

9. Livestock Management

Livestock management directs to run livestock operations and produce milk, meat, egg, fish and other products. Their studies include Chemistry, Nutrition, Health and Safety.

Nutrition

Feed database: Indian Livestock Feed Portal showcases information on feed resources, nutrient requirements, feed standards and assessment, market dynamics and exim indices.

- Supplementation of 100 ppm Zn and 100 IU vitamin E was effective in amelioration of adverse effects of Cd in guinea pigs.
- Study on chelated copper and zinc using methionine (*in vitro* and *in vivo*) indicated more than 95% chelation. The bioavailability using purified diets supplementing Cu and Zn methionates in rat at either 50 or 100 ppm of requirement replacing inorganic salts showed higher gut absorption, higher Cu and Zn content in liver, muscle and improved antioxidant as well immune status.
- Two essential oils, viz. *ajwain* (AjO) and cinnamon leaf oil (CiLO), showed 18% and 27% reduction in methane production, respectively, without disturbing total organic matter digestibility of feed and other fermentation parameters. Saponins isolated from *Chlorophytum borivillianum* leaves when evaluated for rumen fermentation showed reduction in ammonia, methane and protozoa.
- Both methanolic and aqueous extracts of sea buckthorn leaves showed good antioxidant activity.
- In *neelgai*, *chinkara* and *gaur*, the major group of methanogens was *Methanobrevibacter* spp., followed by *Methanobacterium* spp.
- Oils of *Thymus vulgaris*, *Cinnamomum zeylanicum*, *Syzygium aromaticum*, *Eucalyptus globulus*, *Lavendula officinalis* inhibited growth of *Aspergillus parasiticus* (NRRL 2999) fungi, 70–100% in media. They also inhibited fungal growth by 56–83% and aflatoxins production by 71–92% in feed.

Prebiotic supplementation: Sodium hydroxide treatment, followed by steam application was effective for enhancing the recovery of xylan from corn husk. An enzyme dose of 6.625 units, pH 6.0, and temperature 50°C for 16 hr of incubation yielded highest concentration (1.88 mg/ml) of xylobiose.

Lignin degradation: White rot fungi *Pycnoporous sangeus* and *Coriolus versicolor* were immobilized

on five different types of matrices for lignolytic enzymes production. Polyurethane foam (a synthetic matrix) cubes proved as the most promising immobilization matrices in terms of production of the three lignolytic enzymes, followed by sugarcane bagasse and wood shavings.

Cattle

Feed safety and biosecurity: Soil, fodder and dung samples were analyzed for lead, cadmium and arsenic from the dairy-zones of urban and peri-urban fringes of Bengaluru for monitoring livestock related drug residues and environmental pollutants. Lead was in the range of 7.4–9.5 ppm in soil, 14–16 ppm in fodder and 20–24 ppm in dung. The cadmium in soil is 0.07–1.1, fodder 1.9 and dung 0.76–1.2 ppm and arsenic is non-detectable in all the samples. Across Karnataka 14% water samples had more than 1.0 ppm fluorine in the study on metabolic effects of fluorosis and its mitigation.

Sheanut cake (SNC) has more than 6% hydrolyzable tannins. The energy content was more than 7 MJ/kg dry matter (DM). *In vitro* incubation of SNC indicated significant reduction in methane emission. It appeared that SNC could be incorporated in ruminant feeding as a source of energy and protein and also to suppress methanogenesis.

Supplementation of protected fat to high yielding dairy cows improved milk yield (19.0 kg vs. 17.8 kg/cow/day), reproductive efficiency and was economical to farmer.

Buffalo

- Feeding 10% *mahua* seed cake and 2% *harad* in the ration of buffaloes resulted in 17.48% reduction in methane production *in vivo*.
- Buffalo and caprine fetal stem cells were isolated and characterized from very early stage fetus. Caprine fetal stem cells, when differentiated, formed beating cardiomyocytes.

Sheep

Crop residue-based complete feed: Ram lambs fed on complete feed having crop residues maize straw, legume straw and groundnut haulms at 50% level in mash type, achieved higher weight of about 25 kg.

- Detoxified *jatropha* meal could replace conventional protein moiety of soybean meal in the concentrate up to the level of 37.5% on iso-nitrogenous diet without any apparent adverse effect in lambs.
- Diets supplemented with condensed tannins



Lambs feeding on complete feed manufactured at Rural Feed Processing Unit

(1–2% of diet) improved nutrient utilization, growth, metabolic profile, antioxidant status, immune response and FCR and decreased methane emission and gastro-intestinal parasitic load in lambs.

