



# KVASU

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# Research Digest

QUARTERLY RESEARCH NEWSLETTER OF KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY

## Pharmacogenomics emphasised as a tool in the development of new generation drugs

The scientific deliberations during the XIX Annual Conference of Indian Society of Veterinary Pharmacology and Toxicology held at CVAS, Mannuthy emphasized on the use of herbal remedies to overcome drug resistance and employing pharmacogenomics as a tool in the development of new generation drugs. The Annual Conference of ISVPT and National Symposium on Pharmacogenomics in the Development and Validation of Indigenous Drugs was held from 18<sup>th</sup> to 20<sup>th</sup> December 2019. The conference was inaugurated by Dr. H. D. Narayanaswamy, Hon'ble Vice Chancellor, Karnataka Veterinary Animal and Fisheries



Sciences University in a function presided over by Dr. A.M. Thaker, President, ISVPT. Prof. (Dr). M. R. Saseendranath, Hon'ble VC, KVASU released the compendium. Dr.N. Ashok, Registrar, KVASU, Dr. Ajith Jacob George, Director of Academics and Research, KVASU and Dr. M. K. Narayanan, Director of Entrepreneurship, KVASU addressed the gathering. Dr.C. Latha, Dean, CVAS, Mannuthy welcomed the gathering and Dr. Usha P.T.A, Organizing Secretary, ISVPT 2019 delivered the vote of thanks. Dr. B. D. Garg Outstanding Pharmacologist Award was presented to Dr. N. Gopakumar, Former Professor, KVASU. Dr. M. Sabir oration was done by Dr. Gopikrishna Assistant Professor, Department of Agadatantra, V.P.S.V. Ayurveda College, Kottakkal. and Chellappa memorial oration was done by Dr Jayakrishnan Thayyil, Professor, Department of Community Medicine, Govt: Medical College, Kozhikode, Kerala.

A pre-conference workshop on "In silico and cell culture techniques as an alternative to the animal use" was held on 17<sup>th</sup> December 2019. Fifteen participants attended the pre-conference workshop. Around 250 participants from different states took part in the deliberations on Pharmacogenomics/ Pharmacogenetics, Pharmacokinetics/ Toxicokinetics. Ethnopharmacology, Antimicrobials and Antimicrobial resistance, Education in Veterinary Pharmacology & Toxicology, Clinical/Regulatory Pharmacology and Toxicology, Toxicology of Xenobiotics, Animal Welfare and alternate to animal use, Molecular and Neuropharmacology and Industry Academia interaction. Dr.Mohan Kunnammal, Hon'ble Vice Chancellor, Kerala University of Health Sciences was the chief guest for the valedictory session with the august presence of Dr. Prof. (Dr).M.R.Saseendranath, Hon'ble VC, KVASU.



**ICAR Scheduled Caste - Sub plan 20-21 project on establishment of goat nursery and satellite goat farm in scheduled caste clusters of Wayanad, Kerala launched**



The programme “ICAR Scheduled Caste- Sub Plan 20-21 on “Establishment of Goat Nursery and Satellite goat farm in Schedule Caste clusters of Wayanad, Kerala” was inaugurated by Prof (Dr). M.R. Saseendranath, Hon’ble Vice Chancellor, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, on 16<sup>th</sup> March, 2021 at College of Veterinary and Animal Sciences, Pookode, Wayand, Kerala. The main objective of this project to promote Scheduled Caste beneficiary’s economic development through cluster-oriented satellite goat farm by providing adult female and bucks of Malabari breed to the Schedule caste families living below the poverty line. The project conceived the idea on need based human resource development, and 5 days training and capacity building program on Goat farming for improving livelihood security of Scheduled Caste Clusters of Wayanad, Kerala was inaugurated by Dr.Winny Joseph, District Animal Husbandry Officer, Kalpetta, Wayanad.

**Awareness regarding zoonotic diseases is imperative**

“In the era of emerging zoonotic diseases, the importance of bringing awareness regarding zoonotic diseases is imperative” said the Hon’ble minister of Animal husbandry and Dairy Development, zoos, dairy cooperatives and Kerala Veterinary and Animal Sciences University, Smt. J. Chinchu Rani. She also added that there is need for serious thought on safe food, elimination of toxic chemicals from the food chain and reduction in antibiotic resistance in animal farming as well

as in food chain. She was speaking during the inaugural session of 13<sup>th</sup> Kerala Veterinary Science Congress (KVSC) at College of Veterinary and Animal Sciences, Mannuthy campus, Thrissur. Hon’ble Minister for Land revenue, Survey and Land records, Land Reforms and Housing Adv. K. Rajan addressed the gathering as Chief Guest. The Kerala Veterinary Science Congress 2021 was organized jointly by Indian Veterinary Association, Kerala Chapter and Kerala Veterinary and Animal Sciences University in hybrid mode on the 12<sup>th</sup>, 13<sup>th</sup> and 14<sup>th</sup> of November 2021.



The Guest of Honour of the inaugural function Dr. Praveen Malik, Animal Husbandry Commissioner DAHD, Ministry of Fisheries Animal Husbandry and Dairying, Govt. of India detailed on the need for species independent disease detection techniques and importance of trans disciplinary research including agriculture, wild life, human and poultry sciences and the field functionaries in identifying the critical gaps in bringing One health and future shaping up the National plan on reducing antibiotic resistance in the country and how to eliminate indiscriminate use of antibiotics from the poultry and livestock sector in phased manner. Hon. Vice chancellor of the Kerala Veterinary and Animal Sciences University, Prof. (Dr.) M.R. Saseendranath expressed the need for research on redefining thermal comfort zone under Kerala state context and emphasized the need for having weather station catering the need for 152 blocks of the state to correlate the emergence of diseases and to have a better forecast on the diseases and also to bring better solution for climate related livestock production issues. Director Animal Husbandry Dr. A. Kowsigan IAS released the compendium of the veterinary science congress comprising nearly 250 research articles/ abstracts received from research scholars, scientists and veterinarians all over India. Dr. P. Sudheer Babu, Registrar,



Kerala Veterinary and Animal Sciences university, Dr. C. Latha, Dean, College of Veterinary and Animal Sciences, Mannuthy and Dr. Jose James, Managing Director, Kerala Livestock Development Board offered felicitation. Dr. M.K. Pratheepkumar, President, Indian Veterinary Association offered the presidential address. Dr. S. Maya Chairperson, organizing committee of the congress offered welcome address, Dr. Sreeranjini A.R., Organising secretary, KVSC 2021 briefed the concept of KVSC 2021 and Dr. V.K.P. Mohankumar offered vote of thanks.

Six scientific sessions were held during the two day deliberations. Dr. V Usha Menon, Clinical Professor, Dept. of Endocrinology and Diabetics, Amrita Institute of Medical Sciences, Kochi, Dr. R S Sethi, Prof. and Head, Dept. of Animal Biotechnology, School of Animal Biotechnology, GADVASU, Ludhiana, Dr. M.R. Saseendranath, Honourable Vice Chancellor, KVASU, , Dr. Sreekumar Pillai, Prof. & Head, Dept. of Oncosurgery, Jubilee Mission Medical College, Thrissur, Dr. Annie Varghese, former Assistant Director, AHD, Kerala, Dr. Sudhir C. Roy, Principal Scientist (Biochemistry-Animal Science) Molecular Biology Laboratory, Animal Physiology Division, ICAR-National Institute of Animal Nutrition and Physiology and Dr. Sahadeb Dey, Head (Acting), Veterinary Medicine Division ICAR-Indian Veterinary Research Institute Izatnagar – 243122, Bareilly, Uttar Pradesh presented lead papers during the Congress.

### Vilwadri cattle proven as genetically different

Research on genetic diversity analysis by a group of geneticists from KVASU has proved that the native Vilwadri cattle of Thrissur district is genetically divergent from the rest of the cattle population in Kerala. The two-year project, “Genetic diversity analysis among cattle genetic groups of Kerala using microsatellite genetic markers”, was carried out with financial assistance of the Kerala State Council for Science, Technology and Environment. The cattle population, comprising cross-bred cattle, Vechur, Kasaragod, Vadakara, and Vilwadri were studied using 25 microsatellite genetic markers chosen from the Food and Agriculture Organization and the International Society for Animal Genetics panel, which were amplified from genomic DNA using multiplex polymerase chain reaction. The final result showed that the native cattle breed of Kerala — Vechur, along with Kasaragod and

Vilwadri cattle — separated into distinct populations with more genetic distance from others. This finding will definitely boost up the efforts for the conservation of these animals.

### Multi- trait selection criteria for crossbred cattle of Kerala was developed

Research carried out at Centre for Advanced Studies in Animal Genetics & Breeding, Mannuthy could develop multi-trait selection criteria for milk production, fertility and mastitis resistance was developed for crossbred cattle of Kerala A set of major functional genetic markers in *PRL*, *B4GALT*, *DEFB4A*, *CAST*, *POUIF1*, *STAT5A*, *CD14*, *FSHR*, *TLR4* genes among crossbred cattle population that were associated with production, reproduction and disease resistance traits in crossbred dairy cattle were identified. The research programme entitled “Development of multi trait selection criteria for crossbred cattle of Kerala” was funded by Department of Science and Technology, Govt. of India.

### Breed genetic traceability system for native goats of Kerala was established

Population genomics study carried out in native goats of Kerala has led to the development of breed genetic traceability system using breed specific markers for Malabari and Attappady goats of Kerala. Breed genetic traceability permits the assignment or exclusion of the breed of origin to an animal or its product. The other outcome of this genomic research in native goats is the identification and validation of genetic markers useful for multiple birth enhancement in native goats of Kerala. The research programme entitled “Addressing the Production Challenges of Native Goat Breeds of Kerala through Genomic Approach” was funded by Animal Husbandry Department of Kerala.

### Supporting dairy farmers for doubling their income through ICAR-Field Progeny Testing (FPT) Scheme

The genetics division is carrying out ICAR- Field Progeny Testing Scheme (FPT) in which 4813 registered dairy farmers are provided with good quality crossbred Holstein Friesian semen, veterinary services and supply of inputs like dewormers, feed / feed supplements and expert advice of scientists to farmers. The FPT scheme has made an outstanding achievement in the genetic improvement of dairy cattle of farmers registered



in the scheme in that the cows born out of FPT scheme had significantly higher milk production in their first lactation (3232.99 ± 35.23 Kg/lactation; 400 to 500 kg more) compared to its contemporaries and remarkably lower age at first calving. They developed prediction equations for lactation yield for dairy cattle from peak yield/test day milk yield which has practical applications under field conditions.

### **KVASU scientists coordinate the implementation of community based breeding programme amongst small holder Malabari goat farmers through AICRP on goat improvement (Malabari) scheme**

The Genetics and Breeding division is the frontrunner in the moulding of Malabari goat breed of Kerala, the best dual purpose goat breed in India, through the implementation of AICRP on goat improvement (Malabari) scheme among goat farmers in the breeding tracts of Malabari goats. The project is instrumental in the implementation of community based breeding programme for Malabari goats by which the mean inbreeding of Malabari goats was kept to a low level of 0.52%. The programme had recorded per cent of singles, twins, triplets and quadruplets born among Malabari goats as 47.03, 45.52, 7.28 and 0.17, respectively and reduced mortality rate of below 3.30 percent in the project area. Ten trainings with two days duration on goat rearing were organized to 470 goat farmers including 264 tribal farmers. The scheme is also contributed to the development of new treatment regime for contagious pustular dermatitis or orf and model goat shed for 20 goats for high rain fall area.

### **Rumen metagenome profile of Vechur cattle reveals significantly higher microbial diversity among Vechur cattle**

Post graduate research programme on rumen Metagenomics among Vechur and Crossbred cattle revealed significant difference in total microbial species abundance between the crossbred and Vechur cattle. Vechur, the dwarf cattle of Kerala displayed significantly higher microbial diversity compared to crossbred. As per literature, this is presumably the first report of rumen metagenome profile of Vechur cattle, a unique short breed of India.

### **Scientists identified differentially expressed genes in placenta associated with litter size and early kid survival in Malabari Goats**

Post graduate research programme identified a set of functional genes *EGR2*, *IGF1*, *WNT2*, *WNT9B*, *IL6*, *MARCH1* and *CD163L1*, *LIPG*, *FASN* and *LPL* in Malabari goats that were differentially expressed and associated with early kid mortality in Malabari goats. They also developed a novel and simple HRM protocol for the detection of an *indel* of seven base pair located in the promoter region of *MARCH1* gene that was associated with litter size in goats.

### **Chicken Growth Differentiation Factor 9 gene was analysed for markers associated with egg production traits**

DNA of White Leghorn chicken were screened for polymorphisms (SNPs / *indels*) in *GDF9* gene. A total of seven genetic variants and two *indels* were identified and most of them were associated with different egg production traits.

### **Double digest restriction associated DNA (ddRAD) sequencing revealed prolificacy related single nucleotide polymorphism markers in goats**

Post graduate research programme on ddRAD sequencing of goats, first of its kind in goats revealed numerous novel genetic variants influencing the complex traits in the genome of native goats of Kerala.

### **Genetic variants influencing the temperament of traits of Vechur cattle unravelled**

*Bos indicus* species inclusive of Vechur cattle are known for their aggressive behaviour. Post graduate study conducted in various candidate genes associated with behaviour in Vechur cattle revealed that genetic variants in *Plexin A2 (PLXA2)* and *Monoamine Oxidase A (MAO A)* had a significant association with temperament character in Vechur cattle.



### Best prediction model of lactation curve for crossbred cattle of Kerala developed

Lactation curve provides valuable information about the pattern of milk production during lactation. Post graduate research programme had developed best model of lactation curve in crossbred cattle of Kerala. This could be utilised for the prediction of milk yield in a lactation by using monthly test day milk yields in crossbred cattle with reasonable accuracy.

### Association of genetic variants in *ghrelin* and *inhibin alpha* genes with growth traits in goats established

Post graduate research programmes on genetic variability and expression profiles of *ghrelin* and *inhibin alpha* genes in goats revealed the significant influence of genetic variants of both genes with growth traits of native goats of Kerala.

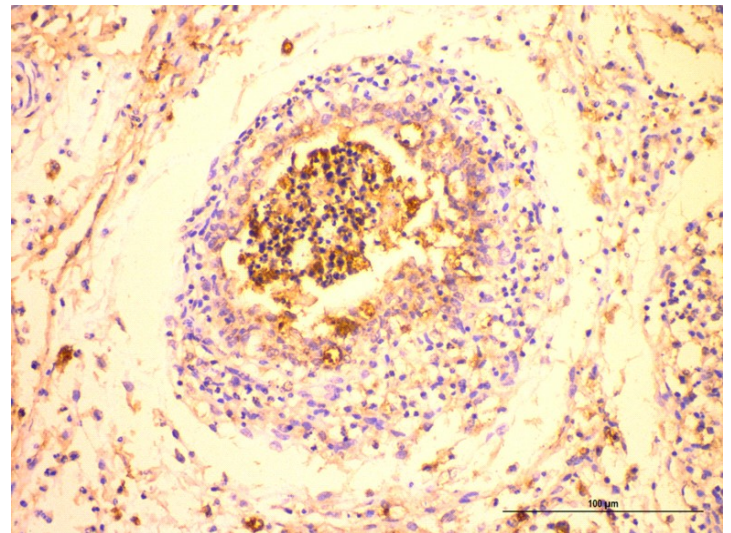
### Genetic studies reveal the uniqueness of Nilgiri Langur (*Trachypithecus johnii*)

Geneticists from College of Veterinary and Animal Sciences, Pookode had come up with the molecular evidence revealing the divergence of Nilgiri Langur (*Trachypithecus johnii*) from other Langur (*Semnopithecus* sp.) of Western Ghats. Their study was based on molecular and phylogenetic analysis of mitochondrial cytochrome b (*CYTB*) gene in the various species of Langur in the Western Ghats.

### Lymphoid depletion and infiltration of heterophils observed in bursa of chicks affected with subclinical IBD, while gross changes in bursa were absent

The study undertaken by the Department of Veterinary Pathology, CVAS, Mannuthy in unvaccinated poultry chicks below 3 weeks of age revealed that in contrast to clinical IBD where bursa is enlarged and oedematous, chicks affected with subclinical IBD did not exhibit any gross changes in bursa. Instead, the gross lesions exhibited were splenomegaly, hemorrhagic typhlitis and swollen congested

kidneys. The microscopic lesions noticed in subclinical IBD included lymphoid depletion and infiltration of heterophils in bursa, lymphoid depletion in spleen as well as congestion and decrease in thymocyte density in thymus. The study also revealed that the organ of choice for demonstrating IBD virus Ag by IHC was Bursa of Fabricius, in comparison to other lymphoid organs. Moreover, the study demonstrated an influx of CD4+ and CD8+ lymphocytes in the bursa of affected chicks.



(IBD: Bursa – Depletion of lymphocytes)

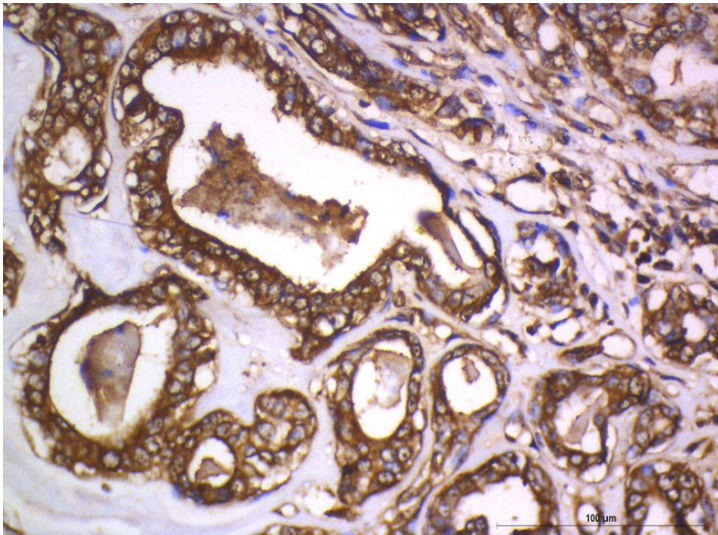
### Depletion of mucosa associated lymphoid tissue (MALT) and bone marrow found in porcine circovirus type 2 (PCV2) infected piglets

The study conducted by the Department of Veterinary Pathology, CVAS, Mannuthy on analysing MALT and bone marrow of PCV 2 infected animals revealed lymphoid depletion in various lymphoid organs. PCR targeting ORF-2 gene yielded 481bp product. Genotypic analysis revealed different PCV-2 genotypes – PCV-2d, PCV-2b and PCV-2h. PCV-2 associated systemic disease was confirmed in the study based on PCR results, history, clinical signs, gross, histopathological and ultra-structural findings as well as sequence data.

