# Annual Report 2003-2004

राष्ट्रीय अश्व अनुसंधान केन्द्र

National Research Centre on Equines

ICAR

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# Director's Foreword

I feel immense pleasure in forwarding the Annual Report of the Centre for the year 2003-2004. The focus of this Centre is on major problems confronting equine health and production. The efforts of the Centre during the recent years have been concentrated on generation of indigenous and cost-effective technologies for diagnosis, prevention and control of major equine diseases.

As is reflected in this report, this period has been quite productive for the Centre. A patent filed by the Centre for EHV-1 diagnostic kit was granted by the patent office, government of India. A patent application entitled *Complement fixation test based COFEB-Kit for diagnosis of Babesia equi infection in equines* was submitted to the patent office during the year. We moved forward towards developing an effective EHV-1 vaccine for controlling abortions in pregnant mares.

A kit for sero-diagnosis of pregnancy in mares is on anvil and an improved sensitive and specific diagnostic kit for equine herpes virus-1 is in the final phase of development. The work towards characterization of indigenous breeds of equines is in progress. Field trials on artificial insemination using cryopreserved semen gave encouraging results. Many new states, *viz,* Jammu & Kashmir, Madhya Pradesh, Meghalya, Manipur, *etc,* were included in our nation-wide sero-monitoring programme in order to assess the status of important equine diseases in different geographical regions of the country.

The 10th Five Year Plan proposal for NRCE was approved by Standing Finance Committee with an outlay of Rs. 13.50 crores including a budgetary provision for development of state-of-the-art infrastructure including microbial containment (BSL-III) facility.

During this period, the infrastructural facilities at NRCE have been evaluated by various expert committees including Department of Animal Husbandry & Dairying (DAHD) for OIE recognition; QRT for last seven years and by the Committee of Agricultural & Scientific Experts, DAHD, Ministry of Agriculture for evaluating the worth of research work being done at NRCE. It is a matter of great satisfaction that these high-powered committees applauded the technical excellence of this Centre. The recommendations made by these expert committees will be guiding force for the future research and development at the Centre.

A short course on *Cryo-preservation of semen, artificial insemination and pregnancy diagnosis in equines* was organized at Bikaner Campus and a workshop on *Information technology for dissemination of scientific knowledge in agriculture* was conducted. Almost entire administrative staff was sent to various trainings for their skill up-gradation.

I would like to record my sincere thanks to Indian Council of Agricultural Research, New Delhi, particularly Dr. Mangla Rai (DG, ICAR and Secretary, DARE), Dr. V.K. Taneja (DDG, Animal Sciences) and Dr. Lal Krishna (ADG, Animal Health) for their continuous support to this centre to improve equine health and production. I compliment the efforts of publication committee for giving new look and for timely printing of this report.

25 July 2004

S.K. Dwivedi

# Executive Summary

Of about 2.0 million equines in India, approximately 98% comprises indigenous equids including donkeys, mules and ponies. These animals provide livelihood to the landless, small and marginal farmers and other section of our rural and semi-urban society. These animals are used for draught and transport especially in hilly, arid and semi-arid zones where motorable roads are inadequate or not feasible. Remaining 2% equines, belonging to elite group, are kept in organized sectors and provide services to the army, police, border security force, racing industry, sports and for ceremonial parades. Efficient performance of equines for these activities depends upon a sound health and freedom from various ailments including infectious diseases. Therefore, during the year, efforts of National Research Centre on Equines were focused to improve health and production of equines, development of improved diagnostics and biologicals for major equine ailments, nation-wide monitoring of equine diseases and to provide advisory & consultancy services to the equine farmers and breeders. A brief account of achievements of NRCE during the year 2003-2004 is given below:

Patent has been granted by the Patent Office, Government of India on an application (2199/DEL/96) entitled *"A method for preparation of a diagnostic kit useful for forecasting Equine Herpes Virus-1 disease".* This has been notified on October 25, 2003 in the Gazette of India, classified as 55E4 1891278. Another patent application entitled "*Complement fixation test based COFEB-Kit for diagnosis of Babesia equi infection in equines"* was submitted to the patent office during the year.

Neutralizing monoclonal antibodies-based blocking ELISA (B-ELISA) kit developed at this Centre for serodiagnosis of equine herpes virus-1 (EHV-1) infection was further evaluated during the year for its shelf life and user-to-user variations when used in different laboratories. Minor variations in the results of the kit were observed when the kit was used by the new user for the first time. The shelf life of the freeze-dried reagents of the kit was evaluated to be 6 months. However, it was recommended that once the freeze dried reagents (supplied along with the kit) are dissolved, they should be used the same day. Efforts are under way to stabilize the reagents in buffer liquid using various stabilizers to further improve its acceptability and shelf life.

A sandwich ELISA for pregnancy diagnosis in mares is in the process of development that will act as an alternate to rectal or ultrasonographic examination. This serum based test is animal and farmer friendly as it does not involve the transport of pregnant animal to diagnostic centre. With the help of this test, pregnancy can be detected between days 35 and 120 of gestation in mares used for horse production only. All the 73 serum samples collected between days 35 and 45 of gestation were detected pregnant by ELISA, as these contained eCG levels ranging from 18.75 to 150.75 IU/ml serum. However, this test gave unequivocal results with the serum samples from mares covered for mule production. The efficacy of this ELISA for pregnancy diagnosis between days 20 and 35 of gestation is under evaluation.

An EHV-1 killed vaccine incorporating indigenous strain (Hisar-90-7) of EHV-1 developed at this Centre was evaluated in

pregnant BALB/c mice for immune response and protection against challenge. Mice immunized with 12.5 µg and 25 µg of immunogen showed initiation of humoral immune response from 14 days post-vaccination and cell mediated immune response as measured by lymphocyte proliferation assay showed an increasing trend in these mice 28 days post-vaccination. After EHV-1 challenge of pregnant mice with Raj-98 EHV-1 strain, 60-70% of immunized mice delivered normal healthy pups, while 4 out of 6 (66.66%) of non-vaccinated mice aborted after challenge. Severe pathological lesions were observed in non-vaccinated animals following challenge, however, vaccinated animals did not show such changes. Optimum dose of EHV-1 immunogen that provided good immune response and protection against challenge in BALB/c mice was observed to be 25 µg per mice through intra-peritoneal route. Experimental trial of this vaccine in equines is under progress.

Among the indigenous horses, Marwari breed is known for their endurance potential, sturdiness, stiffness and relatively better disease resistance. The average height at withers of Marwari stallions was 153.0±0.93 cm whereas it was 149.1+0.43 cm in mares. Genetic characterization using 16 different microsatellite markers by polymerase chain reaction (PCR) indicated that two primers (AHT16 and AHT44) were monomorphic and could be used to differentiate Marwari horses from the exotic breeds of horses. Further characterization of Marwari horses on three more loci is under progress.

In order to establish the normal seminal characteristics of Marwari horses, physico-biochemical characteristics of filtered gel-free semen were studied. Evaluation of different extenders indicated that lactose-glucose-egg-yolk extender gave better (25-40%) post-thaw motility followed by sugar based extender (20-30%) and HF-20 extender (20-30%). However, pre-freezing motility was observed comparatively high with sucrose solution.

Field trial of artificial insemination with frozen semen of jack was undertaken during the year 2003-04 in different villages of Karnal and Panipat districts of Haryana state. Out of 95 females inseminated with frozen semen, 39 were pregnant with a conception rate of 41.05%.

Seromonitoring of important equine diseases is being undertaken with special emphasis on indigenous equines to study the magnitude of existing and emerging equine diseases in different states of the country. During the year, sero-surveillance was conducted in 12 States/ UTs of India, namely Bihar, Delhi, Haryana, Himachal Pradesh, Jammu and Kashmir, Karnataka, Madhya Pradesh, Meghalaya, Rajasthan, Punjab, Uttar Pradesh and Uttaranchal. EHV-1 antibodies were detected in 81 out of 1483 (5.46%) samples, equine influenza (A/equi-2) antibodies in four of 1424 samples, *Mycoplasma equigenitalium* in 40 out of 548 (7.3%) samples, while *Babesia equi* sero-prevalence was detected in 387 (27.04 %) out of 1431 serum samples tested. None of the 1517 samples tested for equine infectious anemia, glanders and *Salmonella* Abortus equi was detected positive. Similarly, 348 samples tested for African horse sickness were found negative. In order to evaluate the effect of toxic environmental contaminants in equines, regular monitoring of fluoride and cadmium has been undertaken during the year.

Infectious diarrhoea is known to pose a challenge to the equine breeders during each foaling season. About 50 diarrheic stool samples collected from foals below 2 months of age from organized farms around Hisar (Haryana) were tested for various pathogens. Rotavirus was detected in 4 (8%) samples by ELISA and RNA-PAGE. Electrophoretic profile of the isolated

rotaviruses indicated that at least two different rotavirus strains are circulating in the region. twenty-eight (54.9%) *E. coli* were isolated from stool samples, of which 9 were cytotoxigenic in vero cells.

Twenty-nine streptococcal isolates of equine origin were characterized using the antibiogram and mice pathogenicity. All the *S. equi* isolates, including the standard strain, were highly pathogenic, however, seven of the *S. zooepidemicus* isolates were less pathogenic to mice.

Fifty eight mares and jennies with known history of infertility were investigated to find out the causes of infertility. Cytological studies and cultural examination established that 45 (77.6%) cases of infertility were associated with microbial agents. There was good agreement (86.67%) between cytological findings on the uterine aspirate and the results of isolation in these cases.

A PCR has been standardized for identification of *Trypanosoma evansi* infection. DNA fragment of *T. evansi* (227 bp) was amplified in this PCR that is being further evaluated for its sensitivity and specificity for detection of *T. evansi* in blood samples of equines.

A short course on *Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines* was organized from September 1-10, 2003 at Equine Production Campus, Bikaner. A total of 22 participants from different states were imparted training in artificial insemination and pregnancy diagnosis using ultrasonography & other serum-based techniques.

NRCE in collaboration with National Information Centre, Hisar organized a one-day workshop on *Information technology for dissemination of scientific knowledge in agriculture* on September 17, 2003. Scientists and employees of various ICAR and central government institutes participated in it. The important recommendations of the workshop included rapid dissemination of laboratory information to the end-users through IT tools, creation and development of web-enabled databases, keeping in view the requirement of the farmers and the use of Hindi and other regional languages for dissemination of knowledge.

During the year, the scientists of the Centre published 22 original research articles in international and national journals, presented 16 research papers in different conferences and symposia and contributed 15 articles in different training manuals, books, *etc*.

Clinical camps were organized at Katra (J&K) from 10$^{th}$ to 12$^{th}$ June 2003 and at Churu (Rajasthan) on October 29, 2003. Major ailments that were observed in animals of these regions and given treatment included lameness, colic, retention of urine, *etc*. In addition, deworming, mineral mixture and vitamin supplements were distributed to the needy equine farmers based on the evaluation report of animals on clinical examination. Blood and serum samples were collected from all the animals for serological testing of various viral, bacterial and parasitic infections.

During the year, the Centre generated a revenue of Rs. 26.49 lacs from its internal sources, mainly through the diagnostic services rendered and sale of livestock to the farmers.

# Introduction

National Research Centre on Equines (NRCE) was established on 26th November 1985 at Hisar (Haryana) under the aegis of the Indian Council of Agricultural Research. The main objective of the Centre was to improve the health, performance and production potential of equines in India. In a short span, NRCE has been recognized as a premier equine research centre in the area of equine health and production.

## Mandate of NRCE

- 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.

The main campus of NRCE is located at Hisar (Haryana) and has state-of-the-art laboratories for undertaking research in areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry and biotechnology. In addition, NRCE has a sub-campus at Bikaner (Rajasthan) where research laboratories for genetics and breeding, reproduction, physiology and nutrition are established to undertake research on equine production. Research activities are carried out by a team of 18 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, library and internet facility. The Centre has well-maintained herd of Marwari & Kathiwari horses and exotic donkeys at Bikaner. Efforts are being made to create facilities for various equestrian events for the benefit of equine lovers and those interested in equine sports. In addition, the Centre has requisite bio-containment facilities and is in the process of development of BSL-III laboratory.

### Major Achievements of the Centre

The Centre has made the following salient achievements in a short span since its inception:

- 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* Abortus equi. Equine Herpes Virus-1 vaccine is under experimental trial in equines.
- Disease Diagnosis: The Centre has been recognized as National Referral Centre for diagnosis of important equine infectious diseases by Department of Animal Husbandry and Dairying, Ministry of Agriculture (Government of India). The Centre has developed diagnostic kits for equine herpes virus-1 (HERP kit) and *Babesia equi* (COFEB kit) infections. In addition, the Centre has developed various tests for diagnosis of equine diseases including equine influenza, equine rhinopneumonitis, equine infectious anaemia, equine piroplasmosis, equine viral arteritis, leptospirosis, mycoplasmosis, *etc*.
- Equine disease surveillance: NRCE is involved in nation-wide disease monitoring and surveillance of important equine diseases particularly those that are included in list “A” and “B” of *Office International des*

*Epizooties* (OIE). The database generated on prevalence of equine diseases from different geographical locations is helping in their effective management. For instance, the Centre contributed significantly in the control of equine influenza outbreak of 1987 involving 83000 equines. Effective influenza vaccine was developed subsequent to this outbreak. The equine babesiosis and equine herpes virus infection is currently endemic in our country and reported by sero-surveillance in 16 different states of the country. Therefore, development of control strategies against these diseases is the main priority of the Centre. Control of EIA in India was done by timely diagnosis and adopting package of practices formulated by NRCE. The disease is not reported from India since 1997 in our active surveillance programme.

- Immunobiologicals: Monoclonal antibodies have been developed for diagnosis and characterization of equine herpes and equine influenza viruses.
- Molecular characterization of pathogens: DNA finger printing of EHV-1 virus and sequencing of antigenically important genes of equine influenza virus was done to identify different strains prevalent in equines of India.
- Artificial insemination: The technique of artificial insemination using frozen semen for production of superior quality mules and donkeys has been perfected. The pure germplasm of endangered indigenous breeds of horses is being conserved using this technology.
- 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 are being established.
- Baseline data has been generated on some of the important haematological, physiological and biochemical indices of Kathiawari horses 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 is being 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 on application (2199/DEL/96) entitled *"A method for preparation of a diagnostic kit useful for forecasting Equine Herpes Virus-1 disease".*
- A patent has been filed for *"Complement fixation test (CFT) based diagnostic COFEB-Kit developed for the detection of Babesia equi antibodies".*

### Services

NRCE provides following services to the farmers and equine breeders:

- Disease diagnosis: 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 mules and donkeys.
- Quality jacks and jennies are supplied to various states, breeding societies and farmers for production of superior quality mules and donkeys.
- Regulation of movement of equines: NRCE is providing health certification for

movement of equines within and outside the country. This facility has helped in promotion of export of horses.

- Extension activities: 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.

## Technologies being developed

- Neutralizing monoclonal antibody based blocking ELISA diagnostic kit for detection of equine herpes virus-1 specific antibodies.
- Inactivated vaccine against EHV-1 infection.
- ELISA-based pregnancy diagnosis kit for equines.
- Latex agglutination test for serodiagnosis of *Salmonella* Abortus equi infection.
- Development of indigenous herbal formulations for equine trypanosomosis.

## Thrust Areas

- Surveillance and monitoring of important equine diseases including emerging and existing diseases with special emphasis on foal mortality and production losses.
- Development of effective and preferably field based diagnostics and potent immunoprophylactics against major equine diseases threatening equine population in India.
- Development of comparatively effective plant-based products for management of some economically important equine diseases and to enhance performance in equids.
- To provide diagnostic and consultancy services for beneficiaries particularly equine farmers and breeders.
- Application of artificial insemination techniques in horse production using frozen semen of true to breed indigenous stallions for the conservation of threatening species in India.
- Breed characterization and *in situ* conservation of various indigenous breeds of horses.
- Exploiting importance of equine draught power for economically weaker section of the society.
- Achieving the status of 'OIE International referral laboratory' for diagnosis of equine rhinopneumonitis and piroplasmosis.

### Staff Position

| Name of the post | Number of posts | | |
|---|---|---|---|
| | Sanctioned | Filled | Vacant |
| Director | 1 | 1 | - |
| Scientific | 25 | 18 | 7 |
| Technical | 23 | 23 | - |
| Administrative | 11 | 11 | - |
| Supporting | 23 | 22 | 1 |
| Total | 83 | 75 | 8 |

## Summary of Expenditure and Revenue Generation

| Summary of Expenditure | 2002-03 | (Rupees in Lacs) 2003-04 |
|---|---|---|
| NON-PLAN | | |
| 1 a. Establishment charges including LSP/PF | 91.53 | 110.08 |
| b. Wages | - | - |
| c. O.T.A. | 0.03 | 0.05 |
| 2. a. Traveling allowances | 2.00 | 2.11 |
| b. HRD | - | - |
| 3. Other charges including equipments | 63.14 | 94.11 |
| 4. Information & Technology | - | - |
| 5. Works | 28.66 | 23.17 |
| *Non-Plan Total* | *185.36* | *229.52* |
| PLAN | | |
| 1. a. Establishment charges including LSP/PF | - | - |
| b. Wages | 0.48 | 0.59 |
| c. O.T.A. | 0.26 | - |
| 2. a. Traveling allowances | 2.00 | 1.91 |
| b. HRD | 0.18 | - |
| 3. Other charges including equipments | 96.87 | 36.77 |
| 4. Information & Technology | 2.36 | - |
| 5. Works | 7.81 | 119.99 |
| 6. One time catch up grant | - | - |
| *Plan Total* | *109.96* | *159.26* |
| Total Expenditure | 295.32 | 388.78 |

| Summary of Revenue Generation | 2002-03 | (Rupees) 2003-04 |
|---|---|---|
| 1. Sale of farm produce & auction of dry trees | 78542 | 5304 |
| 2. Sale of livestock | 591200 | 318582 |
| 3. Sale of publications and advertisements | 1550 | 1050 |
| 4. License Fee | 54363 | 64535 |
| 5. Interest on loans and advances | 222 | 23760 |
| 6. Interest on short term deposits | 68234 | 95900 |
| 7. Income from internal resource generation (EIA service) | 1422500 | 1190600 |
| 8. Auction of old materials | - | 92617 |
| 9. Receipt from services | - | 11652 |
| 10. Other misc. receipts | 125145 | 845676 |
| Total Revenue | 2341756 | 2649676 |

# Research Achievements

## Efficacy of equine herpes virus-1 vaccine in experimental BALB/c mice

An equine herpes virus-1 (EHV-1) killed vaccine incorporating indigenous strain (Hisar-90-7) of EHV-1, developed at this Centre, was evaluated in pregnant BALB/c mice for immune response and protection studies. Humoral immune response was assessed by complement-dependent virus neutralization (VN) test and ELISA, while cell mediated immunity (CMI) was assessed using lymphocyte proliferation assay employing radioactive thymidine. The protective efficacy of the vaccine was assessed by challenging the immunized and control group of mice with heterologous EHV-1 (Raj-98) strain.

