दृष्टि से महत्वपूर्ण माइक्रोबियल कल्चर के प्रमाणीकरण, संरक्षण और वितरण का एक उत्कृष्ट संस्थान है। वर्ष 2021 के दौरान कुल 82 बैक्टीरिया, 31 विषाणु, 92 बैक्टीरियोफेज को भण्डार में शामिल किया गया, जिससे पशु चिकित्सा सूक्ष्मजीवों का कुल संग्रह 3138 हो गया। डेयरी माइक्रोब और रूमेन माइक्रोब्स घटक ने संचयी कल्चर संग्रह बनाकर क्रमश: 48 डेयरी और 80 रूमेन बैक्टीरिया का परिग्रहण किया। एनसीवीटीसी रिपॉजिटरी में 4505 माइक्रोब्स इस वर्ष शामिल किए गए। प्रमुख विषाणुओं में लम्पी त्वचा रोग वायरस, न्यूकैसल रोग वायरस, SARS-CoV2, ब्लूटॉन्ग वायरस, संक्रामक ब्रोंकाइटिस वायरस, एवियन नेफ्रैटिस

disease virus, Newcastle disease virus, SARS-CoV-2, Bluetongue virus, Infectious bronchitis virus, Avian nephritis virus and Fowl adenovirus. Significant new bacterial additions in the repository include *Stenotrophomonas maltophila*, *Aeromonas hydrophila*, *A. veronii*, *A. jandaei*, *Pseudomonas alkaliphila*; *Arcanobacterium pluranimalium*, *Brucella tritici*, *Leucobacter celer* ssp. *Celer*, *Morganella morganii*, *Shewanella algae*, *Shewanella khirikhana* and *Aggregatibacter actinomycetemcomitans*. When it comes to microbial distribution, all in all nearly 75 bacterial cultures, three viruses and 9 cell lines were distributed to different government research institutions, universities and private institutes across the country this year.

Besides working on authentication, accessioning and distribution of microbes, the scientists of NCVTC are also engaged in various basic and applied research works related to microbes. Role of p38 mitogen-activated protein kinase (MAP Kinase) in buffalo pox virus replication was evaluated and it has been found that buffalopox virus (BPXV) exploits p38 mitogen activated protein kinase (a cellular protein) to effectively synthesize its protein and an inhibitor targeting p38 kinase (SB239063) exerts a potent *in vitro* and *in ovo* antiviral effect. Studies on epitranscriptomic regulation of SARS-CoV-2 replication revealed that heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1, an RNA-binding protein) regulates early translation to replication switch in SARS-CoV-2 life cycle. This study also sheds light on epitranscriptomic machinery (methylation) that might represent potential targets for new antiviral drugs against COVID-19. Further, isothermal recombinase polymerase amplification (RPA) assays are being standardized for development point-of-care diagnostics for detection of PCV2 and PCV3 viruses associated with reproductive failure in pigs. An mRNA vaccine construct targeting the immunogenic protein of SARS-CoV-2 has also been prepared for the development of

वायरस और फाउल एडिनोवायरस शामिल हैं। भंडार में महत्वपूर्ण नए जीवाणु जैसे– *स्टेनोट्रोफोमोनस माल्टोफिला, एरोमोनस हाइड्रोफिला, ए वेरोनी, ए जंडेई, स्यूडोमोनास अल्कलीफिला; आर्कनोबैक्टीरियम प्लुरैनिमेलियम, ब्रुसेला ट्रिटिसी, ल्यूकोबैक्टर सेलेर स्पीशी सेलेर, मॉर्गनेला मोर्गेनी, शीवनेला ऐल्गी, शीवनेला खिरीखाना और एग्रीगेटिबैक्टर एक्टिनोमाइसेटेमकोमिटन्स* परिवर्धन में शामिल हैं। माइक्रोबियल वितरण के संदर्भ में इस साल देश भर के विभिन्न सरकारी अनुसंधान संस्थानों, विश्वविद्यालयों और निजी संस्थानों में लगभग 75 जीवाणु कल्चर, तीन वायरस और 9 सेल लाइनों को वितरित किया गया।

प्रमाणीकरण पर काम करने, रोगाणुओं के वितरण को जोड़ने के अलावा, एनसीवीटीसी के वैज्ञानिक रोगाणुओं से संबंधित विभिन्न बुनियादी और अनुप्रयुक्त अनुसंधान कार्यों में भी लगे हुए हैं। भैंस पॉक्स वायरस प्रतिकृति में p38 माइटोजेन–सक्रिय प्रोटीन काईनेज़ (एमएपी काईनेज़) की भूमिका का मूल्यांकन किया गया था और यह पाया गया है कि भैंस वायरस (बीपीएक्सवी) अपने प्रोटीन और अवरोधक लक्ष्यीकरण को प्रभावी ढंग से संश्लेषित करने के लिए p38 माइटोजेन सक्रिय प्रोटीन काईनेज़ (एक सेलुलर प्रोटीन) का शोषण करता है। p38 kinase (SB239063) इन विट्रो और इन ओवो में एक शक्तिशाली एंटीवायरल प्रभाव डालता है। SARS-CoV2 प्रतिकृति के एपिट्रांसस्क्रिप्टोमिक विनियमन पर अध्ययन से पता चला है कि विषम परमाणु राइबोन्यूक्लियोप्रोटीन A1 (hnRNPA1, एक RNA-बाध्यकारी प्रोटीन) SARS-CoV2 जीवन चक्र में प्रतिकृति स्विच के लिए प्रारंभिक अनुवाद को नियंत्रित करता है। यह अध्ययन एपिट्रांसस्क्रिप्टोमिक मशीनरी (मिथाइलेशन) पर भी प्रकाश डालता है जो COVID-19 के खिलाफ नई एंटीवायरल दवाओं के संभावित लक्ष्य का प्रतिनिधित्व कर सकता है। इसके अलावा, सूअरों में प्रजनन विफलता से जुड़े PCV2 और PCV3 वायरस का पता लगाने के लिए विकास बिंदु–देखभाल निदान के लिए एक इज़ोटेर्मल रीकॉम्बिनेज़ पोलीमरेज़ एम्प्लीफिकेशन (RPA) assays को मानकीकृत किया जा रहा है। SARS-CoV2 के इम्युनोजेनिक प्रोटीन को लक्षित करने वाला एक mRNA वैक्सीन निर्माण भी mRNA आधारित प्लेटफॉर्म का उपयोग करके SARS-CoV2 के खिलाफ वैक्सीन उम्मीदवारों के

vaccine candidates against SARS-CoV-2 using mRNA based platform. Research work has also been initiated on generation of immortalized bat cell lines from primary cultures, which will be of use in isolation of bat viruses and studies on host-virus interactions.

The current pandemic of COVID-19 is constantly pressing on the need for one health approach to control and eradicate zoonotic diseases. The detection of SARS-CoV2 and related coronavirus in different animal species warrants the need for testing our animal population for the presence of different coronaviruses. ICAR-NRCE has responded to this challenge by initiating a research programme at the Centre to estimate the prevalence of bovine and equine CoVs in cattle and equines at different districts of Haryana and Rajasthan states to decipher the inter-relatedness of CoVs in different animals, to forecast emergence of pandemics and to assess their zoonotic potential. Under this programme a total of 368 nasal swabs and 301 faecal samples, collected from bovines were tested for the presence of Bovine coronavirus (BCoV) and of which 33 nasal samples and 3 faecal samples were positive for BCoV. Amongst the equine faecal (n=150) and nasal samples (n=238) tested by gene specific PCR, 2 fecal samples turned to be positive for ECoV while all the nasal samples were negative for ECoV. These results indicate the circulation of coronaviruses in indigenous equine and bovine population.

The detection of SARS-CoV-2 antibodies/ virus in different animal species indicates that the virus can also pose threat to animal health due to reverse zoonosis. Under these circumstances, it is imperative to have a standardized serological test for mass screening of domestic animals for the presence of SARS-CoV-2 antibodies. In this regard, to screen the presence of SARS-CoV2 antibodies in canines, ICAR-NRCE has developed a recombinant nucleocapsid protein based indirect ELISA. This

विकास के लिए तैयार किया गया है। प्राथमिक कल्चर से नष्ट न होने वाली बैट सेल लाइनों के निर्माण पर भी शोध कार्य शुरू किया गया है, जो बैट वायरस के अलगाव और होस्ट वायरस इंटरैक्शन पर अध्ययन में उपयोग किया जाएगा।

वर्तमान में फैली हुई COVID-19 महामारी तथा अन्य जूनोटिक रोगों को नियंत्रित करने के लिए पशु-जन स्वास्थ्य संबंधित दृष्टिकोण की आवश्यकता है। विभिन्न जानवरों की प्रजातियों में SARS-CoV2 और संबंधित कोरोना वायरस का पता लगाने हेतु पशु आबादी का परीक्षण करने की आवश्यकता महसूस की जा रही है। भा.कृ.अनु.प.-रा.अ.अनु.के. ने हरियाणा और राजस्थान जैसे राज्यों के विभिन्न जिलों में मवेशियों और अश्वों में SARS-CoV2 के प्रसार का अनुमान लगाने के लिए केंद्र में एक शोध कार्यक्रम शुरू करके इस चुनौती का जवाब दिया है ताकि विभिन्न जानवरों में SARS-CoV2 की अंतर-संबंधितता को समझा जा सके। महामारियों के उद्भव का पूर्वानुमान लगाने और उनकी जूनोटिक क्षमता का आकलन करने के लिए भी इस कार्यक्रम के तहत गोजातीय कोरोना वायरस (बीसीओवी) की उपस्थिति के लिए पशुओं से एकत्र किए गए कुल 368 नासिका स्वाब और 301 मल के नमूनों का परीक्षण किया गया, जिनमें से 33 नासिका और 3 मल के नमूने बीसीओवी के लिए सकारात्मक थे। जीन-विशिष्ट पीसीआर द्वारा परीक्षण किए गए अश्व मल (एन=150) और नासिका नमूनों (एन=238) में से, 2 मल नमूने इक्वाइन कोरोना वायरस (ईसीओवी) के लिए पॉजिटिव पाए गए, जबकि सभी नमूने ईसीओवी के लिए नकारात्मक थे। ये परिणाम स्वदेशी अश्व और गोजातीय आबादी में कोरोना वायरस के प्रसार का संकेते देते हैं।

विभिन्न जानवरों की प्रजातियों में SARS-CoV2 एंटीबॉडी/वायरस का होना यह दर्शाता है कि वायरस रिवर्स जूनोसिस के माध्यम से जानवरों के स्वास्थ्य के लिए भी खतरा पैदा कर सकता है। इस परिस्थिति में, SARS-CoV2 एंटीबॉडी की उपस्थिति के लिए घरेलू पशुओं की सामूहिक जांच के लिए मानकीकृत सेरोलॉजिकल परीक्षण होना अनिवार्य है। इस संबंध में, श्वानों में SARS-CoV2 एंटीबॉडी की उपस्थिति की जांच करने के लिए, भा.कृ.अनु.प.-रा.अ.अनु.के. ने अप्रत्यक्ष एलिसा पर आधारित एक पुनः संयोजक न्यूक्लियो कैप्सिड प्रोटीन विकसित किया है। इस

ELISA has validated with serum neutralization assay and has been found to have 95.66% sensitivity and 89.76% specificity. Research has also been carried out to ascertain whether pre-existing immunity to vaccination or prior infection would be able to protect against the newly emerging SARS-CoV-2 variants or not. For this purpose, growth characteristics and cross neutralization of two SARS-CoV2 (wild type and Delta variant) isolated at NCVTC was studied in detail. The study suggested that the Delta variant replicates significantly faster than the wild type (WT) SARS-CoV-2 and antibodies elicited by vaccination are more efficacious in neutralizing WT virus but significantly less potent against the Delta variant.

Much progress has been made in the basic bacteriology research this year. Molecular characterization of 59 *staphylococci* isolates accessioned at NCVTC repository classified the bacteria into 19 different species. Among these 17 belonged to *Staphylococcus* spp, and the rest two were identified as *Mammalicoccus* spp. Antimicrobial sensitivity profiling was also performed on 73 cultures of *staphylococci* which identified 20 *Staphylococcus* cultures and five *Mammaliicoccus* cultures are penicillin resistant. A survey has been carried out in different fish ponds near Hisar to study the prevalence of motile aeromonads as these bacteria represent important food-borne pathogens and cause opportunistic infections in humans. Study conducted at 66 fish ponds yielded many *Aeromonas* isolates (n=182) and antibiotic sensitivity studies on these isolates revealed the prevalence of virulent antimicrobial resistant *Aeromonasspp.* in village ponds used for fisheries. The emergence of antimicrobial resistance (AMR) in bacteria of food animals is an important risk to humans, which needs routine surveillance in order to measure the prevalence. In this direction, samples collected from different food producing animals such as cattle, buffalo, poultry and were

एलिसा को सीरम न्यूट्रलाइजेशन परख के साथ मान्य किया गया है और इसमें 95.65% संवदेनशीलता और 89.76% विशिष्टता पाई गई है। यह भी शोध किया गया है कि टीकाकरण या पूर्व संक्रमण के लिए पहले से मौजूद प्रतिरक्षा नए उभरते SARS-CoV2 वेरिएंट से रक्षा करने में सक्षम होगी या नहीं। इस उद्देश्य के लिए, एनसीवीटीसी में पृथक किए गए दो SARS-CoV2 (वाइल्ड प्रकार और डेल्टा प्रकार) के विकास विशेषताओं और क्रॉस न्यूट्रलाइजेशन का विस्तार से अध्ययन किया गया था। अध्ययन से पाया गया कि डेल्टा संस्करण वाइल्ड प्रकार SARS-CoV2 की तुलना में काफी तेजी से प्रतिकृति करता है और टीकाकरण द्वारा प्राप्त एंटीबॉडी वाइल्ड प्रकार के वायरस को निष्क्रिय करने में अधिक प्रभावशाली है लेकिन डेल्टा संस्करण के खिलाफ काफी कम शक्तिशाली है।

इस वर्ष बुनियादी बैक्टीरियोलॉजी अनुसंधान में काफी प्रगति हुई है। 59 *स्टेफिलोकोकस* के आणविक लक्षण का वर्णन एनसीवीटीसी भंडार में परिग्रहण को अलग करता है तथा बैक्टीरिया को 19 विभिन्न प्रजातियों में वर्गीकृत करता है। इनमें से 17 *स्टैफिलोकोकस* एसपीपी के थे, और बाकी दो की पहचान *मैमालिकोकस* एसपीपी के रूप में की गई थी। *स्टैफिलोकोकाई* की 73 कल्चरों पर रोगाणुरोधी संवेदनशीलता प्रोफाइलिंग भी की गई थी, जिसमें 20 स्टैफिलोकोकस कल्चरों की पहचान की गई थी और पांच स्तनपायी कोकस कल्चरों पेनिसिलिन प्रतिरोधी हैं। गतिशील एरोमोनैड्स की व्यापकता का अध्ययन करने के लिए हिसार के पास विभिन्न मछली तालाबों में एक सर्वेक्षण किया गया है क्योंकि ये बैक्टीरिया महत्वपूर्ण खाद्य जनित रोगजनकों का प्रतिनिधित्व करते हैं और मनुष्यों में अवसरवादी संक्रमण का कारण बनते हैं। 66 मछली तालाबों में किए गए अध्ययन से कई *एयरोमोनास* आइसोलेट्स (एन=182) प्राप्त हुए और इन आइसोलेट्स पर एंटीबायोटिक संवेदनशीलता अध्ययनों से विषाणुरोधी रोगाणुरोधी प्रतिरोधी *ऐरोमोनास* एसपीपी की व्यापकता का पता चला। मत्स्य पालन के लिए उपयोग किए जाने वाले गाँव के तालाबों में, खाद्य पशुओं के जीवाणुओं में रोगाणुरोधी प्रतिरोध (एएमआर) का उद्भव मनुष्यों के लिए एक महत्वपूर्ण चुनौती है जिसकी व्यापकता को मापने के लिए नियमित निगरानी की आवश्यकता होती है। इस दिशा में, विभिन्न खाद्य उत्पादक जानवरों जैसे गाय, भैंस और मुर्गी

subjected for isolation of *Escherichia coli*, and *Staphylococcus* spp. and the obtained microbial isolates were tested for AMR. Out of 36 *E. coli* poultry isolates tested, 34 (94.5%) isolates were resistant to one or more classes of antimicrobials and one poultry isolate (Ana29), was found resistant to 10 antimicrobials out of the panel of 15 antimicrobials used for testing. Out of 15 staphylococcal isolates obtained from animals, 8 (53.4%) isolates were resistant to at least 1 antimicrobial out of 9 drugs tested. This study indicates the presence of antibiotic resistant bacteria in food producing animals and the potential threat it poses to human health. Efforts are also going in the direction of development of a repository of mycobacteria at NCVTC repository and many samples from different animal species including bats are routinely screened for the presence of mycobacteria.

Trueperella pyogenes is a Gram-positive opportunistic pathogen that causes severe cases of mastitis, metritis, and pneumonia in a wide range of animals, resulting in significant economic losses. Comparative genomics of *T. pyogenes* isolate available at NCVTC identified that an open pan genome of *T. pyogenes* comprises 3214 genes, a core genome of 1520 genes, an indispensable genome of 1093 genes and strain specific genes in the range of 2-63. In addition, an inventory of virulence related genes, 190 genomic islands, 31 prophage sequences, and 40 antibiotic resistance genes that could play a significant role in organism's pathogenicity were also detected. The investigation has provided unique insights into pan genome, virulome, mobiliome, and resistome of *T. pyogenes* genomes and has laid the foundation for future investigations.

Along with bacteria and viruses, the bacteriophages constitute an integral part of the microbiome. Research on the potential use of bacteriophages on biofilm formation has yielded fruitful results. A bacteriophage cocktail to ameliorate

से एकत्र किए गए नमूनों को *एस्चेरिकिया कोलाई* और *स्टैफिलोकोकस* एसपीपी के अलगाव के अधीन किया गया था और प्राप्त माइक्रोबियल आइसोलेट्स का एएमआर के लिए परीक्षण किया गया। परीक्षण किए गए कि 36 ई. कोलाई पोल्ट्री आइसोलेट्स में से 34 (94.5%) आइसोलेट्स एक या एक से अधिक वर्गों के रोगाणुरोधी के लिए प्रतिरोधी थे और एक पोल्ट्री आइसोलेट (एना 29), परीक्षण के लिए इस्तेमाल किए गए 15 एंटीमाइक्रोबियल के पैनल में से 10 एंटीमाइक्रोबियल के लिए प्रतिरोधी पाया गया था। जानवरों से प्राप्त 15 स्टेफिलोकोकल आइसोलेट्स में से 8 (53.4%) आइसोलेट्स परीक्षण की गई 9 दवाओं में से कम से कम 1 एंटीमाइक्रोबियल के प्रतिरोधी थे। यह अध्ययन हमारे खाद्य उत्पादक जानवरों में एंटीबायोटिक प्रतिरोधी बैक्टीरिया की उपस्थिति और मानव स्वास्थ्य के लिए संभावित खतरे को इंगित करता है। एनसीवीटीसी रिपोजिटरी में माइकोबैक्टीरिया के भंडार के विकास की दिशा में भी प्रयास चल रहे हैं और माइकोबैक्टीरिया की उपस्थिति के लिए चमगादड़ सहित विभिन्न जानवरों की प्रजातियों के कई नमूनों की नियमित जांच की जाती है।

ट्रूपेरेला पायोजीनस एक ग्राम- पॉजिटिव अवसरवादी रोगजनक जीवाणु है जो जानवरों की विस्तृत श्रृंखला में मेस्टाइटिस, मेट्राइटिस और निमोनिया के गंभीर मामलों का कारण बनता है, जिसके परिणामस्वरूप महत्वपूर्ण आर्थिक नुकसान होता है। एनसीवीटीसी में उपलब्ध *टी. पाइजेन्स* आइसोलेट के तुलनात्मक जीनोमिक्स ने पहचाना कि *टी. पाइजेन्स* के एक खुले पैन जीनोम में 3214 जीन, 1520 जीनों का एक कोर जीनोम, 1093 जीनों का एक अनिवार्य जीनोम और 2-63 की सीमा में प्रजाति विशिष्ट जीन शामिल हैं। इसके अलावा, विषाणु संबंधी जीन, 190 जीनोमिक द्वीप, 31 प्रोफ़ेज अनुक्रम और 40 एंटीबायोटिक प्रतिरोध जीन जो जीव की रोगजनकता में महत्वपूर्ण भूमिका निभा सकते हैं, की एक सूची का भी पता लगाया गया है। जांच ने पैन जीनोम, वायरुलोम, मोबिलिओम और *टी. पायोजेन्स* जीनोम के प्रतिरोध में अद्वितीय अंतर्दृष्टि प्रदान की है और भविष्य की जांच के लिए नींव रखी है।

बैक्टीरिया और वायरस के साथ, बैक्टीरियोफेज माइक्रोबायोम का एक अभिन्न अंग है। बायोफिल्म निर्माण पर बैक्टीरियोफेज के संभावित उपयोग पर अनुसंधान ने

Pseudomonas aeruginosa infections in biofilms have been formulated and upon testing, this cocktail efficiently inhibited the bacterial biofilm. In similar direction, lytic bacteriophages capable of killing Extended-spectrum β-lactamases (ESBLs) producing bacteria of bovine origin has also been isolated and these phages showed strong biological efficacy suggesting that they have potential to inhibit or significantly reduce the spread of drug-resistant bacteria in livestock.

A monoclonal antibody-based ELISA kit for detection of equine influenza (H3N8) antigen was released by Hon'ble Union Minister of Agriculture and Farmers Welfare, Govt. of India on May 31, 2021. Technology for collection and cryopreservation of equine semen and production of customized artificial vagina was also commercialised and transferred to equine farmers/ breeders. In addition, an encapsulated phage formulation carrying *Salmonella* phages for therapeutic treatment in poultry has also been developed and tested during the period under report.

The scientists of the Centre have published 55 research articles in international journals and national peer-reviewed journals in this year. In addition, 19 Book chapters/ Technical bulletins/ Popular articles and 13 Research abstracts were also published by the scientists in various conferences/ symposia. Prestigious awards were also bestowed on ICAR-NRCE scientists and staff during this year which include National Academy of Veterinary Sciences (NAVS) Fellowship to Dr Sanjay Kumar; Outstanding scientist award to Dr Anju Manuja and Dr Harisankar Singha; International Research Award on New Science Invention-2021 to Dr Harisankar Singha and Best reviewer award to Dr Taruna Anand. Besides, our scientists were also selected as editorial board members of internationally reputed journals (Dr TR Talluri) and executive members (Dr SC Mehata) of the professional societies (Indian Society of

उपयोगी परिणाम प्राप्त किए हैं। बायोफिल्म में *स्यूडोमोनास एरुगिनोसा* संक्रमण को कम करने के लिए एक बैक्टीरियोफेज कॉकटेल तैयार किया गया है और इस कॉकटेल का परीक्षण करने पर बैक्टीरिया बायोफिल्म को कुशलतापूर्वक बाधित किया गया है। इसी तरह, गोजातीय मूल के बैक्टीरिया पैदा करने वाले विस्तारित-स्पेक्ट्रम -लैक्टामेस (ईएसबीएल) को कारने में सक्षम लाइटिक बैक्टीरियोफेज को भी अलग कर दिया गया है और इन चरणों ने मजबूत जैविक प्रभावकारिता दिखाते हुए सुझाव दिया है कि उनमें दवा प्रतिरोधी के प्रसार को रोकरने या काफी कम करने की क्षमता है।

पशुओं में इक्वाइन इन्फ्लुएंजा (H3N8) एंटीजन का पता लगाने के लिए एक मोनोक्लोनल एंटीबॉडी-आधारित एलिसा किट मानवीय केंद्रीय कृषि और किसान कल्याण मंत्री, भारत सरकार द्वारा 31 मई, 2021 को जारी किया गया था। अश्व के वीर्य के संग्रह और क्रायोप्रिजर्वेशन और अनुकूलित कृत्रिम योनि के उत्पादन के लिए प्रौद्योगिकी को भी किसानों/ प्रजनकों को हस्तांतरित किया गया। इसके अलावा, रिपोर्ट अधीन अवधि के दौरान पोल्ट्री में चिकित्सीय उपचार के लिए साल्मोनेला फेज को ले जाने वाला एक इनकैप्सुलेटेड फेज फॉर्म्युलेशन भी विकसित और परीक्षण किया गया है।

केंद्र के वैज्ञानिकों ने इस वर्ष अंतर्राष्ट्रीय पत्रिकाओं और राष्ट्रीय सहकर्मी-समीक्षित पत्रिकाओं में 55 शोध लेख प्रकाशित किए हैं। इसके अलावा, वैज्ञानिकों द्वारा विभिन्न सम्मेलनों/संगोष्ठियों में 19 पुस्तक अध्याय/तकनीकी बुलेटिन/लोकप्रिय लेख और 13 शोध सार भी प्रकाशित किए गए। इस वर्ष के दौरान रा.अ.अनु.के. के वैज्ञानिकों और कर्मचारियों को भी प्रतिष्ठित पुरस्कार प्रदान किए गए जिनमें डॉ. संजय कुमार को राष्ट्रीय पशु चिकित्सा विज्ञान अकादमी (एनएबीएस) फैलोशिन शामिल हैं; डॉ. अंजू मनुजा और डॉ. हरिशंकर सिंघा को उत्कृष्ट वैज्ञानिक पुरस्कार; डॉ. हरिशंकर सिंघा को नए विज्ञान आविष्कार-2021 पर अंतर्राष्ट्रीय अनुसंधान पुरस्कार और डॉ. तरूना आनंद को सर्वश्रेष्ठ समीक्षक का पुरस्कार। इसके अलावा, हमारे वैज्ञानिकों को आंतरिक रूप से प्रतिष्ठित पत्रिकाओं (डॉ. टी आर तल्लूरी) के संपादकीय बोर्ड के सदस्य और प्रबुद्ध वर्ग (इंडियन सोसाइटी ऑफ एनिमल जेनेटिक्स एंड

Animal Genetics and Breeding -ISAGB).

ICAR-NRCE encourages its staff for capacity building in advanced areas of science, administration and skill development. In the year 2021, 8 scientists and one administrative staff updated their skills by attending different training programmes relevant to areas of their field. ICAR-NRCE also imparted training to different stakeholders (veterinarians, medical professionals, students and equine keepers) and the centre organized 14 training programmes during the period under report. Scientists of the Centre also delivered 35 expert/invited lectures on various workshops and training programmes. Besides, Memorandum of Understanding (MOU) has been signed with the four institutes/ organisations/ equine breeders in 2021 for co-operation in the areas of research, education, extension, consultancy, capacity building and other areas of national interest.

Various institutional activities were organized during the period that includes (1) An awareness program on "Emergence of scourge of Antimicrobial resistance in bacteria" was organized for high school students 04th January, 2021 ; (2) Covid-19 vaccination drive for frontline corona warriors of ICAR-NRCE involved in COVID-19 testing on 19th January 2021; (3) Republic day celebration on 26th Jan, 2021; (4) International Webinar on "Preventing future zoonotic pandemics: Interventions at the wildlife–livestock–human interface on world zoonosis day (6th July 2021); (5) Four *"Azadi ka Amrit Mahotsav Webinar Series"* through Aug-Dec, 2021; (6) Antimicrobial Resistance (AMR) awareness programme for high school students on 04th Jan, 2021; (7) Essay writing competition on "Innovative ideas for water conservation" on World Water Day 22nd March, 2021; (8) Tree plantation on *Van mahostav* campaign at EPC Bikaner on 16th July, 2021; (9) *Poshan Vatika* and *Vriksharopan* at EPC, Bikaner campus on 17th Sept, 2021; (10) Foundation

ब्रीडिंग–आईएसएजीबी) के कार्यकारी सदस्य (डॉ. एससी मेहता) के रूप में भी चुना गया था।

केंद्र अपने कर्मचारियों को विज्ञान, प्रशासन और कौशल विकास के उन्नत क्षेत्रों में क्षमता निर्माण के लिए प्रोत्साहित करता है। वर्ष 2021 में, 8 वैज्ञानिकों और एक प्रशासनिक कर्मचारी ने अपने क्षेत्रों से संबंधित विभिन्न प्रशिक्षण कार्यक्रमों में भाग लेकर अपने कौशल को अद्यतन किया। केन्द्र ने विभिन्न हितधारकों (पशु चिकित्सक, चिकित्सा पेशेवर, छात्रों और अश्व पालकों को भी प्रशिक्षण प्रदान किया और रिपोर्ट की अवधि के दौरान केंद्र द्वारा कुल 14 प्रशिक्षण कार्यक्रम आयोजित किए गए। केंद्र के वैज्ञानिकों ने विभिन्न कार्यशालाओं और प्रशिक्षण कार्यक्रमों पर 35 विशेषज्ञ/ आमंत्रित व्याख्यान भी दिए। इसके अलावा, अनुसंधान आउटरीच गतिविधियों के लिए अनुसंधान, शिक्षा, विस्तार, परामर्श, क्षमता निर्माण और राष्ट्रीय हित के अन्य क्षेत्रों में सहयोग के लिए 2021 में चार संस्थानों/ संगठनों/ अश्व के प्रजनकों के साथ समझौता ज्ञापन (एमओयू) पर हस्ताक्षर किए गए हैं।

इस अवधि के दौरान विभिन्न संस्थागत गतिविधियों का आयोजन किया गया जिसमें शामिल हैं- (1) हाई स्कूल के छात्रों के लिए 'बैक्टीरिया में रोगाणुरोधी प्रतिरोध के संकट का उद्भव' पर जागरूकता कार्यक्रम जो कि 04 जनवरी, 2021 को आयोजित किया गया; (2) 19 जनवरी 2021 को कोविड–19 परीक्षण में शामिल केंद्र के कोरोनो योद्धाओं के लिए कोविड–19 टीकाकरण अभियान; (3) 26 जनवरी, 2021 को गणतंत्र दिवस समारोह; (4) ''भविष्य में जूनोटिक महामारी की रोकथाम: विश्व जूनोसिस दिवस पर वन्यजीव–पशुधन–मानव इंटरफ़ेस पर हस्तक्षेप'' पर अंतर्राष्ट्रीय वेबिनार (6 जुलाई 2021); (5) अगस्त–दिसंबर, 2021 में ''आज़ादी का अमृत महोत्सव'' के अंतर्गत चार वेबिनारों की श्रृंखला; (6) विश्व जल दिवस 22 मार्च, 2021 पर ''जल संरक्षण के लिए अभिनव विचार'' पर निबंध लेखन प्रतियोगिता; (7) वन महोत्सव अभियान पर 16 जुलाई, 2021 को ईपीसी बीकानेर में वृक्षारोपण; (8) ईपीसी बीकानेर परिसर में 17 सितंबर, 2021 को पोषण वाटिका और वृक्षरोपन; (9) स्थापना दिवस और 28 सितंबर, 2021 को ईपीसी, बीकानेर परिसर में जलवायु तन्यक किस्मों, प्रौद्योगिकियों और प्रथाओं पर पीएम का

Day and PM's Programme on Climate Resilient Varieties, Technologies and Practices at EPC, Bikaner campus on 28th Sept, 2021; (11) *Mahila Kisan Diwas* on 15th Oct, 2021; (12) World Food day on 16th Oct, 2021; (13) Vigilance awareness week during 26th Oct-1st Nov 2021; (14) Independence day celebrations on 15th Aug, 2021; (15) ICAR-NRCE Foundation day celebration on 26th Nov, 2021 and (16) Special *swachhata* awareness campaign at ICAR-NRCE during 16th-31st Dec, 2021. The centre organized equine health camps, *kisan gosthis* and interactive farmer meets to educate equine owners on various aspects of equine health, production, disease control and management.

Under infrastructure development, a Jenny dairy unit of Halari donkeys has been established at the centre and the same was inaugurated on 30th Jul, 2021 by Dr BN Tripathi (Hon'ble DDG, Animal Sciences). Besides these, repair of small animal house, renovation/repair of animal shed, pathology lab of ICAR-NRCE, clinical examination hall, security check post, external furnishing of NCVTC Building, construction of additional vehicle parking stand and electrification of NCVTC small animal house has also been carried out.

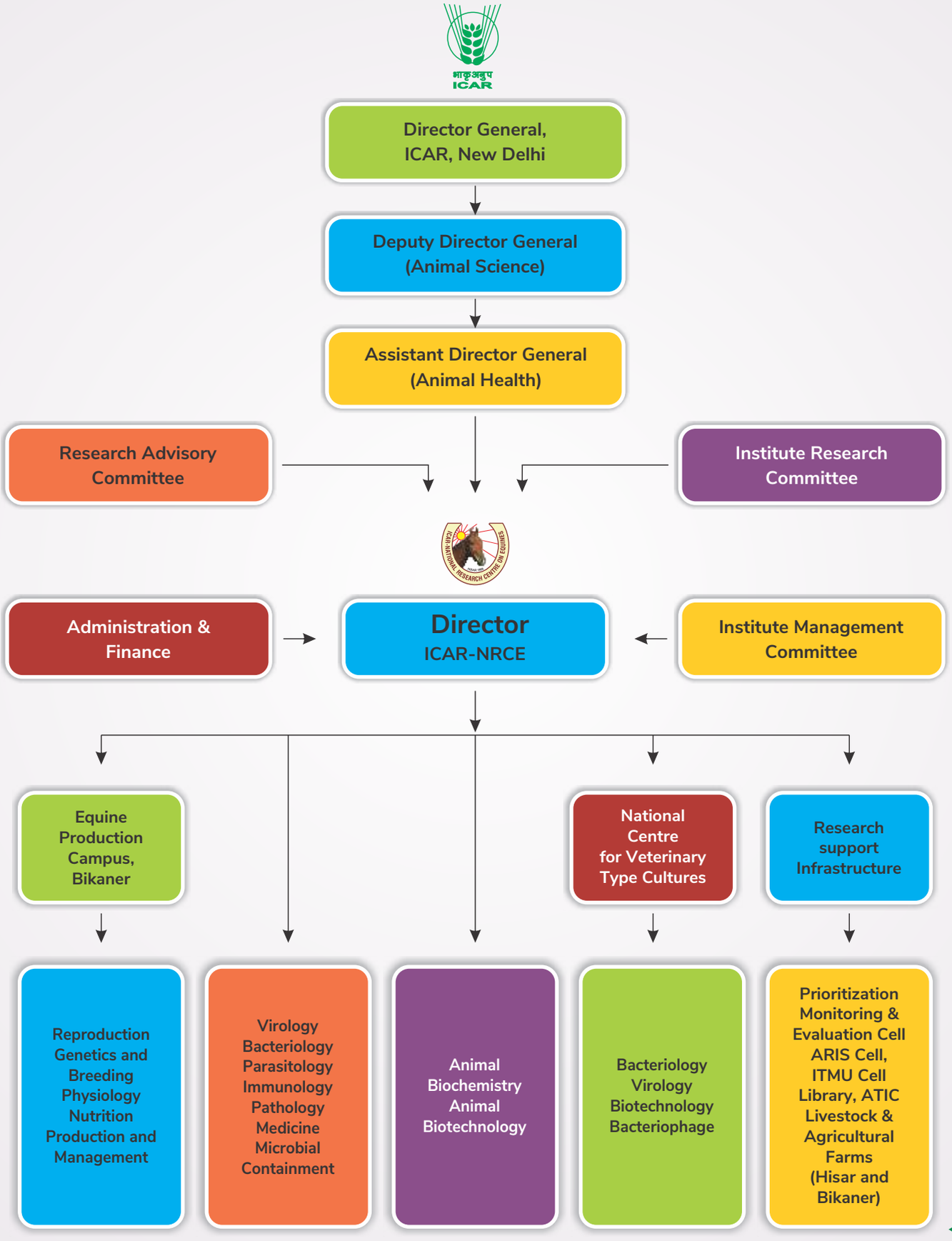
One of the important services rendered by ICAR-NRCE is contractual testing of samples from equine breeders and race courses. During the current year, centre has generated revenue of rupees 75.95 lakhs through testing of 9937 serum samples for various equine diseases. Besides, revenue has also been generated through sale of farm produce and livestock to the tune of 18.66 lakh.

कार्यक्रम; (10) 15 अक्तूबर, 2021 को महिला किसान दिवस; (11) 16 अक्तूबर, 2021 को विश्व खाद्य दिवस; (12) 26 अक्तूबर से 1 नवंबर, 2021 के दौरान सतर्कता जागरूकता सप्ताह; (13) 15 अगस्त, 2021 को स्वतंत्रता दिवस समारोह) (14) 26 नवंबर, 2021 को आईसीएआर-एनआरसीई स्थापना दिवस समारोह; (15) 16-31 दिसंबर, 2021 के दौरान आईसीएआर-एनआरसीई में विशेष स्वच्छता जागरूकता अभियान। केंद्र ने अश्व के स्वास्थ्य, उत्पादन, रोग नियंत्रण और प्रबंधन के विभिन्न पहलुओं पर अश्व के पालकों को शिक्षित करने के लिए अश्व के स्वास्थ्य शिविर, किसान गोष्ठी और इंटरैक्टिव किसान बैठकें आयोजित कीं।

बुनियादी ढांचे के विकास के तहत, केन्द्र में हलरी गर्दभों की एक जेनी डेयरी इकाई स्थापित की गई और इसका उद्घाटन 30 जुलाई, 2021 को डॉ. बी.एन. त्रिपाठी (माननीय डीडीजी पशु विज्ञान) द्वारा किया गया था। इनके अलावा, छोटे पशु घर की मरम्मत, पशु शेड का नवीनीकरण/मरम्मत, आईसीएआर-एनआरसीई की पैथोलॉजी लैब, क्लिनिकल परीक्षा हॉल, सुरक्षा जांच पोस्ट, एनसीवीटीसी भवन की बाहरी साज-सज्जा, अतिरिक्त वाहन पार्किंग स्टैंड का निर्माण और एनसीवीटीसी छोटे पशु गृह का विद्युतीकरण भी किया गया है।

अश्वों के प्रजनकों और रेस कोर्स से प्राप्त नमूनों का संविदात्मक परीक्षण केंद्र द्वारा प्रदान की जाने वाली महत्वपूर्ण सेवाओं में से एक है। इस वर्ष के दौरान, केंद्र ने विभिन्न अश्वों की बीमारियों के लिए 9937 सीरम नमूनों के परीक्षण के माध्यम से 75.95 लाख रूपये का राजस्व अर्जित किया है। इसके अतिरिक्त, कृषि उपज और पशुधन की बिक्री से 18.66 लाख रूपये का राजस्व भी अर्जित किया गया है।

Organizational SET-UP



01

Introduction

"He's of the colour of the nutmeg. And of the heat of the ginger, he is pure air and fire, and the dull elements of earth and water never appear in him, but only in patient stillness while his rider mounts him; he is indeed a horse, and all other jades you may call beasts."

– William Shakespeare

Humans and horses have a primeval relationship, which began around 6000 years ago when humans first domesticated them. Due to their vigor and agility, horses have been an important part of various ancient civilisations. Historical tales and poems describe heroic victories of brave warriors and their fearless horses. The Sanskrit word of horses is *Ashva*, there are several elaborate descriptions of horse and their role in ancient hindu civilizations in the Vedas (c. 1500 - 500 BC). Out of the four Vedas, the *'Rigveda'* has a vivid description of equestrian scenes, often associated with chariots. These majestic animals aided humans in exploring the world providing the ease of travel and transport.

With the advent of modern means of transportation and mechanization, the utility of equines decreased gradually. Despite this, equines play an imperative part in the transportation of products within the craggy regions, carrying of agrarian products to the markets, religious ceremonies, wedding ceremonies and building works and subsequently contributes to the daily earnings to the numerous people in the country.

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 have been undertaking research in areas of equine health, equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry and biotechnology. The research activities are supported by centralized services such as animal and agriculture farms, experimental animal facility, microbial containment laboratory, AKMU cell, ATIC, library and Info-equine museum.

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. The National Centre for Veterinary Type Cultures (NCVTC) was established in the year 2005 at ICAR-NRCE, Hisar main campus 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.

MANDATE OF ICAR-NRCE

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

OBJECTIVES OF ICAR-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 36 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. The Centre is striving hard for the conservation and characterization of Indian breeds of equines in the country and has even established nucleus herds of representative breeds of equines in its Bikaner campus. Some of the major achievements and accolades of the Centre are enlisted below:

Development of diagnostics for equine diseases

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

- HERP kit for field diagnosis of equine herpesvirus 1 (EHV1) infection.
- COFEB kit for diagnosis of *Theileria equi*.
- A monoclonal antibody-based diagnostic kit 'EquiherpesB-ELISA' for EHV1 antibody detection.
- A type-specific ELISA and real-time PCR for differentiation of EHV1 and EHV4 infections.
- Complement fixation test and r-protein-based ELISA for diagnosis of glanders.
- A monoclonal antibody-based sandwich ELISA and RT-PCR for detection of equine rotavirus (ERV) from faecal samples.
- RT-PCR and real-time RT-PCR based assays for typing and diagnosis of equine influenza virus.
- A recombinant antigen based-ELISA for detection of antibodies to *Theileria equi*.
- An indirect ELISA using whole cell lysate antigen and PCR for detection of *Trypanosoma evansi*.
- ELISA and RT-PCR for diagnosis of Japanese encephalitis.
- A recombinant protein-based indirect ELISA for 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.
- Standardized a nested (gB-nPCR) and real-time PCR (gB- qPCR) targeting gB for detection of EHV 1 latency.
- Indirect ELISA using recombinant protein for detection of JEV specific antibodies in horse and pig.
- 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 to detect *T. evansi* antibodies in multiple species.
- Monoclonal antibody-based ELISA kit for detection of equine influenza (H3N8) antigen.

Development of vaccines and immuno-biologicals

- Inactivated EHV1 vaccine *"Equiherpabort"* using an indigenous isolate of EHV1 for prevention of abortions in mares.
- Inactivated equine influenza vaccine using an indigenous isolate(A/equi-2/Ludhiana/87). This vaccine was updated in the year 2008-09 by incorporating a 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*.
- Formulation of inactivated EHV1 vaccine using montanide adjuvant and tested in murine model.
- Encapsulated phage formulation carrying

Salmonella phages for therapeutic application in poultry.

Surveillance and monitoring of equine diseases in India

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

- India has gained OIE 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. Clinical cases of equine infectious anemia (EIA) have not been reported since 2010 (other than two seropositive cases from Uttarakhand & Haryana).
- The re-emergence of Glanders in equines have been detected since 2006-07 from different states and control measures are being adopted for preventing their further spread.
- Effective control of 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 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.

