8. Livestock Management

Animal nutrition

Deconstruction of ligno-cellulosic biomass: Lignolytic enzymes (MnP manganese peroxidase, LiP lignin peroxidase and laccase) were produced from white rot fungi. PUF (polyurethane foam) cubes were the most effective biocatalyst for producing heat and pH stable enzymes with superior kinetic properties. *In vivo* and *in vitro* studies in sheep and cattle revealed that the enzyme treatment significantly improved the digestibility of the treated straws.

The laccase enzyme gene of *Schizophyllum commune* was cloned into *Pichia pastoris*, which was used to produce recombinant laccase enzyme. The bioactive recombinant enzyme could be produced extracellularly in the culture medium.

Canine-origin probiotic

A canine-origin probiotic (*Lactobacillus johnsonii* CPN23; cPRO) improved fibre digestibility, antioxidant level, cell mediated immunity and favourably altered the hind gut fermentation in dogs. It was superior to the dairy-origin probiotic. The cell-mediated immune response improved in the probiotic fed group. Evaluation of this probiotic in rats yielded similar results.

Cattle

Feeding of sugarcane mud up to 20% level in the concentrate mixture to growing calves as a feed ingredient had no adverse effect on growth, nutrient utilization, nutrient balance, rumen fermentation, microbial protein synthesis and blood biochemical profile of growing calves. Its inclusion in the feed, therefore, would further reduce feeding cost of both pre- and post- ruminant calves.

Supplementation of specific mineral mixture developed for temperate hills of Uttarakhand in oak leaves (*Quercus leucotricophora*) based diet (concentrate: roughage:: 40:60) improved nutrient utilization especially protein, fat and fibre in lactating crossbred cows. The milk yield and FCM yield in cattle improved by 8 and 10%, respectively.

Buffalo

In vitro rumen fermentation studies indicated that both sesame oil and mustard oil can reduce ruminal methane production; however, mustard oil exerted greater inhibitory effects on feed degradability and microbial biomass production. Hence, sesame oil proved better than mustard oil as methane inhibiting agent. Chelates of zinc, copper and manganese using different physio-chemical conditions were prepared to be tested for their *in vivo* effect on growing buffalo calves.

A feeding module on economic calf starter was developed using precision feeding approach. The precision feeding of calves improved growth rates and saved ₹ 10.40 on cost of feed/kg weight gain.

Sheep

Supplementing milk replacer: A milk replacer powder was developed (dried skim milk,350g; sesame cake,70g; groundnut cake, 80g; soy flour, 100g; wheat flour,100g; corn flour,100g; rice flour,100g; soybean oil,30g; linseed oil, 30g; mineral mixture, 20g; citric acid, 2g; butyric acid, 0.2ml; and Hyblend, 200mg). The cost of milk replacer powder and liquid milk comes to ₹ 115/kg and ₹ 19.50/litre. The milk replacer is fed to lambs @ 250ml/day from 15 to 90 days of age. Average daily gain in lambs was 170g and feed efficiency 2.13 in milk replacer supplemented lambs compared to 156g and 2.40 in lambs on conventional system. Cost of feed for 1 kg of body weight gain in milk replacer fed lambs was ₹ 51 compared to ₹ 68.10 under conventional system.

Deficiency biomarker: Copper chaperone for SOD (CCS) was evaluated as a sensitive biomarker of copper deficiency in sheep.

Goat

Methane production potential: Azolla *(Azolla microphylla)*, full fat mustard *(Brassica juncea)* seed, gram *straw (Cicer arietinum)* and concentrate mixture used for goat feeding were evaluated for methane production potential. The net gas production (ml/0.2g substrate) was the lowest in azolla (11.33 ml) and was the highest with concentrate mixture (44.0 ml), however concentration of methane in gas was almost similar among feed resources. The azolla produced lowest methane 1.52 ml/100 mg truly digestible substrate, followed by mustard seeds (2.29 ml), gram straw (4.33 ml) and concentrate mixture (4.89 ml).

***Moringa olifera* biomass based feed:** The biomass production of *Moringa* under cultivation is much higher than any other fodder crop. Complete feed was prepared using dry biomass of wild type *Moringa* tree (leaves and twig portion) and concentrate mixture for feeding of goats. The units of antioxidant property of plasma were higher and serum cholesterol level lower in moringa biomass based pelleted feed group than that in the goats fed traditional ration. The feeding of moringa based diet to goats, was cost effective and depended upon the production cost of moringa biomass.

Camel

Pelleted complete diet: Male camel calves fed on iso-caloric (65% TDN) complete feed pellet diets (50:50::R:C; 8.34% CP) showed better growth and nutrient utilization compared to normal feeding.

Rumen microbes in camel: From the C1 compartment fluid collected from camel, 4 microbes were isolated. Based on morphological, biochemical characteristics and on nucleotide homology and phylogeny, three were identified as *Bacillus subtilis* strain B11(1297 bp), *Bacillus subtilis* strain FS2 (1283 bp), *Bacillus subtilis* strain WZ3 (1299 bp), *Bacillus subtilis* strain FS2(1279 bp) and *Clostridium bifermentans* strain E051 (1323 bp).

Assessment of work efficiency in yak

Yak is used as pack animals by the highlanders. The work efficiency of yak was evaluated in different seasons through experimental study, besides recording of quantifiable parameters namely rectal temperature, pulse rate and respiration rate. During summer these parameters were the highest when animals carried load up to 20% of their live body weight. On the contrary during winter there was shift of quantifiable parameters when animals carried 34% of their live body weight. This could be concluded that during winter threshold level of yaks pertaining to carry load increase, which may be attributed towards fall of ambient temperature.

Mithun

Keeping-quality and shelf-life of the feed blocks containing different combinations of grass, tree leaves and fodders were studied up to 24 months. Comparatively a higher microbial i.e. fungus and bacteria, load was observed in the feed blocks containing either congo signal grass or tree leaves than those containing either napier or green maize.