In order to determine the dose of this immunogen, 8-10 weeks old pregnant BALB/c mice were immunized intra-peritoneally with EHV-1 vaccine containing 6.25 µg (group 1) and 12.5 µg (group 2) of viral protein, respectively. Booster dose of the vaccine in group 1 and 2 mice was given on day 21. Two days after booster (i.e. on 23$^{rd}$ day of first immunization) mice were put for mating in ratio of 3 females to one male. The pregnant BALB/c mice were challenged with $10^{7.0}$ $TCID_{50}$/25µl of EHV-1 virus (Rajasthan strain) through intranasal route on 14$^{th}$ day of gestation (i.e. 37$^{th}$ day of first immunization). The mice immunized with 6.25 µg immunogen did not provide good immune response till 14 days post-immunization and the protective response was also less in these mice as compared to the group immunized with 12.5 µg immunogen. Hence, 6.25 µg dose of immunogen was not used in further studies.

In the main experiment, mice seronegative for EHV-1 antibody by ELISA and VNT were divided in 4 groups. Mice belonging to group 1 and 2 were immunized with 12.5 µg and 25 µg immunogen, respectively. Control group 3 mice were inoculated oil adjuvant while control group 4 mice were kept as such. Booster vaccine was given on day 21 and mice were challenged (except group 4) with Raj-98 EHV-1 on 14th day of gestation, as described for previous experiment. The mice were observed for clinical symptoms and abortion/ parturition.

Mice from group 1 and 2 showed initiation of humoral immune response from 14 days post-vaccination. Both group 1 and 2 mice showed almost similar pattern of humoral immune response (Table 1). Cell mediated immune response as measured by lymphocyte proliferation assay showed a marginal increase in animals of groups 1 and 2 on 28 days post-vaccination (Table 1).

**Table 1. Immune response in mice immunized with EHV-1 immunogens**

| Assay used | Days post vaccination | Response in mice at different days | | | |
|---|---|---|---|---|---|
| | | Group 1 12.5 ug (n=16) | Group 2 25 ug (n=18) | Goup 3 Non-vaccinated (n=6) | Group 4 Non-vaccinated (n=6) |
| VNT | 14 | 0.9 (n=2) | 1.1(n=2) | - (n=1) | - (n=1) |
| | 28 | 1.4 (n=2) | 1.1 (n=2) | - (n=1) | - (n=1) |
| ELISA | 14 | 3.6 | 3.3 | - | - |
| | 28 | 3.6 | 3.6 | - | - |
| Lymphocyte proliferation assay | 14 | 1.005 | 1.160 | - | |
| | 28 | 2.230 | 2.250 | - | - |

Fig. 1. Aborted mice fetuses with attached placenta following EHV-1 challenge

After challenge of pregnant mice with Raj-98 strain of EHV-1, the protective efficacy was observed by recording signs like abortion, mortality, dyspnoea, weight loss, crouching in corners, vaginal discharge, virus clearance and histopathological lesions in different groups (Table 2). After virus challenge, 6 out of 10 (60%) immunized mice belonging to group 1 delivered normal healthy pups, while 7 out of 10 (70%) mice belonging to group 2 delivered normal pups. However, 4 out of 6 (66.66%) of non-immunized mice of control group 3 aborted after challenge (Fig 1). Marked dyspnoea and crouching in corners were also recorded in this group of mice till 5 days post-challenge (Table 2). All non-challenged pregnant mice of group 4 delivered normal healthy pups.

Histopathological studies on virus challenge in non-vaccinated animals (group 3) revealed severe changes in lungs characterized by congestion of blood vessels, infiltration of polymorphonuclear cells in the interstitium, presence of eosinophilic intranuclear inclusion bodies in bronchial and alveolar epithelium, hyperpalasia of bronchial epithelium, ballooning and rounding of cells in initial stages followed by necrosis (Fig. 2a), lymphocytic infiltration and syncitia formation up to 7 days post-challenge in lungs, whereas perivascular and peribronchial accumulation of lymphocytes was more pronounced in the sections of the lungs from mice of group 1 and 2 till 7 days post-challenge (Fig. 2b).

Virus antigen could be demonstrated in bronchi till 5 days post-challenge in animals vaccinated with 12.5 µg immunogen by immunoperoxidase technique (IPT) (Fig. 3a). However, it could not

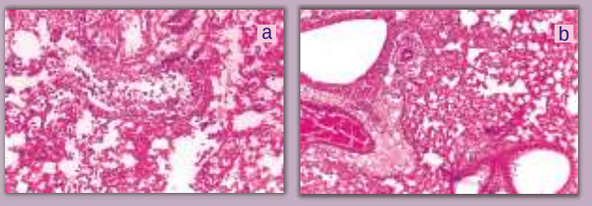


Fig. 2. Section of lung showing complete bronchial epithelial necrosis and infiltration in lumen from animal of group 3 (a) and group 2 mice showing perivascular and peribronchial lymphocytic infiltration (b)

be detected in any tissues of group 2 mice post-challenge. Antigen was demonstrated in both bronchi and alveoli of control mice till 7 days post-challenge (Fig. 3b).

There was severe congestion of chorionic blood vessels in placenta from early abortions of control group 3 mice. Chorionic plate and nuclei of trophoblastic tissue appeared pyknotic along with focal areas of necrosis in trophoblastic tissue. The blood vessels showed focal necrosis of endothelial lining with severe congestion. Placental chorionic plate was severely necrosed in mice aborted around 5 days post-challenge

Table 2. Clinical signs in pregnant BALB/c mice on challenge with EHV-1 (Raj-98 strain)

| Groups | Days post-challenge | Numbers of mice showing signs | | | | |
|---|---|---|---|---|---|---|
| | | Dyspnoea | Crouching in corners | Vaginal Discharge | Abortions | Mortality |
| Vaccinated | 3 | 5 | 7 | 3 | 2 | 1 |
| Group 1 (n=10) | 5 | 2 | 3 | 1 | 2 | 1 |
| Vaccinated | 3 | - | - | 4 | 2 | - |
| Group 2 (n=10) | 5 | - | - | - | 1 | - |
| Unvaccinated | 3 | 6 | 6 | 4 | 2 | 2 |
| Group 3 (n=6) | 5 | 4 | 3 | 2 | 2 | - |
| Unvaccinated, | 3 | - | - | - | - | - |
| unchallenged | 5 | - | - | - | - | - |
| Group 4 (n=6) | | | | | | |

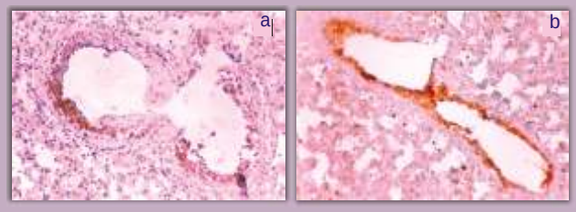


Fig. 3. Indirect immunoperoxidase technique showing the presence of brown coloured antigen in the bronchial epithelial lining in animal from group 2 at 3dpi (a) and in bronchi and alveoli in mice of group 3 (b)

(Fig. 4a). Antigen was also demonstrated in trophoblastic and other tissues by indirect immunofluore scence test in the cases of abortion from all the groups (Fig. 4b).

After challenge, no virus could be isolated from lungs of mice immunized with 25 µg (group 2) immunogen on days 3 and 5 post-challenge, however, placenta from a case of abortion was positive for virus isolation on day 5 post-challenge. Virus was isolated from lungs, brain, placenta and fetuses at days 3 and 5 post-challenge in non-vaccinated animals (group 3). No virus was recovered from any tissues of mice belonging to group 4.

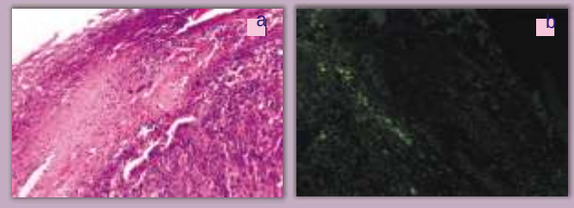


Fig. 4. Necrosis of chorionic plate and presence of pyknotic nuclei in group 2 mice aborted 4 days post-challenge (a) and IIFT showing antigen in the chorionic plate in mice of group 3 (b)

These findings indicate that the EHV-1 immunogen (25 µg per mice) provides good immune response and protection against challenge in BALB/c mice.

(B.K. Singh and Nitin Virmani)

# Seromonitoring of important diseases in indigenous equines

Seromonitoring of important equine diseases is being undertaken with special emphasis on indigenous equines to study the magnitude of existing and emerging equine diseases in different states of the country and to make strategies for effective prevention and control of equine diseases. The long term objective is to improve the health of equines in the country, prevention of diseases through epidemiological modeling, disease forecasting and identification of disease-free zones. During the year, sero-surveillance was conducted in 12 States/ UTs of India, namely, Bihar, Delhi, Haryana, Himachal Pradesh, Jammu and Kashmir, Karnataka, Madhya Pradesh, Meghalaya, Rajasthan, Punjab, Uttar Pradesh and Uttaranchal.

Haemagglutination inhibition (HI) test for equine influenza (EI) was conducted on 1424 samples and four of these samples (2 from U.P, 1 from Karnataka, 1 from M. P) were sero-positive for equine influenza (A/equi-2). A total of 1483 serum samples from indigenous equines were tested for EHV-1 and of these 81 (5.46 %) samples were positive for EHV-1 antibodies. Out of 1431 serum samples tested for *Babesia equi* infection by complement fixation test, 387 (27.04 %) were found positive (Table 3).

For equine infectious anaemia (EIA), 1517 serum samples from indigenous equines were examined by Coggins test, however, none of the samples tested was found positive. In our continuous surveillance and monitoring of EIA,

Table 3. Sero-prevalence of various diseases among indigenous equines

| State/U.T. | Number of tested (positive) | | |
|---|---|---|---|
| | E.I. | EHV-1 | *B. equi* |
| Karnataka | 143(1) | 166 (12) | 166 (32) |
| Haryana | 7 (0) | 13 (0) | 13 (0) |
| Meghalaya | 0 (0) | 21 (0) | 21 (6) |
| Rajasthan | 408 (0) | 414 (10) | 408 (127) |
| J&K | 422 (0) | 422 (50) | 422 (108) |
| Punjab | 57 (0) | 58 (3) | 57 (4) |
| Uttaranchal | 17 (0) | 17 (2) | 17 (2) |
| *U.P.* | 152 (2) | 154 (1) | 109 (26) |
| *H.P.* | 73 (0) | 73 (0) | 73 (11) |
| *Bihar* | 48 (0) | 48 (0) | 48 (26) |
| *M.P.* | 97 (1) | 97 (3) | 97 (45) |
| Total | 1424 (4) | 1483 (81) | 1431 (387) |

not a single positive case has been recorded since 1999. Similarly, none of the samples (n=348) tested for African horse sickness by ELISA was found positive. Out of 548 samples of examined for *Mycoplasma equigenitalium* by indirect ELISA, 40 (7.3%) were positive. Serum samples tested for glanders (n=1519) by complement fixation test, brucellosis (n=1517) by plate/tube agglutination test and *Salmonella* Abortus equi infection (n=1517) by tube agglutination test and all were found negative for these diseases.

(S.K. Dwivedi, S.K. Khurana, A.S. Panisup, A.K. Gupta, B.K. Singh, S. Dey, B.R. Gulati, Y. Pal, R. Kumar, P. Malik and N. Virmani)

# Validation of EHV-1 blocking ELISA kit

This Centre has developed a neutralizing monoclonal antibodies-based blocking ELISA (B-ELISA) kit, which is rapid, simple, highly sensitive, specific and accurate for serodiagnosis of EHV-1 infection, by testing single dilution of horse sera. The results of the kit were further validated during 2003-2004 using purified EHV-1 antigen for coating ELISA plate. The shelf life of the kit was determined and user-to-user variations were assessed by demonstrations in different laboratories.

A total of 360 equine serum samples from 11 states (Haryana; Himachal Pradesh; Jharkhand; Maharastra; Manipur; Meghalaya; Punjab; Rajasthan; Tamil Nadu; Uttaranchal; West Bengal) were collected and used for validation of B-ELISA. The B-ELISA was 73.68 and 93.06 per cent sensitive and specific, respectively, when compared with VNT (Table 4). The percent agreement between these two tests was 90 percent indicating that the present B-ELISA is an alternative to VNT which is considered gold standard for testing serum samples for detection of EHV-1 antibodies.

The shelf life of the freeze-dried reagents of the kit was 6 months. However, if the freeze-dried reagents supplied along with the kit are once dissolved, should be used the same day. To further increase the acceptability of the kit, efforts are under way to stabilize the diagnostic reagents in buffer liquid using various stabilizers and to study the shelf life of the reagents in this buffer.

The kit was demonstrated at Central Military Veterinary Laboratory (CMVL), Meerut in the month of August 2003. The kit worked satisfactorily with minor variations in the result when a new person handled this test for the first time (Table 5).

(This work was done under NATP MM Project on *Veterinary diagnostics for prevalent and emerging disease)*

(B.K. Singh)

Table 4. Analysis of sensitivity and specificity of B-ELISA kit as compared to VNT

| Screening test | Screening test result | Serodiagnosis based on VNT | | Sensitivity per cent | Specificity per cent |
|---|---|---|---|---|---|
| | | Positive | Negative | | |
| B-ELISA | Positive | 42 | 21 | 73.68 | 93.06 |
| | Negative | 15 | 282 | | |

Table 5. Results of B-ELISA Kit with equine serum samples (n=88) when tested by two different persons

| Person performed the test from place | Results of serum samples tested by B-ELISA kit | | |
|---|---|---|---|
| | Negative | Positive (%I ≥ 21-50) | Adequate positive (%I ≥ 51-64) |
| NRCE officer | 40 | 40 | 8 |
| CMVL, Meerut officer | 50 | 29 | 9 |

Percent agreement between tests conducted simultaneously was 87.5%.

# Epidemiology of foal diarrhoea

Infectious diarrhoea poses a challenge each foaling season to farm managers and veterinarians in intensive horse breeding areas throughout the world. The major etiological agents associated with foal diarrhoea are rotavirus, *Cryptosporidium, Clostridium, Salmonella* and *E. coli.* However, there is no information about the magnitude of the diarrhoea in foals of India. About 50 diarrheic stool samples collected from organized farms around Hisar (Haryana) were tested for rotavirus, bacteria (*E. coli, Salmonella* and *Clostridium)*, parasitic oocysts and protozoa (*Cryptosporidium)*.

Rotavirus was detected in 4 (8%) samples by ELISA and RNA-PAGE. Rotavirus from four positive stool samples could be adapted to grow in MA104 cell cultures producing characteristic cytopathic effects in cell culture. RNA electropherotypes of the isolated equine rotaviruses were analyzed to get the evidence for the genetic diversity and heterogeneity among the isolated rotaviruses. The electrophoretic profile of the equine rotavirus isolates from the outbreak in the month of June 2003 (FRV2 and FRV3) differed from those isolated from the July outbreak (FRV25 and FRV28) on the same farm. Migration of RNA segment 5 in case of FRV 2 and FRV3 was slower than that of isolates FRV25 and FRV28 (Fig. 5), indicating that at least two different rotaviruses are circulating in the region.

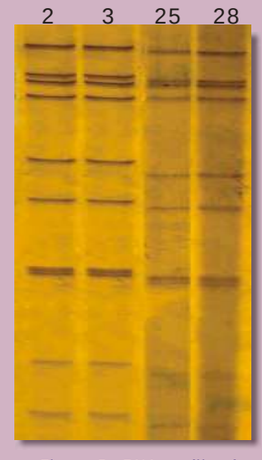

Figure 5. RNA profile of isolated foal rotaviruses

No parasitic oocysts and *Cryptosporidium* were detected in the diarrhoeic foal samples examined by microscopy and acid-fast staining.

On processing the samples for the isolation of bacteria, 28 (54.9%) yielded *E. coli.* No *Salmonella* or *Clostridium* could be isolated. Antibiotic sensitivity of *E. coli* isolates revealed that maximum number of isolates were sensitive to chloramphenicol followed by ampicillin. *In vitro* cytotoxigenicity in Vero cells indicated that only 9 *E. coli* isolates were cytotoxigenic in nature.