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. Phylogenetic analysis based on Unique Short segments classified our isolates into clade 5 along the reference isolates V592.
- Genotypic characterization of *Burkholderia mallei* isolates recovered from recent

glanders outbreaks revealed that they significantly differ from the older Indian isolates (Isolates from pre-independent period).

Phenotypic and genotypic characterization of Indian equine breeds

- 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.
- Phenotypic parameters of Kachchhi-Sindhi horses were analyzed.
- Microsatellite marker based genetic diversity analyzed for proposing effectual population breeding and management strategies for future.

Establishment of nucleus herd

- ICAR-NRCE has initiated *in-situ* conservation programme in the form of developing an equine sanctuary at EPC, Bikaner where nucleus herds of different Indian horse breeds are being maintained.
- Marwari horses from Rajasthan; Kathiawari horses from Gujarat; Zanskari ponies from Zanskar valley (Jammu & Kashmir) and Manipuri ponies from Imphal (Manipur) and herds of indigenous and exotic donkeys are being maintained.
- Large white (Halari) donkeys for their conservation and improvement are being maintained.
- Poitou donkey herd for production of superior mules are also maintained at the Centre.

Improvement in production potential of equines

- In order to conserve the germ plasm of indigenous equine breeds, cryopreservation of semen of Marwari, Kathiawari, Zanskari and Manipuri stallions and Halari & Poitou donkeys has been done.
- Artificial insemination using frozen semen has been perfected for the 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 in 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 carried out. Expression of SPATA1, PLCz and CRISP3 fertility genes have 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, excessive growth of granulation tissue (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 this technology was also transferred to equine farmers/ breeders.
- Production of donkey milk based products (bathing soap, body butter and lip balm) has been standardized.

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.
- Utility of mules in chaff cutting operation has been studied with average output capacity of 660 kg/hour.
- The technique of vermi composting of equine dung has been optimized for use in agricultural fields.

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).

Patents filed

- A highly sensitive kit for detection of antibodies against *Theileria equi* in serum of equids. Application No. 2763/DEL/2012, dated 06.09.2012
- 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. Application No. 201811048899, dated 24.12.2018 (Provisional).
- Polymeric metal nanocomposites and methods of synthesis there of. Application No. 201911009696, dated 13.03.2019.
- 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

Services

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

- Disease diagnostic services for various infectious and non-infectious diseases to equine owners, breeders, state animal husbandry departments, police and army horses.
- Surveillance, monitoring and control of equine infectious diseases in India.
- Health certification for movement of equines within and outside the country to promote export of horses.
- Clinical and diagnostic (including pregnancy diagnosis) services for equine diseases.
- Artificial insemination to augment the production of superior quality horses, mules and donkeys.
- Provision of quality jacks and jennies to various states, breeding societies and farmers, for production of superior quality mules and donkeys.

- 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 pandemic of COVID-19, ICAR-NRCE served as a COVID-19 testing facility amongst one of the 4 institutes of ICAR.

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 ICAR-NRCE Hisar, dairy microbes at ICAR-NDRI, Karnal and rumen microbes at ICAR- NIANP, Bengaluru.

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

At present, NCVTC repository is maintaining a total of 4505 accessioned microbes, which include veterinary microbes (n=3138), rumen microbes (n=642) and dairy microbes (n=725). The year-wise progress in culture collection can be seen in the table below.

Year-wise progress of the NCVTC repository

Year	2009-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22	Total
Veterinary Microbes									
Bacteria	927	110	164	70	123	95	50	63	**1602**
Virus	156	14	28	27	31	44	24	31	**355**
Bacteriophage	32	44	29	24	8	8	48	92	**285**
Recombinant clone	466	45	10	36	16	8	-	-	**581**
Phage library	27	-	-	-	-	-	-	-	**27**
Genomic DNA	223	57	0	8	0	0	-	-	**288**
Total	**1831**	**270**	**231**	**165**	**178**	**155**	**122**	**186**	**3138**
Rumen microbes									
Anaerobic bacteria	142	74	37	37	49	46	62	80	**527**
Fungi/Yeast	107	0	0	0	0	0	-	-	**107**
Meth. Archae	8	0	0	0	0	0	-	-	**8**
Total	**257**	**74**	**37**	**37**	**49**	**46**	**62**	**80**	**642**
Dairy microbes									
Bacteria	468	39	40	30	36	44	20	48	**725**
Total	**468**	**39**	**40**	**30**	**36**	**44**	**20**	**48**	**725**
Grand Total	**2556**	**383**	**308**	**232**	**263**	**245**	**204**	**314**	**4505**

Some of the salient achievements of the NCVTC are listed below.

Veterinary Microbes

- First laboratory confirmed camelpox virus zoonosis.
- First report on isolation and genetic characterization of swine poxvirus from India.
- First isolation of Lumpy skin disease virus from India.
- Accessioning of vaccine strains of viruses *viz.,* *Peste des petits ruminants* virus, Sheeppox virus (Srinagar strain), Goatpox virus (Uttarkashi strain), Orf virus (Mukteswar strain), NDV(R2B strain) and NDV(F strain).
- Complete genome sequencing of Classical swine fever virus (n=2), chicken astro virus (n=2), porcine circo virus (n=4), SARS CoV-2 (n=14), Foot and mouth disease virus (n=3), Newcastle disease virus (n=3), Lumpy skin disease viruses (n=4), Jaagsiekte sheep retrovirus (n=1) and buffalopoxvirus (n=5).
- First isolation and characterization of *Bordetella bronchiseptica*, *Actinobacillus equilli*, *Staphylococcus hyicus Moraxella* (*Branhamella) ovis*, methicillin-resistant coagulase negative *Staphylococcus sciuri* and *Trueperella pyogenes.*
- Whole genome sequencing of *Pasteurella multocida* ssp *.multocida* B:2 serotype, *Trueperella pyogenes*, *Bordetella bronchiseptica*, *Pasteurella multocida*, *Actinobacillus equuli* and *Salmonella* Gallinarum.
- Accessio*ning of rare strains of* bacteria: *Campylobacter* spp., *Bacillus megaterium, Enterococcus casseliflavus, E. cecorum, Barrientosiimonas humi, Corynebacterium amycolatum, Enterococcus devriesei, E. hirae, E. faecium, Nocariopsis alba, Ignatzschineria larvae, Escherichia hermanii, Actinobacillus hominis, Mannheimia caviae, Enterococcus cecorum, Staphylococcus saprophyticus* ssp. *bovis*, *Staphylococcus xylosus*, *Corynebacterium efficiens*, *Shigella*

dysentriae, *Mammalicoccus sciuri*, *Staphylococcus argenteus*, *Aeromonas hydrophila*, *Stenotrophomonas maltophila*, *Aeromonas veronii*, *Pseudomonas alkaliphila*, *Arcanobacterium pluranimalium*, *Brucella tritici*, *Leucobacter celer* ssp. *celer*, and *Morganella morganii*.

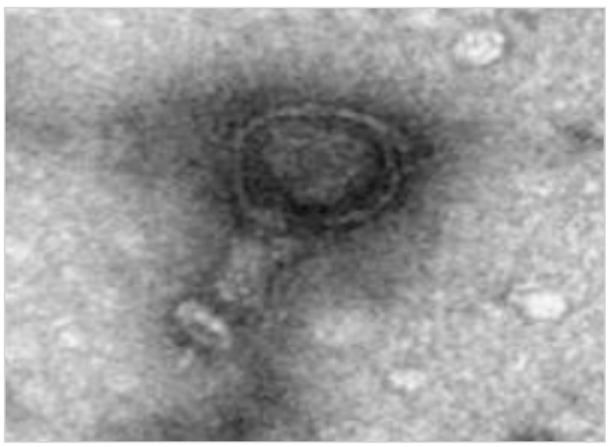
- Isolation and accessioning of thermo tolerant bacteriophage from Ganga river water.
- The whole genome sequencing of *Proteus mirabilis* phage VTCCBPA139 against MDR *Proteus mirabilis*.
- Phage therapy against MDR *K. pneumoniae* in mouse model.

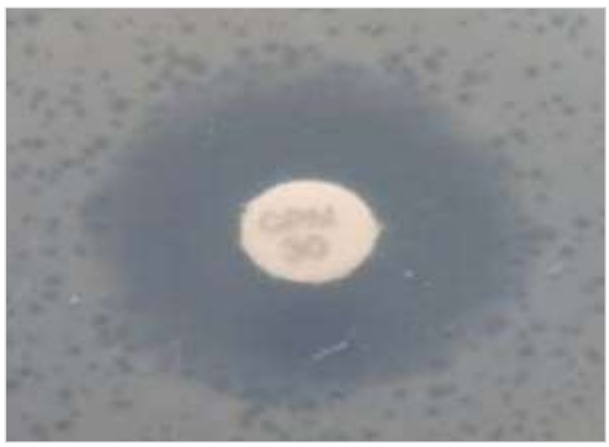
Rumen Microbes

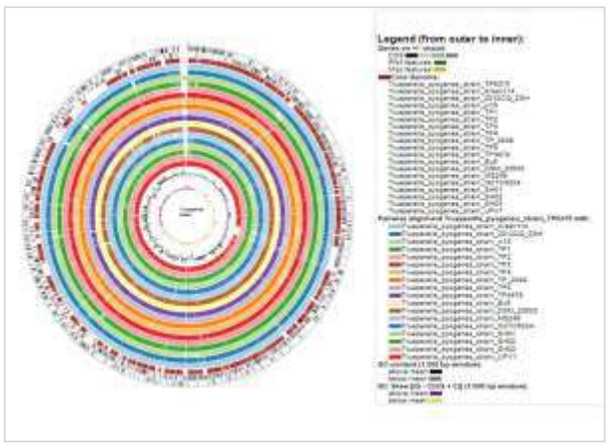
- Isolation and characterization of seven tannin degrading bacteria- *Streptococcus gallolyticus* from goat; fibre degrading bacteria *Ruminococcus flavefaciens*, *Prevotella* sp. and *Butyrivibrio* sp. from buffaloes and cattle; nitrate reducing and cellulose degrading *E. coli* from buffalo.
- Isolation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

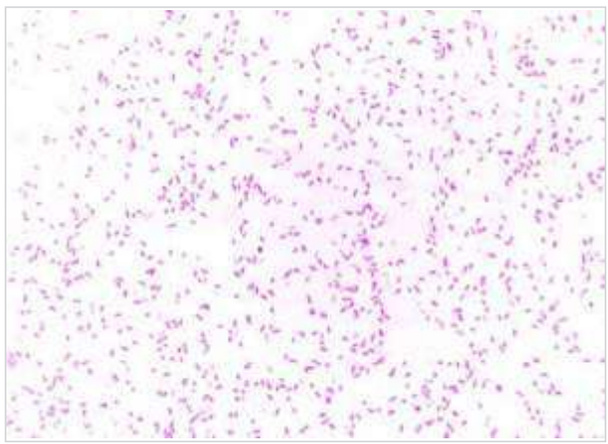
Dairy Microbes

- Preservation of important dairy microbes, *viz*, *Lactococcus lactis* ssp. *lactis, Lactococcus lactis*. ssp. cremoris, *Streptococcus thermophilus*, *Leuconostoc* sp.,. *Bifidobacterium dentium*, *Bifidobacterium longum*, *Kluyveromyces lactis* and *Saccharomyces bisporus* in the repository.
- 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.









LANDMARK ACHIEVEMENTS

Year	Achievement
1985	Foundation of ICAR-NRCE, Hisar
1987	Detection of first outbreak of Equine Influenza in northern India
1989	Establishment of Equine Production Campus, Bikaner
1995	Cryopreservation of Jack semen for AI
1996	Establishment of a herd of Marwari horses
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
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
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
2016	Organisation of SAARC trainings on equine influenza and glanders under OIE twinning program
2016	Methodology for isolation of positive sense 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	Release of Japanese Encephalitis (JE) virus antibody test kit
2021	Commercialisation of customised artificial vagina for semen collection in equines
2021	Commercialisation and transfer of semen collection and cryopreservation technology
2021	Jenny dairy unit for Halari donkeys established at Hisar
2021	Development of a novel vaccine formulations to prevent SARS-CoV-2 infection in animals
2021	Release of monoclonal antibody ELISA kit for detection of EIV (H3N8) antigen
2021	Development of an attenuated novel lumpy skin disease virus (LSDV) with potential to serve as a vaccine candidate

SUMMARY OF EXPENDITURE & REVENUE GENERATION

Details	Year 2021 (Jan. 2021 - Dec. 2021) (in Lakhs)		
Summary of Expenditure			
	NRCE	**NCVTC**	**Total (in Rs.)**
Establishment charges including LSP/PF, wages, OTA	114526442	0	114526442
Traveling allowances and HRD	607969	0	607969
Others charges including equipments& recurring charges	67302908	23115626	90418534
Works	1211931	351209	1563140
Loans and Advances	1000000	0	1000000
Total Plan Expenditure	**184649250**	**23466835**	**208116085**
Summary of Revenue Generation			
	NRCE	**NCVTC**	**Total (in Rs.)**
Sale of farm produce	970613	0	970613
Sale of livestock	896400	0	896400
Sale of publications and advertisements	0	0	0
License fee	148278	0	148278
Interest on loans and advances	895562	0	895562
Interest on short term deposits	572573	0	572573
Contractual diagnostic services	75956005	0	75956005
Receipt from services	0	0	0
Other miscellaneous receipts	5386216	0	5386216
Eco-tourism	61700	0	61700
Total revenue generation	**16526942**	**0**	**16526942**

STAFF POSITION AT ICAR-NRCE & NCVTC

Name of the post	NRCE			NCVTC		
	Sanctioned	**Filled**	**Vacant**	**Sanctioned**	**Filled**	**Vacant**
Director	01	--	01	--	--	--
Scientific	23	15	08	10	8*	02
Technical	25	20	05	01	--	01
Administrative	19	11	08	--	--	--
Supporting	20	15	05	--	--	--

* on deputation

RESEARCH
ACHIEVEMENTS

Equine Health

- Diagnostics
- Vaccines
- Drug Development
- Disease surveillance

Equine Production

- Production Enhancement
- Nutrition
- Breed Characterization
- Breed Conservation

Microbial Conservation

- Microbial Isolation
- Characterization
- Accession
- Conservation

02

Research Achievements

Equine Health

Prevalence studies on coronaviruses in equine and bovine populations

Multiple species of coronaviruses (CoV) are circulating in birds, wildlife, domestic and pet animal species. Since these animals are constantly interacting with each other, host-species expansion or inter-species transmission of new CoV to humans seems to be inevitable. Hence, a surveillance network needs to be established for investigation of the CoV amongst different animal species to decipher their inter-relatedness, to forecast and to prevent future emergence of pandemics. Therefore, a study was initiated to assess the prevalence of CoVs in equine and bovine populations in Haryana and Rajasthan.

***Bovine Coronavirus (BCoV)*:** The bovine nasal swabs (n=368) and faecal samples (n=301) were collected from calves below 6 months of age from different farms of Haryana and Rajasthan **(Table 1)** and tested by real-time PCR for BCoV. A total of 33 nasal samples and 3 faecal samples were positive for BCoV (**Table 1**). Real-time PCR positive samples were passaged in NLBK cells for isolation of BCoV and six viruses (2 from fecal samples and 4 from nasal swab) were isolated during this period. All theses virus isolates have been submitted to NIHSAD, Bhopal for whole genome sequencing and data of one isolate (BFS93) has been received and is being analyzed.

Table 1: Month-wise bovine sample collection during 2021

S. No.	Location	No. of fecal samples		No. of nasal samples	
		Tested	Positive	Tested	Positive
1	CIRB, Hisar, Haryana	103	3	136	24
2	GLF, Hisar, Haryana	38	-	72	3
3	Shree Krishna Gaushala, Kabrel, Hisar	35	-	35	-
4	Shree Krishna Gaushala, Meham, Haryana	27	-	27	-
5	Shree Krishna Gaushala, Khidwali, Rohtak, Haryana	36	-	36	1
6	Aadi Badri Gaushala, Bilaspur, Yamunanagar, Haryana	27	-	27	5
7	Palwal, Haryana	20	-	20	-
8	Cattle farm, Bikaner, Rajasthan	15	-	15	-
	TOTAL	**301**	**3**	**368**	**33**

Equine Coronavirus (ECoV): The nasal swabs (n=238) and faecal samples (n=150) of foals (below 6 month of age) were collected from different farms of Haryana and Rajasthan. ECoV was detected in 2 out of 150 fecal samples tested during the study **(Table 2)**.

Table 2: Month-wise equine sample collection and testing for ECoV during 2021.

Location	No. of fecal samples		No. of nasal samples	
	Tested	Positive	Tested	Positive
April, 2021	25	-	92	-
May, 2021	22	2	39	-
June, 2021	16	-	20	-
July, 2021	16	-	16	-
October, 2021	27	-	27	-
November, 2021	18	-	18	-
December, 2021	26	-	26	-
TOTAL	**150**	**2**	**238**	**-**

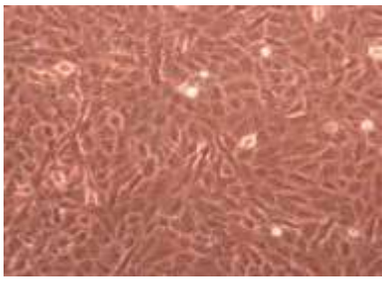
(Gulati BR, Kumar N, Shanmugasundaram K and Riyesh T)

Studies on growth characteristics and cross neutralization of wild type and Delta SARS-CoV-2 from Hisar (India)

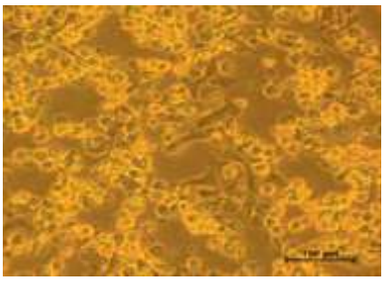
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly evolved to generate several antigenic variants. These variants have raised concerns whether pre-existing immunity to vaccination or prior infection would be able to protect against the newly emerging SARS-CoV-2 variants or not. We isolated SARS-CoV-2 from the COVID-19 confirmed patients in the beginning of the first (April/May 2020) and second (April/May 2021) wave of COVID-19 in India (Hisar, Haryana). Upon complete nucleotide sequencing, these viruses were found to be genetically related with wild-type (WT) and Delta variants of SARS-CoV-2 respectively. The Delta variant of SARS-CoV-2 produced a rapid cytopathic effect (24-36 h as compared to 48-72 h in WT) **(Fig. 1A)**, had bigger plaque size **(Fig. 1B)**, and a shorter life cycle (~6 h as compared to the ~8 h in WT). Furthermore, the Delta variant achieved peak viral titres within 24 h as compared to the 48 h in WT. This evidence suggested that the Delta variant replicates significantly faster than the WT SARS-CoV-2. The virus neutralization experiments indicated that antibodies elicited by vaccination are more efficacious in neutralizing WT virus but significantly less potent against the Delta variant. Our findings have implications in devising suitable vaccination, diagnostic and therapeutic strategies, besides providing insights into understanding virus replication and transmission. The reagents (virus/ hyperimmune serum) generated will facilitate SARS-CoV-2 research at local level.

(A). Cytopathic effect

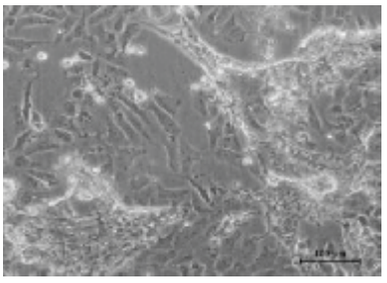
Mock-infected



SARS-CoV-2 (WT)-infected



SARS-CoV-2 (Delta)-infected



(B). Plaque morphology

Fig. 1 A-B. Growth characteristics of Wild type and Delta SARS-CoV-2. Nasopharyngeal swabs positive for COVID-19 with cT value of <20.0 in qRT-PCR were considered for virus isolation in Vero cells. The samples were filtered in 0.45 µm syringe filter and 500 µl of the filtrate was used to infect Vero cells. Samples that produced CPE within three successive passages in Vero cells were authenticated and accessioned. **(A)** Characteristics of the CPE produced by WT and Delta variant of SARS-CoV-2 at passage level 5 is shown. **(B)** Plaque morphology of WT and Delta variant of SARS-CoV-2 is also shown.

(Kumar N, Barua S, Riyesh T, Gulati BR and Yash Pal)

Indirect ELISA for detection of antibodies against SARS-CoV-2 in canines

There have been many reports of detection of SARS-CoV-2 antibodies/ virus in animals during the current COVID-19 pandemic. Reports of infection in animals such as tigers, lions, cats, dogs and minks indicate the virus can pose threat to animal health due to reverse zoonosis and it is also possible that this spillover hosts, namely, various feline or canine species will act as reservoir hosts, which may lead to zoonotic threat. Thus, there is an urgent need to screen our animal population, especially the canine and feline population for SARS-CoV-2 and to develop specific and sensitive diagnostics for it.

In this process, ICAR-NRCE has developed a recombinant nucleocapsid protein based indirect ELISA for the detection of antibodies against the SARS-CoV-2 in canines. More specifically, the synthesized codon optimized N gene of SARS-CoV-2 was cloned into an expression vector and transformed into a prokaryotic host (*E. coli*) and produced large-scale recombinant NP protein (rNP) **(Fig. 2A and 2B)**. In western blot analysis, the rNP was able to detect antibodies against SARS-CoV-2 from canine serum samples **(Fig. 2C)**. Subsequently, rNP protein also showed reactivity in ELISA for specific detection of antibodies against SARS-CoV-2. The developed assay has been tested for detection of antibodies in serum samples collected from canines. A total of 30 canine pre-covid sera and 423 sera samples (collected in 2021) were tested with this indirect ELISA. Of which, all pre-covid sera were found to be negative for SARS-CoV-2 antibodies and the 423 serum samples collected in 2021 showed a positivity of 32.22% at the relative percentage of positivity value of 25%. The assay has also been validated with serum neutralization assay and has been found to have 95.66% sensitivity and 89.76% specificity, respectively.

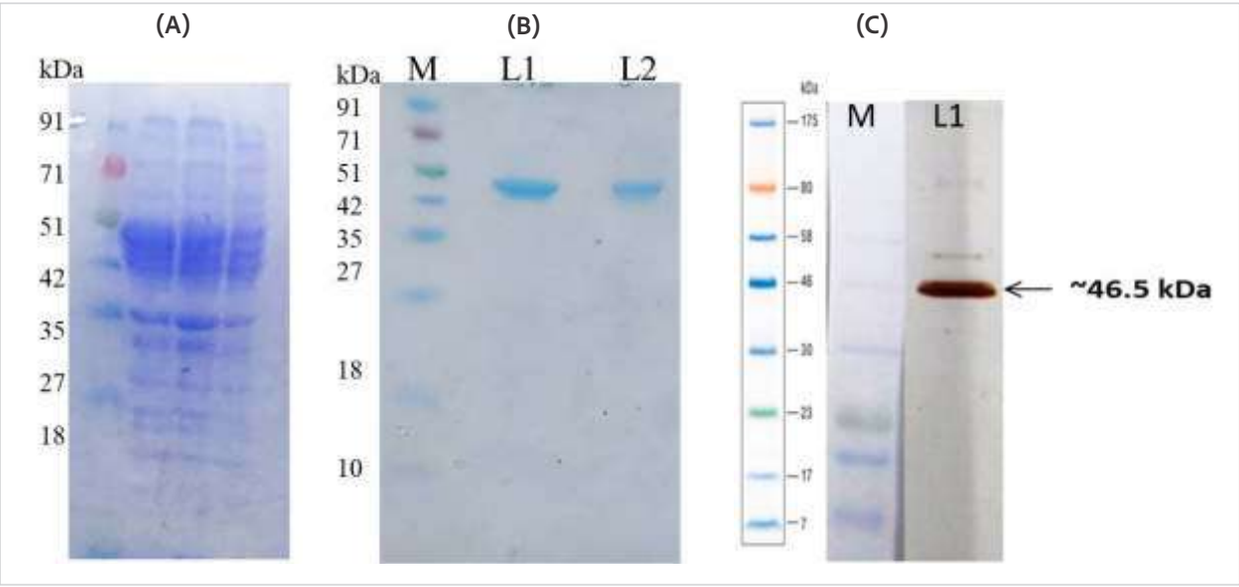


Fig. 2A-C. Expression and western blot of Codon optimised full length rN protein. **(A)** Induction of His-tagged rN protein. M= prestained protein ladder, L1-L3 = induced rN protein; **(B)** Expression of His-tagged rN protein. M= prestained protein ladder, L1 & L2 = purified rN protein; **(C)** Western blot of purified rNprotein. M= pre-stained protein ladder, L1 = reactivity of rN protein with SARS-CoV-2 positive canine serum.

(Virmani N, Bera BC and Anand T)

Complete genome analysis of Equine Rotavirus A (ERVAs)

A wide host range, a segmented genome with the capability of reassortment, have made rotavirus a globally prevalent diarrheal pathogen in young animals and humans. From the year 2014 to 2017, a total of 274 equine samples were tested at ICAR-NRCE, out of these 88 were found to be positive for equine rotavirus. From the positive samples, complete genomes of four rotaviruses isolated from Hisar (ERV3, ERV4 and ERV6) and Tohana (ERV2) regions of India were sequenced and analyzed to gain insights on the genomic constellations circulating in India. The genotypic constellation of the samples, elaborated in the order VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/NSP6 was found to be unique among equines. In ERV2, the constellation was inferred to be G6-P [1]- I2-R2-C2-M2-A3-N2-T6-E2-H3 while ERV3, shared the similarities in all the segments with ERV2 except VP7 with the constellation as: G6-P [1]- I2-R2-C2-M2-A3-N2-T6-E2-H3. Furthermore, most of the segments (n=6) of ERV2 and ERV3 belonged to DS-1 like genotype, signifying the bovine origin of these ERVAs. However, ERV4 and ERV6 both were found to have G3-P [3]-I8-R3-C3-M3-A9-N3-T3-E3-H6, similar to strain RVA/Bat-tc/MSLH14/2012/G3P [3] isolated from insectivorous bat from Yunan Province, China. As per the whole genome, nomenclature of RWCG, the genogroup ERV4 and ERV6 could be AU-1 like genogroup, which is a minor genogroup found in humans. The genotypic constellation of equines samples in this study have been found to be very different from the genotype constellation G3/G14-I2/I6-R2-C2-M3-A10-N2-T3-E2-H7 that has been found to be a largely conserved among equines of various continents in the previous studies.

(Gulati BR and Pathak A)

Sero-surveillance and monitoring of equine infectious diseases in India

Surveillance and monitoring of equine infectious disease is one of the continuous service projects of the institute. During the year 2021, a total of 2000 equine serum samples from 7 states were tested for various diseases like Equine infectious anaemia (EIA), Equine influenza (EI), Equine herpesvirus-1 (EHV-1), Japanese

encephalitis/ West Nile virus (JEV/WNV), *Trypanosoma evansi* (Trypanosomosis), Piroplasmosis, *Salmonealla* Abortus equi and Brucellosis**.** Total number of positive cases and sero-positive percentage are indicated in **(Table 3)**. Highest sero-prevalence was observed for equine *piroplasmosis* (28.40%) followed by EHV-1 (7.80%), JE/WNV (7.40%) and *Trypanosoma evansi* (2.15%). None of the equines were found positive for equine influenza, equine infectious anemia, brucellosis and *Salmonella Abortus equi.*

Table 3: Sero-prevalence of important equine diseases among indigenous equines (Jan-Dec 2021).

State/UTs	EIA	EI	Piroplasmosis	EHV-1	*T. evansi*	JE/WNV	*Salmonella Ab. equi*	Brucellosis
Haryana	398	398	398 (103)	398 (37)	398 (18)	398 (39)	398	398
Uttarakhand	43	43	43 (18)	43 (3)	43	43 (3)	43	43
Uttar Pradesh	995	995	995 (319)	995 (53)	995 (19)	995 (59)	995	995
Rajasthan	16	16	16 (12)	16 (4)	16	16 (7)	16	16
Chhattisgarh	100	100	100 (17)	100 (13)	100	100 (5)	100	100
Madhya Pradesh	400	400	400 (99)	400 (43)	400 (6)	400 (35)	400	400
Jammu	48	48	48	48 (3)	48	48	48	48
Total	**2000**	**2000**	**2000 (568)**	**2000 (156)**	**2000 (43)**	**2000 (148)**	**2000**	**2000**
Sero-prevalence(%)	**-**	**-**	**28.40**	**7.80**	**2.15**	**7.40**	**-**	**-**

*Number in parenthesis indicates sero-positive samples.

Number of samples tested under disease investigation is shown in **Table 4**. For EIA, 14757 serum samples obtained from 17 states were found negative by Coggin's and ELISA test. State wise distribution of samples tested for EIA surveillance is shown in **Table 5**. No EIA positive cases has been reported in India since the last 11 years. This surveillance data would be of immense help for obtaining EIA free status of the country. For annual reconfirmation of AHS free status, a total of 37 samples from 4 states were tested and found negative. Samples tested for other diseases under disease investigation were found negative.

Table 4: Number of samples tested under disease investigation (Jan-Dec.2021)

Disease Investigation	No. of samples tested
Equine infectious anemia (EIA)	14757
African horse sickness (AHS)	37
Equine influenza	4
Piroplasmosis	8
T. evansi	11
EHV-1	11
JE/WNV	5
Histopathology	6
Covid-19	1
Total	**14840**

Table 5 : State wise distribution of equine samples tested for EIA under disease investigation

Sr. No.	State	Sample No.
1	Uttar Pradesh	10801
2	Uttarakhand	824
3	Delhi	364
4	Jammu	496
5	Haryana	484
6	Maharashtra	335
7	Rajasthan	170
8	Himachal Pradesh	265
9	Karnataka	60
10	Chandigarh	33
11	Madhya Pradesh	399
12	Gujarat	105
13	Punjab	125
14	Jharkhand	158
15	Andhra Pradesh	98
16	Bihar	39
17	Tamil Nadu	1
	Total	**14757**

Revenue generation through contractual diagnostic services and consultancy

Under contractual diagnostic services, a total 9937 samples were received from race courses, turf club, stud farm, riding schools, animal quarantine & certification services (AQCS) and other organized sectors during the year 2021. These samples were tested for various notifiable and exotic diseases to check ingression of diseases from abroad and monitoring of elite horses in the private sectors. A total of 4402 sera samples for EIA and 3930 samples for glanders were found negative. Among exotic diseases, 545 swab samples from for Contagious equine metritis (CEM), 303 samples for equine viral arteritis (EVA), 264 samples for African Horse Sickness (AHS) and 271 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. 75.95 lakhs was generated through contractual diagnostic service **(Table 6)**.

Table 6 : Number of samples tested and revenue generation through contractual diagnostic services

Diseases/infection diagnosis	Number of sample tested	Revenue (Rs.)
Equine infectious Anemia (EIA)	4402	2421100
Glanders	3930	2751000
Contagious equine metritis (CEM)	545	872000
Dourine	271	298100
African horse sickness (AHS)	264	290400
Equine viral arteritis (EVA)	303	606000
West Nile virus	6	12000
Equine influenza	39	21450
Equine Herpes virus-1 (EHV-1)	55	110000
Rota virus	2	1100
T. equi	48	96000

B. caballi	53	106000
T. evansi	12	6600
Salmonella Abortus *equi*	7	3850
Total	**9937**	**75,95,600**

(Singha H, Shanmugasundaram K, Gulati BR, Virmani N, Kumar R, Kumar S, Barua S, Vaid RK, Dedar R, Manuja A, Manuja B, Pathak A, Raj A and Yash Pal)

National action plan on glanders for control and eradication of glanders in India

The Ministry of Fisheries, Animal Husbandry and Dairying, Government of India launched the National Action Plan on Glanders for control and eradication of glanders in India in 2019. The overall objective is 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 OIE Terrestrial Code and the OIE Terrestrial Manual.

In 2021, 26257 equine sera from 220 districts of 18 states were collected and tested for glanders. State wise glanders surveillance data is shown in **(Table 7)**. Out of these, 148 glanders positive cases were reported in 46 districts of 10 states. Glanders affected states include Uttar Pradesh (n=100), Uttarakhand (n=15), Haryana (n=9), Jammu (n=1), Himachal Pradesh (n=3), Punjab (n= 5), Gujarat (n=3), Maharashtra (n=7), Madhya Pradesh (n=4) and Telangana (n=1) **(Table 7, Fig. 3)**. It was found that above 60% of the samples and glanders positive cases originated from Uttar Pradesh. In zoonotic point of view, 104 sera from occupationally exposed humans (veterinary officers, equine handlers, and laboratory workers) from Haryana, Uttar Pradesh, Madhya Pradesh and Gujarat were tested and none of them were found positive.

For rapid and efficient execution of surveillance activities, State laboratories are using commercially available glanders ELISA kit manufactured by Genomix Diagnostic Pvt. Ltd. Out of the total samples, 9929 equine samples were screened by ELISA at 8 State Lab/RDDLS (Gujarat, Haryana, Himachal Pradesh, Madhya Pradesh, Punjab, Rajasthan, Maharashtra and Jammu) during the year. As per guidelines, ELISA positive samples were retested and confirmed by complement fixation test (CFT) at ICAR-NRCE.

Taking account of past four year surveillance data, it was observed that only 10-11 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 programme is necessary to assess state wise sero-prevalence for devising future strategies for control and eradication of glanders in India.

Fig. 3. Representative photographs of glanders affected equines.

Table 7 : Nation-wide Glanders surveillance data (Jan-Dec 2021)

Sr. No.	State/UT	No of samples tested at NRCE	No of samples tested at State Lab/RDDLs	No. of districts surveyed	Positive cases
1	Uttar Pradesh	14442	-	75	100
2	Haryana	802	535	16	9
3	Punjab	9	398	9	5
4	Himachal Pradesh	175	2449	8	3
5	Uttarakhand	229	-	8	15
6	Delhi	113	-	3	0
7	Jammu	129	2854	4	1
8	Madhya Pradesh	27	1112	22	4
9	Gujarat	65	975	19	3
10	Maharashtra	13	1246	20	7
11	Rajasthan	4	360	11	0
12	Chhattisgarh	114	-	8	0
13	Chandigarh	3	-	1	0
14	Telangana	8	-	1	1
15	Karnataka	8	-	1	0
16	Bihar	147	-	12	0
17	Jharkhand	9	-	1	-
18	Tamil Nadu	1	-	1	0
	Total	**16328**	**9929**	**220**	**148**

(Singha H, Shanmugasundaram K and Yash Pal)

Bacteriological analysis of equine biological samples

Microbiological analysis was carried out on 442 clinical samples including nasal swab, tissue, abscess, aborted fetus, semen, water, feed, fecal etc. originating from Haryana, Uttar Pradesh, Himachal Pradesh Rajasthan, Chhattisgarh, Punjab, Maharashtra, Andhra Pradesh and Madhya Pradesh were analyzed. Clinical samples were streaked in the suitable bacteriological media for the isolation and yielded 133 bacterial isolates including *Klebsiella pnuemoniae* (n=51), *E.coli* (n=46), *Rhodococcus equi* (n=5), *Streptococcus equi* sub species *zooepidermicus* (n=15) and *Burkholderia mallei* (n=6) **(Table 8)**.

Table 8 : Bacteria isolated from equine clinical samples

Organism	No. of isolates	Sample type/ swab site	Place
Klebsiella pneumoniae	51	Feed (5), Blood (1), Fecal (4), Vaginal Swab (1), Water (1), Fresh Semen (1), Heart (4) , Kidney (1), Large & Small Intestine (2), Liver (3), Lung (4), Nasal (12), Serum (1), Soil (4), Spleen (3), Stomach (2), Throat Swab (1), Whole Blood (1)	Andhra Pradesh (1), Chhattisgarh (2), Haryana (21), Jammu (6), Maharashtra (1), Madhya Pradesh (1), Punjab (3), Rajasthan (11), Uttarakhand (2), Uttar Pradesh (3)
E. coli	46	Liver (5), Kidney (4), Small & Large Intestine (7), Tissue (1), Heart (2), Spleen (4), Lung (2), Rectal Swab (1), Fecal swab (4), Nasal (5), Stomach (8), Trachea (1), Vaginal Swab (2)	Rajasthan (31), Haryana (13), Chhattisgarh (2)

Streptococcus equi subsp *zooepidemicus*	15	Serum (1), Nasal (9), Pus Swab (3), Fresh Semen (1), Eye Swab (1)	Rajasthan (2), Haryana (11), Punjab (1), Uttarakhand (1)
Rhodococcus equi	5	Nasal Swab (2), Feed Sample (1), Pus (2)	Rajasthan (1), Haryana (3), Himachal Pradesh (1)
Burkholderia mallei	6	Nasal (4), Water (1), Feed (10), Soil (8), Wound Swab (1)	Haryana (6)
Streptococcus equi subsp *equi*	8	Nasal (4), Pus Swab (1), Throat Swab (1) Wound Swab (2)	Rajasthan (2), Haryana (6)
Streptococcus spp	2	Nasal (2)	Rajasthan (2)

(Singha H, Shanmugasundaram K and Yash Pal)

Phylogenetic analysis of *Klebsiella pneumoniae* isolated from equine abortion cases

Klebsiella pneumoniae causes digestive, urinary, reproductive and respiratory tract and septic infections in humans and a wide variety of domestic animals. In equines, it causes pneumonia, epidemic metritis, cervicitis and septicaemia. Little information is available on the impact of *K. pneumoniae* infections in equines, disease epidemiology, molecular epidemiology and antimicrobial resistance profiles. *K. pneumoniae* (n=15) were isolated from aborted fetuses and cervical swab of mares. Identity of the bacteria was confirmed by PCR and sequencing. 16S rDNA genes of *K. pneumoniae* were sequenced and a phylogenetic tree was constructed to know the relationships among *K. pneumoniae* species. In addition, some of the 16S rDNA sequences *K. pneumoniae* (n=13) available in the GenBank were also used for phylogeny to determine the diversity of *K. pneumoniae* isolated from equine and other species. The 16S rDNA phylogenetic tree was constructed by the N-J method in the CLC workbench. These isolates were clustered into two branches **(Fig. 4)**. A total of 12 global and 5 equine *K. pneumoniae* of Indian origin (4589, 4987, 4771, 4826 and 4968) grouped in the branch 1 and rest of the 9 *K. pneumoniae* of Indian origin (4841, 4767, 4775, 4793, 4836, 4802, 4846, 4186, and 4766) and one global origin grouped in branch 2. Taken together, the analysis revealed that *K. pneumoniae* recovered from Indian equines are closely related, but genetically diverse from global *K. pneumoniae* strains.

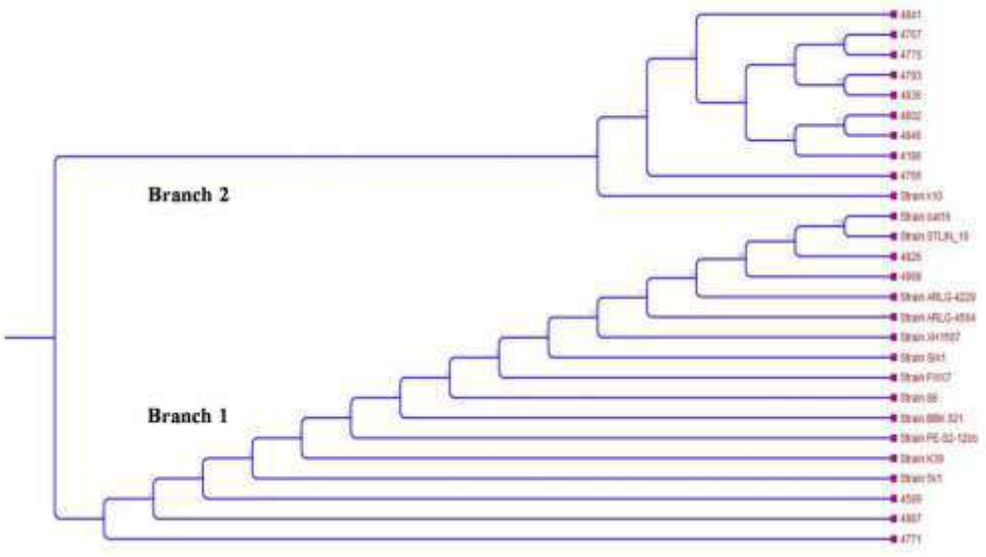


Fig. 4. 16S rDNA Phylogeny of *Klebsiella pneumoniae* isolated from equine abortion cases

(Singha H, Shanmugasundaram K and Yash Pal)

Development of Immunoassay for the sero-surveillance of strangles in horses

Streptococcus equi subspecies *equi* (*S. equi*) causes the serious and highly contagious respiratory disease strangles in horses over most of the world. Clinically silent carrier state after recovery from the disease is a key reason for its persistence in the horse population, and spread to immunologically naive groups of horses. Differentiation of these silent long-term carriers from non-carrier herd-mates is essential for disease management. Bacterial culture methods and PCR of nasopharyngeal washes and guttural pouch lavages are used routinely to test clinical and carrier animals for the presence of *S. equi* but no definitive or gold standard test has been shown to be optimal.

With an aim to develop an immunoassay for detection of specific antibodies for *S. equi,* N terminal sequences of *SeM* and *SeQ 2190* genes coding for specific proteins of diagnostic importance for *S. equi* were amplified using gene specific primers. These PCR amplicons have been cloned into plasmid vector and transformed into E. *coli*. These specific proteins will be expressed to develop a sensitive and specific immune assay for sero-surveillance and detection of silent long-term carriers from non-carrier herd-mates.

(Kumar B, Vaid RK, Manuja A, Shanmugasundaram K and Singha H)

Development of a polymerase spiral reaction (PSR) based point of care assay for rapid detection of genomic DNA of *Trypanosoma evansi*

In India, *T. evansi* infection is endemic in different parts of the country and is of paramount economic importance in the livestock sector. New PCR based diagnostic approaches are specific and sensitive, however, field level diagnosis is not feasible due to their requirement of expensive equipment. Isothermal nucleic acid techniques such as Loop-mediated isothermal amplification (LAMP) and Polymerase spiral reaction (PSR) have provided opportunities for diagnosis of various pathogens at field level at lower cost. Polymerase spiral reaction (PSR) opens new avenues for specific diagnosis of pathogens known for cryptic infection at field level and its application is still unexplored in the field of parasitology. The present study aimed to explore and optimize colorimetry based PSR technique for the detection of *T. evansi* in the blood of the host by targeting the 196bp Invariable Surface Glycoprotein (ISG) gene of *T. evansi*. The specificity of the test was determined against *Theileria equi, Theileria annulata, Babesia caballi, Burkholderia mallei* and Equine herpes virus. The *T. evansi* DNA was extracted from purified parasites and serially diluted from 2.8 ng to 2.8 x 10^{-8} pg. The detection limit of PSR was found to be as low as 2.8 x 10^{-6} pg of *T. evansi* DNA, which will aid in detection of surra infection in areas having very low incidences. The duration of reaction for determination of result of field sample is 1 h and result can be read by naked eyes **(Fig. 5)**. In addition, PSR assay was also performed on DNA extracted from 28 field equine samples; out of which 1 was found positive by microscopy and ISG-196 targeted PCR assay and 2 were recorded positive by PSR assay. Data generated shows colorimetric PSR is a convenient, rapid, sensitive and specific tool for the diagnosis and monitoring of surra infection in livestock at field level.