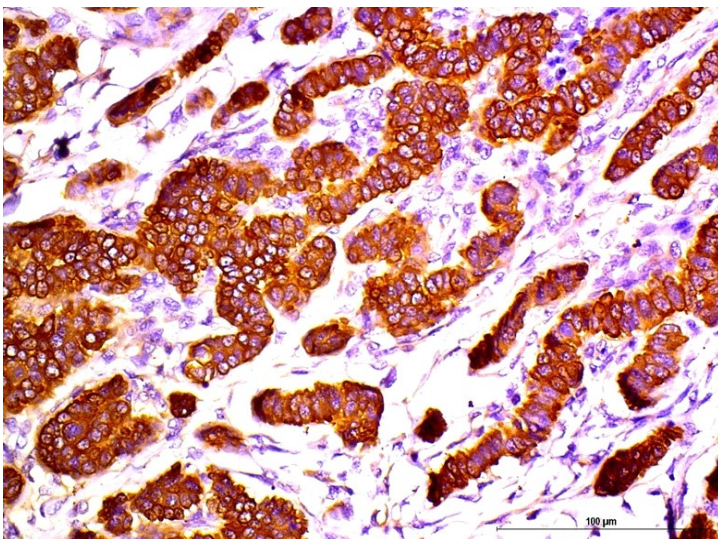


**The two proteins in cell signalling pathway – mTOR and DEPTOR are present in canine mammary and superficial tumours**

Immunohistochemical studies carried out in the Department of Veterinary Pathology, CVAS, Mannuthy confirmed the presence of two proteins in cell signalling pathway—mTOR and DEPTOR in canine mammary and superficial tumours. The expression of mTOR was significantly higher than that of DEPTOR in both the types of tumours studied. Furthermore, the expression of mTOR and DEPTOR were found to be positively correlated in canine mammary and superficial tumours. Thus, the study was instrumental in establishing the oncogenic role of these proteins in canine mammary and superficial tumours.



CABMT- DEPTOR strong expression (IHCx200)



Trichoblastoma - mTOR strong expression (IHCx400)

**Bile treated omental scaffold exhibited better decellularisation efficiency and remodelling response**

Three different biomaterials (bile treated omental scaffold - BBO, triton X-100 treated omental scaffold - TBO and unprocessed omental scaffold - UBO) were studied by Department of Veterinary Pathology, CVAS, Mannuthy to compare for comparing the efficiency of decellularisation as well as host cellular and tissue response. The study results revealed that bile treated scaffold exhibited better decellularisation efficiency and remodelling response. The results suggested that bile is an effective decellularising agent with less tissue toxicity compared to chemical detergent. The research could recommend bile treated omental scaffold as a promising candidate for application in human and veterinary medicine.



UBO – 14 days post implantation



BBO – 14 days post implantation

**TBO – 14 days post implantation*****Erythrina variegata******Tribulus terrestris* (NJERINGIL) protects against cyclophosphamide toxicity on reproductive system of male rats**

Protective effect of *Tribulus terrestris* (Njerinjil) in cyclophosphamide toxicity on reproductive system of male rats was investigated by scientists at Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy. Administration of cyclophosphamide significantly reduced body weight, testicular parameters, sperm motility, count, SOD, GSH, ACP and ALP. There was also significant increase in abnormal sperms, LDH, SDH and LPO levels. Histopathological studies in testis, seminal vesicles and epididymis confirmed toxicity in the form of degeneration and loss of germinal epithelium within the seminiferous tubules. *T. terrestris* extract supplementation significantly ameliorated all the above-mentioned parameters.

***Hordeum vulgare* and *Pergularia daemia* possess anticancer activity**

The anticancer activity of methanol extract of leaves of *Hordeum vulgare* and *Pergularia daemia* and the mode of anticancer action of the MDA-MB231 cell line was evaluated in Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy. Both the extracts showed superoxide anion radical scavenging activity and antioxidant activity. It was found that Hordenine in *Hordeum vulgare* and lupeol in *Pergularia daemia* are the compounds that contributed to their anticancer property.

***Erythrina variegata* (MURRIKKU) and *Mimosa pudica* (THOTTAVADI) possess anticancer potential**

The study conducted in Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy revealed the *in vitro* anticancer potential of *Erythrina variegata* (Murikku) and *Mimosa pudica* (Thottavadi) in MDA-MB-231 and MCF-7 breast cancer cell lines. Both the plant extracts possessed potent antioxidant activity *in vitro* when assessed using total antioxidant assay, DPPH free radical scavenging assay, superoxide free radical scavenging assay and nitric oxide free radical scavenging assay. The methanol extract of *E. variegata* and *M. pudica* were found to be having potent cytotoxic action.

***Mimosa pudica***

*Hordeum vulgare**Basella alba**Pergularia daemia**Artocarpus hirsutus*

**2, 3, 7, 8 Tetrachlorodibenzo paradioxin (TCDD) contamination detected in milk may increase oestrogen secretion**

The study performed in Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy detected the presence of TCDD in packaged, heated packaged and raw cow milk. Treatment with TCDD on human adrenocortical carcinoma (H295R) cell lines caused an increase in the oestrogen secretion by H295 R cells with maximum increase in cells treated with 3.1 and 1nM concentrations of TCDD upto 72 hours. There was also increase in pregnenolone and total cholesterol content of the cells. Treatment with 1nM TCDD caused a 53.82 fold increase in the expression of CYP1A1 mRNA where as a 6.27 fold increase was noted with P450sc.

**Methanolic extracts of *Basella alba* and *Artocarpus hirsutus* have anticancer activity**

A study conducted at Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy established that methanol extracts of whole plant of *B. alba* and seeds of *A. hirsutus* exhibited a concentration dependent anticancer activity with significant down regulation in the antiapoptotic Bcl-2 gene and protein expression in MDA-MB-231 and MCF-7 cell lines in vitro, indicating its potential to induce apoptosis. Phytochemical analysis of the extracts showed high levels of phenols, flavonoids and diterpenes which might be responsible for anticancer effect of plants.





### Lemongrass oil and citral alleviated methicillin/oxacillin resistance in *Staphylococcus aureus* isolates from bovine mastitis

The potential of Lemongrass oil (LGO) and citral in combating methicillin/oxacillin resistance in *Staphylococcus aureus* isolates from bovine mastitis was confirmed by a group of scientists from Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy. It was also identified that downregulation of *mecA* and antibiofilm activities by LGO and citral have ascribed to the antibiotic resistance modulation.



### *Crataeva nurvala* and *Garcinia gummigutta* possess anticancer activity in triple negative breast cancer cell line

The anticancer properties of methanol extract of *Crataeva nurvala* (Neermathalam) and *Garcinia gummigutta* (Kudampuli) in MDA-MB-231 cell line was explored at Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy. Methanol extracts of *C. nurvala* and *G. gummigutta* exhibited a concentration dependent cytotoxic activity in TNF- $\alpha$  stimulated MDA-MB-231 breast carcinoma cell lines. The plant extracts were effective in inducing apoptotic cell death via intrinsic and extrinsic pathways and might be considered as a source for isolating therapeutic molecules for the treatment of inflammatory breast cancer with TNBC phenotype.



### *Tamarindus indica* seed coat protects acetaminophen induced hepatotoxicity and nephrotoxicity

Study conducted in Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy revealed significant hepatoprotective, antioxidant and nephroprotective potentials of aqueous fraction of methanolic extract of *T. indica* seed coat against acetaminophen/paracetamol toxicity with a preliminary safety profile. Hence, *T. indica* seed coat might be recommended for advanced efficacy and toxicity studies to develop it as a promising therapeutic agent for the effective management of paracetamol toxicity in both humans and animals.



(a)



(b)

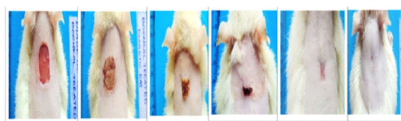


(c)

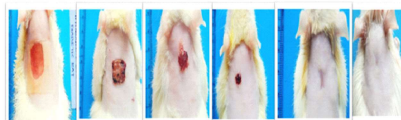
Fig : (a) pod, (b) seed and (c) seed coat of tamarind

### Borneol incorporated $\hat{E}$ -Carrageenan hydrogel shows promising wound healing activity

Wound healing activity of borneol and borneol incorporated  $\kappa$ -carrageenan- gelatin hydrogel coos linked with glutaraldehyde in normal rats and diabetic rats was assessed by scientists at Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy. It was found that the gel possess ambient morphological, physical chemical and biomedical characteristics suited for its use in animals and has the propensity to quickly heal wounds in normal and diabetic rat models without producing any systemic toxicities.



C. Borneol treated diabetic group



D. Borneol incorporated Hydrogel treated diabetic group

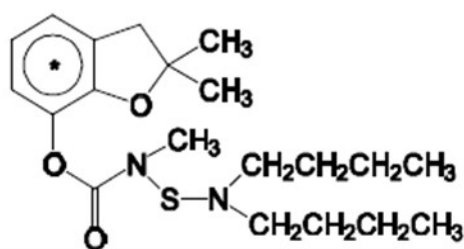
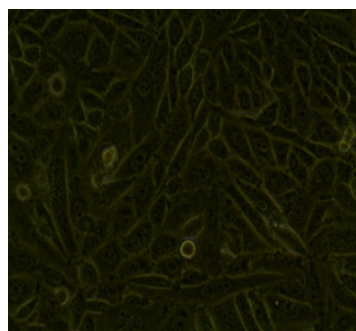
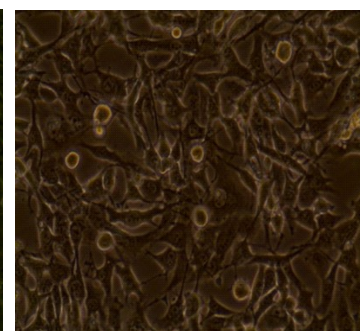


### Alcoholic extracts of *Asparagus racemosus* and *Boerhavia diffusa* modulated the concentration of oestrogen secretion from MCF-7 cells

The study conducted at Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy showed that methanolic extracts of *A. racemosus* increased the concentration of oestrogen secretion from MCF-7 cells whereas of *B. diffusa* and Naringenin significantly reduced the concentration of oestrogen in a dose dependent manner. *B. diffusa* and Naringenin significantly downregulated the expression of CYP19 gene compared to control groups whereas there was upregulation in *A. racemosus* treated cells. The extract of *B. diffusa* and Naringenin significantly downregulated the expression of StAR gene.

### Carbosulfan exhibit cytotoxic, carcinogenic and genotoxic effects in cultured mammalian cells

The study done by scientists at Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy observed that Carbosulfan treatment can cause time dependent carcinogenicity in BALB/c 3T3 clone A31 cells using cell transformation assay, while *in vitro* chromosomal aberration test revealed significant concentration and time dependent chromosomal aberrations by carbosulfan. *In vitro* micronucleus formation test also showed significant concentration and time dependent increase in the number of binucleate cells with micronuclei.

**Carbosulfan****CHO-K1 cells****BALB/c 3T3 clone A31 cells**

### Baicalein and piperlongumine nanoparticles show antineoplastic activity

Antitumour activity of nanoparticles synthesised from baicalein and piperlongumine was investigated at Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy on experimentally induced DLA model in mice. Biosynthesis and characterisation of nanoparticles of baicalein and piperlongumine was done and *in vitro* and *in vivo* antitumour properties of the biosynthesised nanoparticles were assessed. The biosynthesised nanoparticles showed anticancer activity with significant induction of apoptosis by down regulation of *Bcl-2* and up regulation of *Caspase-3*, *p53* genes. Generation of Reactive Oxygen Species also contributed to their anticancer property. Piperlongumine nanoparticles showed more potent antineoplastic activity than baicalein nanoparticles.

### *Campylobacter* spp. survived or exhibited increase in count in sterile water samples

Water samples from seven rivers of Thrissur district were subjected to study the survivability of *Campylobacter* spp. by a team from Department of Veterinary Public Health, CVAS, Mannuthy. The organism survived or exhibited an increase in count by around one log in sterile water samples at both 4°C and 25°C. It showed an almost sharp dip in the count in unsterile samples. Excepting the unsterile sample from Chalakudy, *Campylobacter* spp. could not be detected from other sources by 160<sup>th</sup> day of storage. *Campylobacter* spp. could not survive in sterile water at 160 days of storage at 25°C.



### ***Campylobacter* spp. Critical control points in beef production chain was identified as dung and hide of the animal**

The study conducted at Department of Veterinary Public Health, CVAS, Mannuthy observed prevalence of *Campylobacter* spp. in dung sample in a cattle farm in Thrissur district as 8 per cent. The overall occurrence of *Campylobacter* spp. in the meat processing plant I and II were 9.16 and 15.9 respectively. The primary source of contamination in the processing unit were the dung and hide of the animal.

### **Occurrence of *Campylobacter* spp. in swine production facilities and pork processing lines**

Prevalence of *Campylobacter* spp. in pigs was identified by a team in Department of Veterinary Public Health, CVAS, Mannuthy as 33.3 per cent and 21.70 per cent in Farm 1 and Farm 2 in Thrissur district, respectively. Migratory pathway of infected wild reservoirs resulted in cross-transmission among birds and pigs. Freezing and scalding were established as Critical Control Points in pork processing plants. *C. coli* isolates showed cent per cent resistance against Ceftazidime, Co-trimoxazole and Ofloxacin. Cent per cent of the *C. jejuni* isolates showed resistance against Ceftazidime. The Multiple Antimicrobial Resistance (MAR) index of isolates was in the range of 0.21-0.87.

### **Enterohaemorrhagic *Escherichia coli* on meat contact surfaces was mitigated by phytochemicals**

The study done in Department of Veterinary Public Health, CVAS, Mannuthy revealed that plant essential oils viz., cinnamaldehyde, eugenol and allicin exhibited maximum activity against enterohaemorrhagic *E. coli* (EHEC) by incorporation in beef which provides safe alternatives. EHEC isolates comprising of 30 isolates from cutting board and 10 isolates from knife surfaces were obtained.

### **Residues of commonly used antibiotics were detected and quantified in raw milk**

The quantification of antibiotics in milk samples by scientists of Department of Veterinary Public Health, CVAS, Mannuthy revealed that the oxytetracycline, enrofloxacin and cloxacilin had mean concentration of 1430, 530 and 118.77 ng/mL which was much above the MRL levels. The survey conducted as the part of the project revealed that seventy eight per cent of the people were aware of the use of antibiotic and 43 per cent of people had knowledge about the withdrawal period.

### **Oxytetracycline and enrofloxacin residues in milk samples in Palakkad district was assessed**

The occurrence of tetracycline and enrofloxacin residues in pooled raw milk samples was found to be 1.05 and 1.31 per cent using charm assay in the study done by Department of Veterinary Public Health, CVAS, Mannuthy. The mean concentration of oxytetracycline and enrofloxacin residues in pooled raw milk samples were estimated to be 272.11 and 163.79 ng/mL using HPLC, respectively.

### **Seroprevalence of leptospirosis in cattle and human of Alappuzha district was assessed**

A seropositivity of 52.23 and 29.5 per cent in cattle and 29.4 and 15.6 per cent in humans were observed in Mannancherry and Mararikulam panchayats of Alapuzha district by a study conducted by Department of Veterinary Public Health, CVAS, Mannuthy. By MAT, an overall seroprevalence of leptospirosis in cattle of 40 per cent was observed in Mannancherry and Marrarikulam panchayats of Alapuzha district at a titre between 1:50 and 1: 200.

### ***Moringa oleifera* and *Citrullus lanatus* treated water showed highest acceptability in sensory evaluation for odour and taste**

A study conducted in Department of Veterinary Public Health, CVAS, Mannuthy showed the minimum inhibitory concentration of *M. oleifera* against *E. coli* and Faecal *Streptococci* as 14 and 12 mg/mL, respectively. In a comparative study with chlorine and iodine, *M. oleifera* and *C. lanatus* seed extracts showed satisfactory results on water quality. However complete reduction of microbial count occurred with chlorine and iodine treatments. *M. oleifera* treated water showed highest acceptability in sensory evaluation for odour and taste. Enteroaggregative *Escherichia coli* were prevalent in animals, human infants and associated environmental sources in Ernakulam.

Occurrence of Enteroaggregative *E. coli* in young animals, human infants and associated environment sources in Ernakulam district were assessed and reported by Department of Veterinary Public Health, CVAS, Mannuthy as 21.5, 5.89 and 15.69 per cent. The study showed that 9.52, 9.52 and 4.76 per cent of *E. coli* isolates from calf and piglet diarrhoeal samples harboured *astA*, *pic*, *aggR* and *fimA* genes, respectively suggestive of EAEC.



### ***Campylobacter* spp. were detected in duck, quail, backyard chicken and associated environmental samples**

Occurrence of the organism in duck rearing facility was studied by Department of Veterinary Public Health, CVAS, Mannuthy as 10, 8 and 2 per cent from cloacal swabs, soil and drinking water samples respectively. The overall occurrence in quail rearing facility was 10.45 per cent. Multiple antimicrobial resistance (MAR) index of the *Campylobacter* isolates was within the range of 0.06-0.89.

### **Extended spectrum beta lactamase (ESBL) producing *Klebsiella pneumoniae* and *Salmonella* spp. were isolated from environmental samples**

Out of the 420 water (pond, river and paddy field) and soil samples collected from Thrissur district, 58.57 per cent and 12.62 per cent samples, respectively contained *Klebsiella pneumoniae* and *Salmonella* spp. as per the study conducted by Department of Veterinary Public Health, CVAS, Mannuthy. Totally 68 *K. pneumoniae* and 20 *Salmonella* spp. isolates were confirmed as extended spectrum beta lactamase producers.

### **Cloacal carriage and antimicrobial resistance profiling of extended spectrum beta-lactamase producing *Escherichia coli* and *Salmonella Enteritidis* was identified in broiler chicken**

*Escherichia coli* and *Salmonella* spp. were detected in 77.8 and 9.2 per cent of broiler chicken cloacal samples from Kottayam and Kollam in a study conducted by Department of Veterinary Public Health, CVAS, Mannuthy. *Escherichia coli* isolates showed resistance to ampicillin, cephalosporins and amoxicillin-clauvanic acid.