(Baldev R. Gulati, Praveen Malik and Rajender Kumar)

# Studies on *Streptococcus equi*

Most clinical reports and research involving beta haemolytic Lancefield group C streptococcal infections in equines have been concerned with *Streptococcus equi* subspecies *equi,* the causative agent of strangles. In contrast, infections caused by *Streptococcus equi* subspecies *zooepidemicus* have received very little attention with respect to relevance in clinical cases and research concerning its pathobiology. This subspecies is present as resident microflora on skin, nasopharynx, gastro-intestinal tract and vagina of many healthy equines and is sometimes isolated as the only microbial pathogen in many disease conditions like abortions, endometritis, cervicitis, pneumonia, abscesses, joint infections, etc. Foal pneumonia and lower respiratory disease in young horses are principally associated with *S. zooepidemicus.*

Twenty-nine streptococcal isolates of equine origin were characterized using the antibiogram and mice pathogenicity. The isolates included 11 *S. equi* and 18 *S. zooepidemicus* which originated from both clinically diseased as well as apparently healthy equines. All the *S. equi were* isolated from clinical cases, whereas *S. zooepidemicus* originated from abortions (3),

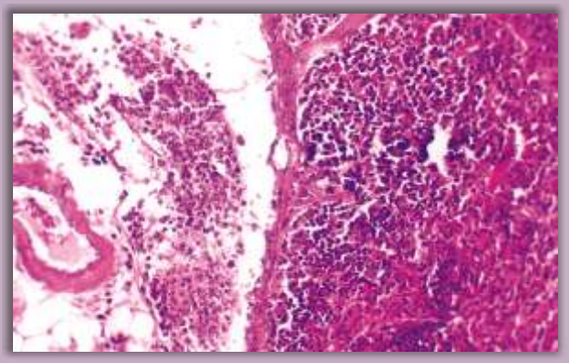

Fig. 6. Spleen showing thickened capsule and neutrophilic infiltration

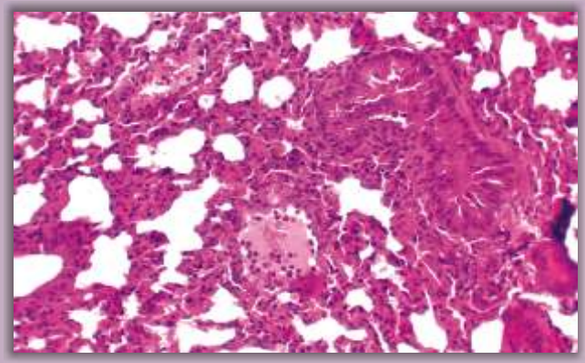

**Fig. 7. Section of lung showing serous exudate and neutrophilic infiltration in alveolar parenchyma**

repeat breeders (5), nasal discharges (2), foal pneumonia (1), strangles like lesion (1), ocular discharge (1), pus (2) and apparently normal equines (2). Remaining were two standard cultures, one each of *S. equi* and *S. zooepidemicus.* Antibiotic sensitivity and mice pathogenicity of these isolates were evaluated during the year.

Antibiotic sensitivity test was conducted using 18 antimicrobials. Most isolates were sensitive to ciprofloxacin, lincomycin, amoxycillin, trimethoprim and penicillin while resistant to kanamycin, nalidixic acid and sulphadiazine.

To assess the pathogenicity of these isolates (n=29), all isolates were grown individually in Todd Hewitt broth at 37°C for 18 h. Three Swiss-albino mice per isolate were inoculated using a dose equal to ten $LD_{50}$ in 0.2 ml of the broth culture by intra-peritoneal (IP) route. For negative control, three mice were injected sterile broth given similar incubation conditions. Following inoculation, mice were observed every 12 h till their death or for seven days (whichever is earlier). Mice that died were necropsied and histopathology conducted. The re-isolation of organism from the heart blood of dead mice was taken as indicator for the specificity of etiology of the mortality.

The results of mice pathogenicity indicated that all the *S. equi* isolates, including the standard strain, were highly pathogenic to mice, killing all the three mice in less than 24 h. Though all the *S. zooepidemicus* isolates were also pathogenic to mice, 7 of them were less pathogenic, taking upto 48-72 h to kill the mice. All these mildly pathogenic isolates originated from mild clinical conditions or from apparently healthy equines, whereas isolates from conditions like abortion, foal pneumonia *etc* were also highly pathogenic to mice.

The histo-pathological examination of the tissues collected from the dead mice revealed that the spleen had thickened capsule along with presence of leukocytic infiltration (Fig. 6). Other organs including lungs (Fig. 7), heart, kidney and liver also showed severe congestion, necrosis and leucocytic infiltration indicating that the isolates were pathogenic in nature.

(Praveen Malik, S.K. Khurana, Nitin Virmani and B.R. Gulati)

## Detection of *Trypanosoma evansi* by PCR

Trypanosomosis caused by *Trypanosoma evansi* is the most important protozoan disease of equines causing high morbidity and mortality. Although trypanosomosis has been studied since the beginning of the century, the diagnostics used for trypanosome infection still suffer from low sensitivity and specificity. The demonstration of the parasites by parasitological and standard trypanosome detection methods is not foolproof. Therefore, there is an intense need to develop

and validate improved diagnostic tools. In the present study, PCR has been standardized for identification of *Trypanosoma evansi* collected from experimentally infected rats. At the peak of peripheral parasitaemia, blood was collected for separation of trypanosomes through DEAE cellulose column. The purified trypanosomes pellet was used for DNA extraction. PCR was standardized using a set of primers (5'-TGCAGACGACCTGACGCTACT-3' and 5'-CTCCTAGAAGCTTCGGTGTCCT-3'). Conditions were optimized for PCR assay using 50 µl reactions containing 200 µM of each dATP, dTTP, dCTP and dGTP, 5 µl reaction mixture buffer, one unit of Taq DNA polymerase, 0.25 µM of each primer and 2 µl template (extracted DNA from one ml blood sample, dissolved in 50 µl of TE buffer). The sample was pre-incubated at 95°C for 5 min to completely denature the DNA. This was followed by 30 cycles of 1 min at 94°C (to denature), 1 min at 60°C (to anneal) and 1 min at 72°C (to extend) and one extensive polymerization at 72°C for 5 min in a thermal cycler. At the end of thermal cycling, the tubes were kept at 4°C before further analysis. Ten µl of PCR product was analyzed by 1.4% agarose gel electrophoresis. *T. evansi* DNA fragment (227 bp) was visualized on UV gel doc system (Fig.8). This PCR is being further evaluated for its sensitivity and specificity for detection of *T. evansi* in blood samples of equines.


Fig. 8. PCR product of *T. evansi*

(Rajender Kumar, A.K. Gupta, S. Dey and S.K. Dwivedi)

# Animal disease monitoring and surveillance

Diseases of livestock cause huge economic losses to livestock owners. In India, outbreaks of major diseases occur frequently and inadequate zoo-sanitary and control measures have result in large number of livestock deaths. In addition, several chronic or sub-clinical diseases add to economic losses by decrease in production and fertility, insufficient weight gain, inefficient feed utilization and reduced draught power. Further, some of the zoonotic diseases have significant impact on public health. This demands accurate information about the health status of nation's livestock population, which is critical in control of endemic diseases.

This study was done to measure disease prevalence and incidence in various livestock species through active surveillance and to strengthen the disease database of the country and to develop specific livestock health systems for promotion of animal health.

During the year 47 cattle, 409 buffalo, 29 sheep and 22 goat sera were screened for brucellosis, out of these 5 (10.6 %) cattle and 28 (6.84%) buffalo serum samples were found to be positive. Sixteen horse serum samples were screened at NRCE for ten diseases (Leptospirosis, *Salmonella* Abortusequi, glanders, brucellosis, equine infectious anaemia, equine herpes virus infection, equine viral arteritis, *Babesia equi, Mycoplasma equigenitalium* infection, and equine influenza) and all were found negative.

(This work was done under NATP Mission Mode Project on *Animal Health Information System through Disease Monitoring & Surveillance*)

(S.K. Dwivedi, S.K. Khurana and S. Qureshi)

# Cadmium in blood of Indian horses

The importance of cadmium (Cd) as a toxic metal and environmental pollutant has long been recognized. Industrial and agricultural processes have resulted in release of this toxic metal in the environment. There is every possibility that horses may be exposed to this toxic metal that could cause adverse effects like painful osteoskeletal diseases, abnormal testicular /ovarian function and neurological disorders. A cross-sectional study was, therefore, undertaken to record the Cd concentration in blood of horses.

Blood samples (n=288) were obtained from horses in urban as well as rural areas. The samples were digested and Cd concentration was estimated using atomic absorption spectrophotometer. The profile of Cd concentration in blood is given in Table 6. The mean Cd concentration in blood samples was 0.064±0.003 and 0.034±0.002 ppm in urban and rural areas, respectively. Most of the samples from horses of the urban locality contained blood Cd above 0.02 ppm (maximum physiological limit in blood), however, no overt signs of toxicity were observed in these animals.

Table 6. Blood cadmium concentrations in Indian horses

| Cadmium Concentration (ppm) | No. of horses (%) in area | |
|---|---|---|
| | Urban | Rural |
| < 0.02 | 5 (2.27) | 26 (38.24) |
| 0.03-0.04 | 10 (4.55) | 22 (32.35) |
| 0.04-0.05 | 20 (9.09) | 18 (26.47) |
| 0.05-0.06 | 70 (31.82) | 2 (2.94) |
| 0.06-0.07 | 50 (22.73) | 0 (0.00) |
| 0.07-0.08 | 35 (15.91) | 0 (0.00) |
| 0.08-0.09 | 30 (13.63) | 0 (0.00) |
| >0.09 | 0 (0.00) | 0 (0.00) |
| Total | 220 (100) | 68 (100) |
| Medium | 0.064 | 0.035 |
| Mean | 0.064* | 0.034 |
| Standard Error | 0.003 | 0.002 |

** Differs significantly ($p \leq 0.01$) from that of samples from rural area*

The mean Cd concentration in forage fed to these horses was 12.74±2.46 and 5.27±0.88 ppm in urban and rural areas, respectively. However, the Cd concentration in water samples used for these horses in both areas was below the detection limit (less than 0.001 ppm).

The results provide evidence that horse population reared in urban areas is exposed to excessive environmental Cd resulting in higher concentration of this toxic metal in their blood. The Cd concentration in forage samples routinely used by these horses in both urban and rural areas is appreciably higher than the permissible limit of this mineral for equines (below 0.5 ppm). Intake of Cd contaminated forages could be a contributing factor for higher body Cd burden in these horses. Continuous monitoring is, therefore, required to avoid adverse effects of this metal on equine health.

(S. Dey and S.K. Dwivedi)

# Studies on fluoride levels in equine serum

Fluoride is a highly reactive element and abundantly distributed in earth's crust, which many a times leaches into soil and sub-soil water. Due to intense industrial and agricultural activities, the concentration of this metal in water, air and vegetation has increased tremendously. Continuous intake of fluoride-rich water affects man and other domestic animals in certain endemic localities. Since horses are also inhabited in such localities, there is a possibility of excessive fluoride exposure to horses. A study was, therefore, undertaken to find out the blood fluoride concentration of horses inhabited in areas rich in water fluoride content.

Samples of water were collected from different parts of India and were analyzed for fluoride content. Based on water mean fluoride content study, areas were divided into 3 categories. A: 1-2 ppm; B: 2-3 ppm; C: >3 ppm (Fig. 9). Blood samples were collected from horses of these areas and serum fluoride content was estimated using ion selective potentiometry.

The fluoride content in serum and water is provided in Table 7. The background values (the level usually found in blood of healthy equine) is reported to be less than 0.20 ppm and sub clinical health hazards have been reported at serum concentration >0.50 ppm. In this study, blood fluoride level in 83 out of 411 equines was =0.50 ppm and these horses were from areas where drinking water contained more than 2.0 ppm of fluoride. Further studies are needed to ascertain the health status of equines in areas with fluoride content more than 2 ppm in drinking water.

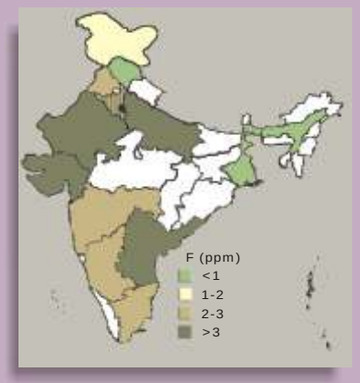


Fig. 9. Areas selected based on water fluoride content in the study

Table 7. Fluoride in serum of equines from areas with different water fluoride concentrations

| Water fluoride (ppm) | Equine serum | No. of samples |
|---|---|---|
| 1-2 | 0.117-0.294 | 127 |
| 2-3 | 0.292-0.682 | 196 |
| >3 | 0.421-0.832 | 215 |

S. Dey and S.K. Dwivedi

# Studies on infertility in mares

There are many infectious and non-infectious causes of infertility that act either alone or in combination and lead to reduced reproductive performance. In the present study, fifty eight mares and jennies with known history of infertility were investigated through exfoliative cytology and microbial studies to find out the causes of infertility. These included 51 mares from organized sector and 7 animals (three jennies and four mares) from unorganized sector.

Exfoliative studies on the uterine aspirate of the affected animals revealed medium to large number of neutrophils, degenerating polymorphonuclear cells, cellular debris, lymphocytes, monocytes, epithelial cells, erythrocytes, bacterial and fungal organisms signifying inflammatory responses. Cytological studies revealed 24 cases (53.33%) to be in acute stage showing live neutrophils, cellular debris and epithelial cells (Fig. 10). Twenty one cases (46.66%) could be identified to be chronic in nature and revealed predominantly lymphocytes, macrophages, plasma cells, cellular debris, mucin threads. On the basis of cytological studies and isolation patterns 45 (77.6%) cases were found associated with microbial agents.

Organisms isolated from uterine and vaginal swabs are shown in Table 8. Majority of the agents identified (40, 84.45%) were bacteria (Fig. 10a & b), however, fungi either alone or in association with bacteria (two cases associated with *Streptococcus zooepidemicus* infection) could be identified in 7 (15.55%) cases (Fig. 10c).

Table 8. Isolation of micro-organisms from equine infertility cases (n=58)

| Organisms isolated | Number |
|---|---|
| *Bacteria* | |
| *Streptococcus zooepidemicus* | 18 |
| α–haemolytic Streptococci | 4 |
| *Staphylococcus* spp. | 10 |
| *E. coli* | 3 |
| Unidentified bacteria | 5 |
| *Fungus* | |
| *Candida* spp. | 5 |
| Fungi with branched septate hyphae | 2 |

In addition, anatomical defects such as pneumovagina (four cases), urovagina (eight cases) and poor perineal conformation (four cases) were found associated in 31.1% cases with infectious causes of infertility. There was good agreement (86.67%) between cytological findings on the uterine aspirate and the results of isolation in these cases. In 13.33% cases, cytological smears indicated the possibility of infection, however, isolation attemps did not yield

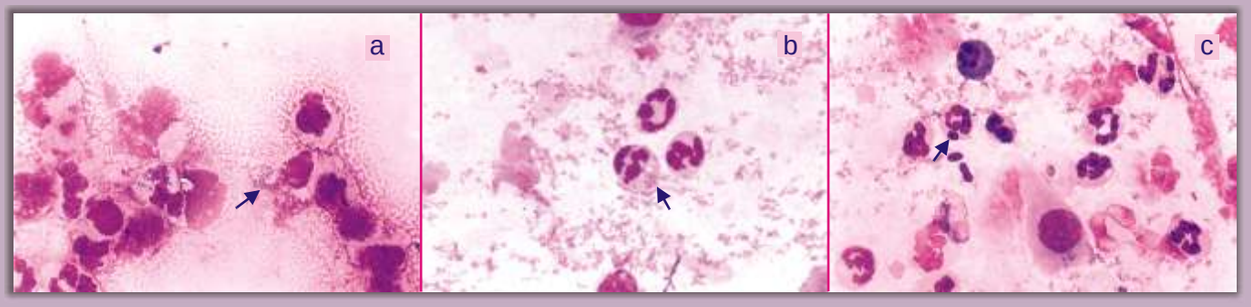


Fig. 10. Exfoliative cytology on uterine aspirate showing neutrophils with phagocytosed cocci (a), bacilli organisms (b) and yeast (c)

any infectious agents. This might be due to anti-microbial treatment in these animals or anaerobic bacterial infection that was not studied in the present investigations. Cytological examination of uterine aspirate was found to be an effective tool in establishing the cause of infertility.

(Nitin Virmani, A.S. Panisup
Praveen Malik and S. Dey)

# Pregnancy diagnosis in mares by ELISA

Pregnancy diagnosis in mares is a major problem faced by poor equine owners and equine breeders who do not have facility for ultrasound scanning of their mares for pregnancy or do not have expert veterinarian within approachable limits or can not afford the cost of the examination fee. This problem has been solved to a greater extent by a sandwich ELISA being developed at this Centre. With the help of this test, pregnancy can be detected between days 35 and 120 days gestation in mares covered by horse stallions. However, this test has a limitation as it can not be used for the same purpose in mares covered by donkey stallion. This serum based test is animal friendly as it does not involve the transport of pregnant animal to diagnostic centre.

Serum samples (n=720) were collected for pregnancy diagnosis from mares covered by horse or donkey. Sandwich ELISA was used to detect the eCG contents in 246 samples collected at different gestation intervals (between days 35 and 150) from mares, covered by horse stallion. The eCG content ranged from 18.75 to 211.25 IU/ml serum in these samples. A rapid increase in eCG content was observed during 45 to 90 days of gestation and thereafter a decrease was observed in all the serum samples (Fig. 11).


Fig.11. Serum eCG content at different gestation intervals in pregnant mares

All the 73 serum samples collected between days 35 and 45 of gestation were detected pregnant by ELISA, as these contained eCG which ranged from 18.75 to 150.75 IU/ml serum. ELISA results were confirmed by ultra-sonography or by rectal examination findings, which in turn indicated that with the help of this sandwich ELISA, pregnancy can be detected in mares from 35 days of gestation.

Further, 30 out of 53 sera collected between days 20 and 35 of gestation were also observed to contain eCG ranging from 2.25 to 67.5 IU/ml of serum, indicating that this ELISA could be used for pregnancy diagnosis even at earlier gestation intervals. The efficacy of ELISA for this purpose is being further studied.

This ELISA was also evaluated for pregnancy diagnosis in mares covered for mule production. Serum samples (n=291) collected at regular interval between 21 and 60 days of gestation from pregnant mares covered by donkey-stallion were evaluated for their eCG contents. eCG levels was within detectable levels in 57 serum samples only. In these samples, it ranged between 1.25 and 72.5 IU/ml of serum. No specific increase or decrease in eCG content with increased gestation period was observed. These results revealed that this test is not suitable for pregnancy detection in the mares covered for mule production.

(A. K. Gupta, Yash Pal and S. K. Dwivedi)

# Characterization of horses of Marwari breed

Marwari horses are known for their endurance potential, sturdiness, stiffness and disease resistance. Quality of these animals is deteriorating due to lack of sound breeding practices, which necessitated the conservation and characterization of horses true to this breed. For characterization of horses of this breed, three different approaches i.e. bio-metrical, bio-chemical and molecular characterization, were followed.