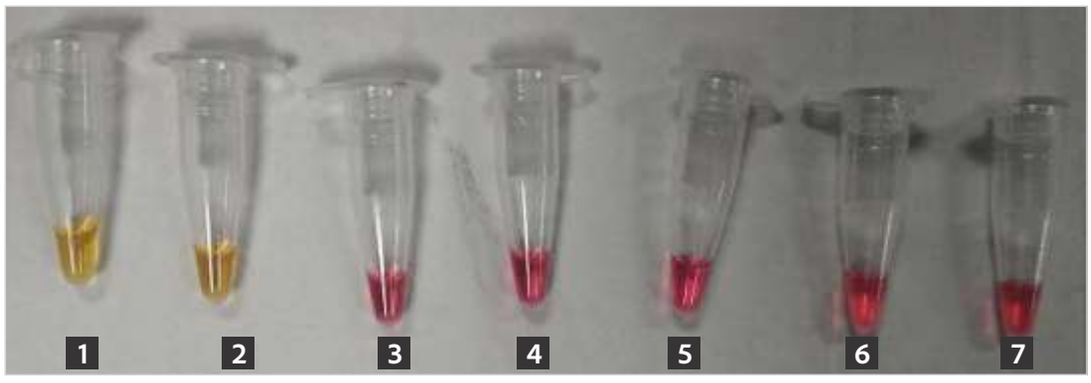


Fig. 5. Visual detection of negative and positive PSR amplification products. 1, 2 Positive control; 3-6 Negative DNA control; 7 Blank (without DNA); yellow represents positive and pink represents negative result.

(Sharma D, Gupta S, Sethi K, Kumar S and Kumar R)

Sero-prevalence of *Trypanosoma evansi* infection in livestock from four agro-climatic zones of Himachal Pradesh, India

Trypanosoma evansi, a unicellular, haemoflagellate parasite is responsible for causing a highly debilitating disease termed as surra in various host species and has a significant negative impact on livestock industry. Himachal Pradesh is an agriculture-intensive state with 89.96% of its population being rural and dependent on agriculture for its livelihood. However, due to a lack of organized epidemiological study of surra in the state, the prevalence status of the disease is still obscured. In the present study, sero-prevalence status of *T. evansi* infection in livestock of four agro-climatic Zones of Himachal Pradesh, India was determined, which would help state veterinarians and policymakers in control of this disease. A total of 440 equines and 444 cattle serum samples were collected from four agro-climatic zones. Further, serum samples of 280 buffaloes from three different agro-climatic Zones of Himachal Pradesh were also collected and evaluated for presence of *T. evansi* infection by indirect ELISA. Data generated showed higher prevalence in buffalo (23.57%) followed by cattle (22.52%) and equines (1.82%). Disease was found to be more prevalent ($P < 0.05$) in cattle of lower altitude as compared to those of higher altitudes. No significant variation was seen in prevalence of disease on the basis of age and sex of the animals. Serum biochemical analysis revealed increased levels of BUN in *T. evansi* infected equines. Levels of liver function enzymes such as ALT/GGT and AST were found to be significantly elevated ($P < 0.01$) in infected animals whereas glucose levels were significantly lower in surra infected animals as compared to non-infected animals. Animal trypanosomosis was found to be highly prevalent in livestock of Himachal Pradesh and thus there is dire need for designing proper control strategies against surra.

(Sharma D, Gupta S, Sethi K, Kumar S and Kumar R)

Identification and evaluation of target specific novel drug molecules against *Trypanosoma evansi* infection using nanotechnology approach

Naphthoquinones (NTQs) are wide spread phenolic compounds present in different families of plants and their molecular structures bestow the property of oxido-reduction. In traditional medicine, especially in some parts of Asia (India and China) and South America, such phyto bioactive naphthoquinones are extensively used for the treatment of various cancers and parasitic diseases. Juglone, lawsone, and plumbagin are the most widespread bioactive naphthoquinones owing to their various biological and pharmacological activities such as antibacterial, anticancer, antifungal, anti-inflammatory, antileishmanial, antimalarial and antitrypanosomal.

Nanoencapsulation is a promising approach to enhance the therapeutic potential of a drug. Herein, three selected naphthoquinone (NTQ) derivatives out of six, based on the IC_{50} value against *T. evansi*, were encapsulated using gum damar as biocompatible and biodegradable natural gum via nanoprecipitation method. Nanoformulation of NTQs (NNTQs) was less than 150 nm in size, was found to be stable and released the drug in a sustained manner. All the three NNTQs exhibited significant anti-trypanosomal effect **(Fig. 6)** and morphological changes at approximately two to three times lesser drug concentrations. The nanoformulations exhibited enhanced production of reactive oxygen species (ROS) in the axenic culture of *T. evansi* and less cytotoxic effect on horse peripheral blood mononuclear cells relative to pure NTQs. As evidenced by flow cytometry, the NNTQs showed dose-dependent and time-dependent increased transition of live cells (AV^-PI^-) to early apoptotic cells (AV^+PI^-), late apoptotic cells (AV^-PI^+), and necrotic cells (AV^+PI^+) using annexin V/propidium iodide probe analysis. The results concluded that NNTQs induced more ROS, apoptosis and necrotic effects that exhibited more inhibitory effect on the growth of *T. evansi* with respect to NTQ by themselves **(Fig. 7)**.

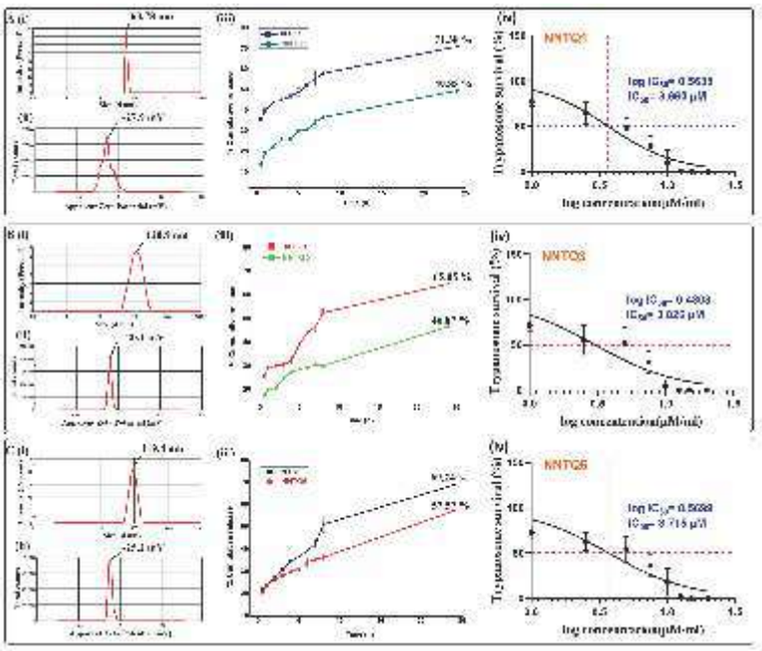


Fig. 6. Particle size image (i), zeta potential (ii), *in vitro* drug release (iii), and growth inhibition curves depict log IC_{50} valueand IC_{50} of NNTQ1 (A), NNTQ3 (B), and NNTQ6 (C), respectively.

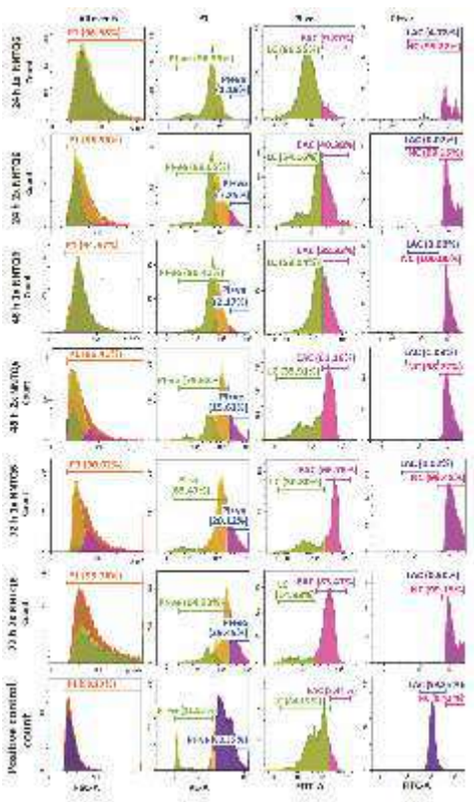


Fig. 7. Flow cytometry apoptosis analysis of NNTQ1-treated *Trypanosoma evansi* using Annexin V/propidium iodide staining. Flow cytometric histogram of 1x and 2x-NNTQ1-treated cells at 24 h, 48 h, and 72 h showing (i) all events; (ii) P1 cell population having PI -ve cells and PI +ve cells; (iii) PI -ve cells including live cells (LC, AV^-PI^-) and early apoptotic cells (EAC, AV^+PI^-); and (iv) PI + ve cells including late apoptotic cells (LAC, AV^-PI^+) and necrotic cells (NC, AV^+PI^+) with respect to % parent population. x represents the IC_{50} value of the nanoformulation.

(Rani R and Kumar R)

In-vitro growth inhibitory efficacy of *Artemisia scoparia* plant extracts against *Theileria equi* in MASP culture system

Ethnoveterinary approach for treatment and prevention of animal disease is based on the use of traditional knowledge of plants regarding its phytochemical constituent, their pharmacological properties and their effect on the biological system. Use of *A. scoparia* for the extraction and purification of artemisinine (an antimalarial drug) has been popularized during recent years. Use of artemisinine as an adjuvant therapy for treating malaria is well known but their use against hemoprotozoan disease in animals has not been established yet. We evaluated the *in-vitro* parasite inhibition efficacy of *A. scoparia* plant extract against the *Theileria equi* under MASP culture system. Plant material was collected from its natural habitat, dried and grinded to make powder. Four different fractions (A, B, C and D) were prepared from the powder by liquid-liquid column fractionation and these fractions were lyophilized to obtain dry form. Stock solution of 50 mg/ml was prepared to make different working concentrations ranging from 1000 µg/ml to 100 µg/ml by serial dilution. *In-vitro* bioassay was performed under MASP culture system and data were analyzed by using online software. These plant extracts were tested at 1000, 500, 250 and 100 µg/ml concentrations in triplicate. After 96 hour of incubation period, the percentage of parasitemia (Mean ± SE) in control well was 8.76±0.854, while the percentage of parasitemia in wells treated with different fraction at their highest concentration was 2.56±0.591, 3.30±1.05, 3.57±0.0.541 and 1.26 ± 0.613 in fraction A, B, C and D respectively (**Fig. 8**). Analysis of data suggested that fraction D is significantly effective against *Theileria equi* at all the concentration as indicated by their parasitemia value, which is significantly differ from that of control well. Current study indicates that plant extract may contain various phytochemicals components, which are having growth inhibitory effect on *Theileria equi.* Further identification of active phytochemicals and *in-vivo* bioassay is required for detailed analysis.

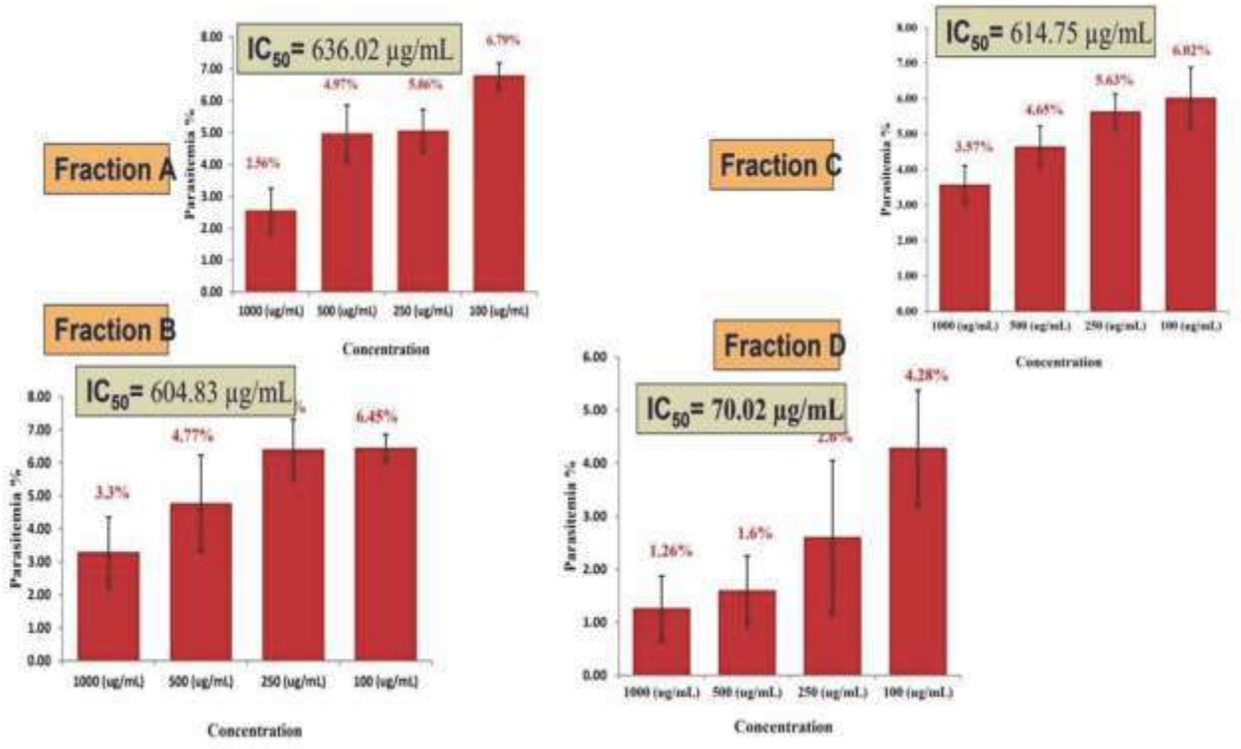


Fig. 8. *Theileria equi* growth inhibiting efficacy of different *Artemisia scoparia* plant extracts in in-vitro cultures and their IC_{50} values

(Gupta KK, Singh L, Saxena N, Dey S, Kumar R, Yash Pal and Kumar S)

ZnO-Alginate/gum acacia and iron oxide nano matrices show enhanced biocompatibility and permeability to intestinal barrier

Zinc oxide is often used as a dietary supplement because zinc is an important dietary and trace element that contributes immensely to the body's functions. Iron and zinc deficiencies are the most common in the world. They often combine with phytate, which interferes with iron and zinc absorption. Nanoscale moieties improve the bioavailability of zinc and iron but also pose an unknown threat to human health. To render them biocompatible, we encapsulated ZnONPs in alginate/gum-acacia hydrocolloids and further conjugated them with iron oxide. The nanomatrices are stable and less than 100 nm in size as determined by transmission electron microscopy and biocompatible with human intestinal Caco-2 cells. SEM images of SAGA ZnONPs and Fe SAGA ZnONPs showed porous structure **(Fig. 9)**. ZnONPs are unstable but their encapsulation in alginate and gum matrix makes them stable as determined by their zeta potentials **(Fig. 10)**. Conjugation with iron oxide also revealed stability of hydrocolloids. We observed Fe/SAGA ZnONPs release higher zinc at acidic pH (stomach) than in the intestinal pH. We determined the effect of alginate/gum /iron oxide ZnONPs matrices on various digestive processes using the intestinal cells (Caco-2) on permeable support. Uptake efficiencies of Zn/Fe using Fe/SAGA ZnONPs are higher than bare ZnONPs. Our results demonstrated that alginate and gum acacia are promising polysaccharides for ZnONPs to protect them against harsh digestive milieu. Further conjugation with iron oxide is shown to be an efficient delivery system.

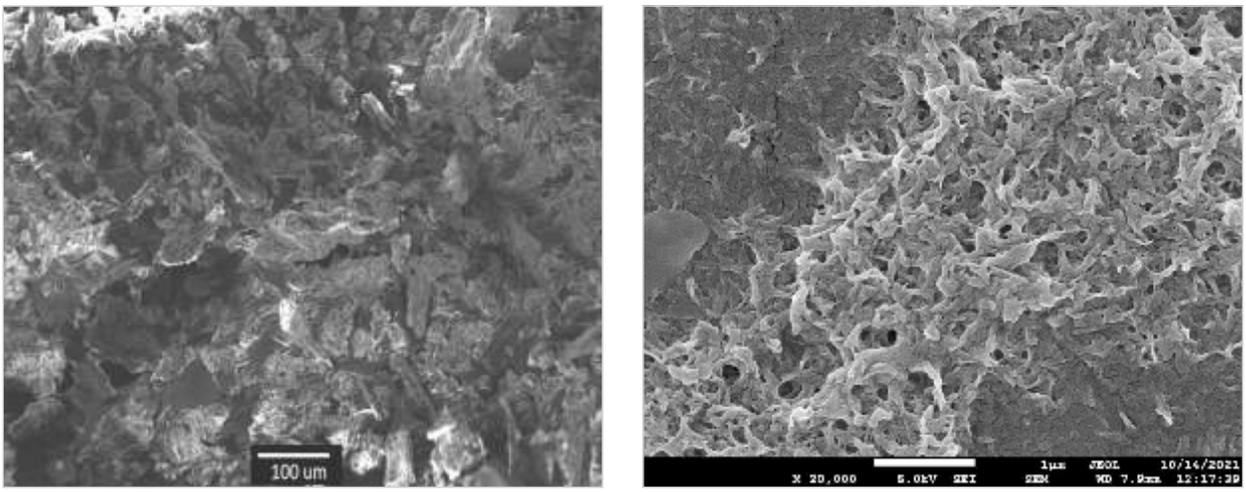


Fig. 9. Scanning electron microscopy images of synthesized (a) SAGA ZnONPs and Fe-SAGA ZnONPs showing porosity.

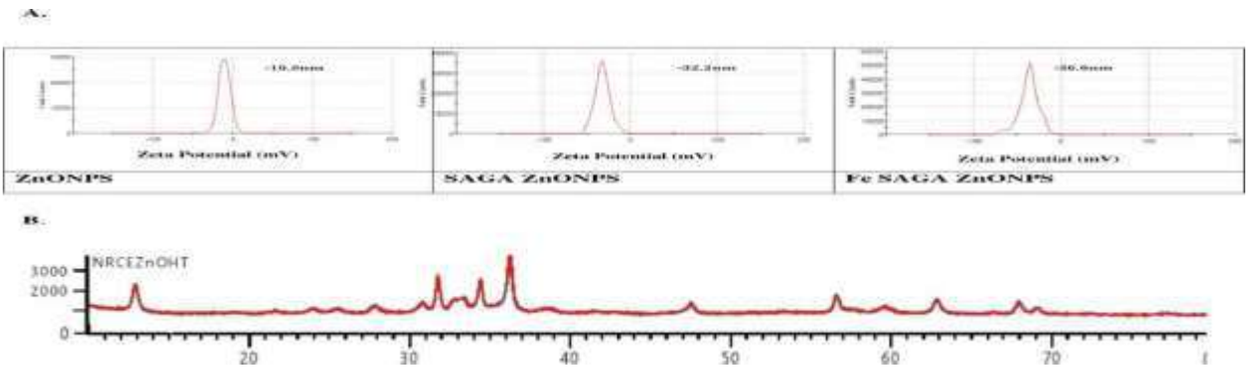


Fig. 10. Graph showing zeta potential of ZnONPs, SAGA ZnONPs and Fe-SAGA ZnONPs indicating stability of SAGA ZnONPs and Fe-SAGA ZnONPs.

(Manuja A, Kumar B, Riyesh T and Mann B)

Effect of desert herbal extracts on the expression of TGF-β in horse dermal fibroblasts

Excessive growth of granulation tissue (proud flesh in horses and development of keloids in human beings) is an important clinical problem. In our earlier work we found that extract of *Aerva javanica* was able to successfully treat proud flesh in horses. So to know the mechanism of action, effect of aqueous and ethanol extracts of *A. javanica*, *Capparis decidua*, *Phoenix dactylifera* and *Ziziphus mauritiana* was seen on the expression of TGF-β1 and TGF-β2 in the dermal fibroblasts originated from horse. A non-cytotoxic concentrations of the aqueous and ethanol extracts of these herbs were added to the fibroblast cells culture for 72 hour and then expression of TGF-β1 and TGF-β2 were evaluated by quantitation of their mRNA in the cell lysates by qRT-PCR. Both ethanolic and aqueous extracts from *A. javanica*, *P. dectylifera* and aqueous extract from *Z. mauritiana* significantly upregulated TGF-β1 mRNA levels **(Fig. 11)**. However, TGF-β2 was shown to be upregulated only with the extract from *A. javanica*. Extract(s) from *C. deciduas* had no effect, neither on TGF-β1 levels nor on TGF-β2 **(Fig.12)**. Extracts prepared from *A. javanica* , *P. dectylifera* and *Z. mauritiana* have potential to be utilized for TGF beta like activities and need further studies for their clinical use in the treatment of equine skin diseases.

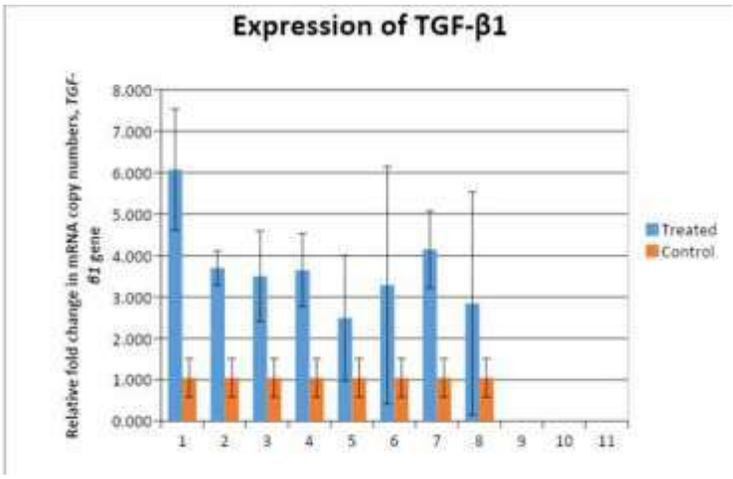


Fig. 11. Effect of aqueous and ethanolic extracts, respectively of desert herbs *A. javanica (1, 2); C. deciduas (3, 4); P. dectylifera (5, 6)* and *Z. mauritiana (7, 8)* on non-cytotoxic concentrations max. m RNA expression level of TGF- β1 gene.

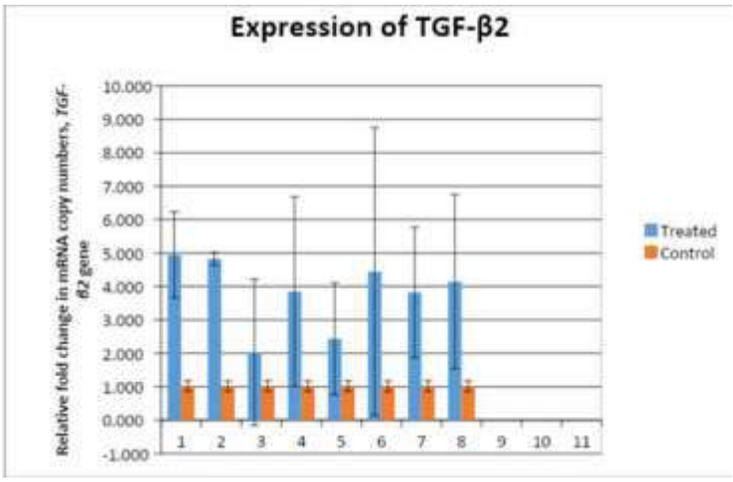


Fig. 12. Effect of aqueous and ethanolic extracts, respectively of desert herbs *A. javanica (1, 2); C. deciduas (3, 4); P. dectylifera (5, 6)* and *Z. mauritiana (7, 8)* on non-cytotoxic concentrations max. m RNA expression level of TGF- β2 gene.

(Dedar RK, Karela P, Kumar R, Suvidhi, Chahar A, Talluri TR and Legha RA)

Effect of diosmin and hesperidin supplementation on haematological and serum-biochemical profile in donkeys

Donkeys have higher risk for hyperlipidemia due to a number of factors including their metabolic efficiency with ability to utilize poor forage and subsequent weight gain if fed inappropriately. Hesperidin (HS) is a flavanone glycoside ($C_{28}H_{34}O_{15}$) flavonoid. Hesperidin has been reported for its hypoglycemic and hypolipidemic properties. Hence, these flavonoids may have potential to be used for the treatment of hyperlipidemic donkeys. Twelve adult donkeys were selected on the basis of their higher levels of serum triglycerides and divided into two groups, control and treatment having 6 animals in each. Treatment group was given oral supplementation of diosmin and hesperidin@ 10 mg/kg body weight, orally daily for 30 days. Serum total lipids, serum triglyceride and serum cholesterol significantly reduced ($p<0.01$) in diosmin and hesperidin supplementation group compared to control group on day 10. Supplementation day 20th onwards did not show any significant effect on lipid profile. MDA levels were significantly lower in the treatment group on day 20 and GGT was found significantly higher on day 20 in serum samples of the treatment group. Decrease in serum MDA levels and increase in serum GGT levels suggested decrease in oxidative stress in donkeys after supplementation of hesperidin and diosmin. Hence, hesperidin and diosmin can be supplemented to the donkeys @ 10mg/Kg body weight to reduce serum triglyceride and cholesterol levels in donkeys, further supplementation of the diosmin and hesperidin reduces oxidative stress but show adverse effect on liver biochemical profile and hematological parameters.

(Dedar RK, Suvidhi, Karela P, Legha RA, Talluri TR, Mehta SC and Yash Pal)

Equine Production

Genetic diversity analysis in indigenous horses and ponies with use of genome-wide SNPs

The admixture, individual relationship, kinship, genetic closeness, and population structure were determined in Marwari and other indigenous horses through microsatellites and SNPs and F-statistics, POPGENE and Structure software. The present study conducted with a panel of 24 polymorphic microsatellites revealed a high number of alleles and heterozygosity in horses. We found about 1789642 total SNiPs for 98 samples and 1698013 total biallelic SNPs, out of which 1510815 biallelic SNPs at RD10 were taken further into analysis. For in depth SNP mining, the SnpEff variant analysis software was applied and 21197 unique SNPs were identified for differentiation among Marwari, Kathiawari, Kachchhi-Sindhi and Thoroughbred horses along with Zanskari and Manipuri ponies **(Fig. 13)**. These led to a total number of 108367 effects, out of which 0.006% are high impact effects and the majority are classified as modifiers. The admixture studies showed mixed structure and individual relationship among the indigenous horses.

Genome	equine
Date	2019-09-13 11:13
SnpEff version	SnpEff 4.3L (build 2017-11-24 15:10), by Pablo Cingolani
Command line arguments	SnpEff -stats equine.html equine equine LD.vcf
Warnings	13,818
Errors	0
Number of lines (input file)	21,197
Number of variants (before filter)	21,197
Number of not variants (i.e. reference equals alternative)	0
Number of variants processed (i.e. after filter and non-variants)	21,197
Number of known variants (i.e. non-empty ID)	0 (0%)
Number of multi-allelic VCF entries (i.e. more than two alleles)	0
Number of effects	108,367
Genome total length	2,506,966,135
Genome effective length	2,411,291,078
Variant rate	1 variant every 113,756 bases

Fig. 13. SnpEff variant analysis of SNPs in Indigenous equines

(Bhardwaj A, Kumar J, Sarika, Iqbal MA, Nayan V, Kumar D, Legha RA, Talluri TR, Yash Pal and Tripathi BN)

Unravelling the selection signatures equine populations in India

Studies on the identification of genomic signatures in Indian equine populations were carried out, and 1631 positive selection signatures were found to be common among all horse breeds **(Fig 14)**. Most of these selection signatures possessed genes that were found to have vital biological roles like neurological development by *GRM8* and *GRIK3*, muscle and tendon strength maintenance by *VDR* and *TNC* genes, respectively, which influence athletic performance in horses. Genes such as *ANK2*, *BCL2*, and *RYR2* were found to have positive selection signatures and act as hub genes that involve transportation of calcium ion and cardiac regulation, which might be a reason for lowering heart rate in horses to cope with its high performance. The high-quality SNP data generated from this work can be used in genome-wide association studies to explore qualitative trait loci and SNPs related to economically and agronomically important traits in equines.

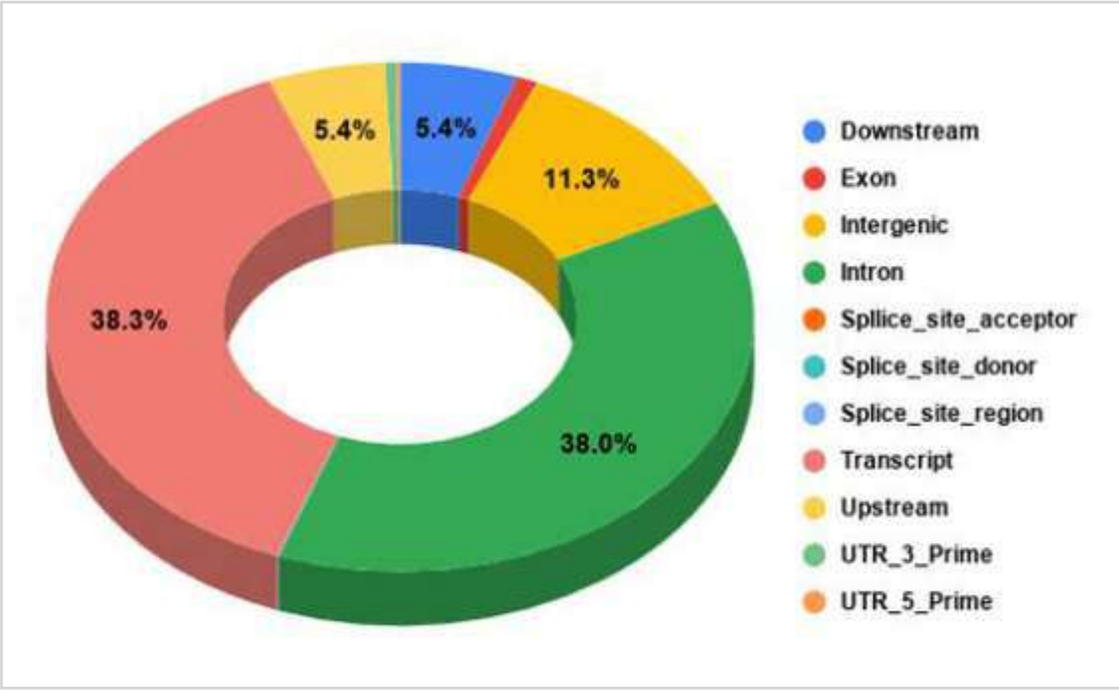


Fig. 14. Gene wise mapping of SNPs and InDels

(Bhardwaj A, Kumar J, Sarika, Iqbal MA, Nayan V, Kumar D, Legha RA, Talluri TR, Yash Pal and Tripathi BN)

Characterization of halari donkey mitochondrial DLOOP gene

The Halari donkey is a distinct breed of India that comes from the community of migration from the regions of Halar, Bharwad, and Rabaris of Gujarat. They have a brief history of travel and migration from the arid and semi-arid regions of India. The DLOOP gene of 542bp size belonging to mitochondrial DNA was amplified by PCR and characterized for genetic comparison with the complete mitochondrial genome of *Equus asinus* (NC_001788.1). The obtained good quality sequences of 23 Halari donkeys were aligned with the reference genome of mitochondria using the *Clustal W* of MEGA 7.0 and were further subjected to diversity analysis using DNASP V6. In the present study, a total of 8 haplotypes were identified with a haplotype diversity of 0.8152 and a nucleotide diversity of 0.12811, indicating that the population has high genetic diversity. The Median joining network tree and neighbour-joining trees revealed close clustering of the Halari donkeys **(Fig. 15)**.

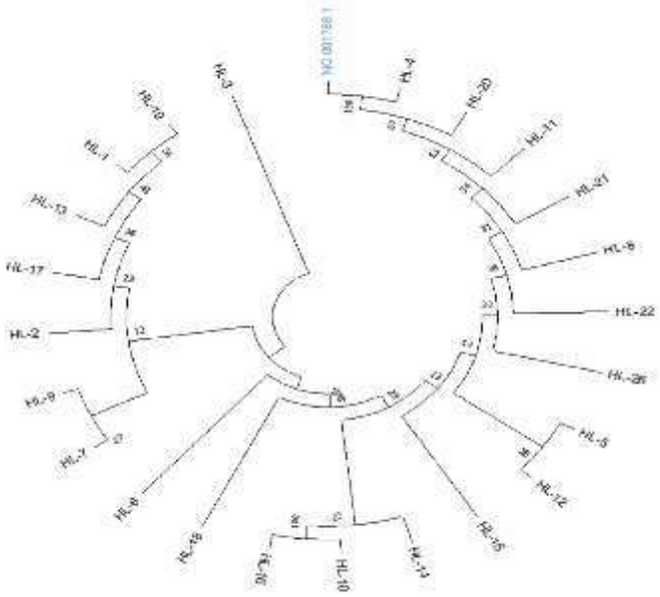


Fig. 15. Halari Donkey Mitochondrial DLOOP Gene

(Bhardwaj A, Kumar J, Unnati, Sonali, Nayan V, Legha RA, Yash Pal and Tripathi BN)

Characterization of horse myostatin gene in Indian horse breeds

Myostatin (MSTN), also known as GDF-8, is a member of the transforming growth factor β super family that regulates both the number and growth of muscle fibres to reduce skeletal muscle mass. In the present study, a total of 50 samples of 5 breeds of horses (Kathiawari, Spiti, Bhutia, Zanskari, and Manipuri, (n =10)) were selected from Gujarat, Himachal Pradesh, Sikkim, Ladakh, and Manipur. Horses of both sex and age >2 years were selected for the sampling and for PCR amplicons were sequenced. The obtained sequences were aligned in MEGA using the *Clustal W* alignment along with a reference sequence (AY840554) and were subjected to generating haplotype datasets using DnaSP v6. The haplotype dataset contained a number of haplotypes, haplotype diversity, and nucleotide diversity **(Table 9)**. The phylogeny tree has been conducted using MEGA 7.0 with a neighbor-joining network.

Table 9 : Haplotype diversity of Myostatin gene acros indigenous equine breeds

Sr. No.	Breed	No. of samples	Haplotype	Haplotype diversity	Nucleotide diversity
1	Kathiawari	11	11	1.000	0.08584
2	Zanskari	11	8	0.8909	0.28099
3	Spiti	11	5	0.6182	0.02930
4	Bhutia	11	1	0.000	0.0000
5	Manipuri	10	10	1.000	0.13427

The phylogeny results suggested that the MSTN gene has been equally distributed among the breeds of equines (**Fig. 16**). Also, MSTN variation in a breed indicates a great way of understanding their distribution among livestock animals.

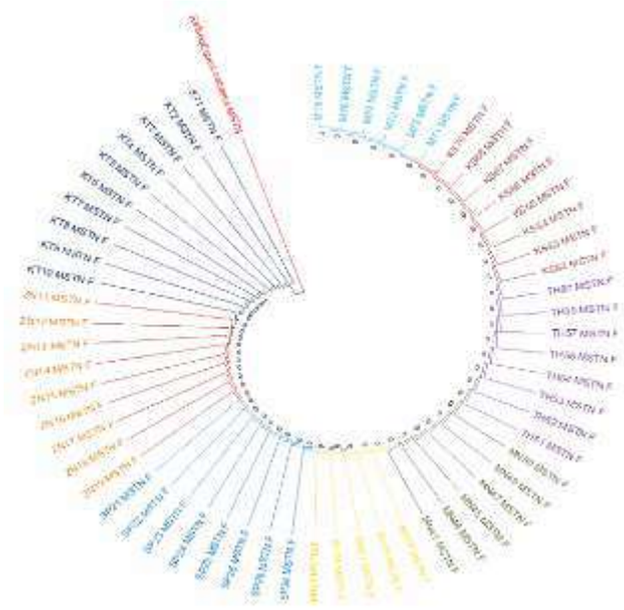


Fig. 16. Phylogenetic tree for the myostatin gene across the indigenous breeds

(Bardwaj A, Sonali, Giri V K, Nayan V, Legha RA and Yash Pal)

Determination of donkey milk rheological properties and techno-functional parameters

Freeze-dried donkey milk powders of French Poitou donkey milk (FPM) and Halari donkey milk (HDM) were subjected to quality evaluation in terms of their physico-chemical and techno-functional properties. Proximate composition analysis revealed that FPM powder had higher milk fat compared to HDM powder. On the contrary, a slightly higher protein was found in HDM powder samples compared to FPM powder. There were no major differences observed between the lactose contents of both powders. Among the techno-functional properties, the bulk density (g/cm3), compressibility index, Hausner ratio, Carr's index, and occluded air content of FPM powder were slightly higher compared to HDM powder **(Table 10)**. The particle density (g/mL), interstitial air content (mL/100g), porosity (%), and flow ability were all higher for the HDM powder. The water-binding capacity (g/g of protein), oil-binding capacity (g/g of protein), and hygroscopicity (g/100 g of solids) were found to be significantly higher ($P<0.05$) for FPM powder compared to HDM powder. The wet ability of FPM powder (127.5 seconds) was significantly higher compared to HDM powder (42.5 seconds). The FTIR curves revealed stretching of N-O compounds at 1500 / cm and stretching of C-H (alkanes and aldehydes) at 3000 /cm associated with both the powders **(Fig. 17)**.

Table 10 : Techno-Functional Properties of Donkey Milk Powders

Parameters	FPM	HDM
Tapped bulk density (g/cm3)	0.425	0.387
Loose bulk density (g/cm3)	0.500	0.440
Compressibility index	15	12
Hausner ratio	1.176	1.136
Particle density (g/mL)	1.003	1.138
Interstitial air content (mL/100 g)	100.93	139.41
Occluded air content (mL/100 g)	19.77	9.92

Parameters	FPM	HDM
Porosity (%)	50.11	61.35
Carr's index	14.99	11.99
Flowability	45.94	48.38
Water binding capacity (g/g protein)	7.88 ± 0.13	2.89 ± 0.08

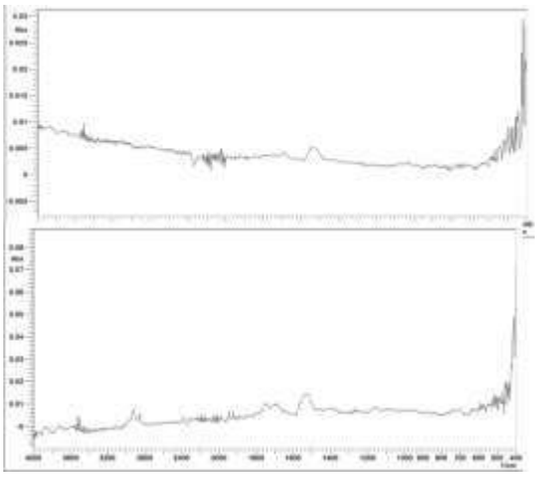


Fig. 17. FTIR Spectra of Donkey Milk Powders

(Bhardwaj A, Yash Pal, Nayan V, Legha RA, Vaid RK, Panjagari NR, and Singh AK)

Physico- biochemical properties of Halari donkey milk

The milk samples were collected from different lactation stages of donkeys of the Halari breed, with good healthy body conditions, at ICAR-NRCE, Hisar. The milking was done manually once per day, yielding up to 1.0 litres of milk per day. Fresh milk samples were sent to the FICCI Research and Analysis Centre, Delhi for their organoleptic, physicochemical and microbial analysis. The organoleptic tests of donkey milk samples reported the taste as characteristic, which were free from odour, having an off-white colour and being off-white in appearance. The physicochemical properties of donkey milk are presented in the **Table 11**.

Table 11 : Physico-chemical properties and microbial load in halari donkey milk

Sr. No.	Composition	Range	Mean+SD Values
1	Moisture Content (%)	90.31 – 90.62	90.45 ± 0.11
2	Total solids (%)	9.38 – 9.69	9.55 ± 0.11
3	SNF (%)	8.51 – 8.96	8.73 ± 0.16
4	Fat (%)	0.73 – 0.89	0.82 ± 0.07
5	Protein (%)	1.95 – 2.33	2.08 ± 0.15
6	Carbohydrates (%)	5.92 – 6.45	6.15 ± 0.19
7	Lactose (%)	5.65 – 7.06	6.08 ± 0.58
8	Ash (%)	0.46 – 0.56	0.50 ± 0.04
9	Casein (%)	0.83 – 0.97	0.90 ± 0.05
10	Titratable acidity	0.03 – 0.31	0.10 ± 0.12
11	Energy (kcal/100ml)	40.02 – 40.57	40.30 ± 0.27

Sr. No.	Composition	Range	Mean Values
12	Water activity (aw)	0.971 – 0.975	0.973 ± 0.001
13	Sugar	4.78 – 5.23	4.83 ± 0.26
14	Cholesterol	nd	nd

Samples were further subjected to vitamin and minerals analysis (**Table 12 and Table 13**). The fatty acid profiles of donkey milk samples showed the values of saturated fatty acids, polyunsaturated and monounsaturated fatty acids in the range as 0.52±0.11 g/100g and 0.17±0.06 g/100g and 0.24±0.10 g/100g, respectively. While the presence of trans fat was not reported in milk samples.

Table 12 : Vitamin concentration in Halari donkey milk

Sr. No.	Vitamins	Range	Mean Values	SD
1	Vitamin A (mcg/100ml)	nd	-	-
2	Vitamin B1 (mg/100 ml)	0.019 – 0.026	0.023	0.003
3	Vitamin B2 (mg/100 ml)	nd	-	-
4	Vitamin B3 (mg/100 ml)	nd	-	-
5	Vitamin B5 (mg/100 ml)	0.341 – 0.348	0.345	0.003
6	Vitamin B6 (mg/100 ml)	0.007 – 0.016	0.012	0.004
7	Vitamin B7 (mcg/100ml)	17.14 – 17.65	17.33	0.23
8	Vitamin B9 (mcg/100ml)	nd	-	-
9	Vitamin B12 (mcg/100ml)	nd	-	-
10	Vitamin E (mg/100ml)	nd	-	-
11	Vitamin C (mg/100ml)	nd	-	-

SD: Standard deviation; nd: Not detected.

