ANNUAL REPORT 2007-2008



राष्ट्रीय अश्व अनुसंधान केन्द्र National Research Centre on Equines





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The achievements and activities of the centre from April, 2007 to March, 2008 are presented in this report. Mention of trademark, proprietary product, or firm in text or figures does not constitute an endorsement and does not imply approval to the exclusion of other suitable products or firms.

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The potential of the centre for equine welfare is set apart by the distinctive amalgam of its Vision, Values and Vitality. It represents a mix of constancy and change; of a timeless core and constantly evolving strategies and processes built around the core.



Director's Foreword



Horses have been known since time immemorial as one of the most elegant and master-friendly creatures. This centre is dedicated to the welfare of horses, mules and donkeys and their utilization for elevating the socio-economic status

of poorest of the poor equine owners. NRCE has a greater role to play in promoting utilization of equines in agricultural operations and transport of post-harvest produce in several parts of India, including hilly terrains, arid and semi-arid zones.

Not only during the period under report (2007-08) but also during my whole tenure as Director, the emphasis of the centre has been on value based R&D activities for development of equine sector under fast changing environmental and food production system. Considering expected crisis of fossil fuel and environmental pollution by green house gases, this appears a necessary proposition in years to come. Towards this end, we at NRCE took initiative in utilizing equines in agricultural operations. Such an innovative approach can lead to creation of exclusive model and conservation of indigenous equine population, generating newer opportunities in farm sector.

We at NRCE have been working relentlessly to provide solutions to some of the most serious health problems threatening existence of equines in India. It is heartening to note that due to whatever small steps taken by the centre in this endeavor, India has been recognized by the World Animal Health Organization to be free from African horse sickness, a dreaded disease of equines. Infectious equine anemia has not been reported from the country since 1997 till date. The centre also contributed immensely in timely control of glanders outbreak during the year. Our efforts are bringing fruits with increased utilization of equines in tourism, especially tourists moving in the desert of Rajasthan on the Marwari horseback.

Development of diagnostics and vaccines for infectious equine diseases has been the mainstay of your centre. The inactivated vaccine for the control of equine herpes virus-1 developed at the centre is being evaluated in the field. It has been our constant endeavor to improve and refine diagnostic tests for equine diseases. Monoclonal antibody based diagnostics for equine herpes virus-1 and equine rotavirus have been added in the arsenals of the centre. Sensitive diagnostics for EHV-4, Japanese encephalitis, piroplasmosis and trypanosomosis have been developed by the centre. These steps have helped in nation-wide surveillance and monitoring of equine diseases and in certification of equines for international movement for trade and sports.

Our centre is contributing immensely in phenotypic and molecular characterization of indigenous equine breeds, including Marwari, Kathiawari, Manipuri and Spiti breeds. Transmission of artificial insemination technology to end users needs more emphasis for conservation of indigenous breeds of horses and ponies and for production of quality mules and donkeys, for effective load carrying and eco-friendly agri-operations. This will be a small step in our efforts to improve the sustainability of subsistence level livelihood systems.

It is deeply gratifying to report that not only did this centre meet the consequential challenges successfully, but also sustained the growth rate. We grew in number (adding more scientists for R&D), we grew in our size (Veterinary Type Cultures facility is our additional mandate) and more importantly we grew in your hearts too.

I will leave NRCE by October 2008 with tremendous respect and admiration for the centre, its mandate, its vision and its staff, particularly scientists who are the most dedicated individuals and very committed professionals. Additionally, I am deeply indebted to the ICAR and series of leaders in this great organization for giving me opportunity to serve my country in a challenging role as NRCE director. I can only hope that in some small measure I have repaid their trust and confidence.

Regards and peace for all of you!



(S.K. DWIVEDI)

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Executive Summary

During 2007-08, NRCE continued efforts towards development & refinement of biologicals and diagnostics for improvement in health and production potentials of equines, nation-wide monitoring of equine diseases & consultancy services to the equine farmers and breeders. The centre also established working laboratories for Veterinary Type Cultures facilities. A brief account of achievements of NRCE in year 2007-08 is outlined below:

An equine herpes virus-1 (EHV-1) killed vaccine, incorporating indigenous strain of EHV-1 developed by the centre was undertaken for the field trial in an organized equine breeding farm at Hisar. The efficacy of the indigenous vaccine was compared with the commercial 'Pneumobort-K' vaccine in mares. The reciprocal antibody titre with both vaccines during primary vaccination was up to 16-32 and increased to 32-128 after booster dose. NRCE EHV-1 vaccine produced better VN antibody booster immune response than mares vaccinated with 'Pneumobort K'. There was no post-vaccination untoward effect in mares. Further vaccine efficacy study is under progress.

To know the status of EHV-4 infection among equines in India, a study was initiated by the centre. Out of 138 nasal swabs from the young stocks suffering from respiratory infection from two organized farms, 14 were detected positive for EHV-4 virus by multiplex PCR. EHV-4 virus was isolated from 11 of these samples using equine embryonic lung cells. These results were confirmed by sequencing of PCR products. This is the first report of isolation of EHV-4 from India.

Japanese encephalitis (JE) is an endemic disease in India. In order to study the prevalence of JE among equines, serum neutralization test (SNT) was standardized for specific differentiation of two related arboviruses i.e., JE and West Nile virus (WNV). Haemagglutination inhibition (HAI) and SNT were compared by parallel testing of 213 serum samples in both assays. The sensitivity of HAI was 96.29% (92.18%-100%, p=0.05) and specificity of HAI was found to be 100%. In addition, a comparative sero-prevalence of JE in different animal species (equine, cattle, buffalo, pigs) was done in different regions of Haryana. JE sero-prevalence was the highest in buffaloes (12.6%, n=182), followed by pigs (11%, n=163), horses (9.7%, n=185) and cattle (7.2%, n=263). The prevalence was higher in the animals in the rice cultivating areas (10.6%, n= 601) as compared to those in the non-rice cultivating areas (7.3%, n=192) of Haryana.

Equine babesiosis is an economically important disease of equids. An ELISA has been developed employing recombinantly expressed merozoite surface protein EMA-2, for detection of *Babesia equi* specific antibodies. The sensitivity and specificity of the ELISA was 94% and 96%, respectively, in comparison to commercially available OIE approved CI ELISA kit.

Trypanosoma evansi is responsible for the majority of trypanosome infections in equines. During the year, a technique was standardized for *in-vitro* cultivation of *T. evansi* using artificial media. SDS-PAGE of sonicated antigen revealed five major polypeptides in the molecular wt. range of 41-81 kDa. Further, *T. evansi* proteins of 35-41 kDa exhibited proteolytic activity.

Rhodococcus equi, a pathogen causing bronchopneumonia in foals was isolated from 4 of the 42 samples from equine foals and their environment. Hyperimmune serum to sonicated antigen of *R. equi* was raised in rabbits.

In our unique program of nation-wide active equine disease surveillance, sero-survey was conducted in the states of Rajasthan, Haryana, Punjab, Uttar Pradesh, Madhya Pradesh, J&K during 2007-08. Antibodies to EHV-1 were detected in 49 (7.1%) of the 685 samples, whereas *Babesia equi* was detected in 170 (24.3%) of the 698 sera tested; Japanese encephalitis in 35 (5.5%) of the 640 serum samples tested. None of the serum samples tested was positive for equine infectious anemia, African horse sickness, equine influenza and *Salmonella*



Abortusequi.

Glanders surveillance revealed that 43 out of 9237 equines tested were positive for glanders in the country during 2007-08. Maximum cases were reported from the state of Uttarakhand (20) followed by Andhra Pradesh (16), Himachal Pradesh (6) and Haryana (1). During the year, three different glanders antigens (mallein PPD, commercial CFT antigen and recombinant DRDE protein) were evaluated as diagnostic antigen in an indirect ELISA. Of these three antigens, only commercial CFT antigen gave good agreement with CFT for known glanders-positive and negative samples.

To study the polymorhphism of the MHC class II gene in Marwari horses, regions of MHC class-II (DRB-2a and 2b) gene fragments of 276 bp and 229 bp were amplified using Be1 and 2 primers, respectively in 22 DNA samples of Marwari horses. Restriction analysis revealed that MHC-DRB2 (276 bp fragment) on digestion with *Hin*fl exhibits polymorphism in 48.39% genotypes.

The effect of the heparin and gelatin binding proteins (HBP and GBP) from stallion seminal plasma on semen post-thaw motility was evaluated. Non-significant effect of HBP treatment on post-thaw motility was observed.

In an inter-institutional research project on characterization of indigenous breeds of equines, biometric indices of Marwari and Kathiawari horses were recorded. The study indicated that Kathiawari horses have prominent orbital fossa, concave back and nasal bone with broad face. DNA of representative animals of these breeds is being analyzed for molecular characterization of the breed.

During the year, the Veterinary Type Cultures facility of the centre established working laboratories for microbiological work and initiated work on isolation of microorganisms from clinical samples.

During 2007-08, the scientists of the centre published 13 original research articles in international and national journals and 8 research articles have been accepted for publication. In addition, 5 popular articles and 9 abstracts were published by the scientists. A training course was coordinated at the centre on monoclonal antibody production and expert lectures (4) were delivered by the scientists in various advanced training/refresher courses organized by various state universities and national institutes. Scientists participated in 15 different national and international conferences/symposia. Thirteen scientists and staff members participated in different training or refresher courses for upgradation of their skills.

The centre extended equine welfare activities in different parts of the country by organizing equine health camps and farmer meets (*Ashwa Palak Goshthis*) to educate the equine owners on various aspects of disease control and management. In addition to the treatment of major equine ailments in these camps, deworming and tetanus vaccination was done in equines. Feedback from farmers was obtained for further research and development in equine health and production. The 22nd Foundation Day of the centre was celebrated with great zeal and enthusiasm on 26th November 2007. On this occasion, a horse show was also organized in which indigenous horses from various states participated in different equestrian events.

The centre also offered consultancy and diagnostic services for important infectious diseases of equines. Under this programme, 4457 equine serum samples were tested for equine infectious anemia, 9237 for glanders in addition to other diseases. The centre generated revenue of Rs. 58.61 lakh from its internal sources, mainly through the contractual diagnostic services.



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Introduction

Homo sapiens made Equus caballus its strongest companion thousands of years ago and as this friendship grew, emerged a dependency on each other along with a mutual faith and trust. A glance on human civilization shows how strong bonding between the two species evolved and how they have co-existed. Modern age mechanization has surely decreased the dependency of the more opportunistic of the two friends but when it comes to tough terrains, poor landless, small and marginal farmers searching for their livelihood and fuel security he still requires the help of his age old companion. For National Research Centre on Equines, this bonding strengthens the commitment to improve health and production of equines for which it is working since its establishment on 26th November 1985. India has 1.70 million equines comprising 0.8 million horses and ponies, 0.2 million mules and 0.7 million donkeys.

Mandate of the centre

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

Our efforts have been concerted to understand infectious diseases confronting equines, and from that knowledge improve the efficiency and sustainability of equine farming. The invaluable research and scientific achievements of the centre since its inception continue to improve health and diminish diseases of equines in India. The vision of the centre is the enhanced utilization of equines for agricultural and transport purposes through *in situ* equine development programmes in order to elevate socio-economic status of under privileged.

The main campus of NRCE is located at Hisar

(Haryana). It has specialized laboratories for undertaking research in areas of equine virology, bacteriology, pathology, parasitology, immunology, medicine, biochemistry and biotechnology. In addition, NRCE has a sub-campus at Bikaner (Rajasthan) where a new building has come up with research laboratories for genetics and breeding, reproduction, physiology and nutrition to undertake research on equine production and management. Research activities are carried out by a team of 21 dedicated scientists under the dynamic leadership of Dr. S.K. Dwivedi, Director NRCE. The research activities are supported by centralized services like animal and agriculture farms, experimental animal facility, a small library and internet facility. The centre has well-maintained herd of Marwari & Kathiawari horses and exotic donkeys at Bikaner subcampus. In addition, the centre is in the process of development of BSL-III laboratory, agricultural technology information centre (ATIC) and Veterinary Type Cultures (VTC) facility.

OUR MISSION

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

Thrust Areas

 Surveillance and monitoring of important equine diseases including emerging and existing diseases giving special emphasis on indigenous breeds.