Biometrical studies were carried out with sixty-six mares and eight stallions and various indices namely height at withers, body weight, heart girth, leg length, height at knee, face length, face width, ear length, tail length and body weight were measured. Statistical analysis of the data of both mares and stallions revealed significant difference due to sex only in average height at withers (Table 9). The average height at withers of Marwari stallions was 153.0±0.93 cm whereas it was 149.1+0.43 cm in mares. This information will serve as baseline data for these biometrical indices for horses of Marwari breed.

Different biochemical indices like enzymes (SGOT, SGPT, ALP, LDH, CK) and metabolites (glucose, albumin, total serum protein, triglyceride and blood urea) were evaluated in horses of both the sexes. Significant difference due to sex was observed in triglyceride, SGPT and SGOT. The GOT was higher in adult animals whereas ALP was higher in young ones. The tri-glycerides and GPT were significantly higher in females than in the males (Table 10). The data generated will be helpful in health monitoring of these horses.

Table 9. Biometrical analysis of Marwari horses

| S. No. | Parameters (in cm) | Mares (n=66) | Stallions (n=8) |
|---|---|---|---|
| 1. | Height at withers | 149.1±0.43 | 153.0±0.93 |
| 2. | Body length | 146.6±0.67 | 144.9±1.22 |
| 3. | Heart girth | 169.8±1.11 | 168.1±1.95 |
| 4. | Leg length (fore) | 99.5±0.56 | 99.6±3.07 |
| 5. | Leg length (hind) | 98.0±0.53 | 101.4±0.98 |
| 6. | Height at knee | 45.3±0.26 | 46.5±1.02 |
| 7. | Face length | 62.9±0.52 | 60.4±2.10 |
| 8. | Face width | 19.7±0.18 | 21.3±0.65 |
| 9. | Ear length | 15.4±0.25 | 12.8±0.37 |
| 10. | Ear width | 8.9±0. | 128.3±0.37 |
| 11. | Tail length | 45.5±0.37 | 46.3±0.92 |

Genetic variability in 45 horses of this breed was evaluated using 16 different microsatellite markers by polymerase chain reaction (PCR) amplification, followed by evaluation of PCR products on denaturing polyacrylamide gel electrophoresis. The gels were dried and documented. The genotypes for each set of microsatellite were recorded manually from the dried silver stained gels. Out of the 16 primer pairs, two (AHT16 and AHT44) were found

Table 10. Biochemical parameters of Marwari horses

| Parameters | Male (n=9) | Female (n=21) | Adult (n=19) | Young (n=11) |
|---|---|---|---|---|
| Glucose mg/dl | 92.2±6.71 | 94.5±5.20* | 90.0±4.87 | 108.7±5.76* |
| Albumin mg/dl | 3.89±0.09 | 4.25±0.05 | 4.22±0.05 | 4.01±0.10 |
| T.Protein g/dl | 8.03±0.34 | 8.18±0.29* | 7.98±0.27 | 8.46±0.39* |
| Cholesterol mg/dl | 93.3±2.14 | 90.5±2.29 | 95.2±2.04 | 94.3±3.33 |
| Tri-glycerides mg/dl | 57.7±2.88 | 66.3±2.48** | 61.3±2.44 | 67.4±3.45** |
| Urea mg/dl | 44.0±3.75 | 39.2±2.15* | 43.6±2.55 | 35.1±2.07** |
| GOT U/L | 399.8±59 | 7450.0±48.60* | 508.5±51.86 | 355.0±34.77 |
| GPT U/L | 23.9±1.90 | 38.7±3.01** | 34.2±3.26 | 33.8±4.01 |
| ALP U/L | 552.1±85.26 | 441.0±38.73* | 430.9±39.72 | 562.3±73.51** |
| LDH U/L | 818.0±22.29 | 920.3±28.73* | 878.6±31.02 | 909.4±26.99* |
| CK U/L | 150.4±15.38 | 153.0±13.41* | 154.9±16.86 | 148.6±10.06 |

*P<0.05, ** P<0.01, n=number of observations

monomorphic and UM015 was unscorable. All these 16 microsatellites including these three, were selected on the basis of polymorphic nature with exotic breeds, namely Thoroughbred and Quarterbred horses. These observations indicated that these two primer pairs, AHT16 and AHT44, can be used to differentiate Marwari horses from the above mentioned exotic breeds. The genotypic data of 13 polymorphic microsatellite primers was analyzed by population genetics software POPGENE version 1.31. The statistical analysis indicated the observed number of alleles in the range of 2 (HTG2) to 8 (AHT17, UCDEQ412, LEX68, TKY19). The effective number of alleles were in the range of 1.21 (HTG2) to 4.46 (LEX68) (Fig. 12). Observed heterozygosity ranged from 0.12 (UCDE-Q502) to 0.89 (AHT17). Expected heterozygosity ranged from 0.18 (HTG2) to 0.78 (AHT17), which indicated that high genetic variability exists in Marwari horse population.

Fig. 12. PCR products at microsatellite locus LEX68 in Marwari horses

Genetic characterization of Marwari horses on three more loci is under progress to study the genetic variability among the individuals of Marwari horses, for their further differentiation from other Indian breeds of horses.

(S.N. Tandon, A.K. Gupta, R.A. Legha, Mamta and R.C. Sharma)

## Cryopreservation of stallion semen in Marwari horses

Horses of Marwari breed are known for their majestic look and endurance potential. This breed belongs to Marwar region of Rajasthan. Marwari, Kathiawari and Sindhi horses have co-existed in Rajasthan over last few decades. Co-existence as well as lack of awareness among their owners resulted in inter-breeding, which has brought the true-to-breed Marwari horses on the verge of extinction. Cryopreservation of semen is one of the important and easiest tools for breed conservation. Cryopreserved doses of quality semen can be used for breed improvement as well as to meet out the demand of equine owners residing in far off places.

In order to establish the normal seminal characteristics of Marwari horses, physico-biochemical characteristics of filtered gel-free semen were studied. The average volume of semen and gel-free semen was 85.50 ml and 38.0 ml, respectively. Average sperm concentration was 156 $x10^6$ per ml in Marwari stallions. Live:dead ratio of spermatozoa in frozen semen was 66:34. Mean activity of GOT and GPT was 146±17 and 13.5±1.9 IU/l. Mean activity of LDH and CK was 698±83 and 724±89 IU/l. Average glucose and total protein content was 15.7 mg/dl and 4.0 g/dl. Average cholesterol and triglyceride concentration was 5.7 and 37.7 mg/dl. The semen was also tested and found negative for the presence of pathogens namely; *Salmonella* Abortus equi, *Pseudomonas* spp., *Brucella* spp., *Streptococcus* spp., *Taylorella equigenetalis, Mycoplasma.*

Six primary extenders *viz.*, BSA primary extender, citrate EDTA extender, glucose EDTA extender, skimmed milk and sugar extender, sucrose solution (11%) and HF-20 extender were used for washing the spermatozoa and secondary extenders, *viz.*, skimmed milk egg yolk extender, lactose-glucose-egg-yolk extender, glycine-egg-yolk-extender, skimmed milk and sugar extender, sugar-based extender and HF-20 extender were used as freezing media during the year. Lactose-glucose-egg-yolk extender gave better (25-40%) post-thaw motility followed by sugar-based extender (20-30%) and HF-20 extender (20-30%). However, pre-freezing motility was observed comparatively high with sucrose solution.

(Yash Pal, R.A. Legha, S.K. Khurana and S.N. Tandon)

# Field trial of artificial insemination with cryopreserved jack semen

Artificial insemination (AI) is one of the important techniques used for the genetic improvement of animals at faster pace, because enough spermatozoa produced by selected males can be inseminated in thousands of females per year. It involves mainly collection of semen from selected males, its evaluation and ultimately deposition of semen into a sexually receptive female at the time of ovulation. AI with frozen jack's semen is an important aspect in production of superior mules. Natural breeding or AI with fresh liquid semen may not be of much advantageous because of certain limitations such as difficulties in transportation of animals, limited use of good stallion, *etc*.

Field trial of AI with frozen semen of jack was undertaken during the year 2003-04 in the villages of Karnal and Panipat districts of Haryana state. A total of 105 females were inseminated with frozen semen during the months of June-Aug 2003. After one month of AI, pregnancy diagnosis in 95 of the inseminated females showed that 39 were pregnant with the conception rate of 41.05%.

In another AI trial in the animals at E.P.C., NRCE, Bikaner, a total of 11 jennies were inseminated with frozen semen out of which 4 conceived.

(R.A. Legha, S.N. Tandon and R.C. Sharma)

# Detection of angiotensin-1-converting enzyme gene (ACE) in indigenous equines

Angiotensin-1-converting enzyme gene (ACE) is well known in humans for its relation with endurance potential. One variant of the gene is found in the humans of limitless stamina. In horses, studies to detect polymorphism, if any, in this gene were initiated with a set of primers for horses (F-GCCAGGATGTTTAAGGA, R-CTTGCCGTTGTAGAAGTCCCA). A part of ACE gene was amplified by polymerase chain reaction in 41 Marwari, 14 Thoroughbred, 24 Kathiawari and 24 Spiti horses. Electrophoresis of the amplified PCR products on 2% agarose gel showed a single band of ~180 bp on amplification (Fig 13), indicating that there is no polymorphism at this level in the amplified ACE gene of Indigenous horses under study.

Fig. 13. PCR products of ACE gene in Marwari horses

(Mamta and A.K. Gupta)

# Technologies Assessed

## Validation of improved kit for EHV-1 diagnosis

Neutralizing monoclonal antibodies-based blocking ELISA (B-ELISA) kit developed at this Centre for serodiagnosis of EHV-1 infection was further evaluated during the year for its shelf life and user-to-user variations when used in different laboratories. Minor variations in the results of the kit were observed when the kit was used by the new user for the first time. The shelf life of the freeze-dried reagents of the kit was 6 months. However, if the freeze-dried reagents are once dissolved, should be used the same day. Efforts are under way to stabilize the reagents in liquid buffer to further improve its acceptability and shelf life.

## Artificial insemination in Equines

To cater to the need of farmers for breed improvement of equines, this Centre provided artificial insemination services using superior quality semen of Marwari and exotic donkey stallions. Fifteen mares were artificially inseminated using semen from Marwari stallion (12), exotic donkey (3) of the Centre for the production of superior quality horses and mules. The expected time of ovulation in these mares was determined using ultrasonography (Fig. 1) and the insemination was performed twice to each mare at 24 hours interval around expected time of ovulation. Ten out of these 15 mares were conceived (Fig. 2) and delivered healthy foals (Fig. 3). The conception rate was recorded as 66.66%. The use of ultrasonography made it possible to improve reproductive performance of equines.

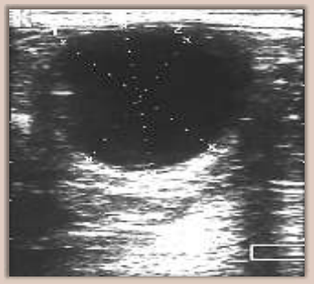

Fig.1. Ultrasonograph showing mature graffian follicle in the right ovary

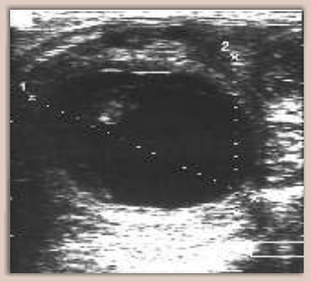

Fig. 2. Uterine body showing 40 days of pregnancy

Fig. 3. Mule foal-at-feet produced by artificial insemination using cryopreserved semen

# Education and Training

One scientist of the Centre acquired training in advanced molecular techniques for characterization of *Babesia equi* from Japan. One scientist completed his PhD research at NRCE and another scientist is presently working for her PhD degree under memorandum of understanding signed with CCS Haryana Agricultural University, Hisar. In addition, a number of students from state universities acquired trainings from this Centre.

### Post-doctoral Fellowship

Dr. Sanjay Kumar, Scientist of the Centre was deputed to National Research Centre for Protozoan Diseases, University of Agriculture and Veterinary Medicine, Obihiro, Japan for post-doctoral fellowship from November 23, 2001 to March 11, 2004. During his PDF, Dr. Sanjay worked on cellular localization and expression behaviors of equi merozoite antigen (EMA) -1 and -2 of *Babesia equi* during the asexual erythrocytic-developmental cycle of merozoite using the anti-EMA-1 or -2 mono-specific mouse serum. Indirect fluorescent antibody test demonstrated that the EMA-1 and EMA-2 were not expressed in all the erythrocytic-developmental stages of the merozoites and these two antigens were co-expressed during the early developmental stages. Additionally, it was shown that the EMA-1 and EMA-2 were mutually expressed on the surface of extraerythrocytic merozoite and also that the intraerythrocytic merozoite shed only EMA-2 antigen in the infected erythrocytic cytoplasm or inside membrane surface (Fig. 1). The specific binding of EMA-2 to Triton X-100-insoluble horse erythrocyte membrane fraction was also demonstrated. Chromosomal localization of EMA-1 and 2 in *B. equi* genome was also studied and further analysis is in

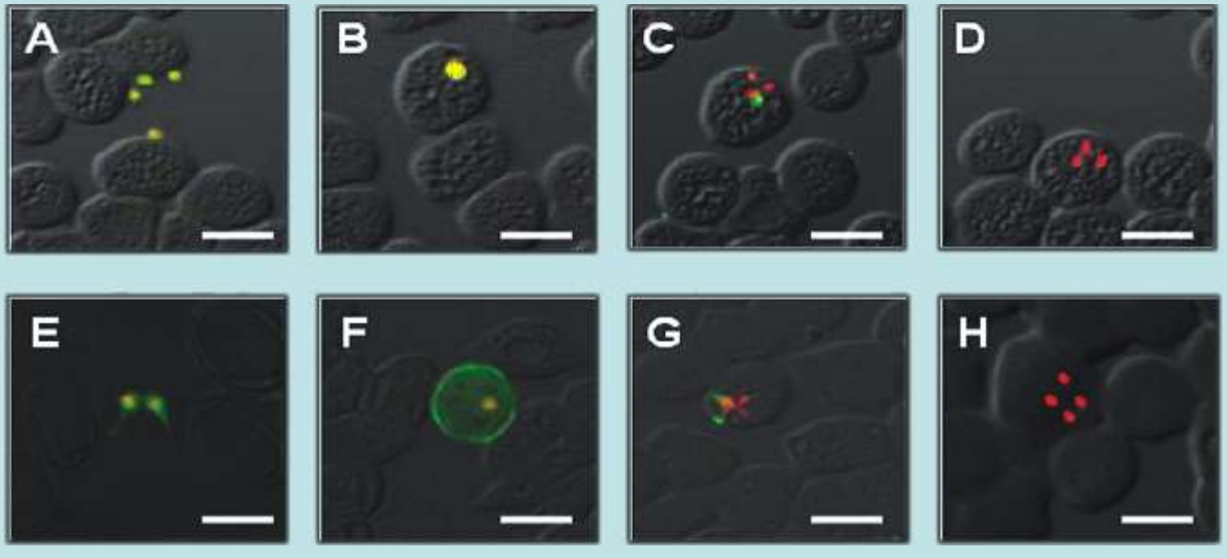


Fig. 1: Specific expressions of EMA-1 and -2 during the different developmental stages of merozoite detected by IFAT with methanol fixed smears. Infected erythrocyte smears were incubated with either anti-EMA-1t (Panels A-D) or anti-EMA-2t (Panels E-H) mouse immune serum and then observed with confocal laser scanning microscopy. The serum-antigen reaction (green) and nucleus reaction (red) were visualized with the Alexa-Fluor 488 conjugated secondary antibody and PI staining, respectively. Bar = 5µm. Note: A and E: extra-erythrocytic merozoites ready to invade to fresh erythrocytes; B and F: internalized merozoites in the erythrocytes after invasion; C and G: Maltese-cross form in the erythrocytes showing an initial phase of merozoites multiplication; D and H: fully developed and separated merozoites ready to escape from the infected erythrocytes.

progress. These findings would facilitate the understanding the biological role of merozoite surface proteins of B. equi and henceforth quest for searching new drug targets.

PhD thesis completed at NRCE

Praveen Malik: Characterization of streptococci of equine origin with special reference to M protein

Summary of work: The study was undertaken to study the variability of M protein among Indian isolates of *Streptococcus zooepidemicus* and its protective value *vis-a-vis Streptococcus equi*. To achieve the objectives, various field samples were collected from healthy and clinically affected equines. Of the total 311 samples collected, 35 streptococcal isolates were obtained including S. equi (6), *S. zooepidemicus* (16) and *S. equisimilis* (13). Of the total samples, 185 were collected from apparently normal equines, which yielded 16 isolates, while 126 samples from clinically affected equines yielded 19 isolates. Of 262 samples originated from organized farms, 25 yielded streptococci, while 49 from unorganized sector, 10 isolates were recovered. All the streptococcal cultures, except 6 *S. equisimilis* isolates, were pathogenic to mice. Antibiotic sensitivity indicated that 38 were sensitive to Lincomycin while 35 were resistant to Nalidixic acid. Colistin showed resistance in only 14 isolates. Variability among profiles of crude extracts and M proteins of *S. zooepidemicus* were noticed, while those of *S. equi* were found to be almost homogenous. One isolate of *S. equi* was, however, showing a slightly different pattern in crude enzyme extract and immunoblot developed by convalescent horse serum. Immunoblot studies indicated a common protein of ~ 30 kD, detectable by anti-*S. zooepidemicus* hyperimmune serum raised in rabbit, whereas with anti- *S. equi* hyperimmune rabbit serum, this protein showed variability in size. Results on *in vivo* mouse protection assay indicated the protective ability of *S. zooepidemicus* M-protein against homologous challenge. However, it was not found protective against infection with *S. equi. In vitro* bactericidal assay also indicated the reduction in number of bacterial cells by homologous antiserum only in both *S. zooepidemicus* and *S. equi.* Results suggested a protective and opsonogenic value of M protein of *S. zooepidemicus* against homologous infection and not against *S. equi*.

Ongoing PhD Research titles at NRCE:

Mamta Chauhan: "Molecular characterization and establishment of genetic relationship between two different breeds of indigenous equines using microsatellite markers".