Table 13 : Minerals in Halari donkey milk

Sr. No.	Minerals (mg/100ml)	Range	Mean Values	SD
1	Magnesium	7.34 – 7.89	7.65	0.23
2	Sodium	6.17 – 6.49	6.33	0.13
3	Calcium	75.29 – 75.73	75.5	0.18
4	Manganese	nd	-	-
5	Potassium	40.26 – 40.63	40.5	0.17
6	Zinc	0.15 – 0.17	0.17	0.02

SD: Standard deviation; nd: Not detected.

Donkey milk samples were also tested for pesticide residues; it was found that none of the pesticides were reported in milk samples. Similarly, results the for presence of metals, crops and other contaminants namely melamine were negative.

(Bhardwaj A, Yash Pal, Nayan V, Legha RA, Vaid RK, Panjagari NR, and Singh AK)

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

In order to initiate molecular selection and to identify the animals at an early age for endurance potential and fertility, the associated SNP markers were tested in Indigenous breeds of horses, *viz.* Marwari, Kathiawari, Sindhi, Manipuri, and Zanskari for polymorphism. Out of 13 SNP markers, BIEC2-1022884 (A > G) was monomorphic and the remining 12 SNPs were found to be polymorphic in the Indigenous horse breeds. In order to carry out the association study, the endurance racing events held at Pugal and Surat were covered. In 68 samples were collected from Pugal, Surat, Bhuj, Anjar, Jaisalmer, Morvi and nearby areas for further analysis. Prior to this, the racing event held at Tilwara was also covered and the samples were collected. The performance of the animals in different racing events was also collected and the animals were grouped into different performance groups for the purpose of studying the association with the markers. The SNP BIEC2-11782, BIEC2-977605, BIEC2-952439 and PLCZ1-3 were relatively less polymorphic in indigenous horse breeds. The rest of the 8 SNPs exhibited good polymorphism. The SNP genotyping and association study is in progress.

(Mehta SC and Talluri TR (on deputation))

Characterisation and recognition of Bhimthadi horse

A proforma for the recording of morphometry and general information on rearing of Bhimthadi horses in the breeding tract has been prepared. A panel discussion with the horse breeders (n=10) of Pune and Baramati was organised and a visit was made in the breeding tract with the panellist to know about the origin, history, distribution and characteristics of the Bhimthadi horses. Fifteen enumerators were trained for biometric measurements of these horses. Basic socio-economic status of the horse breeders along with recording of morphometric traits was carried out for 120 Bhimthadi animals of Pune, Ahmednagar, Satara and Nashik districts. It was observed that tribal people, who are still nomadic, mostly rear the Bhimthadi horses. The predominant colour is chestnut; but other colours such as white, piebald, skewbald etc. are also acceptable. Among the facial markings, star, strip, snip and blaze is common. These animals are relatively smaller; with an average wither height of about 128 cm, body length of 127 cm and heart girth of 139 cm. These animals are very docile in nature.

(Mehta SC and Sorate SD)

Analysis of quantitative traits for genetic improvement of indigenous equines estimation of breeding value

The Best Linear Unbiased Prediction (BLUP) estimate of breeding value for the height at withers (150.45 cm), body length (151.97 cm), heart girth (169.73 cm) and body weight (366.3 kg) for Marwari horses was estimated and was –0.059 cm, –0.079 cm, 0.096 cm and 3.526 kg, respectively. The effect of sex and interaction of sex and tiers was non-significant, but that of tier on the estimated breeding value for body weight was significant, indicating that body weight received favour in breeding programme. The heritability of height at withers, body length, heart girth and body weight has been estimated to be 0.396±0.586, 0.370±0.777, 0.507±1.95 and 0.597±0.612, respectively.

Evaluation of Annual Growth Performance: In order to evaluate the annual growth performance of the equine herd maintained at the campus, analysis of variance was carried out for the birth, 6-, 12-, 18-, 24-, 30- and 36-months' weight of Marwari horses for the period from 2018 to 2022 and was observed to be 38.45±1.21 (31), 163.43±2.99 (30), 213.59±3.80 (22), 257.28±5.45 (18), 296.79±7.75 (14), 336.40±8.37 (10) and 353.00±7.28 (6) kg, respectively. The male animals were consistently heavier than the females, and the animals born in the year 2020 were observed to be heavier than those born in the rest of the years under study. However, the effect of sex and year was found to be non-significant (P>0.05).

The growth performance of the Kathiawari, Manipuri and Zanskari horses during the period from 2012 to 2021 was analysed. One, two & three years birth weight in Kathiawari was 40.00±1.30 (5), 233.25±17.09 (4), 320.67±29.19 and (3) 363.33±23.54 (3); in Manipuri was 24.25±3.47 (4), 145.75±8.64 (4) and 194.50±5.68 (4)

from 235.00±17.36 (4) and in Zanskari was 29.14±3.03 (7), 162.38±4.69 (8), 225.00±9.88 (5) and 262.83±8.38 (6), respectively.

(Mehta SC)

Development of fatigue cum fitness score card for working equines

Three adult mules were selected for the draught ability trials. Payloads equal to 2.0X, 2.5X, 3.0X, 3.5X, and 4.0X of their body weights were tested in mules using conventional pneumatic wheel carts on pucca road at mules' normal speed for four hours. Physiological responses were studied before, during and after the work and 20 min post work. Rectal temperature, respiration rate, and pulse rate levels increased significantly during work at intervals. After 4 hours of work, the physiological responses attained their highest value. There was a significant decrease in the values after a rest of 20 min, but these values were significantly higher than control values. A significant decrease in the speed of mules was observed after every hour of work. All the physiological indices increased significantly after work and come to normal range in the next morning.

(Legha RA and Yash Pal)

Purification, molecular characterization and ligand binding properties of the major donkey seminal plasma protein DSP-1

A study was carried out to identify the major proteins in the seminal plasma of exotic donkeys. In the present study, a major FnII protein has been identified and isolated from donkey (*Equus hemionus*) seminal plasma, which was referred as Donkey Seminal Plasma protein-1 (DSP-1). The amino acid sequence determined by mass spectrometry and computational modelling studies revealed that DSP-1 is homologous to other mammalian seminal plasma proteins, including bovine PDC-109 (also known as BSP-A1/A2) and equine HSP-1/2. High-resolution LC-MS analysis indicated that the protein is heterogeneously glycosylated and contains multiple acetylations, occurring in the attached glycans. Structural and thermal stability studies on DSP-1 employing CD spectroscopy and differential scanning calorimetry showed that the protein unfolds at ~43 ◦C and binding to phosphorylcholine (PrC) – the head group moiety of choline phospholipids – increases its thermal stability. Intrinsic fluorescence titrations revealed that DSP-1 recognizes lysophosphatidylcholine with over 100-fold higher affinity than PrC. Further, interaction of DSP-1 with erythrocytes, a model cell membrane, revealed that DSP-1 binding is mediated by a specific interaction with choline phospholipids and results in membrane perturbation, suggesting that binding of this protein to the sperm plasma membrane could be physiologically significant.

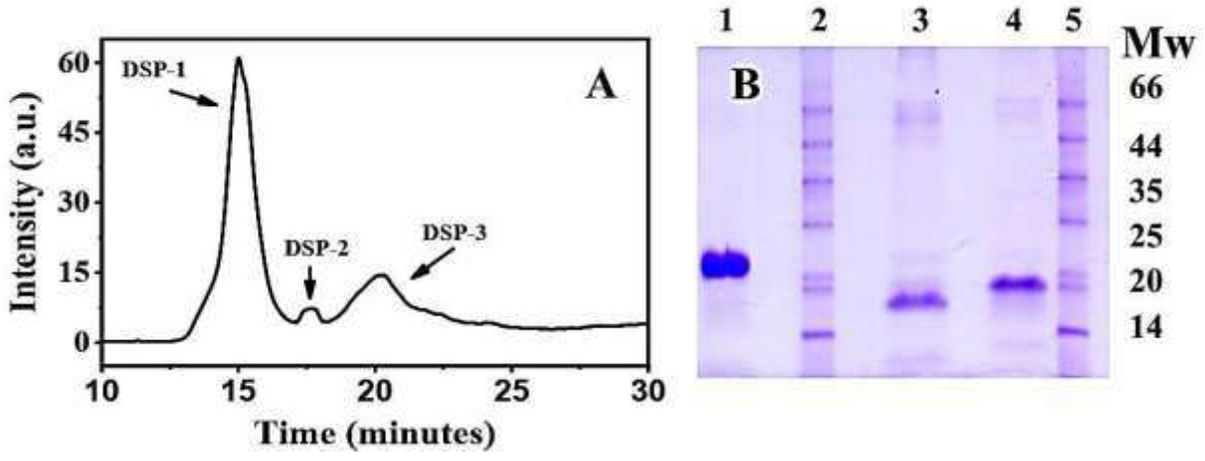


Fig. 18 (A) RP-HPLC chromatogram of Heparin-bound and PPC-agarose-bound fraction showing three major peaks. (B) SDS-PAGE of purified donkey seminal plasma proteins: lane 1, DSP-1; lanes 2 & 5, molecular weight markers; lane 3, DSP-2; lane 4, DSP-3.

(Talluri TR and Swamy MJ)

High throughput deep proteomic analysis of seminal plasma from stallions with contrasting semen quality identifies proteins associated with sperm motility

Seminal plasma from six stallions (three high-and three low-motile) producing semen with contrasting sperm motility were utilized for proteomic analysis. We found 1687 proteins in stallion seminal plasma, with 1627 and 1496 proteins expressed in stallions' high- (HM) and low-motile (LM) sperm, respectively **(Fig. 19)**. A total number of 1436 proteins were co-expressed in both the groups; 191 (11%) and 60 (3.5%) proteins were exclusively detected in the HM and LM groups, respectively. A total of 220 proteins were up regulated (>1 fold change) and 386 proteins were down regulated in SP from LM group stallions as compared to HM group stallions, while 830 proteins were neutrally expressed in both the groups. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed dysregulation of the important proteins related to mitochondrial function, acrosome and sperm cytoskeleton in the seminal plasma of stallions producing ejaculates with low sperm motility. High abundance of peroxiredoxins and low abundance of seminal Chaperonin Containing TCP1 Complex (CCT) complex and Annexins indicate dysregulated oxidative metabolism, which might be the underlying aetiology for poor sperm motility in LM group stallions **(Fig. 20 & 21)**. The current study opens up new avenues for the identification of potential markers of stallion fertility/infertility associated with sperm motility.

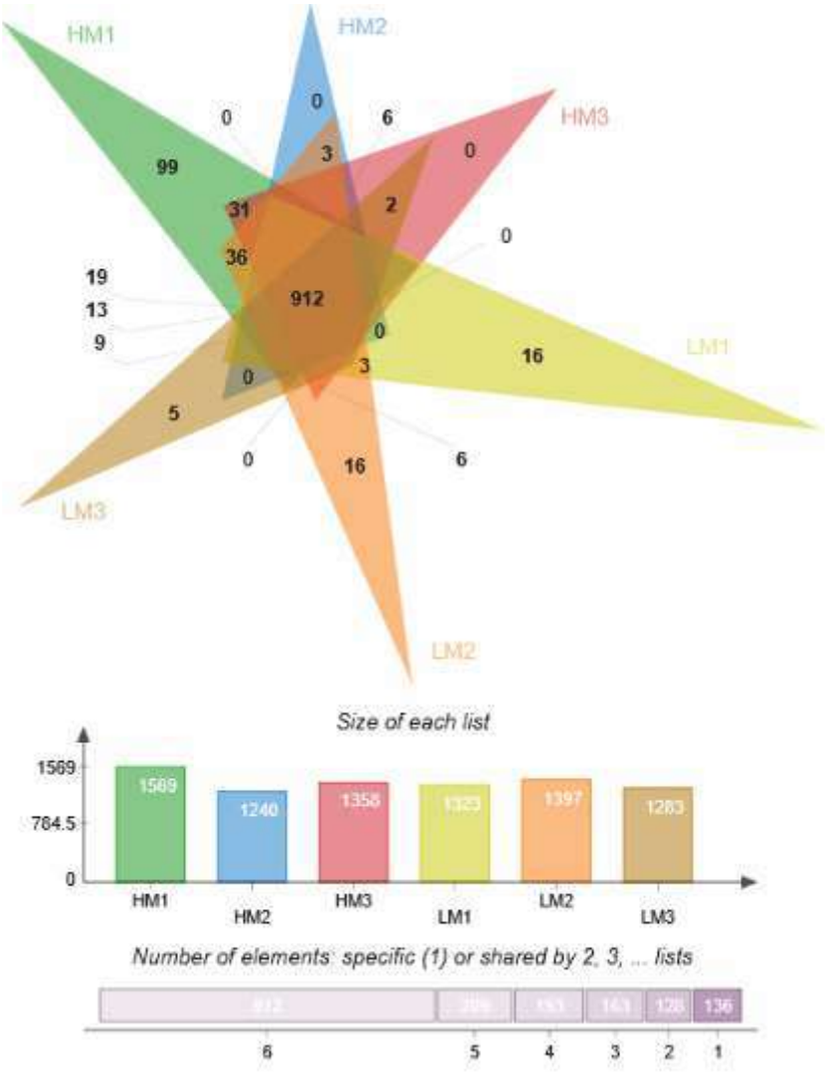


Fig. 19. Venn diagram representing the proteome of the individual stallions of low-motile and high-motile sperm producer.

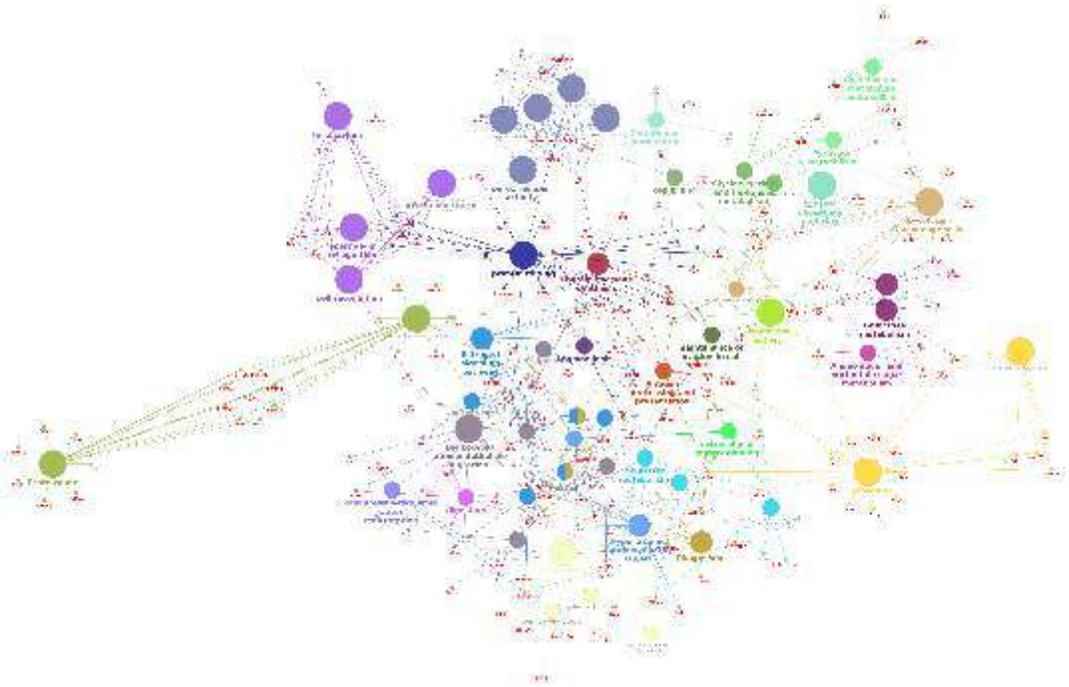


Fig. 20. Complete network analysis of GO terms and KEGG pathways of stallion seminal plasma proteins

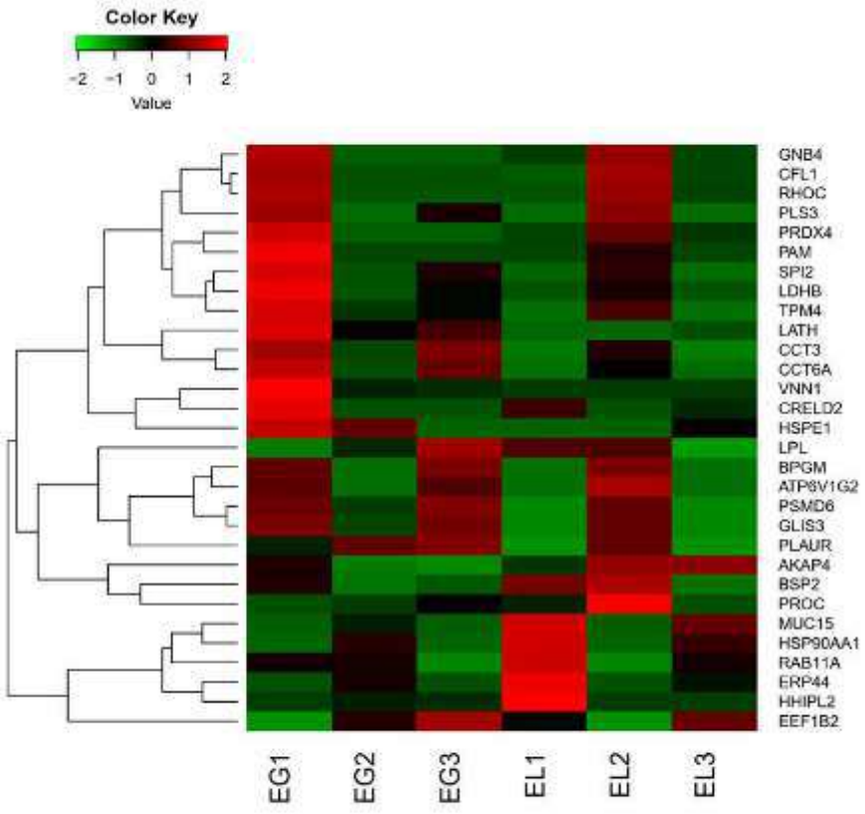


Fig. 21. Heatmap of representing the top 15 proteins in the seminal plasma of high- and low motile sperm producing stallion groups. Heatmap analysis revealed that the sequence of proteins was differentially expressed in low motile to that of high motile group of stallions

(Talluri TR, Kumaresan A, Yash Pal and Legha RA)

Integrated multi-omics analyses reveals molecules governing sperm metabolism potentially influence bull fertility

Bull fertility is of paramount importance in the bovine industry because semen from a single bull is used to breed several thousand cows. However, so far, no reliable test is available for bull fertility prediction. In the present study, spermatozoa from high- and low-fertile bulls were subjected to high-throughput transcriptomic, proteomic and metabolomic analysis. Using an integrated multi-omics approach the molecular differences between high- and low-fertile bulls were identified. We identified a total of 18,068 transcripts, 5041 proteins and 3704 metabolites in bull spermatozoa, of which the expression of 4766 transcripts, 785 proteins and 33 metabolites were dysregulated between high- and low-fertile bulls **(Fig. 22)**. At the transcript level, several genes involved in oxidative phosphorylation pathways were found to be downregulated; while at the protein level genes involved in metabolic pathways were significantly downregulated in low-fertile bulls **(Fig. 23)**. We found that metabolites involved in taurine and hypotaurine metabolism were significantly downregulated in low-fertile bulls. Integrated multi-omics analysis revealed the interaction of dysregulated transcripts, proteins and metabolites in major metabolic pathways, including butanoate metabolism, glycolysis and gluconeogenesis, methionine and cysteine metabolism, phosphatidylinositol phosphate, pyrimidine metabolism, and saturated fatty acid beta oxidation. These findings collectively indicate that molecules governing sperm metabolism potentially influence bull fertility.

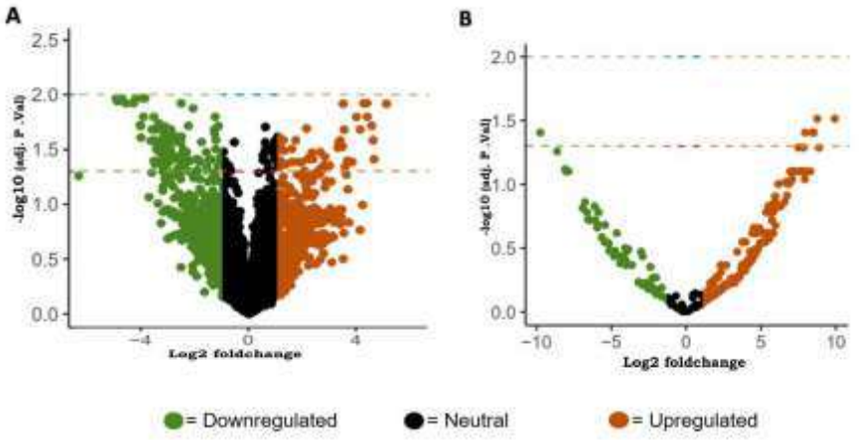


Fig. 22. Volcano plot comparison of A) transcripts and B) proteins of high fertility and low fertility bulls

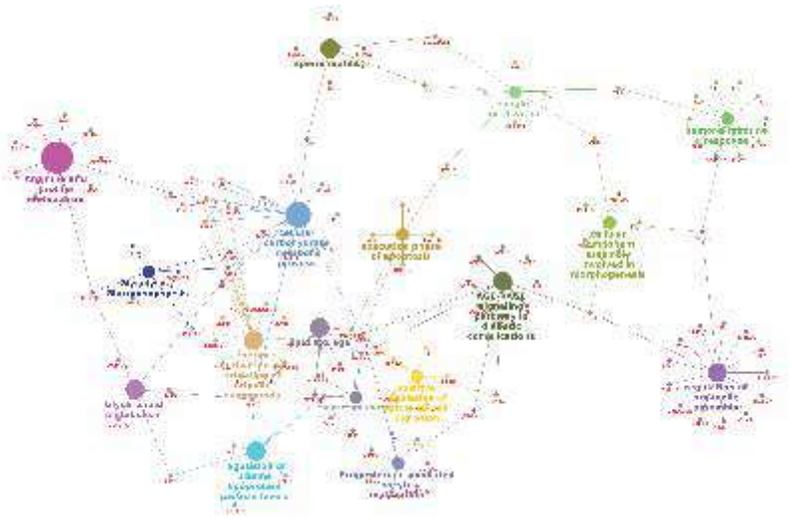


Fig. 23. Network analysis of interactions of GO terms and KEGG pathways in the dysregulated proteome of low fertility bulls

(Talluri TR and Kumaresan A)

Freezability and fertility rates of stallion semen supplemented with trehalose in lactose extender

In the current study, our group aimed to investigate the protective role of trehalose supplementation to the semen extender on stallion sperm quality and enumerate the field fertility rates subjected to freezing. Six adult Marwari breed stallions were used for semen collection. Fresh semen collected from these stallions was divided into three different treatments in a final concentration of 150 x 10^6 sperm / mL by using lactose based extender containing 0, 50, and 150 mM of trehalose and then processed for cryopreservation after equilibration. Sperm total and progressive motility, acrosome integrity, plasma membrane integrity, mitochondrial membrane potential, DNA integrity and oxidative stress related parameters were analysed. Thirty reproductively healthy mares were inseminated with frozen-thawed semen either supplemented with (treatment) or without (control) trehalose to evaluate the field fertility. Results of the current study indicated that the extender supplemented with 50 mM trehalose has enhanced the functional plasma membrane, acrosomal, DNA integrities and augmented the mitochondrial membrane potential **(Table 14)**. Trehalose supplementation to the semen extender not only ameliorated the semen quality parameters, but also protected the stallion sperm from oxidative stress by reducing the levels of reactive oxygen species and lipid peroxidation. The semen extender having 50 mM trehalose resulted in significant (P< 0.05) enhancement in the post-thaw progressive motility and viability compared to the control group and achieved higher pregnancy rates compared to the control. In conclusion, supplementation of 50 mM trehalose resulted in better quality stallion semen after cooling and freezing in terms of reducing the oxidative stress **(Table 15)** and increasing the sperm motility, acrosome integrity, plasma membrane integrity, mitochondrial potential and DNA integrity as well as improved the fertility rates.

Table 14 : Effect of different concentrations of trehalose on the qualitative seminal parameters of stallion semen at various stages of cryopreservation.

Stage of cryopreservation	Seminal attributes	Control	Trehalose (50mM)	Trehalose (150mM)
Pre-freeze	Progressive motility (%)	61.68^{a}±1.55	72.31^{b}±1.58	58.8^{a}±1.46
	Live sperm count (%)	66.85^{a}±1.46	77.91^{c}±1.31	66.29^{a}±1.28
	Acrosomal integrity (%)	78.96^{b}±0.59	82.27^{c}±0.58	75.53^{a}±0.62
	HOST (%)	40.29^{b}±0.38	47.48^{d}±0.52	38.27^{a}±0.54
	DNA intactness (%)	91.44^{a}±0.37	93.32^{b}±0.44	91.32^{a}±0.38
	High MMP (%)	64.82^{a}±1.40	75.54^{b}±1.49	64.75^{a}±1.19
Post-thaw	Progressive motility (%)	42.51^{a}±0.98	54.76^{b}±1.11	40.61^{a}±0.98
	Live sperm count (%)	48.36^{a}±0.89	60.03^{c}±1.28	48.01^{a}±1
	Acrosomal integrity (%)	72.87^{b}±0.5	76.89^{d}±0.49	70.16^{a}±0.46
	HOST (%)	32.65^{b}±0.43	39.62^{d}±0.61	30.65^{a}±0.45
	DNA intactness (%)	87.21^{a}±0.28	90.25^{c}±0.44	87.09^{a}±0.29
	High MMP (%)	45.75^{a}±0.93	59.16^{d}±1.11	45.44^{a}±0.82

{*Mean values in the same columns with different superscripts (a, b, c and d) differ significantly (P<0.05)}.

Table 15 : Levels of LPO and ROS at different stages of cryopreservation

Stage of Cryopreservation	Fresh	Pre-Free			Post-thaw		
		C	T1	T2	C	T1	T2
LPO (MDA) Levels	143.57±5.25	201.13^{sc}±2.14	180.02^{a}±2.27	186.87^{ab}±2.26	499.02^{d}±5.27	445.99^{a}±5.6	469.97^{bc}±5.05
ROS (H2O2) Levels	80.75±2.37	129.8^{ab}±3.26	108.21^{a}±3.1	118.51^{b}±3.12	213.96^{d}±4.76	170.94^{a}±5.64	198.89^{bc}±4.58

Note: *Mean values with different superscripts between treatment groups differ significantly (P<0.05). Group C, T_1, T_2, containing 0mM, 50 mM trehalose and 150 mM trehalose, respectively.*

(Talluri TR, Jhamb D)

Effect of addition of lyophilized heterologous seminal plasma (SP) and Colostrum (COL) to semen extender on cooled and post-thaw stallion semen quality

A study was conducted to investigate the sexual behaviour and normal semen parameters during the non-breeding season and to investigate the effects of SP (2 mg/ml), COL (2 mg/ml) and combination of both (SP 1 mg/ml + COL 1 mg/ml) on cooled and post thaw semen quality parameters in Marwari stallions. Fresh semen was collected from four Marwari stallions aged between 4 to 9 years and a total of 20 ejaculates (5 ejaculates from each animal) were collected during the non-breeding season. Simultaneously, sexual behaviour parameters *viz,* reaction time, erection time, ejaculation time and number of thrusts were also recorded. Each ejaculate was evaluated for various seminal attributes *viz,* colour, consistency, total ejaculate volume, gel volume, gel free volume, pH, progressive sperm motility, sperm plasma membrane integrity, sperm viability, acrosomal integrity and MMP. Semen can be collected from the Marwari stallions during the non-breeding season with the seminal quality parameters in acceptable range for cryopreservation. Supplementation of seminal plasma in semen extender (2 mg/ml) may have detrimental effects on cooled and post-thaw semen quality. Supplementation of colostrum in semen extender (2 mg/ml) may have beneficial effects on cooled and post-thaw semen quality **(Table 16)**. Supplementation of seminal plasma and colostrum in combination in semen extender (1mg/ml+ 1 mg/ml) does not alter the cooled and post thaw semen quality **(Table 16)**.

Table 16 : Post-thaw semen parameters of Marwari stallions supplemented with different concentrations of seminal plasma and Colostrum

Seminal parameters	Control	SP (2mg/ml)	COL (1mg/ml)	SP + COL (1mg/ml each)
Progressive Motility	37.1^{a}±1.63	45.91^{ab}±1.47	49.83^{b}±1.41	47.07^{ab}±1.50
HOST	39.5^{y}±0.88	36.25^{x}±0.83	40.45^{y}±0.83	38.7^{y}±0.85
Viability	46.3^{ab}±0.90	43.6^{a}±1.05	48.1^{b}±0.94	45.5^{ab}±0.93
Acrosome integrity	52.15±1.21	52.3±1.32	56.7±1.40	54.3±1.38
MMP	55.45^{a}±0.90	56.55^{ab}±1.10	61.15^{c}±1.03	58.85^{bc}±1.07

(Talluri TR and Mehra R)

Effect of zinc and gold nanoparticles on cooled and post thaw quality of stallion semen

A study was conducted to investigate the sexual behaviour and normal semen parameters in Marwari stallions during the non-breeding season and to investigate the effects of zinc nanoparticles (ZnNPs) and gold nanoparticles (AuNPs) on cooled and post-thaw semen quality parameters in Marwari stallions. Fresh semen was collected from four Marwari stallions aged between 4 to 9 years and a total of 20 ejaculates (5 ejaculates from each animal) were collected during non-breeding season. Simultaneously, sexual behaviour parameters *viz,* reaction time, erection time, ejaculation time and number of thrusts were also recorded. Each ejaculate was evaluated for various seminal attributes *viz*, colour, consistency, total ejaculate volume, gel volume, gel free volume, pH, progressive sperm motility, sperm plasma membrane integrity, sperm viability, acrosomal integrity and MMP **(Table 17)**. Among the sexual behavior parameters, reaction time significantly varied among the stallions. Seminal characteristics of Marwari stallions during the non-breeding season remain in acceptable range for cryopreservation. Addition of ZnNp (2 mg/ml) to the freezing extender improves stallion post-thaw semen quality but not the cooled semen. Addition of AuNp (2 mg/ml) to the freezing extender does not alter stallion cooled and post-thaw semen quality.

Table 17 : Post-thaw semen parameters of Marwari stallions treated with Zinc and Gold nanoparticles.

Seminal parameters	Control	ZnNP	AuNP
Progressive Motility	42.76±0.89^{x}	47.57±1.02^{y}	42.05±1.38^{x}
HOST	39.25±0.72^{x}	41.80±065^{y}	37.10±1.01^{x}
Viability	50.25±1.19	52.20±1.24	49.60±1.43
Acrosome integrity	65.35±0.88^{xy}	67.35±0.91^{y}	62.70±1.07^{x}
MMP	53.15±1.37	55.95±1.67	51.65±1.70

(Talluri TR, Manuja A and Sultan T)

Attitude of horse keepers towards its management

The rapid mechanization of transportation and agriculture after industrial revolution overshadowed the immense role played by horses in human civilization. According to the Indian livestock census 2019, there is a decline of horses and ponies population of about 45.58 per cent from the Indian livestock census 2012. This population decline of upto 50 per cent is contributed by various factors like increasing management costs, modern means of transportation and lack of organized scientific breeding practices. Looking into this heavy decline in horse population, the attitude of horse stakeholders towards horse management was studied. In this context, an attitude scale has been constructed, standardized and administered to measure the attitude towards horse management. Large number of statements conceived to be related to horse management were gathered from literature, discussion with scientists and veterinarians. These statements were screened by following Edward's informal criteria for attitude statements. Out of these large numbers, 22 generalised attitude statements which were unique and relevant were chosen and arranged for judges' rating. The final statements for inclusion in the attitude scale (**Table 18**) were selected by following the criteria given by Thurstone and Chave.

Table 18: Final set of attitude statements selected

S. No.	Item No.	Scale value	Q value	Statement	Nature of statement
1.	7	7	7	Vaccination is beneficial for horse health.	Positive
2.	19	19	19	Insufficient medicines available for horse treatment.	Negative
3.	9	9	9	Sufficient income can be generated by the sale of horses.	Positive
4.	17	17	17	Adequate veterinary facilities for horses in India.	Positive
5.	4	4	4	Horse management is cost-effective.	Positive
6.	15	15	15	Exotic breeds should be promoted in our country.	Negative
7.	11	11	11	Artificial Insemination (AI) is better than natural services in horses.	Positive
8.	1	1	1	Horses are reared for pleasure over business.	Negative

The constructed scale was then incorporated in an interview schedule for 60 horse stakeholders. After recording the responses of horse stakeholders, the scoring was done with the help of the method proposed by Eysenck and Crown. Each of the statements in the scale were provided with a 4-point continuum response from 'strongly agree', 'agree', 'disagree' to 'strongly disagree'. It was found that more than half of the respondents had unfavourable attitude towards horse management which can be directly correlated with the declining horse population in India. Moreover, the lockdown due to COVID–19 also triggered the unfavourable attitude of horse stakeholders towards horse management when access to, for example, veterinary care, horse feed and adequate outdoor exercise spaces was limited. Around 26 per cent of the respondents had favourable attitude towards horse management **(Table 19)**. These respondents have easy access to veterinary facilities and are young. Only 6 per cent of the respondents had highly favourable attitude towards horse management because of their entrepreneurial capability.

Table 19 : Frequency of respondents under various degrees of attitude towards horse management

S. No.	Attitude score	Degree of attitude	Horse keepers		Veterinarians		NGOs		Total	
			No.	Percent	No.	Percent	No.	Percent	No.	Percent
1.	65 to 80	Unfavourable	25	65.78	13	65	2	100	40	66.66
2.	81 to 96	Favourable	10	26.31	6	30	0	0	16	26.66
3.	97 to 112	Most favourable	3	7.8	1	5	0	0	4	6.66
		Total	**38**	**100**	**20**	**100**	**2**	**100**	**60**	**100**

Table 20 reveals that majority (96.60%) of the horse stakeholders agreed or strongly agreed that vaccination is vital for maintaining a horse healthy and 83.40 per cent of horse stakeholders believed that sufficient income can be generated from horse keeping. Most of the 60 horse stakeholders acknowledged the efficiency of Artificial Insemination over natural services in horses (75% agreed or strongly agreed). Among the interviewed horse stakeholders, almost 50 per cent indicated the advantages of exotic horse breeds in Indian horse industry whereas the remaining half per cent were having problems with the promotion of exotic horse breeds in India. Regarding accessibility to medicines and veterinary services, the respondents had divided opinion with equal share of them having favourable and unfavourable attitude. This finding agrees that most of the veterinary services received by farmers are curative in nature, rather than preventive.

Table 20 : Attitude of horse stakeholders on each attitude statement

S. No.	Statements	Strongly agree	Agree	Disagree	Strongly disagree
1.	(+) Vaccination is beneficial for horse health.	29 (48.30%)	29 (48.30%)	1 (1.70%)	1 (1.70%)
2.	(-) Insufficient medicines available for horse treatment.	4 (6.70%)	26 (43.30%)	23 (38.30%)	7 (11.70%)
3.	(+) Sufficient income can be generated by the sale of horses.	10 (16.70%)	40 (66.70%)	9 (15%)	1 (1.70%)
4.	(+) Adequate veterinary facilities for horses in India.	9 (15%)	23 (38.30%)	26 (43.30%)	3 (5%)
5.	(+) Horse management is cost-effective.	6 (12%)	40 (66.70%)	11 (18.30%)	0
6.	(-) Exotic breeds should be promoted in our country.	3 (5%)	25 (41.70%)	19 (31.70%)	13 (21.70%)
7.	(+) Artificial Insemination (AI) is better than natural services in horses.	14 (23.30%)	31 (51.70%)	12 (20%)	3 (5%)
8.	(-) Horses are reared for pleasure over business.	3 (5%)	27 (45%)	23 (38.30%)	7 (11.70%)

When queried about the entrepreneurial opportunities in horse keeping, 50 per cent of the respondents agreed with the business arena present in this animal. The remaining 50 per cent of the respondents denied the remunerative nature of horse keeping.

(Raj A, Makarabbi G and Dedar RK)

National Centre for Veterinary Type Cultures

National Centre for Veterinary Type Cultures (NCVTC) is working towards the conservation of the animal microbial diversity in the country. The repository activities consist of acquisition, authentication, preservation, documentation, and database management of animal microbes. The repository is being populated through a nationwide network of 15 functional units located across nine different states *viz.,* Assam, Gujarat, Himachal Pradesh, Haryana, Karnataka, Kerala, Rajasthan, Tamil Nadu and Uttar Pradesh. These network units are contributing towards the conservation of animal microbial diversity in three specialized areas i.e. Veterinary microbes at NRCE Hisar, Dairy microbes at NDRI, Karnal and Rumen microbes at NIANP, Bengaluru.

In the current year, 314 microbes were accessioned thereby leading to a cumulative strength of 4505 (till March, 2022) microbes in NCVTC repository. The bacterial repository added 82 cultures in its repertoire and increased the total strength to 1602 bacterial isolates. The important bacterial isolates accessioned in the current year include *Aeromonas hydrophila, Stenotrophomonas maltophila*, *Aeromonas veronii*, *A. jandaei*, *Pseudomonas alkaliphila* (from pond water), *Arcanobacterium pluranimalium* (from buffalo), *Brucella tritici*, *Leucobacter celer* ssp. *celer* (from donkey mare milk) and *Morganella morganii* (from human urine sample). In the virus repository, a total of 46 virus cultures were processed of which 31 virus isolates were accessioned, thereby increasing the strength of the virus repository to 355 virus isolates. The important virus isolates accessioned include, Lumpy skin disease virus (n=1), Newcastle disease virus (n=16), SARS-CoV-2 (n=5)and Bluetongue virus (n=9). Furthermore, 92 bacteriophages were also accessioned during the year to have a total collection of 285 bacteriophages in the NCVTC repository. The rumen microbial repository at ICAR-NIANP Bengaluru, added 80 rumen bacteria during the year and making the total strength to 642. Similarly, the dairy microbe's repository at ICAR-NDRI increased its strength to 725 with the accession of 48 bacteria **(Table 21)**.

Table 21 : Year-wise progress of the repository

Statements	2009-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22*	Total
Veterinary Microbes									
Bacteria	927	110	164	70	123	95	50	63	**1602**
Virus	156	14	28	27	31	44	24	31	**355**
Bacteriophage	32	44	29	24	8	8	48	92	**285**
Recombinant clone	466	45	10	36	16	8	-	-	**581**
Phage library	27	-	-	-	-	-	-	-	**27**
Genomic DNA	223	57	0	8	0	0	-	-	**288**
Total	**1831**	**270**	**231**	**165**	**178**	**155**	**122**	**186**	**3138**
Rumen microbes									
Anaerobic bacteria	142	74	37	37	49	46	62	80	**527**
Fungi/Yeast	107	0	0	0	0	0	-	-	**107**
Meth. Archae	8	0	0	0	0	0	-	-	**8**
Total	**257**	**74**	**37**	**37**	**49**	**46**	**62**	**80**	**642**
Dairy microbes									
Bacteria	468	39	40	30	36	44	20	48	**725**
Total	**468**	**39**	**40**	**30**	**36**	**44**	**20**	**48**	**725**
Grand Total	**2556**	**383**	**308**	**232**	**263**	**245**	**204**	**314**	**4505**

* The data presented above for the year are from April, 2021- March, 2022 (Financial year basis). In the subsequent years the data will be presented in calendar year basis (Jan-Dec).

Role of p38 mitogen-activated protein kinase (MAP Kinase) in buffalo pox virus replication

Much less is known about the kinases required for virus replication, particularly the kinases that regulate BPXV replication. From the literature survey it was found that SB239063, a potent inhibitor of p38MAPK and was identified as one of the components that blocked BPXV replication. This inhibitor was used for identifying the role of p38MAPK in buffalopox virus replication.

***p38 MAPK supports BPXV replication*:** In order to evaluate the role of p38 in BPXV replication, primarily we employed SB239063-a chemical inhibitor of p38-α and p38-β. SB239063 did not produce any significant cell

death up to the concentration of 50 µg/ml. However, higher concentrations were found to be toxic to the cells **(Fig. 24)**. The CC50 was determined to be 57.6 µg/ml. We used sub-cytotoxic concentrations (≤15 µg/ml) of SB239063 (p38 inhibitor) in subsequent experiments. To determine the effective concentration 50 (EC50), virus yields in BPXV-infected Vero cells were measured in the presence of various sub-cytotoxic concentrations of SB239063 wherein a dose-dependent suppression of BPXV yield was observed **(Fig. 25)**. The EC50 was determined to be 0.15 µg/ml. To analyze the virucidal effects on extracellular virions, BPXV was incubated with indicated concentrations of SB239063 for 1.5 h and then residual infectivity was titrated on Vero cells. Infectious viral titers were comparable in both SB239063- and vehicle control-treated cells **(Fig. 26)**, suggesting that SB239063 exerts no direct virucidal effect on BPXV and that the antiviral activity of SB239063 is presumably due to the inhibition of viral life cycle in the target cells.

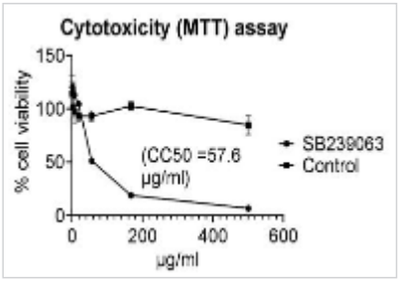


Fig.24. p38 supports BPXV replication. (A) Determination of the cytotoxicity of SB239063 (MTT assay). Indicated concentrations of SB239063 or equivalent volumes of vehicle control (DMSO), in triplicates, were incubated with cultured Vero cells for 96 h and percentage of the cell viability was measured by MTT assay. CC50 was determined by Reed-Muench method.

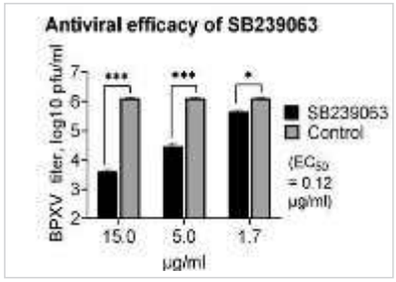


Fig. 25. In vitro antiviral efficacy of SB239063. Vero cells, in triplicates, were infected with BPXV at MOI of 0.1 in the presence of indicated concentrations of SB239063 or equivalent volumes of vehicle-control. At 48 hpi, Infectious virus particles released in the infected cell culture supernatants were quantified by plaque assay. The EC50 was determined by Reed-Muench method.