Camel

Improvement of feed resources and nutrient utilization: Lactating camels fed on complete feed blocks having 6.97% DCP and 107.21 ME MJ, showed better milk yield, body weight maintenance and nutrient utilization. *In-vitro* fermentation study of local camel feeds and fodders indicated maximum gas production in *bajra* grains; its production varied between 94 and 97 ml in *guar phalgati*, groundnut haulms and *guar chur*, and it was lower in complete feed blocks having local feeds in different proportions. The effect of area specific mineral mixture supplementation was beneficial over grazing alone on reproductive performance in female camels.

Yak and mithun

Yak calves fed complete feed block (CFB) with area-specific mineral mixture at Mandala, Arunachal Pradesh at an altitude of 3,048 m above msl, showed significantly higher average daily gain in body weight than other groups. The digestibility of ether extract and crude fibre was significantly higher in animals fed CFB than other groups.

Highland pasture development for yaks: Suitable temperate grasses and legumes were tested for highland pasture development. *Dactylis glomerata*, *Lolium perenne*, *Setaria* spp. and *Trifolium repens* were grown in trial plots to test their suitability and production potential.

Ensiled-spent grain in diet: Incorporation of higher level of ensiled-spent grain in the diet of mithun has a negative effect on total feed intake.

Poultry

Nutrient requirements and feeding schedules for backyard poultry: Calcium and non-phytin phosphorus (NPP) contents in the diet of Vanaraja breeder chicks

could be reduced from 0.9 to 0.8 and 0.45 to 0.4%, respectively, by supplementing vitamin D₃ @ 1,200 ICU/kg during 0–6 weeks of age. In Vanaraja female and male lines, the requirements for metabolizable energy, crude protein, lysine, methionine and NPP were established during juvenile stage. Performance of Vanaraja chicks till 42 days of age was reduced by incorporation of *guar* meal at 20% in diet. Supplementation of mannanase (4,000–6,000 units/g) and xylanase (3,250–4,875 units/g) significantly increased weight gain and feed efficiency in chickens when fed with *guar* meal.

Zn levels in broiler breeder diets: Zinc (organic) supplementation at 60 ppm level to diet was essential for optimum egg production, persistency and hatchability in broiler breeders. Increase of Zn (80 or 100 ppm) had no significant effect.

Quality protein maize in poultry diet: Feeding value of quality protein maize (QPM) was superior to normal maize (NM) in White Leghorn layer chicken diet, whereas that of Nityashree hybrid maize (NHM) was similar to NM. Quality protein maize-based layer diet produced 3.3% higher egg production and recorded 4.9% improvement in feed conversion compared to NM. The egg quality in terms of Haugh unit (77.54 in QPM vs. 72.96 in NM) and yolk colour (7.36 vs. 6.38) of eggs laid by the QPM-fed layers was significantly higher compared to those fed NM.

Nutritional and management strategies were developed to increase egg production. Artificial lighting at the poultry houses using longer wavelength lights (red spectrum) than incandescent lighting improved egg production in commercial layer flocks by about 6.33%. The level of stress during the entire process of oviposition affects egg production and inclusion of garlic in the grower and layer rations reduced this stress and improved egg production in birds from 56 weeks onwards. Feeding of the garlic resulted in sustained egg production beyond 72 weeks, up to 90 weeks of age.

Updating nutrient requirements of poultry: Lysine dense diet (17 g/kg) improved body weight gain, feed conversion ratio, and reduced feedcost of production of meat type Japanese quails. The requirements of folic acid (1 mg/kg) in the diet of Japanese quails, copper (16 mg/kg) and zinc (40 mg/kg) in broiler chicken were established.

Intervention on egg cholesterol reduction: Dietary supplementation of a combination of organic chromium (chromium picolinate) (1 mg/kg) and spirulina (2 g/kg) reduced egg cholesterol by about 20%.

Herbal products for broilers during extreme summer: Addition of dried fresh root powder of *sarpagandha* (*Rauwolfia serpentina*) 0.1 to 0.3% or *ashwagandha* (*Withania somnifera*) 0.2% or dried stem powder of *gelo* (*Tenospora cordifolia*) 0.1% or dried *amla* (*Emblica officinalis*) fruit powder 0.2% improved performance (assessed through HL ratio, immune-competence and oxidative profile) of coloured broiler chickens during peak summer in north India.

Fumaric acid as mould inhibitor in poultry feed:

Fumaric acid @ 0.2% was effective in complete inhibition of aflatoxins production in poultry feed having 13% moisture level, while its 0.5% concentration brought about complete inhibition of these mycotoxins in feed having 15–17% moisture level.

Commercial chicken rearing at high altitude:

CARI-Dhanraja, a promising commercial coloured broiler chicken, exhibited better production performance when reared under cage system as compared to floor rearing, during rainy season at high altitude (about 2,438 m) in Kumaon hills. Similarly, production performance of White Leghorn layers was significantly better in cage system.