Poultry

Linseed oil: Significantly higher total body weight gain was observed in the birds fed linseed oil and natural antioxidants. Linseed oil feeding improved the omega-3 content of meat by more than two folds and the supplementations of curry leaf, turmeric and commercial antioxidant enhanced the meat shelf life.

Coconut xylo-oligosaccharides: Xylo-oligosaccharides were produced from green coconut husks, which mainly comprised higher degree oligomers such as xylopentose and xylohexose. The supplementation of the coconut xylo-oligosaccharides (0.5%) in the diet of broilers increased the abundance of beneficial microbes in the caecum.

Fungal phytase: Phytase enzyme was produced from fungal isolate *Aspergillus foetidis* (MTCC 11682). Incorporation of partially purified fungal phytase in the diet (1000FTU/kg) of broiler chicken was effective in replacing 0.12% non-phytate phosphorus.

Processed protein meals: Processed rape seed meal (RSM) with 80% reduction in glucosinolate concentration could be safely used in commercial broiler chicken diet at 10% level, partially replacing soybean meal. Srinidhi chicks could tolerate raw RSM up to 5% level in diet. *Guar* meal in diet at graded levels (5 to 20%) progressively reduced feed efficiency in commercial broilers. Dimethyl carbonate treated *karanj* cake showed no adverse effect at 3% level in diet, but significantly depressed performance of broiler chicken at higher levels (6 or 9% in diet), even with late introduction at 11 or 21 days of age. Isopropyl alcohol treated *karanj* cake could be used in the feed of Vanaraja chicks at 6% during 5 to 6 weeks of age.

Value addition of chicken meat: Dietary incorporation of fish oil at 3% level during finisher phase improved the bird's performance and enriched the meat with n-3 fatty acid without affecting the sensory attributes of meat. Conjugated linoleic acid (CLA) accumulation in meat significantly increased with increasing CLA level in the diet.

Utilization of alternate feed resources: Inclusion of rice DDGS (dried distillers grains with solubles) up to 10% level did not exert any adverse effect on growth, feed conversion ratio, carcass traits and development of immune organs of broiler chickens. Similarly, high protein *guar korma* could be effectively and economically used up to 10% level in broiler diet replacing costlier soybean meal. Further, irrespective of source of protein commercial protease incorporation in diet was beneficial for improved feed utilization and reduction in feed cost.

Precise nutrient supply: A dietary concentration of 0.35 and 0.25% available phosphorus during starting (0-21 d) and finishing phase (21-42d), respectively, and 1,500 IU/kg vitamin D_3 with supplementation of phytase (500FTU/kg diet) was optimum to obtain maximum growth performance, immune response and leg bone mineralization in broiler chicken.

Turkey

Dietary energy level of 2,600 kcal ME/kg was found optimum for growing turkey poults during 8-16 weeks. Similarly, supplementation of selenium (Se) @ 0.4 mg/kg basal diet was beneficial for lowering age at sexual maturity, improving egg production, egg quality traits, parthenogenetic characters in females and physio-biochemical characteristics of semen in male turkeys.

Animal Physiology and Reproduction

Cattle

Genetic polymorphism of heat shock protein genes: The expression pattern of ATPase beta subunit genes (ATPase B1, ATPase B2, and ATPase B3) among the crossbred bulls under different ambient temperatures (20–44°C) was studied and compared with the relationship of HSP70. Among beta family genes, transcript abundance of ATPase B1 and ATPase B2 was significantly higher during the thermal stress. The expression of ATPase B1, ATPase B2, and ATPase B3

is highly correlated with HSP70.

OAS1 gene related to establishment of pregnancy: PCR-SSCP method was used to scan for mutations within the amplified regions of four exons of 2,5-oligoadenylate synthetase 1 (OAS1) gene 240 animals, comprising 81 Sahiwal and 159 Frieswal animals. Variations were identified in SSCP patterns of OAS1, 5, and 6A exonic regions, whereas monomorphic pattern was detected in OAS4 and OAS6B regions.

Buffalo

Expression and localization of the ghrelin and receptor GHSR-1a in ovarian follicles increases with ovarian follicle development and maturation. Ghrelin had inhibitory effect on estradiol secretion, aromatase expression and apoptosis and stimulatory effect on cell proliferation on GCs in bubaline species.

Pregnancies established from cloned embryos: Transferable stage cloned embryos were produced from somatic cells of a superior bull and established 2 pregnancies in recipient buffaloes. Calvings for the same completed in November 2015. A protocol was tested for superovulation of anoestrus buffaloes that are otherwise normal and have a good body condition score.

For the first time endogenous level of osteopontin in buffalo seminal plasma was estimated, which ranged from 6- 30 pg/ml. Liposome was found to be a better alternative to egg yolk as buffalo bull semen extender.

Sheep

Supplementation of ITS (insulin-transferrin-selenium) and FGF2 (fibroblast growth factor 2) in the maturation medium improved the maturation and cleavage rates of sheep oocytes. GDF9 (growth differentiation factor 9) was crucial for the maturation of metabolically active sheep oocytes. Poor development of the metabolically active sheep oocytes was attributed to disrupted activin/BMP (bone morphogenetic proteins) signalling. Whole transcriptome analysis revealed 914 and 945 significantly up-regulated and down-regulated genes respectively, in the metabolically active compared to silent sheep oocytes.

Production of 3 lamb crops in 2 years: The accelerated lambing system was adopted in Malpura ewes. Ewes were bred at 8 monthly intervals. Ewes not shown estrus in time were induced for estrus with progesterone impregnated intra vaginal sponges and PMSG protocol. It was found that 75% of ewes lambed at every 8 month (i.e. 3 lambs in 2 year).These lambs attained puberty at the age of 337 days and mated within 360 days. The adoption of accelerated lambing system in Malpura sheep produced 32.58% more lambs in comparison to one lamb in a year under conventional system.