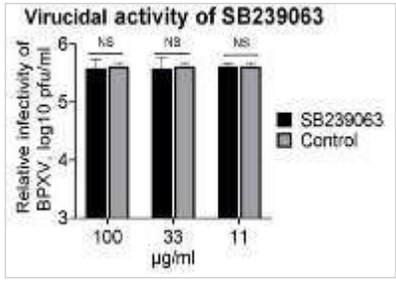


Fig. 26. Virucidal activity. Indicated concentrations of the SB239063 or equivalent volumes of vehicle control, in triplicates, were mixed with BPXV (106 PFU) and incubated for 90 min at 37°C after which the virus was diluted (1/1000) and the residual viral infectivity was determined by plaque assay

p38 inhibition impairs BPXV replication at post-entry steps: In order to examine which specific step(s) of BPXV life cycle could be affected by SB239063, initially a time-of-addition assay was performed in the setting of one-step growth curve. Vero cells were infected with BPXV and the SB239063- or vehicle-controls were applied at a timely interval from 1 to 36 hpi. The yields of infectious virus in the infected cell culture supernatant were quantified when one full cycle of the BPXV was likely to be completed, i.e. at 36-48 hpi. The application of SB239063 resulted in almost similar levels of BPXV inhibition, either applied before infection (pre-treatment) or at 1 and 6 hpi **(Fig. 27)**, suggesting that SB239063 does not inhibit early step of BPXV life cycle (i.e. entry and attachment). The addition of inhibitors at later time points exhibited low (18 hpi) or no inhibition (24 and 30 hpi), suggesting that SB239063 has no significant effect on the late stages of the BPXV life cycle (i.e. assembly/release of viruses). However, addition of SB239063 between 6 to 18 hpi resulted in a significant reduction in the virus yield, thereby suggesting that SB239063 may target the post-entry but pre-budding stages of BPXV life cycle.

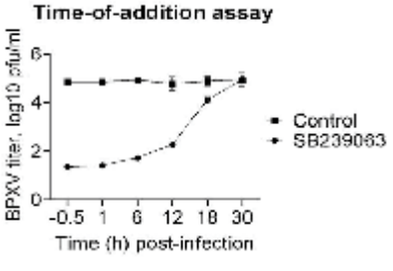


Fig. 27. Time-of-addition assay. Pre-treated (10 µg/ml of SB239063) or un-treated Vero cells were infected, in triplicates, with BPXV for 1 h. Inhibitor (SB239063) or DMSO were periodically applied over the life cycle of BPXV at indicated time points. Supernatants were collected at 48 hpi and yields of infectious progeny virus particles were quantified by plaque assay

***p38 inhibition suppresses levels of viral proteins and DNA in the infected cells*:** In order to determine the effect of SB239063 on synthesis of viral proteins, Vero cells were infected with BPXV at MOI of 5 and the inhibitor was applied at 4 hpi, a time point when early steps of the viral replication cycles (attachment/entry) were expected to occur. As shown in **(Fig. 28**) addition of SB239063 resulted in a reduced BPXV protein synthesis without affecting housekeeping control proteins. The reduced protein levels (viral polymerase), in turn, can impair BPXV DNA synthesis as well. Nevertheless SB239063 was also shown to significantly decrease (~60%) viral DNA levels in the infected cells in our study **(Fig. 29).**

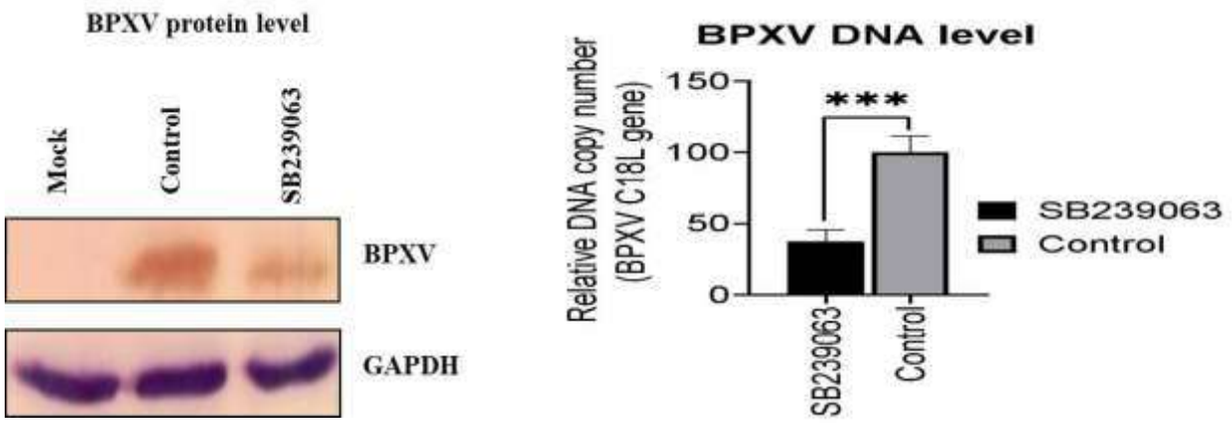
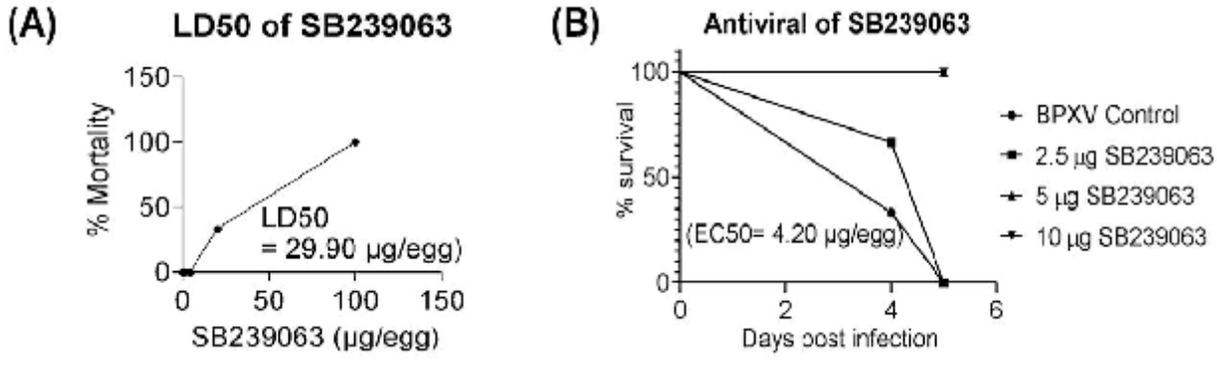


Fig. 28-29. p38 inhibitor impairs BPXV genome and protein synthesis. Confluent monolayers of Vero cells were infected with BPXV at an MOI of 5. The inhibitor or vehicle control were applied at 3 hpi and the cells were scrapped at 24 hpi to examine the levels of viral proteins (A) and DNA (B). Error bars indicate SD. Pair-wise statistical comparisons were performed using Student's t-test. *** = P<0.001. Values are means ± SD and representative of the result of at least 3 independent experiments.

***Antiviral efficacy of SB239063 in providing protection to embroyonated chicken eggs against lethal BPXV infection*:** Members of the family *Poxviridae* can infect chicken embryos and cause distinctly visible lesions (pocks) on CAM of the embryonated chicken eggs. We exploited this model to evaluate the inovo efficacy of SB239063 against BPXV. Mortality of the embryo was observed at SB239063 concentration ≥20 µg/egg but not at 4 µg/egg **(Fig. 30A)**. The LD50 was determined to be 29.90 µg/ egg. For evaluation of anti-BPXV efficacy of SB239063, eggs were infected with BPXV at 100 EID50 along with three different concentrations (10, 5, and 2.5 µg/egg) of SB239063 provided protection from the BPXV-associated mortality in a dose-dependent manner **(Fig. 30B)**. The EC50 was determined to be 4.20µg/egg. As compared to the vehicle-control, no obvious pock lesions could be observed in SB239063 inoculated eggs (protected groups) **(Fig. 30C)** Taken together, it was concluded that SB239063 prevents the development of BPXV-induced pock lesions on CAM as well as the associated mortality.



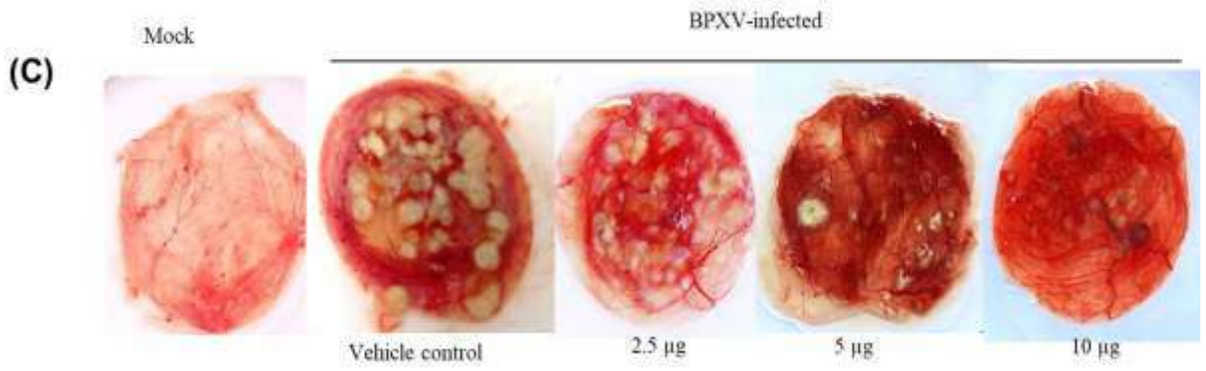


Fig. 30 A-C: In ovo antiviral efficacy of SB239063 against BPXV. (A) Determination of the LD50 of SB239063. LD50 was determined by inoculating 5-fold serial dilutions of SB239063 (concentration ranging from 100-0.16 µg/egg) or DMSO (vehicle control), in 10 days old embryonated SPF eggs, in a total of 100 µl volumes via CAM. Eggs were examined for the viability of the embryos up to five days post-inoculation to determine the LD50 by the Reed-Muench method. **(B) In ovo antiviral efficacy (EC50) of SB239063.** SPF embryonated chicken eggs, in triplicates, were inoculated with 2-fold dilutions (10 to 2.5 µg/egg) of SB239063 or DMSO via CAM route, followed by infection with BPXV at 100 EID50. At 6 days post-infection, eggs were examined for pock lesions and/or death of the embryos. EC50 was determined by the Reed-Muench method.Values are means ± SD and representative of the result of at least 3 independent experiments**. (C) Reduction in BPXV induced pock lesions on CAM of chicken embryo at various doses of SB239063.**

(Barua S, Kumar N and Riyesh T)

Generation of immortalized bat cell lines from primary cultures

Most of the commercially available cell lines do not support the growth of viruses of bat origin. Hence, there is a need for development of stable (immortalized) bat cell lines for in vitro studies to gain insights into the mechanisms of infection and pathogenesis. Different strategies can be employed for immortalization of primary cell cultures. One such strategy is introduction and stable expression of the catalytic subunit of the human telomerase reverse transcriptase (hTERT) in primary cell cultures. The ectopic expression of hTERT in primary cells will prevent the common problem of shortening of telomeres associated with repeated cell divisions and thereby prevent cells entering a state of senescence and death. Unlike other immortalization approaches (eg, SV40T immortalization), this approach results in minimal phenotypic and genotypic changes to cells and therefore preserves more characteristics of the original primary cell line. In this regard, we decided to immortalize two primary bat cell cultures (kidney cells and heart cells) available at NCVTC by ectopic expression of hTERT employing lentiviral mediated transduction.

***Generation of lentiviral psuedotyped viruscarrying hTERT gene*:** As a first step, lentivirus based psuedotyped virus carrying hTERT gene were generated using the plasmids (pLV-hTERT-IRES-hygro, pMDLg/pRRE and pRSV-Rev) procured from M/s Addgene, Watertown, USA. Plasmid expressing envelope G protein of vesicular stomatitis virus (available at NCVTC) was used for psuedotyping. Briefly, all the plasmids were bulk produced and authenticated at NCVTC. The confirmed plasmids were transfected in T-293 cells (using lipofectamine 2000) grown in a 100 mm cell culture dish for generation of psuedotyped viruses as per manufacturer's protocol (M/s Invitrogen , USA). The generated psuedotyped viruses were harvested by collecting the cell culture supernatant at 24 and 48 h post transfection and stored at -70°C till use. The generation of psuedotyped viruses was confirmed by psuedotyped virus infectivity assay in Vero cells and quantitation of hTERT transgene in transfected T-293 cell culture supernatant at different time intervals post transfection **(Fig. 31-32)**. Virus titrationwas also carried out in a 6 well cell culture plate and the titre of psuedotyped virus was found to be ~10^5 TU/ml.

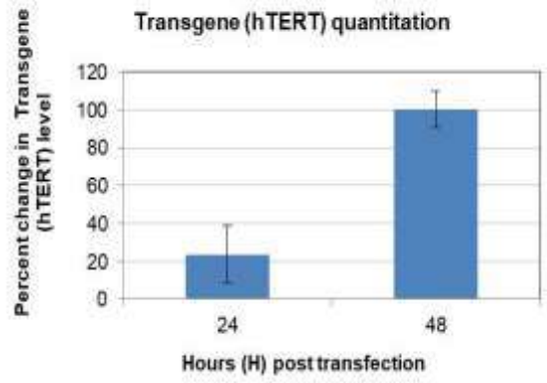


Fig. 31. Quantitation of hTERT transgene in tranfected cell culture supernatant: 293T cells, in triplicates, were transfected with plasmids (pLV-hTERT-IRES-hygro, pMDLg/pRRE and pRSV-Rev, pVSV-G) and control plasmid. At indicated time points, RNA isolated from cell culture supernatant and cDNA was prepared. Quantification of hTERT gene in the transfected cell culture supernatant at different time points (post transfection) was carried out by RT-qPCR. The results are expressed as relative increase in percent change in comparison to RNA at 12 h post-transfection. Error bars indicate SD. Pair-wise statistical comparisons were performed using Student's t-test (*** = P<0.001).

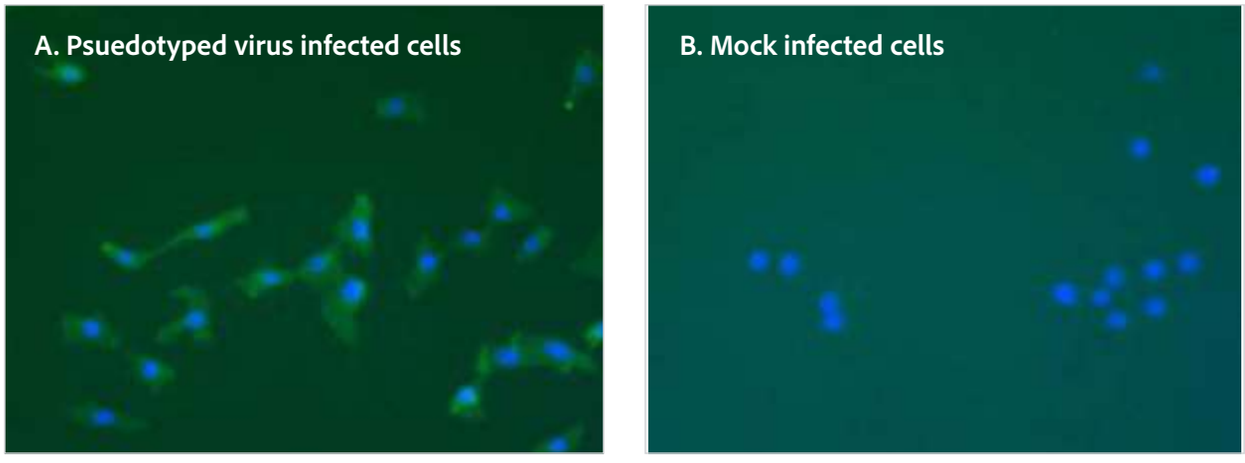


Fig. 32. Pseduotyped virus infectivity assay: Initially psuedotyped viruses were produced by transfection of lentiviral plasmids in 293-T cells as described elsewhere. The supernatant was harvested, clarified and used for virus infectivity assay. For virus infectivity assay, the Vero cells were infected with psudotyped virus at MOI of ~ 5 and incubated overnight to allow virus entry. Later cells were fixed with paraformaldehyde and performed immuno-fluroscence staining using VSV antibodies to detect the presence of pseudotyoed virus in target cells. **(A):** The green fluorescence (arrow heads) represents the presence of pseudotyped virus in cytoplasm of target cells. **(B):** No fluorescence detected in mock infected cells.

***Antibiotic selection of cell clones expressing hTERT*:** Transduction assay followed by antibiotic screening (using hygromycin) was used for selection of cell clones expressing hTERT. Briefly, prior to transduction experiment an antibiotic kill curve experiment was also performed using hygromycin antibiotic to identify the appropriate concentration of antibiotic required for selection of transduced clones of cells. Hygromycin @ 400 µg/ml was identified as a suitable concentration for selection of transduced clones based on MTT assay and visual reading. For transduction experiment cells were prepared on 6 well cell culture plates and infected with pseudotyped virus at MOI of 1. Uninfected control was also kept. 48 h post transduction, hygromycin antibiotic @ 400 µg/ml was used for selection of antibiotic resistant colonies. The antibiotic resistant colonies identified (11 days post transduction) were later trypsinized and limiting dilutions were made. The diluted cell suspensions were plated in the wells of a 96 well tissue culture plate with a cell density of 1 cell/well. These cell colonies are being expanded in our laboratory. Once sufficient growth is achieved, these cells will be checked for the expression of the telomerase gene and their ability to divide even after 20 passages. Each selected clone will be later cryopreserved in liquid nitrogen. The cell lines generated in this study will be useful for studying bat innate immunity and virus-host interaction

(Riyesh T, Kumar N, Shanmugasundaram K, Vaid RK and Barua S)

Epitranscriptomic regulation of SARS-CoV-2 replication

RNA modifications are found in all life forms and have been linked to development, health and diseases. Heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1, an RNA-binding protein) was shown to mediate deposition of N6-methyladenosine (m6A) in internal SARS-CoV-2 RNA. The levels of hNRNPA1 expression and extent of methylation varied, depending on the course of SARS-CoV-2 life cycle. The recruitment of eIF4E (translational initiation factor) facilitated viral RNA translation at 1 hour post infection (1 hpi). However, at 2 hpi, methylation of internal SARS-CoV-2 RNA recruited hNRNPA1 which facilitated viral RNA transcription but resulted in translational repression **(Fig. 33)**, a phenomenon contributing in understanding the early translation to replication switch in the viral life cycle. Besides, the abrogation of methylation also produced a defective 5' cap of viral RNA which failed to interact with eIF4E, thereby resulting in a decreased synthesis of viral proteins. Blocking SARS-CoV-2 RNA methylation resulted in reduced virus yield, suggesting epitranscriptomic machinery (methylation) facilitates SARS-CoV-2 replication and might represent potential target for new antiviral drugs against COVID-19.

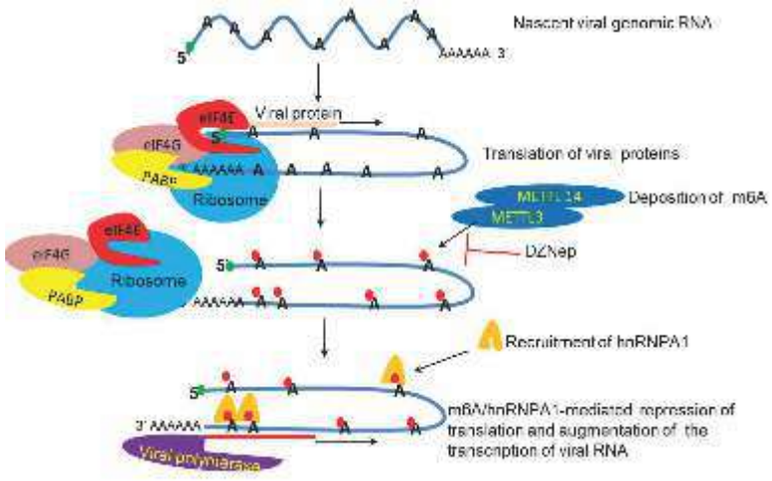


Figure 33. Epitranscriptomic regulation of SARS-CoV-2 replication: hnRNPA1 regulates early translation to replication switch in SARS-CoV-2 life cycle. Immediately following infection (~1h), the nascent positive sense SARS-CoV-2 RNA interacts with cap-dependent translational initiation machinery to directly translate the viral polyprotein which is further cleaved to produce 16 NSPs. After sometime (~2h), viral RNA is subjected to m6A modifications (eight m6A sites in SARs-CoV-2 genome) via cellular writers such as METTL3 and METTL14. m6A deposition facilitates recruitment of hnRNPA1 (three hnRNPA1 binding sites-two at 3' end and one in "S" gene) which eventually repress translation and facilitate transcription-switch of viral RNA from translation to transcription. DZNep treatment inhibits deposition of m6A mark on SARS-CoV-2 RNA which eventually inhibits recruitment of hnRNPA1 and hence reduced synthesis of the viral RNA.

(Kumar N, Barua S, Riyesh T, Gulati BR and Yash Pal)

Development of repository of respiratory viruses of livestock

The research work was conducted with the aim of development of repository of authenticated respiratory viruses of livestock, which will serve as resource for exploring these biological resources for future development of diagnostics, immunobiologicals, as reference material, comparative biological studies, etc. Biological samples collected for identification and isolation of respiratory viruses of livestock species. The biological samples (n=109) in the form of nasal swabs (n=45) and post-mortem tissue samples (n=64) of cattle and goats were collected from veterinary clinics, LUVAS, Hisar and Gaushala, Hisar. PM samples like – lung, trachea, and kidney were collected from dead animals. Immediately after collection, all samples were preserved in the LN2 container and transported to the laboratory. The tissue samples were triturated in cell

culture media, prepared 10% suspension, centrifuged and supernatant were filtered through 0.45 ㎛ filter. The clarified tissue suspensions were subjected to molecular detection of respiratory viruses by multiplex RT-PCR assay. None of the samples screened for detection of pathogens like BRSV, BPIV3, BCoV, and BoHV was positive for the targeted viruses.

(Bera BC, Anand T, Virmani N, Riyesh T and Gulati BR)

Development of isothermal "Recombinase Polymerase Amplification" (RPA) based assays for detection of Porcine circovirus 2 (PCV2) and 3 (PCV3) in pigs

The isothermal recombinase polymerase amplification (RPA) assays are being standardized for development point-of-care diagnostics for detection of PCV2 and PCV3 viruses associated with reproductive failure in pigs. The reproductive failure and piglet mortality are being reported in pigs which incurs huge economic losses in the swine industry. The prime advantages of RPA are the potentially higher degree of fidelity of the assay, requirement of less laboratory equipment and field level application. Currently available diagnostics are time-consuming, require expensive laboratory settings and well-trained personnel, hence RPA assay will help for rapid and highly sensitive detection of PCV2 and PCV3 viruses at field. We have standardized the isothermal amplification of targeted conserved regions of PCV2 and PCV3 viruses using DNA samples isolated from field samples. The RPA assay was standardized using specific reagents from TwistDX, UK and results indicated that the designed primer sets successfully amplified the targeted regions of PCV2 and PCV3 viruses. The detection limits of the assays were estimated using serial dilutions of the known quantity of the standards.

(Bera BC, Anand T and Virmani N)

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

The mRNA vaccine construct was designed targeting the immunogenic protein of SARS-CoV-2 including various regulatory sequences at both ends for stabilization of mRNA structure and strong expression of the protein. The designed construct got synthesized upon codon optimization for better expression. The synthesized construct is cloned into a suitable vector and confirmed by restriction enzyme digestion and confirmed by sequencing. Subsequently, the good quality recombinant plasmids of mRNA constructed from the confirmed clone were purified, linearized and purified linearized plasmids. The linearized mRNA plasmid construct was used as template for in vitro synthesis of RNA employing commercial kits. Upon RNA synthesis, the template plasmid DNA was degraded by DNaseI treatment followed by purification of the synthesized RNA. The quality and quantity of the synthesized RNA was estimated. High quantity of good quality RNA was synthesized and stored at-80°C for further use for liposome preparation.

(Bera BC, Anand T and Virmani N)

Development of bacteriophage cocktail to ameliorate *Pseudomonas aeruginosa* infections in Biofilms

Pseudomonas aeruginosa is a member of ESKAPE group of pathogens. It is gram negative, rod shaped facultative anaerobic bacteria which is an opportunistic pathogen preferentially colonizing immunocompromised patients in healthcare settings. It is a common cause of cystic fibrosis and burn infections. *P. aeruginosa* is resistant to many antimicrobial drugs due to intrinsic carriage of virulence factors and lower permeability of cell wall. The biofilms formed by this pathogen provide additional protection to it and these bacteria are difficult to control in formed biofilms. Bacteriophages provide an alternative means of antibiotics against this bacteria as it can easily penetrate biofilms and thus are more effective for the control of resistant infections. We elaborate the design of a bacteriophage cocktail carrying selected bacteriophages, which can effectively kill a broad range of *P. aeruginosa* isolates in vitro in biofilms. The bacteriophage cocktail was assessed for biofilm inhibition efficiency and biofilm eradication efficiency and it was observed that the phage cocktail efficiently inhibited the bacterial biofilm formation as compared to the control in micro test plates **(Fig. 34).**

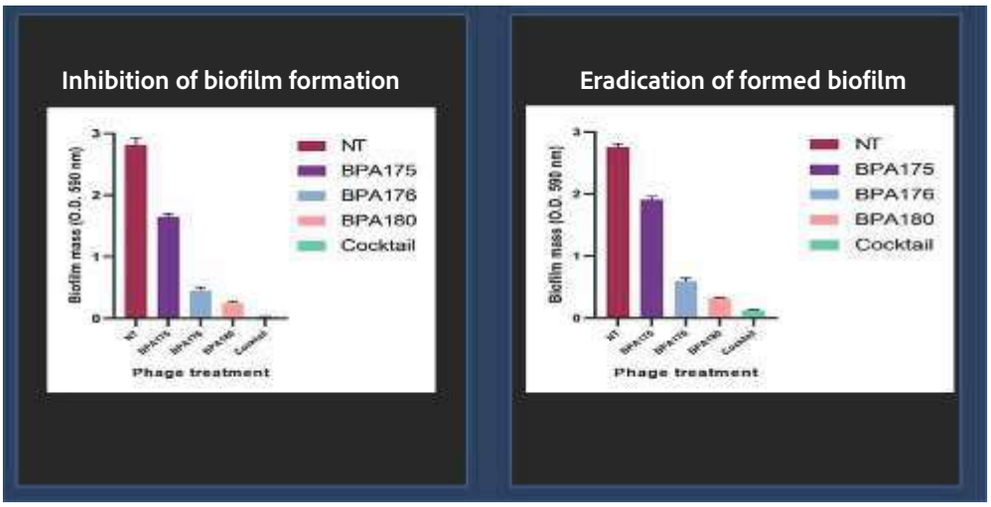


Fig. 34. Assessment of biofilm inhibition efficiency and biofilm eradication efficiency of the bacteriophage cocktail: Phage cocktail efficiently inhibited the bacterial biofilm formation as compared to the control in micro test plates

(Vashisth M, Yashveer S, Virmani N, Bera BC, Vaid RK and Anand T)

Characterization of bacteriophages against ESBL producing bacteria for targeting biofilms in bovines

The existence of ESBLs producing bacteria increased rapidly throughout the world in the recent decades. Extended-spectrum β-lactamases (ESBLs) encoded on plasmids and chromosomes of these bacteria provide resistance to a wide variety of antibiotics. ESBLs are present in gram negative bacteria belonging to *Enterobacteriaceae, Pseudomonadaceae* and *Vibrionaceae* family and in gram positive bacteria belonging to *Staphylococcaceae* family. The bacteriophages (predators of bacteria) are viruses, which provide an effective means to tackle these ESBLs producing bacteria by infecting and killing them. The present study was aimed to isolate lytic bacteriophages capable of killing ESBLs producing bacteria. The targeted ESBLs producing bacteria were of bovine origin and were selected using ESBL agar base supplemented with ceftazidime, cefotaxime, ceftriazone, astreonam and fluconazole. The bacteriophages were isolated (VTCCBPA211 and VTCCBPA212) from sewage samples and serially purified three times using double agar layer method in presence of host bacteria **(Fig. 35).** These bacteriophages showed strong biological efficacy suggesting that they have potential to inhibit or significantly reduce the spread of drug-resistant bacteria spreading through oral and nasal route in livestock.

Fig. 35. Bacteriophage VTCCBPA211 and VTCCBPA212 producing clear circular plaques on nutrient agar.

(Bala A, Ravikant, Vashisth M, Virmani N, Bera BC and Anand T)

Assessment of synergy of *Acinetobacter baumannii* phage with Cephalosporin

Acinetobacter baumannii is aerobic, gram negative member of ESKAPE group of pathogens which is responsible for an immense number of medical problems including bacteremia, pneumonia, meningitis, urinary tract- and wound infections. We isolated a bacteriophage (VTCCBPA182) against *A. baumannii* and examined its antibacterial efficiency in conjunction with commonly used antibiotics. It was observed that cefepime showed synergistic action with bacteriophage as signified by the increased plaque size near the antibiotic disc on bacterial lawn plated with bacteriophages in it **(Fig. 36 A & B).**

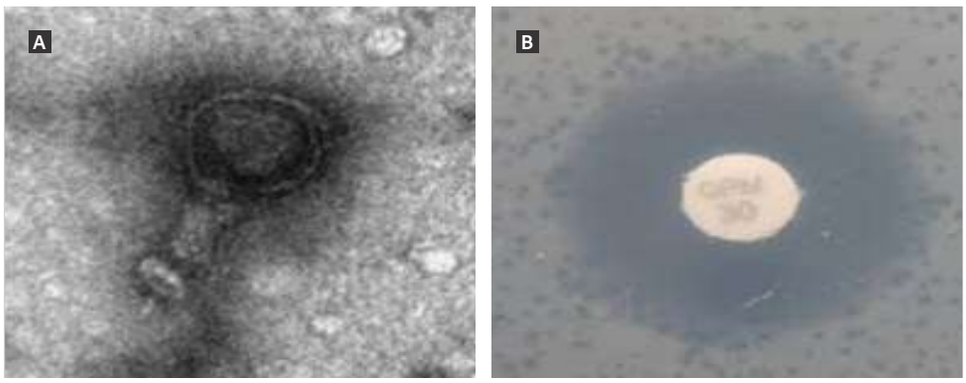


Fig 36. (A) : TEM characterization of bacteriophage BPA182 revealed that it possesses a contractile tail and belong to the family *Myoviridae*. (B) Larger plaques in vicinity of cefepime antibiotic disc

(Vashisth M, Bala A, Yashveer S, Virmani N, Bera BC, Vaid RK and Anand T).

Authentication and accessioning of bacteria

During the year of 2021, among the processed cultures, 82 cultures were accessioned in the bacterial repository which has led to total strength of bacterial culture collection to 1583 veterinary bacteria. Cultures were mainly submitted from IVRI, Izatnagar; AAU, Khanapara; CSWRI, Avikanagar; however a majority of accessions during this period were from NCVTC Bacteriology laboratory. Besides this, a total of 27 sample lots were received comprising of 83 pathological/other samples submitted/collected at NCVTC bacteriology laboratory from different species of animals viz., equines–(Horse/Jenny/Poitu donkey/Nukra mare nasal swab, tissues, stomach contents and abscess); buffalo (13-post-mortem samples/nasal swabs and mastitic milk); donkey/mare milk (n=11), Wild avian fecal samples (n=10); Shrimp samples (n=5) and bat sample (n=1) were processed for bacterial isolation and a total of 281 bacteria were isolated. All these bacteria have been preserved in the repository under the general category of preservation.

Some of the significant accessioned cultures are *Actinobacillus hominis* (As15), *Mannheimia caviae* (Ch18), *Enterococcus cecorum* spp. (Eq426A), *Escherichia coli* (Eq432A), *Pseudomonas aeruginosa* (Mm21), *Staphylococcus saprophyticus* ssp. *bovis* (Mm21A), *Staphylococcus xylosus* (Mm21B), *Corynebacterium efficiens* (RR268), *Staphylococcus xylosus* (RR2015_212), *Shigella dysentriae* (RR28), *Mammalicoccus sciuri* (RR2015230 and RR2016-332); *Staphylococcus argenteus* (RR2016-134), *Staphylococcus hemolyticus* (RR2016-136), *Mammalicoccus fleuretti* (RR2015-88) and *Shigella flexneri* (RR2015-120). Significant new additions in the repository include *Aeromonas hydrophila* (BAA1510), *Stenotrophomonas maltophila* (Aq91, BAA1515), *Aeromonas veronii* (BAA1511), *A. jandaei* (Aq26, BAA1540), *Pseudomonas alkaliphila* (BAA1516) from pond water; *Arcanobacterium pluranimalium* (Bu91, BAA1517) from buffalo, *Brucella tritici* (As14E, BAA1518), *Leucobacter celer* ssp. *celer* (As14D, BAA1519) from donkey milk and *Morganella morganii* (Hs8, BAA1539) from human urine samples.

(Vaid RK, Anand T, Riyesh T and Shanmugasundaram K)

Isolation of *Shewanella* and *Vibrio spp*. from shrimp (*Litopenaeus vannamei*) intestine

Whiteleg shrimp (*Litopenaeus vannamei)* is an important aquatic pisciculture product meant for human consumption, which has been introduced in India recently in last decade due to its high export value and ability to grow in inland saline waters, due to which, it is being cultivated in large scale in Punjab and Haryana. Bacterial diseases of shrimps are important from economic and public health perspective **(Fig. 37)**. The shrimp industry has suffered serious economic losses due to infectious diseases world over, caused by such as *Vibrio harveyi, Photobacterium damsel, Vibrio alginolyticus, Bacillus cereus,* and *Shewanella khirikhana*. In order to survey the prevalence of *Vibrio* species among retail samples, samples obtained from Shrimp farm at Chandigarh were dissected to isolate bacteria from the intestinal tract of shrimp. Many species of *Shewanella* (n=18) and *Vibrio* spp. (n=14) were isolated showing a high diversity of *Vibrio* spp. prevalence as identified from shrimp samples. Among *Vibrio* spp., *Vibrio hyugaensis* (Si5B), *Vibrio metoecus* (Si3), *Vibrio neocaledonicus* (Si3B), *Vibrio hyugaensis* (Si5B), and *Vibrio alginolyticus* (Si6C; Si10B and Si10A) were identified. *Vibrio* spp. microscopic morphology is typical comma shaped **(Fig. 38)**. Nearly 14 cultures of *Vibrio* spp. belong to probable novel taxa and are being identified with a strain LCUEs (LCUE01000032) **(Fig. 39)**. Among *Shewanella* spp. taxa identified were *Shewanella algae* (Si1A, Si3A, Si4A, 4B, Si6B, Si10, 10C), and *Shewanella khirikhana* (Si5, Si5A) among the 18 *Shewanella* isolated, seven *S. algae* strains were divided into two clades and had close similarity with Type strain of *S. algae* JCM21037 **(Fig. 40)**. *Shewanella khirikhana* is an important pathogen of shrimps. *Shewanella algae* is an opportunistic pathogen of humans associated with exposure to marine environments.

Fig. 37. Diseased Whiteleg shrimp (*Litopenaeus vannamei)*

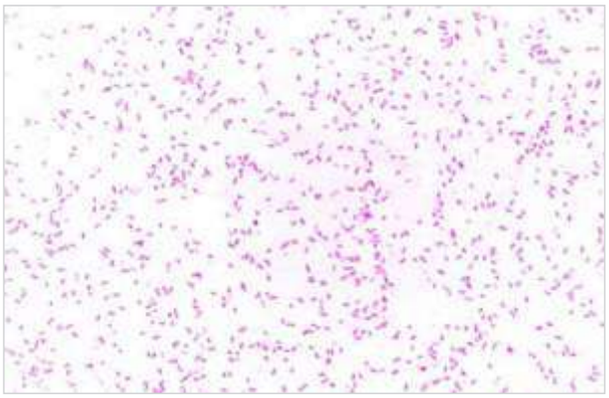


Fig. 38. Vibrio spp. isolate showing Gram negative comma shaped rods 100X oil immersion

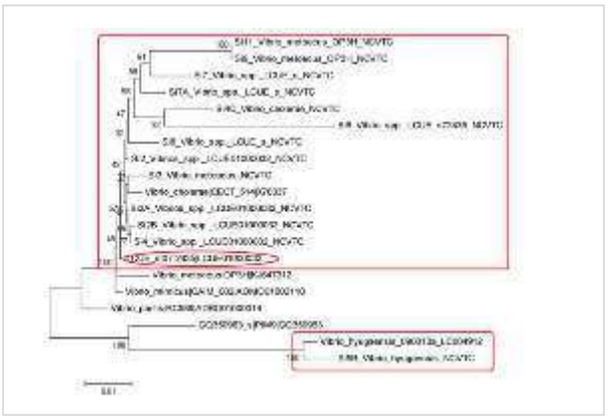


Fig. 39. Phylogenetic analysis of *Vibrio* spp. isolates from shrimp identified with LCUE01000032, a probable novel taxa

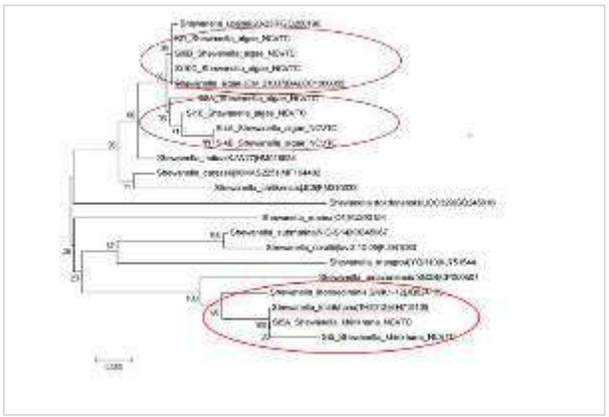


Fig. 40. Phylogenetic analysis of *Shewanella algae* and *S. khirikhana* 16S rRNA sequences (1355 bp) by Neighbour joining method

(Vaid RK, Karuna and Anand T)

Molecular identification and antimicrobial sensitivity profiling of *Staphylococcus* spp.

Detection of Staphylococcus intermedius group (SIG) members in horse

The bacteria of genus *Staphylococcus* are the natural inhabitants of skin and mucous membranes of animals, birds and human beings. Consequently they are important as causative agents of economically important diseases in domestic animals such as mastitis of milch animals. Moreover, the emergence of antimicrobial resistant strains of staphylococci has increased the impact of zoonotic transmission. It is therefore important to identify the *Staphylococcus* spp. in order to understand their epidemiology and to manage infections caused by them. Genus *Staphylococcus* presently includes 61 species. We used 16S rDNA PCR and sequencing to identify 59 staphylococci isolates which are accessioned cultures of NCVTC, originating from different domestic animals. Due to rapidly changing taxonomy of the family *Staphylococcaceae*, and due to incomplete identification of deposited strain, it is important to identify the strain at species level. The 59 *Staphylococcus* spp. cultures were identified up to species level taxa using near complete 16S rRNA sequence by homology searching. A total of 19 different species were identified, of which 17 were of *Staphylococcus* spp, and the rest were identified as *Mammalicoccus* spp. Total forty nine isolates were identified as 19 strains of *S. argenteus*, 4 strains each of S. *chromogenes* and *S. hemolyticus*, 3 strains each of *S. delphini*, *S. simulans*, 2 strains each of *S. intermedius, S. hominis, S. agnetis,* and *S. aureus*; and a single strain each of *S. borealis, S. condiment, S. hyicus, S. pseudointermedius, S. cornubiensis, S. saprophyticus*, and *S. xylosus*. Due to taxonomic changes in staphylococcal nomenclature, 8 isolates, which were previously of *Staphylococcus* spp. are now included in a novel genera i.e., *Mammalicoccus* spp. These were identified as *Mammaliicoccus sciuri* (6), and *Mammaliicoccus fleurettii* (2) **(Table 22).**

Table 22 : Characteristics of strains identified as Mammalicoccus spp. from different animals

S. No.	Accession No.	Year of Deposit, State	RR/NO./ Strain	Source	Species Name after Molecular Identification	Completeness (%)	Similarity (%)
1.	VTCCBAA752	2014, Karnataka	NIVEDI/DBT/ YaK/339Y	Yak	*Mammaliicoccus fleurettii*	*94.9*	*99.79*
2.	VTCCBAA854	2015, Tamilnadu	TNV/2015/003	Cattle	*Mammaliicoccus sciuri*	*94.9*	*99.93*
3.	VTCCBAA861	2015, Tamilnadu	TNV/2015/016	Cattle	*Mammaliicoccus sciuri*	*94.1*	*100*
4.	VTCCBAA916	2015, Haryana	Eq64	Horse	*Mammaliicoccus sciuri*	*94.6*	*99.93*
5.	VTCCBAA982	2015, Assam	RR/2015/230	Cow (milk)	*Mammaliicoccus sciuri*	*99.7*	*95.38*
6.	VTCCBAA984	2015, Assam	RR/2015/232	Cow (milk)	*Mammaliicoccus sciuri*	*99.5*	*98.15*
7.	VTCCBAA1036	2015, Uttar Pradesh	RR/2015/88	Cattle (milk)	*Mammaliicoccus fleurettii*	*98.9*	*98.08*
8.	VTCCBAA1146	2016, Assam	RR/2016/332	Pig	*Mammaliicoccus sciuri*	*99.00*	*92.53*

A probable novel species which has not yet been named in literature was identified as CP02288_sM0911. Antimicrobial susceptibility testing was performed on 73 cultures. Twenty *Staphylococcus* cultures and five *Mammaliicoccus* cultures were found to be penicillin resistant. The species identification revealed biodiversity of *Staphylococcus* spp. present in animals. Members of the *Staphylococcus intermedius* group (SIG) include *S. intermedius*, *S. pseudintermedius*, *S. cornubiensis*, *S. delphini* and *S. ursi*. Many horse isolates were identified which belonged to *Staphylococcus intermedius* group (SIG), thus underlining the importance

of this pathogenic group in equines. Isolates earlier identified as *Staphylococcus aureus* were identified as those belonging to *S. argenteus* taxa.

