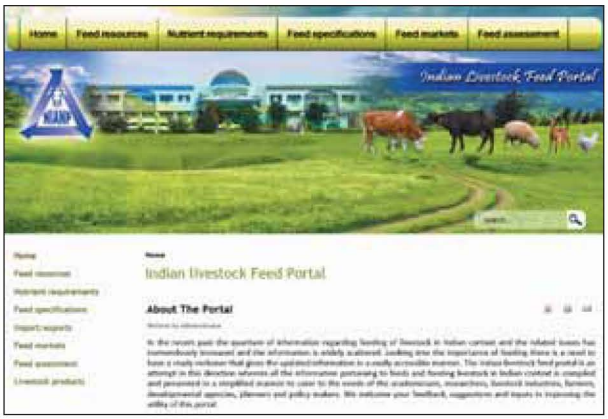
9. Livestock Management

Effective livestock management, requires proper feeding rations, breeding practices, adequate shelter, sound health etc. Novel advanced technological innovations are required for rearing livestock economically with increased productivity and profitability.

Animal nutrition

Livestock feed resources: 'Indian Livestock Feed Portal' with recent information pertaining to various feed resources, feeding standards for livestock and other related issues was made available online for various stakeholders. This portal will play a vital role in refining management of feed resources and planning livestock development schemes.



Indian Livestock Feed Portal

Enteric methane production and mitigation: Methane production potential (MPP, ml/100 mg DDM) of several feed samples was established and categorized based on their MPP. Lowest MPP was recorded in tree leaves (1.34) followed by cereal grains (2.44), de-oiled cakes (2.47) and cultivated fodders (2.83). Maximum MPP was recorded in cereal by-products (5.92) and straws (6.01). A Significant correlation between fibre fraction (NDF and ADF) and IVDMD with MPP of the samples was observed, which was used to develop a prediction equation to calculate MPP based on nutrient composition. Based on the MPP value, the livestock census and feeding practices, district-wise methane production data were generated for Karnataka.

Tamarind seed husk reduced methane production by more than 70%. Cashew nut seed coat oil showed anti-methanogenic property (45%). With a high tannin bioactivity value leaves of *Azardirachta indica* (48%) and *Ficus benghalensis* (70%) were good methane suppressants.

Goat

Methane emission and mitigation strategies: Methane emission from goats under different feeding systems was studied to develop suitable mitigation strategies.

In goats: (*i*) mustard cake inclusion reduced methane production by 21.09%, (*ii*) concentrate pellet containing *guar korma* and urea produced 4.83% less methane in comparison to linseed cake pellets, (*iii*) concentrate mixture replacing 50% conventional protein source with mustard cake reduced methane production as glucosinolate content of mustard-cake has antimicrobial properties for several microorganisms.

Quality of feed especially the protein content is responsible for the level of methane production. Methane gas production significantly got reduced with increasing proportion of azolla in goat feed.

Buffalo

Inhibition of methanogenesis: Feeding of EO-1 (0.02 ml/kg body weight) essential oil extracted from *Trachyspermum ammi* seeds increased body weight gain by 6% (587 vs 555 g/d), reduced methane emission by 16% (174.2 vs 145.1 L/d) and improved feed conversion efficiency by 7% (7.04 vs 6.60 unit feed/unit gain) as compared to control animals. Supplementation of EO-1 positively influenced rumen fibrolytic bacteria, viz. *Ruminococcus flavefaciens* (1.95 times), *Ruminococcus albus* (3.3 times), *Fibrobacter succinogenes* (3.25 times), *Butyrivibrio fibriosolvens* (5.45 times) and *Prevotella ruminicola* (1.1 times). Better feed conversion efficiency in animals fed essential oils was attributed to increased number of fibre-degrading microbes in the rumen.

Precision feeding: Buffalo starter containing 18% crude protein (CP) and 72% total digestible nutrients (TDN) along with limiting the amino acids lysine and methionine, and vitamin A and D produced better growth as compared to high CP concentrate based diet.

A feeding module was developed for 18–24 months old replacement buffalo heifers. The feeding module comprising concentrate mixture (18.3% CP) and wheat straw @ 1.24 kg and 0.89 kg/100 kg body weight/day, respectively, achieved 747.8 g/day growth rate with saving of 20% wheat straw (635 g/day) as compared to control.

Sheep

Rumen bypass fat (RBF) for maximising body weights: Weaner Malpura lambs were fed on *ad lib.* concentrate and roughage (lobia hay) from 3 to 6 month of age. Incorporation of RBF in ration (4%) of growing lambs enhanced body weight by 12% at 6 month (32.5 vs 29.0 kg) and increased feed conversion ratio (FCR) by 13.56% (4.94 vs 5.61 kg).

Camel

In vitro evaluation of crop residues and grass samples with camel rumen liquor indicated that among crop residues the gas production was lower from wheat straw (20.67 ml/0.2 g) and maximum from *guar phalgati* (30.00 ml) whereas, among grasses lowest gas production was from sewan grass (19 ml/0.2 g) and maximum from *dhaman* grass (28.67 ml/0.2 g).

Pigs

Feed for economic swine production: Bakery waste, water hyacinth and tapioca meal were used to substitute maize in pig feed formulation up to 25%, 5% and 15%, respectively, without affecting the growth, nutrient utilization, FCR and economy of feeding.