Reproduction in ruminants: An attempt was made to develop pregnancy associated glycoprotein (PAG) based immunodiagnostic in buffaloes. The PAG7 is expressed predominantly during early pregnancy in buffaloes. The recombinant protein and synthetic peptide corresponding to PAG7 were generated and used for anti-sera production, which were suitable for immunoassay development.

Mithun

Study on expression profiling of *KiSS1R* genes revealed a higher relative abundance of the transcripts encoding *KiSS1* and *KiSS1R* genes in mithun that were in the transition phase from prepubertal to pubertal phase than those in pre-pubertal stage. A trial was conducted to determine the optimum osmolarity of hypo-osmotic solution to assess the functional membrane integrity of mithun sperm. Osmolarity of 150 mOsm was the most suitable for hypo-osmotic swelling test in mithun.

Poultry

Effect of bisphenol-A (BPA) on reproduction in quail: Evaluation of effect of endocrine disruptors (ED) such as bisphenol-A (BPA) on reproductive function of male Japanese quail revealed that it has adverse effect on reproductive functions.

Quick detection of *Brucella melitensis*

A Taqman probe based OMP-31gene realtime PCR assay was developed for the diagnosis of *Brucella melitensis* in vaginal washings/swab, aborted contents, preputial swab, milk etc. The oligos and probes were designed in the coding region of the OMP31 gene specific to *B. melitensis*. The assay has a very high sensitivity that detects positive *B. melitensis* DNA spiked to clinical samples with concentration as low as 100 femtograms. The advantage of this assay is that it is specific to *B. melitensis*, which is the most common abortion causing agent in small ruminants and can be assayed approximately in 30 minutes using the suspected DNA sample.

LIVESTOCK PROTECTION

Epidemiology and disease informatics

During period under report, forecasts for the 15 economically important animal diseases were released. Records (5,577), originating from 30 states pertaining to various diseases were reported to the National Animal Disease Referral Expert System (NADRES); and 2,151 serum samples received from Jharkhand, Madhya Pradesh, Maharashtra, Meghalaya, Odisha, Punjab and Tamil Nadu etc., were catalogued in the National Livestock Serum Repository.

The economic analysis in cattle and buffaloes in selected endemic states of India revealed that estimated mortality losses due to haemorrhagic septicaemia (HS) was ₹ 27,647 and ₹ 31,901 per animal, respectively. A cluster map of HS and FMD outbreaks in Tamil Nadu and Kerala, was prepared. The socioeconomic impact study revealed that total estimated direct loss due to FMD was ₹ 23,193 crore in the reported period. A parametric regression model with threshold for southern and eastern region of the country for *peste des petitis ruminants* (PPR)

outbreak was developed. At the optimum incremental level of 10%, the estimated loss due to PPR in sheep and goats in India was ₹ 1,611 crore.

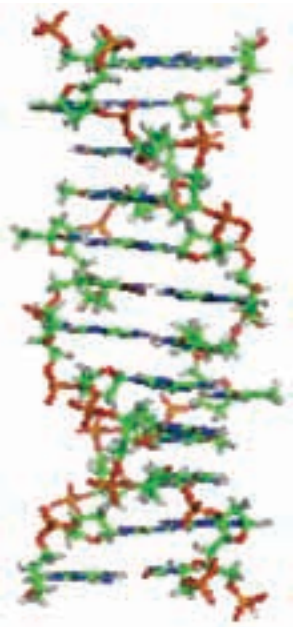
The hemagglutinin (H) and nucleocapsid (N) protein of PPR virus were expressed in prokaryotic system and on evaluation of indirect ELISA based on recombinant N and H antigen showed 97.97% sensitivity and 99.49% specificity. A total of 391 serum samples were collected from sheep and goats in seven states of NE region. On screening for PPRV antibodies, 17.90 and 63% of seroprevalance was recorded in suspected and random population of goats. Phylogenetic analysis carried out with 24 E2 gene sequence revealed that all the recent CSFV 24 E2 sequence could be grouped into subtype 2.2, which is now gradually dominating the traditional 1.1 group. The porcine tissue samples from Udupi, Karnataka, screened for TTV gene groups 1 and 2, were found positive for gene group 2. Out of 1,022 bovine samples from 14 states of the country, 31.5% cases were positive for IBR antibodies; Arunachal Pradesh had the highest (90%) and West Bengal the lowest (43.30%). In yak, a high percentage (95.23%) was found positive for IBR antibodies.

Under epidemiological study of rabies in livestock, out of 124 samples taken from animals from Uttar Pradesh, Gujarat, Karnataka and Kerala, 45 were found positive by fluorescent antibody technique. The N gene sequences of the rabies-positive samples revealed that all the isolates belonging to gene type 1 of rabies virus are of arctic lineage. Analysis of pox outbreak records revealed that, there was an increased trend from 2005-13 followed by a declining trend. The highest number of outbreak was reported from Andhra Pradesh. The number of deaths is directly proportional to number of outbreaks and number of attacks in each year. The outbreaks were mostly recorded during Dec-May. In goats, 70.31% morbidity and 46.87% mortality were reported.

Risk path analysis of notifiable avian influenza (NA, HPNAI, LPNAI) was identified for the import of chicken, meat and by-product and also live birds; the process includes the hazard identification, release, exposure and consequent assessment. Previously, reported HPAI outbreaks were mapped based on GIS coordinates as point dot maps. Temporal data analysis suggested three different introductions of disease in 2008 in different places. Majority outbreaks were recorded in the adjoining districts with Bangladesh and Nepal, which are endemic to H5N1 avian influenza. The outbreaks in crows reported during 2011-12 from Jharkhand, Maharashtra, Odisha and Bihar. This study suggested the spreading of the disease in different places/locations since, the crows are found near human habitations.

Under the All India Network Program on Blue Tongue (BT), screening of 562 serum samples for antibodies of BTV from 9 districts of Maharashtra, revealed 87.54% prevalence. The age-wise analysis of the prevalence showed that the number of affected animals increase with the increase in age.