Quail

Acidifiers as alternative of antibiotics feed additives:

Addition of either sodium acetate 0.2% or tartaric acid 0.1% in feed or glacial acetic acid 2.5 ml/litre of drinking water improved body weight gain, feed efficiency and immune response with decreased microbial load of caecal/faecal contents of growing Japanese quails.

Physiology and reproduction

Cattle

Loss of superoxide dismutase (SOD), leakage of lactate dehydrogenase (LDH) and glutamic oxaloacetic transaminase (GOT) enzyme activity was significantly higher in non-freezable than freezable quality semen of crossbred bulls, indicating their significant role in freezing of semen.

Buffalo

Parthenogenetic embryonic stem (ES) cells were generated and propagated up to seventh passage. Both parthenogenetic and IVF embryos derived stem cells expressed similar pluripotent markers.

Azolla as a protein supplement at 5% DM level improved follicular growth. On the contrary, a high protein ration increased levels of ammonia, urea and blood urea nitrogen which have a negative effect on ovulation and fertilization.

Antioxidants and prostaglandin E₂ prevented early embryonic losses. An isoform of glutathione peroxidase GPx-8 was discovered in buffaloes which is different from that of cattle. *In-vitro* studies revealed that IGF-I improved progesterone production from luteal cells thus improving embryonic survivability.

Sheep

The mRNA expression of IL-1 α and TLR 4 was 6- and 11-fold higher, respectively, in subclinical/clinical endometritis as compared to normal ewes, indicating their application for early diagnosis of sub-clinical endometritis.

The lambing rate of 64.4% was achieved with insemination of short-term preserved semen of young ram's per-os for two cycles in Malpura ewes exhibiting

natural oestrus. In improving reproductive efficiency of sheep, three lamb crops in two years were achieved in 57.2 and 72.7% ewes within the target period of 243 and 636 days.

Camel

Use of human chorionic gonadotrophin in artificial insemination:

Highly purified-human chorionic gonadotrophin (HPHCG) preparation 5,000 IU, was used as ovulating agent and artificial insemination was done at 30 and 45 hr after the injection. Three out of five female camels were tentatively pregnant.

Bio-stimulation and post-parturient breeding:

The bio-stimulation and sexual stimulation showed positive effect on male reproduction and were effectively used to augment rut before the onset of breeding season.

For attempting pre-seasonal breeding, 32 females were examined and follicles were observed in 69% females; 20% females conceived from 21 females given service. After attempting post-parturient breeding (within 30–60 days calving) in 15 females, a reduction of 300 days in the calving interval in more than 50% of females was achieved.

Equines

Draughtability studies with equines under arid conditions:

Exotic female donkeys with body weight of 345 kg were used in carting with 6, 8 and 10 q of load under continuous work for 3 hr. Changes in physiological indices and physical changes (frothing, profuse sweating, watery discharge from nostrils and eyes) were recorded. Animal was reluctant to continue to work after 2 hr. No specific changes in haematological values (Hb, PCV, TEC and TLC) after work were observed during 6 and 8 q load. However, under 10 q load, the animals showed total fatigue in 1 hr of work with clear physical changes (frothing, in-coordination of legs, profuse sweating, watery discharge from nostrils and eyes, reluctant to continue work) indicating their inability to carry this much load. The similar results were obtained by work-rest-work cycle technique.

Use of exotic Poitu donkeys for agricultural operations:

Four male donkeys (body weight: ~298 kg) reared on standard feeding and management were trained and used in ploughing operation. A single animal-drawn matching plough was designed and both male and female adult donkeys were trained for ploughing operation.

The whole operation was planned as 2 ploughing sessions of 1.5 hr each with 1 hr rest in between. Physiological indices increased significantly during ploughing operation. Average land ploughed was 0.07 ha/hr by the donkey. Donkeys were able to plough 0.21/ha in two sessions at an average speed of 2.60 kmph.

Similarly, three exotic donkeys were utilized for 2 hr continuous work regimen and used in sowing operation. Single animal-drawn matching plough with

two furrows was designed. In 2 hr, one donkey sowed 0.27 ha at an average speed of 2.635 kmph. Physiological indices increased significantly during sowing but reached normal physiological levels after 1 hr of rest. Slight increase in Hb, PCV, TEC and TLC values was observed during sowing.

Use of indigenous donkey in ploughing operation: Average body weight of local donkeys was 112 kg. Single animal-drawn matching plough was designed and four male adult donkeys were trained for ploughing operation. Donkeys were employed in 4 sessions of 2 hr each with 2 hr rest in between each session. Donkeys were able to plough an average of 0.036 ha/hr and total land ploughed was 0.15 ± 0.072 ha at an average speed of 1.835 kmph.