Prebiotic production/evaluation: Prebiotic was produced successfully from cotton stalks. Treatment of cotton stalks with sodium hydroxide (4%) yielded 14.4% and 12.7% xylan under steam application and overnight incubation, respectively. Almost complete recovery of xylan was possible with potassium hydroxide (8%) treatment. Inulin is a potential alternative to antibiotics in pig as its supplementation ensured better gut health by promoting the growth of beneficial gut microflora.

Yak

Locally available tree fodder generally fed to yak were analyzed and highest protein content was recorded in *Phrengpa* (*Quercus walliasehiana*) tree containing 20% protein while *Baggar* (*Berberis* sp.) tree leaves possessed highest cellulose content. Feeding maize silage improved body weight gain in growing yaks and milk yield (940 vs. 360 ml) in lactating yaks. Silage or complete feed blocks (CFB) feeding has beneficial effects over free grazing in lactating and growing yaks during winter feed crisis.

Poultry

Processed *karanj* cake: Solvent extracted *karanj* cake (SKC) could be used up to 9% replacing soybean meal without adversely affecting egg production in laying birds of 50 to 61 weeks of age. Dimethyl carbonate treated SKC could also be used up to 3% in broiler chicken diet without any adverse effect. However, SKC beyond 3% level in diet was detrimental for the growth of Krishibro and Srinidhi chickens. The diet with ME of 2,600 kcal/kg along with 16% CP was found optimum for harvesting the maximum production from PD-3 layers.

Feed quality and safety: Fungal isolates *Aspergillus awamori* (NICM 885) and two species of *Aspergillus foetidus* (including MTCC 11682) were isolated from soil and used for bulk production of phytase. Phytase activity of 80–100 ftu/ml in production media was obtained on 6–8 days post incubation and enzyme was stable over a wide range of temperature (30–70°C) and pH (3.5–6.5). Immobilized crude phytase was efficient in replacing 0.12% non-phytin phosphorus in broiler chicken diet.

A farmer with his backyard poultry unit

Alternate feed resources: Azolla meal up to 7.5% in the diets of growing meat type and laying Japanese quails reduced the feed cost. Similarly, use of decorticated cottonseed meal, up to 15%, containing 44% protein, 2.9% fat and 10.57% fibre on DM basis was beneficial for economic egg production in laying hens without affecting the production performance, nutrient utilization and egg quality traits. Addition of commercial protease in diets was beneficial for improving feed and protein utilization efficiency in broiler chickens.

Management of mycotoxicosis: Aflatoxin production in mixed feed during storage was inhibited completely by adding propionic acid @ 0.25%, benzoic acid @ 0.30% or tartaric acid @ 0.40% at 13% moisture level. However, at 15% moisture level propionic or benzoic acid @ 0.5%, or tartaric acid above 0.5%

Success story

Specialized integrated farming system for landless and marginal farmers

Forty-two villages of Barabanki and Raebareli districts of Uttar Pradesh constituted nearly 67% of rural population of these two districts. A specialized integrated farming system (SIFS) was developed which included opening of small dairy with low initial cost, rural poultry production integrating *in situ* azolla production as an alternate source of protein for feeding, mass infertility control programme, prevention and therapeutic strategy for control of mastitis, and plant bio-growth enhancer for increasing production and improvement in soil health. Under the various interventions 5,940 farmers were covered directly and a large number of farmers adopted the technologies horizontally. Average annual income was ₹ 14,750 for landless and ₹ 26,500 for marginal farmers. The survey showed that average annual income of landless farmers increased to ₹ 88,560 and of marginal farmers to ₹ 1.8 lakh/acre land holding. In pre-intervention period, farmers spent 71.4% of their income on food and clothing, 1.2% on education, 5% on health and 22.4% on household expenses, and borrowing was a regular feature. After the intervention the expenditure on food and clothing, education, health and household expenses was 23.2%, 10%, 12.8% and 21.7%, respectively. Families were able to spend 32.3% of their income on infrastructures like house, farm machinery etc., which was not possible earlier. Drop out school children due to financial constraints were re-admitted.

was required for complete inhibition of aflatoxin production. Similarly, addition of 40 ppm Zn (zinc) or 0.1% methionine in diet (above NRC recommendation) to the aflatoxin contaminated diet (250 ppb) ameliorated the ill effects of aflatoxicosis on production performance, gut health and immunity of the broilers.

Mineral supplementation: Supplementing the organic form of Zn, Se and Cr at 20, 0.3 and 1 or 40, 0.15 and 2 mg/kg, respectively, was required to harvest the optimum performance and improved anti-oxidant status in commercial broiler chickens. Supplemental vitamin E (α-tocopherol acetate, 200 mg/kg) elicited optimum retention in meat (42 mg/kg).

Improving reproductive performance: Supplementation of chromium (Cr as chromium picolinate) @500–750 µg/kg and 750 µg/kg level in diet of adult male turkeys improved semen biochemical and physical characteristics respectively.

Rumen

Microbiome of cattle and buffalo rumen: Metagenomic analysis of rumen microbiome indicated that cattle and buffalo with respect to total bacteria, fungi and methanogens were similar in their rumen microbial community structure and ability to degrade fibre. Also there was no difference in three major classes of carbohydrate active enzymes, glycoside hydrolases (GHs), carbohydrate esterases (CEs) and cellulose binding modules indicated no difference in the ability of fibre degradation in the two animals.