- Development of effective and preferably field based diagnostics and potent immunoprophylactics against major equine diseases threatening equine population in India especially those affecting reproduction, work performance, foal mortality and morbidity.
- Development of traditional herbal products for enhancement of performance in equids and management of arthropod borne equine diseases.
- To provide diagnostic, consultancy services and training to equine farmers, veterinarians and breeders of India and SAARC countries.
- Improvement and updation of the techniques involving artificial insemination and cryopreservation of the semen and embryos of true to breed indigenous horses.
- Transfer of AI technology for superior quality mule production to end users.
- In situ conservation of true to breed horses/ponies of Indian origin through AI using frozen semen of proven stallions.
- Breed characterization of indigenous breeds of horses.
- Identification and demarcation of disease-free zones with special reference to some of the important equine diseases endemic in India.
- To conduct explorative research using byproducts of equines, namely blood/serum, dung, urine, milk, placenta and hair.
- To develop balanced rations for equines of different regions based on the local resources for the benefit of underprivileged equine owners.
- Studies on utilization of equine energy (donkey, mule & ponies) for agricultural operations and transport purposes using suitable agricultural implements.
- O Promotion of equine sports activities.
- Development of linkages with Army, Ministry of Agriculture, State Animal Husbandry Departments and Agricultural Universities for integrated research programmes.

- Achieving the status of 'OIE International referral laboratory' for diagnosis of equine infectious diseases.
- Commercialization of technologies with the help of pharmaceutical and drug houses viz. kits and vaccines for equine herpes virus and eCG based ELISA for pregnancy diagnosis.

Major achievements

The salient achievements of the centre since its inception are:

- Vaccines for the control of equine diseases: The centre has developed equine influenza vaccine using indigenous isolate (A/equi-2/Ludhiana/87). Improved bacterin and outer membrane protein-based vaccines have been developed for *Salmonella* Abortusequi. Experimental as well as field trials of equine herpesvirus-1 inactivated vaccine have been completed and vaccine is ready for commercialization and release.
- Disease diagnosis: The centre has been Ο recognized as national referral centre for diagnosis of important equine infectious diseases by Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture (Government of India). The centre has developed diagnostic kits for equine herpes virus-I (HERP kit) and Babesia equi (COFEB kit) infections. In addition, the centre has developed various tests for diagnosis of equine diseases including equine influenza, EHV-1 & EHV-4, equine rotavirus diarrhoea, equine infectious anaemia, equine piroplasmosis, trypanosomosis, equine viral arteritis, Japanese encephalitis, leptospirosis, mycoplasmosis, glanders, rhodococcal and streptococcal infections.
- Equine disease surveillance: NRCE is involved in nation-wide monitoring and sero-surveillance of important equine infectious diseases, with a view to manage, control and eradicate diseases. For instance, the information generated by NRCE helped in declaring India free of African horse sickness in 2006 by World Animal Health

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Organization. Outbreaks of equine glanders in country during 2006 were timely detected and its control measures were taken to prevent its further spread. Our centre took timely control measures for equine influenza outbreaks in northern India (1987). The last outbreak of equine infectious anaemia in India was reported in 1998-99 and the centre is continuously monitoring the disease since then.

- Immunobiologicals: Monoclonal antibodies have been developed for diagnosis and characterization of equine herpes, equine influenza and equine rotaviruses. Monoclonals have also been developed against equine chorionic gonadotropin hormone.
- Molecular characterization of pathogens: DNA finger-printing of EHV-1 virus, sequencing of antigenically important genes of equine influenza virus was done to identify different strains prevalent in equines of India. Sequencing of outer surface proteins (VP4 and VP7) of equine rotaviruses for their genotyping and molecular epidemiology was done.
- Repository of veterinary pathogens: The centre has initiated efforts for establishment of a culture repository of veterinary pathogens under Veterinary Type Cultures facility.
- Artificial insemination: The technique of artificial insemination using frozen semen for production of superior quality Marwari horses, superior mules and donkeys has been perfected. The pure germplasm of endangered indigenous breeds of horses is being conserved using this technology. Seminal plasma proteins have been isolated, characterized and their role in equine fertility is being evaluated.
- Indigenous breed characterization: Phenotypic and molecular characterization of indigenous breeds of horses has indicated the existence of genetic variability within Marwari breed and molecular markers for breed identification have been established. Characterization of Manipuri



ponies is in progress.

- Baseline data has been generated on some of the important haematological, physiological and biochemical indices of Marwari and Kathiawari horses, Manipuri ponies as well as local donkeys.
- Early pregnancy diagnosis: Pregnancy diagnosis between days 14 and 18 post-insemination has been achieved using ultrasonography in donkey and horse mares. An ELISA for pregnancy diagnosis in mares using serum samples has been developed.
- Donkey fibre has been used to produce carpets by mixing with sheep fibres in the ratio of 40:60.

Patents

- Patent has been granted by the Patent Office, Government of India entitled "A method for preparation of a diagnostic kit useful for forecasting Equine Herpes Virus-I disease".
- A patent has been filed for "COFEB-Kit for diagnosis of *Babesia equi* infection in equines".
- A patent has been filed for "A method for preparing complement fixation test based (COFEB) kit for the diagnosis of *Babesia equi* infection in equines".
- The centre has filed a patent for "A kit for detection of pregnancy in equines and assay thereof".

Services

- The centre provides disease diagnostic services for various infectious and non-infectious equine diseases to equine owners, breeders, state animal husbandry departments, police and army horses.
- Artificial insemination to augment the production of superior quality Marwari horses, mules and donkeys.
- Quality jacks and jennies are supplied to various states, breeding societies and farmers, for production of superior quality mules and donkeys.
- NRCE is providing health certification for movement of equines within and outside the

country. This facility has helped in promotion of export of horses.

- Assessment and transfer of technology using the latest know-how of information technology is also given due importance to extend the technologies to the end-users. The scientific and technical staff provides clinical and diagnostic (including pregnancy diagnosis) services and consultancy to the farmers on demand in the areas of equine health and production. Farmers are imparted trainings and supplied education materials for equine management, production and health.
- Extension activities: To receive feedback from the equine owners, various activities like health camp, awareness and farmers meets are organized on regular basis in different areas of the country.

Veterinary Type Cultures facility at NRCE

lsolation, preservation and conservation of veterinary pathogens, their identification and molecular characterization can help in better risk assessment and management of risks associated with emerging and reemerging veterinary pathogens, majority of which also cross species barrier. The need for a veterinary microbial genetic resource center was felt to fulfill resource gap in the fast developing knowledge based society facing new challenges in the field of animal health. Therefore, Indian Council of Agricultural Research entrusted NRCE the responsibility of establishing Veterinary Type Cultures during the 10th plan period. The Veterinary Type Cultures became functional in June 2005 with the following objectives:

- National repository of microorganisms of animal origin including recombinant cultures and plasmids.
- Identification, characterization and documentation of microorganisms.
- Conservation, maintenance and utilization of microorganisms.
- Surveillance of indigenous/ exotic microorganisms.
- O Human resource development.

STAFF POSITION							
		NRCE		VTC			
Name of the post	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant	
Director	1	1	-	-	-	-	
Scientific	25	18	7	10	3	7	
Technical	23	23	0	1	1	0	
Administrative	11	11	0	0	0	0	
Supporting	22	21	1	0	0	0	
Total	82	74	8	11	4	7	



Expenditure & Revenue

Rs. in lacs

Sum	mary of Expenditure :	2006-07	20007-08					
	NON-PLAN							
1.	Establishment charges including LSP/PF, wages, OTA	165.80	181.09					
2.	Traveling allowances	2.90	3.89					
3.	Others including equipments & recurring charges	90.79	110.15					
4.	Works	44.81	24.99					
	Total Non-Plan Expenditure	304.37	320.12					
	PLAN							
1.	Establishment charges including LSP/PF, wages, OTA	9.36	14.62					
2.	Traveling allowances & HRD	3.62	2.25					
3.	Others including equipments & recurring charge	193.13	251.54					
4.	Works	126.23	139.42					
	Total Plan Expenditure	332.47	407.83					
	Total Expenditure (Plan and Non-Plan)	636.84	727.95					

Figures in Rs.

Sum	mary of Revenue Generation:	2006-07	20007-08						
	NON-PLAN								
1.	Sale of Farm Produce & auction of dry trees	101262.00	-						
2.	Sale of Livestock	132500.00	102700.00						
3.	Sale of Publication and advertisements	2300.00	600.00						
4.	License Fee	67885.00	70154.00						
5.	Interest on loans and advances	115606.00	81655.00						
6.	Interest on short term deposits	77593.00	484340.00						
7.	Income from internal resource generation	2359050.00	3263221.00						
8.	Receipt from services	350.00	3800.00						
9.	Other misc. receipts	837385.00	1854396.00						
	Total Revenue	3693931.00	5860866.00						



Research Achievements

Field trials of equine herpes virus-l vaccine

Field trials of EHV-1 vaccine developed by the centre was undertaken in an organized equine breeding farm at Hisar. A total of 48 equines (36 pregnant mares and 12 naïve fillies) were included in this study. The mares were divided into 4 groups. Group I (pregnant mares, n=24) were inoculated killed oil emulsion mannide monooleate-based NRCE vaccine and group II (pregnant mares, n=12) were inoculated a commercial vaccine (Pneumoabort K). Non-pregnant fillies (n=6) inoculated NRCE vaccine constituted group III while non-vaccinated fillies (n=6) served as control group IV. The first dose of vaccination to mares was done at 5 month of gestation in November 2007 and followed by two boosters each at an interval of 2 months (January and March 2008). There was no post-vaccination untoward effect in mares of any group. On primary



Antibody response to EHV-I vaccination

immunization, naïve fillies that were not vaccinated earlier produced slightly less virus neutralizing (VN) antibodies than those vaccinated earlier with EHV-I vaccine. The reciprocal antibody titre during primary vaccination was up to 16-32. After first booster, the antibody titre raised up to 32-128. The immune response following vaccination with both NRCE Vaccine and Pneumabort 'K' was comparable. However, NRCE EHV-I vaccine produced better VN antibody booster response than those mares received booster inoculation of Pneumabort 'K' (Fig.). Further immune response study is under progress.

There were two abortions in Pneumabort "K" vaccinated mares (GII) and one mare died during experiment. Similarly, two abortions and one unnoticed abortion was recorded in mares inoculated NRCE vaccine (GI). No infectious agent was recovered from any aborted fetuses of this group of aborted mares. Naïve non-pregnant fillies (n=6) vaccinated with NRCE vaccine remained healthy and showed good immune response during primary and first booster vaccination. Serum samples of second booster are being processed. Two healthy foals (one foal from each vaccinated group of mares) have delivered by vaccinated mares. The results will be analyzed for vaccine efficacy on completion of the experiment.

(B.K. Singh, Nitin Virmani & Baldev R. Gulati)

Epidemiology of EHV-4 infection in India: Isolation, characterization and serology

The status of EHV-4 infection among equines in India is not known. A study was initiated by the centre to assess the prevalence of EHV-4 infection among young ones of equines in organized equine farms and in the field. For this purpose, a recombinant protein based ELISA was developed that could specifically detect antibodies to EHV-1 and EHV-4 in the equine sera. In addition, for confirmation of EHV-4 infection, isolation system and multiplex PCR were also standardized. The detailed findings of the surveillance are as follows:

Isolation of EHV-4 virus

Out of 138 nasal swabs from the animals suffering from respiratory infection from two organized farms, 14 were detected positive for EHV-4 virus by multiplex PCR designed for detection of EHV-1 and EHV-4. PCRpositive samples were passaged in equine embryonic

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EHV-4 infected **EEL** cells

lung (EEL) and RK-13 cell cultures. Eleven out of 14 PCR-positive samples showed cytopathic effects (CPE) in EEL cells including rounding, ballooning and formation of grape like clusters followed by detachment of the cells from the surface. No CPE was observed in RK-13 cells.

PCR and sequencing to confirm EHV-4 isolation

DNA was extracted from the EEL cell lysates showing CPE and multiplex PCR was done for confirming the identity of the isolate. These samples were showing



Multiplex PCR showing 504 bp EHV-4 amplification

bands at position at 507 bp and 580 bp in multiplex PCR using glycoprotein C gene and nested PCR using glycoprotein B gene, respectively.