Yak and mithun

Ovum pick up standardized in yaks: On an average, 1.53 oocytes/animal were recovered from 13 yak females. Of the total oocytes, 85% were A and B categories. Subsequently, A and B category oocytes were subjected to *in-vitro* maturation and fertilization which resulted in 70% fertilization rate.

Ovarian folliculogenesis/follicle maturation pattern in mithun: Follicular dynamics for each mithun cow were studied at least for three consecutive cycles. Ovaries in each cow were scanned with a linear array trans-rectal probe (7.5MHz transducer). Of the 24 inter-ovulatory cycles studied, 70.8% were of two-wave cycles.

Standardization of boar semen collection: Semen collection by Gloved hand and Simple fist method was standardized. Boars of Hampshire, Duroc and Ghungroo breeds were used. Artificial insemination (AI) was undertaken in pigs widely in the institute and neighbouring area within a radius of 65 km. Total AI born piglets were 1,516.

Synchronization was carried out with progesterone priming making 20 mg capsules in the laboratory for oral feeding. A period of seven days priming was done by feeding one capsule orally at 24 hr interval. Estrous was detected within 5–7 days and AI was carried out on the second day of estrous. In gilt, the induced precocious puberty was eliminated from doing AI. In other groups of studies eCG was injected for follicle stimulation and GnRH or HCG was injected for ovulation. Progesterone priming alone was very economic in inducing estrous.

Poultry

Climate change and poultry production: The high ambient temperature during summer significantly decreased the fertility and hatchability in chickens. High ambient temperature adversely affected the semen quality of broiler breeders. The body weight of normal broiler chicken was higher as compared to naked neck chickens in low ambient temperatures.

Reproductive efficiency in broiler breeding hens: Phytoestrogen feeding had beneficial impact on both egg size and number, besides advancing the sexual

maturity irrespective of the body weight of hens. The rampant reproductive abnormalities prevailing at the phase of initial laying period were checked by phytoestrogen supplementation.

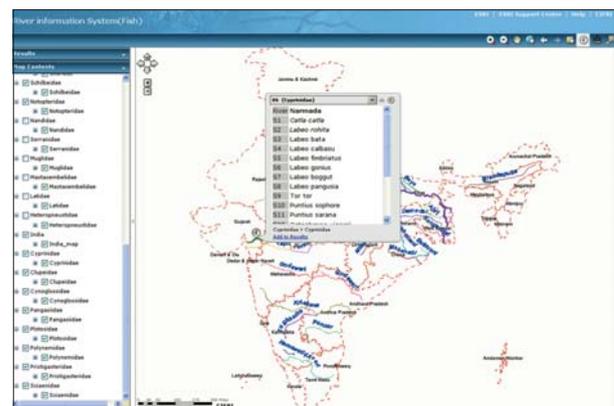
Fertile period of stored and freshly ejaculated semen: Use of freshly ejaculated diluted (1 : 3) and stored semen, resulted in maximum fertility (nearly 80% and above) between 3 and 7 days in White Leghorn hens.

Heat shock protein-70 gene expression: *HSP-70* expression was observed highest in duodenum when compared to proventriculus, ileum and jejunum under varied stressors like heat, feed withdrawal and restraint in broiler and layer chickens. Feed withdrawal affected highest expression of *HSP-70* at three weeks, whereas after six weeks, restraint stress induced peak *HSP-70* expression in both male and female broiler chickens. Of all stress conditions, feed withdrawal was most effective in inducing higher expression of *HSP-70* gene in various gastrointestinal segments. This trend was reversed by administration of cycloheximide, a protein synthesis inhibitor in broilers.

Fish

Capture fisheries

Fish species distribution map of rivers: Major rivers of North and South India, namely Ganga, Yamuna, Chambal, Betwa, East Banas, Son, Ken, Rupnarayan, Ajay, Subarnarekha, Kangsabati, Tapti, Narmada, Godawari, Krishana, Kaveri, Tava, Tungabhadra, Hemawati, Mahanadi and Pennar were delineated using TNT Mips software for preparing species distribution map on rivers. Data structure of 45 families of 11 fish orders, namely Zeiformes, Torpediniformes, Tetraodontiformes, Syngnathiformes, Synbranchiformes, Stomiiformes, Squaliformes, Rajiformes, Pristiformes, Polymixiiformes and Heteropneustidae, were created in MS-Access. For popularizing the database, archive data on river Narmada and Ganga were used.



Fish species distribution map

CMFRI marine fish landing statistics recognized as official statistics of Government of India: The Central Marine Fisheries Research Institute has been steadfastly executing the primary mandate of assessing marine resources off Indian coast for the past six

decades. This demanding task was carried out by keeping the tab on the resources and their divergence in terms of abundance and diversification successfully. A sampling design was perfected—it is self-improving and most practical and is a result of years of statistical reasoning. The multi-stage stratified random sampling scheme was refined for the estimation of landings.