Effect of multi-nutrient blocks on milk yield of ruminants

Multi-nutrient blocks (MNB) were offered to the bovines @ 250 g/head/day and multi nutrient mixture (MNM) to goats @ 100 g/head/day in 12 selected villages of Nagaur district under NRAA project. The average daily milk yield of cows and buffaloes increased to the tune of 7.70 and 5.80%, respectively, with supplementation of MNBs. The B: C ratio of the intervention was higher (4.48) in buffaloes than cows. The daily milk yield of goats ranged from 1.4 to 1.8 litre, and average improvement in daily milk yield was 14%.

Performance of Tharparkar cattle fed on thornless cactus

A feeding trial of thornless cactus (*Opuntia ficus indica*) @ 2.5 kg/calf/day along with *ad lib* offering of *Cenchrus ciliaris* hay and 1.05 kg concentrate, decreased dry matter intake by 5.4 g/kg $W^{0.75}$/day compared to calves maintained with *Cenchrus ciliaris* hay plus concentrate (98.97 g/kg$W^{0.75}$/day) at Jodhpur, Rajasthan. Feeding cactus reduced daily water intake by 74.45 ml/kg$W^{0.75}$/day compared to animals maintained with hay plus concentrate only (488.09 ml/kg$W^{0.75}$/day). Feeding of cactus increased total digestible nutrients from 55.95 to 70.43%.

Rumen biotechnology: To improve digestibility of fibrous feeds, genes encoding feruloyl esterase (FAE), endoglucanase and exoglucanase were cloned in *Butyrivibrio fibrisolvens* and yeast. Recombinant FAE enzyme, recombinant microbes (*B. fibrisolvens* encoding FAE and yeast encoding FAE and exoglucanase) as well as the mixed culture of the microbes improved *in vitro* digestibility (5–10%) of finger millet/wheat/paddy straws. Fibre digestibility (17% NDF and 24% ADF) also increased in crossbred cattle fed FAE enzyme.

Nutritive value of tree leaves, shrub and bush forages

Six uncommon and less commonly used trees leaves, shrubs and bush forages of Himachal Pradesh were evaluated for feeding goats. Based on the nutritive values of the leaves, these forages could be ranked as follows: *Salix alba, Salix tetrasperma, Litsea monopetala, Rubus ellipticus, Pyrus pashia* and *Pinus sylvestris. In vivo* validation studies showed that *S. tetrasperma* mature tree leaves could replace 50% of the local grass (brachiaria and setaria) for feeding to growing Gaddi goats without any adverse effects during winter when there is fodder scarcity in the region.

Salix tetrasperma mature tree leaves could replace 50% of the local grass for feeding to growing Gaddi goats without any adverse effects

Inclusion of *Cordia dichotoma* leaves at graded levels (5–20% of the substrate DM) with wheat straw improved true degradability of dry matter and organic matter of composite diet and reduced methane production up to 13.86% under *in vitro* system. Supplementation of *Cordia dichotoma* leaves @ 5% in DM, increased body weight gain (17%) in lactating buffaloes although milk yield and composition remained unchanged.

Animal physiology

Expression profile of *HSP70* gene in sheep: Heat shock proteins are the chaperone proteins, which help in the proper folding and protection of the stress exposed

proteins. Ewes were exposed to heat stress from 38°C 42°C to 44°C and reduced slowly to 40°C. *HSP 70* gene expression in ewes increased with increase in temperature and decreasing thereafter. It is thus concluded that *HSP 70* is very sensitive and quickly responds to temperature changes.

Cattle

Semen: During the year 245,900 semen doses were frozen from Frieswal bulls, and 70,733 doses were distributed to Military Farms while 25,680 semen doses were sold to para vets, SAUs, State Animal Husbandry departments and NGOs for improvement of field animals. In addition, semen doses were frozen for Ongole (184,375), Kankrej (54,640), Gir (14,651) and Sahiwal (23,895), under the Indigenous Breed Project. Frieswal bulls having less scrotal skin fold thickness (<4.5 mm) showed significantly higher sperm concentration/ml, initial progressive sperm motility per cent and total sperm yield per ejaculate as compared to bulls having thicker scrotal skin fold (≥ 4.5 mm). There were no significant differences in luteinizing hormone (LH), testosterone and estradiol levels, between good and poor semen producer bulls. However, the duration of onset in attainment of peak after gonadotropin release hormone (GnRH) challenge was delayed significantly in poor semen producer bulls.

Buffalo

Regulation of ovarian function: Presence of ghrelin and its receptor was documented in the buffalo ovarian follicles and corpus luteum. Ghrelin inhibited estradiol and progesterone secretion from the ovarian follicles and corpus luteum respectively.

Enhancing buffalo blastocyst production *in vitro*: Buffalo oocytes were matured, fertilized and presumptive zygotes were cultured in modified synthetic oviductal fluid (mSOF) in 50 and 100% WJ-MSC-CM while keeping basal media of stem cell culture as control (DMEM+SR). The cleavage rate did not differ significantly but blastocyst rate improved significantly and total cell number per blastocyst also significantly increased in CM supplemented groups.

Reproduction in ruminants: For screening sub-fertile buffalo bulls, four putative fertility/motility associated proteins, viz. CatSper3, TIMP-2, BSP-5 and PLA2 were characterized for the first time in buffalo semen. CatSper3, TIMP-2 and BSP-5 have the potential to serve as motility/fertility markers of buffalo semen.

Development of early pregnancy detection kit: One of the IFN-τ stimulated genes (ISG) in peripheral blood leukocytes and its serum protein levels indicated distinguishing pattern during early pregnancy. This may open new avenues for development of a cow side test for early pregnancy diagnosis in buffaloes.