The PCR products were got sequenced commercially. Sequence alignment of PCR product revealed 98% homology with glycoprotein C fragment of EHV-4 (strain NS80567). The findings confirmed that EHV-4 virus has been isolated from respiratory equine infections in EEL cells. This is the first report of isolation of EHV-4 from India.

(Nitin Virmani, B.K.Singh & Baldev R. Gulati)

Development of Diagnostics for Japanese encephalitis and sero-prevalence in equines

Japanese encephalitis (JE) is endemic in India. In order to study the prevalence of JE among equines, efforts were made to develop diagnostics for JE seroprevalence and also for agent identification. During this year, serum neutralization test (SNT) was standardized for specific differentiation of two related arboviruses i.e., JE and West Nile virus (WNV). In addition, a RT-PCR was also developed for specific detection of JE virus in the affected tissue specimens.

SNT for JEV

The cell culture adapted JE virus at passage level 5 was titrated in porcine stable (PS) cells using 96-well tissue culture plate. The titer of the virus was estimated to be approximately 106 TCID50 / ml. The constant virus-varying serum dilutions method was standardized for VNT using 60, 100, 300 and 600 TCID50 / 25 μ l of the



virus and the optimum results were observed with 300 TCID50 / 25 μ I of JEV. All the known positive, hyper immune rabbit and guinea pig sera were serially diluted (two-fold) along with known negative animal sera and their VNT titer were determined. At 1:8 dilutions, all negative sera were detected negative and positive sera as positive. Hence, a cut off value of 8 was taken for the test sera. Guinea pig and rabbit positive sera gave VNT titer of 64 and above. Similarly, SNT for WNV was standardized.

Comparison of SNT and HAI

A total of 213 serum samples were included for parallel testing by both HAI and VNT. Out of 213 serum samples tested, 81 were positive by VNT and 78 were tested positive in HAI test. Since VNT is considered as golden standard serological test, the comparative sensitivity and specificity of the HAI was calculated considering VNT as a reference test. The sensitivity of

HAI was 96.29% (92.18%-100%, p=0.05) and specificity of HAI was found to be 100% (Table).

Comparison between HAI and VNT for detection of Japanese encephalitis antibodies									
Accov Booult		V	NT	Tetel	Soncitivity	Specificity			
Assay	Result	+	-	Total	Sensitivity	opecificity			
HAI	Positive	78	0	78	96.2%	100			
	Negative	3	132	135	(92.18-100%)				
	Total	81	132	213					

Standardization of RT-PCR

Primers against different regions of JEV genome including NSI and 3' NTR have been used for standardization of RT-PCR. The 3' NTR-region specific primers amplified a flavivirus-specific product of 146 bp. The RT-PCR using 3' NTR amplified both WNV and JEV. To make RT-PCR specific for the detection of JEV, the PEI/PE2 primer pair (developed for envelope protein) were used. This primer set amplified only JEV with the product size of 291 base pairs and did not show any product in WNV (Fig.). This RT-PCR is being further





tested for its specificity in differentiating JE from WNV infection in equines.

Sero-prevalence of JEV

Equine serum samples from seven different states collected during 2006-07 revealed that equines in Manipur had maximum sero-prevalence (91.67%) To assess the JE status in Manipuri equines further, the retrospective JE sero-prevalence was done by testing the archived serum samples of Manipuri equines collected during year 2005 by the centre. Out of 40 equine sera collected during year 2005, 14 (35%) were detected JE-positive. The findings of this study indicate that JE is highly endemic among equines in Manipur.

In addition, a comparative sero-prevalence of IE in different animal species (equine, cattle, buffalo, pigs) was done in different regions of Haryana. Parallel testing of serum samples by VNT and HAI showed that seventyeight (9.8%) of 793 animals tested were positive for |E antibodies. JE sero-prevalence was the highest in buffaloes (12.6%, n=182), followed by pigs (11%, n=163), horses (9.7%, n=185) and cattle (7.2%, n=263). The prevalence was higher in the animals in the rice cultivating areas (10.6%, n=601) as compared to those in the non-rice cultivating areas (7.3%, n=192) of Haryana. The corresponding respective values for the two areas were 12.5% and 3.7% in pigs, 10.6% and 5.7% in horses, 7.6% and 6% in cattle, and 13.6% and 10.9% in buffaloes. Sero-prevalence of JE in animals suggested their role in the transmission of infection which can be correlated to the outbreaks of human disease in the state.

> (Baldev R. Gulati, B.K. Singh & Nitin Virmani)



Recombinant antigen based ELISA for detection of equine piroplasmosis

Equine babesiosis is an economically important disease of equids and sporadic outbreaks are not uncommon. A significant segment of the equine population has carrier status, due to which the draugtability of these animals gets lowered and poor farmers suffer economically. Microscopic demonstration of intra-erythrocytic parasite is still the best, most reliable, economical and sustainable method for confirmative diagnosis of equine babesiosis, but not suitable for detection of carrier animals. Among serological tests, CFT used to be preferred test (as per CiE guidelines), but due to its inherent disadvantages of giving false positive results and low sensitivity this test has now been considered by OIE as alternate test for antibody detection and ELISA is now being preferred. An ELISA was developed using recombinant antigen expressed from the EMA-2 gene. The results of this ELISA were compared with OIE approved CI ELISA. Sixty serum samples of known disease status were selected (33 known disease positive and 27 known disease negative). These sera were tested

Sensitivity, specificity and predictive value efficacy of ELISA in comparison to commercial CI-ELISA

Test Status	Disease Status					
	CI ELISA		EL	ISA		
	+	-	+	-		
+	32	I	31	I		
-	I	26	2	26		
Sensitivity	0.	97	0.94			
Specificity	0.	96	0.96			
Positive predictive value	0.	97	0.97			
Negative predictive value	0.	96	0.93			





separately with ELISA and CI ELISA. Results were compared in terms of sensitivity, specificity, positiveand negative- predictive values.

The results indicated a very high correlation between CI ELISA and ELISA. This ELISA is being transformed into a user friendly ELISA kit. The initial findings indicated comparable results between the kit format and the earlier ELISA (Fig.). Further standardization of ELISA kit component is in progress.

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

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In vitro cultivation of Trypanosoma evansi

Trypanosoma evansi is most widely distributed trypanosome and endemic throughout South-East Asia. The principal hosts are equine, camel and ruminants



Trypanosoma evansi growing in artificial media

and transmitted mechanically by tabanid flies. The simplest and most direct means for the detection of protozoan found in the bloodstream is by examination of a Giemsa/ Wright stained blood

smear under the microscope. The goal of cultivation was to devise *in vitro* conditions that would retain the bloodstream morphology and physiology of the trypanosomes thus minimizing use of laboratory animals for maintenance of this organism. This is of particular importance in testing drugs for trypanocidal activity, where antimicrobial sensitivities can be affected by metabolic and synthetic pathways. Presently, there is no system for cultivation of *T. evansi* in laboratory and for its maintenance, laboratory animals (rats, mice, rabbits, etc) are in common use. Therefore, in present study an attempt was made to standardize technique for in-vitro cultivation of *T. evansi*

Blood stream forms of *T. evansi* have been cultivated in culture system by using Iscove's modified DMEM based HMI-9 medium supplemented with bathocuproinedisulphonic acid, L-cysteine, hypoxanthine, 2-mercaptoethanol, pyruvate, thymidine and 20% adult horse serum. Parasite concentration was monitored daily and further sub-culturing was done so as to maintain the growth/multiplication of parasites. Bloodstream forms continued to proliferate in the culture media and the trypomastigotes retained their morphological characteristics and further infectivity to mice (Fig.).

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

Characterization of Trypanosoma evansi antigens

In order to develop immunodiagnosis and control measures against trypanosomosis in equines, characterization of T .evansi antigen was carried out using immunobiological tools. Cell free T. evansi parasites from blood of mice pertaining to first parasitaemic wave were isolated by DEAE cellulose chromatography. SDS-PAGE profile of sonicated antigen revealed five major poly peptides among the 12-15 visible bands in the molecular wt. range of 26-81 kDa along with several other minor polypeptides. The major polypeptide bands were of 81, 78, 66, 62, 55 kDa, along with cluster of at least 4 minor closely separated poly peptides bands in the range of 35-41 kDa and two in the range of 26-28k Da. Further, proteases were analysed by gelatin substrate SDS-PAGE. The areas where the proteolytic activity had degraded, the gelatin did not stain and form clear bands (zone of clearance). The T. evansi antigen revealed strong proteolytic activity in vitro in acidic range between pH 3.0 to 5.0 while at pH

8.0 and pH 10.0, no activity was seen suggesting presence of cysteine proteases. The prominent zone of clearance were evident at molecular wt. ranging from 26 to 28 kDa,35 to 41 kDa, 67 to 69 kDa and as well as at







higher ranges also between 95 to 170 kDa in the form of multiple bands. The enzymatic activity was more intense at 26-28 kDa and 35-41 kDa size at pH 5.0. Further, studies on identification, purification, and

Development of diagnostics for Rhodococcus equi infection in foals

During the year, forty two samples (nasal, faecal, soil) collected from different parts of Haryana were tested for *Rhodococcus equi*. These included 20 nasal swabs, 20 faecal samples and two soil samples. In all 4 isolates of *R. equi* were obtained from nasal swabs of foals with respiratory problems. These isolates were subjected to in vitro antibiotic sensitivity testing to 17 antimicrobial agents which were Amoxycillin, Gentamycin, Ampicillin, Trimethoprim, Chloramphenicol, Sulphadiazine, Cloxacin, Oxytetracycline, Amikacin, Streptomycin, Cotrimoxazole, Cephalexin, Kanamycin Erythromycin,

characterization of *T. evansi* proteases for diagnostic purposes are in progress.

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

Ciprofloxacin, Neomycin and Rifampicin. Chloramphenicol, Erythromycin, Ciprofloxacin, Neomycin and Rifampicin were found to be sensitive.

Sonicated *R. equi* antigen was prepared. Protein profile was done using SDS-PAGE. Hyperimmune serum was raised in rabbit using this antigen. This was tested on agar gel immuno diffusion and showed precipitin lines. Further work for developing diagnostics is under progress.

(S. K. Khurana & Praveen Malik)

Seromonitoring of important equine diseases

During the period of report (2007-08), equine disease sero-survey was conducted in the states of Rajasthan, Haryana, Punjab, Uttar Pradesh, Madhya Pradesh, J&K. A total of 685 serum samples from indigenous equines were tested for detection of antibodies against EHV-1. Of these 49 (7.1 %) samples were positive for EHV-1. Besides, sera samples were tested for equine viral arteritis. Various samples were also subjected to virus

Sero-prevalence of diseases in various states									
State	Number tested (positive) for disease								
	EHV-I B.equi JE								
Punjab	51(1)	51 (42)	-						
J&K	319 (20)	319(15)	319(0)						
Rajasthan	48 (4)	48 (6)	48 (0)						
Haryana	12(0)	12(5)	12(3)						
U.P.	51 (0)	51 (20)	51 (4)						
M.P.	204 (24)	217 (82)	210 (28)						
Total	685 (49)	698 (170)	640 (35)						



isolation.

Out of 698 equine sera tested for *Babesia equi* antibodies by ELISA, 170 (24.3%) were found positive. Sero-surveillance of JEV antibodies showed 35 (5.5%) out of 640 equines positive.

HI test for equine influenza was conducted on 698 samples from indigenous equines yielded negative results. None of the 698 serum samples tested was found positive for *Salmonella* Abortusequi (H antigen), glanders and equine infectious anemia.

In addition, samples from various private organizations, quarantine stations and other establishments were tested for various diseases. For EIA, 4457 serum samples from thoroughbred as well as indigenous equines were examined by Coggins test, however, none of the samples tested was found positive.

Cultural examination

Bacteriological analysis was done of 144 samples. These samples including nasal swabs, vaginal/cervical swabs, aborted foetus and contents, PM tissues, pus/wound/ other lesions, eye swab, blood, faecal swab/sample and soil samples. Twelve isolates including Burkholderia mallei (6), E. coli (2), Streptococcus equi subsp. zooepidemicus (1), Group C Streptococci (3) were obtained. A total of 324 samples from animal quarantine

Isolates recovered and their origin							
Isolate	No.	Place of Origin					
E. coli	2	Haryana (2)					
B. mallei	6	A.P.(5), HP(1)					
S.equi subsp. zooepidemicus	I	Rajasthan (1)					
Group 'C' S. equi	3	Rajasthan (3)					

centres including 242 vaginal swabs and 82 preputial swabs tested for CEM were negative.