Trainings for post-graduate students:

- Training on *Cultivation of viruses in cell culture and identification of foal rotavirus* was conducted for two students of Guru Jambeshwar University, Hisar from June 10-July 20, 2003.
- Training on *Production of monoclonal antibodies and their application for diagnosis of animal rotaviruses* was conducted from January 5-May 25, 2004 for students of Guru Jambeshwar University.
- Training on Studies on *Profiling of bacterial and viral proteins and nucleic acids* was conducted from February 21-May 26, 2004 for students of Guru Jambeshwar University.
- Training on *DNA polymorphism in Marwari horses and revival of EHV-II clones* was conducted from January 5-April 29, 2004 for students of Guru Jambeshwar University

# Awards and Recognitions

### Dr. Dwivedi nominated as member of ICAR society

Indian Council of Agricultural Research has nominated Dr. S.K. Dwivedi, Director NRCE as one of the members of the prestigious ICAR Society as well as Governing Body of the ICAR Society for a period of three years w.e.f. October 8, 2003.

### Two Laboratories being considered for OIE Recognition

The Department of Animal Husbandry and Dairying (DAHD), Ministry of Agriculture, Government of India is considering recognizing two laboratories of this Centre, *viz.*, the equine rhinopneumonitis and equine piroplasmosis laboratories as *Office International des Epizooties* (OIE) International Reference Laboratories. OIE is a world organization for animal health with 164 member countries and on getting its recognition; these laboratories will be referred by the entire South-East Asian Region for diagnosis, control and management of these equine diseases. A high-powered committee appointed by the DAHD visited this Centre on July 14-15, 2003. The Committee headed by a renowned virologist of International repute also included experts from National Accreditation Board for Testing & Calibration Laboratories (NABL) under the aegis of Department of Science & Technology, New Delhi. The Committee critically evaluated the infrastructure, facilities and expertise available in order to meet the stringent requirements for international recognition. The Committee was of the opinion that this Centre must implement quality control system and biosafety practices as per international norms for the international recognition.

Members of the expert committee evaluating equine piroplasmosis laboratory for OIE recognition

The Director NRCE, Dr. S.K. Dwivedi informed the experts that this centre is according high priority to biocontainment and biosafety issues to safeguard the laboratory workers and the environment. For safe handling of potentially hazardous equine pathogens, bio-safety level 3 (BSL-3) microbial containment facilities are being established at this centre, for which the Indian Council of Agricultural Research has already given the financial sanction to this centre. Measures are also being taken to develop a quality control system at the centre including third party validation of laboratories from international agencies.

### Young Scientist Award

Dr. A. Arangasamy received Young Scientist Award for best paper presentation during XIX Annual Convention of ISSAR and National Symposium on Current reproductive technologies for improvement of livestock production in India held at Kolkata from August 22-24, 2003.

### Sh. R.A. Parashar wins Zonal Chess Competition

Sh. R.A. Parashar, AF&AO represented NRCE chess team in ICAR Zone V Sports Meet held at Central Soil Salinity Research Institute, Karnal from December 15-18, 2003 and won the first position in Chess Competition.

# List of Publications

Research articles


1. Banerjee, D.P., Kumar, R., Kumar, S. and Sengupta, P.P. 2003. Immunization of crossbred cattle (*Bos indicus x Bos taurus*) with fractionated midgut antigens against *Hyalomma anatolicum anatolicum. Tropical Animal Health and Production* 35: 509-519.
2. Bhatia, V., Gupta, A. K. and Nainawatee, H. S. 2003. Characterization of equine influenza A/Equi-2/Ludhiana/87 ($H_3$ $N_8$) isolate by nucleotide sequencing of its neuraminidase gene. *Indian Journal of Biotechnology* 2: 203-213.
3. Bork, S., Yokoyama, N., Ikehara, Y., Kumar, S., Sugimoto, C and Igarashi, I. 2004. Growth inhibitory effect of heparin on *Babesia equi* parasites. *Antimicrobial Agents and Chemotherapy* 48: 236-241.
4. Dey, S. and Dwivedi, S.K. 2004. Lead in blood of urban Indian Horses. *Veterinary and Human Toxicology* : Accepted.
5. Gupta, A. K., Ahlawat, A. and Bhatia, V. 2003. Detection of equine influenza viral genome by RT-PCR and RNA:DNA hybridization. *Indian Journal of Biotechnology* 2: 214-219.
6. Gupta, A. K., Singh, R., Mamta, Pal, Y., Singh, M. K. and Yadav, M. P. 2002. Influence of season on characteristics of Jack semen. *Indian Journal of Animal Scences* 73: 986-991.
7. Joshi, A., Naqvi, S. M. K., Bag, S., Dang, A. K., Sharma, R. C., Rawat, P.S. and Mittal J.P. 2003. Sperm motion characteristics of Garole rams raised for a prolonged period in a semi-arid tropical environment. *Tropical Animal Health and Production*, 35: 249-257.
8. Khurana, S.K., Garg, D.N and Kumar, A. 2004. Seroprevalence of *Mycoplasma equigenitalium* antibodies in equines by indirect ELISA. *Journal of Immunology and Immunopathology* 6 (S-1): 77-78.
9. Kumar, R., Banerjee, D.P. and Gupta, A.K. 2003. Immunization of rabbits with midgut antigens against *Hyalomma anatolicum anatolicum tick. Journal of Parasitic Diseases* 27: 53-63.
10. Kumar, S., Kumar, Y., Malhotra, D.V., Dhar, S. Nichani, A.K. 2003. Standardisation and comparison of serial dilution and single dilution enzyme linked immunosorbent assay (ELISA) using different antigenic preparations of the *Babesia (Theileria) equi* parasite. *Veterinary Research* 34:71-83.
11. Kumar, S., Gupta, A.K., Pal, Y., Dwivedi, S.K. 2003. *In-vivo* Therapeutic efficacy trial with artemisinin derivative, buparvaquone and imidocarb dipropionate against *Babesia equi* infection in donkeys. *Journal of Veterinary Medical Science* 65: 1171-77.
12. Kumar, S., Sharma, R. C., Mishra, A. K. and Arora, A. L. 2003. Production performance of sheep and certain management practices in farmers' flocks of south-east Rajasthan. *Indian Journal of Small Ruminants* 9: 103-105.
13. Pal, Y. and Gupta, A. K. 2004. Effect of transient feed withdrawal stress on physiological indices and acid base balance in equid. *Annals of Arid Zone* 43: 1-6.
14. Pal, Y. and Gupta, A.K. 2004. Comparative physiological and biochemical studies in equids under short term feed deprivation stress. *Indian Journal of Animal Sciences* 74: Accepted.
15. Patnayak, D.P., Gulati, B.R., Sheikh, A.M. and Goyal, S.M. 2003. Cold adapted avian pneumovirus for use as live, attenuated vaccine in turkeys. *Vaccine* 21: 1371-1374.
16. Sen, A. R., Karim, S.A. and Sharma, R.C. 2003. Mutton production potentiality and meat quality traits of crossbred wool strains. *Indian Veterinary Journal* 80: 1149-52.
17. Sharma, R. C. Arora, A. L., Mishra, A. K. and Kumar, S. 2003. Production performance of Malpura sheep in an

organized farm in Rajasthan. *Indian Journal of Small Ruminants* 9: 119-121.

18. Sharma, R. C., Arora, A.L., Kumar, A. and Kumar, R. 2003. Evaluation of mutton type sheep in farmers' flocks of Rajasthan. *Indian Journal of Animal Sciences* 73: 785-787.
19. Singh, B.K., Shalini, A. and Gulati, B.R. 2004. Development of neutralizing monoclonal antibody-based blocking ELISA for detection of equine herpes virus-1 antibodies. *Veterinary Research Communications* 28: Accepted.
20. Singh L.P., Arangasamy, A. and Silvester, S.A. 2004. Characterization of Epididymal fluid protein of Buffalo bulls. *Indian Journal of Animal Sciences* : accepted.
21. Virmani, M., Gupta, A. K. and Garg, S.K. 2003. Extraction, purification and characterization of equine chorionic gonadotropin (eCG) from pregnant mare's serum. *Indian Journal of Animal Sciences* 73: 1224 - 1228.
22. Virmani, N, Panisup, A.S. and Singh, B.K. 2004. Immunohistochemical diagnosis of Equine Herpes Virus-1 infection employing microwave irradiation for reducing the processing time. *Indian Journal of Veterinary Pathology*: Accepted.

Abstract in Conferences, Symposia, etc.

1. Arangasamy, A., Singh, L.P. and Ahmed, N. 2003. Isolation and characterization of heparin and gelatin binding proteins in buffalo seminal plasma. In: XIX Annual Convention of ISSAR and National Symposium on Current reproductive technologies for improvement of livestock production in India, Kolkata, August 22-24.
2. Batra, M., Pruthi, A.K., Virmani, N. and Verma, P.C. 2003. Detection of antigens of *Pasteurella multocida* A: 1 in the tissues of experimentally infected chicken. In: XX$^{th}$ Annual Conference of Indian Association of Veterinary Pathologists and National Symposium on Basic pathology and animal diseases- A need for fresh approach in Indian scenario, JNKVV, Jabalpur, M.P, November 12-14.
3. Dadarwal, D., Purohit, G.N., Tandon, S.N., and Pareek, P.K. 2003. Ultrasonographic evaluation of uterine involution in french jennies (Equus Asinus) In: Proceedings of XIX Annual convention of ISSAR and National Symposium on Current reproductive technologies for improvement of livestock production in India, Kolkata, August 22-24.
4. Dey, S., Dwivedi, S.K. and Jitender Singh. 2004. Chronic cyanide poisoning in Horses In: Proceedings of Symposium on Latest approaches and biotechnological tools for health management of farm and companion animals, IVRI, Izatnagar, Feb 11-13.
5. Gulati, B. R., Kumar, R. and Malik, P. 2004. Prevalence, isolation and preliminary characterization of group A rotaviruses from diarrhoeic foals. In: 4th Indian Veterinary Congress, IVRI, Izatnagar, February 27-28.
6. Gupta, A. K. 2003. DNA based diagnostics for equine diseases. In: Frontiers in DNA Application - Proceedings : Seminar on 50 years of DNA research : Unfolding Biological Imperative and Mysteries (Eds R.K.Behl, A. K. Chhabra and Satyavir), CCS HAU, Hisar, May 30.
7. Gupta, A. K. 2004. DNA Vaccines: An overview. In: National Seminar on "Role of Biochemistry in Modern Day Agriculture", Dept. of Biochemistry, CCS HAU, Hisar, January 24.
8. Khurana, S. K., Malik, P., Srivastava, S. K. and Panisup, A. S. 2003. Isolation of Acholeplasmas from indigenous equines. Presented at Xth Annual conference of Indian Association for the Advancement of Veterinary Research and National Symposium on "Challenges and Strategies for Sustainable Animal Production in Mountains", College of Veterinary and Animal Sciences, HPKVV, Palampur, April 14-15.
9. Legha, R. A., Sharma, R. C. Tandon, S. N. and Pal, Y. 2004. Preservation of jacks semen and AI for mule production in the field. In: Proceedings of Annual Conference of ISAG&B and National Symposium on Conservation and Propagation of Livestock and Poultry, GB PU A& T, Pantnagar, Feb. 26-28.
10. Mamta, Gupta, A. K., Tandon, S. N. and

Dhillon, S. 2004. Phenotypic and genetic characterization of indigenous Spiti ponies. In: National Symposium on 'Livestock Biodiversity *vis-à-vis* resource exploitation: An introspection', NBAGR, Karnal, February 11-12.

11. Pal, Y, Legha, R .A., Khurana, S. K., Tandon, S. N., Sharma, R. C. and Gupta, A. K. 2004. Cryopreservation of Marwari stallion semen for breeds conservation. In: National Symposium on 'Livestock biodiversity *vis-a-vis* Resource Exploitation: An Introspection', NBAGR, Karnal, February 11-12.
12. Singh, L.P. and Arangasamy, A. 2003. Effect of gelatin bound seminal plasma proteins on *in vitro* fertilizing ability of buffalo cauda spermatozoa. In: XIX Annual Convention of ISSAR and National Symposium on Current reproductive technologies for improvement of livestock production in India, Kolkata, August 22-24.
13. Singh. L.P and Arangasamy. A. 2003. Effect of heparin binding seminal plasma protein on in vitro fertilizing ability of buffalo cauda spermatozoa. In: National Seminar on frozen semen technology, Kerala Livestock Developement Board Phalghat, Kerala, December 4-6.
14. Singh, L.P and Arangasamy, A. 2004. *In vitro* assessment of fertilizing ability of buffalo cauda spermatozoa treated with seminal plasma proteins. In: 22nd Convention of ISVM and National Symposium on latest approaches and biotechnological tools for health management of farm and companion animals, IVRI, Izatnagar, February 11-13.
15. Virmani, N., Singh, B.K., Verma, P.C., Batra, M. and Panisup, A.S. 2004. Immune responses to Equine Herpes Virus-1 primary infection in pregnant BALB/c mice. In : 4th Indian Veterinary Congress, XIth Annual Conference of Indian Association for advancement in Veterinary Research and National Symposium on Newer concepts and challenges in Animal Health and Production in the wake of WTO, IPR, Trade Barriers and certification of standards, IVRI, Izatnagar, February 27-28.
16. Virmani, N., Verma, P.C., Panisup, A.S., Singh, B.K., and Batra, M. 2003. Comparative studies on tissue tropism of indigenous strains of EHV-1 in murine model. In : XXth Annual Conference of Indian Association of Veterinary Pathologists and National Symposium on Basic pathology and animal diseases- A need for fresh approach in Indian scenario, JNKVV, Jabalpur, November 12-14.

Articles in books, manuals, etc.

1. Dey, S. 2003. Diagnostic approach and management strategies for iodine deficiency in dairy animals. In: Advanced diagnostic techniques and therapeutic approaches to metabolic and deficiency diseases in dairy animals, Division of Medicine, IVRI, Izatnagar, July15-Aug 4, pp 75-77
2. Dey, S. 2003. Estimation of Fluoride in biosamples using Ion selective Potentiometry. In: Advanced diagnostic techniques and therapeutic approaches to metabolic and deficiency diseases in dairy animals, Division of Medicine, IVRI, Izatnagar, July15-Aug 4.
3. Dey, S. 2004. Biomarker strategies for detecting effects of pollution in man and animals. In: Proceedings of Symposium on Latest approaches and biotechnological tools for health management of farm and companion animals, IVRI, Izatnagar, Feb 11-13, pp71-74.
4. Gulati, B.R. and Grover, Y.P. 2004. Genetic information in microbes. In: Laboratory manual on 'Development of instructional materials for General Veterinary Microbiology. Centre of Advanced Studies, Department of Veterinary Microbiology, CCS Haryana Agricultural University Hisar, February 10-March 1, pp.209-213.
5. Gulati, B.R. and Grover, Y.P. 2004. Mechanisms of Microbial Pathogenicity. In: Laboratory manual on 'Development of instructional materials for General Veterinary Microbiology. Centre of Advanced Studies, Department of Veterinary Microbiology, CCS Haryana Agricultural University Hisar, February 10-March 1, pp.209-213.
6. Gulati, B.R., Singh, B.K. and Sehgal. N. 2003. Western blotting of viral proteins.

In: Laboratory manual on 'Production and use of instructional material on Veterinary Immunology and Serology. Centre of Advanced Studies, Department of Veterinary Microbiology, CCS Haryana Agricultural University Hisar, December 16-January 6, pp.209-213.

7. Gupta, A. K. 2003. DNA based diagnostic for equine diseases. In : Refresher Course on "Application of Biotechnological Techniques in Agriculture", Department of Biotechnology & Molecular Biology, CCS HAU, Hisar. (Eds : V.K.Chowdhury, R. K. Jain and R.K. Patel), December 4-24, pp174-177.
8. Gupta, A. K. and Mamta. 2003. Animal genomics: An overview. In: Refresher Course on "Application of Biotechnological Techniques in Agriculture", Department of Biotechnology & Molecular Biology, CCS HAU, Hisar. (Eds: V.K.Chowdhury, R. K. Jain and R.K. Patel), December 4-24, pp 178-181.
9. Gupta, A.K. 2003. Serum based techniques for pregnancy diagnosis and their importance in equids. In: Compendium of Short Course on "Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines" Equine Production Campus (NRCE) Bikaner, September 1-10, pp 18-27.
10. Legha, R.A. and Sharma, R.C. 2003. Factors involved in cryopreservation of semen in equids. In: Compendium of Short Course on "Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines" Equine Production Campus (NRCE) Bikaner, September 1-10, pp 62-69.
11. Pal, Y and Mamta. 2003. The influence of age, frequency of collection and season on equine semen production and its freezability In: Compendium of Short Course on "Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines, Equine Production Campus (NRCE) Bikaner, September 1-10.
12. Sharma, R. C. and Legha, R. A. 2003. Evaluation of stallion semen for artificial insemination. In: Compendium of Short Course on "Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines" Equine Production Campus (NRCE) Bikaner, September 1-10, pp 41-47.
13. Singh, B.K., Kanojia, R.A. and Virmani, N. 2003. Microbial causes of equine abortions-their diagnosis and control. In: Compendium of Short Course on "Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines" Equine Production Campus (NRCE) Bikaner, September 1-10, pp 70-79.
14. Singhvi, N. M. and Tandon, S.N. 2003. Sexual behavior of male and female horses. In: Compendium of Short Course on "Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines" Equine Production Campus (NRCE) Bikaner, September 1-10, pp13-17.
15. Tandon, S N. and Arangasamy, A. 2003. Physiology of pregnancy and pregnancy diagnosis in mare. In: Compendium of Short Course on "Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines" Equine Production Campus (NRCE) Bikaner, September 1-10, pp8-12.

Thesis, books:

1. Malik, P. 2003. Characterization of streptococci of equine origin with special reference to M protein. Thesis, Ph. D. submitted to CCS Haryana Agricultural University, Hisar. 96, xxiv p.
2. Malik, P., Kumar, Rajender, Kulshreshtha, M. P. and Kumar, Akhilesh. 2003. Compendium of lectures on One day workshop on 'Information technology for dissemination of scientific knowledge in agriculture', NRCE, Hisar, September 17.