Goat

Parthenogenetic goat embryos: Parthenogenesis, a form of reproduction in which the ovum develops into a new individual without fertilization, could lead to the production of superior livestock without the need for male counterpart. Parthenogenetic embryos were produced and the *in vivo* development was observed up to 23 and 28 day in two recipients.

Exploring growth hormone and fecundity gene: Fragments 245 bp and 472 bp of growth hormone gene were amplified. Alleles A, B and C were identified. The 245 bp fragment genotypes had significant effect on birth weight. Animals having AC genotype had 47% more weight than the animal having CC genotype. Genotypes of 472 bp fragment had significant effect on body weight at both 6-month and 9-month of age in Black Bengal goat. Animals having AC genotype had 65% more weight than the animal having CC genotype at both 6- and 9-month of age.

Fecundity genes like *BMP15* and *GDF9* were found monomorphic in Black Bengal goat whereas *BMPR1B* gene was found polymorphic. *BMPR1B* locus in Black Bengal goats revealed GG and GA genotypes and consequently two allelic variants A (wild) and G (mutant). Animals having homozygous mutant genotype, i.e., GG gave 2.1 kids/kidding whereas carrier animal, i.e. heterozygous animal (GA) gave 1.6 kids. Twinning percentage in homozygous mutant (67.66%) was much higher than the heterozygous carrier (54.75%) animals.

Success story

Vermiculture technology:
For recycling of animal and farm waste

Livestock and crop production activity in India generate about 300 crore tonnes of solid bio-waste. The safe disposal of agro-waste in short period is the major concern at national and international levels. Traditional methods of recycling of animal and farm waste take 6 months duration and yield inferior quality organic manure. The exotic earthworm species, viz. *Eisenea foetida* and *Eudrilus eugeniae* used for vermicomposting generally, do not survive at high temperature and humidity, have low fecundity and poor vermicast quality. An indigenous earthworm, viz. *Perionyx ceylensis* species that has the ability to (*i*) thrive on cow dung and crop residues, (*ii*) adapt under variable temperature (0°–44°C), (*iii*) multiply beneficial soil and fermentation microorganisms, and (*iv*) is highly prolific. The vermiculture of this species was very rich in microorganisms beneficial for soil and fermentation. This earthworm species has been given name as "Jai Gopal" and technology as "Jai Gopal Vermiculture Technology".

The vermiculture technology was commercialized and transferred to 16 entrepreneurs/universities/institutes/NGOs/KVKs/Government agencies spread across the country. Farmers were benefited by this technology in Uttar Pradesh, Uttrakhand, Bihar, Madhya Pradesh, Haryana, Gujarat, Rajasthan, Himachal Pradesh and Asom. This technology besides being low cost is also pro-poor, pro-women and pro-nature and beneficial to agriculture and industry.

Poultry

Designed siRNA: Modulation of prolactin (PRL) levels by partial knockdown of prolactin gene using siRNA technology was attempted to augment egg production. Designed siRNA suppressed PRL and PRL-mRNA expression specifically in the transfected cells derived and cultured from chicken anterior pituitary gland.

LIVESTOCK PROTECTION

Sheep

Breeding sheep for resistance/resilience to gastrointestinal nematodes: Two divergent lines (resistant or susceptible to *Haemonchus contortus*) of sheep were developed through selection using faecal egg count as phenotypic marker. In spite of no anthelmintic treatment in resistant line on majority of occasions, the monthly mean FECs remained significantly lower compared to susceptible line where anthelmintic treatment was given. On nucleotide sequencing of Ovar-DRB-1 coding region, several unique SNPs were identified in R and S line individuals. Most of the SNPs in peptide binding regions (PBRs) are non-synonymous in nature.

Camel

Prevalence of trypanosomes: Examination of blood samples collected from different places of Rajasthan, revealed that camels were positive for *Trypanosome* infection. PCR amplification of all these blood samples revealed trypanosome specific distinct 500 bp band. Besides, VSG genes partial amplification of 205 and 448 kb were observed, which is used for the accurate diagnosis of cameline surra. Kinetoplast DNA sequence was also exploited as "signature sequence" for the diagnosis of *T. evansi* infection.

Equines

Sources of stem cells: Tendon injuries are common in race horses and mesenchymal stem cells (MSCs) isolated from adult and fetal tissues were used for tendon regeneration. Evaluation of equine amniotic fluid (AF) and umbilical cord blood (UCB) as alternate sources of MSCs in horses revealed that these cells expressed mesenchymal surface markers as detected by RT-PCR and immune-cytochemistry, but did not express haematopoietic markers. AF and UCB were easily accessible and effective sources of MSCs.

Glanders: Glanders is a notifiable disease. Four cases of glanders were observed in Himachal Pradesh, while a single case of disease was detected in Chhattisgarh. All of the infected equines were tested serologically positive by CFT (titer 8–64) and in-house immune-assays. The causative agent *Burkholderia mallei* was isolated from two infected mules in Himachal Pradesh.

Traditional herbs

ITK known herbs, viz. fruits of *kantkari* (*Solanum xanthocarpum*) and *vidangh* (*Embelia jerium cottam*), and bark of *siris* (*Albezia lebbek*) and *harshringar* (*Nyctanthes arbortristis*) were screened for their anthelmintic activity. Out of various combination of extracts tested, chloroform-methanol extract of the fruit extract of *Embelia jerium cottam* was most effective in killing up to 82.6% of L3 larvae of *Haemonchus contortus*.