### ***Escherichia coli* pathotypes in drinking water were isolated and characterized**

*Escherichia coli*, ETEC, EAEC and EHEC were detected in 51.09, 0.20, 12.87 and 5.35 per cent of water samples in a study done by Department of Veterinary Public

Health, CVAS, Mannuthy. Occurrence of *E. coli* in drinking water had highly significant association with the distance of well from the septic tank

### **Pathogenic leptospires were detected in slaughtered cattle of Thrissur**

Seropositivity of leptospirosis in slaughtered cattle and slaughterhouse workers from two organized slaughter houses in Thrissur were identified as 44.24 and 32 per cent by a team from Department of Veterinary Public Health, CVAS, Mannuthy. Among cattle slaughtered in Thrissur, the predominant serovars detected were Sejroe, Grippotyphosa and Australis. The predominant serovars detected in slaughterhouse workers were Djasiman and Bataviae

### ***Campylobacter* spp. were identified and characterised in sea foods and associated environment of Kerala**

Occurrence of *Campylobacter* spp. in seafoods was identified as 12.24 per cent, 11.76 per cent, 13.51 per cent in marine fishes, crustacean and molluscs respectively in the study conducted by Department of Veterinary Public Health, CVAS, Mannuthy. The *C. jejuni* NCTC 11168, and *C. coli* survived for 150 days in autoclaved water at 25°C. Sanitisers like hot water, chitosan, bleaching powder and aqueous extract of *Eichhornia crassipes* (water hyacinth) were effective on biofilms of the *C. jejuni* and *C. coli* at various combination treatments.

### **Drug resistant and biofilm forming food borne pathogenic bacteria were detected in retail chicken and the *Couroupita guianensis* extract could combat these drug resistant bacteria**

The prevalence of *E. coli*, *Salmonella* spp. and *Campylobacter* spp. from retail chicken of Kerala was detected as 54.17, 14.33 and 17.17 per cent, respectively in the study done at Department of Veterinary Public Health, CVAS, Mannuthy. The highest per cent of resistance was detected to nalidixic acid in *E. coli* and to tetracycline in *Salmonella* spp. and *Campylobacter* spp. The aqueous cold extract of *C. guianensis* was found to have good antibacterial property against drug resistant *E. coli*, *Salmonella* spp. and *Campylobacter* spp.

**Pathology of tumours of canine digestive system with special reference to galectin-3 expression**

A study was conducted by the Department of Veterinary Pathology, CVAS, Pookode, to obtain the incidence of tumours of digestive system in dogs and to evaluate the expression of Galectin-3, a putative tumour marker of different tumours. The incidence of tumours involving digestive system was found to be 7.14 per cent with highest incidence in dogs of age group 7 to 9 years. The study encountered benign tumours such as oral papilloma, acanthomatous ameloblastoma, desmoplastic ameloblastoma, peripheral odontogenic fibroma and hepatoid gland adenoma, while malignant tumours such as oral melanoma, oral fibrosarcoma, squamous cell carcinoma, basal cell carcinoma, extramedullary plasmacytoma, hepatic lymphoma and hepatocellular carcinoma were also reported. Immunohistochemical assessment of galectin-3 expression in different tumours and corresponding normal tissues showed that the marker was overexpressed in oral melanoma and downregulated in other oral tumours of epithelial and mesenchymal origin.

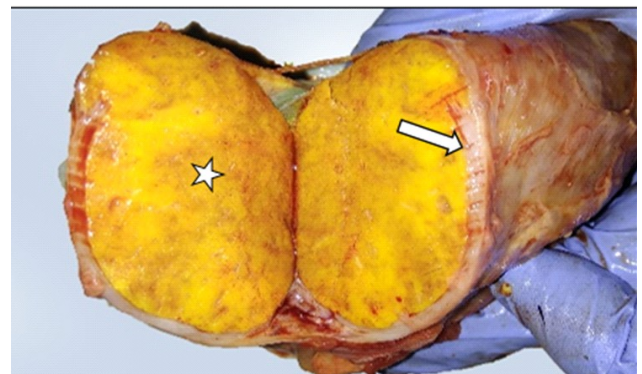
**Oral melanoma - strong expression of galectin-3 (DAB, 400X)****Application of molecular diagnostic tools for detection of nervous infections of viral etiology in dogs and cats**

A comprehensive study was performed in the Department of Veterinary Pathology, CVAS, Pookode, aimed at detecting the presence of rabies virus, canine distemper virus, feline

parvovirus and feline infectious peritonitis virus in dogs and cats in Kerala. PCR/RT-PCR was employed for the detection of viral nucleic acid. The application of the molecular diagnostic tools could detect the presence of rabies, canine distemper and feline panleukopenia viruses. The sequence analyses showed that these viruses were closely related to other Indian isolates. Lesions like Negri body formation, Babes nodules, perivascular cuffing, hypoplasia of pyramidal cells and necrosis of Purkinje cells were the important histopathological findings observed in brain tissues of positive cases.

**Pathological studies of entero-hepatic affections in ducks and screening for specific viral etiology**

The study encompassed screening in ducks showing hepatic and intestinal lesions for viruses such as duck enteritis virus, duck hepatitis A virus and duck astrovirus I, by PCR. The study couldn't detect any viruses, however, it was interesting to notice the widely present hepatic amyloidosis (61 per cent of the birds examined). It was observed that the ducks farmed under intensive system of rearing had a higher incidence of amyloidosis, particularly in White Pekin ducks.

**Amyloidosis-Liver- Diffuse capsular thickening (arrow) and yellow parenchyma (star) in cut section of liver.****Extended spectrum  $\beta$ - lactamase producing enteric-bacterial pathogens detected from raw milk samples**

Emergence of Extended spectrum beta lactamase (ESBL) producing organisms are a major concern in medical practice. The distribution status of ESBL producing *E. coli* and *Salmonella* spp. from raw milk samples was studied. A total of 50 raw milk samples were analyzed by the Department of



Veterinary Public Health, CVAS, Pookode, for the *E. coli* and *Salmonella* through conventional culture method and confirmed by PCR using *uidA* gene and *invA* gene, respectively. The occurrence of *E. coli* and *Salmonella* spp. in raw milk was found to be 60 per cent and 18 per cent, respectively. Of these, 21 *E. coli* (70%) and 7 *Salmonella* (77.77%) were ESBL producers and resistant to third generation cephalosporins. The ESBL genes,  $bla_{TEM}$ ,  $bla_{CTX-M}$  and  $bla_{SHV}$  were present in 56.66 per cent, 40 per cent and 26.66 per cent of the *E. coli* isolates, respectively. The occurrence of  $bla_{SHV}$ ,  $bla_{CTX-M}$  and  $bla_{TEM}$  genes in *Salmonella* isolates was 55.5 per cent, 44.4 per cent and 11.1 per cent, respectively. Thus, the present study highlighted the need of hygienic milk production and antibiotic stewardship to prevent spill over of resistant pathogenic organisms among the public and the environment.

### Enteroaggregative *Escherichia coli* detected from diarrhoeal samples of human infants and calves

Enteroaggregative *Escherichia coli* (EAEC) has been implicated as an emerging cause of persistent infantile diarrhoea in developing countries. In a study conducted in the Department of Veterinary Public Health, CVAS, Pookode, a total of 120 diarrhoeal samples were collected from human infants (60) and calves (60). All the samples were subjected to isolation and identification of *E. coli* and were further characterized for EAEC by PCR amplification of plasmid-borne (*aggR*) and chromosomal associated (*fimA*) genes. Out of 91 *E. coli* isolates, 28 were found positive for *fimA* (30.76%) gene and 7 were positive for *aggR* (7.69%) gene. Ten isolates showed cell surface hydrophobicity by the salt aggregation test. Antimicrobial susceptibility testing by disc diffusion method revealed that the typical EAEC isolates exhibited maximum resistance towards methicillin, tetracycline and nalidixic acid. The study will provide preliminary data of EAEC from human infants and calf in Wayanad district of Kerala which could form a base for future research.

### Nasal carriage of *Staphylococcus aureus* and MRSA among companion animals and livestock

Antimicrobial resistance (AMR) is one of the leading threats in healthcare management system. The Department of Veterinary Public Health, CVAS, Pookode, ventured to evaluate the nasal carriage of *S. aureus* and methicillin-

resistant *S. aureus* (MRSA) among apparently healthy companion animals and livestock. A total of 160 nasal swabs were collected from dogs (30), cats (30), cattle (40), goats (30), and pigs (30). All the samples were initially screened for prevalence of *S. aureus*, which was confirmed by PCR using species specific primer (*nuc* gene). There was significant difference in nasal carriage of *S. aureus* among animal population (Chi-square value – 33.206, p-value < 0.001), which was found as 76.67 per cent, 23.33 per cent, 53.33 per cent, 62.5 per cent, and 86.67 per cent among dogs, cats, cattle, goats, and pigs respectively. All the *S. aureus* isolates were screened for occurrence of MRSA, both phenotypically (by double disc diffusion using cefoxitin and oxacillin discs) and genotypically (by PCR targeting *mecA* gene). None of the nasal swabs from animals were positive for MRSA. The present study signifies the occurrence of nasal carriage of *S. aureus* and absence of MRSA among domestic animal population of Wayanad district.

### Expression and purification of an immunogenic SUMO-OmpC fusion protein of *Salmonella* Typhimurium in *Escherichia coli*

*Salmonella* is found to be a major cause of food borne diseases globally. Poultry products contaminated with this pathogen is one of the major sources of infection in humans. Outer membrane protein C (OmpC) of *Salmonella* Typhimurium is a promising DNA vaccine candidate to mitigate *Salmonella* infection in poultry. However, the large-scale production of bioactive recombinant OmpC (rOmpC) protein is hindered due to the formation of inclusion bodies in *Escherichia coli*. In a study conducted in the Department of Veterinary Public Health, CVAS, Pookode, high level expression of rOmpC protein was obtained. The *ompC* gene was optimized and fused with small ubiquitin-related modifier (SUMO) gene. The fusion protein with ~58/ kDa molecular weight was observed on SDS-PAGE gel. The expression levels of rOmpC fusion protein reached maximum of 38 per cent of total soluble protein (TSP) after 8/ hours of 0.2 per cent rhamnose induction. Protein purification was carried out using nickel nitrilotriacetic acid (Ni-NTA) purification column. Western blot were performed to analyse expression and immunoreactivity of rOmpC fusion protein. The results indicate that SUMO fusion system is ideal for large scale production of functional rOmpC fusion protein by expression in *E. coli*.



### **ESBL producing *Yersinia enterocolitica* detected among companion animals in Wayanad district**

Antibiotic resistant strains in *Yersinia* spp. among companion animals is one of the unexplored sectors and a major public health concern. In a study conducted by the Department of Veterinary Public Health, CVAS, Pookode, a total of 100 faecal samples/swabs from dogs (50) and cats (50) were analyzed through conventional culture and molecular methods using 16S rRNA PCR-RFLP. The occurrence of *Y. enterocolitica* was recorded in 12 samples. Species specific-PCR revealed that 12 per cent of samples were identified as *Y. enterocolitica* (ystA). Further, characterization of isolates for ESBL production through phenotypic disc diffusion method revealed that 41.66 per cent of *Y. enterocolitica* isolates were ESBL producers and on genotypic PCR assay it was observed that 33.33 per cent of isolates in dogs harboured the *bla*<sub>TEM</sub> gene. Likewise, 50 per cent of *Yersinia* isolates (each) recovered from dogs were found to harbour *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> ESBL genes. Thus, the present study signifies the importance of virulent *Yersinia* strains harbouring ESBL resistance genes and their dissemination in companion animals, which have the potential to infect humans.

### **Assessment of knowledge of rabies among school children**

Rabies is a 100 per cent fatal and a 100 per cent preventable zoonotic disease, caused by rabies virus, a *Lyssavirus*. Although all age groups are affected, dog bite is most common in children in India. Hence, awareness among the young generation is the key component of successful control programs. A survey on rabies awareness was undertaken by the Department of Veterinary Public Health, CVAS, Pookode, among 545 school students in six randomly selected schools of Calicut District, Kerala. A total of 63.3 per cent of the students recognized Rabies as a major public health threat. Survey also revealed that 51.2 per cent of the students were aware about the transmission of rabies and 45.7 per cent of the students recalled that they have been taught about rabies in their school. Nearly, one third of the students (33%) were unaware about the clinical symptoms shown by rabid dogs and 28.8 per cent were unaware about the clinical symptoms

shown by affected humans. A total of 79.7 per cent students were aware about anti-rabies vaccination for humans. The work revealed the need to create more awareness on importance of rabies among school children as the first step towards disease control.

### **Detection of antibiotic resistant bacteria from the enteric tract/ faecal samples of chicken**

A study was carried out by the Department of Veterinary Microbiology, CVAS, Mannuthy, in which a total of fifty samples, thirty from dead chicken and twenty from healthy chicken were collected. *Staphylococcus aureus* (5), *Klebsiella pneumoniae* (7) and *Escherichia coli* (32) were isolated and tested for antibiotic sensitivity pattern by disc diffusion method. Most of the isolates exhibited high degree of resistance to antibiotics such as enrofloxacin, ofloxacin, clindamycin, ciprofloxacin and methicillin. Among the isolates, one *E. coli* was resistant to all the antibiotics tested. Eight isolates including one *K. pneumoniae* and seven *E. coli* were found to be resistant to one or more than one carbapenems tested viz., imipenem, meropenem and ertapenem.

### **Detection of leptospira in seroreactive goats by isolation and polymerase chain reaction**

In a study conducted by the Department of Veterinary Microbiology, CVAS, Mannuthy, seroprevalence of leptospirosis in goats in five districts of Kerala, Thrissur, Malappuram, Palakkad, Kozhikkode and Kannur, was studied by microscopic agglutination test and it was found to be 10.80 per cent. Serogroup Pomona was the most prevalent one.

### **Detection of Carbapenem resistant *E. coli* in cattle and pigs**

The Department of Veterinary Microbiology, CVAS, Mannuthy confirmed the presence of carbapenem resistant *E. coli* in cattle and pigs. Faecal samples and mastitic milk samples from cattle as well as faecal samples and slaughter house samples from pigs were examined and 23 *E. coli* isolates were obtained. The sensitivity pattern against carbapenems viz., ertapenem, imipenem and meropenem were analysed for all the isolates. Eight isolates were found to be resistant to carbapenems.



### Molecular detection of infectious bronchitis virus in chicken

This study was carried out by the Department of Veterinary Microbiology, CVAS, Mannuthy, among 95 samples collected from poultry with suspected cases of respiratory ailments. Out of 95, 45 samples were found positive for the presence of infectious bronchitis virus (IBV). The isolates revealed more than 95 % similarity with the vaccine strain as well as with other Indian isolates. On phylogenetic analysis of hypervariable regions 1 and 2 of the S1 subunit of S gene, all isolates clustered with each other, whereas, on phylogenetic analysis of hypervariable region 3, all the isolates were branched separately among themselves and also with the IBV isolates from different regions of the country.

### Detection of carbapenem resistance among *Esherichia coli*, *Kliebsiella pnuemoniae* and *Pseudomonas aeruginosa* associated with skin and urogenital tract infections in dogs

This study was conducted by the Department of Veterinary Microbiology, CVAS, Mannuthy on 100 samples collected from dogs. Out of these 100 samples, fifty three *Staphylococcus* spp., two Gram positive bacilli, thirty *E. coli*, eleven *K. pneumoniae* and three *P. aeruginosa* were isolated. Forty-two Gram negative bacilli were found to be multidrug resistant. A high prevalence of carbapenem resistance (31 per cent) was noted among the isolates and on phenotypic confirmation tests; the presence of major carbapenemase (KPC and NDM) was detected.

### Comparative efficacy of loop-mediated isothermal amplification (LAMP), real-time PCR and immunochromatography for the detection of *Brucella* in bovines

The study was conducted by the Department of Veterinary Microbiology, CVAS, Mannuthy. LAMP showed a higher sensitivity (100 per cent) and specificity (86 per cent) in comparison to real time PCR (sensitivity 60 per cent and 86 per cent) by taking immunochromatography as the standard test. More numbers of positive cases of brucellosis were reported from private farms compared to government farms when analyzed by both LAMP and real time PCR.

### Comparison of molecular methods for determination of genetic variation among *Riemerella anatipestifer* isolates

Different molecular methods were compared at the Department of Veterinary Microbiology, CVAS, Mannuthy, to determine the genetic variations among the *Riemerella anatipestifer* isolates. Repetitive-sequence based PCR (REP-PCR) and Polymerase chain reaction- Restriction fragment length polymorphism (PCR-RFLP) showed similarity with pulsed field gel electrophoresis (PFGE), which distinguished the isolates into three pulsotypes. Although all methods displaying broadly similar discriminatory powers, REP-PCR subtyping proved to be a much easier, cheaper and more rapid method. It was also concluded that to group the local isolates with universally categorised isolates, one or more of the different typing methods need to be applied in conjunction with conventional methods.

### Identification of Gram-positive agents involved in bovine mastitis

A study conducted by the Department of Veterinary Microbiology, CVAS, Mannuthy, to identify Gram-positive agents involved in bovine mastitis revealed *Staphylococcus aureus* as the major organism involved, followed by *Streptococcus uberis*. Antibiotic sensitivity test revealed that all the *S. aureus* isolate were sensitive to gentamicin, amikacin, norfloxacin, imipenem and piperacillin/tazobactam and hence could be recommended as effective drugs to treat mastitis in the field. They showed resistance against the currently used drugs to treat mastitis like tetracycline, enrofloxacin and third generation cephalosporins. One of the *S. aureus* isolates was resistant to methicillin (12.5 per cent) and might be a methicillin resistant *Staphylococcus aureus* (MRSA).

### Isolation and identification of bacterial agents associated with polyarthritis in calves

A study was conducted by the Department of Veterinary Microbiology, CVAS, Mannuthy on isolation and identification of bacterial agents associated with polyarthritis in calves. Based on cultural, morphological and biochemical tests, the bacteria agents were identified as *Staphylococcus aureus* (38.9 per cent), *S. epidermidis* (11.1 per cent), *Streptococcus* spp. (11.1 per cent), *Corynebacterium jeikeium* (5.6 per





cent), *Escherichia coli* (5.6 per cent), *Klebsiella* spp. (5.6 per cent), *Pseudomonas aeruginosa* (5.6 per cent) and *Salmonella* spp. (5.6 per cent). On antibiogram, Gentamicin was found to be the most effective drug against the isolates obtained. Polymerase chain reaction (PCR) was standardised for the rapid identification of *Corynebacterium* spp. and *Staphylococcus* spp.

### Preparation and evaluation of a subunit vaccine against *Riemerella anatipestifer*

A subunit vaccine employing recombinant outer membrane protein A (rOmpA) of *Riemerella anatipestifer* was prepared by the Department of Veterinary Microbiology, CVAS, Mannuthy. Evaluation of comparative efficacy of the developed vaccine with that of a standardised inactivated vaccine was performed. It was found that the inactivated vaccine was superior in terms of results obtained from the challenge study, antibody titre, cell mediated immune response and gene expression analysis than the subunit one. Hence, it was desirable to advocate the use of inactivated vaccine in field condition owing to its easiness to prepare and low cost.