Probiotics-based shrimp seed production: A probiotic-based shrimp seed production technique was developed using allochthonous probiotic strains, *Lactobacillus rhamnosus* (JCM 1136) and yeast *Saccharomyces cerevisiae* (IAM 14383T). Optimum dose of probiotic bacteria was 10^5 to 10^6 cfu/ml for seed production of tiger shrimp *P. monodon*. These probiotics showed a positive influence on larval development, metamorphosis and survival of the shrimp larvae.

Development of cell lines: A fibroblastic-like cell line was established from the ornamental fish red-line torpedo (*Puntius denisonii*). The growth rate of red-line torpedo fin (RTF) cells increased as the fetal bovine serum (FBS) proportion increased from 5 to 20% at 28°C with optimum growth at the concentration of 10% FBS. The morphology of RTF cell was predominantly fibroblastic-like. The cell line was cryopreserved in liquid nitrogen and could be recovered from storage after six months with good cell viability.



Puntius denisonii and its fin cells at 52nd passage

Cobia breeding on land-based broodstock system:

Trials were conducted to develop captive broodstock and captive maturation in black king fish cobia (*Rachycentron canadum*), one of the fastest growing marine fishes. Fishes of 5–20 kg caught from the wild were stocked at a density of 1 kg/m³. Successful maturation of the fish was achieved in a pond-based broodstock system. This is a breakthrough since earlier success by other institutions is based on broodstock held in marine cages. Matured fishes were maintained in recirculation aquaculture system (RAS) and induced bred with hormonal administration and about 1.4 lakh larvae were produced. This success will greatly simplify hatchery operation of this species and will facilitate entry of entrepreneurs to produce cobia seed for propagation of its farming.

Frontline demonstration of farm-made feeds: The farm-made feed, prepared using locally available cost

Success story

Nursery rearing of seabass as a livelihood option for aqua farmers

Rearing seabass fry to stockable size seed was identified as a livelihood option for aquafarmers. Seabass were reared in net cages (hapas) in farmer's site at Kottur, Mannargudi Taluk, and Thiruvarur district. Seabass fry produced in hatchery was stocked @ 400/m³. The average survival rate was 62% and size of a fingerling varied between 12 and 14 cm after 40 days of rearing. The total expenditure incurred by farmers for rearing 50,000 fry was ₹ 2.8 lakh, which included cost of hapa net cages, feed, water supply, fuel charges, labour charges, etc. Seedlings were sold for a price ranging from ₹ 12 to 15. The revenue realized by the farmer was ₹ 4.32 lakh with a profit of ₹ 1.52 lakh from a pond water spread area of 2,000 m².



Nursery rearing of seabass as a livelihood option

effective ingredients (poultry offal, mustard cake, soybean cake, some low value fish and other essential ingredients) was tested in a farmer's pond of 1,400/m² area at Madanganj, Namkhana Block, South 24 Paraganas. Wild seabass fry (initial weight 0.9 g) were weaned with farm made feed for one month and 700 weaned fry (average body weight 1.9 g) were stocked. After 318 days of culture, the crop was harvested and

Revival of small mechanized purse seine fishery

Introduction of large mesh purse seines facilitated by the CIFT has led to the revival of small mechanized purse seine fishery in Kerala. The changeover of mesh size in the purse seine from the conventional 20 mm to 45 mm showed good results and the purse seiners were able to land larger size catches of high value species. Experimental fishing operations carried out in the depth range of 50 to 220 m revealed that the catch mainly comprised large sized mackerels (62.08%), followed by tunas (16.08%), pomfrets (1.93%), carangids (14.43%) and miscellaneous fishes (5.47%). The purse fleet has increased to more than 100 compared to 17 earlier and started operating in deeper waters. The use of hydraulic power block in purse seine operations was demonstrated for the first time in small-scale mechanized purse seine sector.

seabass attained an average body weight of 865 ± 87.3 g. Use of farm made feed for this culture resulted in a survival rate of 61.4% with a feed conversion ratio of 1.98 indicating that use of farm-made feed for culture seabass is feasible.

Livestock health management

Foot-and-mouth disease

Clinical specimens were collected from 253 suspected outbreaks of which 180 outbreaks could be confirmed as foot-and-mouth disease (FMD). In all the geographical regions, serotype 'O' was most prevalent. Though majority of the outbreaks involved cattle, disease also occurred in buffaloes, pigs, sheep, goats, yak and mithun. Gradual reduction in the occurrence of FMD and significant reduction in its severity has been possible owing to application of a vaccine carrying appropriate vaccine strains, and sero-monitored by sensitive and specific companion diagnostics. At present, the National FMD virus Repository holds a total of 1,712 isolates (O-1102, A-276, C-15 and Asia 1-319). Studies on antigenic relationship of the field outbreak strains showed close antigenic match with respective vaccine strain.