Glanders seromonitoring

Glanders re-surfaced in the country during 2006-07 when equine cases were reported from Maharashtra, Punjab, Uttarakhand and Uttar Pradesh. NRCE continued its efforts throughout the country to monitor the disease during 2007-08. During this year, glanders was reported from Uttarakhand, Andhra

Pradesh, Himachal Pradesh and Haryana. In Uttarakhand 20 more serum samples out of 284 tested were found positive by CFT during the year. From Andhra Pradesh 16 out of 2021 equines were positive and 5 isolates of *Burkholderia mallei* were obtained. In Himachal Pradesh 6 out of 252 equines tested were positive and one isolate was made. One serum sample out of 292 serum samples tested was found positive in Haryana. Overall out of 9237 equines tested throughout the country 43 were positive by CFT and 6 isolates were obtained.

Miscellaneous

On the etiopathological front, PM/biopsy/morbid material revealed cases of granulomatous pneumonia and bronchopneumonia (4), sarcoid (1), anoxia (2), disseminated intravascular coagulation due to multiple thrombi formation (1).

A software program for depiction of diseases has also been developed under the project. The software aptly describes the disease status in various states of the country during different periods of time.

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

RFLP-based genotyping of major histocompatibility complex class II genes in Marwari horses

Twenty two blood samples of Marwari horses (16 from field and 6 from EPC farm) were collected to study MHC class II genes. The DNA was isolated from the blood samples using phenol-chloroform extraction method. The PCR conditions for amplification of MHC-DQA loci were optimized and a fragment of 246 bp was successfully amplified using DQA 2e & DQA 2f primers in Marwari horses. Digestion of MHC- DQA fragment was carried out with *Mspl*, *Nsil*, *Pvull* and *Hinfl* enzymes but no RE site was found.

Digestion of MHC- DRA fragment (229 bp) was carried out with Nsil, HindIII, Mspl, Pvull, EcoRI enzymes but no RE site was found. Whereas, digestion of above loci fragment with *Hae* III enzyme resolved two bands of 165 bp & 64 bp and with *Hin*fl enzyme resolved 200 bp & 29 bp.

Digestion of amplified fragment (276 bp) of MHC-DRB2 with *Hin*fl revealed polymorphism. The homozygous genotypes were 51.61% with band size of 226 bp and 50 bp. The heterozygous genotypes were 48.39% and the bands were of 276 bp, 226 bp and 50 bp. The restriction enzyme *Mspl* digestion resolved all animals to be homozygous with 161 bp plus more than one fragment of remaining part. RFLP with *Ha*elll enzyme resolved that all the animals were homologous with 212 bp & 64 bp and 160 bp &116 bp fragments whereas, with *Rsa*l





enzyme, multiple cutting sites yielding fragments of \leq 50 bp were observed. RFLP analysis showed its potential to group the animals into different classes and genotypes

so obtained will be sequenced to know their bands pattern in a more precise way.

(R.C. Sharma & S.C. Mehta)

Effect of herparin and gelatin binding proteins on maintenance of post-thaw motility of cryopreserved horse semen

The effect of the isolated heparin binding proteins (HBP, five proteins ranging from 17 to 83 kDa) and gelatin binding proteins (GBP, four proteins ranging from 18 to 83 kDa) from stallion seminal plasma as semen additives on maintenance of post thaw motility (%) during cryopreservation was evaluated. Using a protein dose of 40 μ g of HBP and GBP, non-significant difference were observed in the maintenance of post-thaw motility % between HBP treated and control groups. The GBP treated group showed decline in the maintenance of post-thaw motility % which was significant (P<0.01).

motility of sperms								
S.No	Post-thaw motility (%)							
	Control	HBP(40µg)	GBP (40µg)					
I.	40	45	30					
2	40	40	25					
3	45	45	20					
4	40	40	25					
Mean±SE	41.25±1.25	42.5±1.44	25.00±2.04					

The isolated stallion seminal plasma proteins (40 μg of the HBP and GBP) were used as semen additives for



Chromatin integrity using acridine orange stain

maintenance of post-thaw chromatin integrity (%) during cryopreservation. Chromatin integrity status was assessed with acridine orange test and observed in fluorescence microscopy (Fig.). Non-significant differences were observed in the maintenance of postthaw chromatin integrity between proteins (HBP and GBP) treated and control groups.

(A. Arangasamy)



Isolation, maintenance and characterization of bacterial pathogens and their molecular identification

Under this project, during 2007-08, 24 horse samples were processed for isolation studies. Out of these 16



CAMP test : Typical shovel heamolysis confirming Rhodococcus species

nasal swab, 6 vaginal, one Lung sample and one ocular sample were microbiologically processed by plating on Sheep Blood Agar for pathogen isolation. A total 43 isolates were picked up, out of which 25 were Grampositive, 13 were Gram-negative, 4 were unclassified due to unclear Grams reaction and one was a fungal isolate.

Out of 13 Gram-negative isolates, 3 were Pseudomones aeruginosa (one α -hemolytic and 2 are β -hemolytic) two Escherichia coli, one Aeromones. All 3 pseudomonads produced green pigment and were multi-drug resistant. Among the isolates 2 are Escherichia coli. Seven unclassified Gram-negative rods remain to be characterized up to species level. Among the 29, Gram-positive isolates, 3 belonged to pigmented Kocuria spp., 6 Staphylococcus spp (out of which is one β -hemolytic was used in CAMP test (Fig.) for *Rhodococcus* spp. diagnosis), 6 α and β -hemolytic Streptococcus spp. including Streptococcus equi isolates. Six isolates of Coryneform bacteria have also been isolated which include strains of Rhodococcus equi isolates from pneumonic lung of a foal. There are 2 Bacillus spp. and 2 unclassified Gram-positive cocci. Four isolates remain to be classified.

> (Rajesh Kumar Vaid, Sanjay Barua & Mamta Tigga)



Inter-institutional Research Projects

Molecular characterization of Indigenous breeds of horse for genetic diversity within and between different breeds

A research project in collaboration with National Bureau of Animal Genetics Resources, Karnal for characterization of indigenous breeds of equines is under progress at the Centre. During the year, phenotypic characterization of Marwari and Kathiawari horses was undertaken along with collection of blood samples for molecular characterization work. For this, fifty unrelated Kathiawari horses, true to their breed were selected among equines maintained in different farms around Junagarh, Gujarat while 65 true-to-breed Marwari horses from the farms around Jodhpur and Bikaner (Rajasthan) were also included in the study. Salient findings are:

Phenotypic characterization: The phenotypic characteristics of Kathiawari and Marwari breed

than in mares. Important features of Kathiawari breed included prominent orbital fossa, concave back and nasal bone with broad face. Out of 18 stallions, bay was quite prominent (11) coat colour followed by chestnut (4) and grey (3) among stallions while in mares chestnut was the most common colour (16) followed by bay (8), grey (5) and skew bald (3). In Marwari horses, average values of almost all the phenotypic indices except body length and, ear length and ear width were appreciably higher in stallions than in mares. Bay, chestnut, grey and skewbald were important coat colours (Table).

A comparison of overall phenotypic characteristics of both Kathiawari and Marwari horses revealed that all the indices except height at hock and face width were higher in Marwari horses.

Mean p	Mean phenotypic characteristic of Kathiawari and Marwari horse (in mm)											
Animal details	Parameters	Body length	Height at wither	Heart girth	Hind leg length	Height at hock	Foreleg length	Height at knee	Face length	Face width	Ear length	Ear width
Stallion	Kathiawari (18)	153.2	150.9	167.7	97.5	60.4	99.1	45.9	66.6	19.6	11.7	8.4
	Marwari (15)	155.4	157.0	173.8	102.6	57.4	101.1	48.4	65.9	19.1	13.0	9.6
Mares	Kathiawari (32)	150.4	148.1	164.9	96.6	59.5	96.3	45.2	63.6	19.1	11.8	9.9
	Marwari (50)	157.2	155.0	174.1	98.0	55.0	98.8	46.9	65.6	18.2	13.5	10.1
Overall	Kathiawari (50)	151.3	149.3	165.9	96.8	59.6	97.1	45.5	64.8	19.4	11.7	9.2
	Marwari (65)	156.8	155.4	174.0	99.1	55.5	99.4	47.2	65.6	18.4	13.4	10.0

are presented in the Table. No appreciable difference was observed in animals of both the sexes in Kathiawari horses. However the average values of most of the features was higher in stallion Molecular characterization: Blood samples of both Kathiawari and Marwari animals were processed for DNA isolation for further use in PCR standardization protocol. PCR standardization





with 27 microsatellite has been done using different PCR conditions (Fig). Studies on multiplexing with fluorescence labeled microsat and polymorphism with different primers is under progress.

(A.K. Gupta, S.N. Tandon, Mamta Chauhan, S.C. Gupta & Neelam Gupta)

Gel electrophoresis of PCR products

Usefulness of recombinant protein for serodiagnosis of glanders

A research project in collaboration with Defence Research & Development Establishment (DRDE), Gwalior to evaluate the usefulness of a recombinant protein for serodiagnosis of glanders is under progress at the Centre. We previously evaluated the two different batches of recombinant protein provided by



Indirect ELISA for glanders using CFT antigen

DRDE by complement fixation test (CFT) for diagnosis of glanders. In our study, the recombinant proteins did not work satisfactorily in CFT.

During the year, three different glanders antigens (mallein PPD, commercial CFT antigen and a recombinant protein provided by DRDE) were evaluated as diagnostic antigen in an indirect ELISA. A total of 255 known negative and 15 known glanderspositive equine serum samples were used in the evaluation. The indirect ELISA using CFT antigen showed a clear cut difference in positive and negative samples. Though mallein PPD and recombinant protein could differentiate the positive and negative samples, the difference in the ODs of positive and negative samples was not always significant.

The results using recombinant protein did not appear to work in the CFT satisfactorily. Further evaluation of the recombinant protein in other immunoassays is under progress.

(Praveen Malik & Santosh Kumar)



Technologies Assessed

Field assessment of monoclonal antibody based immunoassay for detection of rotavirus in different animals

The centre has developed a sensitive and specific enzyme-linked immunosorbent assay (ELISA) employing a monoclonal antibody (mAb) raised against group-specific protein VP6 of equine rotavirus for detection of equine rotavirus (ERV) from stool samples. This assay specifically detected rotavirus and did not react with other representative equine viruses. The ELISA has been found to be 100% sensitive specificity of 0.96 (0.85410.9932, P<0.05) in comparison to virus isolation. This assay could detect rotavirus in the stool of various animal species including bovines, porcines, ovines and human infants.

Field assessment of the diagnostic for detection of equine rotavirus in diarrhoeic foals has already been done. The assessment of efficacy of the rotavirus diagnostic in other species of animals was done by two national laboratories working on bovine and ovine rotaviruses.

The Department of Animal Biotechnology, CCS Haryana Agricultural University, Hisar tested a total of 455 faecal samples collected from five organized buffalo farms in northern India utilizing this monoclonal antibody based ELISA. A total of 33 samples were positive for group A rotavirus and per cent positivity ranged from 3.22 to 28% with an overall prevalence of 7.25%. The same set of samples was also tested by RNA-PAGE and RT-PCR. The sensitivity and specificity of ELISA, RNA-PAGE, and RT-PCR was found to be 100%, 66.67% and 71.43% and 100%, 98.63%, 98.43% respectively.

This rotavirus diagnostic was also employed to test the ovine samples for rotavirus by the Department of Veterinary Microbiology, Shere-Kashmir University of Animal Sciences & Technology, Jammu). Of the 241 stools samples collected from diarrheic lambs, 11 (4.56%) were detected positive for rotavirus. The ELISA positive ovine samples were also confirmed for rotavirus infection by other assays including RT-PCR.

These findings of external laboratories confirm that the monoclonal antibody based immunoassay developed by the Centre is a sensitive and specific assay for routine diagnosis of rotavirus in different species of animals.