Pig

Porcine circovirus and porcine parvovirus: Considering the negative impact of porcine circovirus (PCV) type 2 infection and porcine parvo virus (PPV) infections on pig production, the intensively managed pigs were screened in Asom and Mizoram for the presence of antibodies against PCV and PPV. Out of 186 animals examined, 23 (12.36%) were positive for PCV and 25 (13.44%) for PPV. Prevalence of antibodies against PCV in piglets (up to 2 months), growers (3–6 months) and adults (above 6 months) were 4.30, 3.76 and 4.30%, respectively, whereas the corresponding values for PPV were 4.83, 5.91 and 2.68%, respectively. Antibodies against both these viruses were also present in 11 (5.91%) animals.

Poultry

ALV typing: ALV strains (24) were isolated and identified in chicken; three isolates were ALV-A, seven were ALV-B and remaining were mixture of ALV-A, B and C. The envelop gene of one ALV-A isolate (DPRE32) was sequenced and compared with reference strains. The isolate was closely related to ALV-A reference strain. TVB receptor status of Aseel (from field) and Red Jungle fowl (RJF) was analyzed by PCR-RFLP. In Aseel, two alleles and three genotypes were found while in Red Jungle fowl only one allele and one genotype were found. Red Jungle fowl was susceptible to ALV subgroups B, D and E. In Aseel, genotypes S1/S1 and S1/S3 were susceptible to ALV subgroups B, D and E, while genotype S3/S3 was susceptible to ALV-B and ALV-D and resistant to ALV-E.

Epidemiology and diseases informatics

Twelve samples tested positive for H5N1 avian influenza virus. Ten H9N2 viruses were confirmed by real time RT-PCR, and virus were isolated from Chhattisgarh (1), Odisha (5) and Rajasthan (4). Out of RNA (57) and tracheal samples (88), received from Nepal, 43 RNA samples were positive for H5N1 and 3 RNA were positive for AIV. Although 84 tracheal samples tested positive by real time RT-PCR yet H5N1 virus could be isolated from only 29 samples.

BVDV-3 infection in dairy cattle, clinical malignant catarrhal fever (MCF) in a buffalo from Tamil Nadu, and PRRSV in pigs in Mizoram were reported for the first time in India.

Extensive study was carried out on economically important livestock diseases, viz. *Peste des petits ruminant* (PPR), brucellosis, infectious bovine

rhinotracheitis (IBR), bluetongue (BT), trypanosomiosis, fascioliosis, sheep- and goat-pox, haemorrhagic septicemia (HS), rabies, mastitis, avian influenza, foot and mouth disease (FMD), classical swine fever (CSF), porcine respiratory and reproductive syndrome (PRRS), malignant catarrhal fever (MCF) etc.

The in-built interactive, dynamic web based software NADRES (National Animal Disease Referral Expert System) is having both animal health information system (AHIS) and weather based animal disease forecast (WB_ADF).Thus the software provides both disease forecasting services and animal health information to professionals engaged in animal health sector.

The retrospective analysis showed that FMD and sheep and goat pox remained top viral diseases, HS and black quarter (BQ) remained top bacterial diseases, and fascioliosis and babesiosis were top parasitic diseases. Methodology was developed for assaying the risk of introduction of notifiable avian influenza (NAI: HPNA1 and LPNA1) in India. A retrospective epidemiological study of HPAI revealed that the majority of outbreaks were reported in West Bengal (55) followed by Asom (18). In a cross sectional surveillance study of MCF, 24.44% samples were positive for MCF infection among sheep (356) in Karnataka where Raichur district showed highest positivity. Among 6,327 bovine serum samples, 52% were positive for antibodies against IBRV in ELISA. In a retrospective study, Chhattisgarh showed highest seroprevalence (66%) and overall 36.52% seroprevalence in the country.

Forecasting of PPR incidence in Andhra Pradesh, Karnataka, Kerala and Maharashtra with the help of SAS NL MIX procedure was done. Besides, the methodology for deterministic model as enhanced solution for effective vaccination was developed. The retrospective study, revealed that 649 outbreaks were reported during 2003–13 in Karnartaka. The economic loss analysis depicted that in India an estimated annual loss was ₹ 818.65 crore whereas the same in Karnataka was ₹ 60.13 crore. An overall 11.63% prevalence was recorded among goats in NE region indicating that the area is endemic for PPR. A PPR clinical score card for the assessing clinical disease pattern was developed, which will be very useful for field investigation of outbreak among sheep and goats in vaccinated and unvaccinated areas. Analysis of disease data on BT during 2001–14, revealed that the highest incidence was recorded during 2005–06 and most of the outbreaks occurred from September to December. Spatial distribution of BT outbreak in Andhra Pradesh (AP) was mapped and as many as 7 serotypes were reported from AP.

An overall 42.8% pig sample (collected from Madhya Pradesh, Andhra Pradesh, Kerala, Asom, Manipur, Meghalaya and Karnataka) were found positive for antibodies against CSFV by ELISA. The overall seroprevalance of 35.4% was recorded. Besides, 29.9% samples were recorded for the presence of antibodies against PRRS virus infection among 352 pig serum samples collected from Asom, Arunachal Pradesh, Meghalaya, Manipur, Nagaland, Maharashtra and Karnataka. In Karnataka, during 2013–14, FMD outbreaks were recorded in all districts except Gulbarga and Yadgir districts, a total of 5,514 villages of 29 districts were found affected; the highest number of bovine were affected in Kolar district (9,817) and lowest in Bijapur district (43). HS occurred mostly during July, August and September but FMD occurred throughout the year.