(Ankush and Vaid RK)

Isolation and identification of *Aggregatibacter* spp. from bat

Samples from a live juvenile insectivorous bat was collected for isolation and identification of bacteria. Four types of colonies were observed after 24 hours and 48 hours of incubation. In 24 hours, 2 Gram positive and 1 Gram negative bacterial isolates were identified. The isolated Ff1 grew as 2-3 mm grey circular haemolytic flat colonies causing pitting on agar surface, which showed morphology of Gram positive filamentous rods. These were identified as *Bacillus altitudinis*. The Ff1A isolate colonies were 1-2 mm grey shiny non-hemolytic colonies on SBA, which showed a dark lemon yellow pigmentation after 2-3 days. This strain was closest in identity to *Arthrobacter saudimassiliensis* strain 11W110_air with 94.89% similarity at 98.9% completeness of 16S rRNA sequence. The Gram-negative non-motile isolate which produced gas on HL media was identified as *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*. However, very slow growing colonies were discerned after 48 hours of incubation on 5% SBA. The minute pinpoint colonies were of non-hemolytic nature and upon culture staining Gram-negative coccobacillary rods with bipolarity were observed. More growth of bacteria was obtained after 78 h incubation. The isolate 16S rDNA sequence was searched by homology matching and it was identified as a strain closest to *Aggregatibacter actinomycetemcomitans,* which was closest to strain ATCC 33384, with 94.22% identity at 99% completeness. This bacterium is associated with Localised Juvenile Periodonitis (LJP), a human disease in which destruction of tissues around incisors and first molars is reported in which Serotype b strains of *A. actinomycetemcomitans* are found. Since the near complete 16S rDNA sequence showed a percent identity of 94.22%, this strain may belong to novel taxa, pending polyphasic characterization. On phylogenetic analysis, using sequences of type strains of Pasteurellacease family genera, the strain Ff1E was placed close to *Aggregatibacter actinomycetemcomitans* type strain ATCC33384 **(Fig. 41)**. Family *Pasteurellaceae* is an economically important taxa, whose representatives are inhabitants of mucosal membranes of a variety of domestic, wild animal species. Many members of this family are agents of important diseases in animals such as *Pasteurella multocida, Mannhemia hemolytica, Actinobacillus equilli,* in small and large ruminants and birds.

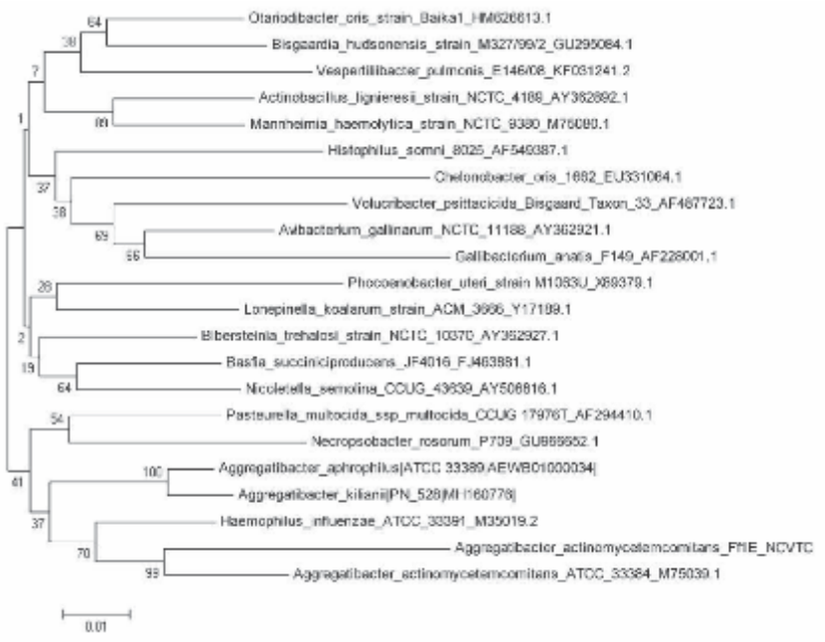


Fig. 41. Phylogenetic tree of Aggregatibacter Ff1_species

(Vaid RK, Riyesh T and Anand T)

Survey of fish ponds near Hisar for prevalence study of motile aeromonads

Motile aeromonads are widespread, Gram-negative, rod-shaped, facultatively aerobic microbes commonly found in aquatic environments. They are important pathogens which cause furunculosis, hemorrhagic septicemia or motile aeromonas septicemia (MAS), hemorrhagic enteritis, epizootic ulcerative syndrome, red sore disease, tail and fin rot, and lethargy in finfishes. They are also important food-borne pathogens and opportunistic pathogens in humans. Therefore, this study surveyed the prevalence of *Aeromonas* spp. in the natural freshwater village ponds (66 Nos) near Hisar. Among the presumptive *Aeromonas* isolates (n=182), 99 isolates were randomly selected and examined phenotypically which were Gram-negative rods, oxidase and catalase positive. The bacteria produced positive reaction to catalase, oxidase and D-glucose while variable reactions were observed with utilization of citrate, malonate, lactose and sucrose.

For species level identification, a total of 97 isolates were further analyzed by PCR amplification of housekeeping gene *gyr*B. The phylogeny of 85 *Aeromonas* isolates from water samples based on *gyr* B sequence determination was performed. The isolated bacteria were closely related and included *A. veronii* (64 isolates), *A. hydrophila* (11 isolates), *A. jandaei* (5 isolates), *A. caviae* (4 isolates), *A. punctata* (2 isolates), *A. sobria* (1 isolate) and *A. culicicola* (1 isolate). *Gyr*B sequencing also identified 3 isolates as *Stenotrophomonas maltophilia*, *Pseudomonas alcaliphila* and *Pseudomonas sediminis* (1 isolate each) among those sequenced. All *Aeromonas* spp. isolates had minimum one virulence gene out of 7 genes tested, whereas highest frequency of the isolates (33.67%) were positive for 4 virulence genes out of 7. Examination of resistance profiles of 98 confirmed *Aeromonas* isolates to 15 common antimicrobial agents (8 classes) was performed by disc diffusion assay. The overall multiple antibiotic resistance (MAR) index ranged from 0.01 to 0.45, corresponding to the highest value of 44.9% isolates resistant to at least two antimicrobial agents. Out of motile aeromonads tested, 11.2% were Extended-Spectrum β-Lactamase (ESBL) producers and 35.7% were AmpC β-Lactamase producers. The survey showed prevalence of virulent antimicrobial resistant *Aeromonas* spp. in village ponds used for fisheries.

(Nokhwal A, Vaid RK and Anand T)

Comparative genomics of *Trueperella pyogenes* including Indian strain (Bu5) isolated from water buffalo

Trueperella pyogenes is a Gram-positive opportunistic pathogen that causes severe cases of mastitis, metritis, and pneumonia in wide range of animals, resulting in significant economic losses. This ubiquitously found opportunist bacterium, is the aetiological agent of abortions and chronic suppurative infections like mastitis, pyometra, pneumonia, and abscesses leading to significant losses in livestock industry, especially in intensive system of animal husbandry. Nevertheless, very little is known about the dynamic regulation of the virulence factors involved in the disease pathogenesis. Moreover, a comprehensive comparative genome analysis of *T. pyogenes* genomes has not been performed till date. In order to understand the genetic level composition of *T. pyogenes*, this analysis was carried out to characterize and compare 19 *T. pyogenes* genomes originating in different geographical origins across the world including an Indian origin strain *T. pyogenes* Bu5 isolated from water buffalo (*Bubalus bubalis*). Additionally, candidate virulence determinants that could be crucial for their pathogenesis were also detected and analyzed by using various bioinformatics tools. The average genome size of the investigated genomes is 2,327,522.5 Mb, ranging from 2,187,257 (*T. pyogenes* DSM 20630) to 2,427,168 (*T. pyogenes* TP4). The average GC% is 59.54% ranging from 59.33% of *T. pyogenes* strain jx18 to 59.8% of *T. pyogenes* strain MS249. The circular plot visualization of others investigated 18 *T. pyogenes* genomes with reference to *T. pyogenes* str. TP6375 depicts varied GC content and GC skew along with core-region similarity **(Fig. 42)**. The pan genome calculations revealed an open pan genome of *T. pyogenes* comprising 3214 genes, a core genome of 1520 genes, an indispensable genome of 1093 genes and strain specific genes in the range of 2-63. In addition, an inventory of virulence related genes, 190 genomic islands, 31 prophage sequences, and 40 antibiotic resistance genes that could play a significant role in organism's pathogenicity were also detected. The synteny plots depicted synteny and large-scale genomic

rearrangements like inversion, duplication, and relocation **(Fig. 43)**. The study highlights an open pan genome as well as differential distribution and diversity of candidate virulence determinants such as fimbrial genes, genomic islands, prophages and antibiotic resistance genes. The identified core genome can be further used for screening of drug and vaccine targets whilst strain specific genes can be utilized for strain level bacterial typing with further experimental inputs. In addition, identified putative virulence factors can be further experimentally investigated to unravel underlying mechanisms involved in infection development. The investigation has provided unique insights into pan genome, virulome, mobiliome, and resistome of *T. pyogenes* genomes and laid foundation for future investigations.

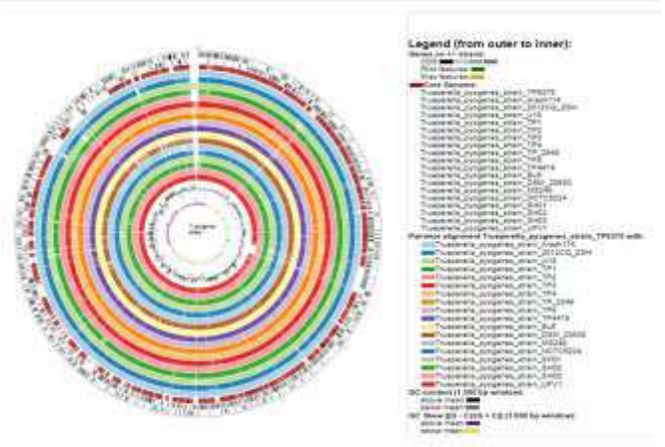


Fig. 42. Circular representation of nineteen investigated *T. pyogenes* genomes with reference genome as *T. pyogenes* TP6375.

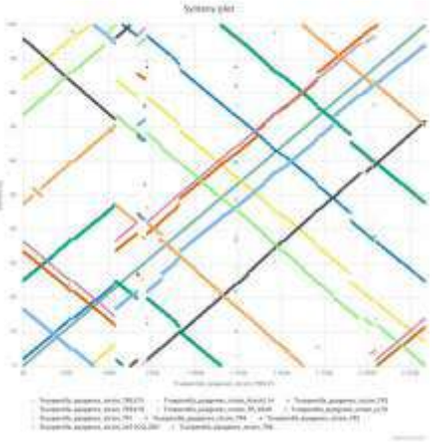


Fig. 43. Synteny plot visualization of investigated complete *T. pyogenes* genomes compared against reference genome *T. pyogenes* TP6375

(Thakur Z, Vaid RK, Anand T)

Indian Network for Fisheries and Animal Antimicrobial Resistance (INFAAR)

The emergence of antimicrobial resistance (AMR) in bacteria of food animals is an important risk which needs routine surveillance in order to measure the prevalence. In this direction, milk samples from cattle/buffalo (11), rectal swabs from Murrah buffalo calves (11) and cloacal swabs from poultry (23) were collected for isolation of *Escherichia coli* and *Staphylococcus* spp. and microbial isolates were tested for AMR. From 23 poultry cloacal swabs, 36 *E. coli*; from 11 milk samples 15 *Staphylococcus* spp. isolates; and from 11 buffalo calves diarrheal swabs, 9 *Escherichia coli* isolates were obtained. *E. coli* isolates (46), were confirmed biochemically and by duplex PCR (*lac*Y &*pho*A positive). The *Staphylococcus* spp. isolates were confirmed phenotypically and for *Staphylococcus aureus* taxa by *nuc* PCR.

Disc diffusion has been performed on 36 *E. coli* poultry isolates. Out of 36 isolates tested, 34 (94.5%) isolates were resistant to one or more classes of antimicrobials. Out of 36 isolates, 3 (8.34%) isolates were resistant to at least 1 antimicrobial tested, whereas 4 (1.1%) isolates were resistant to at least 2 antimicrobials; whereas 10 (27.8%) isolates were resistant to at least 3 antimicrobials; whereas 7 (19.5%) isolates were resistant to at least 4 antimicrobials. It is noted that at least 10 (27.8%) isolates were resistant to either 5 or more antimicrobials tested. One poultry isolate, Ana 29 was resistant to 10 antimicrobials out of the panel of 15 antimicrobials used for testing.

Among the milk isolates, there was isolation of 15 *Staphylococcus* species out of which 3 were positive for *nuc* gene by PCR and were identified as *Staphylococcus aureus*. Rests were identified as Coagulase negative staphylococci (CoNS). Out of 15 Gram positive staphylococcal isolates, 8 (53.4%) isolates were resistant to at least 1 antimicrobial out of 9 drugs tested. Significantly 4 isolates (26.7%) isolates were resistant against cefoxitin. An AMR diagnostic kit received from IVRI, Eastern Station was evaluated by testing the kit for ESBL

positive and ESBL negative testing. Under the EQAS (External Quality Control Scheme) program of INFAAR, 12 unknown bacterial cultures in a batch of 4 each were analysed for AMR testing and bacterial identification and results were sent to VIT, Vellore.

(Vaid RK, Anand T, Singha HS and Pathak A)

Isolation, characterization and generation of repository of mycobacteria

A total of 45 biological samples were collected for screening and isolation of mycobacterial species. The collected samples include fecal samples from cattle (n=16), buffaloes (n=2) and bats (n=25) and milk samples from buffaloes (n=2). Two soil samples were also collected from an unorganized dairy farm. Fecal samples from cattle and buffaloes (n=18) were tested for the presence of acid fast bacilli by PCR targeting *afb* and *hspX* genes and all fecal samples were found positive for acid fast bacilli by *afb* gene based by *PCR*. The milk samples were negative for *afb*and *hspX* genes based PCR.

All the 18 fecal samples were decontaminated, processed for isolation, inoculated in duplicate in HYEM and Middlebrook 7H11 agar base and incubated at 37°C. in addition, a total of 12 intestinal samples collected from sheep were also processed for the isolation of *Mycobacterium avium* subspecies *paratuberculosis*. In brief, 250 mg (approximately) of intestines were triturated with sterile PBS and sand in a mortar and pestle. Triturated samples were decontaminated with 0.75% HPC and thereafter, washed twice with sterile PBS to remove the decontaminating agents. Afterwards, pellets were resuspended in 2 mL of sterile PBS and 200 µl samples in duplicate were inoculated in HEYM and Middlebrook 7H11 agar base supplemented with mycobactin J and incubated for isolation. In addition, an aliquot of 200 µl of decontaminated samples were used to detect the presence of mycobacterial species by PCR targeting *afb* and *hspX* genes and of which 10 samples were found to be positive by *afb* and *hspX* genes. Moreover, slides were also prepared and stained with acid-fast staining to detect the presence of acid fast-bacilli. Acid-fast bacilli were detected in 6 intestinal tributes. One *Mycobacterium* species (**Fig. 44 A & B**) was isolated after 4 weeks of incubation at 37 C from an intestinal sample and further characterization is under progress.

Out of 25 bat fecal samples tested, 20 yielded expected amplicon in afb and *hspX* genes based PCR. The positive samples were further tested for the presence of *Mycobacterium tuberculosis* complex by conventional PCR with MTBC primers and all samples found negative. In addition, all 30 samples were screened for the presence of acid- fast bacilli by Ziehl-Neelsen stain and found negative for the presence of acid-fast bacilli. Further, three mycobacterial species (*M. smegmatis, M. abscessus* and *M. kansasii)* stored under cold chains (4°C and -80°C) were processed to check bacterial survival. It has been observed that these three bacterial species can be stored in 4°C in slant cultures for up-to 6 months time without loss of viability. All three species stored in -80°C also revived without any loss. However, we have noticed that cultures stored at 4°C take longer time to revive in the first sub-culturing.

Fig. 44 (A). *Mycobacterium* isolated from sheep intestine

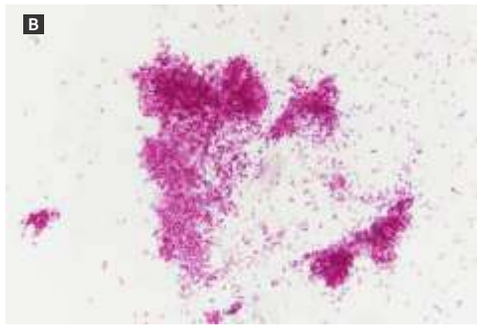


Fig. 44 (B). Acid-fast bacilli of *Mycobacterium* isolated from sheep intestine

(Shanmugasundaram K, Vaid RK and Bera BC)

Distribution of microbes from NCTVC

During the year 2021, multiple requests for cultures were received from government research institutions, universities and private institutes across the country and the following cultures were distributed for research and teaching purposes.

Distribution of bacterial cultures

The following bacterial cultures were distributed for research and teaching purposes.

Bacterial culture	Distributed to
Shigella flexneri, Salmonella enterica serovar Typhimurium	College of Veterinary Sciences, Palampur, Himachal Pradesh
Staphylococcus aureus, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Klebsiella pneumoniae, Proteus vulgaris, Bacilus subtilis, Clostridium perfringens, Enterobacter* spp, *Yersinia* spp, *Corynebacterium* spp, *Actinobacillus* spp, *Actinomyces* spp, *Fusobacterium* spp, *P. multocida, E. coli*	Faculty of Veterinary and Animal Sciences, Institute of Agricultural Sciences, BHU, Varanasi
Salmonella enterica, Salmonella Enteritidis, *Salmonella* Gallinarum, *Clostridium sporogenes, Clostridium perfringens, E. coli* O135; O108, *Shigella flexneri,*	Department of Microbiology Central University of Punjab, Bathinda
Salmonella Gallinarum Cultures (5 strains)	Nagpur Veterinary College, Nagpur
Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, S. aureus, and *Enterococcus* spp.	CMVL, Meerut
Escherichia coli, *Bacillus cereus*, *S. aureus* and *Salmonella enterica* cultures	CSWRI Avikanagar
Staphylococcus hyicus, *Brucella abortus* S99, *B. melitensis* Rev 1 cultures	ICAR-NE Complex Meghalaya

Furthermore, *Pasteurella multocida* culture was replaced by the Centre for Medical Biotechnology, MDU Rohtak. All in all nearly 75 cultures were distributed to researchers

Distribution of viruses and cell lines:

The following virus cultures were distributed for teaching and research purposes to different stakeholders.

Viral culture/Cell line	Distributed to
Bovine herpesvirus-1	Kamdhenu University, Gujarat
Newcastle disease virus and Porcine circovirus-2	M/s Reliance Life science, Mumbai
RK-13, MDBK and BHK-21 cells	Central Military Veterinary Laboratory (CMVL), Meerut
BHK-21, Vero and PK-15	LUVAS, Hisar
PK-15, Vero and BKH-21	RAJUVAS, Bikaner

(Vaid RK, Bera BC, Riyesh T, Shanmugasundaram K, Anand T, Kumar N, Barua S and Yash Pal)

03

Technology Development, Transfer and Commercialization

The Centre has made focused efforts for the development of advanced technologies in equine health and production. Also, suitable methodologies were designed to uplift the livelihoods of equine keepers and all stakeholders of equine sector. Many diagnostic kits, vaccines and biologicals developed by the scientists of ICAR-NRCE are being used in the field while several other technologies are either commercialized, transferred or in pipeline.

Technologies developed by ICAR-NRCE

Vaccines

- Inactivated Equine herpes virus – 1 vaccine (Equiherpabort)
- Updated Equine Influenza vaccine

Diagnostic kits

- Equiherpes B-ELISA kit for diagnosis of EHV – 1 infection
- Recombinant antigen-based ELISA kit for diagnosis of *Theileria equi*
- LFA for diagnosis of Equine Piroplasmosis
- LFA for diagnosis of equine Trypanosomosis
- Recombinant protein-based ELISA kit for diagnosis of Glanders
- Recombinant protein-based ELISA kit for diagnosis of EIA
- Recombinant gG-based type-specific ELISA for differentiation of EHV 1 and EHV 4 infection
- Monoclonal antibody-based ELISA kit for diagnosis of Rotavirus infection
- Japanese Encephalitis virus antibody test kit, iELISA for equids and pigs
- Monoclonal antibody-based ELISA kit for detection of equine influenza (H3N8) antigen

Reproduction and Dairy technologies

- Pregmare kit for pregnancy diagnosis in mares
- Collection and cryopreservation of equine semen
- Customised Artificial vagina for semen collection in horses
- Donkey milk based products (Bathing soap, Body butter and Lip balm)

Technology released in 2021

ICAR-NRCE has developed *"A Monoclonal antibody-based ELISA kit for detection of equine influenza (H3N8) antigen"* and the same was released by Hon'ble Union Minister of Agriculture and Farmers Welfare, Govt. of

India Sri NS Tomar ji during "Dedication of ICAR Technologies to Farmers and *Kritagya* Hackathon Award Ceremony" on May 31, 2021.

Release of 'ELISA kit for detection of equine influenza antigen' by Shri N S Tomar, Hon'ble Union minister, Ministry of Agriculture and Farmers Welfare

Technologies commercialized in 2021

Semen collection and cryopreservation in indigenous horses

Equine Production Campus (EPC), ICAR-NRCE has standardized the formulations and technology for collection and cryopreservation of indigenous stallion, exotic and indigenous jack semen. The population of indigenous equines is drastically declining at a rapid pace due to advancements in the mechanisation and hence necessary steps have to be taken to conserve this elite indigenous equine germplasm. In this endeavour, scientists at EPC have made efforts in standardizing the methodology of collection of the semen from the stallions, formulated various combinations of chemicals for successful cryopreservation of semen. These technologies are now made ready for transfer and distribution of this package of practices to the agripreneurs, stakeholders, equine owners and breeders. This year, this technology has been transferred to two equine farmers/ breeders and imparted training in stallion semen collection and cryopreservation.

Technology of semen collection and cryopreservation in horses developed at ICAR-NRCE

(Talluri TR, Yash Pal and Legha RA)

Customised Artificial Vagina for semen collection from stallions

The currently available Artificial Vagina (AV) for semen collection in stallions is being imported from other countries and they are expensive. These AVs are not equipped with temperature monitoring. The maintenance of optimum temperature in AV is very much critical for successful collection of semen from stallions. In this direction, to address these issues in the existing AV, scientists at EPC have designed, assembled and developed inexpensive hand-made AV from locally available, easily obtainable, and cheaper materials and is suitable for collecting the semen from indigenous stallions. In the current year, this technology has been transferred to two equine farmers/ breeders.

Prototype of customised Artificial Vagina for semen collection in horses

(Talluri TR, Yash Pal and Legha RA)

MoU with Brooke India, New Delhi

MoU on semen collection and cryopreservation signed with Mr Balender Kumar Goswami, Hanumangarh

Technologies Developed in 2021

Encapsulated phage formulation carrying *Salmonella* phages for therapeutic treatment in poultry

Salmonella sps are the most important pathogens of poultry. It causes foodborne illness in humans such as gastroenteritis when contaminated food is consumed. The ability of different types of *Salmonella* sps to infect birds and contaminate eggs makes it a potent infectious agent for humans. At NCVTC, we have developed a product containing selected bacteriophages combined in specific proportion in a cocktail and by encapsulating them in alginate beads to provide a suitable targeted oral delivery system for prevention of *Salmonella* infections in poultry. This improved encapsulation formulation could be used for reducing

intestinal colonization of *Salmonella* infections in poultry easily. It can be given orally to poultry birds. The phage product has shown positive effects against major infectious agents (*S.* Typhimurium, *S.* Paratyphi and *S.* Gallinarum) in *in-vitro* studies. When used against targeted pathogens *in-vivo* experiments, the product has shown beneficial effects in prevention of *Salmonella* Typhimurium and *S.* Paratyphi in poultry birds and also the preparation has worked well by spray applications on meat products. The encapsulated phage formulation will also be beneficial in prevention of *S.* Gallinarum infections in poultry as active phages against this pathogen have been included to prevent direct losses to poultry industry simultaneously addressing the issue of public health by prevention of *S.* Typhimurium and *S.* Paratyphi.

(Anand T, Barnele M, Virmani N, Bera BC, Vaid RK, Vashisth M and Tripathi BN)

A novel lumpy skin disease virus (LSDV) with potential to serve as a vaccine candidate against LSD

We isolated LSDV from outbreak(s) from cattle in Ranchi (India) from the scabs (skin lesions) in the primary goat kidney cells. The phylogenetic analysis suggested that the isolated virus (LSDV/India/2019/Ranchi, referred to as P0 virus hereafter) is closely related to Kenyan LSDV strains. Later, the virus was adapted to Vero cells. The time taken to produce CPE progressively decreased on successive passages (P) in Vero cells. At P50, the virus grew at a titer of ~10^7 $TCID_{50}$/ml. On complete genome sequence analysis, it was observed that as compared to P0, 50-times passaged LSDV (referred to as LSDV/P50 hereafter) virus has significant modifications in terms of deletion/fragmentation in at least 3 virulence associated genes viz; Ankyrin repeat domain-containing protein, Kelch repeat protein and Envelope phospholipase F13. Therefore, the original LSDV (P0) which we modified by continuous cell culture passage, namely LSDV/P50, is potentially attenuated and may serve as a vaccine candidate.

(Naveen Kumar, Barua S and Tripathi BN)

A novel vaccine formulation to prevent SARS-CoV-2 infection in animals

Besides humans, SARS-CoV-2 has been shown to infect cat, dog, lions and minks which also includes mortality in lions and minks. Jumping of SARS-CoV-2 from humans to animals might accelerate its evolution and hence affecting surveillance and control strategies of COVID-19 in humans. Therefore, implementing effective risk management measures to prevent the transmission of SARS-CoV-2 between humans and susceptible animals is a major task of veterinary services. Whereas extensive research efforts have been made for human vaccine for COVID-19, very less attention has been paid to develop COVID-19 vaccines for animals. In India, SARS-CoV-2 (Delta variant) infection has recently been reported in lions; other pet animals (dog, cat) are also at high risk due to their close contact with human population. This invention includes isolation of the Delta variant of SARS-CoV-2 from COVID-19 confirmed patients and its subsequent adaptation (modification) into the cell culture system by continuous passage (P) in Vero cells. As compared to the original virus (P0; SARS-CoV-2 Delta) which produced low vial titer (~10^4 pfu/ml), modified virus (P10) produced significantly higher viral titers (~10^7pfu/ml) and therefore it was used to develop an inactivated vaccine, termed as Ancovax. A single dose of the vaccine (Ancovax) induced a potent antibody response against Delta SARS-CoV-2. At 21 days-post-infection, in rabbits (n=3), the titers ranged from 32 to 128 whereas in mice (n=3) it ranged from 64 to 512. Following booster dose, the antibody titer in the rabbits were increased up to 1024. In dogs (n=8), at 21 days post-vaccination, the antibody titer induced by Ancovax were in the range of 16-128. These titres increased (range varied from 32-256) upon booster dose which was given at 21 days post-primary vaccination.

(Naveen Kumar, Barua S, Tripathi BN, Gulati BR and Yash Pal)

Patents filed in 2021

S. No.	Title of patent	Authors	Application No. & date
1.	Modified vaccine construct for EHV 1 and methods of preparing the same	Virmani et al.	Application No 202111000312, dated 05.01.2021
2.	Monoclonal antibody based immunoassay for detection of equine influenza (H3N8) antigen	Virmani et al.	Application No 202111004847, dated 04.02.2021
3.	Mutated EHV-1 (TOH Strain) genome based vaccine construct and method for preparation	Bera et al.	Application No.202111057300, dated 09.12.2021
4.	Recombinant nucleocapsid protein based indirect ELISA kit for detection of anti SARS-COV-2 antibodies in canines	Virmani et al.	Application No.202111057358, dated 09.12.2021
5.	Hydroxychloroquine/chloroquine zinc oxide nanoparticle formulation.	Manuja et al.	Application No.202111057698, dated 11.12.2021

04

Education and Trainings

Training on "Entrepreneurship Development in Equine Husbandry"

A three days training programme titled "Entrepreneurship Development in Equines" under the theme "Genetic Improvement of Indigenous Horses" was organized from February 25th to 27th, 2021 for the field veterinarians at Equine Production Campus, Bikaner, Rajasthan. During this training program, 6 practical sessions and 7 expert lectures and various interactive sessions have been organised. Participants were appraised of various methods in cryopreservation of equine semen, artificial insemination in mares and pregnancy diagnosis. For this training programme 14 veterinarians from different states like Gujarat, Uttar Pradesh, Haryana and Rajasthan have attended.

Inauguration of training on "Entrepreneurship Development in Equine Husbandry"

Training on "Laboratory Diagnosis of Avian Influenza and Brucellosis"

A two day workshop cum hands-on training program on "Laboratory Diagnosis of Avian Influenza and Brucellosis" was organised at ICAR-NRCE, Hisar from March 8th to 9th, 2021. In this training program, 57 trainees (22 Veterinary and 35 Medical professionals) from various districts of Haryana were trained on different laboratory diagnostic methods.

Release of training manual during the inaugural session

Distribution of certificates to participants

Training on "Prevention, Control and Eradication of Equine Glanders"

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 2021, three training programmes in three batches (Batch 1: October 21-23, 2021; Batch 2: October 28-30, 2021; Batch 3: November 10-12, 2021) were organized at ICAR-NRCE, Hisar on "Prevention, Control and Eradication of Equine Glanders". It was conducted in collaboration with Haryana Veterinary Training Institute (HVTI), Department of Animal Husbandry, Govt. of Haryana. A total of 57 Veterinary Officers from 22 districts of Haryana participated in the training programs.

A series of lectures on glanders in the area of epidemiology, disease transmission, surveillance, management of glanders outbreak and practical demonstration on sample collection, and laboratory diagnosis (CFT and ELISA) was the major focus of the training programs. Dr B. R. Kamboj, Vice-Chancellor of CCS HAU and GJU S&T, Hisar, Dr Praveen Malik, Animal Husbandry Commissioner, Department of Animal Husbandry & Dairying, Govt. of India and Dr Jasbir Singh, Principal, (HVTI), Hisar were participated as chief guests in these training programmes. They have appreciated the works of ICAR-NRCE on glanders surveillance, development of ELISA test and diagnostic services rendered for glanders surveillance in India. Dr Harisankar Singha, Senior Scientist and Dr Shanmugasundaram K, Scientist organized these three training programmes under the chairmanship of Dr Yash Pal, Director, ICAR-NRCE.

Participants of Training on "Prevention, Control and Eradication of Equine Glanders (Batch – 2)

Participants of Training program on "Prevention, Control and Eradication of Equine Glanders (Batch – 3)

The comprehensive list of various trainings organized by ICAR-NRCE in 2021 is given below in chronological order.

S. No.	Name of the training	Period	No. of participants
1.	Training on "Entrepreneurship Development in Equine Husbandry" for Veterinary Officers	25 - 27 February, 2021	14
2.	Training on "Laboratory diagnosis of Avian Influenza and Brucellosis"	8 - 9 March, 2021	57
3.	Training Camp under SC/SP scheme organised at 1 NZM, Sri Ganganagar, Rajasthan	21 March, 2021	25
4.	Training for job role of Animal Health Workers under ASCI (Agriculture Skill Council of India)	25 March to 19 April and 22 July to 7 August, 2021	20
5.	One month training to M Tech student (Mr. Himanshu Kamboj from Amity University, Noida) on cell culture by Dr Naveen Kumar	1 - 30 April, 2021	1
6.	Equine Health Camp under SC/SP scheme at Village Rohina and Degana district Nagore	27 June, 2021	14
7.	Training Camp under SC/SP scheme organised at 6 JKM, Sri Ganganagar, Rajasthan	17 July, 2021	25
8.	Hands-on training to Mr Pankaj Baloda, M Sc (Biotechnology) from Chandigarh University, Chandigarh on Bio nanotechnology by Dr Anju Manuja	6 August - 23 October, 2021	1
9.	Training Camp under SC/SP scheme organised at 9 AS and 10 AS, Sri Ganganagar, Rajasthan	20 August, 2021	26
10.	Training Camp under SC/SP scheme organised at Malerkheda, Hanumangarh, Rajasthan	20 August, 2021	11
11.	Equine Health Camp under SC/SP Scheme at Peerkamdia, Hanumangarh, Rajasthan	21 August, 2021	20
12.	Training on characterization and conservation of Bhimthadi horse	8 - 10 September, 2021	27
13.	Training on "Upgradation of knowledge and skill of Scheduled Caste Livestock Farmers" in collaboration with Directorate of Extension Education, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar at village Bhodia Khera, Fatehabad, Haryana	10 - 14 September, 2021	35
14.	Training on "Upgradation of knowledge and skill of Scheduled Caste Livestock Farmers" in collaboration with Directorate of Extension Education, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar at village Ghespur, Yamunagar, Haryana	18 - 22 September, 2021	35
15.	Training on "Upgradation of knowledge and skill of Scheduled Caste Livestock Farmers" in collaboration with Directorate of Extension Education, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar at village Hamzapur, Fatehabad, Haryana	22 - 26 September, 2021	35
16.	Training on "Upgradation of knowledge and skill of Scheduled Caste Livestock Farmers" in collaboration with Directorate of Extension Education, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar at village Potli, Yamunagar, Haryana	24 - 29 September, 2021	35
17.	Training on "Prevention, Control and Eradication of Equine Glanders" (Batch – 1)	21 - 23 October, 2021	17

18.	Training on "Prevention, Control and Eradication of Equine Glanders" (Batch – 2)	28 - 30 October, 2022	21
19.	Training on "Prevention, Control and Eradication of Equine Glanders" (Batch – 3)	10 - 12 November, 2021	19
20.	Hands-on training on Application of Molecular Markers for Improvement of Livestock Production	6 - 7 December, 2021	9

Valedictory program of Training for job role of Animal health workers under Agriculture Skill Council (ASCI) of India

Distribution of training kits to trainees of SCSP training organised in collaboration with LUVAS, Hisar

Director, ICAR-NRCE along with scientific staff distributing the medicines and mineral mixture to the beneficiaries at health camps organised under SC/SP scheme

Expert/ invited lectures NRCE scientists in training programmes

- **Taruna Anand** delivered an invited talk on "Bacteriophages as a potential life-saving alternative in the post-antibiotic era" in international webinar on 'Alternative Therapies to Mitigate Microbial Resistance' held during February 23-24, 2021.
- **Taruna Anand** delivered an oral presentation on "Isolation and characterization of bacteriophage against *Salmonella* from poultry litter" in the 2nd International conference on 'Bacteriophage research' held during July 22-24, 2021.
- **Anuradha Bhardwaj** delivered a lecture on "Gender equality and women issues" on 'Women equality day 2021 at ICAR-NRCE, Hisar on August 26, 2021.
- **BR Gulati** delivered an expert lecture on "Diagnosis and Control of equine viral diseases" during training programme for Field Veterinary Officers' on 'Prevention, Control and Eradication of Equine Glanders' organized by ICAR-NRCE, Hisar from October 21- 23, 2021.
- **BR Gulati** delivered an expert talk on "Risk Assessment & Biosafety in Research Laboratories" in RUSA sponsored 2-week online subject refresher course on Life sciences organized by UGC-HRD Centre, Guru Jambeshwar University of Science & Technology, Hisar on September 21, 2021.
- **BR Gulati** presented an expert lecture on "Role of animals in the origin and transmission of SARS-CoV2" in the World Veterinary Day Function on 'Veterinarian's response to the COVID-19 crisis' organized by Faculty of Veterinary Sciences and Animal Husbandry, SKUAST, Alusteng, Srinagar on April 23, 2021.
- **BR Gulati** presented an expert lecture on "Role of animals in the origin and transmission of SARS-CoV2" in the World Veterinary Day Function on 'Veterinarian's response to the COVID-19 crisis' organized by Institutional Development Plan Cell, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana on April 24, 2021.
- **BR Gulati** presented an invited lecture on "Role of animals in the origin and transmission of SARS-CoV2" on World Veterinary Day function at ICAR-NRCE, Hisar on April 24, 2021.
- **Naveen Kumar** delivered an invited lecture on "Cellular kinases as target for antiviral therapy" during a training program on 'Biotechnological Tools in Animal Health and Production' organized by Department of Animal Biotechnology, LUVAS, Hisar from September 9 - 29, 2021.
- **Naveen Kumar** delivered an invited lecture on "Contribution of Veterinary Research Institutes in COVID-19 related research and challenges faced by ICAR-NRCE" during National symposium on 'Veterinary response to the COVID-19 crisis' organized by Lala Lajpat Rai University of Veterinary and Animal sciences, Hisar on April 24, 2021.
- **Naveen Kumar** delivered an invited lecture on "Detection and management of viral coinfections" during workshop on 'Skill development in laboratory diagnosis of livestock diseases through advanced tools and techniques' organised by Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana from December 13 - 17, 2021.
- **Naveen Kumar** delivered an invited lecture on "Detection and management of viral coinfections" in a training program on 'Skill development in animal viral disease diagnosis and prophylaxis' conducted under DBT at GADVASU-Canine Research Centre and Networks from January 5 - 11, 2021.
- **Naveen Kumar** delivered an invited lecture on "Targeting cellular kinases for antiviral drug development" in First National Conference on 'Biotechnology – A link to research on the prevention and control of diseases of public health importance' organized by GLA University, Mathura on February 25, 2021.
- **Naveen Kumar** delivered an invited lecture on "The COVID-19 Pandemic: Scientific and Social Introspection" at Guru Jhambheswar University of Science and Technology, Hisar, Haryana on March 25, 2021.
- **Naveen Kumar** delivered an invited lecture on "Viral coinfections" in a training program on 'Recent techniques in nucleic acid-based diagnostics and cell culture' organised by Department of Animal Biotechnology, LUVAS, Hisar from January 11 - 31, 2021.
- **Anju Manuja** delivered an invited lecture on "Nanotechnology approaches for drug delivery against Trypanosomiasis" in the international online conference on 'Macromolecules: Synthesis, morphology, processing, structure, properties and applications' organized by Mahatma Gandhi University, Kottayam,

Kerala & Gdansk University of Technology, Gdansk, Poland & Wroclaw University of Technology, Wroclaw, Poland from September 10 - 11, 2021.

- **SC Mehta** delivered a lecture on "Tools and techniques for molecular characterization" under 'National Higher Education Project' organized by SKRAU, Bikaner on October 23, 2021.
- **SC Mehta** delivered a lecture titled "Entrepreneurship development in Equines" organized to commemorate the 75 years of Indian Independence *'Azadi ka Amrut Mahotsav'* by ICAR-NRCE, Hisar on October 18, 2021.
- **SC Mehta** delivered an expert talk on the topic "Scientific breeding and management of horses" in 'e-Pashupalak chopal' organised by RAJUVAS, Bikaner on May 26, 2021.
- **K Shanmugasundaram** delivered an expert lecture on "Diagnosis of glanders: serological and molecular methods" during training programme for Field Veterinary Officers' on 'Prevention, Control and Eradication of Equine Glanders' organized by ICAR-NRCE, Hisar from October 21 - 23, 2021.
- **K Shanmugasundaram** delivered an expert lecture on "Diagnosis of glanders: serological and molecular methods" during training programme for Field Veterinary Officers' on 'Prevention, Control and Eradication of Equine Glanders' organized by ICAR-NRCE, Hisar from October 28 - 30, 2021.
- **K Shanmugasundaram** delivered an expert lecture on "Diagnosis of glanders: serological and molecular methods" during training programme for Field Veterinary Officers' on 'Prevention, Control and Eradication of Equine Glanders' organized by ICAR-NRCE, Hisar from November 10 - 12, 2021.
- **K Shanmugasundaram** delivered an expert lecture on "Sample collection, processing and dispatch for glanders diagnosis" during training programme for Field Veterinary Officers' on 'Prevention, Control and Eradication of Equine Glanders' organized by ICAR-NRCE, Hisar from October 21 - 23, 2021.
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- **HS Singha** delivered a lecture on "Containment of Glanders: Guidelines for field functionaries" on 'World Zoonosis day 2021' organized by LUVAS, Hisar on July 6, 2021.
- **HS Singha** delivered a lecture on "National Action Plan on Glanders 2019: Genesis, Progress and Issues" during a workshop organized by Directorate of Animal Husbandry, Govt. of Uttar Pradesh at Lucknow on January 29, 2021.
- **HS Singha** delivered an expert lecture on "Glanders surveillance status and National Action plan" during training programme for Field Veterinary Officers' on 'Prevention, Control and Eradication of Equine Glanders' organized by ICAR-NRCE, Hisar from October 21 - 23, 2021.
- **HS Singha** delivered an expert lecture on "Glanders surveillance status and National Action plan" during training programme for Field Veterinary Officers' on 'Prevention, Control and Eradication of Equine Glanders' organized by ICAR-NRCE, Hisar from October 28 - 30, 2021.
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- **TR Talluri** delivered an expert lecture on "Basics in Equine Reproduction" organized to commemorate the 75 years of Indian Independence *'Azadi ka Amrut Mahotsav'* by ICAR-NRCE, Hisar on December 9, 2021.
- **TR Talluri** delivered an expert lecture on "Estrus detection and artificial insemination in Equines" at the Department of Veterinary Gynecology and Obstetrics, College of Veterinary Sciences, GADVASU, Ludhiana on March 24, 2021.

Participation of Scientists and Staff in Online training programmes

ICAR-National Research Centre on Equines encourages its staff for capacity building in advanced areas of science, administration and skill development. In the year 2021, the staff of ICAR-NRCE obtained following trainings.