Assessment of recombinant protein based ELISA for EHV-1 and EHV-4 diagnosis

Equine herpes virus infections are a cause of world wide epidemics of equine rhinopneumonitis. Two viruses EHV-1 and EHV-4 collectively contribute to this disease entity. EHV-1 causes outbreaks of abortions, respiratory disease, perinantal foal mortality and neurological disorder whereas EHV-4 is primarily a respiratory pathogen. These two viruses have extensive cross reactivity and can not be differentiated using routine diagnostic techniques. To address this problem and to know the status of EHV-4 infections in India, a serological assay was developed using recombinant proteins from a fragment of glycoprotein G gene of EHV-1 and EHV-4. This indirect type specific ELISA was shown to be capable of distinguishing the antibodies against EHV-1 and EHV-4.

The assay developed was assessed for field utility and antibodies against EHV-4 were surveyed in 611 serum samples which included 259 fresh samples and 352 samples from retrospective studies. The test is further being developed into a kit for the benefit of the endusers. The findings indicate 463 out of 611 (75.70%) equines in the field have antibodies against EHV-4 infection.



Validation of the recombinant protein-based ELISA for diagnosis of *Babesia equi* in equine

Equine babesiosis is recognized as a serious problem of major economic importance. The affected animal manifests decreased working capacity, loss of appetite, etc. Two different protozoa, *Babesia caballi* and *Babesia equi* are known to cause infection. *Babesia equi* infections are more wide-spread and pathogenic than those caused by *B. caballi*. In *B. equi*, two kinds of merozoite surface proteins, equi merozoite antigen (EMA) -1 (34 kDa) and -2 (30 kDa), have been identified as most immunodominant antigens. EMA-1 and -2 genes have 52 % amino acid identity with each other.. We expressed the truncated ORF of the gene EMA-2 (merozoite surface of *B. equi*) in *E. coli* and harvested the specific protein/antigen. This protein

was used in ELISA for specific diagnosis of *B. equi* antibodies. We standardized this ELISA for specific diagnosis and no cross-reaction was observed with *B. caballi* antibodies.

Further, the assay was validated on serum samples of known disease status, which had been collected after experimental infection with *B. equi* parasite. The assay detected the specific antibodies as early as 6 days post-infection. We collected field samples from identified areas of Haryana and Rajasthan state and assessed the status of B. equi antibodies with this assay. A total 74 and 128 serum samples were collected form Haryana and Rajasthan and we recorded 68.9 % and 21.8% seropositivity, respectively by this assay.



Consultancy & Commercialization of Technology

Consultancy

This centre offers consultancy and diagnostic services for investigation of equine diseases in the country. Under this programme, equine disease investigation is done on-farm by sending teams of experts to different parts of the country. In addition, veterinarians, equine owners and quarantine officers submit equine samples to the centre for disease investigation. The results of such investigations are conveyed along with advice to the concerned for taking necessary measures for treatment and control of equine diseases. During 2007-08, a total of 4457 equine serum samples from animal quarantine stations and equine farms were examined for equine infectious anemia (EIA) by Coggins test. None of the samples tested was positive for EIA.The centre also tested 9237 equine serum samples for glanders by CFT. In addition, samples from equines were received in the centre for contagious equine metritis, piroplasmosis, rhinopneumonitis, equine influenza and bacterial infections.

Commercialization of technology

The technologies developed at the centre including diagnostic services helped in generation of revenue for the centre. During the year, Equine Health Unit of the centre has generated revenue to the tune of Rs.39,92,703/- by testing samples for various diseases including equine infectious anaemia (Rs.14,78,103/-), contagious equine metritis (Rs.2,93,000/-), glanders (Rs.21,08,500/-), equine viral arteritis (Rs. 2000/-),

EHV-1 (Rs.16,000/-), piroplasmosis (Rs.36,400/-), Equine Influenza (Rs.57,000/-), *Salmonella* Abortus equi antibodies (Rs.1000/-) and bacteriological analysis (Rs.700/-). In addition, improved germplasm of equines was also provided to the farmers in different parts of the country. Artificial insemination using exotic jack semen was done in field in different states to improve mule production in the country.



Education and Training

Trainings and Short Courses Organized at NRCE

An advanced training was organized by the centre in collaboration with the Centre of Advanced Studies in Veterinary Microbiology, CCS Haryana Agricultural University, Hisar during January 23- February 12, 2008. Dr. Baldev R. Gulati, Senior Scientist, coordinated practicals and lectures at the centre in this 21-day training course on "Techniques in Murine Monoclonal Antibody Production".



Participants in the training at NRCE

Participation in Scientific Training

- 1. Dr. Rajender Kumar, Sr. Scientist participated in First TrainingcumWorkshop on "IP and Technology Management in ICAR System", held at NAARM, Hyderabad from May 28-30, 2007.
- 2. Dr. A. Arangasamy, Scientist participated in the training programme on "Ultrasonography for the Veterinary Practitioner" held at Department of Clinics, Madras Veterinary College, Chennai from July 2-6, 2007.
- 3. Dr. Ramesh Kumar Dedar, Scientist and Dr. Jitender Singh, Veterinary Officer, attended a training on "Use of Endoscope and Ultrasonograph in Equines" at Kunigal Stud Farm, Distt. Tumkur and Bangalore Turf Club, Bangalore from September 17-29, 2007.

- 4. Dr. Mamta Tigga, Scientist and Dr. Ramesh Kumar Dedar, Scientist NRCE, Hisar participated in the practical training course on, "DNA based diagnostics" organized at Department of Animal Biotechnology, CCS HAU, Hisar from November 13 December 4, 2007.
- 5. Dr. Niranjan Lal, Scientist participated in the training course on "Empowerment of rural youth through participatory extension methodology" organized at Division of Agriculture Extension, Indian Agriculture Research Institute of New Delhi from December 12, 2007-January 19, 2008.
- 6. Dr. Yash Pal, Senior Scientist, participated in the short course on "Application of Embryonic Stem Cells in Livestock Research and Production" organized at CIRB, Hisar from December 10-19, 2007.
- Dr. Nitin Virmani, Sr. Scientist participated in training course on "Road to Toxico-pathology" organized by Charles Louis Davis D.V.M. Foundation in association with Society for Toxicologic Pathology in India at Pune from March 13-15, 2008.
- 8. Dr. Rajender Kumar, Sr. Scientist participated in training programme on "Capacity building for Intellectual Property Protection and Technology Licensing in Agriculture under Indo-US Agricultural knowledge initiative" organized at CCS HAU, Hisar from February 11-13, 2008.
- 9. Dr. Rajender Kumar, Sr. Scientist attended Training-cum-Workshop on "IP and Technology Management in ICAR system" organized by National Academy of Agricultural Research Management, Hyderabad from May 28-30, 2007.

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- Dr. Sanjay Kumar, Senior Scientist participated in the EU-INDIA Grid Workshop on "Applications in Computational Biology" (under TEQIP) organized by Maulana Azad National Institute of Technology, Bhopal from May 5-9, 2008.
- Dr. A.K. Gupta, Pr. Scientist, Dr. B.K. Singh, Pr. Scientist, Dr. R.C. Sharma, Sr. Scientist, Dr. Rajender Kumar Sr. Scientist and Dr. A. Arangasamy, Scientist participated in Trainingcum-Workshop on "IP and Technology Management in the ICAR systems" organized by CCSHAU from May 19-21, 2008.

Participation in Administrative Training

 Sh. Hawa Singh, Assistant and Sh. S. P. Kaushik, Assistant participated in training programme on "Purchase Procedures" organized at ISTM, New Delhi from May 14-16, 2007.

Training for Post-Graduate Students

- Dr. Baldev R. Gulati, Senior Scientist as coadvisor guided M.V.Sc student of College of Veterinary Sciences, CCS HAU, Hisar, on "Seroepidemiological studies on Japanese encephalitis among animals in Haryana"
- 2. Dr. P. Malik, Senior Scientist as co-advisor guided M.V.Sc student of College of Veterinary

Sciences, CCS HAU, Hisar, on "Studies on serodiagnosis of Glanders using various antigens".

Lectures, Practical Trainings and Demonstrations for Advanced Training, Refresher Courses

- Dr. Sanjay Kumar, Senior Scientist delivered an invited lecture on 'Recent advances in diagnosis of *Babesia equi* and *Trypanosoma evansi* infection in India' in the Ist International Meeting for Protozoan Diseases organized by National Research Centre for Protozoan Diseases, Obihiro, Hokkaido, JAPAN, from September 6-7, 2007.
- 2. Dr. P. Malik, Senior Scientist delivered an invited lecture on 'Glanders- the current status, pathogenesis, diagnosis and control measures to be adopted for containing the disease' during a seminar organized by GKVS-BHA Equine Welfare Project, Haldwani, Nainital on April 16, 2007.
- Dr. P. Malik, Senior Scientist, delivered a lecture on 'Glanders- its diagnosis in field and lab' during the meeting of Directors of Animal Husbandry Departments held at NRCE Hisar on October 30, 2007.

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RAC, IRC & IMC Meetings

Research Advisory Committee Meeting The 9th RAC meeting of NRCE was held under the chairmanship of Dr.S.K. Garg, former Vice-Chancellor, Veterinary University, Mathura on May 5th 2007 to discuss various scientific, administrative and policy matters. On the matter of filling up of vacant posts, the RAC again recommended to submit the same along with additional posts as per the recommended Staff Ratio (Scientific to Technical to Administrative to Supporting - 1:1.5:0.75:2) in 11th five-year Plan document to the



RAC discussing research priorities of NRCE

Council. RAC recommended upgradation of the Centre to National institute. On HRD issue, RAC recommended that the international trainings must be specifically permitted to NRCE personnel viewing limited availability of the experts in the specialized field in the country and for developing this centre as a Centre of Excellence for R&D in equine sciences in India. The RAC approved four new research projects and two research projects were recommended to discuss in IRC meeting. The Chairman RAC appreciated the work on cryo-preservation of stallion semen of Marwari stallions at farmers' door for the conservation of threatened breeds. He also applauded the Director and NRCE staff for newly extended laboratory facility at Hisar.

The 10th RAC meeting was held under the chairmanship of Dr.S.K. Garg, Director, College of Applied Education & Health Sciences, Meerut on Oct. 6th 2007 at EPC (NRCE), Bikaner. The committee appreciated the efforts of NRCE in containing the glanders outbreak. The RAC suggested organizing a meeting of the Directors of state Animal Husbandry departments to apprise them of the seriousness of the glanders and control measures.

The chairman RAC highlighted the need and importance of proper recording/maintenance of raw research data in each laboratory to avoid any discrepancy or conflicts in presentation of research results and IPR issues.

Institute Research Committee Meeting

The IRC meeting was held under the chairmanship of Dr.S.K. Dwivedi on May 16th - 17th, 2007 to discuss the progress made in various on-going research projects. Four new research project proposals were approved by the IRC. The chairman emphasized that the technologies ready for transfer should be popularized through centre's website, press, media and publicprivate interface. The chairman also emphasized the need of sharing of resources for optimal use.

IRC met again at EPC, Bikaner on July 23rd, 2007 at EPC (NRCE), Bikaner to discuss the research related to equine production. The Chairman again emphasized that new project should be formulated keeping in view priority of the end-users, vision and mandate of the centre, availability of manpower & facilities and necessity/demand of the nation. Projects should be formulated to explore use of equine energy in agricultural and transport purposes in view of depleting fossil fuels, rapid climatic change and global warming.

IMC Meeting

The 28th meeting of the Institute Management Committee (IMC) of the National Research Centre on Equines, Hisar held on December 26, 2007 at NASC Complex, Pusa, New Delhi under the chairmanship of Dr. S.K. Dwivedi, Director. The IMC approved revised rates for glanders testing and and approved providing free-of-cost artificial insemination services to indigenous equines at NRCE, in the best interest of nation.





Workshop, Seminars & Institutional Activities

State Animal Husbandry Directors' Meet on Glanders

A meeting of Directors of State Animal Husbandry Departments was organized at the Centre on October 30, 2007 to discuss the emergence of glanders and steps to be taken for containment of the disease in the country. The meeting was chaired by Dr. S.K. Dwivedi,



Delegates discussing the status of glanders in India

Director NRCE and was attended by the State Animal Husbandry Directors (or their representatives) representing Haryana, Punjab, Himachal Pradesh, Uttar Pradesh, Madhya Pradesh, Rajasthan, Andhra Pradesh and Manipur. Addressing the delegates, Dr. Dwivedi explained the recent status of glanders in the country and emphasized the need to curtail its spread.