Whole genome shotgun sequencing was completed for one each of field isolate, *Brucella melitensis* ADMAS-G1 (Accession # NZ_ AUTT00000000) and reference strain *Brucella abortus* S99 (Accession # AWTU00000000); and genome sequences are available in NCBI GenBank. Among 1,360 samples collected from different species of animals, more than 10% were found positive by screening through RBPT and ELISA. A newly developed lateral flow assay (LFA) kit for detection of brucellosis in animals showed 87.1% sensitivity and 92.6% specificity. The recombinant proteins of *Brucella* namely rBLS (36 KDa) and rBP26 (44 KDa) were expressed and analysed, which showed immunoreactivity of the proteins. Standardization of ELISA using such recombinant proteins also carried out. Standardization of fluorescent polarization assay for detection of brucellosis in animals was carried out and it showed 90% sensitivity and 78.33% specificity. Besides, 3,459 animal serum samples received from seven AICRP collaborating centres were screened for brucellosis. Out of them, 0.54% cattle, 0.33% buffalo and 0.97% goat were found positive for the presence of *Brucella* antibodies. In a questionnaire survey, 86% veterinarians opined in favour of brucellosis vaccination in animals whereas, 14% were against the vaccination.



Brucella abortus S19, whole genome shotgun sequencing

On the study of methicillin-resistant staphylococci organism, out of 97 goat samples, two isolates showed amplification for *mec*A gene by PCR. The antibiotic sensitivity test (ABST) showed one of the isolates as intermediate resistant to cefoxitin but another isolate was sensitive to both methicillin and cefoxitin. One of the *mec*A positive isolate was identified as *Staphylococcus epidermis*. The study on *in vivo* pharmacological properties of membrane- active glycoprotein antibiotic (YV11455) against MRSA (methicillin-resistant *Staphylococcus aureus*), revealed that effective dose response of the drug in 50% maximal bacterial killing (ED_{50}) was 1.43% mg/kg. The beta lactamase producing *Enterobacteriaceae* organisms were also studied.

Leptospira serovars (19) were used for MAT (macroscopic agglutination test) screening for the detection of *Leptospira* antibodies in the samples. For sero-epidemiology of leptospirosis, this year 887 animal serum samples from Odisha, West Bengal and Karnataka were screened and overall 36.87% seroprevalence was recorded with 36.45% in cattle, 54.28% in buffalo, 28.33% in goat, 44.44% in sheep and 31.37% in horse. Among human, 5.55% cases of leptospirosis were

detected by MAT screening. A lateral flow kit for the diagnosis of *Listeria* species was designed using listerolysin-o (LLO) and colloidal gold conjugated protein G and the test was evaluated with 405 serum samples showing 100% agreement with LLO based indirect-ELISA.

Serum samples (2,093) from cattle, buffaloes, horse, donkey and camel from Karnataka, Odisha, West Bengal and Rajasthan, screened for *Trypanosoma evansi* by ELISA, revealed that, 586 samples were positive for *T. evansi* antibodies. The buffalo showed highest seroprevalance (36%) followed by cattle (28-31.25%), camel (31%), donkey (6.89%) and horse (5.10%). Analysis of faecal samples (222) collected from cattle, buffalo, sheep and goats form Karnataka, showed that 19.81% were positive for parasitic infections; *Fasciola* 17%, Strongyles 12%, Amphistome 15%. On screening of *Lymnea* species snails, an overall 13% were found positive for *Fasciola* infection. During this year, 148 human samples collected from West Bengal, Karnataka and Andhra Pradesh were screened for the presence of *Toxoplasma gondii* antibodies by agglutination test. An overall 12.16% seroprevalance was recorded with 25.49% in Andhra Pradesh and 17.85% in Karnataka.

Development and improvement of diagnostics

Molecular diagnostics developed for animal diseases include real-time PCR assay for detection and quantification of porcine circovirus 2 load in tissues; DNA bar-coding of *Brucella* using *rpo*B gene; PCR for VNTR analysis of *Brucella* isolates; multiplex PCR for detection and differentiation of *Salmonella* serovars; and PCR assays for detection of *Clostridium difficile.*

Recombinant antigen based diagnostics for animal diseases include ESAT6-CFP10 fusion protein based indirect ELISA for differentiation of *Mycobacterium bovis* and non-tuberculous mycobacteria in cattle; listeriolysin O (rLLO) and recombinant phosphatidyl-inositol phospholipase C (rPI-PLC) based indirect ELISA for listeriosis; and BgP12 protein based indirect ELISA and dot-ELISA for diagnosis of *Babesia gibsoni* infection in dogs.

Surface plasmon resonance (SPR) biosensor assay for detection of *B. abortus* specific antibodies was developed using a recombinant p17 kDa protein (6, 7-dimethyl-8-ribityllumazine synthase-2). The SPR assay was used to record interactions between recombinant p17 protein and *Brucella* specific antibodies in real time. Since, SPR is a label-free technique and does not require any species specific conjugate or fluorophore, this assay using may be employed for providing rapid, real-time and label-free serodiagnosis of brucellosis in different species of animals.

Diagnostic vigilance

Avian influenza (AI): The avian influenza viruses were detected and isolated from poultry which included H5N1 (17 from Kerala, 6 from Uttar Pradesh and 1 each from Chandigarh and Odisha), H9N2 (2 from Kerala), H6N2 (1 from Kerala) and H3N8 (4 from Kerala) from a total of 45,980 morbid materials received from various parts of the country. Eighteen sera (14 chicken and 4 ducks) were positive for antibodies to avian influenza virus (subtype H5) and 20 sera were positive for H9N2 AIV antibodies (17 and 3, respectively, from Kerala and Chandigarh) of the 5,496 serum samples tested.