### Isolation and genotypic characterisation of infectious bronchitis virus isolates from Kerala

This study was carried out as part of the DST-SERB PROJECT in the Department of Veterinary Microbiology, CVAS, Mannuthy. In the study, RT-PCR assays targeting the 5'UTR and partial region of S1 subunit of spike gene was standardised for the direct detection of virus from clinical samples and also, two Taqman® probe based real time RT-PCR assays targeting 5'UTR and conserved region of N gene were developed for the rapid detection of the virus from cases of field outbreaks. We have developed RT-PCR assays targeting complete S1 subunit of spike gene, N gene and M gene and the representative amplicons from different zones of Kerala were sequenced and the sequences were compared with that of vaccine strain and other Indian isolates. Nucleotide variations were noted in the isolates belonging to different zones of Kerala and also with reference strain and other sequences from India.

### Development of a latex agglutination test for on-farm diagnosis of contagious caprine pleuropneumonia (CCPP) in goats

Isolation of *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) and development of a latex

agglutination test for diagnosis of contagious caprine pleuropneumonia (CCPP) in goats was carried out as part of the AHD research project in the Department of Veterinary Microbiology, CVAS, Mannuthy. For this, nasal swabs and postmortem tissue samples collected from goats with respiratory infection. Genus specific 16S rRNA PCR was carried out and positive samples obtained were later subjected to *Mycoplasma mycoides* subsp. *capri* (Mmc) specific PCR and nucleotide sequencing. None of the samples were found to be positive in Mccp specific PCR. Nucleotide sequencing revealed the presence of *M. ovipneumoniae*, *M. conjunctivae*, *M. agalactiae* and *M. capri*. Two of the samples produced minute colonies on modified Hayflick's solid medium and typical fried egg appearance of colonies could be appreciated under inverted microscope. Later these were identified as Mmc on PCR.

### Development of an antigen detection Latex agglutination test for the diagnosis of mycoplasmosis in goats

This study was conducted in the Department of Veterinary Microbiology, CVAS, Mannuthy, as continuation of the research project. One hundred nasal swabs and twenty six post mortem tissue samples were taken for the study. On Mycoplasma 16S rRNA genus specific PCR, 69 samples turned positive. The isolation rate was found to be 2.94 %. An antigen detection LAT was standardised by sensitising latex beads with hyperimmune serum raised against *Mycoplasma mycoides* subsp. *capri*. In comparison with PCR, LAT had a sensitivity of 70 per cent and specificity of 100 per cent.

### Serological and molecular diagnosis of campylobacteriosis associated with bovine abortion

The Department of Veterinary Microbiology, CVAS, Mannuthy, conducted a study on "Serological and molecular diagnosis of campylobacteriosis associated with bovine abortion" in which PCR and real time PCR were standardised using reference cultures of *Campylobacter fetus* subsp. *fetus* and *Campylobacter fetus* subsp. *venerealis*. However, on performing these tests for the diagnosis of campylobacteriosis in bovines at Thrissur district, none of the clinical samples tested were positive. Similarly, ELISA was standardised for detecting antibodies from clinical samples of bovines and all the serum samples from Thrissur district were found to be



negative for campylobacteriosis. The study indicated that the infection caused by *Campylobacter foetus* rarely caused abortion in bovines in Thrissur district.

### Isolation of *Leptospira* spp. from infected animals and use of multilocus sequence typing for serovar identification

A study was undertaken by the Department of Veterinary Microbiology, CVAS, Mannuthy, to isolate *Leptospira* from infected animals and rats and to perform multilocus sequence typing (MLST) for the serovar level identification. The seroprevalence recorded using microscopic agglutination test (MAT) was 20.97 per cent. Among the 205 samples, 15 were confirmed to have the presence of pathogenic *Leptospira* in PCR and ten isolates could be obtained. The isolates were further subjected to *secY*, *icdA* and *GyraseB* PCR and sequenced. Among the ten isolates, nine could be identified at the serovar level. The serovar of the isolates identified by MLST were perfectly matching with that of MAT. It was concluded that MLST could be used as an alternative to the whole genome sequencing and other cumbersome procedures for the serovar level identification of *Leptospira*. Further, it is recommended that for getting more promising results in MLST, the sequence data from at least seven genes need to be employed.

### Comparison of Giemsa, Leishman and Acridine orange staining techniques for diagnosis of bacterial infection in blood

A comparison of different staining techniques, Giemsa, Leishman and Acridine orange for diagnosing bacterial infection in blood was carried out by the Department of Veterinary Microbiology, CVAS, Mannuthy. Blood smears from sixty animals were taken *i.e.*, two smears for each of the three stains and thus a total of 360 smears were examined for the study. Based on the analysis of data with respect to visualisation characteristics, Acridine Orange stain was found to be superior compared to Giemsa and Leishman.

### Serological and molecular diagnosis of *Peste des Petits Ruminants* (PPR) in Kerala

The Department of Veterinary Microbiology, CVAS, Mannuthy carried out a study on serological and molecular diagnosis of peste des petits ruminants (PPR) in Kerala. Serum

samples were collected from 300 goats, including 150 healthy animals maintained in government as well as private farms across Kerala and 150 animals with clinical signs of PPR. Among these, only 30 healthy animals were vaccinated. Nasal swab samples, faecal samples, blood samples were also collected from 150 goats with clinical signs of PPR. Eleven samples were positive in real time PCR. Four were subjected to sequencing and on phylogenetic analysis, it was found that they clustered together and also with other Indian isolates belonging to lineage IV. An indirect ELISA was standardised for the detection of anti-PPR antibodies. Eight out of 150 samples from clinically ill animals and one among the 150 healthy animals were found to be positive. This study helped in detecting the magnitude of the disease and to find the evolutionary relationship of the virus with other isolates.

### Characterisation of the New Castle disease virus isolates prevalent in Kerala by RT-PCR

The New Castle disease virus isolates prevalent in Kerala were characterised by RT-PCR, intra cerebral pathogenicity index and mean death time in the Department of Veterinary Microbiology, CVAS, Mannuthy. Samples collected from dead and ailing birds showing lesions and signs suggestive of ND formed the materials for the study. Out of 63 samples, 8 revealed the virus by RT-PCR targeting the F gene directly from the tissue samples. The virus could be isolated from all the samples and confirmed by haemagglutination inhibition test. Based on the intra-cerebral pathogenicity index and mean death time, three of the isolates were classified under velogenic group and the remaining under mesogenic group. On phylogenetic analysis of the fusion protein cleavage site of the F gene, all the isolates were grouped in to genotype II of class II viruses. In this study, NDV isolates with lentogenic FPCS region belonging to velogenic and mesogenic pathotypes were obtained.

### Detection and molecular characterisation of emerging viral pathogens of pigs

An externally aided project on emerging viral pathogens of pigs could detect the presence of porcine reproductive and respiratory syndrome virus (PRRSV) in pigs in North Kerala. Now, after the inclusion of PRRSV in the diagnostic panel of infectious agents of pigs (along with classical swine fever, porcine circovirus 2, porcine parvovirus and rotavirus of pigs which are prevalent in Kerala), accurate diagnosis can be carried out. Under the project, facility for diagnosis of the



above mentioned viral diseases has been created in the University. This is a great help to the pig farmers and to the clinicians as they can chart out specific control measures more effectively. Under the project, the genotyping of rotaviruses of pigs in North Kerala was carried out for the first time in the State.

### Detection and molecular characterisation of rotavirus of pigs

One of the important health problems in suckling and recently weaned piglets is neonatal diarrhoea. A study was undertaken to detect the presence of rotavirus in faecal samples of piglets by reverse transcriptase polymerase chain reaction (RT-PCR) and to genotype the virus by nucleotide sequencing. A total of 100 diarrhoeic faecal samples were collected from piglets reared in organized farms in Wayanad, Kozhikode, Palakkad, Thrissur and Ernakulam districts of Kerala. All these faecal samples were screened for the presence of porcine rotavirus (PRV) by RT-PCR. Of the 100 diarrhoeic faecal samples, 12 (12 per cent) samples was found to be positive for VP6 gene and positive samples were obtained from Palakkad and Wayanad districts only. Some of the sequences showed close similarity to rotaviruses isolated from humans and from bovines. The results of the study indicated that the rotaviruses of pigs in Kerala are genetically diverse.

### Molecular detection and characterisation of Infectious Bursal Disease Virus (IBDV) in poultry

A study on molecular detection and comparative genetic analysis of the hypervariable region of the VP2 gene of IBD virus from the field outbreaks in Kerala was carried out. Genetic analysis of 22 isolates from 41 suspected field outbreaks of IBD was done. In phylogenetic analysis, the obtained field isolates fell into genogroup 1 and 3. In genogroup 3, all very virulent IBDV isolates shared a common ancestor with other south Indian isolates but isolates 9/CVASP/IBDV, 10/CVASP/IBDV, 12/CVASP/IBDV, 14/CVASP/IBDV and 17/CVASP/IBDV are most recently evolved and is diverged from the south Indian isolates and other isolates obtained in the study. Two isolates (15/CVASP/IBDV and 18/CVASP/IBDV) were similar to intermediate plus vaccine strain. The isolates 8/CVASP/IBDV and 19/CVASP/IBDV had amino acids unique for

the intermediate vaccine with mutations observed at H253Q and V256I in 19/CVASP/IBDV, T270A and novel mutation N279Y in isolate 8/CVASP/IBDV. These two isolates had non-virulent classical heptapeptide sequence 'SWSARGS' nevertheless they produce field outbreaks of IBD. This is the first genetic characterisation study of field IBDV isolates in Kerala, India.

In a separate study, molecular characterisation of VP1 gene of virulent IBDV was carried out in Kerala. The phylogenetic analysis of the partial VP1 gene sequences revealed the clustering of IBDV isolates into vvIBDV and non vvIBDV. Eighteen isolates (11 isolates from vaccinated flock and 7 from non-vaccinated flocks) clustered with very virulent strains. Four isolates (3 isolates were from vaccinated flock and one from non-vaccinated flock) clustered with non-virulent IBDV strains of classical virulent, classical attenuated and antigenic variant along with serotype 2 IBDV. The amino acid analysis of these 22 isolates revealed that 17 isolates possessed the characteristic vvIBDV TDN amino acid triplet while the four isolates had non vvIBDV NEG amino acid triplet at 145/146/147 position. The remaining isolate 1/CVASP/IBDV/VP1 shows unique PDN triplet instead of TDN. Two vvIBDV isolates (15/CVASP/IBDV/VP1 and 18/CVASP/IBDV/VP1) showed 100 per cent nucleotide and amino acid similarity with IL4 strain which is an attenuated very virulent vaccine strain. Four vvIBDV isolates showed neutral amino acid substitution K251R which was earlier reported in Indian strains. The novel amino acid substitution observed in our study were neutral E269D amino acid substitution in 12 isolates, neutral amino acid substitution T329S in five isolates, neutral T174N and non-polar to polar amino acid substitution A178T in isolate 10/CVASP/IBDV/VP1, non-polar to polar amino acid substitution P360R in isolate 17/CVASP/IBDV/VP1 and non-polar to polar amino acid substitution P188S in isolate 1/CVASP/IBDV/VP1. These novel mutations in our study reveals that evolution of vvIBDV occurred by genetic drift. The isolate 2/CVASP/IBDV/VP1 from non-vaccinated flock shows VP1 gene of non-vvIBDV, but possessed VP2 of vvIBDV type, indicates this is evolved by genetic shift of segment A and B.



**Genotypic characterisation of Porcine Reproductive and Respiratory Syndrome virus and development of a real-time reverse transcriptase polymerase chain reaction for its detection**

Porcine reproductive and respiratory syndrome (PRRS) is one of the emerging viral disease conditions. A study was undertaken with the objectives of characterisation of PRRS virus (PRRSV) by reverse transcriptase polymerase chain reaction (RT-PCR) followed by sequencing, and development of rapid and specific assay for the detection of PRRSV. Nucleotide analysis revealed that, all isolates belonged to genotype 2 with similarity to Indian sequences reported previously and to sequences of highly-pathogenic PRRSV from China. Phylogenetic analysis based on ORF5, ORF7 and Nsp2, revealed close clustering with Assam, Meghalaya and Mizoram isolates. Sequence analysis revealed amino acid changes in all three regions when compared to other Indian sequences which reflected high genetic diversity of the virus and most of the amino acid changes were unique to Kerala isolates. Based on the nucleotide sequences of the ORF7, primers and TaqMan probes were designed for detection of PRRSV prevalent in Kerala. The detection limit was estimated using cloned template and was found to be 3298 copies. The designed TaqMan real time PCR was used to test 40 samples and 17 samples (42.5 per cent) were positive. Percentage positivity of TaqMan real-time assay was higher compared to that of conventional ORF6 based RT-PCR (35 per cent), indicating its higher sensitivity.

**Grass based complete feeds incorporating coffee husk in early lactating cows**

Grass based complete feeds for lactating dairy cows, with the optimum level of NDF viz., 35 per cent, containing the unconventional protein rich feed ingredient coffee husk, was successfully developed for the first time in Kerala, with economic benefits, as part of the M.V.Sc research work.



**Soy milk as a cost effective milk replacer in calves.**

The technology to extract soy milk from raw soybeans was first developed in Kerala and it was proved that soy milk can be successfully incorporated upto 50 per cent as a milk replacer for calves, with economic benefits, as part of the M.V.Sc research work.

Soy milk preparation photographs



**Ayurvedic medicinal residues can be used as unconventional feeds for Malabari kids**

*In vitro* and *in vivo* studies on effect of dietary incorporation of ayurvedic pharmaceutical gritham residues, which are rich in energy, protein and fibre as replacement for costly energy feeds like maize and protein supplements like



soybean meal were carried out and it was proved that Panchagavya Gritham residue and Brahmi gritham residue could safely and economically be incorporated in the rations of Malabari kids, with beneficial effects on their growth performance. The research was carried out as part of M.V.Sc research project.



Dhanvantharam  
kashayam residue



Vilvadi lehyam residue



Panchagavya gritham  
residue



Kottamchukkadi  
thailam residue

### Extraction of bypass fat rich oil from rape seed and its incorporation in the rations of lactating dairy cows

Studies on extraction of bypass fat rich oil from rape seed and its successful and economic incorporation in the rations of lactating dairy cows was carried out.

### Dietary substitution of palm oil by rapeseed oil in broilers

Studies on dietary substitution of costly palm oil with cheaper rape seed oil was carried out and found that substitution at different levels had no adverse on growth performance and economics of broilers.

### Phenotypic and genotypic characterisation of Kuttanad ducks

The research project funded by DBT, Government of India aims in the characterization of indigenous duck germplasm, improvement of meat type ducks and to develop improved location specific duck strains. Studies to develop a meat line of Kuttanad ducks is also being continued as part of the project. Ducklings of 6<sup>th</sup> generation were produced through Artificial Insemination from the parent stock through individual selection based on body weight at eighth week. Crosses from the above selected population will be developed and evaluated for identifying suitable males and females for achieving highest heterosis based on the body weight achieved at the 8th week of age. The work is being carried out at Dept. of Poultry Breeding and Genetics at CASM, Thiruvazhamkundu.

### Bamboo leaves extract for better growth in weaned piglets

Studies on growth performance proved that inclusions of vitamin E, vitamin C and bamboo leaves extracts in daily diet of piglets improved the growth rate as evidenced by increasing body weight gain. Among three anti-stress supplements studied, bamboo leaves extract addition to diets resulted in more body weight gain in piglets than vitamin C and vitamin E groups. Though vitamin E and vitamin C anti-stress supplements also had beneficial effects on growth performance in early weaned piglets, its use cannot be recommended owing to its higher cost. Temperature and humidity index were positively correlated with body weight and there is no correlation between growth rate and wind velocity and light intensity. Supplementing bamboo leaves extracts had no adverse effect on the haematological and bio-chemical parameters of blood profiles in early weaned piglets. Overall, bamboo leaves had improved the growth rate by 14 per cent and performance indices with economic profitability in the weaned piglets.

### Dietary fat supplementation on production and fatty acid profile of milk in Malabari goats

Feeding rice bran oil at a rate of 35 g/day and calcium salts of fish oil at a rate of one per cent of their concentrate diet to Malabari goat reduced the concentrations of short chain



and medium fatty acids which is not good for human consumption and enhanced the concentrations of long chain fatty acids like CLA and DHA which is very good for human consumption. The FASN gene expression was down regulated leading to decrease in the short chain and medium chain fatty acids which is the supportive evidence for the enrichment of milk.

### **Brooding systems and ideal temperature humidity index improves growth performance and behaviour in Large White Yorkshire (LWY) piglets**

Brooding had reduced the mortality rate of the piglets in the farm. Improvement in the production performance of the preweaning and post weaning piglets provided electrical and charcoal brooding apart from the improvement in the feed intake and daily gain. Electrical brooding system had reduced the aggressive behaviour in piglets compared to the control group. Ideal THI for the piglets should be provided for the better performance by providing the comfort zone. Health status of the piglets was found improved in piglets provided brooding facilities. Among the three brooding system provided, electrical brooding was found to be better effective and brooding with 100-watt bulb was economically feasible compared to infrared bulb. Wood charcoal can also be recommended in rainy season and rural areas where electricity is not available.

### **Two stage restricted flow anaerobic baffled biogas digester and gas purification systems for efficient biogas production**

The dry matter content, C/N ration and volatile solid percentage of the substrate was improved by co-digesting kitchen waste and cow dung in 1:1 proportion. The biogas production and composition from two stage restricted flow anaerobic baffled biogas digester was significantly higher than single stage non baffled digester. The biogas production and methane concentration was 15.30 and 11 per cent more than that of single stage. The digester temperature optimisation to 37.76 °C and 36.25 °C in single stage and two stage digester by circulating hot water through water jacket significantly improved biogas production by 70.92 per cent and 67.11

per cent and methane concentration by 6.43 per cent and 10.22 per cent respectively, irrespective of seasons. The removal of water vapour by activated alumina balls, hydrogen sulphide adsorbed by 100 cm bed of activated charcoal and carbon dioxide removal by chemical reaction with 0.25 M sodium hydroxide solution was more efficient in upgrading the biogas to 92.45 per cent methane.