After in-depth deliberations, specific recommendations were made for controlling the disease, including registration of all equines; monitoring the movement of equines; testing sera of equines participating in sports or animal fairs by CFT; surveillance, monitoring & reporting of glanders outbreaks and elimination of infected animals with suitable compensation to the equine owners.

Equine Health Camps in J&K and Punjab

A clinical camp was organized at Katra, Jammu from 22nd to 26th October, 2007. Major ailments observed in animals of this region included lameness, colic, saddle wounds, retention of urine, etc. The sick animals were provided necessary treatment in the health camp. All animals in the health camp were given doses for deworming, mineral mixture and vitamin supplements. Animals were also examined for reproductive disorders by ultrasonography. A total of 319 serum samples collected in this camp were tested for major equine diseases at the centre. EHV-1 infection was prevalent in 20 (6.3%) and *Babesia equi* infection in 15 (4.7%) of the sera tested.



Scientists treating equines in a health camp at Katra

In addition, an equine health camp was organized at Bhagsar village, Muktsar (Punjab) on 29th August 2007, in which 51 equines were treated for various ailments and their samples were collected for disease diagnosis. Prophylactic anti-surra drug was injected to all the equids in the health camp.



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ifr; kfxrk esiFke LFkku iklr fd; kA lekiu lekjkg dkslækf/r djrsgq iHkkjh funs kd MkW v'kkd xtrk usnSud dk; kæsfgUnh dk vf/d lsvf/d iz kx djusdsfy, ifjr fd; kA

InHkkouk IIrkg ds nk§ku dk0; xk\$Bh dk vk; kstu

dbinz ea 19&25 uongj 2007 dks i nHkkouk i irkg euk; k x; k fti ds i eki u volj ij 25 uongj dks, d dk0; xksBh dk vk; kstu fd; k x; kA bi dk0; xksBh eafgi kj dsx.kekU; dfo; ka



dk0; xksBh eadfork i kBu djrsgq fofHkUu dfo

us I nHkkouk fo"k; ij dfork ikB itrr fd; kA dk; De dh vè; {krk gfj; k. kk dsi Fke jkT; dfo] Jh mn; Hkkuqgal usdh rFkk bl lekjkg eal kekftd lå Fkkvkadsi frfuf/; kausHkh Hkkx fy; kA lå Fkku ds funs kd MkW 'Kyshnz f}onh us vius lacks⁄u ea lHkh ukxfjdkal stkr&ikr dh Hkkouk I sÅij mBdj jk"V^a dksfeytgy dj le¼ cukusdsfy, ÑrladYi jgusdh vihy dhA ekuuh; dfo; ka}kjk itrr dh x; hjpukvkadh JkrkvkausHkhj&Hkhj i*t* ka k dhA

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I & FKku ea 26 ruojh 2008 dks 57 okax.krak fnol dk ∨k; kstu fd; k x; k4 funsikd egkn; MKW 'KSykhızf}onsh us èotkjikg.k dj jk"Vh; frjaxs dksl ykeh nh4 l & Fkku ds deipkfj; ka dksl Ecks/r djrsgq funsikd egkn; usnsik dsifr i wk2fu"Bk l sdk; Zdjusdk egkiq "Kkadsin fplgkaij pyusdk ∨kgeku fd; k4 bl ∨olj ij l & Fkku ds deipkfj; kao mudscPpkaus, d jaxkjax dk; Øe i trr fd; k4

Visit of Dignitaries

Dr. Bujarbaruah applauds research achievements of the Centre

Dr. K.M. Bujarbaruah, Deputy Director General (Animal Sciences), ICAR and Dr. C.S. Prasad, Assistant Director General (AN&P) visited the Centre on 24th August, 2007. During the visit, Dr. Bujarbaruah laid the foundation stone for establishment of Agricultural Technology Information Centre (ATIC). Dr. S.K.



Dr. Bujarbaruah laying the foundation stone of ATIC building

Dwivedi, Director highlighted the on-going research and production programs of the Centre to the DDG. Dr. Bujarbaruah was impressed by the infrastructure and research achievements of the Centre in the area of development of diagnostics and vaccines. Addressing the scientists, he emphasized the need of competitive capacity building on a sustained basis, for which the plans may be made by the Centre. Dr. Bujarbaruah visited the laboratories of the Centre and had conversation with scientists in their laboratories.

Gen. Mohanty Felicitated by NRCE

Lt. Gen. Narayan Mohanty, AVSM, VSM, the first Director General Remount Veterinary Services visited the Centre on 4th August 2007. Addressing the scientists, Gen. Mohanty emphasized the need of collaborative efforts for improving the equine productivity and performance. On this occasion, Dr. S.K. Dwivedi, Director NRCE felicitated Gen. Mohanty for his exemplary contributions in the field of equine health and production management. Dr. Dwivedi highlighted that under the visionary



Gen Mohanty being presented citation by Director NRCE

stewardship of Gen. Mohanty, effective scientific linkages and collaborations between RVC and NRCE have been initiated. These efforts have helped us make significant progress towards development of equine disease diagnostics and vaccines.

Arvind Ji Visited Bikaner Campus

Shri Arvind Singh Mewar Ji from Royal family of Udaipur and a renowned equine lover visited Equine Production Campus, Bikaner on 18th August 2007. On this occasion,



Sh Arvind ji being apprised of AI activities

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Dr. S.K. Dwivedi, Director highlighted the role played by NRCE in conservation of Marwari breed. He emphasized the significance of unique policy of fieldlevel cryopreservation of the semen of the prized Marwari stallions for inseminating the mares kept at our farm. Shri Arvind Singh *Ji* was very much impressed to see the quality Marwari foals born from such mares. He extended his help to the Centre for implementing Marwari horse improvement programme in Rajasthan.

Russian Delegation visits NRCE

A four-member Russian delegation, led by Mr. Ivanov Andrey, Vice-President, Russian Academy of



Russian delegates during visit to NRCE

Agricultural Sciences (RAAS), Russia visited centre on 21st November 2007 to explore the possibility of collaborative research between Russia and India. Other members of delegation included Mr. Lukomets Viacheslav, Director, All Russia Science Research Institute of Oil Crops; Mr. Kulikov Ivan, Director, All Russia Research Institute of Horticulture and Seedlings and Ms. Kurnyavko Natalia, Head Specialist of Foreign Relations Department of RAAS, Russia. Dr S.K. Dwivedi, Director apprised the members of the delegates about the research activities of the Centre and possible areas for collaboration.

Visit of other distinguished dignitaries

Dr. S. Ayappan, DDG (Animal Sciences) visited the Centre on 4th April, 2007. He applauded the work being carried out at NRCE, Hisar.

Dr. A. Nour, Director of International Programs and Dr. Augustine Peter, Professor of Veterinary Obstetrics and Gynaecology, School of Veterinary Medicine at Purdue University visited NRCE, Hisar on 7th June, 2007 as a part of four member delegation exploring the possibilities for collaborative research work and education programmes.

Dr. N.K. Tyagi, Member, Agricultural Scientist Recruitment Board, ICAR, New Delhi visited our Bikaner campus on 18th December 2007. Dr. S.N. Tandon apprised him about the efforts of the Centre in producing exotic donkeys for mule production and improvement of Marwari breed.

Dr. R.M. Acharya, former DDG (AS) visited the centre on 29th October, 2007. He had a meeting with the scientists of the centre.

A delegation of board members of Karnataka University, Bidar comprising Prof. S.N. Jayadevappa, Smt. Sheela Tiwari, Sh. M. Rajamna, Sh. Samad Siddique and Sh. N.G. Govindaiah, Special officer visited NRCE, Hisar on 20th November 2007. They were apprised about the research activities of the institute.

Col. N.S. Kanwar, Commandant Equine Breeding Stud, Hisar visited NRCE on 24th January, 2008 and he was shown the infrastructure and facilities available with NRCE for carrying out research for the welfare of the equines. He stressed for close association and collaborations between NRCE and army for the benefit of the horses in the country.



Infrastructure Development

Construction work for BSL-3 Laboratory started

The work on the construction of biosafety level 3 laboratory at Hisar campus started in 2007 and is targeted to be completed by the end of 2008. The facility with an estimated cost of Rs. 3.0 crores will include state-of-the-art biosafety level 3 research laboratories for handling equine pathogens and laboratory animals. The BSL-3 laboratory will cater to the need of working on infectious animal diseases that can spread rapidly from animals to animals and also on diseases that can be transmitted from animals to humans. The BSL-3 laboratory will promote health and safety in the laboratories so that diseases do not become risk for the community and the environment.

As per World Health Organization (WHO) codes of practice for the safe handling of pathogenic microorganisms and DBT guidelines, BSL-3 laboratory facility is essential for working on indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure. Such laboratories have special engineering and design features like controlled double-door access, ducted exhaust air ventilation and have specialized biosafety cabinets to handle these pathogens. Air entering and exiting from the BSL-3 laboratory is passed through special HEPA-filter to make it free from pathogens. These features are essential for the safety of laboratory workers and to prevent the release of pathogens from the laboratory thus safeguarding the community and the environment.



Prototype of BSL-3 laboratory being constructed

Establishment of a Veterinary Type Cultures facility

The centre has initiated construction of laboratory complex for Veterinary Type Cultures facility. In the meantime, two laboratories have been established at NRCE for conducting the research work on microbial cultures in the repository.

Procurement of standard/reference strains of microbial resources from accredited organizations/individuals in the country is being done. As regards the isolation of microbes from field samples, this is being done in the project on "Isolation, maintenance and characterization of bacterial pathogens and their molecular identification". The standardization of different storage protocols is underway. Furthermore, the complete working guidelines for Veterinary Type Cultures have been developed and submitted to the Council.

Agriculture farm production

During 2007-08, fodder crops of *Rabi* and *Kharif* season were produced at the agricultural farm at

Fodder production at NRCE (Hisar and Bikaner)							
Fodder	Productio	on (in QtIs)	Total				
	Hisar	Bikaner					
Lucerne	84.0	613.15	697.15				
Oat	117.0	338.65	455.65				
Millet	74.0	-	74.00				
Sorghum	322.0	306.80	628.80				
Cowpea	49.0	-	49.00				
Berseem	23.00	-	23.00				
Oat + Berseem	199.0		199.00				
Maize	64.0	181.05	245.05				
Paddy straw	70.0		70.00				
Total	1002	1439.65	2441.65				

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Hisar and Bikaner Table. The centre produced

Livestock

The centre is maintaining a representative herd of equines at Bikaner campus comprising indigenous horses of Marwari and Kathiawari breed (46), exotic donkeys (39) and mules (5) (Table).

Equine herd strength at Bikaner campus							
Category	ategory Horses Exotic donkey		lonkeys	Mules		Total	
	Male	Female	Male	Female	Male	Female	
Stock as on 1.4.2007	13	31	18	22	03	02	89
Births during the year	2	06	02	-	-	-	10
Deaths during the year	-	04	-	-	-	-	04
Sold during the year	-	02	03	-	-	-	05
Balance as on 31.3.08	15	31	17	22	03	02	90

The centre is also maintaining some animals at Hisar campus comprising horses of Marwari breed (14), ponies (2), exotic donkeys (2) and mules (3) of different age groups (Table). The centre is also maintaining a

frozen stock of the semen which is being used for artificial insemination purpose. During 2007-08, the centre generated an income of Rs. 102700/- by sale of livestock at Hisar.