Bovine viral diarrhea (BVD): Out of 713 sera of mithun, cattle, buffaloes, sheep and goats from NE region, only five animals were positive for BVDV neutralizing antibodies indicating a low BVDV prevalence in this region.

Malignant catarrhal fever (MCF): The causative agent of MCF, ovine herpesvirus -2 (OvHV-2), was confirmed in blood samples of pigs from Mizoram for the first time in India. Twenty-two samples were positive for OvHV-2 genome of the 179 blood samples of cattle, buffalo and sheep tested from various parts of the country.

Porcine reproductive and respiratory syndrome virus (PRRSV): Out of the 495 porcine whole blood/ serum samples received from North East India, 238 (48%) were positive for PRRSV antibodies. Two sera (one each from Mizoram and Meghalaya) out of 163 inoculated on PAM cultures were positive for virus isolation and were confirmed by immunoperoxidase monolayer assay on MARC-145 cells using PRRSV specific polyclonal serum.

Swine influenza viruses (SIV): Sera samples (162) from pigs from different parts of the country were tested with HI test and 43 samples were found positive for antibodies against H1N1.

Diagnostics for exotic and emerging diseases

RT-PCR ELISA for pestiviruses: A reverse transcription polymerase chain reaction ELISA (RT-PCR ELISA) was developed for detection of ruminant pestiviruses and its diagnostic performance was evaluated on clinical samples obtained from cattle, sheep and goats. The assay had high analytical specificity, good reproducibility with 95.9% diagnostic sensitivity, 98.6% specificity and a strong agreement (97.5% concordance) with the reference virus isolation method.

PCR-array based multiple pathogen diagnostic: PCR arrays for the detection of 17 prioritized exotic and emerging viruses, viz. Crimean-Congo haemorrhagic fever (CCHF), Rift Valley fever (RVF), West Nile fever (WNF), vesicular stomatitis (SV), Japanese encephalitis (JE), Aujeszsky's disease, Ebola virus disease, Marburg, Eastern equine encephalitis (EEE) and Hantaan fever, bovine viral diarrhea (BVD), caprine arthritis encephalitis (CAE) and Schmallenberg virus infection, African swine fever (ASF), Nipah, swine vesicular disease (SVD), transmissible gastroenteritis (TGE), using molecular beacon (MB) and SYBR green realtime PCR chemistry were developed. This technology offers the simultaneous detection of various pathogens by running a panel of real time PCR in different wells of a single PCR plate under uniform reaction and thermal conditions.

AGID test kit for avian influenza: A kit for agar gel immunodiffusion (AGID) test for detection of avian

influenza antibodies in chicken serum samples was developed and evaluated against reference test. The sensitivity is 94.28% and specificity is 94.96% compared to HI test. The intra-laboratory validation on lyophilized antigen was conducted successfully by three independent scientists.

Molecular characterization of pathogens

- Molecular and genetic characterizations based on B2L gene of parapox viruses, ORF 19 of swinepox virus, UL 0.5 gene of bovine herpesvirus 1, VP2 gene of BTV, N and F genes of PPR viruses and omp 16 gene of *P. multocida* isolates, were carried out.
- Complete genome sequencing and phylogenetic analysis of PCV2 revealed presence of natural recombinants arising from PCV2a and PCV2b.
- Methicillin resistant *Staphylococcus aureus* (MRSA) was isolated and characterized from milk samples collected from bovine and caprine mastitis.
- The current phylogenetic analysis of avian influenza viruses showed that the H5N1 viruses isolated till early 2014 grouped with clade 2.3.2.1A, whereas, the recent H5N1 viruses isolated from Kerala, Chandigarh and Uttar Pradesh grouped with a new clade 2.3.2.1 C, which has not been detected so far in Indian poultry. Detailed phylogeny indicated role of migratory birds in the spread of H5N1 virus, although trade of poultry/poultry products cannot be ruled out.
- H5N1 isolates (46) from the year 2007 to 2014 were screened for drug resistance for Oseltamivir and Zanamivir, revealed drug resistance in 3 Indian isolates and decreased susceptibility for two isolates. These findings indicated emergence of antiviral resistance in avian influenza viruses isolated in India.
- The phylogenetic analysis of the 5'-UTR sequence identified five clusters within the BVDV-3 virus clade and demonstrated that Indian BVDV-3 isolates were grouped into two distinct clusters separate from the previously reported BVDV-3 viruses from South America, Europe and Australia. IndBHA5309/12 group of viruses was the most divergent BVDV-3 strain reported so far. Based on phylogeny of N^{pro} gene, three BVDV-3 virus lineages could be identified globally with two from India.
- Genetic analysis of the complete ORF 5, ORF 7 and nsp2 coding regions showed that the Indian PRRSV isolate grouped with genotype-2 viruses reported from China. Analysis of deduced amino acid from ORF 5, ORF 7 and nsp2 (within ORF 1a) sequences showed the presence of 2, 1 and 14 unique amino acid changes, respectively, as compared to the PRRSV genotype-2 currently existing in various parts of the world.

Success story

New indigenous sheep-pox vaccine

Sheep and goat rearing contributes significantly to the livelihood of >75% of small and marginal farmers of the country. Sheep-pox is endemic in India and outbreaks are reported regularly from almost all the states and the high rate of death (50%) causes heavy economic losses. ICAR-IVRI initiated the vaccine development programme using an indigenous isolate (SRIN-38/00 strain) of sheep-pox virus by adapting the virus to Vero cells. The attenuated vaccine strain can be used to produce large quantity of vaccine on commercial scale. Small quantity (1 ml) of virus may yield ~10,000 doses of vaccine. The vaccine was tested for its safety and protective efficacy in >90,000 sheep. A single dose of the vaccine provides immunity up to 4 years, which is sufficient for life time protection. Sheep-pox control is vital enhanced productivity of small ruminants through effective disease control programme using this indigenous vaccine.