Phylogenetic analysis of serotype 'O' virus revealed circulation of different lineages/sub-lineages of Middle East-South Asia (ME-SA) topotype in the country. Virus strains of Ind2001 sub-lineage, which re-emerged in late part of the year 2008, spread to majority of states in northern, eastern, north-eastern and southern India. Strain of Pan Asia II lineage was responsible for sporadic outbreaks/cases in West Bengal, Arunachal Pradesh and Maharashtra. Strains of PanAsia I lineage were identified in Punjab cattle. In serotype A, all the isolates clustered within genotype 18 (VII), are grouped both in the non-deletion and the VP3⁵⁹-deletion sub-lineages. The Asia1 field isolates were grouped in lineage C reiterating its supremacy since 2005.

Vaccines and therapeutics

- TaqMan qPCRs were developed for the potency estimation of live attenuated orf and buffalo pox vaccines.
- rHaa86 based tick vaccine could be used for the control of both *Hyalomma anatolicum anatolicum* and *R. (B.) microplus* infestations.
- Oncolytic potential of VP3 gene of CAV and NS1 gene of canine parvo virus-2 was established in MDCK cells.
- Swine influenza was confirmed by virus isolation in chicken embryos, MDCK cell line, HA test, electron microscopy, RT-PCR for detection of viral genome and cloning and sequencing of all eight genes of the virus. The phylogenetic analysis revealed that the virus was H1N1 subtype with very close homology to the H1N1 virus that caused the human H1N1 pandemic.
- Autogenous bacteria of *Staphylococcus aureus* in combination with vitamin C, or honey were

Success story

DIVA ELISA to differentiate FMD infected from vaccinated animals

This indigenously developed r3AB3 DIVAKIT is first of its kind for any animal disease in the country and has been designed as per WHO guidelines.

ELISA was developed as an easy-to-perform fool proof confirmatory assay in FMD free/controlled zones. Recombinant expressed proteins could be purified to near homogeneity. The optimal concentration of antigens and test serum dilutions were determined by checkerboard titration in ELISA. Cut-off values of 40 and 30% were adopted for 3AB and 3ABC, respectively. Diagnostic sensitivity (DSn) values of 84.14%, 98.23%, 99.118% and 99.118% and diagnostic specificity (DSp) values of 97.89%, 98.42%, 100% and 97.89% were obtained for 2C₁, 3D, 3AB and 3ABC, respectively. The rate of concordance in test results among all four NSP ELISAs was found to be 97.36%, 57.69% and 83.7% for naïve, vaccinated and infected (21–365 dpi) serum samples, and the highest degree of concordance was observed between 3AB and 3ABC-ELISAs.

The economy of this kit is one of its best attributes as it is at least four-fold cheaper at ₹ 25,000 per 250 tests than the commercial DIVA kit available on import. The diagnostic kit has a shelf-life of one year in support of robustness of the recombinant protein antigen.

This kit is for differentiation of FMD virus infected from vaccinated animals (DIVA), and can be effectively used for utilization in the National Control Programme on FMD.

found to be effective against clinical mastitis and enhancement of CD3 positive $\alpha\alpha$ TCRs (T-cell receptors) in diseased mammary parenchyma.

Diagnostics

- LAMP was developed for detection of *Pasteurella multocida*.
- VP6 gene based real-time PCR (SYBR Green) assay was developed for the detection of animal rotaviruses.
- Porcine circovirus 2 recombinant capsid protein (22.5 kDa) expressed in prokaryotic system was confirmed by Western blot analysis, and it was used in ELISA for detection of PCV2 specific antibodies in pig serum.
- Real time PCR was standardized for molecular diagnosis and quantification of bovine papilloma virus-2.
- A combined indirect ELISA was developed for simultaneous detection of *peste des petits ruminants* and bluetongue virus antibodies.
- A diagnostic kit for diagnosis of *Haemonchus contortus* infection was developed.
- Phosphate buffered saline, charcoal associated virus transport medium and normal saline preserved the H5N1 virus for up to six days and is recommended for transport of clinical samples for diagnosis of avian influenza.
- The detection of *Cao* gene from mastitic milk

sample was more accurate than bacterial culture for screening of *Staphylococcus aureus* mastitis in large herd.

Molecular characterization of pathogens

- Sequencing of 20 isolates of H5N1 virus was completed, two samples from 2011 outbreak in ducks of Tripura were phylogenetically new strain of H5N1 (clade 2.3.2). All other virus isolates from different outbreaks (2007-10) belonged to Qinghai-like clade 2.2, which were very close to Bangladesh and Bhutan isolates, indicating persistent circulation of these viruses in the region.
- Nucleotide sequence analysis of 2 H1N1 isolates revealed close relation with pandemic H1N1 2009 human isolates from India, Canada, Argentina, Taiwan and China.
- *C18L* gene of zoonotic buffalo pox virus was genetically characterized.
- 120 rotavirus isolates from buffaloes, cattle calves and human beings were genotyped.
- Protocols for phage display library screening and *in-vitro* biopanning were standardized and biopanning on horn cancer cell line was carried out for identification of bovine tumour cell specific ligands.