Equine Herd strength at Hisar campus (as on 31.03.2008)							
Category Horses		Indigenous	Mules	Donkeys	GrandTotal		
	Marwari	Others	Ponies				
Adult Male	1	2	1	2	2	8	
Adult female	10	1	2	1	-	14	
0-1 yrs	3	-	-	-	-	3	
Grand Total	14	3	2	3	2	25	



List of Publications

Research Articles (authored by NRCE scientists)

- 1. Arangasamy, A. and Singh, L.P. (2007). *In vitro* capacitation of epididymal spermatozoa with added heparin and gelatin binding buffalo seminal plasma proteins. *Indian Journal of Animal Sciences*, 77: 549-552.
- 2. Ghosh, R.C and Tigga, M. (2007). An outbreak of caprine coccidiosis in Chattisgarh. *Indian Veterinary Journal*, 84:767-768.
- Gupta, A.K., Yash Pal, Chauhan, M. and Kumar, S. (2007). Biometrical, physiological and hormonal indices in new born pony and donkey foals at different growth intervals. *Indian Journal* of Animal Reproduction, 28 : 34-39.
- 4. Kumar, S., Kumar, R., Gupta, A.K. and Dwivedi S.K. (2008). Passive transfer of *Theileria equi* antibodies to neonate foals of immune tolerant mares. *Veterinary Parasitology*, 151 : 80-85.
- 5. Kumar, S. and Kumar, R. (2007). Diagnosis of *Babesia equi* infection: an update on the methods available. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 2 : 14.
- Mallick, A.I., Singha, H., Chaudhuri, P., Ahmad Nadeem, Khurshid A. Dar and Owais, M. (2007). Liposomal delivery of a recombinant ribosomal L7/L12 protein protects BALB/c mice against *Brucella abortus* 544 infection. *Vaccine*, 25(18): 3692-3704.
- Mallick, A.I., Singha, H., Khan, S., Ahmad Nadeem, Khurshid A Dar, Chaudhuri, P. Owais, M. (2007). Escheriosome mediated delivery of recombinant ribosomal L7/L12 protein confers protection against murine brucellosis. *Vaccine*, 25(46): 7873-7884.
- Nehra, R., Purohit, G. R., Sharma, T., Sharma, Dhuria, R. K. and Legha, R. A. Nutritional Evaluation of Sewan (*Lasiurus sindicus*) Grass Hay in Marwari Horses of Arid Region. *Animal Nutrition & Feed Technology*, 8(1):105-109.

- 9. Singh, M.K., Gupta, A.K. and Yadav, M.P. (2007). Performance evaluation of donkeys in arid zone in India. *Indian Journal of Animal Sciences*, 77(10): 1017-1020.
- 10. Singh, N., Pathak, K.M.L, Chaudhri, S.S. and Kumar, R. (2007). Cameline trypanosomosis (surra) in dromedaries of Western Rajasthan, India. *Indian Journal of Animal Sciences*, 77: 718-20.
- Tandon, S.N., Vyas, Sumant and Legha, R.A. (2007). Ultrasound detection of twining in Equus asinus- A case report. *Indian Journal of Animal Reproduction*, 28(2): 116-117.
- 12. Virmani, Nitin, Singh B.K., Batra Munish, Verma P.C., Panisup, A.S. (2008). Comparative pathology and tissue tropism of two indigenous strains (H-90 and R-98) of equine herpes virus 1 in pregnant BALB/c mice towards occurrence of respiratory affections and abortions subsequent to intranasal inoculation. *Journal of Immunology and Immunopathology*, 10(1): 14-19.
- Virmani, M., Gupta A.K., Virmani Nitin, Garg S.K. (2008). Haemagglutination inhibition assay for Equine Chorionic Gonadotropin (ECG) detection in pregnant mare serum. *Journal of Immunology and Immunopathology*, 10(1): 55-59.

Accepted Research Papers

- Arangasamy, A., Legha, R.A., Pal Y., Bansal, R.S., Singh, J. and Sharma, R.C. (2008). Cryopreservation of Marwari stallion's semen by custom freezing and its conception rate. *Indian Veterinary Journal.*
- 2. Arangasamy, A. and Tandon, S.N. (2008). Evaluation of frozen semen characteristics of Marwari stallions and Poitou jacks. *Indian Veterinary Journal*.
- 3. Arangasamy, A., Bansal, R.S., Sharma, R.C., Legha R.A., Pal Y. and Tandon, S.N. (2008). Some reproductive characteristic of Marwari

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mares. Indian Journal of Animal Reproduction.

- 4. Arangasamy, A. (2008). Effect of seminal plasma proteins on hypo-osmotic swelling test of stallion spermatozoa. Indian Veterinary Journal.
- 5. Gulati, B.R., Yadav, R.R. and Singh, B.K. (2008). Epidemiological studies on equine rotavirus infection in foals of organized farms in India. *Indian Journal of Animal Sciences.*
- 6. Gupta, A.K., Singh, R., Mamta, Singh, M. K and Pal Y. (2007). Physical and biochemical studies in Jack's semen. *Annals of Arid Zone*.
- Singha, H., Mallick, A.I., Jana C., Isore D.P., Goswami T.K., Srivastava S.K., Vasco A.A. Azevado, Chaudhuri P and Owais M. (2008). Escheriosome entrapped DNA vaccine Coexpressing Cu-Zn superoxide dismuatse and IL-18 confers protection against *Brucella abortus*. *Microbes and Infection*.
- 8. Vaid R.K., Barua S., Kumar Ashok, Dwivedi D., Rana R. and Vihan V.S. (2008). Isolation and Molecular Identification of a Multi-Drug Resistant *Pasteurella multocida* isolate from Sheep. *Vet. Practitioner.*

Popular Articles

- 1. Bansal, R.S. and Arangasamy, A. (2007). Use of ultrasonography in female horses. *Pashudhan*, 33 (6).
- 2. I nnhi [kgikuk (2007)] ?kkk/kadsH; kog jkx] jktHkk"kk Lekfjdk] Hkkjrh; i kqfpfdRIk I hLFkku] bTtruxj] mÙkj i nšk-
- 3. ; 'ki ky , oe-jke ∨orkj y9kk (2007)] ∨'okaeaÑf=ke xHkkl⁄ku dk; D⁄e dksli@y d9 scuk, a\iq; ol q⁄jk Ñf"k ekfld (∨i§y ∨ad) %i"B 30&31-
- 4. ; 'ki ky] jke ∨orkj y3kk , oe~lýt ukjk; .k V&/u (2007) ekjok/Ma uLy dsmRre ?k&/kadsoh; 21 sÑf=ke xHkU/kuA jktLFkkuh [krh (ebZvzd) %i"B 9&10-
- ; 'ki ky] jke vorkj y§kk, oe~lýt ukjk; .k V&lu (2007) xnHkøead\$ sgksldrk g\$uLy lq/kkj A Ñf"k p; fudk (tgykb&flrEcj vad) % i"B 24, oe~ 38&39-

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Abstracts Published in Conference, Seminar and Symposia Proceedings

- 1. Arangasamy, A., Bansal, R.S. and Singh, J. (2008). Cryopreservation of Poitou Jacks semen and its conception rate. In: XVII Annual Conference of Society of Animal Physiologists of India & National Symposium on Current concepts in productivity management in livestock and poultry- Environment, Nutrition and stress, Pantnagar, Uttarakhand, February 7-9.
- Chugh, M, Gulati, B.R. and Gakhar, S. K. (2008). Monoclonal antibodies AC-43 and AC-29 against midgut proteins block malaria parasite *P. vivax* development in *An. culicifacies* (Diptera: Culicidae) and reduce its fecundity. IN: IX International Symposium on Vector and Vector Borne Diseases, organized by National Institute of Malaria Research, Puri, Orissa, February 15-17.
- Gulati, B.R., Singh, B.K., Virmani, N and Khurana, S.K. (2007). Serosurveillance of Japanese encephalitis among equines in India. In: International Conference on emerging and reemerging viral diseases of the Tropics & Sub-Tropics, New Delhi, December 11-14.
- 4. Gulati, B.R., Singh, B.K. and Yadav, R.R. (2007). Molecular epidemiology of equine rotavirus infections in India. In: International Conference on emerging and re-emerging viral diseases of the Tropics & Sub-Tropics, New Delhi, December 11-14.
- Legha R.A., Arangasamy A., Pal, Y., Sharma, R.C. and Tandon, S.N. (2007). Effect of thawing temperature and time on post thaw sperm motility of cryopreserved jack's semen. In: "National Symposium on Recent Trends in Policy Initiatives and Technological Interventions for Rural Prosperity in Small Holder Livestock Production System" held at Tirupati from June 20-22, pp 149.
- Malik, P., Khurana, S.K. and Dwivedi, S.K. (2007). Re-emergence of glanders in India Report of Maharashtra State. 48th Annual Conference of Association of Microbiologists

of India organized at IIT Madras, Chennai from December 18-21.

- Nagaleelavathi, S.P., Gulati, B.R. and Garg, S. R. (2007). Japanese encephalitis seroprevalence in equines and bovines in Haryana, India. In: International conference on emerging and reemerging viral diseases of the Tropics & Sub-Tropics, New Delhi, December 11-14.
- Nagaleelavathi, S.P., Gulati, B.R. and Garg, S.R. (2007). Serosurveillance of Japanese encephalitis among pigs in Haryana, India. In: 6th Annual Conference of Indian Association of Veterinary Public Health Specialists, Anand Agricultural University, Anand, November 29-30.
- 9. Yash Pal, Legha, R.A., Arangasamy, A. and Sharma R.C. (2007). Semen cryopreservation of Marwari stallions at farmer's door. In : "National Symposium on Recent Trends in Policy Initiatives and Technological Interventions for Rural Prosperity in Small Holder Livestock Production System", Tirupati from June 20-22, pp 148-149.

Technical Bulletins/Books

 Training Manual on Techniques in Murine Monoclonal Antibody Production (eds. Kadian S.K., Singh A., Gulati B.R. and Kumar A.), ICAR Centre of Advanced Studies, Department of Veterinary Microbiology, CCS HAU, Hisar, 2008.



Participation in Conferences and Symposia

- 1. Dr. S.K. Dwivedi, Director attended one day interaction on the subject "Animal Genetic Resources and IPR issues" organized by NBAGR and Centre for advancement of Sustainable Agriculture (CASA) at NASC Complex, Pusa, New Delhi on May 11, 2007.
- 2. Dr. Rajender Kumar, Sr. Scientist attended one day interaction on the subject "Animal Genetic Resources and IPR issues" organized by NBAGR and Centre for advancement of Sustainable Agriculture (CASA) at NASC Complex, Pusa, New Delhi on May 11, 2007.
- Dr. Yashpal, Sr. Scientist and Dr. R.C. Sharma, Sr. Scientist participated in "National Conference on Enhancing Effectiveness" organized by Scientists & Engineers Wing, Rajyoga Education & Research Foundation at Mt. Abu (Rajasthan) from May 19-23, 2007.
- 4. Dr. Yashpal, Sr. Scientist and Dr. R.A. Legha, Senior Scientist participated in the "National Symposium of Recent Trends in Policy Initiatives and Technological Interventions for Rural Prosperity in Small Holder Livestock Production System" organized by Indian Society of Animal Production and Management at Tirupati from June 20-22, 2007.
- Dr. S.K. Dwivedi. Director chaired the session on "Herbal medicine and Ethnoveterinary Practices" during International Seminar on "Growth and Development of Animal Industries" at Pragati Maidan, N. Delhi on August 17, 2007.
- 6. Dr. S.K. Khurana attended workshop on "Rabies and its prevention" on World Veterinary Day organized by Department of Veterinary Public Health, COVSc, HAU, Hisar on September 7, 2007.
- 7. Dr. P.A. Bala, Scientist participated in National Symposium on "Rangeland and Forage Resources in Changing Socio-economic Scenario" organized by Range Management Society of India and Indian



- 8. Dr. Baldev R. Gulati, Sr. Scientist participated and presented an invited research paper on "Molecular epidemiology of equine rotavirus infections among diarrhoeic foals in India" in International Conference on Emerging and Re-Emerging Viral Diseases of the Tropics and Sub-Tropics organized by Indian Virological Society at New Delhi from December 11-14, 2007.
- 9. Dr. Praveen Malik, Sr. Scientist participated and presented a research paper on "Re-emergence of Glanders in India-Report of Maharashtra State" in 48th Annual Conference of the Association of Microbiologists of India (AMI) organized by Association of Microbiologists of India at Indian Institute of Technology Madras, Chennai from December 18-21, 2007.
- Dr. Baldev R. Gulati, Sr. Scientists and Dr. Sanjay Barua, Senior Scientists attended the meeting of the committee for framing strategies for conservation of Animal genetic resources, microbes, genes and DNA held under the chairmanship of DDG (AS), ICAR, Krishi Bhawan, New Delhi on January 18, 2008.
- Dr. S.K. Dwivedi. Director, participated in "National Conference on Distance education to animal farmers with emphasis on women self help groups" organized by Indian Association of Animal Production (IAAP) and Institute of Agricultural science BHU, at Varanasi from February 1-3, 2008.
- 12. Dr. Yash Pal, Sr. Scientist, and Dr. R. A. Legha, Sr. Scientist, participated in "National Conference on Distance education to animal farmers with emphasis on women self help groups" organized by Indian Association of Animal Production (IAAP) and Institute of Agricultural Science BHU, at Varanasi from February 1-3, 2008.