Name of the staff	Designation	Title of the training	Duration of the training	Organized institute
Dr Anju Manuja	Principal Scientist	3rd virtual Australian Bioprinting Workshop for Tissue Engineering and Regenerative Medicine	October 4 - 5, 2021	Australasian Society for 3D Bioprinting (AS3B)
Dr Balvinder Kumar	Principal Scientist	Training Programme on RTI	July 15 - 16, 2021	ICAR-National Academy of Agricultural Research Management (NAARM), Hyderabad
Dr Balvinder Kumar	Principal Scientist	MDP on Priority Setting Monitoring and Evaluation (PME) of Agricultural Research Projects	October 25 - 30, 2021	ICAR-National Academy of Agricultural Research Management (NAARM), Hyderabad
Dr RK Vaid	Principal Scientist	Training on Baclink component of the WHONET 5.6 software	February 23, 2021	FAO-ICAR INFAAR Program
Dr SC Mehta	Principal Scientist	Training on Concept and process of formulating standards	January 7 - 8, 2021	Bureau of Indian Standards (BIS), New Delhi
Dr TR Talluri	Senior Scientist	National training programme on Statistical tools in Research and data analysis	August 9 - 14, 2021	Department of Animal Genetics and Breeding, CVAS, Parbhani, Maharashtra
Dr TR Talluri	Senior Scientist	2nd International Flow Cytometry course	September 1 - 4, 2021	Trust for Education and Training in Cytometry (TETC), Jaipur
Dr Taruna Anand	Senior Scientist	International Virtual Workshop Series on Regulatory approaches for Animal Biotechnology on regulatory approaches for genome edited animals	September 23 - 24, 2021	Diane Wray – Cahen, Senior advisor for Animal Health, production and animal products
Dr Yash Pal	Director ICAR-NRCE	Training on "RTI Act, 2005"	July 15 - 16, 2021	ICAR-NAARM, Hyderabad
Ms Ana Raj J	Scientist	Training on ICT and mass media in Agricultural Extension	May 4 - 8, 2021	Collaborative training with MANAGE, Hyderabad and Birsa Agricultural University, Ranchi
Ms Ana Raj J	Scientist	Training on Video production and dissemination skills for Agricultural extension functionaries	June 21 - 24, 2021	National Institute of Agricultural Extension Management (MANAGE), Hyderabad
Sh Om Parkash	Assistant Account Section	Training on Accrual Accounting	July 26 - 30, 2021	ICAR-National Rice Research Institute, Cuttack, Odisha

Post-Graduate Students' Research and Guidance

Sr. No.	Student Name and Organisation	Name of the guide/ co-guide	Title of the thesis	Completed/ Ongoing
Ph D students				
1.	**Aashwina Madhwal** ICAR –IVRI, Izatnagar, UP	Dr Nitin Virmani	Development of modified live EHV1 vectored bivalent vaccine candidate against Equine influenza virus & Equine herpes virus 1: Protective efficacy studies in mouse challenge model	Ongoing
2.	**Ajmer Singh** Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan	Dr Yash Pal	A sociological study of socio-economic aspects of equine farmers in Haryana (India)	Ongoing
3.	**Alka Nokhwal** CCS HAU, Hisar, Haryana	Dr RK Vaid	Isolation and characterization of bacteriophages against aeromonads from fish culture ponds	Ongoing
4.	**Anubala Jaglan** CCS HAU, Hisar, Haryana	Dr Taruna Anand	Exploring bacteriophage derived endolysins against the biofilm forming bacteria	Ongoing
5.	**Assim Verma** GJU S&T, Hisar, Haryana	Dr Sanjay Barua	Study on the antiviral efficacy of Hesperetin against Buffalo Pox Virus	Ongoing
6.	**Dinesh Jhamb** RAJUVAS, Bikaner, Rajasthan	Dr TR Talluri	Effect of L-arginine and trehalose supplementation to semen extender on quality and fertility of cryopreserved stallion semen	Completed
7.	**Indu** CCS HAU, Hisar, Haryana	Dr K Shanmugasundaram	Targeted genome editing using CRISPR-Cas9 approach to decipher the functional role of predicted genes in survival of *Mycobacterium kansasii*	Ongoing
8.	**Karnam Sangwan** Amity University, Jaipur, Rajasthan	Dr Yash Pal	Preparation and optimization of watermelon rind powder based double emulsion and its incorporation in donkey milk derived dairy product	Ongoing
9.	**Medhavi Vashisth** CCS HAU, Hisar, Haryana	Dr Taruna Anand	Characterization of bacteriophages against ESKAPE pathogens and assessment of their synergy with antibiotics	Ongoing
10.	**Priya** CCS HAU, Hisar, Haryana	Dr RK Vaid	Avifaunal diversity assessment and fecal matter screening for bacterial components from selected locations	Completed
11.	**Renu Garhwal** Amity University, Jaipur, Rajasthan	Dr Anuradha Bhardwaj	Characterization of physicochemical qualities of donkey milk and its utilization in value added dairy products	Ongoing
12.	**Snehil Gupta** LUVAS, Hisar, Haryana	Dr Rajender Kumar	Screening, identification and evaluation of some novel target specific therapeutic compounds against *Tryanosoma evansi*	Ongoing

13.	**Sonali Soni** Maharaja Agrasen University (MAU), Solan, Himachal Pradesh	Dr Anuradha Bhardwaj	Comparative genomic studies on horse and donkey performance traits genes	Ongoing
14.	**Stephanie S Pradhan** ICAR –IVRI, Izatnagar, (Uttar Pradesh)	Dr Nitin Virmani	Comparative pathogenicity and immunogenicity of modified live EHV–1 vaccine candidate (s) in mouse model and development of gE protein based ELISA for differentiation of vaccinated and infected animals	Completed
15.	**VK Pal** COVS & AH, Kumarganj, Ayodhya, (Uttar Pradesh)	Dr Rajender Kumar	Molecular characterization and sero-prevalence studies on equine haemoprotozoan diseases with special reference to *Trypanosoma evansi*	Completed
16.	**Yogesh Chander** GJU S&T, Hisar, Haryana	Dr Sanjay Barua	Role of p38 in Buffalopox virus replication	Ongoing
Sr. No.	**Student Name and Organisation**	**Name of the guide/ Co-guide**	**Title of the thesis**	**Completed/ Ongoing**
MVSc/ MSc students				
1.	**Ankush** GJU S&T, Hisar, Haryana	Dr RK Vaid	Molecular identification and antimicrobial sensitivity profiling of *Staphylococcus* spp. isolates originating from different animal species	Completed
2.	**Diksha Sharma** IVRI, Izatnagar, (Uttar Pradesh)	Dr Rajender Kumar	Epidemiological studies of *Trypanosoma* evansi in cattle, buffaloes and equines of Himachal Pradesh and characterization of immunodominant antigens	Completed
3.	**Lokender Singh** LUVAS, Hisar, Haryana	Dr Naveen Kumar	m6A modification of SARS-CoV-2 RNA: Exploring the epitranscriptomic regulation of proinflammatory cytokine production	Ongoing
4.	**Palak Gupta** RAJUVAS, Bikaner, Rajasthan	Dr RA Legha	Effect of dietary supplementation of Linseed oil (*Linum usitatissimum*) on nutrient utilization and semen quality of Marwari Horses	Ongoing
5.	**Prapti Parkhe** HPKVV, Palampur, Himachal Pradesh	Dr RK Vaid	Molecular characterization, antibiogram and plasmid DNA isolation of the *Pasteurella multocida* B:2 isolates.	Completed
6.	**Priyanka Karela** RAJUVAS, Bikaner, Rajasthan	Dr RK Dedar	Effect of various desert herbs on expression of TGF-β in skin fibroblasts of horse	Ongoing
7.	**Rajendra Mehra** RAJUVAS, Bikaner, Rajasthan	Dr TR Talluri	Effect of addition of lyophilized heterologous seminal plasma and colostrum to semen extender on cooled and post thaw stallion semen quality	Completed
8.	**Sakshi Pandita** LUVAS, Hisar, Haryana	Dr Naveen Kumar	Studies on the miRNA response to LSD virus infection	Ongoing

9.	**Supriya** ICAR –IVRI, Izatnagar, UP	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 modified live vaccine candidate	Ongoing
10.	**Suvidhi** RAJUVAS, Bikaner, Rajasthan	Dr RK Dedar	Effect of diosmin and hesperidin supplementation on haematological and serum biochemical profile in Donkey (*Equus asinus*)	Completed
11.	**Tipu Sultan** RAJUVAS, Bikaner, Rajasthan	Dr TR Talluri	Effect of zinc and gold nanoparticles on cooled and post thaw quality of stallion semen	Completed
12.	**Upender, (VPH)** ICAR –IVRI, Izatnagar, (Uttar Pradesh)	Dr RK Vaid	Comparative prevalence of ESBL *Escherichia coli* in poultry farms and retail poultry meat in Hisar and lytic activity of phages against the ESBL *E. coli* isolates	Ongoing
13.	**Vijay Lakshmi** GJU S&T, Hisar, Haryana	Dr K Shanmugasundaram	*Rhodococcus equi* and the threat of *R. equi* infection in foals and other animals	Completed
Post-doctoral Research fellow				
1.	**Dr Ruma Rani** Sponsored by CSIR, New Delhi	Dr Rajender Kumar	Identification and evaluation of target specific novel drug molecules against Trypanosoma evansi infection using nanotechnology approach	Ongoing

MoU for co-operation in Research and Education

ICAR-NRCE, Hisar inked Memorandum of Understanding with the following institutes/ organizations/ equine breeders in 2021 for co-operation in the areas of research, education, extension, consultancy, capacity building and other areas of national interest.

Sr. No.	Institute/ Organization/ Equine breeders/ stakeholders	MoU date
1.	The Brooke India, New Delhi	16 March, 2021
2.	Chaudhary Bansi Lal University (CBLU), Bhiwani, Haryana	27 October, 2021
3.	Mr Ranjit Dilip Kher, Aundh, Pune	24 November, 2021
4.	Dr Balender Kumar, Hanumangarh, Rajasthan	24 December, 2021

MoU signed with Mr Ranjit Dilip Kher, Aundh, Pune

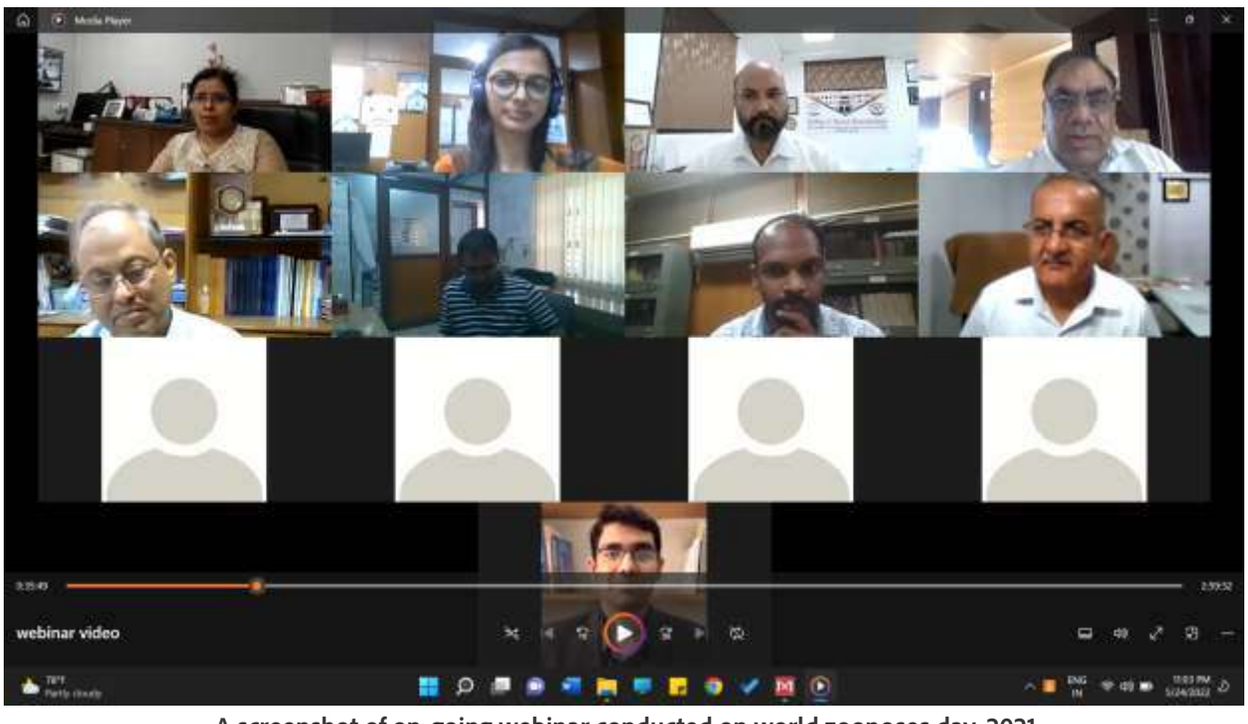
MoU with Chaudhary Bansi Lal University (CBLU), Bhiwani

05

Institutional Activities

International Webinar on "Preventing future zoonotic pandemics: Interventions at the wildlife–livestock–human interface"

On the occasion of World Zoonoses Day 2021 (July 6th, 2021) a webinar entitled "Preventing future zoonotic pandemics: Interventions at the wildlife-livestock-human interface" was organized by ICAR-NRCE in collaboration with Indian Virological Society and National Center for Disease Control. Dr BN Tripathi, DDG (AS) was the chief patron of the event whereas eminent microbiologists *viz.,* Dr RK Singh (President, IVS), Dr Sujeet Kumar Singh (Director, National Center for Disease Control) and Dr Yash Pal (Director, ICAR-NRCE, Hisar) attended the event. Dr Hinh Ly from the University of Minnesota, USA; Dr Pragya Dhruv Yadav from NIV, Pune; Dr HV Murugkar from NIHSAD, Bhopal and Dr Abdul Rahman Omar from Universiti Putra Malaysia, Serdang, Malaysia were the guest speakers for the webinar. A total of 308 attendees registered for this event. Dr Baldev R Gulati was the chairman while Dr Naveen Kumar co-chaired the session. Dr Riyesh T and Dr Anubha Pathak were the organizing secretaries of the event and Dr Harisankar Singha and Dr Shanmugasundaram K acted as co-organizers for the virtual webinar.



A screenshot of on-going webinar conducted on world zoonoses day, 2021

Azadi ka Amrit Mahotsav Webinar Series

Webinar on "Balancing your diet – Need of the hour"

A webinar titled "Balancing your diet – Need of the hour" was organized on August 12th, 2021, to stress the importance of nutritional security. Dr Sangeeta C. Sindhu, HoD, Food & Nutrition, CCS HAU, Hisar delivered the guest lecture after keynote address by Dr A Sahoo, Director, ICAR-NRCC, Bikaner. Dr Yash Pal, Director, chaired the session, which was attended by 40 participants.

Webinar on "Entrepreneurship development in Equines"

A webinar on "Entrepreneurship development in equines" was organized on October 18th, 2021. The lecture was delivered by Dr. S.C. Mehta, Principal Scientist, ICAR – NRCE which was attended by 48 participants including scientists, farmers and veterinarians. The session was chaired by Dr Yash Pal, Director, ICAR-NRCE.

Webinar on "Import and export policy for animals in India with special reference to equines"

A webinar lecture was organized on Oct 26th, 2021, on "Import and export policy for animals in India with special reference to equines". The lecture was delivered by Dr. Praveen Malik, Animal Husbandry Commissioner, Department of Animal husbandry and Dairying, GoI, New Delhi, which was attended by 42 participants including scientists, NGOs and veterinarians. The session was chaired by Dr Yash Pal, Director, ICAR-NRCE.

Webinar on "Basics in equine reproduction"

A webinar on "Basics in equine reproduction" was organized on December 9th, 2021. Dr T R Talluri, Senior Scientist at ICAR-NRCE, Hisar, delivered the lecture. Apart from the staff and students of ICAR-NRCE, equine keepers also attended the lecture to gain knowledge on equine reproduction issues. The session was chaired by Dr Yash Pal, Director, ICAR-NRCE.

Antimicrobial Resistance (AMR) Awareness Programme

ICAR-NRCE organized an awareness programme on "Emergence of scourge of antimicrobial resistance in bacteria" at Govt. School, Behbalpur for 10-12th class students on January 4th, 2021. The facility of smart class was used for the presentations, and showing short movies on AMR problems, depicting real life situations in patients and points on prevention and control of AMR.

Antimicrobial Resistance (AMR) awareness programme at Behbalpur

Covid-19 vaccination of frontline warriors at ICAR-NRCE

ICAR-NRCE was one of the first research centers to start COVID-19 testing among ICAR institutes in India. Covid-19 vaccination program for frontline warriors at ICAR-NRCE was organized on January 19th, 2021. All frontline warriors were given the first dose of the COVISHIELD® vaccine. Scientific, technical and administrative staff of research actively participated in this vaccination program. This vaccination was done by the employees of the Health Department, Government of Haryana under the supervision of Dr. Yash Pal, Director, ICAR-NRCE and Dr. BR Gulati, Principal Scientist, ICAR-NRCE.

Scientist and staff being vaccinated at ICAR-NRCE

Republic Day celebrations at Centre

ICAR-NRCE and EPC Bikaner celebrated Republic Day on January 26th, 2021, with great enthusiasm. 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. To mark the day, children of the employees took pride in glorifying and celebrating the spirit of unity.

Republic Day flag hoisting at ICAR-NRCE, Hisar and EPC, Bikaner

World Water Day celebrated at NRCE

On March 22nd, 2021, an essay writing competition on "Innovative ideas for water conservation" was conducted at the ICAR-NRCE auditorium to celebrate world water day. The participants were gifted a water-saving sprinkler faucet to promote water conservation.

Van mahostav campaign at EPC, Bikaner

As a part of 75 years of independence celebrations of India (*Azadi ka Amrut Mahotsav*) nation-wide campaign on tree plantation and awareness was conducted at EPC, ICAR-NRCE, Bikaner on the eve of ICAR's Foundation Day, July 16th, 2021, with a motto of "*Har Med Par Ped*". On this occasion, 51 trees of Gulmohar, Bel Patra, Pili Kaner, Peepal etc. were planted at the campus.

Equine health camp

An equine health camp was organized at Sadalpur village, Hisar on August 4th, 2021. In the health camp, horses were examined by a multidisciplinary team of scientists and technical officers, the bio-samples were

also collected from the animals for different epidemiological studies at institute laboratories. Based on examination, prescriptions were given to the equine owners and the details of their equines were registered.

Scientist and staff of NRCE Interacting with equine owners at Sadalpur equine health camp

Independence day celebrations

ICAR-NRCE celebrated Independence Day on August 15th, 2021, 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 the 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 endeavor. The institute auditorium reverberated with patriotic fervor and enthusiasm. The children brought the stage alive with their passion and love for the motherland.

Poshan Vatika and *Vriksharopan* programme

EPC Bikaner has organised *Poshan Vatika* and *Vriksharopan* at the campus on September 17th, 2021, by planting 500 trees of *Neem* and *Shisham*. This programme was organized in reference to the declaration of the year 2023 as the International Year of Millets by the United Nations.

हिन्दी सप्ताह

भारतीय कृषि अनुसंधान परिषद के राष्ट्रीय अश्व अनुसंधान केन्द्र में 15.01.2021 से 21.09.2021 तक हिन्दी सप्ताह का अयोजन किया गया। हिन्दी सप्ताह का आरम्भ हिन्दी कार्यशाला से हुआ जिसमें श्रीमती पूजा, सहायक प्रध्यापिका, गवर्नमेंट कॉलेज, आदमपुर थी। मुख्य वक्ता ने हिन्दी राजभाषा: स्थिति एवं भविष्य पर व्याख्यान प्रस्तुत किया। सप्ताह भर में कुल 7 प्रतियोगिताएं आयोजित की गई। इनमें हिन्दी प्रश्नोत्तरी, हिन्दी मुहावरे लेखन, हिन्दी नारा लेखन, हिन्दी टंकण, आशुभाषण प्रतियोगिताएं शामिल थी। अंतिम दिन समापन समारोह के साथ कविता पाठ आयोजित किया गया। समापन समारोह में मुख्य अतिथि के रूप में चौधरी चरण सिंह हरियाणा कृषि विश्वविद्यालय (एचएयू) के कुलपति डॉ. बी.आर. कंबोज उपस्थित थे। मुख्य अतिथि ने हिन्दी राजभाषा के महत्व पर व्याख्ययान प्रस्तुत कर संस्थान के वैज्ञानिक, अधिकारी, कर्मचारी एवं छात्र–छात्राओं को संबोधित किया तथा सप्ताह पर आयोजित की गई प्रतियोगिताओं के प्रतिभागियों को पुरस्कार विरित भी किए। समापन समारोह में प्रतिष्ठित साहित्यिक संस्थान चंदन साहित्य मंच के अध्यक्ष महेन्द्र जैन जीएवं सदस्य श्रीमती ऋतु कौशिक, श्री दीपक प्रताप, डॉ. मीरा सिवाच, श्रीमती रिम्पी

अश्व अनुसंधान केन्द्र में हिन्दी सप्ताह का आयोजन

अंकुर एवं श्री नीरज कुमार मनचंदा भी सम्मिलित हुए। सभी कवि गणों ने अपनी प्रस्तुतियां दी तथा अश्व अनुसंधान केन्द्र के प्रतिभागियों की प्रस्तुतियां में से विजेताओं का चयन भी किया। हिन्दी सप्ताह, केन्द्र के निदेशक एवं हिन्दी कार्यान्वयन समिति के अध्यक्ष डॉ. यशपाल एवं समिति के उपाध्यक्ष डॉ. गुलाटी की देखरेख में सम्पन्न हुआ।

वर्ष 2021 के दौरान राष्ट्रीय अश्व अनुसंधान केन्द्र, अश्व उत्पादन परिसर, बीकानेर में ''सितम्बर हिन्दी मास'' के उपलक्ष्य में हिन्दी व्याख्यान का आयोजन किया गया। इसके अंतर्गत दिनांक 03.09.2021 को मुख्य हिन्दी वक्ता डॉ. डी.डी. ओझा, पूर्व वरिष्ठ वैज्ञानिक एवं प्रभाग अध्यक्ष, भू-जूल विभाग, जोधपुर ने वैज्ञानिक एवं प्रशासनिक क्षेत्र में हिन्दी का उपयोग कैसे बढ़ाया जाए। पर व्याख्यान प्रस्तुत किया इस कार्यक्रम में : डॉ. यशपाल, निदेशक, राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार/बीकानेर विशेष अतिथि के रूप में पधारे दिनांक 14.09.2021 को ''हिन्दी दिवस'' एवं ''हिन्दी राजभाषा सप्ताह शुभारम्भ'' समारोह का आयोजन किया गया। इस समारोह में अधिवक्ता महेन्द्र जैन, सदस्य – न्यायधीश, स्थायी लाक अदालत – बीकानेर ने न्याय प्रणाली में हिन्दी भाषा के बढ़ते योगदान पर अपने विचार प्रस्तुत किया एवं प्रो. जी.एन. पुरोहित, पूर्व अधिष्ठाता – पी.जी. स्टडीज, राजुवास, बीकानेर मुख्य अतिथि के रूप में पधारे एवं श्री पूर्णचन्द राखेजा, पूर्व अध्यक्ष – महावीर इंटरनेशनल, बीकानेर विशिष्ठ अतिथि रहे। दिनांक 20.09.2021 को हिन्दी कार्यशाला एवं ''हिन्दी राजभाषा सप्ताह समापन'' समरोह कार्यक्रम में प्रो. अजय कुमार जोशी – साहित्यकार, रचनाकार एवं कवि, बीकानेर ने ''हिन्दी की लोकप्रियता विभिन्न आयाम'' पर व्याख्यान प्रस्तुत किया। समारोहत में प्रो. आर.पी. सिंह, कुलपति – स्वामी केशवानन्द राजस्थान कृषि विश्व विद्यालय, बीकानेर मुख्य अतिथि के रूप में पधारे एवं डॉ. रेणुका व्यास उर्फ 'नीलम' – व्याख्याता, राजस्थान माध्यमिक शिक्षा विभाग, श्री नदीम अहमद उर्फ 'नदीम' – साहित्यकार, रचनाकार एवं कवि और श्री राजाराम स्वर्णकार – साहित्यकार, रचनाकार एवं कवि विशिष्ठ अतिथि के रूप में पधारे।

अश्व उत्पादन परिसर में हिन्दी सप्ताह का आयोजन

Foundation Day and PM's Programme on Climate Resilient Varieties, Technologies and Practices

The 33rd Foundation Day and farmers scientists' interface on Climate Resilient Varieties, Technologies and Practices was conducted at Equine Production Campus, ICAR-NRCE, Bikaner on September 28th 2021. The online programme of Honourable Prime Minister was attended by the invited guests, farmers and the staff members. Total 95 participants took part in the event.

33rd Foundation day and farmers scientists' interface on Climate Resilient Varieties, Technologies and Practices at EPC-Bikaner

Mahila Kisan Diwas and World Food Day, 2021

The *Mahila Kisan Diwas* on October 15th, 2021, and World Food Day on October 16th, 2021 were observed at the village Mallapur, Hisar district in the village anganwadi center. Around 21 rural women attendees were explained about the importance of healthy food and the significance of rural women in Indian agriculture. A food contest was organised among the women and the institute staff judged the best nutritive food among the food items prepared.

***Mahila Kisan Diwas* celebration at Mallapur, Hisar**

Vigilance awareness week at ICAR-NRCE

The vigilance awareness week was celebrated from 26th October to 1st November 2021 at ICAR-NRCE. The vigilance awareness oath was taken by the employees of the institute under the leadership of the Director, ICAR-NRCE.

Oath taking ceremony during vigilance awareness week at ICAR-NRCE

ICAR-NRCE Foundation Day

The 37th foundation day of ICAR-NRCE was celebrated on November 26th, 2021. Commandant, EBS Brig. SS Balaje was the chief guest of the event. Progressive equine owners from Haryana who have contributed significantly to conservation of equines graced the auditorium to celebrate this event. Dr Yash Pal, Director, ICAR-NRCE presided over the function and apprised the dignitaries about the research activities and accomplishment of the Centre in equine health and production.

Foundation Day Celebrations at ICAR-NRCE

Health camps and *kisan gosthis*

An equine health camp was organized at Gurana village, Hisar on November 23rd,2021. In the health camp, 15 horses were examined by a multidisciplinary team of scientists and technical officers. After the camp, a farmer-scientist interaction meeting (*Kisan gosthi*) was organized in the veterinary dispensary premises for advising the equine owners on different health issues of horses like colic, ticks and nutrient requirements.

Equine Health camp organised by ICAR-NRCE at Gurana village, Hisar

Special *Swachhta Pakhwada* programme

A swachhta campaign was organised at ICAR-NRCE Hisar and EPC Bikaner from 2nd to 31st October, 2021. In this activity, a total of 117 participants such as farmers, school children and staff members of the institute participated. Awareness on waste to wealth management, kitchen garden management, and manure preparation from bio-wastes, plastic free campus and single use plastics were imparted.

Special swachhata awareness campaign at ICAR-NRCE

Swachhta Pakhwada activities

Swachhta Pakhwada activities of ICAR-NRCE, Hisar and EPC Bikaner were organized from 16th to 31st December, 2021. During the program various activities, such as *swachhta* pledge, basic maintenance awareness on cleaning of sewerage and water lines, recycling of waste water, water harvesting for agriculture, horticulture application and kitchen gardens in residential colony and office campus were organised. *Kisan Divas* was organized at MGMG village Himatasar, Bikaner. A total of ten farmers attended the program. Quiz, essay and drawing competitions for school children and village youth were conducted. In pursuance of DARE, New Delhi Office memorandum every Friday "Regular Cleanliness Drive and Disposal of Pendency" activity was also performed.

Swachhta oath taking ceremony at ICAR-NRCE

Cleanliness Drive at EPC Bikaner

Mera Gaon Mera Gaurav (MGMG) activities

Celebrating the birth of girl children

A programme for celebrating the birth of girl children at Anganwari, village Mallapur, Hisar was conducted on January 24th, 2021, in collaboration with anganwari officials. The NRCE scientists motivated the mothers and other women (35) of girl child for proper nutrition and girl's education and honored mothers giving birth to second girl child.

A programme for celebrating the birth of girl children at Anganwari, village Mallapur, Hisar

Honoring mothers giving birth to second girl child

Infertility cum health camp at Salasar, Kolayat

MGMG team of ICAR-NRCE and EPC Bikaner, visited the village Salasar, Kolayat on June 26th, 2021. Meeting with animal owners on advanced animal husbandry practices were organised. Animal health camp focused on infertility in animals was also conducted. Health issues were addressed and medicines were distributed to 32 male and 14 female animal keepers (total 46 animal owners) for 521 animals comprising 158 cows, 83 calves, 3 bulls/bullocks, 2 camels, 150 sheep and 125 goats.

Scientist-farmer interaction at Salasar, Kolayat

Parthenium Week Celebration

ICAR–NRCE Hisar carried out a 'Parthenium Awareness' campaign during August 16th to 22nd 2021 at Government Senior Secondary School, Talwandi, Hisar. Lectures were delivered to create awareness about the menace of Parthenium weed, associated health problems in human beings, animals and deterioration of the environment. The solution to the problem including parthenium uprooting, biological control, use of mexican beetles, planting some plants, reducing this weed, spraying herbicides etc was also informed. The related literature and extension material was distributed to 96 participants.

ICAR-NRCE Scientists delivering lecture on Parthenium Awareness at Government Senior Secondary School, Talwandi, Hisar

Celebration of Nutrition week

As a part of nutrition week celebration, ICAR-NRCE distributed Bajra cakes and oats biscuits among the girl children (90) of Government Senior Secondary School, Talwandi Rana, Hisar, Haryana on September 17th, 2021. The focus of the programme was on nutrition of women and girl children.

ICAR-NRCE scientists interacting with students and teachers of Government Senior Secondary School, Talwandi, Hisar

Equine Treatment Camp at Shobhasar, Bikaner

Dr S. C. Mehta, Sh. Kamal Singh, Dr R.A. Pachouri and Sh. Brij Lal visited the village Shobhasar, Bikaner on September 21st 2021. Total 18 horses were treated for infertility, skin infection, wound, debility, ectoparasites etc.

Online *Kisan Gosthi* on "Entrepreneurship development in equines" for equine keepers

A *Kisan Gosthi* was organised on October 18th 2021 on the topic "Entrepreneurship development in equines. It was attended by 48 participants including scientists, farmers and veterinarians. Different entrepreneurial opportunities available in the equine sector were discussed. The queries of the farmers and veterinarians were taken and addressed online.

Kisan Gosthi on ICAR-NRCE Foundation Day

To mark the 37th foundation day of ICAR-NRCE, a farmer-scientist interaction meet was organized on November 26th 2021. A total of 9 equine keepers participated in the meet and discussed their issues with a team of equine experts. Different equine management practices were taught to the equine keepers based on their need.

Infertility camp and the *Vichar Gosthi* at Chhoti Nal, Bikaner

The team from EPC campus Bikaner visited the village Chhoti Nal, Bikaner on December 31st, 2021. Importance of deworming in sheep and other livestock species was discussed. The role of macro and micro minerals in health, vigour and fertility was also discussed. National policies and government's efforts to increase the income of farmers were discussed in the *Vichar Ghoshthi*. The medicines for deworming, mineral mixture and ecto-parasiticidal were distributed to the 12 farmers possessing 1725 sheep, 349 goats and 80 cows.

***Vichar Gosthi* at Village Chhoti Nal, Bikaner**

***Kisan Gosthi* on ICAR-NRCE Foundation day**

***Poshan Vatika Abhiyaan* celebrations at EPC, ICAR-NRCE, Bikaner**

Training on Balance use of fertilizer to the equine farmers at EPC, ICAR-NRCE, Bikaner

06

IRC, RAC and Review Meetings

Institute Research Committee (IRC) reviews research projects

The annual IRC meeting of ICAR-NRCE was held on 28th -29th April, 2021 under the chairmanship of Dr Yash Pal, Director, ICAR-NRC on Equines to evaluate the various research projects in the areas of equine health, production, NCVTC and extension. Overall, 23 institute research projects, 11 externally funded projects and three new project proposals were presented and discussed during the meeting. The Chairman urged all the scientists to follow the recommendations of Research Advisory Committee (RAC) for better research output. He also urged the scientists to apply for externally funded projects and to publish their research papers in high impact journal so as to get recognition in the scientific fraternity. The necessity for ISO-17025 certification of Glanders and Equine Influenza laboratories of the Centre was also discussed in the meeting. The chairman appreciated the progress made in the various projects and requested all the scientists for timely submission of RPP of the ongoing projects or completed projects for smooth functioning of PME.

Research Advisory Committee (RAC) reviews the research activities of the Centre

The 24th RAC meeting of ICAR- National Research Centre on Equines was held through video conferencing under the Chairmanship of Dr M.C. Sharma (Former Director cum Vice-Chancellor, IVRI) on 07th April, 2021 to review the research achievements for the year 2020-21. The RAC members and all the scientists of ICAR-NRCE, NCVTC, Hisar and EPC, Bikaner were present in the meeting. In-charges of the respective units *viz.,* Equine health, Equine Production Campus (Bikaner) and NCVTC presented the overall progress of the research work. New research proposals were also presented in the meeting. The RAC members applauded the ongoing activities at the Centre and gave specific recommendations for further improving the research activities. The key recommendations of RAC are (1) emphasis must be given to develop point-of-care diagnostic and vaccines against all important equine diseases; (2) research work should be initiated on non-infectious diseases as well; (3) standard operating

RAC meeting in progress

protocols need to be developed for control of important equine diseases; (4) mega programme on diagnosis and management of parasitic diseases of equines needs to be initiated; (5) studies need to be carried out on economics of equine farming with particular emphasis on doubling farmer's income; (6) whole genome sequencing of some important equine breeds should be carried out and (7) training programme for NCVTC network units partners must be organized for collection of biological specimens from the field and dispatch of microbial cultures to the repository.

Annual Review of Network Units of NCVTC

NCVTC Annual Review meeting in progress

The XIth Annual scientific review meet of National Centre for Veterinary Type Cultures (NCVTC) was held on 10th Dec 2021 in virtual mode on zoom platform under the chairmanship of Hon'ble Deputy Director General (AS), Dr BN Tripathi. Besides, Dr Ashok Kumar, ADG (Animal Health), Dr Yashpal, Director (ICAR-NRCE, Hisar) cum Project Coordinator (PC), NCVTC Hisar, Dr Artabandhu Sahoo (Director, ICAR-NRC on Camel), Dr Jyoti Misri, Principal Scientist (AH) at ICAR Headquarters, Dr Sanjay Barua, In-Charge, NCVTC, PIs, Co-PIs and Scientists from different network units also attended the meeting. The key recommendations of the meeting are (1) Two more dairy centers may be included in the NCVTC network; (2) All the cultures available in the NCVTC repository needs to be catalogued; (3) Midterm review of the progress of the NCVTC network units may be conducted; (4) Wild type as well as vaccine strain of viruses should be submitted in the repository; (5) NCVTC should search more partners for working on bacteriophages and should consider making NCVTC a Centre of excellence for bacteriophage research; (6) Pathogenic mycobacterial cultures and *Clostridium* strains available with network units need to be deposited in the NCVTC repository and (7) Deposit form should also be sent along with the cultures at the time of submission.

Members of RAC	
Dr M. C. Sharma Former Director, ICAR-IVRI	**Chairman**
Prof. Mohammed Hafeez Former Head, Division of Parasitology, Sri Venkateswara Veterinary University, Tirupati	**Member**
Dr T. V. Anil Kumar Scientist G and Head, Sree Chithra Tirunal of Institute of Medical Sciences and Technology, Thiruvananthapuram	**Member**
Dr B. K. Joshi Former Director, ICAR-NBAGR, Karnal	**Member**
Dr Ashok Kumar ADG (AH), ICAR Krishi Bhavan, New Delhi	**Member**
Dr Yash Pal Director, ICAR-NRCE, Hisar	**Member**
Dr Balvinder Kumar Principal Scientist, I/C PME Cell	**Member Secretary**

07

Visit of Dignitaries

Director, ICAR-CIPHET, Ludhiana visits Infoequine museum

Dr Nachiket Kotwaliwale, Director, ICAR- CIPHET, Ludhiana visited Infoequine museum of ATIC, ICAR-NRCE on January 11, 2021. He was impressed with the displays in museum and referred it as a 'National Treasure'. He took keen interest in all the items displayed in info equine museum. Dr Yash Pal, Director, ICAR-NRCE accompanied Dr Nachiket Kotwaliwale and detailed about the displays in the museum.

Commandant, Equine Breeding Stud (EBS), Hisar visits the Centre

Brig. Balaje SS, Commandant, Equine Breeding Stud (EBS), Hisar visited ICAR-NRCE campus for an interactive meet with all ICAR-NRCE staff on March 22, 2021. He also visited all laboratories of ICAR-NRCE and appreciated the research achievements and technologies developed by the ICAR-NRCE scientists. He desired to have collaborative research work between EBS and ICAR-NRCE. He also applauded the services extended by ICAR-NRCE towards EBS, Hisar.

DDG (Animal Sciences) applauds ICAR-NRCE research activities

Dr BN Tripathi, Deputy Director General (AS), ICAR Headquarters, New Delhi visited ICAR-NRCE campus on July 30, 2021 and inaugurated the Jenny dairy unit and interacted with scientists of ICAR-NRCE. He encouraged all scientists to work on core areas of equine research and applauded ICAR-NRCE for its significant contribution in COVID-19 testing. He assured to extend full support to ICAR-NRCE from ICAR headquarters and inspired scientists of the centre to conduct impact research studies.

Dr SL Goswami, Vice-Chancellor, visits ICAR-NRCE laboratories

Dr SL Goswami, Former Vice-Chancellor, Banda University of Agriculture and Technology (BUAT), Banda, Uttar Pradesh visited ICAR-NRCE on September 17, 2021. He interacted with all lab in-charges to understand the ongoing research in ICAR-NRCE. He was impressed with various research activities being carried out by the scientists of the Centre.

Vice-Chancellor, GJUST applauds ICAR-NRCE activities

Prof. BR Kamboj, Vice-Chancellor, CCS HAU and VC GJUS&T, Hisar visited ICAR-NRCE on September 21, 2021. He addressed the ICAR-NRCE staff on the occasion of Hindi week celebrations and participated in a tree planting programme at ICAR-NRCE farm.

Vice-Chancellor, CBLU appreciates R & D activities of NRCE

Prof. RK Mittal, Vice-Chancellor (VC), Chaudhary Bansi Lal University (CBLU), Bhiwani, Haryana visited the ICAR-NRCE campus on October 27, 2021. He appreciated the efforts of ICAR-NRCE in equine disease diagnosis and interacted with all lab in-charges of ICAR-NRCE and NCVTC. He was impressed at the display of models and charts in Infoequine museum of ATIC. Dr. Yash Pal, Director, ICAR-NRCE briefed the activities of NRCE to Prof. RK Mittal.

Additional Secretary (DARE) & Financial Advisor (ICAR) visits NRCE

Sh. Sanjiv Kumar, Addl. Secretary (DARE) & Financial Advisor (ICAR) visited the centre on December 6, 2021. He visited the institute laboratories, infoequine museum and animal sheads to understand the various activities undertaken at ICAR – NRCE. The Director, ICAR-NRCE explained the significance of NRCE in animal health research. The Addl. Secretary assured full support to NRCE and congratulated all the scientists for their future endeavours.

Chairman, RAC interacts with ICAR-NRCE staff

Dr MC Sharma, Chairman of ICAR-NRCE Research Advisory Committee (RAC) visited NRCE laboratories on December 31, 2021 to interact with the scientists and staff. During his visit, he got to know about the progress of each scientist in their respective research areas. He appreciated the various research activities going on at ICAR-NRCE. Also, he urged the scientists to work in multidisciplinary team to attain best research outcomes.

08

Infrastructure and Developmental Activities

Establishment of Jenny Dairy Unit of female donkeys of Halari breed

Ten female donkeys and one donkey colt foal have been procured from Horse and Donkey Breeding Farm, Chanasma, Pattan dist., Gujarat and brought to ICAR-NRCE main campus, Hisar in July 2020 for the purpose of establishing the Jenny Dairy Unit for research on donkey milk characterization. The established Jenny Dairy Unit was inaugurated by Dr BN Tripathi, DDG (Animal Sciences), ICAR Headquarters, New Delhi on 30 July, 2021.

Inauguration of Jenny Dairy Unit by DDG (Animal sciences), ICAR, New Delhi

Other infrastructure and developmental activities

The major works undertaken in ICAR-NRCE during the period include construction of Animal Quarantine shed, Jenny dairy shed for 10 animals, repair of small animal house, renovation/repair of animal shed, pathology lab of NRCE, clinical examination hall and security check post. While, other works in NCVTC include; external furnishing of NCVTC building, construction of additional vehicle parking stand, renovation/repair and electrification of NCVTC small animal house, etc. These infrastructural developments have been carried out through the CPWD.

Newly furnished building of NCVTC, ICAR-NRCE, Hisar

Establishment of equine quarantine facility

Equine quarantine facilities were created at the Centre and this facility is approximately 800 meter away from main animal paddock. This facility has a capacity to house 3 large equids at a time. Quarantine unit is well protected from flies and insects. This building was inaugurated by Dr B N Tripathi, Honourable DDG (AS) on 30th July 2021.

Inauguration of equine quarantine facility by DDG (Animal sciences), ICAR, New Delhi

Livestock Strength

At present, a total of 157 equines of various breeds are being maintained at Hisar and Bikaner campuses, including 72 horses, 19 ponies, 63 donkeys and 3 mules **(Table 1 & 2)**. At Bikaner campus, there are 123 equines, including Marwari (n=54) and Kathiawari (n=07) horses; Zanskari (n=09) and Manipuri (n=05) ponies; Poitou (n=34) & indigenous donkeys (n=09) mules (n=03) and nukra (n=02).

Table 1 : Equine herd strength at ICAR-NRCE, Hisar campus

Particulars	Horse				Pony		Donkey				Total
	Stallion	Mare	Colt	Filly	Mare	Colt	Stallion	Mare	Colt	Filly	
Stock as on 01.01.2021	02	06	03	02	04	01	02	12	03	02	**37**
Birth	0	0	0	0	0	0	0	0	03	0	**03**
Auctioned/Sold	01	01	02	0	0	0	0	01	01	-	**06**
Balance as on 31.12.2021	01	05	01	02	04	01	02	11	05	02	**34**
Grand Total	**09**				**05**		**20**				**34**

Table 2 : Equine herd strength at EPC, ICAR-NRCE, Bikaner Campus

Particulars	Horse				Pony				Donkey				Mule		Nukra		Total
	Marwari		Kathiawari		Zanskari		Manipuri		Poitou		Halari						
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Stock as on 01.01.2021	22	31	02	04	03	08	04	03	14	18	03	06	02	01	02	0	**123**
Birth	07	07	0	01	0	0	0	01	03	06	0	02	0	0	0	0	**27**
Purchased	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Mortality	02	0	0	0	0	0	0	02	0	01	0	01	0	0	0	0	**06**
Auctioned / Sold	05	06	0	0	0	02	01	0	06	0	0	01	0	0	0	0	**21**
Balance as on 31.12.2021	22	32	02	05	03	06	03	02	11	23	03	06	02	01	02	0	**123**
Grand Total	**54**		**07**		**09**		**05**		**34**		**09**		**03**		**02**		**123**

Total agricultural production

During the year 2021, 251 acres of land was cultivated (149 acres at ICAR-NRCE, Hisar and 102 acres at EPC, Bikaner). The land was rotationally used for cultivating green fodder, dry fodder and grains for feeding farm equines. Total farm production was 4238.80 quintals, including 2640.65 quintals of green fodder, 828.85 quintals of dry fodder and 769.30 quintals of grains **(Table 3)**.