Veterinary biologicals

- Doses of RD 'F' strain vaccine (148,000), R2B vaccine (116,000), fowl pox vaccine (60,000), lapinized swine fever vaccine (304,880), tissue culture sheep pox vaccine (1,054,700); doses of PPR vaccine (4,640,200), *Brucella abortus* strain-19 (live) vaccine (9,375 ml), enterotoxaemia vaccine (4,250 ml), HS adjuvant vaccine (11,520 ml), tuberculin PPD (55,000); Johnin PPD (55,000); mallein PPD (18,000); *Brucella* agglutination test antigen (68,000 ml); *Brucella abortus* Bang ring antigen (4,620 ml); rose Bengal plate test antigen (18,470 ml); *Brucella abortus* positive serum (142 ml); *S. Pullorum* coloured antigen (5,520 ml); plain antigen (8,750 ml); *S. Pullorum* positive serum (41 ml), and of *S. Abortus equi* 'H' antigen (6,000 ml) were produced, quality tested and supplied to various organizations.
- 6.82 million monovalent doses of FMD vaccine were produced at Bengaluru Campus and 1.44 million trivalent doses of FMD vaccine were sold.
- PPR c-ELISA (14) and PPR s-ELISA (14) kits were produced and supplied.

Herbal medicines

- Antiviral potential against H5N1 virus was identified in some indigenous herbal extracts by *in-vitro* studies.
- A bio-organic formulation containing *Ocimum sanctum*, *Emblica officinalis*, and other bio-organics, viz. *Syzygium aromaticum*, *Commiphora mukul*, *Santalum alum*, *Annona muricata*, *Centella*

asiatica, *Allium sativum*, *Rubia cordifolia*, *Tinospora cordifolia*, have immunomodulatory potential.

Sheep

The PCR protocol was standardized for diagnosis of John's disease, ovine pulmonary adenomatosis and caseous lymphadenitis in sheep. Genetic resistance of sheep to *Haemonchus contortus*, on the basis of sire-wise mean FEC were studied (post-drench), the sires were ranked and progenies were selected for susceptible (S) or resistant (R) line.

Economic losses due to gastro-intestinal parasitism in Rajasthan were ₹ 97.37 crore in adult and ₹ 21.79 crore in yearling sheep. The cost : benefit analysis for strategic control of gastro-intestinal nematodes (single drench schedule) resulted in prevention of losses to the tune of 45.53% in female and 59.00% in male sheep. The economic evaluation revealed better economic impact of target selective treatment (TST), followed by targeted treatment (TT) approach compared to conventional drench schedule.

Camel

Epidemiology of infectious diseases:

Trypanosomosis remains a constant threat to the camel. PCR amplification was detected for *Trypanosoma evansii* in DNA samples from *Stomoxys* fly collected from Bikaner, suggesting prevalence of infection. Schlafen-like protein gene of camel pox virus (1510 bp) was submitted to the NCBI database (Accession number JF975616). Phylogenetic analysis revealed that Indian camel pox virus isolates were clustered with camel pox virus strain CMS and camel pox virus isolates from Kazakhstan. The full length gene sequence of topoisomerase gene of pseudo cow pox virus isolates from camels was submitted to the NCBI database (Accession Number HQ844268). Phylogenetic analysis revealed that pseudo cow pox virus isolates from camels represent a separate entity with regard to topoisomerase gene of ORFV (ORF virus) and PCPV (pseudo cow pox virus) from Reindeer.

Equines

Validation of equine herpes virus-1 vaccine:

Equine herpes virus-1 (EHV-1) vaccine- Equiherpabort, a killed oil emulsion mannide monooleate vaccine (OEMM) developed for control of abortions in mares was validated in field trials. A higher antibody response was noted in Thoroughbred mares as compared to indigenous mares indicating that Equiherpabort vaccine will be effective for large scale vaccination for control of EHV 1 in pregnant mares. Incidences of Japanese encephalitis piroplasmosis, trypanosomosis; as well as outbreak of glanders were reported in Himachal Pradesh and Uttar Pradesh. The disease could be effectively controlled with technical inputs from the NRCE and efforts of State Animal Husbandry Departments of both the states.