# List of Approved and Ongoing Research Projects

| Scheme Code | Title of the Scheme | Team | Date of Start | Date of Completion |
|---|---|---|---|---|
| EQUINE PRODUCTION | | | | |
| 2000414001/ 2.2 | Molecular characterization for studying genetic diversity among Marwari breed of horses | S.N.Tandon, A. K. Gupta R.A.Legha, Mamta Chauhan and R. C. Sharma | Oct., 2001 | Sept., 2004 |
| 2000414002/ 2.3 | Standardisation of procedure and techniques for cryopreservation of Jack's semen | R.A.Legha, S.N.Tandon and R.C. Sharma | April, 1997 | Sept.,2003 |
| 2000414003/ 2.4 | Cryopreservation of stallion semen and perfection of AI in Marwari horses | Yash Pal, R .A. Legha S .K. Khurana and S.N.Tandon. | May, 2002 | June, 2005 |
| 2000414004/ 2.5 | Development of equine chorionic gonadotropin (ecg) based ELISA based test for pregnancy diagnosis in equines | A.K.Gupta, Yash Pal and S.K.Dwivedi | May, 2002 | June, 2004 |
| 2000434001/ 2.6 | Molecular marker based pilot study for detection of Angiotens in-1-converting enzyme gene (ACE) in indigenous equines | Mamta Chauhan and A.K.Gupta | July,2003 | August, 2004 |
| EQUINE HEALTH | | | | |
| 2000443002/ 1.2 | Development of improved vaccine against equine diseases | B.K.Singh, S.K.Khurana, P.Malik and N.Virmani | | |
| 2000443002.2/ 1.2.2 | Development of vaccine(s) against equine herpes virus-I infection | B.K.Singh and N.Virmani | 1-11-1998 | 31.03.2005 |
| 2000448001/ 1.3 | Epidemiological studies on emerging and existing diseases of equines | S.K.Dwivedi, S.K. Khurana, A. S. Panisup, B.K.Singh, A.K.Gupta, S.Dey, B.R.Gulati P.Malik, Yashpal, Nitin Virmani and Rajender Kumar. | Continuous Service Project | Contd. |
| 2000446002/ 1.4 | Chemotherapeutic and diagnostic studies on trypanosomiasis and babesiosis in equines | S.Dey, S.K. Dwivedi, Rajender Kumar and A.S.Panisup | | |
| 2000446002.1/ 1.4.1 | Isolation and characterization of secondary plant metabolites for the development of an antitrypanosomal drug | S.Dey, S.K.Dwivedi, A.S.Panisup and Rajender Kumar | 11-1-2000 | 30-11-2003 |
| 2000446002.2/ 1.4.2 | Development of diagnostic tests for equine protozoal disease-Trypanosomosis (Surra) | Rajender Kumar, S.Dey, A.K.Gupta and S.K.Dwivedi | June, 2003 | March, 2006 |
| 2000442001/ 1.5 | Studies on relative prevalence of various pathogens in foal diarrhoea and development of diagnostics | Baldev R. Gulati, Praveen Malik and Rajender Kumar | June, 2003 | March, 2006 |
| 2000442002/ 1.6 | Development of diagnostic(s) for pathogenic *Streptococcus equi* in equines | Praveen Malik, B. R. Gulati, Nitin Virmani and S. K. Khurana | June, 2003 | March, 2006 |
| 2000441001/ 1.7 | Studies on the prevalence of infertility in female equids | Nitin Virmani, A. S. Panisup, S. Dey and Praveen Malik | June, 2003 | March, 2004 |

# Patents, Consultancy and Commercialization of Technology

## Patents

**Patent granted:** Patent has been granted by the Patent Office, Government of India on application (2199/DEL/96) entitled "A method for preparation of a diagnostic kit useful for forecasting Equine Herpes Virus-1 disease". This has been notified on October 25, 2003 in the Gazette of India, classified as 55E4 1891278.

**Patent filed:** Patent application no 36/Del/2001 entitled "Complement fixation test based COFEB-Kit for diagnosis of *Babesia equi* infection in equines" has been finally submitted after first examination report. A divisional application for patenting of product has also been submitted through ICAR to Patent Office, New Delhi.

## Consultancy

This Centre offers consultancy and diagnostic services for important infectious diseases of equines. Under this programme, 2766 equine sera received from 13 states and union territories were examined for equine infectious anaemia (EIA) by Coggins test. None of the samples tested was found positive. As part of surveillance and monitoring project, 1517 additional samples from indigenous equines were also tested and all the samples were found negative for EIA. So far, 22,671 serum samples have been tested for EIA during last five years and not a single positive case has been recorded since 1999.

Serum samples from equines belonging to various private organizations, quarantine stations and other establishments were also tested for other diseases including 20 samples for EHV-1, 24 for equine influenza, 92 for equine viral arteritis, 61 for glanders, 148 for *Salmonella* Abortus equi and 10 for leptospirosis. All the samples were found to be negative for the disease conditions tested.

Contagious equine metritis (CEM) testing by agent isolation and identification was done for 72 samples including 66 vaginal swabs from animal quarantine station (Delhi: 54 and Chennai: 12) and 6 prepucial swabs from animal quarantine station, Delhi. All samples were found negative for CEM.

Table 1. Important bacterial isolates recovered and their origin

| Isolate | Nos. | Nature of sample | Place of origin |
|---|---|---|---|
| *Streptococcus equi* subsp. *equi* | 1 | Vaginal swab (1) | Haryana (1) |
| *Streptococcus equi* subsp. *zooepidemicus* | 1 | Nasal swab (1) | Rajasthan (1) |
| *Streptococcus equisimilis* | 1 | Pus (1) | Haryana (1) |
| Group C streptococci (α-hemolytic) | 7 | Sub-mandibular lesion (1), testicular lesion (1), nasal swab (4), vaginal swab (1) | Punjab (4) Uttaranchal (2) Haryana (1) |
| α-hemolytic *Streptococcus* | 1 | Aborted foetus (1) | Rajasthan (1) |
| *Staphylococcus* | 4 | Nasal swab (3), vaginal swab (1) | Rajasthan (2), Punjab (1), Uttar Pradesh (1) |
| *Micrococcus* | 1 | Nasal swab (1) | Uttar Pradesh (1) |

Bacteriological examination of 71 other samples, including nasal swabs, vaginal swabs, ocular swab, faecal samples, wound/lesions, exudates, pus samples and aborted foetus yielded 21 isolates (Table 1). Four *Acholeplasma laidlawii* (two each from repeat breed and apparently healthy mares) from 13 vaginal swabs and one *A. oculi* (from a case of conjunctivitis) from 6 eye swabs were also isolated.

Post-mortem examination : Three necropsies on equines revealed the causes of death due to meningo-encephalitis(1), anoxia due to pulmonary congestion and haemorrhage (1). Morbid material received at the Centre revealed the cause of abortion as placentitis (1) and veno-vascular disorder induced abortion.

## Commercialization of technology

The Centre is providing diagnostic services to the equine industry on payment basis. The Centre generated revenue to the tune of Rs. 11.9 lacs during the year by testing samples for various diseases including equine infectious anaemia, equine viral arteritis, contagious equine metritis, equine herpes virus, piroplasmosis, equine influenza, *etc.* In addition, the improved germplasm of equines was provided to the farmers in different parts of the country.

# RAC, Management Committee and SRC Meetings

## Staff Research Council Meeting

The annual SRC meeting was held under the chairmanship of Dr. S. K. Dwivedi on 5-6th May 2003 to discuss the progress of various research

Annual SRC meeting in progress under the chairmanship of Dr. S.K. Dwivedi, Director, NRCE

projects. The house reviewed the research projects currently undergoing in the institute in the area of equine health and production. Five new project proposals were also approved by SRC.

New projects initiated at NRCE:

- Studies on relative prevalence of various pathogens in foal diarrhoea and development of diagnostics
- Development of diagnostic(s) for pathogenic *Streptococcus equi* in equines
- Molecular marker based pilot study for detection of angiotensin-1-converting enzyme gene (ACE) in indigenous equines
- Studies on the prevalence of infertility in female equids
- Development of diagnostics for equine protozoal disease-trypanosomosis (surra)

## Research Advisory Committee Meeting

The 5th RAC meeting was held on May 20, 2003, under the chairmanship of Dr. V. Gnanaprakasam. Various technical, administrative and policy matters related to research work were discussed, including five new research projects and recommendations were made to the Council. The RAC reiterated

5th RAC meeting being held to discuss research activities of NRCE

that the development of containment facilities at the Centre should be the priority of the Centre.

## 23rd Institute Management Committee Meeting

Twenty-third meeting of the Institute Management Committee was held on August 30, 2003 under the chairmanship of Dr. S.K. Dwivedi, Director. Important decisions regarding purchase of equipments for the current financial year, reduction of testing fee for equine infectious anemia to Rs 250/- for indigenous equines belonging to non-profit making bodies, including BSF and police horses.


23rd IMC meeting in progress to discuss important management issues

## 10th Five Year Plan for NRCE approved

Indian Council of Agricultural Research, New Delhi has granted its approval to National Research Centre on Equines for the 10th Five Year Plan. An outlay of Rs. 13.50 crores has been

approved for the plan period 2002-2007. Under the 10th Plan outlay, a provision for construction of office-cum-laboratory building at Bikaner sub-campus and extension of laboratory-cum-office building at Hisar has been made. There is also provision for construction of a microbial containment (BSL-III) laboratory at Hisar for working on hazardous equine infectious agents.

### Quinquennial Review Team (QRT) reviewed NRCE activities

The Indian Council of Agricultural Research constitituted the Quinquennial Review Team (QRT) under the chairmanship of Dr S.S. Rathore, Ex-Dean, College of Veterinary Sciences, PAU, Ludhiana to review the work done by the Centre during 1996-2002. Other members of QRT included Dr. P.N. Khanna, Ex-Joint Director, IVRI, Dr J.M. Nigam, Ex-Dean, College of Veterinary Sciences, Palampur, Dr. R.P. Mishra, Ex-FAO Expert and Dr. H.C. Joshi, former Professor & Head, Division of Veterinary

QRT under the chairmanship of Dr. S. S. Rathore reviewing research activities of the Centre

Medicine, Pantnagar. The first meeting was held at Hisar Campus on September 4-5, 2003. The QRT members visited different laboratories and interacted with the scientists individually about the progress made during the period under review. QRT members appreciated the ongoing research activities under the dynamic leadership of Dr. S.K. Dwivedi and excellent team spirit among scientists of NRCE. The second meeting of QRT was held at Bikaner Centre on February 24, 2004 to review the activities of Equine Production Campus. The final meeting was held at Hisar on March 22, 2004 to finalize the report of the Centre for the period under review.

### Staff Research Council Meeting

The half yearly SRC meeting was held under the chairmanship of Dr. A.K. Gupta, in-charge Director, on December 10, 2003 to discuss the progress of various ongoing research projects in the area of equine health and production. The house reviewed the research projects and made their specific recommendations for different ongoing research projects.

### 24th Institute Management Committee Meeting

The 24th Institute Management Committee meeting was held on March 23, 2004 under the chairmanship of Dr. S.K. Dwivedi, Director. Important decisions regarding writing off losses, fixation of training fee for student trainees at NRCE were taken. The Chairman QRT, Dr. S.S. Rathore had an interactive meeting with members of IMC and apprised the members on the recommendations of QRT.

### NRCE Cup Race

Delhi Race Club organized horse race competition on October 7, 2003. In recognition of the yeoman services rendered by NRCE for the welfare of equines, this competition was named as *NRCE Cup Race*. Smt. Binoo Sen, Secretary, Department of Animal Husbandry & Dairying, Ministry of Agriculture (GOI) was the chief-guest on the occasion and presented the NRCE Cup to the owner of the winning horse. Dr. S.K. Dwivedi was also present on the occasion.

## Members of Research Advisory Committee

| | |
|---|---|
| Dr. V.Gnanaprakasam, Ex-Vice Chancellor, TNUVAS, Chennai | Chairman |
| Dr. S. K. Dwivedi, Director, NRCE, Hisar | Member |
| Dr. R.P. Mishra, Ex-FAO expert, Bareilly | Member |
| Dr. N.N.Pathak, Principal Scientist (Animal Nutrition), IVRI, Izatnagar,U.P. | Member |
| Dr. M.C. Goel, Ex-ADR, CCS HAU, Hisar | Member |
| Dr. O.P. Dhanda, Professor (Animal Production Physiology), CCS HAU, Hisar | Member |
| Dr.Lal Krishna ADG (AH), ICAR, New Delhi | Member |
| Sh. Arvind Yadav, 208, Sector 3, Rewari (Haryana) | Member |
| Sh. Ram Kripal Bhadoria, C-20 Dilkhusha, Lucknow (UP) | Member |
| Dr. Rajender Kumar, Scientist, NRCE, Hisar | Member Secretary |

## Members of Institute Management Committee

| | |
|---|---|
| Dr. S.K. Dwivedi, Director NRCE, Hisar | Chairman |
| Dr.Lal Krishna, ADG(AH), ICAR, New Delhi | Member |
| Sh. B.K. Bansal, Finance & Accounts Officer, NBPGR, New Delhi | Member |
| Dr. S.N. Tandon, Principal Scientist, NRCE, Bikaner | Member |
| Dr. A.K. Gupta, Principal Scientist, NRCE, Hisar | Member |
| Dr. A.S. Panisup, Principal Scientist, NRCE, Hisar | Member |
| Dr. B.K. Singh, Principal Scientist, NRCE, Hisar | Member |
| Sh. R.A. Prashar, AFAO, NRCE, Hisar | Opted member |
| Sh.Arvind Yadav, 208, Sector 3, Rewari (Haryana) | Member |
| Sh.Ram Kripal Bhadoria, C-20 Dilkhusha, Lucknow (UP) | Member |
| Sh. Dilip Kar, AAO, NRCE, Hisar | Member Secretary |

# Participation in Conferences, Symposia, etc.

### Dr. S.K. Dwivedi visited Colombo (Sri Lanka)

Sri Lanka Equestrian Association invited Dr. S.K. Dwivedi to Colombo on September 26, 2003. During his visit, he delivered a talk on various

The President, Equestrian Association of Sri Lanka presenting a memento to Dr. S.K. Dwivedi, Director NRCE

aspects related to infectious diseases of equine, quarantine requirements and monitoring & surveillance of equine diseases to veterinarian and quarantine officers. He also attended an equine show of Marwari horses exported from India. Dr. Dwivedi also met the Minister of Commerce, Govt. of Sri Lanka and discussed about the possibilities of export of Marwari horses from India.

### Participation in Conference/Symposia

1. Arangasamy, A. participated in XIXth Annual Convention & National Symposium on "Current Reproductive Technologies for Improvement of Livestock Production in India" held at Kolkata from Aug. 22-24, 2003.
2. Dey, S. participated in National Symposium on "Latest Approaches and Biotechnological Tools for Health Management of Farm and Companion Animals" organized by Indian Society for Veterinary Medicine at Indian Veterinary Research Institute, Izatnagar, Bareilly, U. P. from Feb. 11-13, 2004.
3. Gulati, B.R. participated and presented a paper in XI Annual Conference of Indian Association for the Advancement of Veterinary Research (IAAVR) being organised at Indian Veterinary Research Institute, Izatnagar, Bareilly, U. P. from Feb. 27-28, 2004
4. Khurana, S.K. attended X$^{th}$ Annual Conference of Indian Association for the Advancement of Veterinary Research and national Symposium on "Challenges and Strategies for Sustainable animal production in Mountains" held at Veterinary College, Palampur, H.P., from April 14-15, 2003 .
5. Kumar, Rajender attended National Seminar on "Patents' Protection, Valuation and Commercialization" held at New Delhi, April 28, 2003.
6. Mamta participated in National Symposium on "Livestock Biodiversity *vis-a-vis* resources exploitation: an introspection" organized by Society for Conservation of Domestic Animal Biodiversity and National Bureau of Animal Genetic Resources at Karnal, Haryana from Feb. 11-12, 2004.
7. Pal, Y attended National Symposium on "Livestock Biodiversity *vis-a-vis* resources exploitation: an introspection" organized by Society for Conservation of Domestic Animal Biodiversity and National Bureau of Animal Genetic Resources at Karnal, Haryana from Feb. 11-12, 2004.
8. Pal, Y. participated in National Workshop on "Iodine requirement and problems in Human

and Dairy Animals" organized at National Dairy Research Institute, Karnal on August 22, 2003.

9. Virmani, Nitin participated in XIXth Annual Convention & National Symposium on "Current Reproductive Technologies for Improvement of Livestock Production in India" held at Kolkata from Aug. 22-24, 2003.
10. Virmani, Nitin participated and presented a research paper in the National Symposium on "Basic Pathology and Animal Diseases- A Need for Fresh Approach in Indian Scenario" held at Jabalpur (M.P.) from Nov. 12-14, 2003.

### Pariticipation in trainings:

1. Arangasamy, A. attended "Foundation Course for Agricultural Research Service" at NAARM, Rajendra Nagar, Hyderabad from October 28, 2003-February 24, 2004.
2. Kar, Dilip (AAO) participated in a training programme on "Establishment and Personnel Matters conducted by Institute of Secretariat Training & Management", New Delhi from November 3-7, 2003.
3. Kumar, Jitendar participated in a short course on, "Cryopreservation, Artificial Insemination and Pregnancy Diagnosis in Equids" at Equine Production Centre -NRCE, Bikaner from Sept. 1-10, 2003.
4. Malik, Praveen participated in the training Course on, "Management of microbes as an instrument of SPS compliance and international livestock trade" organized by Centre of Advanced Studies, Department of Veterinary Microbiology, CCS HAU, Hisar from November 18-December 08, 2003.
5. Pal, Ram (Assistant), Kaushik, S.P. (Assistant) & Chander, S. (UDC) participated in a training programme on "Computer Application for Administrative and Financial Management" organized by National Academy of Agricultural Research Management, Hyderabad, from September 16-23, 2003.
6. Parashar, R.A. (AF&AO) attended a training programme on "New Formats of Accounts prescribed by CGA for central autonomous bodies (non-profit organizations)" organized by National Institute of Financial Management, Faridabad, from October 13-17, 2003.
7. Parashar, R.A. (AF&AO) attended an "Internal Auditor's Training Programme on ISO 9001:2000 QMS" organized by Consultancy Development Centre, New Delhi from August 28-29, 2003.
8. Sharma, D.D. (LDC) participated in "48th Competence and Skill Building Workshop for Personal Secretaries, Personal Assistants, Executive Assistants, Executive Secretaries, Stenos & other secretarial staff" organized by Third World Development Centre at Ootacamund, Tamil Nadu from September 1-5, 2003.
9. Singh, Hawa (Assistant) participated in "41st Advanced Workshop on Implementation of Establishment Rules, Regulations, Procedures & Administration for Govt. Departments, Public Sectors & other Organizations" organized by Third World Development Centre at Ootacamund, Tamil Nadu from September 1-5, 2003.