Real time-PCR assay and multilocus sequence typing of *Brucella* was done. Buffalo showed highest positivity for brucellosis followed by HF crossbred. A battery of recombinant antigens targeting *BLS, bp26, sodc* gene of *Brucella abortus* was developed. Besides, 4.14% serum samples were found positive among 9,195 animals (cattle, buffalo, sheep, goat and pigs) in ELISA. Pooled milk samples (64,818) from the Karnataka were subjected to MRT and 2.55% positivity was observed. A protein G based ELISA kit for detection of brucellosis was found with 88.79% sensitivity and 97.74% specificity. A lateral flow assay for detection of brucellosis was also developed and a good agreement was found when compared with RBPT and ELISA. A recombinant antigen LigB protein (*Leptospira borgpetersonii* Hardzo serovar) based ELISA was developed. This antigen showed 88.24% sensitivity and 95.4% specificity in ELISA when compared with MAT. Seroprevalence of leptospirosis was studied in different coastal regions of India including east, south and west, and 24.96% was found positive among 1,309 samples. The highest prevalence was recorded in West Bengal (80.9%).

During the reporting period, serum samples comprising cattle (5,498), buffaloes (1,824), goat (3,263), pig (520), sheep (1,443) were received, catalogued and arranged by serum bank. Screening of these serum samples revealed presence of antibodies against different pathogens and found positive for *Brucella abortus* 3.44%; *B. suis* 7.9%; *B. melitensis* 11.73% in sheep and 4.46% in goats; BoHV1 47.59%; CSFV 41.89%.

Diagnostics and vaccines

- Molecular beacon probes and primers for diagnostic real time RT-qPCR for exotic/emerging viral diseases were designed and uniform reaction conditions were optimized for SYBR Green based real-time PCR for 22 viral infections.
- A new real-time PCR assay for PCV-2 detection was developed. From dead neonatal piglets PCV-2, porcine enterovirus serotype-9 and porcine teschovirus were detected by molecular methods.
- Developed loop mediated isothermal amplification (LAMP) for detection of *Babesia gibsoni* infection in dogs targeting hyper variable region of the 18S rRNA gene.
- Recombinant BgSA-1 protein was successfully

used to standardize indirect ELISA, dot-ELISA and sandwich-ELISA for diagnosing *B.gibsoni* infection in dogs.

- SPR-based biosensor assay was developed for detecting serum cancer biomarkers associated with canine mammary cancer.
- A deletion mutant of *Brucella abortus* S19 was developed, which is less virulent than S19 vaccine strain, and possesses probable DIVA capability.

Molecular characterization of pathogens

- The HA gene phylogeny of H5N1 avian influenza viruses isolated during 2011–2013 from India, Bhutan and Nepal revealed that they belonged to a new subgroup A within clade 2.3.2.1
- On the basis of phylogenetic analysis in the entire N^{pro} gene, the border disease virus (BDV) isolate originating from migratory sheep in Madhya Pradesh was typed as BDV-3 and was found closely related to the BDV-3 strain in China.
- Molecular characterization showed that PRRSV isolate from Mizoram belonged to genotype 2 with high sequence homology to Chinese highly pathogenic PRRSV with 30 amino acid deletion in NSP 2.
- Whole genome sequencing of three classical swine fever vaccine strains was done; one strain belonged to genotype 2.2 and the others to genotype 1.1.
- Complete genome sequence of *peste des petits ruminants* virus (PPRV) Sungri/96 vaccine strain was determined. The phylogenetic analysis revealed 89–99% identity with the PPRV genome sequence available in NCBI database indicating lineage IV of PPRV.
- Complete genome sequence of F vaccine strain of Newcastle disease virus (NDV) was determined (Accession # KF727980). Phylogenetic analysis of F, HN and complete genome sequences grouped F strain in genotype II category, which are considered as early genotypes and corroborated with its year of isolation.

Herbal medicine

The fraction-2 of ORP-EVM-8 significantly affected the mitigation of super oxide radicals (LPO, SOD and catalase) and was positively correlated with its anti-urolithiatic activity. Chemical characterization of the active principles revealed fraction 2 containing 4 putative compounds.

Stem cell research

An experimental study revealed that culture expanded bone marrow-derived allogenic mesenchymal stem cells (BM-MSCs) can augment the healing of osteochondral defects. Collagen and laminin gels were suitable scaffolds for BM-MSCs in the treatment of osteochondral defects. Addition of growth factors to BM-MSCs further enhanced the healing of osteochondral defects. Xenogenic canine bone marrow-derived MSCs and the conditioned media also enhanced healing of skin wounds in healthy and diabetic rats. Two clinical cases of spinal cord injury showed complete recovery after treatment with canine bone marrow derived mesenchymal stem cells.

Disease monitoring and surveillance in wildlife

Wildlife disease investigations revealed important conditions namely rabies in sambar deer and Himalayan black bear; aflatoxicosis in a thamin deer; tuberculosis in sloth bears and nilgai; carcinomas in leopard, lioness, sloth bear and Rozy pelican; organochlorine poisoning in giraffe, leopard; and hepatitis along with nephritis in Himalayan Griffon vultures. Sero-positivity was observed for leptospirosis in lions, tigers and elephants, and canine distemper in stray dogs around one tiger reserve.