Vaccine development and delivery

FMD: Immunization trial in cattle using recombinant Ad5-FMD constructs with and without adjuvant against three Indian FMDV strains (O, A and Asia-1) showed 100% protection against type O and A when booster vaccination was done. The efficacy of Ad5-FMD Asia-1 vaccine was lower than O and A.

Two new generation adjuvant formulations were evaluated for formulating trivalent FMD vaccines (Type O, A and Asia-1). Cattle were immunized with single 2 ml dose and challenged with homologous type O FMD virus. One of the proprietary formulations protected 100% of animals.

IBR: A mutant BoHV-1 (gE) gene deleted BoHV-1 marker vaccine candidate against infectious bovine rhinotracheitis (IBR) was developed. Guinea pigs immunized with oil-adjuvanted vaccine showed virus neutralization (VN) titer $>2^6$ which increased to $>2^9$ with booster dose and maintained up to $>2^{10}$ till 90 days post-immunization.

Clostridium: The protective efficacy of expressed recombinant immune-reactive proteins of *C. chauvoei* tested in guinea pigs. Enolase elicited better protective

immunity followed by Flagellin, Ribosomal L10 and glycosyl hydrolase proteins.

Trypanosoma: Immunization of rats with gamma irradiated *Trypanosoma evansi* at 450-500 Gy, induced 100% protection against homologous challenge.

Therapeutics

- Atorvastatin showed potential to ameliorate arsenic-induced hypertension.
- Polyherbs containing extracts of *Eclipta alba*, *Solanum nigrum*, *Macrotyloma uniflorum*, *Murraya koenigii* and *Phyllanthus niruri* have potent antioxidant, diuretics and hepatoprotectant effect in compromised liver condition.
- Pre-treatment with alcoholic extract of *Dalbergia sissoo* leaves showed cardioprotective effect in isoprenaline-induced myocardial injury in rats.
- The supplemental feeding of leaves of *Aegle marmelos* and *Murraya koenigii* augmented fertility in delayed pubertal heifers both at farm and field conditions.

Foot and mouth disease

During the year 2015-16, no major outbreak of FMD was reported and only 47 incidences were recorded in the country. Among them, serotype O dominated with 45 incidences while serotypes A and Asia-1 caused only one incidence each. The majority of the incidences were recorded in Karnataka, Kerala, Asom, Odisha, Madhya Pradesh, Uttar Pradesh and Bihar. A few incidences were also recorded in Gujarat and Rajasthan. Serotype A was reported from Gujarat and serotype Asia1 was reported from West Bengal. No disease was reported from Haryana, Himachal Pradesh, Maharashtra and Delhi during this period, while sporadic incidence was recorded in Punjab.

Phylogenetic analysis, carried out to assess genetic variations, inter-strain relationship and to track the movement of the virus in the country, revealed exclusive presence of Ind2001 lineage of serotype O in the field, while presence of PanAsia lineage was also recorded in state of Asom. Vaccine matching exercise revealed that the currently used vaccine strains of serotype O (INDR2/

Success Story

Development and launch of foot-and-mouth disease-decision support system

Foot and mouth disease- decision support system (FMD-DSS), an online decision support system for near real-time surveillance and monitoring, was developed and hosted at www.fmd-dss.res.in. The principal objectives of the system were to compile and automatically analyze the huge data generated from day-to-day activity related to FMD epidemiology in India and to provide refined information and reports to the concerned persons. The system archives the entire information of the samples and handle the raw data sheets of the various serological assays to reduce the manual errors, the system is having application to; map the disease (by GPS mapping up to the village level), map the serological status and analyze the FMD epidemiology, sero-monitoring, and sero-surveillance data using parametric and non-parametric assays. Various activity reports to be submitted to different departments for administrative purposes will automatically be prepared on start of the month, saving valuable time.

An online database management system for FMD in India

will produce a unique bar-coded accession number, through which the associated information of the samples can be accessed. The information of the FMD-DSS was used to generate FMD alerts in the country. For faster transmission of the information, the system is equipped with tools to— handle both emails and text messages; Various data sets such as, livestock demography, species, breed, GIS maps, meteorological parameters, districts, sub-districts and villages (>600,000) were procured and integrated with the system that may also help to build similar systems for other animal diseases in the country.

Success story

Understanding FMD viral ecology and landscape epidemiology towards control and eradication

The possible role of ruminants persistently infected with FMD virus in initiating new outbreaks remains to be understood. With generation of disease free zones in the country, understanding the role of the carrier animals in spreading the disease is crucial. Moreover, determining the probability of the animal becoming a carrier post infection and the time-dependent probability of clearing persistence is very important. A study was carried out in collaboration with Plum Island Animal Disease Centre (PIADC) and USDA. As pilot project three study sites were established having the natural FMD outbreaks in 2013. Regular sampling consisting of serum and oro-pharyngeal fluids were collected and examined. In the clinically infected category, both the farms in Uttarakhand, revealed comparable non-structural protein-antibody (NSP-Ab) positive animals, whereas considerable difference was noticed in the asymptomatic in-contact category despite showing similar proportion of genome PCR positive results. This may be due to older animals having more mature immune systems and seroconversion with more robust response following infection. Overall, the high prevalence of NSP-Ab in asymptomatic animals is important because it indicated that many animals that never show disease are actually infected. From 3 animals in the dairy farm, virus could be isolated and clustered with the O/ME-SA/Ind2001d lineage. In dairy farm at Chhattisgarh, in cattle only up to 7 months after outbreak, while in buffalo up to 13 months virus could be isolated. Virus clearing with time in all the three sampled farms was evident from the gradual decline in the proportion of viral genome positive samples and virus isolation.

1975) provided optimal antigenic coverage to the field isolates. Some isolates were found divergent from the vaccine strain; emergence of such antigenic variants in the field is a regular phenomenon and is not a serious concern at present. In serotype Asia-1, the field isolates analyzed had perfect match with the currently used vaccine strain (IND63/1972).