# Workshops, Seminars, Summer Institutes, Farmers' day, etc.

During the period, the Centre organized a practical training course at Bikaner and a workshop on information technology at Hisar. Besides this, two equine health camps were organized in different states of India to provide health coverage to indigenous equines and to create awareness among the farmers about better management of production and health of the equines.

## Short course on semen cryopreservation, artificial insemination & pregnancy diagnosis at Bikaner

A short course on *Cryopreservation of semen, artificial insemination and pregnancy diagnosis in equines* was organized from September 1-10, 2003


Dr. Parmatma Singh, Vice-Chancellor, RAU, Bikaner addressing the participants during valedictory function

at Equine Production Campus, Bikaner. A total of 22 participants from different states took part in the course. The course was inaugurated by Dr. M.S. Sahani, Director National Research Centre on Camel, Bikaner. During the course, lectures and practicals pertaining to equine behaviour, physiology of reproduction, semen evaluation, artificial insemination, early pregnancy diagnosis, care of young ones, *etc*, were conducted. The participants were given the opportunity to interact with various experts and imparted training in artificial insemination and pregnancy diagnosis using ultrasonography & other serum-based techniques. In the valedictory function, the chief guest, Dr. Parmatma Singh, Vice-Chancellor, Rajasthan Agricultural University, Bikaner emphasized that the training imparted through such short courses will help in the conservation of endangered breeds of indigenous equines.

## Workshop on information technology

NRCE in collaboration with National Information Centre, Hisar organized one-day workshop on *Information technology for dissemination of scientific*

Dr. S.K. Dwivedi, Director NRCE addressing the participants about computer related ailments

*knowledge in agriculture* on September 17, 2003. Scientists and employees of various ICAR and central government institutes participated in it. The Commissioner, Hisar Division, Mr. P.K. Das was the chief guest. He emphasized upon the need for dissemination of information technology (IT) so that the benefits reach to the farmers located in the remote area of the country. Dr. S.K. Dwivedi, Director NRCE highlighted the activities and the achievements of the NRCE and gave an insight on computer related illness and its prevention. Dr. A.K. Jain, Assistant Director General, Agricultural Research Information System, ICAR, New Delhi and Dr. M.V.S. Sharma, Technical Director, National Information Centre, New Delhi discussed the present scenario, gap and thrust areas in IT in India with particular reference to IT Plan and future prospective in agriculture. The workshop concluded with the following conclusions and recommendations:

a. Automation in agriculture is to be given due importance for accurate and speedy decision

making in positive direction through creating knowledge networks. Integration of various information systems (networks) is also need of the hour in agriculture, which would enable the linkage between research, technology and production.

b. Focus should be on creation and development of web enabled databases, knowledge-based management system, data warehouse by IT experts at NIC in coordination with subject matter specialists keeping a view of requirement of the farmers. The appropriate use of GIS, GPS and RS technologies must be considered in the development of such databases.

c. Use of Hindi and other regional languages needs to be encouraged in IT to extend laboratory information to the farmer community.

d. Fundamental as well as customized class-room training is required to be imparted at all level of staff at R&D and extension agencies for proper utilization of the IT tools.

e. An experienced professional Network Administrator/Database Administrator is the prime need of every organization that is dealing with scientific information for extension of knowledge to end users through appropriate IT tools.

f. Intranet within ICAR may be designed for transparency and effective office management.

### Equine Health Camp at Katra (J&K)

A clinical camp was organized at Katra, Jammu from 10th to 12th June, 2003 for health care of about 8000 equids of the region. Major ailments that were observed in animals of this region included lameness, colic, retention of urine, *etc*. In addition, animals were given deworming, mineral mixture and vitamin supplements based on their health status and examination report.

A total of 422 serum samples collected from animals in this camp were tested for major equine diseases. All sera were found negative for antibodies to *Salmonella*


Farmers getting treatment from NRCE scientists for their sick animals in a Health Camp at Katra (J&K)

Abortus equi, equine infectious anemia, glanders, equine influenza A/equi-2 and brucellosis. EHV-1 infection was found prevalent in 52 (12.3%) and *Babesia equi* infection in 108 (25.6%) of the equine sera tested.

### Equine Health Camp at Churu (Rajasthan)

To address the health problems of donkeys, NRCE organized a health camp on October 29, 2003 at Churu (Rajasthan), a donkey populated area of the country. A total of 96 donkeys and 5 mares were examined and treated for various ailments including lameness, wounds, digestive and reproductive disorders. The donkey owners showed great interest in the camp and were educated about the common health problems of donkeys. Literature on management of equines was distributed among farmers. Animal blood and serum samples were collected to test for various viral, bacterial and parasitic infections.


NRCE scientists treating sick animals in an equine health camp at Churu (Rajasthan)

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# Personnel Milestones

## Promotions

- Dr. Yash Pal Sharma (Scientist) promoted to Senior Scientist w.e.f. June 3, 2002.
- Dr. R.A. Legha (Scientist) promoted to Scientist (Senior Scale) w.e.f. August 5, 2001.
- Dr. Jitender Singh (T-3) promoted to T-4 (Veterinary Officer) w.e.f. October 4, 2002.
- Sh. P.P. Chaudhary (T-3) to T-4 (Laboratory Technician) w.e.f. December 10, 2002.
- Sh. Partap Singh (Jr. Clerk) upgraded to the grade of Rs. 4000-100-6000 w.e.f. February 29, 2004.
- Sh. Om Parkash (T-1) promoted to T-2 (Tractor Driver) w.e.f. October 23, 2002.

## New appointments

- Dr. A Arangasamy, Scientist (Animal Reproduction) joined at Equine Production Campus, Bikaner on July 23, 2003.

## Joined on transfer

- Sh. Dilip Kar joined as Assistant Administrative Officer on May 5, 2003.

## Staff on study leave

- Dr. R.S. Bansal, T-9 (Farm Manager) from April 3, 2000 to April 2, 2003.
- Dr. Pramod Singh, Scientist from August 1, 2000 to January 31, 2004.

## Return from abroad

- Dr. Sanjay Kumar, Scientist (Veterinary Medicine) joined back duty in the Centre on March 15, 2004 after availing JSPS post-doctoral fellowship at Japan for two years.

## Obituary

- Sh. Jagminder Singh, SSG-II expired on May 11, 2003.

# Staff at

## Director

Dr. S. K. Dwivedi

## Principal Scientists

Dr. S.N. Tandon

Dr. A.K. Gupta

Dr. A.S. Panisup

Dr. B.K. Singh

## Senior Scientists

Dr. S. Dey

Dr. R.C. Sharma

Dr. S.K. Khurana

Dr. Yash Pal

Dr. B.R. Gulati

## Scientists

Dr. Rajender Kumar

Dr. Nitin Virmani

Dr. Praveen Malik

Dr. Sanjay Kumar

Ms. Mamta

Dr. R.A. Legha

Mr. Pramod Singh

Dr. A. Arangasamy

# NRCE

## Administrative

| | | |
|---|---|---|
| 1. | Sh. R.A. Parashar | AFAO |
| 2. | Sh. Dilip Kar | AAO |
| 3. | Sh. Hawa Singh | Assistant |
| 4. | Sh. Ram Pal | Assistant |
| 5. | Sh. S.P. Kaushik | Assistant |
| 6. | Sh. Ashok Arora | Jr. Stenographer |
| 7. | Sh. Subhash Chander | Sr. Clerk |
| 8. | Sh. Pratap Singh | Jr. Clerk |
| 9. | Sh. D.D. Sharma | Jr. Clerk |
| 10. | Sh. Om Prakash | Jr. Clerk |
| 11. | Sh. Mohinder Singh | Jr. Clerk |

## Technical

| | | |
|---|---|---|
| 1. | Dr. R.S. Bansal, T-9 | Farm Manager |
| 2. | Sh. R.K. Chaturvedi, T-5 | Technical Officer |
| 3. | Sh. K.S. Meena, T-4 | Farm Manager |
| 4. | Sh. K.K. Singh, T-4 | Lab. Technician |
| 5. | Dr. Jitender Singh, T-4 | Veterinary Officer |
| 6. | Sh. P.P. Chaudhary, T-4 | Lab. Technician |
| 7. | Sh. Ajmer Singh, T-3 | Stock Assistant |
| 8. | Sh. Brij Lal, T-3 | Stock Assistant |
| 9. | Sh. D.D. Pandey, T-3 | Lab. Assistant |
| 10. | Sh. Sita Ram, T-3 | Lab. Assistant |
| 11. | Sh. S.K. Chhabra, T-3 | Lab. Assistant |
| 12. | Sh. N.K. Chauhan, T-3 | Farm Technician |
| 13. | Smt. Indu Jyoti, T-3 | Hindi Translator |
| 14. | Sh. Mukesh Chand, T-2 | Lab. Assistant |
| 15. | Sh. Sajjan Kumar, T-2 | Staff Car Driver |
| 16. | Sh. Arun Chand, T-2 | Tractor Driver |
| 17. | Sh. Khiraj Singh, T-2 | Jeep driver |
| 18. | Sh. Suresh Kumar, T-2 | Vehicle driver |
| 19. | Sh. Joginder Singh, T-2 | Lab. Assistant |
| 20. | Sh. Shankar Lal, T-2 | Jeep-cum-tractor driver |
| 21. | Sh. Rajendra Singh, T-1 | Lab. Technician |
| 22. | Sh. S.N. Paswan, T-1 | Livestock Assistant |
| 23. | Sh. Om Prakash, T-1 | Tractor driver |

## Supporting

| | | |
|---|---|---|
| 1. | Sh. Ishwar Singh | SSGr.III |
| 2. | Sh. Guru Dutt | SSGr. III |
| 3. | Sh. Jai Singh | SSGr. III |
| 4. | Sh. Mahabir Prasad | SSGr. III |
| 5. | Sh. Ramesh Chander | SSGr. II |
| 6. | Sh. Sajjan Singh | SSGr. II |
| 7. | Sh. Mardan | SSGr. II |
| 8. | Sh. Balwan Sigh | SSGr. II |
| 9. | Sh. Desh Raj | SSGr. II |
| 10. | Sh. Raghubir Singh | SSGr. II |
| 11. | Sh. Ishwar Chander | SSGr. II |
| 12. | Sh. Om Prakash | SSGr. II |
| 13. | Sh. Deepak Kumar | SSGr. II |
| 14. | Sh. Gopal Nath | SSGr. II |
| 15. | Sh. Satbir Singh | SSGr. I |
| 16. | Sh. Hanuman Singh | SSGr. I |
| 17. | Sh. Subhash Chander | SSGr. I |
| 18. | Sh. Ishwar Singh | SSGr. I |
| 19. | Sh. Ram Singh | SSGr. I |
| 20. | Sh. Dalsher | SSGr. I |
| 21. | Sh. Raju Ram | SSGr. I |
| 22. | Sh. Mahabir Prasad | SSGr. I |

# Distinguished Visitors

## Major General B.S. Panwar visited NRCE

Major General B.S. Panwar, Additional Director General, Remount Veterinary Services, Army HQ, New Delhi visited this Centre on 28th April 2003. During his visit Maj. Gen. Panwar took keen interest in the ongoing

Dr. S.K. Dwivedi apprising Maj. Gen. B.S. Panwar about ongoing research activities at the Centre

research activities of the Centre and appreciated the outstanding research work being done in the area of diagnosis, epidemiology and control of equine diseases. On this occasion, Dr. S.K. Dwivedi highlighted the salient achievements of the Centre in the area of equine health and production and also discussed about the future research priorities of the Centre. Addressing to the scientists, Maj. Gen. Panwar said that Remount Veterinary Corps and this Centre can collaborate to exchange each other's experiences for the welfare of equine population and offered all kind of help to this Centre.

## Dr. J.B. Chowdhury visited the Centre

Dr. J.B. Chowdhury, Chairman, Committee of Agricultural & Scientific Experts, Ministry of Agriculture, Government of India visited on December 6, 2003 to evaluate the worth of research work being done at NRCE. During his visit, he personally evaluated the research work of all the laboratories and interacted with the scientists. Dr. Chowdhury was impressed by the technical excellence that NRCE has achieved in equine health and production. He said that the expertise available at the Centre coupled with the excellent infrastructural facilities and leadership provided by Dr. Dwivedi makes it an ideal research Centre for national and international equine diseases of high priority.

## Secretary DAHD visited NRCE

Smt. Radha Singh, Secretary, Department of Animal Husbandry & Dairying, Ministry of Agriculture (Government of India) visited this centre on December 22, 2003. She was accompanied by Dr. V.K. Taneja, Deputy Director General (Animal Sciences), Ms. Nita

Smt. Radha Singh, Secretary (DAHD) examining experimental animal facilities at the Centre

Chowdhury & Smt. Neerja Rajkumar, Joint Secretaries DAHD, Dr. A.L. Chaudhary, Chairman, and Dr. K.S. Dangi, Managing Director of Haryana Livestock Development Board, Chandigarh. During her visit, Smt. Singh examined the experimental and animal containment facilities at the Centre. She also took keen interest in the ongoing research activities of the Centre.

# Infrastructure Development and Other Activities

## Infrastructure

During the year, the Centre initiated work on construction of laboratory-cum-office building at Equine Production Campus, Bikaner. The work for extension of laboratory building at main campus at Hisar has also been initiated. In addition, civil work for construction of fodder shed and colic box at Bikaner campus and type IV quarter for staff was completed during this period.

## Agriculture production

During the period 2003-04, the fodder production at Hisar and Bikaner Centres is shown in Table 1.

Table 1. Fodder production at NRCE (Hisar and Bikaner)

| Type of fodder | Production in Quintals | | Total |
|---|---|---|---|
| | Hisar | Bikaner | |
| Lucern | 435 | 390.29 | 825.29 |
| Oat | - | 58.70 | 58.70 |
| Millet | - | 200.05 | 200.05 |
| Sorghum | 412 | - | 412.00 |

## Livestock production

The Centre has maintained a representative herd of equines comprising of indigenous horses of Marwari and Kathiawari breed, exotic donkeys and other equines including ponies and mules (Table 2). During the year, there were four foalings, two in horses, one in ponies and one in mules.

Table 2. Equine herd strength at NRCE (Bikaner and Hisar)

| Cetegory | Horses | | Donkeys | Others* | Total |
|---|---|---|---|---|---|
| | Kathiawari | Marwari | | | |
| Adult male | 1 | 5 | 7 | 3 | 16 |
| Adult female | 5 | 6 | 14 | 1 | 26 |
| 2-3 yrs | - | 2 | 2 | - | 4 |
| 1-2 yrs | 1 | 4 | 9 | - | 14 |
| 6M-1yr | 1 | 2 | 3 | 2 | 8 |
| 0-6M | 2 | 1 | 2 | - | 5 |
| Total | 10 | 20 | 37 | 6 | 73 |

* Other equines (mules and ponies) at Hisar Campus

Breeding performance of farm herd at Bikaner centre during the year is given in Table 3. The number of breedable mares and donkeys available were 8 and 14, respectively.