- Dr. S.K. Dwivedi. Director delivered the felicitation address in the SAARC Congress on "Canine Practice" at Tamil Nadu Veterinary and Animal Sciences University, Chennai on February 7, 2008.
- 14. Dr. R.S. Bansal, T-9 participated in National Symposium on "Current Concepts in Productivity Management in Livestock and Poultry-Environment, Nutrition and Stress" organized by G.B. Pant University of Agriculture and Technology and Society of Animal Physiologists of

India at Department of Vety. Physiology, COVSc, Pantnagar from February 7-9, 2008.

15. Sh. R.K. Chaturvedi, T-6 participated in National Symposium on "Redefining role of indigenous animal genetic resources in rural development" organized by Society for Conservation of Domestic Animal Biodiversity (SOCDAB) and Karnataka Veterinary, Animal and Fisheries Sciences University (Bidar) at Bangalore from February 15-16, 2008.



Personnel Milestones

Joinings



Dr. Ramesh Kumar Dedar joined as Scientist (Veterinary Medicine) at this centre on 18th May, 2007 after NAARM training. He has done his graduation and postgraduation from College of

Veterinary Sciences, Rajasthan Agricultural University, Bikaner in 2000 and 2002, respectively.

Dr. Prokasananada Bala joined as Scientist (Animal Nutrition) at Equine Production Centre, NRCE, Bikaner on 18th May, 2007 after NAARM training. He did his undergraduate degree from



Madras Veterinary College, TANUVAS, Chennai in 2003 and Master's degree from NDRI, Karnal in year 2006



Dr (Ms). Mamta Tigga joined as Scientist (Veterinary Pathology) in Veterinary Type Cultures at NRCE, Hisar on 18th May, 2007 after NAARM training. She did her B.V.Sc.& A.H. in 2001 from College

of Veterinary and Animal Sciences, Durg, Chattisgarh. In 2004, she acquired her Master's degree in Veterinary Pathology from Indira Gandhi Agricultural University, Raipur.

Promotions

- Dr. S.C. Yadav (Senior Scientist) has been promoted as Principal Scientist w.e.f. 27th July 2006.
- Dr. Sanjay Barua, Scientist has been promoted as Senior Scientist w.e.f. 27th March, 2006.

- Dr. R.K. Vaid, Scientist has been promoted as Senior Scientist w.e.f. 6th December, 2006.
- Dr. R.A. Legha, Scientist has been promoted as Senior Scientist w.e.f. 5th August, 2006.
- Dr. Mamta Chauhan, Scientist has been promoted as Senior Scientist w.e.f. 5th December, 2006.
- O Sh. Hawa Singh, Assistant has been promoted as Assistant Administrative Officer from 1st March, 2008.
- Sh. Ishwar Singh, SSGr. III to SSGr. IV from 18th February 2008.
- o Sh. Guru Dutt, SSGr. III to SSGr. IV from 18th February 2008.
- Sh. Ramesh Chander, SSGr. II to SSGr. III from 18th February 2008.
- Sh. Mardan, SSGr. II to SSGr. III from 18th
 February 2008.

Superannuation

O Sh.K.K.Chandna, AAO, superannuated on 28th February, 2008.

Appointment

Sh. Sant Ram has been appointed on the post of S. S. Grade-I w.e.f. 17th November, 2007.

Selection

Dr. B.R. Singh (Principal Scientist) was relieved from NRCE on 31st October 2007, subsequent to his selection as Head, Division of Animal Science, Central Agricultural Research Institute, Port Blair, Andaman & Nicobar.



Staff at NRCE

Director Dr. S. K. Dwivedi

Hisar Campus				
1.	Dr. A. K. Gupta	Principal Scientist		
2.	Dr. B. K. Singh	Principal Scientist		
3.	Dr. S. C. Yadav	Principal Scientist		
4.	Dr. S. K. Khurana	Senior Scientist		
5.	Dr. Baldev R. Gulati	Senior Scientist		
6.	Dr. Rajender Kumar	Senior Scientist		
7.	Dr. Praveen Malik	Senior Scientist		
8.	Dr. Nitin Virmani	Senior Scientist		
9.	Dr. Sanjay Kumar	Senior Scientist		
10.	Dr. Mamta Chauhan	Senior Scientist		
11.	Dr.Niranjan Lal	Scientist		
12.	Dr. Ramesh Dedar	Scientist		

EPC, Bikaner				
1.	Dr. S. N. Tandon,	Principal Scientist & I/c EPC		
2.	Dr. Yash Pal	Senior Scientist		
3.	Dr. R. C. Sharma	Senior Scientist		
4.	Dr. R. A. Legha	Senior Scientist		
5.	Dr. A. Arangasamy	Scientist		
6.	Dr. P.A. Bala	Scientist		

VT	C, NRCE, Hisar	
1.	Dr. Sanjay Barua	Senior Scientist
2.	Dr. R. K. Vaid	Senior Scientist
3.	Dr. Mamta Tigga	Scientist

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

Supporting staff (Hisar and Bikaner)				
١.	Sh. Ishwar Singh	SSGr. IV		
2.	Sh. Guru Dutt	SSGr. IV		
3.	Sh. Jai Singh	SSGr. III		
4.	Sh. Ramesh Chander	SSGr. III		
5.	Sh. Mardan	SSGr. III		
6.	Sh. Mahabir Prasad	SSGr. II		
7.	Sh. Desh Raj	SSGr. II		
8.	Sh. Ishwar Chander	SSGr. II		
9.	Sh. Om Prakash	SSGr. II		
10.	Sh. Deepak Kumar	SSGr. II		
11.	Sh. Gopal Nath	SSGr. III		
12.	Sh. Sant Ram	SSGr. II (17.11.07)		
13.	Sh. Satbir Singh	SSGr. I		
14.	Sh. Hanuman Singh	SSGr. I		
15.	Sh. Subhash Chander	SSGr. I		
16.	Sh. Ishwar Singh	SSGr. I		
17.	Sh. Ram Singh	SSGr. I		
18.	Sh. Raju Ram	SSGr. I		
19.	Sh. Mahabir Prasad	SSGr. I		
20.	Smt. Ram Kali	SSGr. I		
21.	Smt. Santra	S		

Technical staff (Hisar and Bikaner)

١.	Dr. R.S. Bansal, T-9	Farm Manager
2.	Sh. R.K. Chaturvedi, T-6	Technical Officer
3.	Sh. K.K. Singh, T-5	Technical Officer
4.	Sh. K.S. Meena, T-5	Farm Manager
5.	Dr. Jitender Singh, T-5	Veterinary Officer
6.	Sh. P.P. Chaudhary, T-4	Lab. Technician
7.	Sh. Ajmer Singh, T-4	Livestock Assistant
8.	Sh. Brij Lal, T-4	Livestock Assistant
9.	Sh. D.D. Pandey, T-4	Lab. Technician
10.	Sh. Sita Ram, T-4	Lab. Technician
Π.	Sh. S.K. Chhabra, T-4	Lab. Technician
12.	Sh. N.K. Chauhan, T-4	Farm Technician
13.	Sh. Joginder Singh, T-3	Lab. Technician
14.	Sh. Mukesh Chand, T-3	Lab. Technician
15.	Sh. Sajjan Kumar, T-3	Driver
16.	Sh. Arun Chand, T-2	Tractor Driver
17.	Sh. Suresh Kumar, T-3	Driver
18.	Sh. Shankar Lal, T-2	Driver
19.	Sh. S.N. Paswan, T-2	Livestock Assistant
20.	Sh. Om Prakash, T-2	Tractor Driver
21.	Sh. Rajendra Singh, T-I	Lab. Assistant
22.	Sh.Raghubir Singh T-1	Vehicle Driver

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Ongoing Research Projects

Bassauch Busiant		Period		
Research Project	Investigators	From	То	
EQUINE HEALTH				
Development of vaccine(s) against equine herpes virus-l infection.	B.K.Singh, B.R. Gulati & N.Virmani	June, 2005	May, 2009	
Studies on the improvement of the Diagnostics for differentiation between EHV-I & 4 infections employing molecular techniques.	Nitin Virmani, A. S. Panisup, B. K. Singh & B. R. Gulati	May, 2004	March, 2009	
Development of sensitive & specific methods for diagnosis of equine rotavirus from diarrhoeic foals	Baldev R. Gulati & B.K. Singh	June, 2003	March, 2007	
Development of diagnostic(s) for pathogenic Streptococcus equi in equines	P. Malik, B.R. Singh, N. Virmani, S.K. Khurana & Mamta Chauhan	June, 2003	March, 2007 (Kept in abeyance)	
Development of diagnostics for <i>Rhodocococcus</i> equi infection in foals	S. K. Khurana, B. R. Singh, Praveen Malik & Nitin Virmani	May, 2004	March, 2009	
Development of sensitive and specific diagnostics for Japanese encephalitis in Equines	Baldev R. Gulati, B.K. Singh & N. Virmani	Oct., 3006	Sept., 2009	
Studies on high-level drug resistant bacteria in equines for search of sentinel microbes to use in predictive disease modeling and microbes with vector potential	B.R. Singh, Baldev R. Gulati, S. K. Khurana, Mamta Chauhan & Niranjan Lal	Oct., 2006	Oct., 2007	
Epidemiological studies on emerging and existing diseases of equines.	S.K.Dwivedi, S.K. Khurana, B.K. Singh, S.C. Yadav, Baldev R. Gulati, Rajender Kumar, P. Malik, Sanjay Kumar, Nitin Virmani, Sanjay Barua, Rajesh Kumar Vaid, A. Arangasamy & Ramesh Kumar Dedar			
Development of diagnostic tests for equine trypanosomosis (Surra).	Rajender Kumar, Sanjay Kumar, S.C. Yadav & S.K. Dwivedi	June, 2003	March, 2009	
Development of sensitive and specific diagnostic tests for detection of equine piroplasmosis	Sanjay Kumar, Rajender Kumar, A.K. Gupta, S.C. Yadav & S.K. Dwivedi	May, 2004	March, 2009	
Inter-institutional collaborative project - Usefulness of recombinant protein for serodiagnosis of glanders	Praveen Malik	Oct., 2006	Sept., 2008	
EXTENSION				
To study the existing donkey and mule production system in three states Haryana, Rajasthan and U.P.	Niranjan Lal, Rajender Kumar, Ramesh Dedar, Praveen Malik & Rajni Kant Chaturvedi	July, 2006	May, 2009	



Dessent Dusis of		Period	
Research Project	Investigators	From	То
νтс			
Establishment of a Repository (Culture collection) of Veterinary pathogens: processes & procedures	Sanjay Barua & Rajesh Kumar Vaid	June, 2007	May, 2010
Isolation, maintenance and characterization of bacterial pathogens and their molecular identification	Rajesh Kumar Vaid, Sanjay Barua & Mamta Tigga	June, 2007	May, 2010
EQUINE PRODUCTION			
Cryopreservation of stallion semen and perfection of Al in Marwari horses.	Yash Pal, R.A. Legha, A. Arangasamy & S.N. Tandon,	May, 2002	June, 2007
Development of equine chorionic gonadotropin (ecg) based ELISA based test for pregnancy diagnosis in equines.	A.K.Gupta, Yash Pal & Sanjay Kumar	May, 2002	June, 2006
RFLP-based genotyping of major histocompatibility complex class II genes in Marwari horses	R.C. Sharma & S.C. Mehta	Oct., 2004	Oct., 2008
Molecular markers based parentage testing in horses of Indian origin.	Mamta Chauhan & A.K. Gupta	Dec., 2004	June, 2007
Isolation of stallion seminal plasma proteins and their effect on in vitro fertilizing ability of spermatozoa	A. Arangasamy	Oct., 2004	March, 2008
Superior mule production in the field through frozen semen of exotic Jacks	R.A. Legha, R.C. Sharma & A. Arangasamy	Dec., 2004	Service Project
Molecular characterization of indigenous breeds of horse for genetic diversity within and between different breeds.	A.K. Gupta, S.C. Gupta, S.N. Tandon, Mamta Chauhan & Neelam Gupta	Oct., 2006	Sept., 2009
Cryo-preservation of embryos for conservation of Marwari horses.	R.K. Chaturvedi & A. Arangasamy	Jan., 2007	March, 2009



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