During this period, 160,243 serum samples collected as per the sampling frame from the states covered under FMDCP were tested by single dilution liquid phase blocking ELISA for estimation of antibody titers against the three serotypes. The percentage protective antibody titre in the serum samples collected at random from FMDCP states were higher when compared to the non CP states. Currently, 88, 77 and 89.1% animals tested are having protective antibody level ($\log_{10}$ 1.8 and above) against serotypes O, A and Asia-1, respectively, in post-vac serum samples in the first phase FMDCP districts, however, the remaining samples are still under testing. Under national sero-surveillance programme, 19,697 serum samples collected at random were tested for the presence of antibodies against non-structural proteins 3AB3 of FMD virus which is an indicator of FMD virus exposure regardless of vaccination status. The test revealed seropositivity in ~ 25% samples/animals.

Sheep

Genetic improvement of resistance to *Haemonchus contortus:* The increase in anthelmintic resistance in gastrointesinal nematodes of small ruminants necessitates identification and adoption of suitable worm management strategy with minimal dependence on anthelmintic. The identification and establishment of genetically resistant animals could be a sustainable non-chemical based option for worm management. Divergent lines (R - resistant and S - susceptible) were created in Malpura and Avikalin sheep through screening of lambs and evaluation of sire for low and high faecal egg counts (FECs). Challenge study in progenies from divergent lines was also conducted and it showed relative resistance to infection in R line.

Camel

Trypanosomiasis in camel: Persistence of low parasitaemia in camel due to development of Quinapyramine resistance was confirmed by RFLP. Alternative drugs prepared from local trees/herbs are being tested for management of drug resistant *Trypanosoma* infection.

Equines

Surveillance, monitoring and control of diseases: The country had no active case of equine influenza after 2009; the seropositivity in few cases is under investigation. EHV-1 is endemic in the country with an overall incidence of 2.53%. All samples tested for EIA were negative for antibodies. The incidence of piroplasmosis in 2014-15 was as high as 32.67%, while that of trypanosomosis was 3.54%. Amongst bacterial diseases, outbreaks of glanders, though of limited scale since 2006, were reported from Uttar Pradesh, Himachal Pradesh and Jammu and Kashmir.

Expression and characterization of expressed recombinant glycoproteins of EHV-1: Abortions due to EHV-1 cause huge economic losses. In quest to develop better immune-prophylactics, glycoprotein D (~48kD) and gM (~52kD) of EHV-1 were expressed in eukaryotic system by transfecting sf9 cells transfected with recombinant bacmid, and the expression of protein is being further optimized with various conditions. For construction of EHV-1 bacterial artificial chromosome (BAC), gene 71 (g71) was selected as targeted region to clone EHV-1 isolate, and transfection of RK-13 cells with virus and linearized plasmid showed desired results.

Neuropathogenic and non-neuropathogenic variants of EHV-1 and associated latency: A real-time PCR assay was standardized for detection of SNP at position 2,254 of ORF30 for differentiation between neurogenic and non-neurogenic EHV-1 infection. The existence of neurogenic EHV-1 was reported in the country. Genetic diversity based on the sequence analysis of partial ORF68 gene of 7 EHV-1 isolates revealed that the Indian isolates belonged to group 4 and group 5.

Equine influenza virus (H3N8) and vaccine efficacy: The continuous drift in equine influenza viruses requires harmonization of vaccines and strains

substitution. The studies were undertaken to develop mouse model for studying pathology of H3N8 influenza A virus (Sublineage Florida clade 2 virus) and to elucidate the protective efficacy of inactivated indigenous H3N8 vaccine eliciting protective immune response in mouse model.

Evaluation of synthetic drug molecules against *Theileria equi:* Equine piroplasmosis is a tick-borne haemoprotozoan disease of equids and there is no drug, which can completely eliminate *T. equi* infection from carrier animals. The target specific drug molecules were tested in MASP culture of *T. equi* in the *in-vitro* system. Of nine drug molecules tested, *in-vitro* growth of *T. equi* was significantly inhibited by HHD, HDTAB, HMC, decamethonium bromide and dodecyltrimethyl ammonium bromide molecules. On *in-vitro* cytotoxicity trials harmaline, decamethonium bromide and NBCN salts were found the most promising drug molecules in inhibiting *T. equi* growth with least cytotoxicity.

Diagnostic assays for *Trypanosoma evansi* infection: A highly sensitive real time PCR with detection level of 0.15pg of genomic DNA of parasite was developed and compared well with gold standard TBR-PCR. Both the techniques could detect the infection after 24 h post infection. Another approach for diagnosis of *T. evansi* using serological assay based on Hsp70 recombinant protein could detect antibodies against *T. evansi* at 10 dpi.

Success story

Eri silkworm: A non-mulberry silkworm as a bioreactor for expression of recombinant proteins

Insect larvae or insect cells that are permissive to baculoviruses are used as host to produce recombinant proteins, for use in diagnostic/pharmaceutical application. There are limited species of insect larve that are naturally susceptible to AcMNPV (Autographa californica multicapsid nucleopolyhedrovirus). The Eri silkworm (*Samia ricini*) is reared in many states of India, Japan and China. It feeds primarily on leaves of the castor plant (*Ricinus communis* L.). Eri silkworms are susceptible to infection by AcMNPV or recombinant AcMNPV (such as the one expressing GFP marker protein) leading to productive infection in larvae when injected by intrahemocoele route. The larvae were exploited for production of FMD virus non-structural protein 3ABC, when infected with recombinant baculovirus. Each larva yielded recombinant protein nearly equivalent to the quantity of protein recovered from 1×10^7 Trichoplusia ni 5 (Tn5) cells infected with the virus under stationary culture conditions. This systematic study demonstrated the experimental infection of the Eri silkworm (*Samia ricini*) larvae by AcMNPV and its utility as a potential bioreactor for expression of heterologous recombinant proteins.