Table 3. Reproductive performance of equine herd at Bikaner

| Parameters | Mares | Donkeys |
|---|---|---|
| Number of adult females | 8 | 14 |
| Number of A.I./N.S. done | 11 | 37 |
| Number conceived | 5 | 12 |
| Number of foalings | 4 | 8 |

# fun'kd dk ikDdFku

jk"Vªh; v'o vul/kku dUn dh okf"kd ifronu 2003&2004 iLrr djuk ej fy, g"kZ dk fo"k; gA v'ok dh LokLF; ,oe mRiknu lECkfU/kr leL;kvk dk lek/kku bl dUn dh e[; ikFkfedrk jgh gA foxr o"kZ e dUn v'o jkxk d funku] cpko ,oe jkdFkke gr Lon'kh o fdQk;rh ik|kfxfd;k d fodkl gr i;Ru'khy jgkA tl k fd bl ifronu e mYyf[kr g] bl vof/k d nkjku dUn u bl fn'kk e dkQh ixfr dh gA bl o"k iVV dk;ky;] Hkkjr ljdkj u bl dUn }kjk fodflr v'o gjiht ¼bñ,pñohñ&1½ jkx funku fdV dk iVV inku fd;k rFkk v'o ccfl;k d funku gr fodflr dh x;h fdV d iVV gr vkonu fd;k x;kA geu v'ok e xHkikr jkdFkke gr bñ,pñohñ&1 Vhdkdj.k d fodkl dh fn'kk e ljkguh; ixfr dhA ?kkfM;k e jDr&tkp l xHkk/kku&funku gr ,d fdV fodflr dh tk jgh g ,oe bñ,pñohñ&1 d funku gr l'kkf/kr] lonu'khy ,oe fof'k"V fdV dk fodkl vfUre pj.k e gA v'ok dh Lon'kh itkfr;k d y{k.k&o.ku dk dk; ixfr ij gA fgehÑr oh; l v'ok e Ñf=e xHkk/kku d Qke ijh{k.k d ifj.kke mRlkgo/kd jgA n'k d fofHkUu Hkkxkfyd {k=k e Qyh v'o tkfr e fofHkUu jkxk dh ;FkkfLFkfr tkuu gr db u, jkT;k tl e/; in'k] tEe ,o d'ehj] e?kky; bR;kfn dk Þv'o jkx jk"Vªh; lo{k.k dk;ØeÞ e bl o"k 'kkfey fd;k x;k gA

jkñ vñ vuñ dñ dh 10oh ip o"kh; ;ktuk e 13-50 djkM #- d foÙkh; ifj0;; dk LFkk;h foÙk lfefr }kjk vuekfnr fd;k x;kA bl ;ktuk e vR;k/kfud lfo/kk;Dr cfu;knh <kpk] ft le chdkuj fLFkr ^v'o ituu ifjlj* e i;kx'kkyk ,o i'kklfud Hkou dk fuek.k rFkk fglkj e i;kx'kkyk Hkou ,oe ck;kl¶Vh yc&3 lfo/kk d fodkl dju dk iko/kku gA

o"k 2003&2004 foÙkh; o"k dh vof/k e jkñ vñ vuñ dñ dh cfu;knh lfo/kkvk ,o vul/kku xfrfof/k;k dk fofHkUu fo'k"kK lfefr;k ¼bl dUn dh nk i;kx'kkykvk dh vUrjk"Vªh; ekU;rk gr Ñf"k e=ky; d lk'kikyu ,o M;jh foHkkx }kjk ukfer lfefr( ip okf"kd leh{kk lfefr ,o Ñf"k e=ky; dh Ñf"k o oKkfud fo'k"kK lfefr½ }kjk eY;kdu fd;k x;kA ;g vfr g"k dk fo"k; g fd bu lfefr;k u bl dUn dh rduhdh mRÑ"Vrk dh ljkguk dh ,o mudh flQkfj'k Hkfo"; e dUn d vul/kku ,o fodkl e ekxn'ku djxhA

bl o"k d nkjku dUn u chdkuj ifjlj e ,d if'k{k.k dk;Øe Þv'ok e oh; dk fgehdj.k] Ñf=e xHkk/kku ,o xHk/kkj.k funkuÞ dk vk;ktu fd;k rFkk fglkj e ÞÑf"k lECkfU/kr oKkfud tkudkfj;k d ilkj gr lpuk rduhdhÞ ij ,d dk;'kkyk dk vk;ktu fd;kA bl o"k yxHkx lHkh i'kklfud depkfj;k dk dk; dk'kyrk e l/kkj d fy, fofHkUu if'k{k.kk gr Hktkx;kA

e Hkkjrh; Ñf"k vul/kku ifj"kn] ub fnYyh] fo'k"kdj Mkñ exyk jk; th ¼egkfun'kd HkkñÑñvuñiñ ,o lfpo] Ñf"k vul/kku ,o f'k{kk foHkkx½] Mkñ fot; dekj rutk th ¼mi&egkfun'kd] i'k foKku½ rFkk Mkñ yky Ñ".k th ¼lgk;d egkfun'kd] lk'k LokLF;½ dk ân; l vkHkkjh g ftUgku v'o LokLF; ,o mRiknu e l/kkj d fy, bl dUn dk fujUrj leFku fn;kA idk'ku lfefr bl okf"kd ifronu d uru :Ik ,o le;c) idk'ku d fy, c/kkb dh ik= gA

25 tykb 2004

'ਡਾਇਰੈਕਟਰ ਜਨਰਲ ਨਿਰੋਗਹ

# Dk;dkjh lkjk'k

Hkkjro"k d yxHkx 20 yk[k v'o itkfr d lk'kvk e l 98 ifr'kr Lon'kh itkfr d v'o g] ft le xnHk] [kPpj ,o VVV Hkh 'kkfey gA ; lk'k Hkfeghu] NkV ,o lhekUr fdlkuk ,o nlj xkeh.k ,o v/k'kgjh ox d fy, thfodk&;kiu dk lk/ku gA igkMh] 'k"d] o v/k&'k"d {k=k e tgk ij lMd ekx miyC/k ugh g] ogk ; lk'k ;krk;kr ,o ck>k <ku e i;kx gkr gA 'k"k 2 ifr'kr v'o lxfBr {k= e luk] ifyl vkj lhek lj{kk cy] ?kM&nkM m|kx] [ky ,o lekjkg ijM e i;kx fd; tkr gA bu xfrfof/k;k e v'ok dh iHkko'kkyh dk;{kerk mud mÙke LokLF; ,o jkxeDr fLFkfr ij fuHkj djrh gA vr% bl o"k d nkjku dUn dh e[; xfrfof/k;k v'ok dh LokLF; ,oe mRiknu e l/kkj] v'o jkxk d funku] cpko ,oe jkdFkke gr Lon'kh o fdQk;rh ik|kfxfd;k dk fodkl djuk] v'o jkxk dk jk"Vh; lo{k.k rFkk v'o ikydk dk lykg ,o ijke'k lok, miyC/k djkuk jghA jk"Vh; v'o vul/kku dUn dh 2003&04 dh miyfC/k;k dk C;kjk l{ki e uhp fn;k x;k gA

iVV dk;ky;] Hkkjr ljdkj u bl dUn d vkonu ¼2199@Mhb,y@96½ ^^v'o gjiht jkx d io&funku gr fdV d fuek.k dh fof/kÞ dk iVV inku fd;kA ;g Hkkjr ljdkj d jkti= e 25 vDrcj 2003 dk vf/klfpr fd;k x;k g] ft ldk oxhdj.k Øe 55b4 1891278 gA bl o"k ,d vU; iVV vkonu ^^v'o ccfl;k d funku gr dEifyeUV fu/kkj.k vk/kkfjr ijh{k.k fof/kÞ iVV dk;ky; dk Hkk tk x;kA

bl o"k v'ok e bñ,pñohñ&1 d funku d fy; dUn }kjk fodflr ekukDykuy ,UVhckMh&i;Dr funku ¼chñ,ykbt½ fdV d HkMkj.k ,o mi;kx gku rd dh vof/k rFkk fofHkUu i;kx'kkykvk e eY;kdu d fy, ijh{k.k fd, x,A bl fdV dk i;kx iFke ckj dju ij fdV d ifj.kkek e xk.k fofHkUurk ik;h x;hA fdV dh HkMkj.k vof/k N% ekg ik;h x;hA rFkkfi ;g vu'klk dh xbg fd fdV dh lkexh ,d ckj [kkyu d ckn mlh fnu mi;kx dh tkuh pkfg,A fdV dh Lohdk;rk c<ku d fy, ble mi;Dr lkexh d rjy voLFkk e fLFkjhdj.k d }kjk HkMkj.k vof/k c<ku d i;kl fd, tk jg gA

v'ok e 'kh?k xHkk/kku igpku d fy; xnk&ijh{k.k vFkok ijk/ofud tkp d fodYi d rkj ij jDr ¼lhje½ vk/kkfjr lUMfop ,ykbtk fof/k fodflr dh tk jgh gA ;g fof/k fdlkuk d fy, ljy ,o ykHknk;d g D;kfd ble lk'kvk dk xHkk/kkj.k tkp gr funku dUn rd y tku dh vko';drk ugh gA bl fof/k d }kjk ?kkfM;k e 35&120 fnu d xHk dh igpku dh tk ldrh gA ?kkfM;k d jDr d 35&45 fnu dh xHkkof/k d lHkh 73 ueu bl fof/k }kjk ldkjkRed ik; x, rFkk bu ueuk e blhth gkeku dh ek=k 18-75&150-75 vkbñ;ñ ifr feñfyñ ik;h x;hA [kPpj mRiknu gr xHkorh ?kkfM;k d fy, ;g fof/k dkjxj ugh gA 20 l 35 fnu dh xHkorh ?kkfM;k e xHk tkp gr bl ,ykbtk fof/k dh {kerk dh tkp dh tk jgh gA

bl dUn e fodflr bñ,pñohñ&1 ¼fglkj&90&7½ d fuf"Ø; fo"kk.k i;DÙk Vhdk dh ifrj{k.k ,o cpko {kerk dk xHkorh ckYc@lh e"kdk e ijh{k.k fd;k x;kA e"kdk e 12-5 vkj 25 ekbØk xkñ ifrtu d Vhdkdj.k ij nkuk rjg d Vhdk u 14 fnu d vUrjky ij vPNk vlj fn[kk;k tk fd dEifyeUV fQfDlx] ok;jl U;Vykbftx rFkk fyEQklkbV iQyu fof/k }kjk ekik x;kA bñ,pñohñ&1 d thfor fo"kk.kvk l lØfer dju ij 60&70 ifr'kr VhdkÑr

xHkorh e"kdk e lkekU; ,o LoLFk cPp ink g, tcfd 6 e l 4 ½66-66 ifr'kr½ fcuk&Vhdk Ñr xHkorh e"kdk e xHkikr gvkA e"kdk e xHkikr dk jkdu d fy, 25 ekñxkñ bñ,pñohñ&1 ifrtu dh ek=k mi;Dr ik;h xbA bl Vhdkdj.k d ?kkfM;k e ik;kfxd ijh{k.k ixfr ij gA

ekjokMh itkfr d Lon'kh ?kkM lgu'khyrk] icyrk ,o jkx&vojk/kd x.kk d dkj.k tku tkr gA ekjokMh itkfr d ?kkMk dh vklr mpkb 153-0$0-93 lVhehVj tcfd ?kkfM;k e 149-1$0-43 lVhehVj ik;h xbA ekjokMh tkfr d v'ok e vuokf'kd y{k.k&o.ku dk dk; cgydhdj.k J[kyk vfHkfØ;k ¼ihñlhñvkjñ½ fof/k l 16 fofHkUu ekbØklVykbV ekdj l dju ij Kkr gvk fd ekjokMh v'o nk ikbejk ¼,ñ,pñVhñ16 o ,ñ,pñVhñ 44½ l ,d:ih g ,o budk i;kx ekjokMh itkfr d v'ok dk fon'kh uLy d ?kkMk l foHknhdj.k gr fd;k tk ldrk gA ekjokMh v'ok d y{k.k&o.ku dk dk; rhu vU; x.kl=k e fpfUgr dju dk i;kl fd;k tk jgk gA

ekjokMh itkfr d ?kkMk d lkekU; tyh&jfgr oh; d vfHky{k.k o.ku gr Hkkfrd&to jl;fud v/;;u fd; x;A oh;k d fgehdj.k lj{k.k d fy, fofHkUu rudkjdk d eY;kdu dju ij ik;k x;k fd yDVkl&Xydkl&vMktnh rudkjd l cgrj rjyhdj.k mijkUr 'kØk.k xfr'khyrk ½25&40 ifr'kr½ feyrh gA 'kDdj&vk/kkfjr rFkk ,pñ,Qñ&20 rudkjd d i;kx l 20&30 ifr'kr 'kØk.k xfr'khyrk ik;h x;hA ;|fi 'kDdj no d i;kx l fgehdj.k io 'kØk.k xfr'khyrk cgrj ik;hA

o"k 2003&04 e fgehdr xnHk oh; l djuky ,o ikuhir ftyk d xkok e eknk v'ok e df=e xHkk/kku d Qke ijh{k.k fd; x,A bu i;klk }kjk 95 e l 39 eknk v'ok e 41-05 ifr'kr dh nj l xHk/kkj.k gvkA

n'k d fofHkUu Hkkxk e Qyh ijh v'o tkfr] fo'k"kdj Lon'kh v'ok e fo|eku jkxk d v/;;u gr Þjk"Vh; v'o jkx lo{k.k dk;Øeþ bl dUn d rRok/kku e py jgk gA bl o"k d nkjku 12 fofHkUu jkT;k] fcgkj] fnYyh] gfj;k.kk] fgekpy in'k] tEe d'eh] dukVd] e/; in'k] e?kky;] jkt LFkku] itkc] mrj in'k rFkk mrjkpy e jDrkn&lo{k.k fd;k x;kA jDr ¼lhje½ d ueuk dh tkp dju ij 1483 e l 81 ½5-46 ifr'kr½ ueu bñ,pñohñ&1 lØfer] 548 e l 40 ½7-3 ifr'kr½ ueu ekbdklyktek xflr ik; x;] tcfd ccfl;k dk lØe.k 1431 e l 387 ½27-04 ifr'kr½ v'ok e ik;k x;kA bl nkjku tkp x, 12 jkT;k d 1517 v'ok e l fdlh e Hkh Xy.Mjl] lkyekuyk] v'o ,fufe;k ¼bñvkbñ,ñ½ dk lØe.k ugh ik;k x;k rFkk lHkh 348 jDr ueu vÝhdu ?kkM dh chekjh l eDr ik; x;A okrkoj.k in"kdk dk v'ok d LokLF; ij fo"ky iHkko dk v/;;u dju d vUrxr jDr ueuk e dMfe;e rFkk ¶lykjkbM dh ek=k dk fujUrj vkdyu fd;k x;kA

v'ok e cPp tuu d ekle e vfrlkj dk lØe.k v'oikydk d fy, ,d cMh leL;k gA fglkj d bn&fxn ?kkMk Qkek l nk ekl l NkV 50 vfrlkfjr v'o cPpk d nLr d ueu ,d= djd fofHkUu jkxtuk d fy, tkp x;A ,ykbtk ,o vkjñ,uñ,ñ&it fof/k }kjk tkp dju ij jkVkok;jl uked fo"kk.k pkj ½8 ifr'kr½ ueuk e ik;k x;kA fV';&dYpj iFkDÑr jkVkok;jl fo"kk.kvk d o|rd.klpyu ij ik;k x;k fd bl {k= d ?kkMk Qkek e de l de nk fofHkUu jkVkok;jl fo"kk.k fo|eku gA nLr d ueuk e 28 ½54-9 ifr'kr½ bñ dkykb thok.k ik; x; ;|fi bue l doy 9 ohjk dkf'kdkvk d fy, foÑfrtud ik; x;A

v'ok d 29 LVIVkdkDl bDokb uked thok.kvk d thok.kjk/kh ,o e"kd&jkxtudrk y{k.k o.ku dk dk; fd;k x;kA lHkh LVIVkdkDl bDokb thok.k

e"kdk e vfr&foÑfrtud ik; x;] ;|fi lkr LVIVkdkDl >,fiMfedl thok.k de foÑfrtud FkA

vBBkou ck>iu l xflr ?kkfM;k e ck>iu d dkj.kk dh tkp&iMrky dh x;hA dkf'kdh; v/;;u ,o thok.k lEo/ku ijh{k.k l ;g rF; lkeu vk;k fd 45 ¼77-6 ifr'kr½ ?kkfM;k e ck>iu dk dkj.k l{etho FkA bu ?kkfM;k d xHkk'; p"k.k dh dkf'kdh; v/;;u ,o thok.k lEo/ku ijh{k.k ifj.kkek e vPNh lgefr ¼86-67 ifr'kr½ ik;h x;hA

v'ok e Vkbiuklkek boUlkb dh igpku d fy, ,d cgydhdj.k J[kyk vfHkfØ;k ¼ihñlhñvkjñ½ dk ekudhdj.k fd;k x;kA bl ihñlhñvkjñ l Vkbiuklkek boUlkb thu d ,d [k.M ¼227 cl tkM½ dk foLrfjr fd;k x;k ,o v'ok d jDr d ueuk e bl fof/k dh lonu'khyrk o fof'k"Vrk dk voykdu fd;k tk jgk gA

v'o mRiknu ifjlj chdkuj e 1&10 flrEcj 2003 dk ,d if'k{k.k dk;Øe Þv'ok e oh; dk fgehdj.k] Ñf=e xHkk/kku ,o xHk/kkj.k funkuÞ dk vk;ktu fd;k x;kA ble fofHkUu jkT;k d 22 ifrHkkfx;k dk Ñf=e xHkk/kku rFkk xnk tkp] jDr o ijk/ofud tkp l xHk/kkj.k funku dh fof/k;k e ijh{k.k fn;k x;kA

jkñ vñ vuñ dUn fglkj u jk"Vh; lpuk dUn fglkj d lg;kx l 17 flrEcj 2003 dk ,d fnu dh dk;'kkyk ÞÑf"k lEcfU/kr oKkfud tkudkfj;k d ilkj gr lpuk rduhdhÞ dk vk;ktu fd;kA ble Hkkñ Ññ vñ iñ ,o dUn ljdkj d lLFkkuk e dk;jr oKkfudk ,o depkfj;k u Hkkx fy;kA bl dk;'kkyk dh e[; lLrfr;k e i;kx'kkyk dh uohure tkudkfj;k dk fdlkuk rd 'kh?k ilkj djuk] fdlkuk dh ekx d vu:lk Ñf"k&lEcfU/kr vk/kkj lkexh r;kj djuk ,o fgUnh o vU; Hkk"kkvk dk Kku&ilkj d fy, vf/kdkf/kd i;kx djuk jgh A

bl o"k dUn d oKkfudk u 20 ey 'kk/k&i= jk"Vh; o vUrjk"Vh; 'kk/k if=dkvk e idkf'kr fd;] 16 'kk/k&i= fofHkUu oKkfud lEeyuk o xkf'B;k e iLrr fd; ,o 15 oKkfud y[k fofHkUu if'k{k.k xUFkk e idkf'kr fd;A

gekj ^v'o&jkx&igpku ,o oKkfud&lykg&lok* dk;Øe d vUrxr dVjk ¼tEe&d'ehj½ e 10 l 12 tu 2003 ,o p: ¼jktLFkku½ e 29 vDrcj 2003 dk v'o LokLF; f'kfojk dk vk;ktu fd;k x;kA bu f'kfojk e e[; v'o jkx tl yxMkiu] mnj'ky] i'kkc dk vojk/ku] bR;kfn dk bykt fd;k x;kA bld vfrfjDr ijh{k.k dju ij t:jren v'ok dk iV d dfeuk'kd] [kfut feJ.k rFkk foVkfeu dh [kjkd nh x;h rFkk v'ok d jDr d ueu fofHkUu thok.kvk] fo"kk.kvk rFkk ijthoh lØe.k dh tkp d fy, ,d=fd;x;A

bl nkjku dUn u viu vkrfjd L=krk l 26-49 yk[k :ñ vftr fd,] ftle v'o&jkx igpku ,o funku lok, rFkk v'o ikydk dk mÙke uLy d v'ok dh fcØh e[; FkhA

NRCE Hisar Campus

The main campus at Hisar is located in Haryana on National Highway No. 10, about 170 km North-West of Delhi and is situated at a distance of about 4 km on Sirsa Road from Bus Stand, Hisar and about 6 km from Railway Station, Hisar. It is situated between latitude $29.10^0$ N and longitude $75.46^0$E.

# Contacts

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