Table 3 : Crop-wise production

Type of Crop	Production (Quintals)	
	NRCE, Hisar	EPC, Bikaner
Green Fodder		
Sorghum	189	904
Oats	188	426.15
Lucerne	370	410.50
Berseem	114	—
Cowpea	39	—
Total green fodder	**900**	**1740.65**
Dry Fodder		
Total dry fodder (Oats, Bajra, Sewan Grass, Gaur, Wheat straw etc.)	**509.65**	**319.20**
Grains		
Oats	133.15	18.10
Wheat	246.90	—
Barley	148.95	138.20
Rapeseed	—	84.00
Total grains	**529**	**240.30**
Grand Total	**1938.65**	**2300.15**
Total Revenue Generated (Rs.)	**3,75,561/-**	**3,27,349/-**

09

Awards, Recognitions and Personal Milestones

Awards and recognitions

- **Dr Anju Manuja** was awarded with "Outstanding scientist award" on 15th August, 2021 for her research contribution in 2020-2021. This award was conferred by ICAR- NRCE, Hisar.
- **Dr Anuradha** was bestowed with the "Award of Honor" by Om Sterling Global University, Hisar on the eve of International Women's day-2021
- **Dr Harisankar Singha** was awarded with "Outstanding scientist award" on 15th August, 2021 for his research contribution in 2020-2021. This award was conferred by ICAR-NRCE, Hisar
- **Dr Harisankar Singha** bestowed with "International Research Award on New Science Invention NESIN-2021" for Best Research conferred by Science Father.
- **Dr Naveen Kumar** has been assigned as a Section Editor of "Virulence"
- **Dr Naveen Kumar** has been nominated by the Honorable Governor (Haryana) as a Member (Eminent Scientist) of the Board of Management (BOM) of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana
- **Dr Naveen Kumar** has been nominated as a member (DG Nominee) of the selection committee for considering promotion cases of scientific personnel of ICAR-Central Institute for Research on Goats, Mathura under ICAR Career Advance Scheme (CAS) in the discipline of Veterinary Microbiology
- **Dr Naveen Kumar** has been nominated as a member, expert panel for evaluation, monitoring and review of COVID-19 related international research projects funded by International Cooperation Division, Department of Science and Technology IDST), Govt. of India (2020-2023).
- **Dr Naveen Kumar** has been nominated as a member Board of Studies, Department of Biotechnology, GLA University, Mathura
- **Dr Sanjay Kumar** was bestowed with the National Academy of Veterinary Sciences (NAVS) Fellowship.
- **Dr SC Mehta** has been selected as the "Executive Member" of the Indian Society of Animal Genetics and Breeding (ISAGB).
- **Dr SC Mehta** acted as panelist in Interface meeting on "Characterisation and documentation of animal genetic resources of Rajasthan: A mission towards zero non-descript populations held at National Bureau of Animal Genetic Resources, Karnal on November 16th, 2021

- **Dr SC Mehta** acted as panelist in Interface meeting on "Characterisation and documentation of animal genetic resources of Maharashtra : A mission towards zero non-descript populations held at National Bureau of Animal Genetic Resources, Karnal on October 25th, 2021
- **Dr Taruna Anand** received the Best reviewer award from Journal- Animal Reproduction Update.
- **Dr TR Talluri** obtained "Reviewer Excellence Award" for reviewing the articles at Indian Journal of Animal Research, ARCC journals, Karnal.
- **Dr TR Talluri** selected as Editorial Board member of International Journal of Equine Science
- **Dr TR Talluri** selected as Editorial board member of Journal of Animal Research.
- **Dr. Yash Pal** was conferred with Vigyan Vagish Samman on 24th December, 2021 for his long term contribution in livestock development, equine production, development of equine products and for up gradation of official language Hindi in the scientific field. This award is conferred by Vigyan Parishad Prayag, Rajasthan Branch, Jodhpur
- **Dr B R Gulati and his team (**Dr Naveen Kumar, Dr Shanmugasundaram and Dr Riyesh T**)** was awarded with a multi-institutional (IVRI, Izatnagar, NIHSAD, Bhopal and NIVEDI, Bengaluru) project entitled "Epidemiological studies and development of antiviral therapeutics against coronaviruses". This project is for 3 years and the budget of the project is Rs.306.42 lakh.

Personal milestones

- **Dr TR Talluri**, completed PGD-TMA with Distinction and obtained PGD-TMA degree from ICAR-NAARM and University of Hyderabad.
- **Sh. Joginder Singh** has been promoted as Senior Technical Officer on 14.09.2021
- **Sh. SN Paswan** has been promoted as Technical Officer on 09.08.2021
- **Sh. Raj Kumar** has been promoted and transferred to ICAR-IIMR, Ludhiana as Senior Administrative Officer on 16.10.2021
- **Sh. Ramesh Chander** was promoted as LDC on 05.11.2021
- **Sh. Sanjeev Kumar** has been promoted as Asst. Chief Technical Officer on 14.09.2021
- **Sh. Sita Ram** has been promoted as Asst. Chief Technical Officer on 23.08.2021

Transfer

- **Sh. PP Chaudhary**, Assistant Chief Technical Officer has been transferred to ERS of ICAR-NDRI, Kalyani on 06.03.2021

Superannuation

- **Sh. Desh Raj**, Skilled Supporting Staff was retired on 31.03.2021.
- **Sh. Om Prakash**, Skilled Supporting Staff was retired on 30.06.2021.

Sh. Om Prakash, skilled supporting staff retired on 30.06.2021 after serving ICAR-NRCE for a long time.

Sh. Desh Raj, skilled supporting staff retired on 31.03.2021 after serving ICAR-NRCE for a long time.

10

Publications

Research Papers

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3. Anand T, Bera BC, Vaid RK, Virmani N & Tripathi BN. 2021. Personalized Phage Therapy: Proving to be a valuable therapeutic approach against Difficult-to-treat infections in humans. ***International Journal of Bacteriophage Research***, 1:49-50.
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5. Badsiwal DK, Chahar A, Kumar L, Kumar S, Legha RA, Pal Y & Dedar RK. 2021. Prevalence of *Rhodococcus equi* in foals of west northern Rajasthan by VAPa and VAPc genes specific polymerase chain reaction. ***Journal of Entomology and Zoology Studies,*** 9(1):291-294.
6. Bhalothia SK, Mehta JS, Kumar T, Prakash C, Talluri TR, Pal RS & Kumar A. 2021. Melatonin and canthaxanthin enhances sperm viability and protect ram spermatozoa from oxidative stress during liquid storage at 4°C. ***Andrologia,*** https://doi. org/10.1111/and.14304.
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2. Gupta KK, Singh L, Saxena N, Dey S, Kumar R, Pal Y & Kumar S. **In-vitro growth inhibitory efficacy of *Artemisia scoparia* plant extracts against *Theileria equi* in MASP culture system**. Proceedings of International Online Conference: Veterinary Science - Sustainable Cooperation in the 60th anniversary of Institute of Veterinary Medicine, Mongolia at Ulaanbaatar, Mongolia. 18 November, 2021. Pp. 62-63.

3. Nilendu P, Talluri TR, Raval Kathan, Kamaraj Elango, Verma A & Kumaresan A. **Nanopurification of bull semen for enrichment of functionally competent spermatozoa**. Compendium of the International Symposium conducted on "Novel Knowledge, Innovative Practices and Research in Theriogenology" organised by College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. 27-29 December, 2021. Pp. 122.

4. Pal VK, Singh A, Kumar R & Singh HK. **Prevalence and relative risk factors associated with equine Trypanosomosis in eastern region of Uttar Pradesh**. Proceedings of 30th National Congress of Veterinary Parasitology & National Symposium on "Fundamentals of Integrated Parasite Management and its Relevance in One Health" organised by College of Veterinary and Animal Sciences, Parbhani, Maharashtra. 14-16 December, 2021. Pp. 149.

5. Ram V, Jhamb D, Talluri TR, Sharma S, Kumar N, Vyas H, Arora S & Gaur M. **Effect of addition of lyophilized heterologous seminal plasma on cooled stallion epididymal sperm quality**. Compendium of the International Symposium conducted on "Novel Knowledge, Innovative Practices and Research in Theriogenology" organised by College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. 27-29 December, 2021. Pp. 209.

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9. Sharma D, Gupta S, Sethi K, Kumar S & Kumar R. **Immunological and epidemiological investigation of *Trypanosoma evansi* infection in livestock of Himachal Pradesh, India**. Proceedings of 30th National Congress of Veterinary Parasitology & National Symposium on "Fundamentals of Integrated Parasite Management and its Relevance in One Health" organised by College of Veterinary and Animal Sciences, Parbhani, Maharashtra. 14-16 December, 2021. Pp. 75.

10. Sinha MK, Kumaresan A, Talluri TR, Ebenezer SKPJ, Prakash MA, Raval K & Aranganathan **V. SNPs study related to genomics differences for fertility in crossbred bull spermatozoa**. Compendium of the International Symposium conducted on "Novel Knowledge, Innovative Practices and Research in Theriogenology" organised by College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. 27-29 December, 2021. Pp. 137.

11. Suthar A, Gopalakrishnan A, Maji C, Dahiya RK, Kumar R & Kumar S. ***Theileria equi* growth inhibition efficacy of quaternary ammonium salts in in-vitro MASP culture**. Proceedings of 30th National Congress of Veterinary Parasitology & National Symposium on "Fundamentals of Integrated Parasite Management and its Relevance in One Health" organised by College of Veterinary and Animal Sciences, Parbhani, Maharashtra. 14-16 December, 2021. Pp. 52.

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13. Vashisth M, Bala A, Yashveer S, Virmani N, Bera BC, Vaid RK & Taruna Anand. **Synergistic effect of a cephalosporin with a broad spectrum bacteriophage against *Acinetobacter baumannii***. Proceedings of International webinar on Alternative Therapies to Mitigate Microbial Resistance organized by IVRI, Izatnagar, UP. 23-24 February, 2021.

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2. Anand T, Vashisth M, Jaglan A, Virmani N, Bera BC & Vaid RK. 2021. **Bacteriophages as a potential lifesaving alternative in the post-antibiotic era**. In the Bulletin of International Webinar on "Alternative Therapies to Mitigate Microbial Resistance", February 23-24, 2021.

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5. Dedar RK, Talluri TR, Legha RA, Singh J & Pal Y. 2021. **Some important non-infectious clinical problems in horses and their feeding management**. In the training manual on "Genetic Improvement of Indigenous Horses" published under the training programme for veterinary officers on "Entrepreneurship Development in Equine Husbandry" organised at EPC, Bikaner, February 25-27, 2021. Pp. 71-77.

6. **Know Your Institute: ICAR-NRCE**, Equine Production Campus, Bikaner. Desert Environment News Letter 22 (1-2) from ENVIS RP, ICAR-CAZRI, Jodhpur.

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8. Legha RA, Pal Y, Talluri TR, Singh J & Dedar RK. 2021. **How to make artificial insemination programme successful**. In the training manual on "Genetic Improvement of Indigenous Horses" published under the training programme for veterinary officers on "Entrepreneurship Development in Equine Husbandry" organised at EPC, Bikaner, February 25-27, 2021. Pp. 30-34.

9. Pal Y, Legha RA, Talluri TR, Bhardwaj A & Dedar RK. 2021. **Halari Donkey**. Monograph. Pp. 1-24.

10. Shanmugasundaram K & Singha H. 2021. **Differential diagnosis of equine glanders**. In the training manual on "Prevention, Control and Eradication of Equine Glanders" published under the training programme for field veterinary officers in collaboration with Haryana Veterinary Training Institute (HVTI), Hisar organised at ICAR-NRCE, Hisar, November 10-12, 2021. Pp. 20-24.

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14. Singha H & Shanmugasundaram K. 2021. ***Burkholderia mallei* as potential biological warfare agent**. In the training manual on "Prevention, Control and Eradication of Equine Glanders" published under the training programme for field veterinary officers in collaboration with Haryana Veterinary Training Institute (HVTI), Hisar organised at ICAR-NRCE, Hisar, November 10-12, 2021. Pp. 28-29.

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16. Singha H & Shanmugasundaram K. 2021. **Laboratory Protocol for Serological (ELISA and CFT) diagnosis of glanders**. In the training manual on "Prevention, Control and Eradication of Equine Glanders" published under the training programme for field veterinary officers in collaboration with

Haryana Veterinary Training Institute (HVTI), Hisar organised at ICAR-NRCE, Hisar, November 10-12, 2021. Pp. 30-34.

17. Singha H & Shanmugasundaram K. 2021. **Management of glanders outbreak**. In the training manual on "Prevention, Control and Eradication of Equine Glanders" published under the training programme for field veterinary officers in collaboration with Haryana Veterinary Training Institute (HVTI), Hisar organised at ICAR-NRCE, Hisar, November 10-12, 2021. Pp. 25-27.

18. Singha H, Shanmugasundaram K & Yash Pal. 2021. **Equine glanders Present status with special reference to Haryana**. In the training manual on "Prevention, Control and Eradication of Equine Glanders" published under the training programme for field veterinary officers in collaboration with Haryana Veterinary Training Institute (HVTI), Hisar organised at ICAR-NRCE, Hisar, November 10-12, 2021. Pp. 4-8.

19. Talluri TR, Legha RA, Pal Y, Mehta SC, Dedar RK & Singh J. 2021. **Collection and cryopreservation of stallion semen**. In the training manual on "Genetic Improvement of Indigenous Horses" published under the training programme for veterinary officers on "Entrepreneurship Development in Equine Husbandry" organised at EPC, Bikaner, February 25-27, 2021. Pp. 53-59.

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Participation, Presentation in Seminar, conferences and Symposia

- Dr Anju Manuja attended Webinar on "APIs/ drugs encapsulation in PLGA" organized by Dolomite-microfluidic on August 26th 2021.
- Dr Anju Manuja attended Webinar on "Animal Coronaviruses and their pandemic potential" organized by ICAR-NIHSAD, Bhopal on September 2nd 2021.
- Dr Anuradha Bhardwaj attended training on "Sensitization on Intellectual Property Rights in Agricultural Research and Education" organized by ICAR-Central Institute for Women in Agriculture, Bhubaneswar, Odisha on August 4th 2021.
- Dr Anuradha Bhardwarj delivered oral presentation on "Genome-wide genetic diversity detection and population structure analysis in native horse breeds" in the ISAGB- National Conference on "Animal Breeding Strategies in the Era of Genomics and Phenomics" organized by ICAR-NBAGR from December 17th-18th 2021.
- Dr Rajender Kumar attended training on "Sensitization on Intellectual Property Rights in Agricultural Research and Education" organized by ICAR-Central Institute for Women in Agriculture, Bhubaneswar, Odisha on August 4th 2021.
- Dr Sanjay Kumar attended training on "Sensitization on Intellectual Property Rights in Agricultural Research and Education" organized by ICAR-Central Institute for Women in Agriculture, Bhubaneswar, Odisha on August 4th 2021.
- Dr SC Mehta attended a creative meet and discussion on "Animal Husbandry and Rural Development" organized by RAJUVAS, Bikaner and NABARD, Rajasthan on February 12th 2021.
- Dr SC Mehta attended National Seminar on "In search of pastoral identity-mainstreaming the pastoral production system in Saurashtra" organized by Sahjeevan (Registered Society-NGO), at Rajkot, Gujarat on March 27th 2021
- Dr SC Mehta attended International Webinar on "Recognising Pastoralists for their Knowledge, Local Breeds and Biodiversity Conservation" organized by RISG-South Asia IYRP (International Year of rangelands and Pastoralists for 2026) - Regional Subgroup for South Asia on May 22nd 2021.
- Dr SC Mehta attended Interactive meet on "Opportunities and constraints in camel production system and its sustainability' organized by ICAR-NRC on Camel, Bikaner and NABARD on August 3rd 2021.
- Dr SC Mehta attended National Webinar on 'Building Nation through Standards 75 years of Independence under *Azadi ka Amrut Mahotsava* organized by Bureau of Indian Standards on August 16th 2021.

- Dr SC Mehta attended 22nd meeting of FAD 05-Animal Husbandry, Feeds and Equipment Sectional Committee Meeting organized by Bureau of Indian Standards on October 29th 2021.
- Dr SC Mehta acted as panelist in Interface meeting on "Characterisation and documentation of animal genetic resources of Maharashtra : A mission towards zero non-descript populations organized by ICAR-National Bureau of Animal Genetic Resources, Karnal on October 25th 2021.
- Dr SC Mehta acted as panelist in Interface meeting on "Characterisation and documentation of animal genetic resources of Rajasthan: A mission towards zero non-descript populations" organized by ICAR-National Bureau of Animal Genetic Resources, Karnal on November 16th 2021.
- Dr Taruna Anand delivered keynote presentation entitled "Strengthening Bacteriophage Bank and Studies: on Application of Novel Phage Cocktails for Therapy in vivo Murine Models" in the 2nd International conference on bacteriophage research from July 22nd- 24th 2021.
- Dr Taruna Anand delivered a keynote presentation entitled "Application of Bacteriophage in Veterinary Sector and Significance of Bacteriophage Repository in the Current Era of Emerging Antimicrobial Resistance" in 2nd International conference on bacteriophage research as the core committee member from July 22nd- 24th 2021.
- Dr TR Talluri delivered a lead paper on "Status and future prospects of ARTs in equine reproduction in India" at the International symposium conducted on "Novel Knowledge, Innovative Practices and Research in Theriogenology" by CVAS, Mannuthy, Thrissur, Kerala, India from December 27th- 29th 2021.
- Dr Yash Pal attended "Regional Committee Meeting VI" on March 13th 2021
- Dr Yash Pal attended meeting on "Covid-19 situation and other administrative & EFC" with all directors of ICAR institutes under the guidance of DDG (AS) on April 22nd 2021.
- Dr Yash Pal attended meeting on for "Repurposing the potential of emetine against COVID-19" under the guidance of DDG (AS) on April 22nd 2021.
- Dr Yash Pal attended meetings of Academic Council of CCSHAU, Hisar as the member of Academic Council.
- Dr Yash Pal attended a joint committee virtual meeting on "The consideration of guidelines for rehabilitation of Race horses after their racing age and to house requisites number of animals in given turf" organized by Animal Welfare Board of India on April 30th 2021.
- Dr Yash Pal attended meeting for "The presentation of scheme on Strategic Research, Veterinary Education and Animal Health Management on June 17th 2021.
- Dr Yash Pal attended training on "Sensitization on Intellectual Property Rights in Agricultural Research and Education" organized by ICAR-Central Institute for Women in Agriculture, Bhubaneswar, Odisha on August 4th 2021.
- Dr Yash Pal attended workshop on "Mission towards Zero Non-descript AnGR of India" through virtual mode on zoom platform organized by ICAR-NBAGR, Karnal on August 11th 2021.
- Dr Yash Pal attended meeting of the Assessment Committee for CAS constituted by the Chairman, ASRB for the Animal Physiology discipline at ASRB premises on September 8th 2021.
- Dr Yash Pal attended meeting on "Hackthon 2.0 Animal Science institutes" in virtual mode on zoom platform under the chairmanship of DDG (AS) on September 24th 2021.
- Dr Yash Pal attended a meeting of the committee constituted for "Assessing the losses of infrastructure, capital, agriculture etc. damaged due to recent flood like situation caused by heavy downpour at Hisar" on October 7th 2021.
- Dr Yash Pal attended meeting of the Animal Science institute Directors with DDG (AS) through virtual mode organized by ASD on October 13th 2021.
- Dr Yash Pal attended Interface Meet on "Characterization and Documentation of Animal Genetic Resources of Maharashtra State: A mission towards Zero Non-Descript Population" on October 25th 2021.

- Dr Yash Pal attended meeting on "LSD Vaccine and other issues" called by DDG (AS) through virtual mode on October 27th 2021.
- Dr Yash Pal attended Interaction Meeting of ICAR Scientists with Secretary, DARE & DG, ICAR on October 28th 2021.
- Dr Yash Pal attended meeting of the Animal Science institute Directors with DDG (AS) mode organized by ASD on December 9th 2021.
- Dr Yash Pal attended XIth Annual Scientific meet of National Centre for Veterinary Type Cultures under the chairmanship of DDG (AS) held on December 10th 2021.
- Dr Yash Pal attended XXV Meeting of ICAR Regional Committee-III on December 11th 2021.
- Dr Yash Pal attended a meeting chaired by Secretary DARE and DG ICAR to discuss the program of Honourable PM, Hosted by ICT Unit, ICAR on December 13th 2021.
- Ms Ana Raj presented a paper on "Women's Empowerment Index in Cassava: An Innovative tool for gender main streaming" in MANAGE International conference on "Agricultural Extension and Advisory Services: Innovations to impact" at National Institute of Agricultural Extension Management (MANAGE), Hyderabad from February 25th-27th, 2021.

Ongoing Research Projects (2021)

Equine Health

Sr. No.	Title	Team	From to	To	PIMS Code/ Page
1.	Surveillance, Monitoring and Control of Emerging and Existing Diseases of Equines	H. Singha*, B.R. Gulati, Rajender Kumar, Sanjay Kumar, Nitin Virmani, Sanjay Barua, R.K. Vaid, R.K. Dedar, Anju Manuja, Balvinder K, Shanmugasundaram K, Anubha Pathak, Ana Raj J and Yash Pal	April, 1997	Continuous Service Project	IXX00257
2.	Development of recombinant EHV1 viruses employing bacterial artificial chromosome mediated mutagenesis and their pathological evaluation in murine model	Nitin Virmani*, B.C. Bera, Taruna Anand and B.N.Tripathi	April, 2017	Sept, 2021	IXX14011
3.	Characterization of Equine herpes virus isolates in India and documentation of their genetic diversity	B.R. Gulati*, Naveen Kumar, Riyesh T.	Sept, 2018	March, 2021	IXX14746
4.	Diagnosis and sequence typing of strains *Streptococcus equi*	Balvinder Kumar*, R.K. Vaid, Anju Manuja, Shanmugasundaram K and H. Singha	April, 2018	Sept, 2021	IXX14584
5.	Biomacromolecules based nanoscaffolds for wound healing using 3D printing	Anju Manuja*, Balvinder Kumar and Riyesh T.	Oct, 2020	Sept, 2023	IXX15412
6.	Development of improved serological diagnostic assays for Surra using *Trypanosoma evansi* recombinant protein	Rajender Kumar*, Sanjay Kumar and B.N. Tripathi	July, 2021	June, 2022	IXX15796

*** Principal Investigator**

Equine Production

Sr. No.	Title	Team	Date of Start	Date of Completion	PIMS Code
1.	Endurance and fertility analysis in indigenous horses using SNP (single nucleotide polymorphisms) markers	S.C. Mehta*, R.K. Dedar and T.R. Talluri	Oct, 2017	Sept, 2021	IXX13995
2.	Studies on antitumor and antiviral potential of some plant extracts	R.K. Dedar*, Naveen Kumar and B.N. Tripathi	Nov, 2018	Oct, 2021	IXX14758
3.	Explicating genomic insights of Indigenous equines breed population through *"Computational Genomics"* and *"Artificial Intelligence"* based approaches	Anuradha Bhardwaj*, Sarika, Yash Pal, M.A. Iqbal and Dinesh Kumar	Dec, 2019	Nov, 2022	IXX15401
4.	Elucidation of physico-chemical, metabolomic and functional attributes of indigenous donkey milk	Anuradha Bhardwaj*, Yash Pal, R.A. Legha, Varij Nayan, AK Singh, PN Raju, Rajan Sharma and R.K. Vaid	July, 2020	June, 2023	IXX15413

5.	Optimisation of procedures for non-surgical recovery and bio-banking of equine embryos	T.R. Talluri*, Yash Pal and R.A. Legha	Oct, 2020	Oct, 2023	IXX15417
6.	Characterization and recognition of Bhimthadihorse	S.C. Mehta* and Sachin D. Sorate	July, 2021	June, 2023	IXX15797
7.	Analysis of quantitative traits for genetic improvement of indigenous equines	S.C. Mehta*, R.A. Legha and J. Singh	April, 2021	March, 2026	IXX15798

*** Principal Investigator**

NCVTC

Sr. No.	Title	Team	Dura-tion	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 Shanmugasundaram K	June, 2015	Service Project	IXX11884
3.	Isolation, characterization and generation of repository of *Mycobacterium* species	Shanmugasundaram K.*, R.K. Vaid, and B.C. Bera	Oct, 2017	March, 2022	IXX13994
4.	Development of repository of respiratory viruses of livestock and isothermal based diagnostics for rapid identification.	B.C. Bera*, Nitin Virmani, Taruna Anand, B.R. Gulati and Riyesh T.	Aug, 2020	July, 2023	IXX15338
5.	Indian network for fisheries and animal antimicrobial resistance (INFAAR)	R.K. Vaid*, Taruna Anand, H. Singha and Anubha Pathak	June, 2018	March, 2025	IXX15418
6.	Isolation and characterization of bacteriophages against important biofilm forming bacteria	Taruna Anand*, R.K. Vaid, Nitin Virmani and B.C. Bera	April, 2021	March, 2024	IXX15795
7.	A study on bat virome for unravelling the viral diversity in India	Riyesh T*, Naveen Kumar, Shanmugasundaram K, R.K. 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, Sanjay Barua and Riyesh T	Jan, 2021	Jan, 2022	IXX16676

*** Principal Investigator**

Extension Project

Sr. No.	Title	Team	From	To	PIMS Code
1.	Impact of social networking in equine extension and advisory services	Ana Raj J*, Gururaj M and R. K. Dedar	Jan, 2021	Jan, 2023	IXX15419

*** Principal Investigator**

Externally 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.	Elucidating therapeutic role of bacteriophages and encoded endolysins against multidrug resistant enteric pathogens of poultry	Taruna Anand*	June, 2018	May, 2021	OXX04448
3.	Exploration of genomic signatures for indigenous horses using next-generation sequencing approaches (DST-SERB)	Anuradha Bhardwaj*	Dec, 2018	Nov, 2021	OXX04453
4.	Investigating mechanism underlying acquisition of antiviral drug resistance against host targeting agents	Naveen Kumar* and Sanjay Barua	March, 2019	March, 2022	OXX04469
5.	National One Health Program on Prevention and Control of Zoonotic Diseases (NOHPPCZ)	**Bacterial Diseases :** H. Singha, Shanmugasundaram K and Anubha Pathak **Viral Diseases :** B. R Gulati*, Naveen Kumar and Riyesh T	June, 2019	On going	OXX04686
6.	Role of p38 MAP kinase in buffalopox virus replication	Sanjay Barua* and Naveen Kumar	29 Jan, 2020	28 Jan, 2023	OXX04792
7.	Development of host-directed anti-coronavirus agents (DST SERB)	Naveen Kumar* and Riyesh T	17 June, 2020	16 June, 2021	OXX04795
8.	Development of Antigen Detection Rapid Diagnostics for Equine Piroplasmosis	Sanjay Kumar* and Rajender Kumar	April, 2021	March 2022	OXX5112
9.	Epidemiological studies and development of antiviral therapeutics against coronaviruses	B.R. Gulati*, Naveen Kumar, Riyesh T and Shanmugasundaram K	June, 2021	May, 2024	OXX4935
10.	Validation and translation of the vaccines as well as diagnostic technologies developed in Phase-I of ADMaC	B.R. Gulati*	April, 2021	March, 2024	OXX4940
11.	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
12.	DBT Network Programme on Anthrax Diagnosis and Control in India	R.K. Vaid*, B.C. Bera and Shanmugasundaram K	Sept, 2021	Sept, 2024	-
13.	Development of Diagnostics for coronavirus infections	Nitin Virmani* and Naveen Kumar	June, 2021	May, 2023	OXX5111
14.	Studies on host pathogen interaction and development of vaccine against zoonotic coronaviruses	B.C. Bera*	June, 2021	May, 2024	-

*** Principal Investigator**

Staff at ICAR-NRCE (as on 31.12.2021)

Sr. No.	Scientific Staff	Designation
Main Campus ICAR-NRCE, Hisar		
1.	Dr. Yash Pal	Director
2.	Dr. B. R. Gulati	Principal Scientist
3.	Dr. Rajender Kumar	Principal Scientist
4.	Dr. Nitin Virmani	Principal Scientist
5.	Dr. Sanjay Kumar	Principal Scientist
6.	Dr. Anju Manuja	Principal Scientist
7.	Dr. Balvinder Kumar	Principal Scientist
8.	Dr. Anuradha Bhardwaj	Senior Scientist
9.	Dr. Harishankar Singha	Senior Scientist
10.	Dr. Anubha Prashant Pathak	Scientist
11.	Ms. Ana Raj. J	Scientist
NCVTC, Hisar		
1.	Dr. Praveen Malik	Principal Scientist (On Deputation)
2.	Dr. Sanjay Barua	Principal Scientist
3.	Dr. R. K. Vaid	Principal Scientist
4.	Dr. Naveen Kumar	Principal Scientist
5.	Dr. Taruna Anand	Senior Scientist
6.	Dr. B. C. Bera	Senior Scientist
7.	Dr. Shanmugasundaram. K	Scientist
8.	Dr. Riyesh. T	Scientist
Equine Production Campus, Bikaner		
1.	Dr. S. C. Mehta	Principal Scientist
2.	Dr. R. A. Legha	Principal Scientist
3.	Dr. R. K. Dedar	Senior Scientist
4.	Dr. T. R. Talluri	Senior Scientist
Technical Staff ICAR-NRCE Main Campus, Hisar		
1.	Sh. Sita Ram	Asst. Chief Technical Officer
2.	Sh. K.S. Meena	Asst. Chief Technical Officer
3.	Sh. P.P. Chaudhary	Asst. Chief Technical Officer
4.	Sh. Sanjeev Kumar	Asst. Chief Technical Officer
5.	Sh. Ajmer Singh	Technical Officer
6.	Sh. Sajjan Kumar	Technical Officer
7.	Sh. Suresh Kumar	Technical Officer
8.	Sh. Joginder Singh	Senior Technical Officer
9.	Sh. Mukesh Chand	Technical Officer
10.	Sh. Raj Kumar Dayal	Technical Officer
11.	Sh. Raghbir Singh	Senior Technical Assistant
12.	Sh. Arun Chand	Senior Technician

Sr. No.	Technical Staff	Designation
Equine Production Campus, Bikaner		
1.	Dr. Jitender Singh	Asst. Chief Technical Officer
2.	Sh. K. K. Singh	Asst. Chief Technical Officer
3.	Dr. R. A. Pachori	Asst. Chief Technical Officer
4.	Sh. Narender Chauhan	Asst. Chief Technical Officer
5.	Sh. Brij Lal	Technical Officer
6.	Sh. Om Parkash	Technical Officer
7.	Sh. S. N. Paswan	Technical Officer
8.	Sh. Rajender Singh	Technical Assistant
9.	Sh. Gopal Nath	Technical Assistant
ADMINISTRATIVE STAFF		
ICAR-NRCE Main Campus, Hisar		
1.	Sh. Raj Kumar	Administrative Officer
2.	Sh. S. P. Kaushik	Assistant Administrative Officer
3.	Sh. Subhash Chander	Assistant Administrative Officer
4.	Sh. Ashok Kumar	Personal Assistant
5.	Sh. Sunil	Assistant
6.	Sh. Dinesh Datt Sharma	Assistant
7.	Sh. Om Parkash	Assistant
8.	Sh. Deepak Kumar	Upper Division Clerk
9.	Sh. Guru Dutta Sharma	Lower Division Clerk
10.	Sh. Ishwar Chander	Lower Division Clerk
11.	Sh. Ramesh Chander	Lower Division Clerk
ICAR-NRCE, Equine Production Campus, Bikaner		
1.	Sh. Mahender Singh	Upper Division Clerk
SKILLED SUPPORTING STAFF		
ICAR-NRCE Main Campus, Hisar		
1.	Sh. Ishwar Singh	Skilled Supporting Staff
2.	Sh. Jai Singh	Skilled Supporting Staff
3.	Sh. Mahabir Prasad	Skilled Supporting Staff
4.	Sh. Mardan	Skilled Supporting Staff
5.	Sh. Hanuman Singh	Skilled Supporting Staff
6.	Sh. Subhash Chander	Skilled Supporting Staff
7.	Sh. Ishwar Singh	Skilled Supporting Staff
8.	Sh. Ram Singh	Skilled Supporting Staff
9.	Smt. Santra	Skilled Supporting Staff
10.	Sh. Lilu Ram	Skilled Supporting Staff
11.	Sh. Sant Ram	Skilled Supporting Staff
12.	Smt. Soma Devi	Skilled Supporting Staff
ICAR-NRCE, Equine Production Campus, Bikaner		
1.	Sh. Mahabir Prasad Meena	Skilled Supporting Staff
2.	Sh. Raju Ram	Skilled Supporting Staff
3.	Sh. Ashok Kumar	Skilled Supporting Staff

ICAR-NRCE IN NEWS

चिंताजनक • जनवरी माह में कुल मिले थे 39 पॉजिटिव केस, लगातार बढ़ती जा रही ग्लैंडर्स पॉजिटिव की संख्या

हरियाणा, यूपी, महाराष्ट्र और एमपी में 8 घोड़े मिले ग्लैंडर्स पॉजिटिव

हिसार एनआरसीई ने संबंधित जिलों के अधिकारियों को सैंपलिंग के लिए लिखा पत्र

जानें कैसे... पॉजिटिव घोड़े को मारा जाता है

वर्ष 2020 में जांच को 34 हजार सैंपल आए, 210 मिले थे पॉजिटिव

जानिए... क्या है ग्लैंडर्स बीमारी और लक्षण

अश्व कल्याण नीतियों को मिलकर बढ़ाएंगे ब्रुक इंडिया और एनआरसीई

जागरण संवाददाता, हिसार : देश में अश्व कल्याण मुद्दों में सुधार, बेहतर अनुसंधान एवं अश्व पालक समुदाय की समस्याओं के समाधान के लिए ब्रुक इंडिया और भारतीय कृषि अनुसंधान केंद्र से संबंधित राष्ट्रीय अश्व-अनुसंधान केंद्र (एनआरसीई) हिसार ने एक समझौते (एमओयू) पर हस्ताक्षर किए।

समझौते पर एनआरसीई के निदेशक डा. यशपाल और ब्रुक इंडिया के मुख्य कार्यकारी अधिकारी ब्रिगेडियर जेम्स धरमाधीरण ने हस्ताक्षर किए। इस समझौते का उद्देश्य वर्तमान में आ रही अश्व कल्याण चुनौतियों का सामना करना एवं संयुक्त रूप से अश्व प्रजाति से जुड़े, अनुसंधान निष्कर्षों की मदद से नीति निर्माताओं तक अपनी बात रखना है।

दोनों संगठन अपने स्टाफ व सदस्यों के लिए संयुक्त कौशल प्रशिक्षण कार्यक्रमों, शैक्षिक सहयोग, संयुक्त अनुसंधान परियोजनाओं के माध्यम से तकनीकी जानकारी का आदान-प्रदान करेंगे।

एमओयू के यह है प्रमुख बिंदु

- लैब टू लैंड टेक्नालॉजी ट्रांसफर। घोड़े की मदद से चलने वाला हल।
- गधी के दूध के पायलट प्रोजेक्ट
- नालबंदी कौशल की वृद्धि के लिए नालबंदी प्रशिक्षण स्कूल का विकास
- पशु चिकित्सकों, पीएचडी छात्रों, राज्य पशुपालन अधिकारियों के लिए विशेष सत्र
- रैपिड ग्लैंडर्स परीक्षण किट का प्रसार
- कामकाजी अश्वों के लिए फीडिंग मानकों का विकास।
- पशुचिकित्सा अधिकारियों, पशु चिकित्सा पैरावेट, स्थानीय सेवा प्रदाता व गरीब पशु मालिकों के लिए कौशल विकास प्रशिक्षण।

जाने क्या है ब्रुक इंडिया

ब्रुक इंडिया एक नॉट फॉर प्रॉफिट कंपनी है। ब्रुक इंडिया का लक्ष्य देश में कामकाजी घोड़ों, गधों और खच्चरों के जीवन को बदलना है। उनकी तत्काल पीड़ा कम करते हैं। ब्रुक इंडिया अश्व पालक समुदायों और संगठनों के साथ काम करके उनका आर्थिक सुधार भी करती है। ताकि काम करने वाले अश्वों और अश्व पालक समुदाय की स्थिति में सुधार हो सके। 2001 में अपनी स्थापना के बाद से ब्रुक इंडिया 11 राज्यों और केंद्र शासित प्रदेशों में अश्व पालक समुदायों के उत्थान के लिए काम रहा है। जिसमें बिहार, दिल्ली, गुजरात, हरियाणा, हिमाचल प्रदेश, जम्मू-कश्मीर, महाराष्ट्र, पंजाब, राजस्थान, उत्तराखंड और उत्तर प्रदेश शामिल है।

राहत : जीनो टाइप टेस्ट के सभी सैंपल की रिपोर्ट आई निगेटिव

10 से अधिक सैंपल भेजने के आदेश

वैज्ञानिकों ने एलाईजा किट तैयार की, मात्र 3 घंटे में साधारण लैब में भी हो सकेगी जांच

केंद्रीय कृषि मंत्री ने ऑनलाइन किट का किया विमोचन

भास्कर खास • राष्ट्रीय अश्व अनुसंधान संस्थान में ग्लैंडर्स पर अंकुश लगाने को लेकर किया विचार-विमर्श

ग्लैंडर्स के अधिक केस मिलने पर सात जिले संवेदनशील घोषित, बिना सैंपलिंग के घोड़े नहीं जा सकेंगे अन्य राज्यों में

ग्लैंडर्स को बढ़ने से रोकने के लिए हर जिले में सैंपलिंग करवाएगा पशुपालन विभाग

जानिए... किन राज्यों में ग्लैंडर्स के कितने केस मिले

उत्तर प्रदेश	75
उत्तराखंड	13
हिमाचल प्रदेश	5
जम्मू-कश्मीर	3
पंजाब	3
हरियाणा	7
गुजरात	1
महाराष्ट्र	4
मध्य प्रदेश	3
कुल	112

बिहार, बंगाल, राजस्थान, और छत्तीसगढ़ में इस बार केस नहीं मिला।

एनआरसीई में पहुंचे डीडीजी, पशु फार्म का किया निरीक्षण

जेनी डेयरी का उद्घाटन करते आईसीएआर के डीडीजी डा. बीएन त्रिपाठी। • एनआरसीई

हिसार : राष्ट्रीय अश्व अनुसंधान केंद्र में भारतीय कृषि अनुसंधान परिषद से उप महानिदेशक डा. बीएन त्रिपाठी निरीक्षण के लिए आए। उन्होंने अनुसंधान प्रयोगशालाओं, घोड़े के संग्रहालय और पशु फार्म का दौरा किया। मुख्य परिसर में उन्होंने डेयरी फार्म में जेनी डेयरी यूनिट व क्वारंटाइन शेड का शुभारंभ किया। निदेशक डा. यशपाल ने संस्थान के वैज्ञानिकों व कर्मचारियों द्वारा किए जा रहे विस्तार कार्यों से अवगत कराया।

दैनिक भास्कर **हिसार** हिसार, रविवार 01 अगस्त, 2021 | 06

शोध • कोरोना की एंटी वायरल ड्रग और डायग्नोसिस किट के लिए प्रोजेक्ट मिला, वैज्ञानिक संक्रमण फैलने के कारण और निराकरण खोज रहे

एनआरसी के वैज्ञानिक कोरोना की दवा बनाने के नजदीक, फेज 2 में पहुंची प्रक्रिया, जल्द क्लीनिकल ट्रायल के लिए अनुमति लेंगे

एंटी वायरल दवा बनाना इसलिए मुश्किल

मनुष्य वाले कोरोना लक्षण पशुओं में नहीं मिले

किसानों के हित में रिसर्च करने को कहा

ICAR-NRCE IN NEWS

उत्तर प्रदेश से दूसरे राज्यों में ग्लैंडर्स फैलने का बढ़ने लगा खतरा

2020 में यूपी में ग्लैंडर्स की स्थिति

11,704

76

28

2019 में यह थी स्थिति

30,000

135

अम्बाला में ग्लैंडर्स से दो घोड़ों की मौत, सैंपलिंग की तैयारी

संबंधित जिलों के अधिकारियों को निर्देश जारी

सेना के कुत्तों पर होगा कोविड वैक्सीन का ट्रायल

अश्व अनुसंधान संस्थान में हलारी नस्ल की गधी के दूध की डेयरी शुरू

गधी के दूध से साबुन, लिप बाम, बॉडी लोशन बनेंगे, एनआरसीई के वैज्ञानिक रिसर्च में जुटे

• देखिए वो लैब : जहां अब तक 1.30 लाख सैंपल जांचे गए

अश्व अनुसंधान केंद्र ने एमओयू साइन किया

हिसार| राष्ट्रीय अश्व अनुसंधान केंद्र और पशुओं के ब्रूक हॉस्पिटल फॉर एनिमल्स के बीच एमओयू साइन किया गया है। जिसके तहत दोनों संस्थाएं अश्वों में होने वाली बीमारियों से बचाव, अश्व पालकों के कल्याण के लिए आवश्यक कार्यक्रम आयोजित करेगा। राष्ट्रीय अश्व अनुसंधान संस्थान की पीआरओ डा. अनराधा शर्मा ने बताया कि एमओयू पर राष्ट्रीय अश्व अनुसंधान केन्द्र के निदेशक डॉ यशपाल और ब्रूक हॉस्पिटल फॉर एनिमल्स (इंडिया) के मुख्य कार्यकारी अधिकारी ब्रिगेडियर जे एस धर्मधीरन द्वारा हस्ताक्षर किए गए। इस ज्ञापन के तहत सहयोगी अनुसंधान और प्रशिक्षण के दौरान अश्व-कल्याण पर आधारित कार्यक्रम संयोजित किये जायेंगे।

ग्लैंडर्स पर अंकुश लगाने वाले तौर का दौरा करेंगे पशु चिकित्सक

दौरा • आईसीएआर के उप महानिदेशक केंद्रीय भैंस और अश्व अनुसंधान पहुंचे

पशुओं को लेकर नई रिसर्च के निर्देश

अश्व अनुसंधान संस्थान में स्थापना दिवस पर हलारी का बुकलेट निकाला

ग्लैंडर्स को लेकर एनआरसीई में शुरू हुई कार्यशाला


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