Gene-deleted DIVA-enabled candidate marker vaccine for bovine brucellosis

Bovine brucellosis, one of the most important zoonotic diseases, is endemic in India and causes huge economic losses to dairy industry due to infertility, abortion, birth of weak offsprings and reduced productivity. A modified strain of *Brucella abortus* S19 was developed at IVRI through gene knock-out approach, which was named as *B. abortus* S19-IVRI-ΔP (S19ΔP). The newly developed S19ΔP strain was characterized and its protective efficacy evaluated in experimental mice model. The developed strain showed cultural, morphological and biochemical properties similar to the parent strain. S19ΔP is less virulent than S19. The strain also conferred high level of protection similar to parent strain in lab animals. On the other hand, the S19ΔP strain possesses DIVA capability and thereby capable of differentiating between infected animals from vaccinated ones. These properties provide superiority to S19ΔP strain over S19 strain. The brucellosis control programme would be facilitated if a safer vaccine with DIVA capability is made available. Once tested and validated in the target host (cattle/buffaloes), the modified S19ΔP strain would be a potential candidate to replace S19 strain for the control of brucellosis in India.

Foot and mouth disease

During the reporting period, 472 FMD outbreaks were recorded in the country and almost 50% of the outbreaks were recorded in Karnataka, Tamil Nadu, Kerala and Andhra Pradesh, where a four-fold increase in outbreak was observed. There was no incidence of the disease in Punjab and Delhi during the period, and few sporadic cases were recorded in Haryana and Himachal Pradesh. Incidence of FMD reduced in the western region compared to previous year possibly due to optimal vaccination coverage/infection immunity.

Success story

Rescue of FMD virus by transfection to improve the rate of diagnosis

RNA transfection using lipofectamine to rescue FMD virus from clinical materials was optimized and its potential application as an alternative to the conventional cell culture isolation was investigated. A significantly higher rate of virus regeneration from clinical materials regardless of their detection status in ELISA or multiplex PCR was achieved by this method (62%; 118 out of 190 samples) compared to the conventional cell culture isolation (16%; only 30 out of 190 samples). Comparison of the results of virus isolation by conventional cell culture vis-á-vis RNA transfection from the clinical samples stored at different temperatures and pH for up to five-week time revealed that the latter outperformed the former in all storage conditions suggesting stability of viral genomic RNA under inappropriate conditions that breakdown viral capsid. Transportation of clinical materials under stringent cold chain is not an easy task always and is practically difficult in case of remote locations in the country. Hence, the possibility of virus recovery by RNA transfection from clinical materials transported without application of cold chain is of immense significance for FMD surveillance and diagnosis programmes in a vast, subtropical country like India.

Serotype O caused maximum numbers of outbreaks (96.2%) and serotypes A and Asia 1 were restricted to only a few outbreaks/incidences. Compared to previous year, number of outbreaks caused by serotype O has greatly increased. Outbreaks due to serotype Asia 1 decreased five-fold compared to the last year and occurrence of serotype A decreased two-fold. Serotype Asia1 that caused many outbreaks in the Southern region during previous year could not be encountered during 2013–14. This year, serotype A was recorded only in the Eastern, North eastern and Western regions.

Phylogenetic analysis based on VP1 coding region was carried out to assess genetic variations, inter-strain relationships and track movement of the virus. During the year, phylogenetic analysis of serotype O virus showed that Ind2001 strains, which re-emerged in late part of the year 2008, nearly out-competed PanAsia lineage in causing outbreaks in the county. The sub-lineage Ind2001 was distributed widely covering all states. In case of serotype A, all the isolates clustered within the genotype 18 in the maximum likelihood tree, and grouped both in the clade 18c of the VP3^{59}-deletion lineage and non-deletion lineage. In case of serotype Asia1, the isolates clustered within the lineage C indicating its exclusive prevalence since 2005.

Vaccine matching exercise was carried out to evaluate antigenic relationship of field isolates with currently used vaccine strains to monitor antigenic variation, if any, occurring in the field, and assessing appropriateness of in-use vaccine strains. In serotype O, the vaccine strain O/INDR2/1975 antigenically covered 80% of the field isolates.

Emergence of antigenic variant in an endemic country is a normal phenomenon and the currently used vaccine strain O/INDR2/1975 provided near optimal antigenic coverage to the field isolates.

Some isolates were found divergent from the vaccine strain, which is not alarming at present. In serotype A, many of the isolates showed lesser match with the vaccine strain, A/IND40/2000. Considering the emerging antigenic diversity, a panel representing various lineages was selected.

National FMD Virus Repository was upgraded with latest/new virus isolates. Virus isolates (77: 32 type O, 19 type A and 26 Asia 1) were added to the repository during the reported period. At present the National FMD virus Repository holds 1,851 isolates (O-1180, A-298, C-15 and Asia 1-358).

Under National FMD Sero surveillance, 52,224 bovine serum samples collected at random from various parts of the country were tested in r3AB3 NSP-ELISA for assessing NSP-antibody (NSP-Ab) response, which is an indicator of FMD virus exposure/circulation regardless of vaccination status. The test revealed overall sero positivity in ~29.2% samples/animals, which is almost equal to the previous year.