### **Satellite piggery unit established in Wayanad district for improvement of rural livelihood security**

The Rashtriya Krishi Vikas Yojana (RKVY) project entitled “Improvement in Rural Livelihood Security by Establishment of Satellite Piggery Unit in Wayanad district” that is being implemented in the College of Veterinary and Animal Sciences, Pookode Total outlay of Rs. 170.0 lakh was sanctioned with specific allocation of Rs.110 lakhs for shed construction with proper disposal and storage of manure and urine pits, Rs.6.30 lakhs for purchase of breeding stock, Rs. 52.92 lakhs for feed purchase and Rs.0.78 for purchase of farm tools. The sanctioned amount of Rs.170.00 lakhs has been utilized. The nucleus stock has been established.

A two storey pig shed is a house with two floors was constructed (454 m<sup>2</sup>). The first floor (227.00 m<sup>2</sup>) consists of 8 pens with dimensions (length × width) of each pen are 4.8 × 4.0 m for 8-pigs group. The ground floor (227.00 m<sup>2</sup>) consists of 10 farrowing pens with dimensions (length × width) of each pen are 4 × 2.3 m and 4 weaner pens with dimension (length × width) of each pen are 4.8 × 4.0 m for 11 weaned piglets group. Breeding gilts (above 7 months’ age) were purchased and artificial insemination of the gilts was completed. The farm tools viz. pregnancy detector for pigs and microchips with syringe and RFID tag reader were purchased for identification of pigs.



**Fig. Two storey pig sty with ramp**

### The skin and feathers of broiler and layer ducks differ in anatomy and its utility

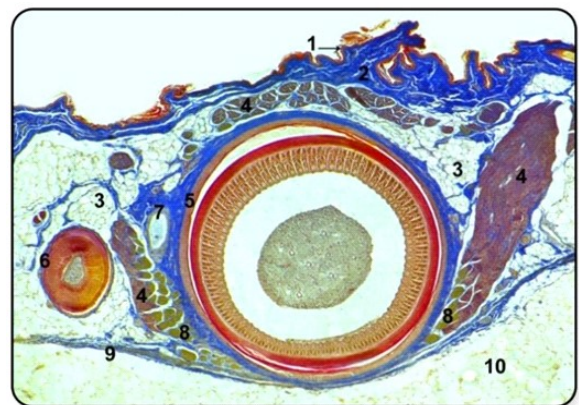
Results of studies carried out in KVASU implied the possibility of duck feather as a natural source of protein and energy and a valuable raw material in the manufacture of bioplastics, biodiesel, biopesticides, textiles, composites, and also can be exploited in the pharmaceutical and cosmetics industries. This in turn reduces the huge environmental pollution caused by the feather waste discharged from the slaughter houses. Moreover, it provides an extra income to the duck farmers. Comparative studies on the skin and feathers of broiler Vigova Super-M ducks of six to eight weeks of age and spent Kuttanad ducks (layer) above 40 weeks of age were conducted using 24 birds comprising of six males and females in each group. Overall skin thickness was more in Kuttanad ducks than broiler ducks and was slightly thicker in male birds. Skin was thicker on the ventral surface of the body especially in ventral abdominal region in both groups which might be an adaptation to resist heat dissipation during swimming. Epidermis was very thin and formed of two layers, *stratum germinativum* and *stratum corneum*. Hydroxyproline and collagen contents of dermis were more in the skin of layer ducks than in broiler ducks, which resists the wear and tear of skin. Average length of barbs (broiler,  $2.32 \pm 0.10$  cm and layer,  $1.83 \pm 0.07$  cm), average diameter ( $0.06 \pm 0.004$  mm) and aspect ratio ( $536.305 \pm 054$ ) were in the desired range to be used in textile manufacturing. The percentage contribution of total feathers to the body weight was maximum in female broiler duck ( $5.47 \pm 0.26\%$ ). The relative density of whole feather fractions in ducks was  $0.65 \pm 0.01 \text{ g/cm}^3$ .

Scanning electron microscopic studies revealed that both rachises and barbs of duck feather have an internal structure of a honeycomb. Crude protein content was 77.59 per cent in broiler and 73.50 per cent in layer whereas moisture content was 9.0 per cent in broiler and 9.5 per cent in layer ducks. Elemental analysis, amino acid analysis, FTIR-ATR studies, X-ray diffractogram, tensile strength and thermal stability were similar in both the groups. Wing feathers of female birds showed more radio-opacity than males, thus can be used for gender differentiation.



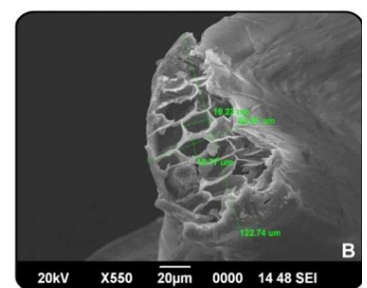
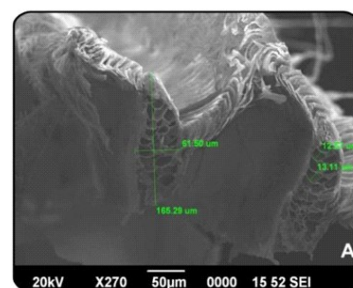
**Fig. 1 Gross skin of broiler duck**

1. Ensheathed feather 2. Neck of feather follicles

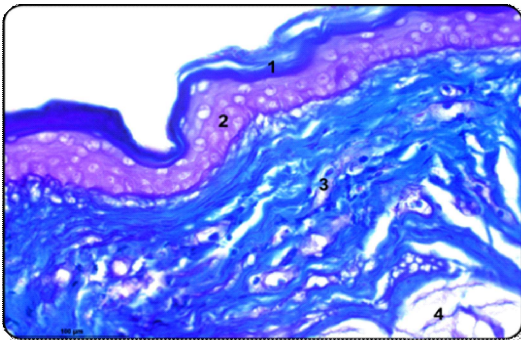


**Fig. 2 Section of skin, Dorsal abdomen, Kuttanad duck (Masson's trichrome method x 40)**

1. Epidermis 2. Stratum compactum 3. Stratum laxum 4. Arrectores plumorum muscle. 5. Contour feather follicle 6. Smaller feather follicle 7. Herbst corpuscle 8. Elastic tendon 9. Lamina elastica 10. Subcutis.

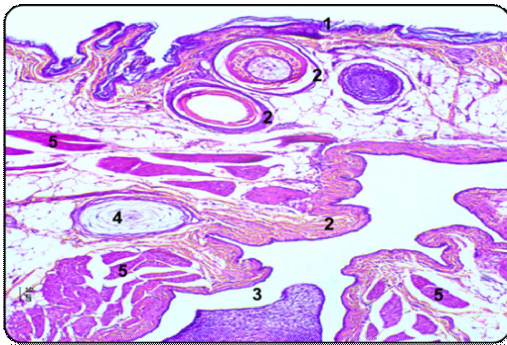


**Fig. 3 SEM image of the cross section of barb of broiler (A) and Kuttanad (B) duck showing hollow honey comb structure**



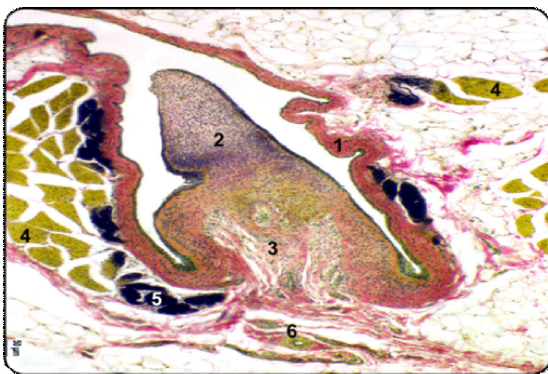
**Fig. 4** Section of skin, Dorsal abdomen, Broiler duck. McManus method for glycogen x 400

1. Stratum corneum, 2. Stratum germinativum, 3. Stratum compactum, 4. Stratum laxum.



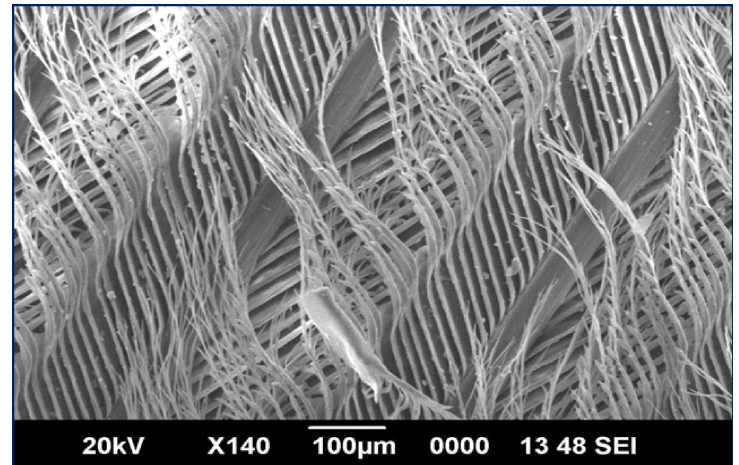
**Fig. 5** Section of skin, Dorsal abdomen, Broiler duck. H&E x 100

1. Epidermis, 2. Feather follicle in dermis, 3. Follicular cavity with dermal papilla, 4. Herbst corpuscle, 5. Arrectores plumorum muscle.



**Fig. 6** Section of skin, Dorsal abdomen, Broiler duck. Ver Hoeff's elastic stain x 100

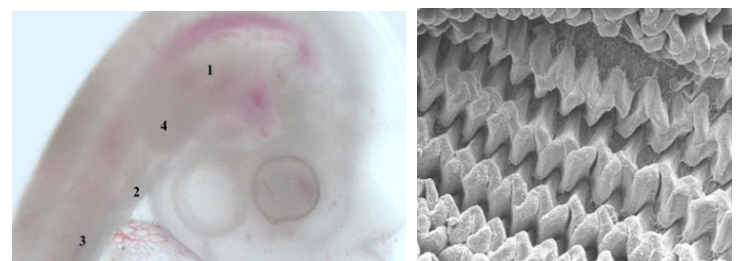
1. Wall of feather follicle, 2. Dermal papilla, 3. Base of dermal papilla, 4. Arrectores plumorum muscle, 5. Elastic tendon, 6. Blood vessels



**Fig. 7** SEM image of Kuttanad duck feather showing rachis and barbs

**Pre-hatch development of lower digestive tract in turkey (*Meleagris gallopavo*) revealed its early maturing nature**

Pre-hatch developmental pattern of lower digestive tract in Beltsville White turkey was studied to investigate the morphogenesis and histogenesis. The divisions of the primitive gut were apparent during the fourth day of incubation and the regional differentiation occurred by sixth day of incubation. There was significant increase in the weight and length of lower digestive tract and that of its segments as the age advanced. The histological examination of the segments revealed well developed tunica mucosa, tunica muscularis, tunica serosa and an inconstant tunica submucosa. The longitudinal folds of the tunica mucosa indicating future villi were obvious by fifteenth day of incubation. All layers of the small and large intestines increased in thickness as age advanced and became similar to the adult structure by twenty-first day of incubation.





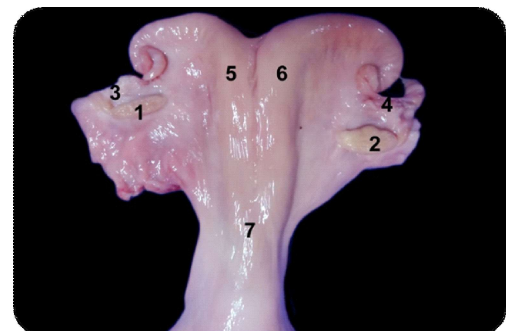


**Anatomical causes of infertility in crossbred dairy cows directly relates to the reproductive system morphology and genetic makeup**

The information gathered by this study will provide insights to the extent and causes of infertility among crossbred cattle of Kerala. Biochemical analysis of follicular fluid is a potent tool for assessing reproductive status. The candidate gene *wnt7a* can be used for genome-wide scans of anatomical abnormality in bovine species at early stages itself so that the route cause can be eliminated by culling of such animals. The findings will also be useful for diagnosing and minimizing the anatomical causes of reproductive disorders so that conception rate of the herd can be increased thus reducing economic loss to the farmers.

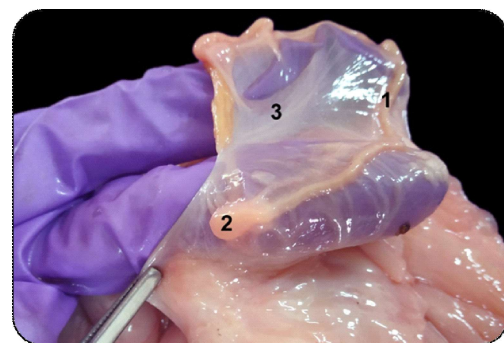
Anatomical causes of infertility in crossbred dairy cows were studied using the genitalia collected from 100 dairy cows / heifers from the Meat Technology Unit, Mannuthy with a known history of infertility. Morphology, morphometry, histology and histochemistry of genitalia, immunohistochemistry of IGFBP-2, scanning electron microscopic peculiarities of various regions of affected genitalia, biochemical analysis of follicular fluid and validation of SNPs of candidate genes were studied. Among the group of 100 animals under study, 14 animals showed anatomical defects in the genitalia. Considering the total number of female animals maintained in the farms, the overall incidence of anatomical abnormalities of genitalia was estimated to be 1.24 per cent. CBHF showed a higher incidence of anatomical defects in the genitalia followed by Frieswal and CBJ. Cervical abnormality was the most common type noticed followed by ovarian hypoplasia, Mullerian duct abnormalities (like aplasia of body of uterus and uterus unicornis) and tubal obstruction. Histological, histochemical and scanning electron microscopic structure of different regions of genitalia with anatomical defects were explored. Immunohistochemically, IGFBP-2 was localized in the primordial and atretic follicles in the hypoplastic ovary and ovary of uterus unicornis and kinked cervix conditions. Biochemical analysis of follicular fluid revealed that the higher mean concentrations of potassium, triglycerides, urea, nitrogen, cholesterol, copper and magnesium in affected group compared to that of control group.

Single nucleotide polymorphisms reported in the study population by ddRADseq in four candidate genes viz., *wnt7a*, *emx2*, *hoxa13* and *lhx1* affecting the development of female reproductive tract were selected for validation. To identify the single nucleotide variations in bovine *wnt7a*, PCR was performed and the sequence analysis of 272 bp amplicon revealed the targeted SNP at position 89 (A'!G transition) and three novel SNPs at positions 119 (A'!G transition), 195 (C'!T transition) and 196 (T'!A transversion) respectively. For identifying the SNP in bovine *emx2*, PCR- RFLP was performed and confirmed the population as homozygous. To identify the SNP in bovine *hoxa13* and *lhx1*, HRM was performed and did not show any polymorphism. Amplification of exon 2 of *foxa2* by PCR revealed polymorphism at 168<sup>th</sup> position (T'!C transition) of the 320 bp product. Thus, analysis of SNPs in five candidate genes revealed four SNPs in *wnt7a* and one in *foxa2*. This highlights the vital role of *wnt7a* in regulation of reproduction in bovines.



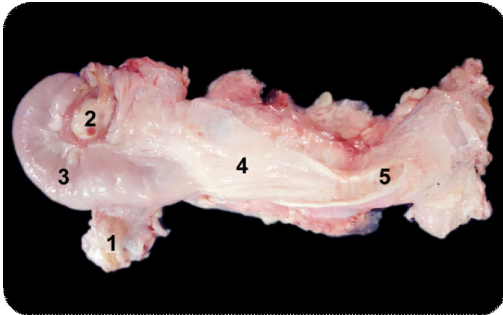
**Fig. 1 Genitalia of a heifer with complete bilateral ovarian hypoplasia**

1. Left ovary 2. Right ovary 3. Left oviduct 4. Right oviduct
5. Left uterine horn 6. Right uterine horn 7. Body of uterus

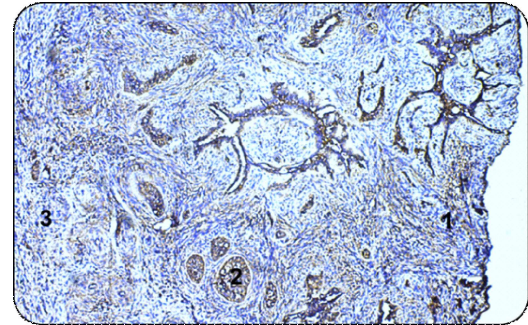


**Fig. 2 Blind end of the left oviduct in a cow with uterus unicornis**

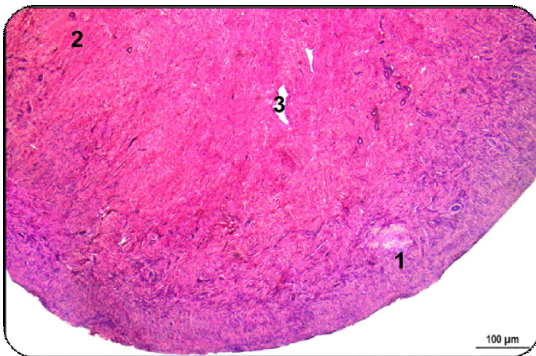
1. Oviduct 2. Blind end of oviduct 3. Mesosalpinx

**Fig. 3 Uterus unicornis in a cow**

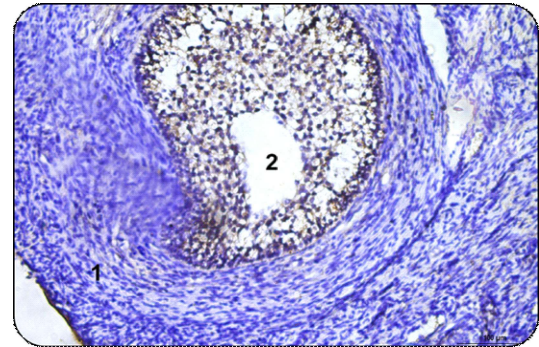
Left ovary 2. Right ovary 3. Right uterine horn 4. Cervix 5. Vagina.