Healthy silkworm larvae

Pig

Rapid detection of mithicillin-resistant *Staphylococcus aureus* (MRSA): Presence of mithicillin-resistant *Staphylococcus aureus* (MRSA) in food-producing animals and retail meat has increased the concern about the exposure of humans through the food chain, and hence, there is a need to use rapid method for its quick detection. The PCR protocol for rapid detection of MRSA from pigs was standardized for routine screening of pigs.

Yak

Sero-prevalence studies, carried out for detection of bovine hepesvirus-1, bovine viral diarrhoea virus and *Pasteurella multocida* in yak breeding tract of Arunachal Pradesh, revealed that percentage positivity was 37%, 21% and 2.4%, respectively. A survey revealed that yak farmers generally use albendazole as dewormer when the animals migrate downwards in winter. Out of 55 samples processed, only 9 (16.36%) samples were positive for albendazole residue. The range of concentration was 4.563 ppm to 19.999 ppm with an average of 14.502 ppm. A primary culture of yak skin fibroblast was established from the biopsy of ear pinna of yak calf. Fibroblasts were cryopreserved for conservation of yak germplasm and for preparation of feeder cell layer for yak spermatogonial stem cell culture.

Mithun

The IgG and IgA concentrations were higher in all the physiological stages of mithun than Tho-Tho cattle. However, IgE was higher in Tho-Tho cattle than that of mithun. Comparatively higher concentration of IgG suggested that mithun might have a better humoral immunity potential. However, a significantly higher concentration of Granzyme B and PRF-1 in Tho-Tho cattle than the mithun suggested a better CMI response in cattle.

In North-Eastern region, the overall seroprevalence of BVDV infection was 13.70% (111/810) in mithun. Clinical case of BVDV infection could not be detected. Further, in all the tested samples including aborted foetuses, neither BVDV nor its genome could be detected by virus isolation, PCR, RT-PCR, real time RT-PCR and IHC.

Differences could not be observed in the partial sequences of MHC (DRA, DRB1, DQA1 and DYB genes) and NRAMP1 gene of mithun with and without FMD and *Brucella* infection.

Genetic characterization of mithun isolate of *Fasciola gigantica* showed polymorphisms in ITS-2 sequence, which can be used as a marker for differentiating *F. gigantica*, *F. hepatica* and intermediate forms of the parasite.

Based on the sequence analysis of ITS-2, ribosomal DNA and Cox 1 gene, *Setaria digitata* was identified first time in mithun.

Based on mitochondrial gene NADH1 sequencing, genotype of the hydatid cyst, isolated from mithun

was characterized as *Echinococcus granulosus* and *E. ortleppi* for the first time.

Poultry

ALV and *Mycoplasma* surveillance: Pure line chickens (4,436), tested for avian leukosis virus (ALV) using group specific antigen ELISA, revealed 5.07% incidence. The genome of ALV-A isolate (DPRE32) was sequenced. Furthermore, the virus was detected in infected CEFs by transmission electron microscopy (TEM). Sequence analysis of *mgc2* genes of *M. gallisepticum* showed similarity ranging from 93.8–100% among 13 field isolates. Identity between field and reference strains was 89.1-100%. Sequence analysis of *vlhA* genes of *M. synoviae* showed identity ranging from 94.6–100% among 19 field isolates. Identity between field and reference strains was 91.6-100%. The chicken RBCs treated with two *M. synoviae* isolates examined under scanning electron micros (SEM) and TEM failed to show invasion of RBCs. *HaeII* PCR-RFLP using *HaeII* enzyme could be used to differentiate field and vaccine strains in our country.

Fish

Marine fish harvests: Marine fish landings estimated for the mainland of India in 2014 amounted to 3.59 million tonnes, registering a 5% decline compared to production in 2013. Gujarat, Tamil Nadu and Kerala have been the top three marine fish producing states in the country, since 2006. These states represented 54% of the total marine fish landings, with Gujarat and Tamil Nadu holding the first and second positions respectively. All the maritime states except Andhra Pradesh, Karnataka, Odisha and Goa recorded reduced landings in 2014 compared to 2013.

Management of hilsa: Hilsa (*Tenualosa ilisha*) is a trans-boundary fish species inhabiting the rivers of India, Bangladesh and Myanmar in Bay of Bengal region. Application of Thompson and Bell bioeconomic model in West Bengal waters, showed that the current fishing effort, has already exceeded maximum sustainable yield (MSY), indicating 20% over exploitation of the stock at Hooghly estuary and near shore areas. There is 40% over exploitation of spawning stock biomass (SSB) of >250-260 mm total length. Further, increase in the exploitation levels might cause serious decline in the fishery.

Gill nets are the major gear used for exploitation of hilsa in both inland and marine sectors. Use of small mesh sized gill nets and large scale capture of spawning stock during breeding period reduce the scope of breeding and recruitment of the fish. Henceforth, about 20% reduction in fishing effort, restriction on use of small mesh sized gill nets and banning of fishing during breeding season may be implemented.

Effect of plant extracts on aquatic leech

Aqueous and methanolic extracts of *Nicotiana* spp. and *Zanthoxylum alatum* showed effective hirudinicidal activity with mean dead time ranging between 2.11±0.111 and 20.56±2.298 min at different concentrations in comparision to the levamisole @ 333µg/ml (12.60±1.348). The methanolic extracts of *Solanum khasianum* showed mean dead time ranging from 24.89±2.342 to 113.67±20.996 at 500 and 50 µg/ml, respectively.

Fish age determination: A well-equipped fish ageing and imaging analysis laboratory was established at ICAR-CMFRI, Kochi. Age of Indian oil sardine (*Sardinella longiceps*), Indian mackerel (*Rastrelliger kanagurta*), Silver pompano (*Trachinotus blochii*) and Mahi mahi (*Coryphaena hippurus*) were determined using hard parts. Otolith morphometric studies for species/stock confirmation were also standardized for tropical marine species.