Success story

Detection of *Theileria equi* antibodies

Recombinant equine merozoite surface antigen-2 (rEMA-2), a 52 kDa recombinant protein based ELISA (r-ELISA) was developed for detection of specific antibodies for diagnosis of *T. equi* infection in equine serum. This kit was validated vis-à-vis OIE approved CI ELISA on 60 serum samples of known disease status (33 known positive and 27 known negative). Diagnostic specificity and sensitivity of these two assays were compared and a very high correlation was observed. The accuracy of the results obtained by r-ELISA was also compared with Western blot analysis on selected number of serum samples which confirmed the results.

Veterinary type culture: The Veterinary type culture centre (VTCC) strengthened its activities for collection of samples from different livestock species across different geographical regions, viz. microbial isolates from different institutes/network units along with their characterization and preservation. The repository has accessioned 358 veterinary microbes including 255 bacterial and 103 viral cultures along with 169 recombinant clones which include isolates like Japanese encephalitis, equine herpes-1, camel pox etc.

Poultry

Breeder chickens (3,828) were tested for avian leucosis virus (ALV); positive were culled to prevent vertical transmission. Thermostable ND vaccine maintained its titre for 14 days without loss at 37°C. In Vanaraja and Gramapriya the thermostable ND vaccine yielded similar immune response to that of commercial vaccine.

Animal disease monitoring and surveillance

Project Directorate on Animal Disease Monitoring and Surveillance (PD-ADMAS) is catering to the needs of surveillance and monitoring of livestock diseases in the country. Analysis of data revealed that FMD, HS, and babesiosis are the top viral, bacterial and parasitic diseases, respectively, reported from the country. The ecopathozones for PPR (peste des petits ruminants), CSF (classical swine fever), BQ (black quarter) and HS (haemorrhagic septicemia) were prepared. A logistic regression analysis for 15 economically important livestock diseases was carried out using NADRES model. Forecast maps for different diseases were prepared for the 12 calendar months.

In temporal epidemiology, the major livestock diseases were studied at zonal level based on 10-year (2001–10) data available in the databank. All the six zones (north, south, east, west, central and north-east) reported HS over the period whereas for BQ and anthrax there are relatively less reports. In the south zone the diseases were consistently recorded, whereas in north-east zone there is low or negligible reporting of the disease. The disease was recorded more in cattle than in buffaloes. The south zone showed a consistent disease

incidence and the other zones showed nil to negligible incidences. Spread sheet modules for economic impact analyses for different diseases were prepared to estimate the 'direct costs' (output loss/resource wastage, treatment and prevention costs) of each disease. Economics on reproductive disorders in bovines of organized farms vis-à-vis nutritional status showed decreased zinc traces in animals with reproductive problems.

Under the outreach programme on zoonotic diseases, epidemiology of brucellosis, leptospirosis and listeriosis in addition to their zoonotic relevance were studied. The burden of these agents in livestock and their products is documented. Zoonotic potentials of brucellosis, leptospirosis and listeriosis in samples collected from risk group (veterinarians, para veterinarians, farmers/workers associated with the animals) and persons showing clinical signs were assessed. The standardization of immunoassay using rLigB/IgG like protein of *Leptospira* spp. is underway which would differentiate pathogenic and non-pathogenic leptospiral infections in cattle herd.

Extensive and in-depth study on epidemiology of mastitis was undertaken with special reference to local and global epidemiology of pathogens and their factors associated in causation of mastitis (under NAIP lead project). The molecular epidemiology of predominant etiological agent *Staphylococcus* revealed that none of the *S.aureus* isolates belonged to ST 398, an important factor in zoonosis. Multiplex PCR was standardized for detection of 14 mastitis pathogens.

In sero-epidemiology of infectious bovine rhinotracheitis (IBR), analysis of serum samples (57,009) from different parts of the country during 1995-2010 revealed that 36% were positive. Molecular epidemiology of BoHV-1 infection, revealed that all the Indian strains used in the study, belonged to subtype 1.1 irrespective of animal species and clinical manifestations. The phylogenetic analysis indicated that BoHV1.1 is the subtype prevalent in India.

Fish

Herbs to inhibit pathogenic fungi: Aqueous extract of dry leaves of marigold, pine, *kalmegh*, *kali sarson* and lemon grass were used to study the impact of these on growth of pathogenic fungi *Saprolegnia parasitica* and *Saprolegnia diclina*, which affect cold-water fishes mahseer and trout. Intensity of the infection in mahseer during downfall of the water temperature was more prominent. *Kali sarson* and lemon grass effectively inhibited the growth of *Saprolegnia*.

Immunoperoxidase-based diagnostics for *Macrobrachium rosenbergii* nodavirus: An immunoperoxidase test was standardized to detect *Macrobrachium rosenbergii* nodavirus (MrNV) in virus infected larval stages of prawn. Recombinant capsid protein hyperimmune serum raised in rabbit was used. This test could be of use in screening large number of samples in a standard histopathology laboratory and also for virus pathogenicity study. ■