**Fig. 6 Immunolabelling of IGFBP-2 in atretic follicle of ovary in a cow with partial bilateral ovarian hypoplasia. IHC x 100.**

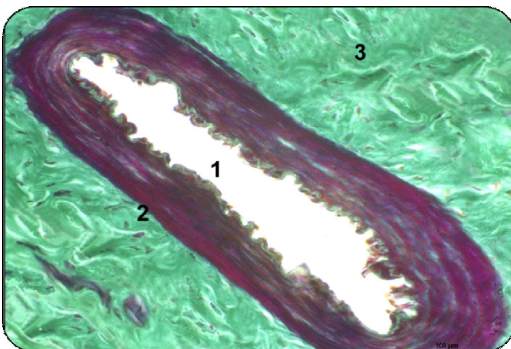
1. Cortex 2. Atretic follicle 3. Medulla

**Fig. 4 Section of ovary in a cow with complete bilateral ovarian hypoplasia. H&E x 100**

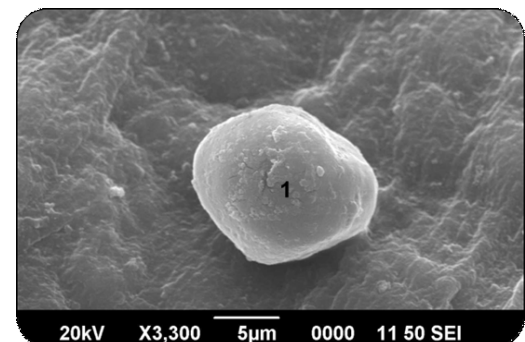
1. Cortex 2. Medulla 3. Venous sinus

**Fig. 7 Immunolabelling of IGFBP-2 in corpus luteum of ovary in a cow with uterus unicornis. IHC x 200**

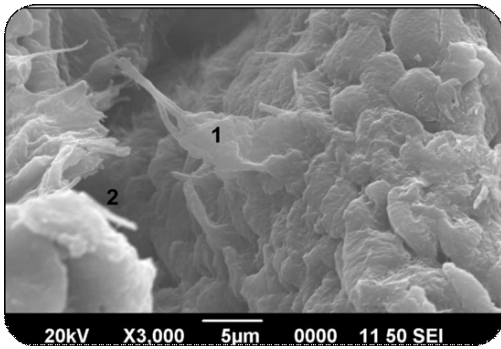
1. Cortex 2. CL

**Fig. 5 Section of cervix in a cow with kinked cervix condition. Masson's trichrome method x 400**

1. Blood vessel 2. Tunica media 3. Connective tissue

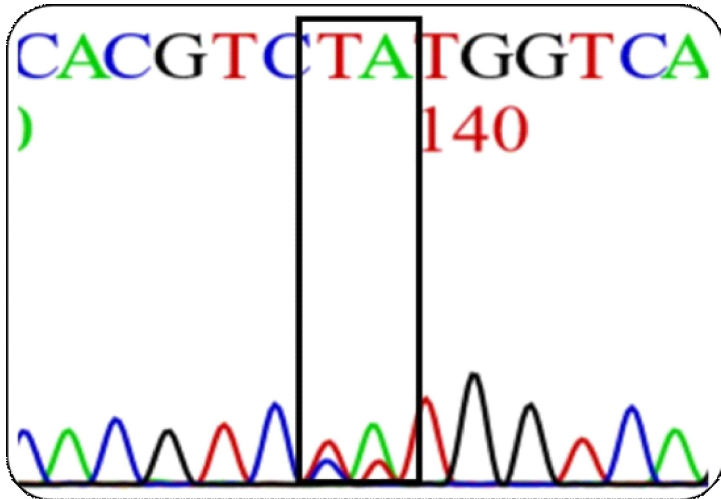
**Fig. 8 SEM image of germ cells of the ovary in a cow with unilateral ovarian hypoplasia (SEM x 3300)**

1. Germ cells

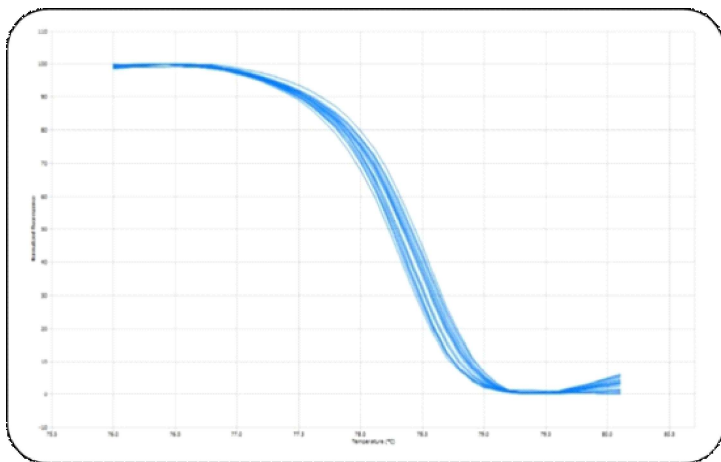


**Fig. 9 SEM image of endometrial glandular opening with ciliated epithelial cells of uterus in a cow with aplasia of body (SEM x 3000)**

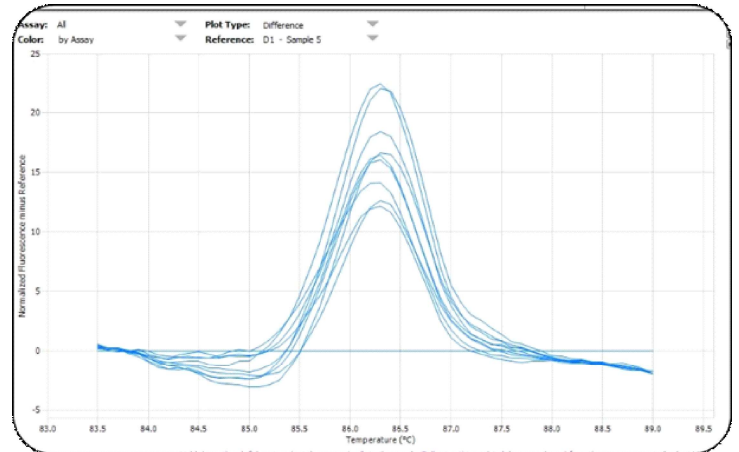
1. Ciliated cells 2. Endometrial glandular opening



**Fig. 10 Sequence map of TC and AT genotype of group 2 (*wnt7a*)**



**Fig. 11. HRM melting curve pattern in *hoxa13***



**Fig. 12. Difference plot of *Ihx1***

**Comparative anatomical studies on hooves of *Cervidae* family conducted**

The morphometric values of hoof of *Cervidae* family were more simulating with that of small ruminants. The similarity in hoof morphometry between deer and small ruminants may be because of the common grazing and sprint pattern followed by them and comparable body size. Hoof ailments are one of the major diseases observed in members of *Cervidae* family under captivity. The results of present study will form an anatomical reference data for further investigation into the hoof care and management as well as for the study of biomechanics of motion in deers.

i. The results of the study will help to develop reference data that will provide reliable baseline information on the normal anatomical structure of hooves of *Cervidae* family which will help the zoo and wildlife veterinarians in managing various hoof ailments of these animals.

ii. The collected specimens of hooves fixed in formalin will be displayed in the department museum which will provide an exposure to farmers, researchers, students and general public who regularly visits the museum.

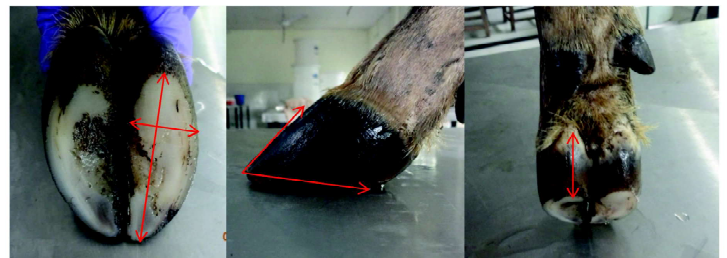
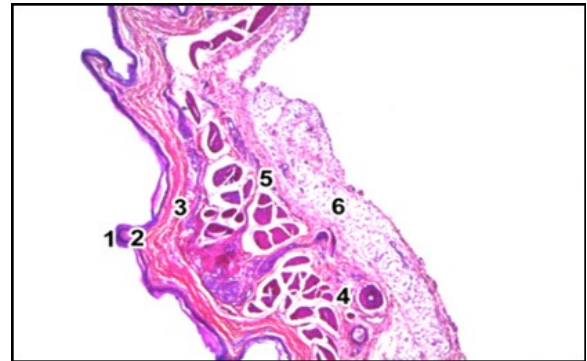


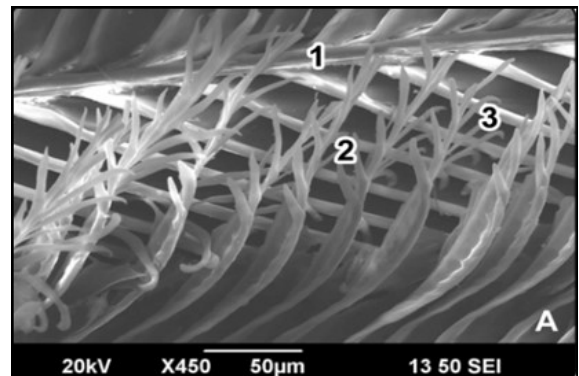
Figure showing the various morphometric values recorded.

### Histomorphological and ultrastructural studies on skin and feathers in turkey (*Meleagris gallopavo*) and its utility

The skin of turkey with its high collagen content of eight per cent may be recommended as a preferred raw material for scaffold production. Feathers fibres of turkey, with high tensile strength, good fineness and high aspect ratio will also form a supplementary reinforcing material for composites and textiles. Histomorphology and ultrastructure of skin and feathers in turkey were studied using samples procured from 12 birds each of either sex. Skin of turkey was pale pink in colour and attached loosely to the underlying structures. It contributed 8.4 to 9.7 per cent to the total body weight, with female birds contributing more. Thickness of skin was significantly high in male than in female. Number of feather follicles was found to be higher in dorsal wing region irrespective of sex. Histologically, skin of turkey comprised of epidermis and dermis (Fig. A). Males had more thickness for the dermal layers than females. Scanning electron microscopic studies of skin exhibited filoplumes and overlapping surface cells. Histochemical studies revealed the presence of carbohydrates, lipids and phosphatases in skin. Morphologically, the feather possessed branched structure with rachis, barb and barbules. Ultrastructural studies of feathers showed hooklets at the tip of barbules (Fig. B). Feather fibre of turkey was characterized by its fineness, hydrophobicity, high aspect ratio, high tensile strength and low apparent density. Feather fibre of females exhibited higher aspect ratio over that of males. Moisture content and moisture regain percentages were nine and 12 respectively for feather. On proximate analysis, feathers were found to be a rich source of protein with low crude fat content, minimum total ash and negligible crude fibre. Elemental composition of feather exposed high content of carbon and nitrogen, but low value for hydrogen and traces of sulphur. Major amino acids contained in feather were alanine, serine, histidine, glycine and aspartic acid. Main functional groups associated with peptide bonds of keratin proteins in feather as per Fourier Transform Infrared Spectroscopic studies were N-H stretching, C=O stretching, C-N stretching and N-H bending. X-Ray diffractogram revealed high crystallinity of barbs. Thermogravimetric analysis of feather suggested drying temperature to be less than 100°C and processing temperature to be less than 250°C.



**Fig. A. 1. Epidermis 2. Dermis, Stratum Superficiale. 3. Dermis, Stratum Compactum 4. Dermis, Stratum Laxum 5. Lamina elastica 6. Subcutaneous tissue.**

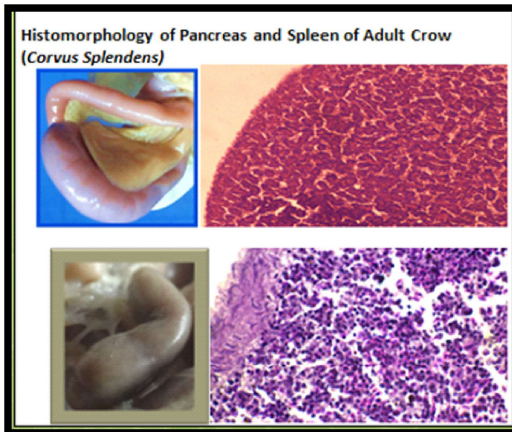


**Fig. B. 1. Barb 2. Barbule 3. Hooklet**

### Anatomical studies on wild birds conducted for forensic identification

The study was envisaged to form a basis for future research especially to the wild life researchers. The findings will also be useful to understand the species specific features of the bones which are of archaeological significance and help to identify the species of wild birds fossils recovered by the paleontologists.





### Comparison of morphometry and histomorphology of the hoof of crossbred cattle and buffaloes carried out

A morphological and morphometric study on the hoof of crossbred cattle and buffalo was undertaken in the work. The morphometric values were determined following standard procedures and protocols. Toe length, toe height, toe angle, sole length, sole width, heel bulb length, heel bulb height and heel bulb width were recorded separately for each claws of forelimb and hindlimb. The proximate analysis of the hoof was also carried out to assess the biochemical properties of hoof.

The proximate analysis of buffalo hoof revealed that it contained 14.82 percentage moisture. The crude protein (CP) content on dry matter basis was 88.04 percentage, total ash on dry matter basis was 1.84 percentage, crude fiber (CF) on dry matter basis was 0.79 percentage, ether extract (EE) on dry matter basis was 0.88 percentage and nitrogen free extract (NFE) on dry matter basis was 8.45 percentage.

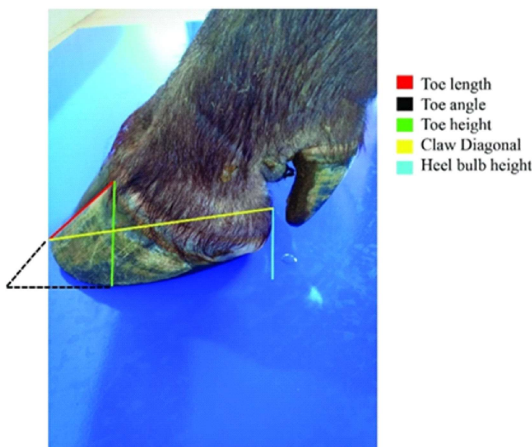


Figure showing the various morphometric values recorded.

### Acellular matrix could be developed from porcine tunica vaginalis

Parietal layer of tunica vaginalis (PTV) collected from the testes of adult healthy pigs were used for the development of the acellular matrix. PTV was decellularised by two protocols using aqueous solution of bovine bile and Trypsin-EDTA. Assessment of the efficiency of decellularisation by these two methods indicated that decellularisation using Trypsin-EDTA was better. Present study revealed that tunica vaginalis which is being discarded from local meat industry can be turned into a biomaterial. Further animal studies are needed to prove the suitability of this acellular matrix as a biologically and medically valuable product.

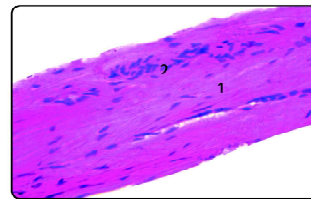


Fig. 1 Cross section of separated parietal layer of tunica vaginalis (Before decellularisation). H&E x 400

1. Collagen 2. Nucleus



Fig. 2 Cross section of decellularised parietal layer of tunica vaginalis (Trypsin-EDTA treated). H&E x 400

1. Collagen

### Development of phytosterol incorporated functional Pre- cheese

Ultrafiltered (UF) pre-cheese incorporated with phytosterol as functional ingredient was developed in the Department of Dairy Technology, College of Dairy Science and Technology as part of M. Tech. research project 'Process development for phytosterol incorporated functional Pre- cheese'. Optimized parameters for low fat UF pre-cheese were 26.83 per cent total solids (TS), 17.95 per cent Fat on dry matter (FDM) and 0.015 per cent rennet. In the case of full fat UF pre-cheese the optimized parameters were 26.82 per cent TS, 48.57 per cent Fat (FDM) and 0.015 per cent rennet. Phytosterol content of the ultrafiltered (UF) pre-cheese was 2.22%. On analyzing the developed products during ripening and storage period of 90 days they were found to be unfit for consumption based on microbiological quality.



### Fat replaced low calorie *Chhana Podo* incorporated with *Punica granatum* peel powder developed

Standardized a process for the preparation of fat replaced low calorie *Chhana podo* incorporated with *Punica granatum* peel powder by partial and complete replacement of fat and sugar with Whey Protein Concentrate (WPC) and sucralose respectively as part of the M. Tech. Dairy Technology research work entitled 'Process Standardisation of Fat Replaced Low Calorie Chhana Podo Incorporated with the *Punica granatum* Peel Powder' carried out at CDST, Mannuthy. Optimized levels of *Punica granatum* peel powder at 3.5 per cent, Sucralose at 550 ppm and Whey Protein Concentrate at 1 per cent were selected for *Punica granatum* peel powder incorporated fat replaced low calorie *Chhana podo* with maximum acceptable sensory tributes. The baking condition for the preparation of product was optimized at 120°C for 50 minutes. The product thus optimized contained 10.77 per cent fat, 26.09 per cent protein, 40.78 per cent moisture, 20.93 carbohydrates, 1.43 per cent ash and 0.24 per cent lactic acid acidity. The expansion volume, antioxidant activity IC 50 value and total polyphenol content of the optimized product was found to be 2.08mm, 1522 mg/L and 0.13%. The shelf life of the optimized product was found to be 3 days at 32 ± 1°C and 17 days at 7 ± 1°C. Cost of production of one kilogram of *Punica granatum* peel powder incorporated fat replaced low calorie *Chhana podo* was estimated to be Rs.344.44.



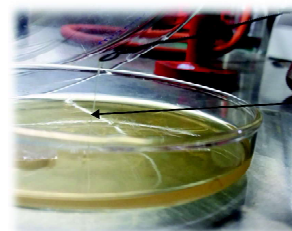
**Fat replaced low calorie *Chhana Podo* incorporated with the *Punica granatum* peel powder**

### Isolation of exopolysaccharide producing *Pediococcus pentosaceus* with antioxidant potential

An M. Tech. research conducted in the Department of Dairy Microbiology, College of Dairy Science and Technology, Mannuthy could successfully isolate lactic acid bacteria (LAB) from different indigenous sources like goat milk, tender coconut water, Vechur cow milk, passion fruit and potato. Growth conditions for exopolysaccharide (EPS) production were optimized for *Pediococcus pentosaceus* DMG01, an isolate selected from the five isolates based on its superior probiotic properties. Antioxidant potential in terms of 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of *P. pentosaceus* and EPS (0.03mg) were found to be 35.48% and 38.71% respectively. *P. pentosaceus* produced EPS maximally when grown in nutrient broth (pH 7.0) supplemented with two percent maltose inoculated at two percent rate of inoculum and incubated at 37°C for 24h.