During the reported period 174,076 pre-and post-vaccinated serum samples were tested under FMD Control Programme (FMDCP) and of which, 42,514 serum samples were from first phase FMDCP districts representing XIII, XIV, XV and XVI phases of vaccinations, and remaining 131,564 from Phases II, III, IV, V and VI.

Veterinary Type Culture Collection

The total collection at VTCC, Hisar up to the last year numbered to 1,630 microbes/clones. During the period under report (2013–14), 367 cultures/clones have been reposited in the VTCC after authentication, and conventional and molecular characterization including GC-FAME and 16S rRNA sequence analyses. These 367 cultures/clones include 73 bacterial isolates, 11 virus isolates,13 bacteriophages, and 59 clones of veterinary significance, 80 rumen microbes comprising anaerobic bacteria and fungi, and 93 dairy microbes. The centre is also maintaining 13 cell lines. More than 70 genera of bacteria are represented in VTCC repository, which include some of the novel taxa such as *Brevibacillus, Brevibacterium, Castellaniella, Comamonas, Delftia, Ignatchschineria, Nocardiopsis, Prolinoborus* and *Trabulsiella.*

The viral isolates include buffalopox virus, swinepox virus, PPR virus and bluetongue virus. The dairy cultures were categorised as exopolysaccharide (EPS) producing and bio-protective cultures (*Lactobacillus rhamnosus,* L. *plantarum, L. paracasei*) based on their functional characteristics. These cultures/clones are being supplied on request to various stakeholders including ICAR institutes and universities.

Fisheries

Marine capture fisheries

The estimate of all India annual marine fish landings for the year 2013 is 3.78 million tonnes as against the all-time high of 3.94 million tonnes during 2012, registering a decline of 1.56 lakh tonnes (4%). Gujarat contributed the highest at 7.17 lakh tonnes followed by Tamil Nadu (6.88 lakh tonnes) and Kerala (6.71 lakh tonnes). Pelagic resources dominated in the landings (56%) followed by demersal resources (26%), crustaceans (13%) and molluscs (5%). Contributions from mechanised were 30.0 lakh tonnes (79.5%), motorised 7.0 lakh tonnes (18.6%) and from non-mechanised crafts 0.73 lakh tonne (1.9%). In all 670 species landed along the Indian coast in 2013.

Satellite telemetry studies on tunas: Satellite telemetry studies on tunas were conducted through tagging of large sized yellowfin tunas. Preliminary information indicated that tunas have remained within the Indian EEZ. The number of dives made during 24 hr period, the temperature preference of the fish and the general track were identified.

Diagnosis of fish and shellfish viral diseases: Commonwealth Scientific and Industrial Research Organisation—Network of Aquaculture Centres in Asia Pacific (CSIRO-NACA) affirmed ICAR-CIBA for accurate diagnosis of seven fish and shellfish viruses of regional concern namely, yellowhead virus (YHV), taura syndrome virus (TSV), infectious myonecrosis virus (IMNV) for shrimp and viral nervous necrosis (VNN), spring viraemia of carp virus (SVCV), Koi herpes virus (KHV) and red seabream irodovirus (RSIV). Disease surveillance of shrimp farms revealed occurrence of WSSV and infectious hypodermal and hematopoietic necrosis (IHHN) disease.

Bacterial pathogen of koi carp characterized: Tail rot in koi carp, *Cyprinus carpio koi,* ascertained

Success story

A step towards sustainable fisheries

Short-neck clam (*Paphia malabarica*) fishery of Ashtamudi Lake has become the first eco-labelled fisheries of India. It is a milestone in Marine Stewardship Council (MSC) certification in India, and Ashtamudi short neck clam fishery is the third fishery in Asia to receive this recognition. This was achieved through the joint efforts of ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), World Wildlife Fund (WWF)-India, and Kerala State Fisheries Department, working with the local fishing community. MSC certification enhances conservation and sustainability of the resource and provides greater economic benefits.

Short-necked clam

Success story

Seabass rearing in low saline areas

In low saline areas of village Thennadar, Nagapattinam, Tamil Nadu, 15,000 seeds (1.0 cm size) of seabass were stocked in a pond of 0.3 ha, fed naturally on available zooplankton. They grew to about 5 to 7 cm in 45 days and at this stage about 8,000 fingerlings were sold for ₹ 160,000; while 3,000 were stocked in another 0.30 ha low saline to freshwater pond for pre-grow out culture. After 2

months of culture fish attained a size of 80 to 100 g. Of these 1,200 advanced fingerlings were stocked in a pond for grow out culture. Within 8 to 10 months about 700 kg fish was harvested as fish attained body weight of 1.2 to 3.8 kg (average 1.5 kg). Depending on its size seabass was sold for ₹ 300–350/kg generating revenue of ₹ 227,500 and profit of ₹ 105,000 on production cost of ₹ 122,500.

Tail rot in koi carp

three bacterial strains *Citrobacter freundii, Klebsiella pneumoniae* and *Proteus hauseri*. *P. hauseri* was the causative agent of disease outbreak.

Diseases encountered in coldwater fishes: Screening of rainbow trout farms spotted symptoms of lactococcosis, unilateral exophthalmia, inflammation and swollen vent, petechial haemorrhages in viscera, and focal haemorrhages and swelling in liver. Opacification and bacterial gill disease was spotted in golden mahseer. The causative agents were *Pseudomonas koreensis* (strain TPEB02) and *Chryseobacterium scophthalmum,* respectively.

Eye opacification in golden mahseer

Golden mahseer with bacterial gill disease

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