**Congo red agar showing black colonies of EPS producing LAB.**



Ropiness formation by EPS producing LAB

**Ropiness formation by *Pediococcus pentosaceus* DMG01**

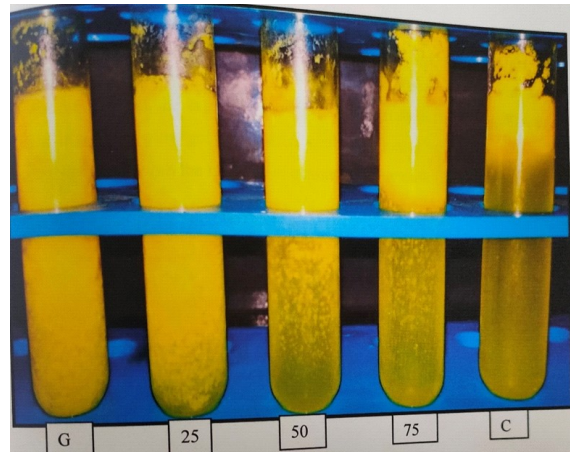


### Lactic acid bacterial metabolites of *Lactobacillus fermentum* MH 782089 as effective biopresevatives for paneer

This study conducted in the Department of Dairy Microbiology, College of Dairy Science and Technology aimed to isolate Lactic acid Bacteria (LAB) from different sources, assess them for antimicrobial metabolite production and to use the metabolites of the most potential isolate for the biopreservation of paneer, an indigenous traditional dairy product. Three different sources namely orange, tomato and curd were screened for the isolation of lactic acid bacteria. Out of the three isolates obtained, *Lactobacillus fermentum* MH 782089 isolated from orange was selected for further studies pertaining to its use for biopreservation of paneer. The study revealed that dipping of paneer samples in the antimicrobial metabolites produced by this isolate for a period of 30 minutes as an effective method of biopreservation of paneer.

### A novel test for detecting adulteration of goat milk with cow milk

Department of Dairy Chemistry, College of Dairy Science and Technology developed a test for detecting the presence of cow milk in goat milk. The physicochemical and compositional properties of cow and goat milk samples were analyzed and significant variation between the samples was observed in terms of fat and chloride content. The ethanol stability was checked for the samples and it was found out that goat milk has lower ethanol stability compared to bovine milk which was improved by addition of cow milk. A blue colour which varies proportionately in intensity from light blue for pure goat milk to dark purple by increased addition of cow milk was developed by addition of bromocresol purple and was verified using colorimeter. Addition of Seliwanoffs reagent to the above said samples led to the development of coagulum with significant differences in appearance such as a completely dispersed coagulum in case of goat milk and a clearly settled coagulum on top portion for cow milk. The rate of settlement of coagulum to the top increases with increase in content of cow milk in goat milk. The above stated differences may be considered as the basis for detection of presence of cow milk in goat milk. The advantages offered by this test are the shorter time requirement and lower quantity of reagents.



**Decreasing rate of coagulation after addition of bromocresol purple and Seliwanoffs reagent**

### Application of ultrafiltration process for manufacture of Emmental-type cheese.

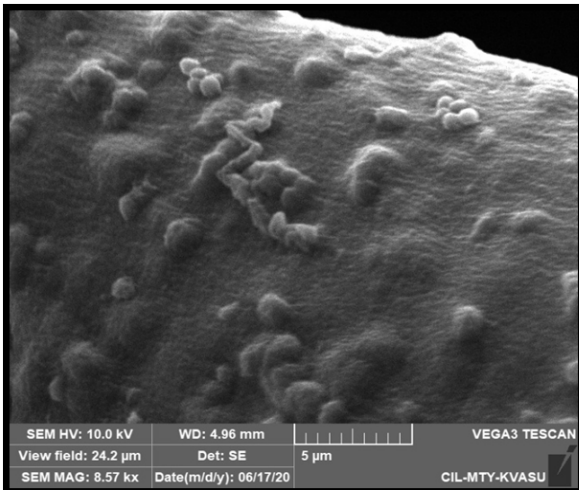
M.Tech. study conducted in the Department of Dairy Technology, CDST, Mannuthy standardized the manufacture of Emmental-type cheese observing pre-cheese technology. Levels of total solids in the retentate (28.00%), Rennet (0.010%), starter culture (0.375%) and adjunct culture (0.005%) were optimized with 84.10% desirability. Optimized product was prepared with and without paraffining and the paraffined cheese had a significantly better microbial quality. The Emmental-type cheese thus prepared had adequate eye formation and propionic acid flavour. The ripening study was carried out for one month at 21 °C and 80-85% RH. The paraffined Emmental type cheese had a significantly lower ripening index ( $17.35 \pm 0.32$ ) than the non-paraffined cheese ( $21.90 \pm 0.15\%$ ).



**Emmental-type cheese prepared through pre-cheese technology**

**Successful encapsulation of a potential probiotic lactic acid bacterium in sodium alginate matrix**

M. Tech. research conducted in the Department of Dairy Microbiology, College of Dairy science and Technology, Mannuthy could successfully encapsulate by extrusion method a potential probiotic human breast milk isolate of *Pediococcus pentosaceus* DM101 in a matrix constituted by 2.0% sodium alginate as gelling agent, 0.1 M CaCl<sub>2</sub> as hardening solution and gelling time of 20 minutes. The encapsulated microflora could be stored for 60 days both under 4°C and -18°C. Observations under this study endorse encapsulation as an effective tool for sustaining viability of probiotic organisms in food matrix.



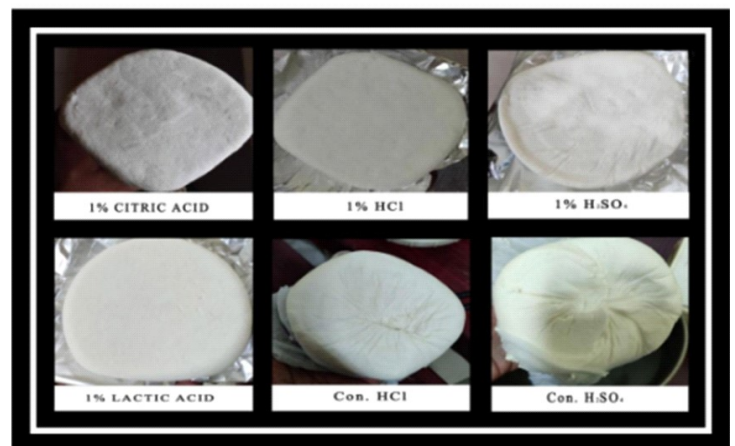
**Scanning electron micrograph of *P. pentosaceus* DM101 in sodium alginate bead**

**Remarkable increase in unsaturated fatty acids in organogel incorporated yoghurt**

Department of Dairy Chemistry, College of Dairy Science and Technology successfully developed skim milk yoghurt with improved textural characteristics by incorporating canola oil organogels prepared with sunflower wax and candelilla wax. Addition of organogels led to a remarkable decrease in syneresis compared to that of control yoghurt. Up to 3% addition of organogel, matched with the sensory score of control yoghurt. GC-MS analysis for fatty acids revealed remarkable increase in unsaturated fatty acids in organogel incorporated yoghurt samples.

**Significant variation between organic and inorganic coagulants in coagulation time and amount of coagulant required for paneer preparation**

Department of Dairy Chemistry, College of Dairy Science and Technology studied effects of inorganic coagulants on the physicochemical and sensory properties of Paneer as part of an M. Tech. research. Main factors that affect the sensory and physico-chemical characteristics of paneer are type and concentration of coagulants used. Commonly used coagulants are citric acid and lactic acid. The unscrupulous addition of inedible non-food grade coagulants like sulfuric acid, HCl etc. for paneer preparation is a matter of great concern in recent times. Consumption of such spurious or synthetic Paneer is highly lethal for health as it causes severe intestinal and stomach infection, which can also be cancerous. Detection of such fraudulent practices adopted by the manufacturers is very much important. The paneer was prepared from buffalo milk by direct acidification process using 1 per cent citric acid, 1 per cent lactic acid, 1 per cent H<sub>2</sub>SO<sub>4</sub>, Concentrated H<sub>2</sub>SO<sub>4</sub>, 1 per cent HCl and concentrated HCl. Physico-chemical, compositional and coagulation characteristics of prepared paneer samples and the resultant whey were analyzed using standard methods. The coagulation time and amount of coagulant required varied significantly (5 per cent level) between organic and inorganic coagulants. The yield was higher when concentrated sulfuric acid was used.



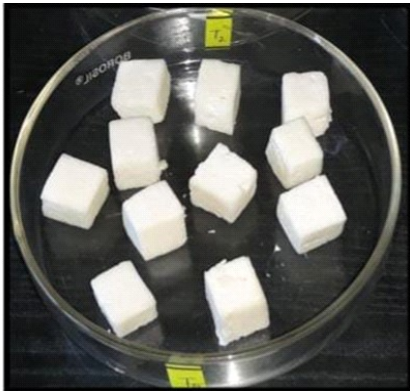
**Paneer prepared using different coagulants**





### Extension of shelf life of paneer on using essential oil incorporated casein based edible coating

Effect of application of edible coating based on sodium caseinate incorporated with clove bud essential oil on paneer was studied. The optimised formulation of coating solution were 13% of sodium caseinate, 3% of glycerol, 1.25% of pectin and 0.35% of clove bud essential oil. There was no significant difference in the proximate composition and acidity between the coated paneer and the control paneer ( $p > 0.01$ ). Shelf life of samples coated with optimized edible coating, packaged in LDPE pouches had more shelf life than the control paneer as the coated paneer kept well for 9 days while the control paneer was spoiled after the 6<sup>th</sup> day of refrigerated storage. The paneer coated with casein based edible coating had good anti-oxidant property with IC 50 of 5878 mg/Kg initially compared to the IC 50 value 10340mg/Kg of control paneer. Anti-oxidant potency of both the coated and control paneer kept decreasing on storage, the rate of decrease was significantly higher for the control paneer.



**Paneer with casein edible coating**

### Significant reduction in bioavailability of milk calcium due to tea polyphenols

A research conducted in the Department of Dairy Chemistry, College of Dairy Science and Technology reported significant reduction in milk calcium and protein and subsequent reduction in overall nutritional value of milk on preparing tea using it. Significant reduction in the bioavailability of calcium in milk tea beverage compared to milk was observed on

simulated gastrointestinal model evaluation. Bioavailability of calcium was found to be lower in milk tea ( $19.14 \pm 0.04\%$ ) compared to milk ( $25.45 \pm 0.009\%$ ). Hence it is suggested that consumption of milk is better than milk tea beverage for getting maximum amount of nutrients specially calcium from milk. The study also reported that preparatory steps like brewing time and level of addition of tea powder enhances the interactive behaviour of polyphenols with milk calcium.

### Extent of degradation of ghee on repeated use as cooking medium

Aim of this M. Tech. study conducted in the Department of Dairy Chemistry, College of Dairy Science and Technology, Mannuthy was to check the extent of thermal degradation in ghee over other edible oils on repeated use as cooking medium. Due to their popularity in Kerala cuisine, coconut oil and sunflower oil were selected for comparison with ghee. Safe extent of repeated usage of ghee and other cooking oils were determined by analysing the fatty acid composition (GC-MS), Total polar compounds (ATR- FTIR and Testo 270) and oxidation products (Fluorescence spectroscopy). It was recommended that up to four repeated heating, coconut oil, sunflower oil and ghee are safe for consumption.

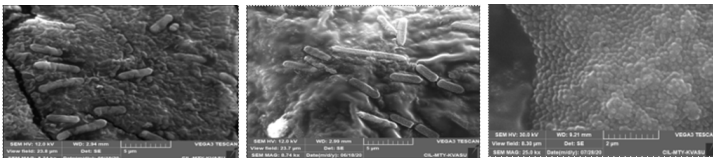
### Isolation of lactic acid bacteria with cadmium and lead bioremediation potential

Five lead and cadmium resistant lactic acid bacteria were isolated in a M. Tech. research work conducted in the Department of Dairy Microbiology, College of Dairy Science and Technology, Mannuthy. The study also analysed the effect of different factors; pH, metal concentration, inoculum rate, temperature and contact time on metal biosorption. Two factor interaction response surface model revealed the optimized condition for cadmium biosorption by *Weissella confusa* as 1% inoculum rate, 50 mg/L concentration of cadmium and 30 h of contact time. Quadratic model fitted for lead biosorption by *Lactobacillus plantarum* recommended optimum conditions as 30p C incubation temperature, 7 pH, 1% inoculum rate, 50 mg/L concentration and 30h of contact. These isolates can be considered as potential bioremediation candidates in for dairy/food industry.



### Isolation of atypical Gram negative pasteurization surviving low temperature growing bacteria in market samples of pasteurized milk collected from Mannuthy, Thrissur, Kerala

A post graduate research work conducted in the Department of Dairy Microbiology, College of Dairy science and Technology, Mannuthy to understand the pasteurization surviving low temperature growing (thermoduric psychrotrophic) bacterial profile of local dairy environment with special emphasis to pasteurized milk, a total of 57 samples constituted by 42 samples of pasteurized milk, nine samples of milk products and six dairy equipment swabs were assessed. Thermoduric psychrotrophic bacteria could be isolated only from pasteurized milk samples and they were present in 14.3 per cent of pasteurized milk samples tested. Out of the six isolates obtained five were Gram negative organisms. 16S rRNA sequencing identified the isolates as *Aeromonas caviae*, *Moraxella osloensis*, *Delftia tsuruhatensis*, *Staphylococcus arlettae* and two strains of *Carnobacterium maltaromaticum*. Isolation of pasteurization surviving low temperature growing strains of Gram negative bacteria not typically reported in pasteurized milk was an interesting outcome of this study.



a

b

c

Scanning electron microscopy images of a. *Aeromonas caviae*, DMV01, b. *Moraxella osloensis* DMV03 and c. *Staphylococcus arlettae* DMV02

### Development of ginger incorporated peda with enhanced sensory quality and fiber content

Ginger incorporated peda with increased fiber content and better sensory attributes was developed. The new product is believed to increase the digestibility and can be considered a value added product. The work was carried out in the Department of Dairy Chemistry of College of Dairy Science and Technology, Mannuthy

### A modified method for increasing the yield of casein from milk

Post graduate research work was carried out in the Department of Dairy Chemistry of College of Dairy Science and Technology, Mannuthy found out that the use of 1 M HCl instead of 5 % acetic acid and 1M phosphate buffer resulted in higher yield of casein. Also there was a difference in the texture and water holding capacity of casein in each case which could be effectively utilized in appropriate foods.

### Better casein solubilising properties of salt than alkali

Solubility studies on casein have shown that salt has better solubilising properties compared to alkalis. Among the different temperatures, solubility was better at a higher temperature of 80° C. At higher salt concentration salting out was observed. Also at refrigerated condition salting out occurs at higher salt concentration. This is a relevant data for optimizing salt content to have soluble protein. The work was carried out in the Department of Dairy Chemistry of College of Dairy Science and Technology, Mannuthy

### Effect of type of coagulants on physicochemical properties of buffalo milk paneer

Organic and inorganic coagulants were used for preparation of paneer and the prepared paneer was checked for its sensory quality, yield and physicochemical properties. Paneer was prepared from buffalo milk by direct acidification process using 2% citric acid, 0.1N Citric acid, 0.1 N HCl. Physicochemical, compositional and coagulation characteristics of the prepared paneer samples and the resultant whey were analysed using standard methods. Textural properties like gumminess, cohesiveness were analysed using textural profile analyser. The work was carried out in the Department of Dairy Chemistry of College of Dairy Science and Technology, Mannuthy



### Physico chemical properties of cow milk alternatives affected by different processing variables

Physico chemical properties of cow milk alternatives and their change according to different processing treatments were analysed. Coconut milk was selected as an alternative for cow milk. Compositional parameters and physico chemical parameters of coconut milk samples also were analysed at similar conditions. The analysis showed that coconut milk contained higher fat percentage (10%) than that of cow milk and on boiling showed a slight increase in amount. The specific gravity value was similar with cow milk while the mineral content and titratable acidity of coconut milk showed a low value. Protein content was significantly lower in coconut milk than cow milk. The work was carried out in the Department of Dairy Chemistry of College of Dairy Science and Technology, Mannuthy

### Paneer prepared with mixed flax seed milk and buffalo milk

Paneer has been prepared with the mixed flax seed milk and Buffalo milk and its physico chemical and anti oxidant properties were studied. The protein content, crude fibre and mineral content of flax seed paneer was higher than the control paneer. There was no significant difference in fat content between flax seed paneer and buffalo milk paneer. Antioxidant activity of flax seed incorporated paneer was higher than that of control sample.



**Paneer prepared using mixed Flax milk and Buffalo milk**

### Aloe vera supplementation increases the antioxidant properties of probiotic yoghurt

Probiotic yoghurt containing Aloe vera was prepared and its shelf life studies were conducted. Antioxidant properties by DPPH assay was also conducted. Sample showed better antioxidant properties than the control.



**Aloe vera supplemented probiotic yoghurt**

### Effect of processing treatments on physico chemical properties of different milks

Different types of milks were subjected to different heat treatments and change in physico chemical properties was studied. Composition and physical properties of cow and buffalo milk under boiling and freezing conditions were studied. Fat was the main compositional parameter which varied significantly according to both the temperature and species.

### Technological properties and probiotic potential of *Lactobacillus fermentum* isolated from different sources

On assessing the technological properties and probiotic potential of *Lactobacillus fermentum* isolated from different sources (milk, curd, orange and tomato), it was found that these properties are highly strain specific. *Lactobacillus fermentum* DMA01 isolated from milk exhibited better probiotic and technological properties. Outcomes of this study highlighted the relevance of accounting strain specificity while reporting research findings and using the appropriate probiotic by both the medical care professionals and the public.

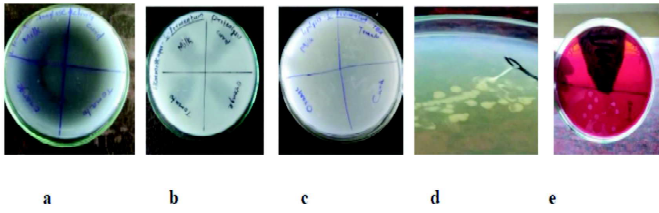


Fig 1. a. Starch agar Petri dish with no zone of clearance b. Skim milk agar Petri dish with zone of clearance c. Tributyrin agar Petri dish with no zone of clearance d. Inoculation loop method showing rope formation e. Congo red agar plate showing black colored growth of *Lactobacillus fermentum* DMA01

### Assessment of microbiological quality of drinking water collected from household water purifiers and characterization of predominant isolates

In a study conducted in Department of Dairy Microbiology, microbiological quality of drinking water collected from household water purifiers was assessed and the predominant isolates were characterized. Observations of the study highlighted the importance of regular cleaning and conditioning of filters to ensure an efficient reduction of microbial load in filtered water and identified *Acinetobacter baylyi* as the predominant isolate in the water samples tested. The study also found that the isolate was completely susceptible to UV light in the wavelength range of 100 to 280 nm (UV-C range) when exposed for 5 and 10 minutes.

### Isolation of a proteolytic and lipolytic *Pseudomonas aeruginosa* strain from domestic compost

This study isolated a proteolytic and lipolytic strain of *Pseudomonas aeruginosa* from domestic compost and confirmed the inherent ability of *P. aeruginosa* to resist antibiotics. The isolate was found to be capable of reducing the pH of kitchen waste water from 4.04 to 7.0 over a period of five days.

### Isolation of a biofilm producing *Chryseobacterium* species from pasteurized milk

A study conducted in the Department of Microbiology isolated a biofilm producing *Chryseobacterium* species from milk which was found to withstand 62.5°C for 40 minutes and 72°C for 1 minute. The isolate was found to be resistant to Ampicillin, Chloramphenicol, Amoxicillin, Azotrenam and Penicillin-G

### National seminar on “Emerging trends in Quality Assurance of Milk and Milk Products”

College of Dairy Science and Technology, Mannuthy in association with Indian Dairy Association (Kerala chapter) conducted a one day National seminar on “**Emerging trends in Quality Assurance of Milk and Milk Products**” on 20<sup>th</sup> May 2019. First session was handled by Dr. B. Surendra Nath, Principal Scientist, Dairy Chemistry and Bacteriology Section, ICAR-National Dairy Research Institute, SRS Adugodi, Bangalore. Second session was handled by Dr. Ramakant Lawanya, Head Operations – Duke’s Thompson on the topic Biofilm (City Of Microbes) The Hidden Threat and the third talk was by Mrs. Rosilint Joseph (Director Duke’s Thompson’s) on the topic Advanced Technologies for the Rapid Detection of Microbial and Non Microbial Contaminants in Dairy, Beverages and Food Processing Industries.





### World Milk Day celebrations

College of Dairy Science and Technology, Mannuthy in association with Indian Dairy Association (Kerala chapter) organized a Milk quality Awareness Training programme named “JEEVA” for college students of science colleges on 1<sup>st</sup> June 2019 in connection with World Milk Day celebrations.



Kerala), Dr. R. Ramkumar (Member, Planning Board) , Dr. M. R. Saseendranath (Hon. Vice Chancellor, Kerala Veterinary and Animal Sciences University), Sri. P. A. Balan Master (Chairman, Kerala Cooperative Milk Marketing Federation), Sri. S. Sreekumar (Director, Dept. of Dairy Development) and Dr. P. Sudheer Babu (Dean, College of Dairy Science and Technology, Mannuthy) elaborated on various dimensions of the agreement and its impacts on dairy sector. A total of 140 participants attended the workshop.



### National seminar on ‘Probiotics - Way Forward to Wellness

Department of Dairy Microbiology, College of Dairy Science and Technology, Mannuthy, Thrissur, Kerala in association with Probiotic Association of India organized National seminar on ‘Probiotics - Way Forward to Wellness’ 02.03.2019 . The seminar was inaugurated by Dr. Joseph Mathew, Registrar, Kerala Veterinary and Animal Sciences University. Four technical sessions, E-poster competition on the theme ‘Popularization of probiotics’ and a Quiz competition were held as a part of this event.



### State Level Workshop on “Regional Comprehensive Economic Partnership (RCEP) Agreement – Impact on Dairy sector

College of Dairy Science and Technology, Mannuthy and Indian Dairy Association (Kerala chapter) jointly organized a state level workshop on “Regional Comprehensive Economic Partnership (RCEP) Agreement – Impact on Dairy sector” on 21<sup>st</sup> October 2019. Eminent Personalities like Sri. K. Krishnankutty (Hon. Minister for Water resources, Govt. of

### Workshop on Dairy Entrepreneurship Development Scheme (DEDS)

NABARD Thrissur in association with College of Dairy Science and Technology Mannuthy, organized a “WORKSHOP ON DEDS” on 20<sup>th</sup> November 2019 at Seminar Hall, Verghese Kurien Institute of Dairy and Food Technology, Mannuthy.



## National Milk Day Celebration

National milk day, to commemoration of Dr. Verghese Kurian’s birthday was celebrated on 26<sup>th</sup> November 2019 at CDST, Mannuthy. In connection with this, multifaceted activities were organized in the campus with the combined effort of various Departments, Indian Dairy Association (Kerala chapter) and Students’ Union ‘19-‘20. A one day training Programme was successfully conducted with the collective effort of Dairy Microbiology Department (CDST, Mannuthy) and Indian Dairy Association- Kerala Chapter (IDA-KC) for selected ten women on “Preparation of Fermented Dairy Products”. The second programme was an All Kerala Level Quiz Competition for the UG and PG students across the state. Fifty students from various colleges across the state participated in the event.



## Training on ‘Food Handler’s Hygiene’

Department of Dairy Microbiology organized a training on ‘Food Handler’s Hygiene’ utilizing the state plan fund 2018-2019 under the state plan project ‘Strategy development to assure safe food for the public by combating microbial threats in the food chain’ at College of Dairy Science and Technology, Mannuthy on 01<sup>st</sup> September, 2018.





## One month training under revolving fund, 'Starter culture', Department of Dairy Microbiology, CDST, Mannuthy

Department of Dairy Microbiology, CDST, Mannuthy organized one month training for students of St. Thomas College, Pala from 17.12.2018 to 17.01.2019 under revolving fund, 'Starter culture'.



## Hands on training programme on "Preparation of milk products for small scale enterprises"

Department of Dairy Technology, CDST, Mannuthy conducted three days Hands on Training programme "Amruth 2019" on "Preparation of milk products for small scale enterprises" in three phases. A total of 53 persons attended the programme held in three phases from 7 to 9<sup>th</sup> May 2019, 19<sup>th</sup> to 21<sup>st</sup> June, 2019 and 24<sup>th</sup> to 26<sup>th</sup> July, 2019.

## Off campus training

Dairy Technology Department of College of Dairy Science and Technology, Mannuthy in association with Agricultural Technology Management Agency conducted an off campus training at Alangad Block Panchayath on 26<sup>th</sup> October 2019.



## One day training on 'Preparation of Fermented Milk Products'

One day training on 'Preparation of Fermented Milk Products' was conducted by Dairy Microbiology Department of CDST Mannuthy on 30<sup>th</sup> November, 2019 for the participants of short term training on 'Clean milk production and preparation of milk products' for SHG/Kudumbasree members organized by Extension Training Centre, Mannuthy from 28/11/2019 to 30/11/2019.





### Training on ‘Starter Cultures for Fermented Milk Products’

Dairy Microbiology Department of College of Dairy Science and Technology Mannuthy conducted a training on ‘Starter Cultures for Fermented Milk Products’ on 4<sup>th</sup> and 5<sup>th</sup> of December, 2019 by Revolving Fund ‘Starter Cultures’. Department also offered a training on Starter Culture technology to an employee of Eden Food Products, Kunnankulam from 8<sup>th</sup> to 9<sup>th</sup> June 2020.

### AICTE Approval for CDST, Mannuthy

College of Dairy Science and Technology has got approved by All India Council for Technical Education, in the approval process 2020-2021 adding another laurel to the more than 25 year old history of the first ever dairy technology college in the state.

### Surgical facilities strengthened for imparting clinical teaching and optimum surgical care

Department of Veterinary Surgery and Radiology, COVAS, Mannuthy enhanced their surgical facilities by adding more equipment for anaesthesia monitoring, cataract and other surgeries. It enables the department to provide better service to public and optimum clinical exposure to the students.

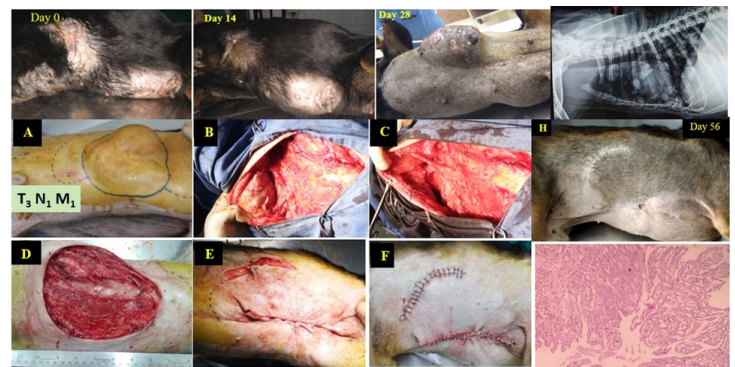
### Improved the existing centre for rehabilitation of stray puppies

Department of Veterinary Surgery and Radiology, COVAS, Mannuthy improved the existing facilities for early neutering of dogs and the training facilities for the veterinarians

### Surgical approaches of skin and subcutaneous neoplasms in dogs got streamlined

Scientists of veterinary surgery and radiology department, COVAS, Mannuthy studied an overall 295 cases of neoplasms in dogs by the during October 2017 to September 2020 and

subjected thirty six cases of mammary and skin/ subcutaneous of neoplasms to detailed study in three groups based on Tumour Node Metastasis (TNM) staging of neoplasms. Group I, II and III were subjected to curative-intent surgery alone, neoadjuvant chemotherapy at biweekly intervals followed by surgical excision and surgical excision followed by adjuvant chemotherapy at biweekly intervals respectively. The neoadjuvant and adjuvant chemotherapies were carried out using (VBL-P) protocol (vinblastine at 2 mg/ m intravenously and prednisolone at 1 mg/kg body weight intramuscularly) and (DOX-P) protocol (doxorubicin at 18 mg/ m<sup>2</sup> and prednisolone at 1 mg/kg bodyweight intramuscularly) in six animals each in both groups. Neoadjuvant chemotherapy with VBL-P protocol was found highly beneficial in defining surgical margins. Surgical approaches to neoplasms were streamlined based on TNM staging which were later correlated with histopathological grading of neoplasms. On Kaplan Meir analysis, the mean survival time was found less in Group III, compared to Group II and Group I.



### Uniplanar bilateral acrylic external skeletal fixation offers an economical, reliable and technically feasible alternative in treating long bone fracture in goats

Scientists of Veterinary Surgery and Radiology department, COVAS, Mannuthy studied six goats with metatarsus fracture for assessing the fracture healing when uniplanar bilateral





external skeletal fixation using acrylic correcting bar being used and thus evaluate their efficacy of this method in long bone fractures in goats. Clinically all the animals started bearing weight on the fracture affected limb from second postoperative day. Postoperative radiograph of all the goats showed good alignment and apposition between the fracture fragments with adequate implant stability throughout the observation period. The study revealed that use of external skeletal fixator using acrylic connecting bar provided excellent implant stability during the period of fracture healing and this can be used as an economical and technically feasible and reliable alternative in treating long bone fracture in goats.

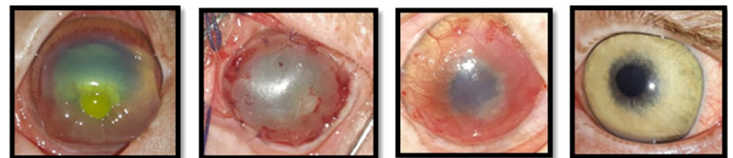
### Proper care and timely diagnosis can reduce the chance of oral and maxillofacial disorders in canines

The study conducted at Veterinary Surgery and Radiology department, COVAS, Mannuthy evaluated occurrence and management of various oral and maxillofacial disorders in dogs. The study involved 87 cases of oral and maxillofacial disorders with major dental disorders encountered as dental tartar, neoplasms, enamel hypoplasia, lacerations, dental attrition, mandibular fractures, oral ulcers, misalignment of teeth in dental arcade, persistent deciduous teeth, pus in antrum, cleft palate, mandibular abscess, supernumerary teeth, glossoplegia and halitosis. They concluded that proper care, management, feeding and timely diagnosis using appropriate diagnostic procedures could reduce the chance of disorders involving the teeth and oral cavity.

### Grafting with decellularised bovine omentum will enhance re-epithelialization of cornea in dogs with corneal injuries

Veterinary Surgery and Radiology department, COVAS, Mannuthy conducted a study to evaluate the efficacy of decellularised bovine omentum alone and in combination with mitomycin-C for the management of corneal injuries in dogs.. Twelve corneas from dogs irrespective of breed, age and sex were randomly divided into Group I and II with six corneas each. Corneal grafting was carried out with

decellularised and gamma irradiated bovine omentum in Group I whereas Group II corneas underwent single time intra-operative application of topical mitomycin-C for 2 minutes before corneal grafting. Corneal oedema subsided by day 14 in all the cases and corneal neovascularization was at the peak on day 7 and day 14 post-grafting which regressed completely by day 60. None of the corneas developed vision impairing corneal melanosis or scarring except one in Group I. From the present study it was concluded that re-epithelialization of cornea was enhanced by corneal grafting with decellularised bovine omentum and application of mitomycin-C was effective in controlling corneal fibrosis.



Day 0

Day of Grafting

Day 7

Day 28

### A balanced intramuscular anaesthetic combination for elective surgery in cats with good quality of induction and faster recovery

This study at Veterinary Surgery and Radiology department, COVAS, Mannuthy evaluated a balanced intramuscular anaesthetic combination using dexmedetomidine, ketamine hydrochloride, midazolam and butorphanol at the dose rate 10µg, 10mg, 0.2 mg and 0.2 mg per kilo gram respectively, mixed in a single syringe in 12 mixed breed adult female cats presented for elective surgical procedure. The duration and depth of anaesthesia were found satisfactory with adequate muscle relaxation. The recovery period was fast and of good quality. Physiological, haematological, serum biochemical, electrocardiographic and blood gas parameters were in normal range, after recovery from surgical plane of anaesthesia. The balanced anaesthetic combination with lower dose of ketamine was found safe, reliable and effective for elective surgical procedures with good quality of induction and faster recovery from surgical plane of anaesthesia.



### Elastic stable intramedullary nailing (ESIN) – A reliable alternative with good implant stability for long bone fractures in young dogs

Surgeons from Veterinary Surgery and Radiology department, COVAS, Mannuthy studied efficacy of stainless steel elastic stable intramedullary nailing (ESIN) for management of long bone fracture in six clinical cases of fracture of radius and ulna in dogs. All the animals showed normal weight bearing on all the limbs on eighth postoperative week. The study concluded that use of elastic stable intramedullary nailing technique using stainless steel elastic nail provided good implant stability and was found to be a reliable alternative over other fracture fixation techniques for treatment of radius and ulna fractures in dogs especially in young dogs where fracture stabilization can be performed without disturbance to growth plate.



### Auto-transplantation of minor labial salivary gland is a good method for managing dry eye in brachycephalic breeds of dogs

Surgeons from Veterinary Surgery and Radiology department, COVAS, Mannuthy evaluated the efficacy of minor labial salivary gland as autograft for treatment of dry eye in brachycephalic breeds of dogs. Six dogs were selected irrespective of age and sex for treatment of the dry eye by auto-transplantation of minor labial salivary gland to the upper conjunctival fornix under general anaesthesia. All the graft materials transplanted to the conjunctival fornix had healed completely and incorporated into the surrounding scleral surface. The graft has healed without any complications as observed during the post-operative period except for elevation

of graft materials from the surface of scleral tissue in two dogs. The study concluded that auto-transplantation of minor labial salivary gland can be recommended as a surgical treatment for the management of dry eye in brachycephalic breeds of dogs without any complications considering its value as a simple method, and its merit in enhancing tear production and tear film breakup time.



### Gastroscopy should be considered as a reliable and non-invasive technique for investigation of chronic vomiting in dogs

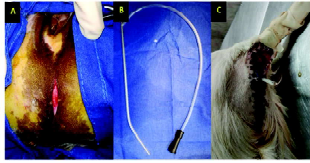
This study at Veterinary Surgery and Radiology department, COVAS, Mannuthy evaluated the efficacy of endoscopy as a diagnostic tool to ascertain the aetiology of chronic vomiting in dogs. Twelve dogs with the history of chronic vomiting and not responding to the routine medical treatment formed the subject of the study. Endoscopy was used for diagnosing the condition and for taking biopsies from gastro-oesophageal lesions in a non-invasive manner. The study concluded that endoscopy should be considered as a reliable and non-invasive technique to elucidate the aetiology of chronic vomiting in dogs by direct visualisation of the mucosa of oesophagus, stomach and duodenum.

### Modified proximal perineal urethrostomy and tube cystostomy got compared in managing chronic obstructive urolithiasis in male goats

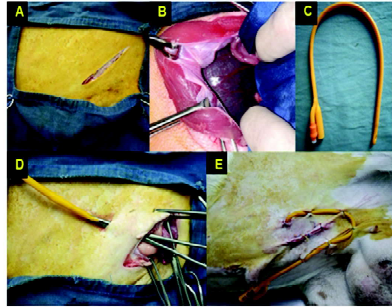
Surgeons from Veterinary Surgery and Radiology department, COVAS, Mannuthy compared the efficacy of two surgical techniques - modified proximal perineal urethrostomy (MPPU) and tube cystostomy in twelve clinical cases of chronic obstructive urolithiasis in male goats. MPPU technique provided a direct access to proximal perineal urethra and was found effective in cases with intact urinary bladder. Tube cystostomy provided direct visual assessment of urinary bladder, more invasive and identified as an effective approach



for correcting cystorrhesis resulted from obstructive urolithiasis. The MPPU technique demanded high levels of skill and accuracy to locate the perineal urethra. Functional patency of normal urethra was regained in five out of six animals of each group by third post-operative week. Microscopic examination of urine sediments revealed the presence of erythrocytes, struvite crystals and epithelial cells.



Modified Perineal Urethrostomy) using direct guided urethral catheterisation technique



Tube cystostomy technique

**A combination of polyethylene glycol and methyl prednisolone sodium succinate offers faster and better recovery in dogs with spinal injuries**

Department of Veterinary Surgery and Radiology, COVAS, Mannuthy conducted a study in fourteen dogs with clinical signs of spinal cord injury (SCI) in two groups. Neurological and radiographic examination suggested that, thoracolumbar region was mostly affected with SCI. All the animals were administered methyl prednisolone sodium succinate (30 mg/kg body weight) IV on the day of presentation. Animals of Group I were treated with 30 per cent solution of polyethylene glycol (M.W. 4000 Da), at the rate of 4ml/kg body weight twice at 48 hours interval, followed by oral administration of prednisolone acetate (1mg/kg body weight) at divided and tapering doses for next 10 days. Group II animals were given with 4-aminopyridine (4- AP) at the rate of 0.5mg/kg body weight intravenously on the day of presentation followed by oral administration of 4-AP at the rate of 1 mg/kg body three times daily for 10 days. All dogs were given supportive therapy with neurotrophic vitamin and analgesic for 30 days. Among Group I animals, 71.4% had recovered from the condition , and 42.8% in Group II by the end of sixth week. The recovery was found to be earlier among the animals of Group I.

**Midazolam – ketamine- isoflurane anaesthesia after butorphanol-xylazine premedication is a safe anaesthetic protocol with adequate unconsciousness, analgesia and muscle relaxation in bovines**

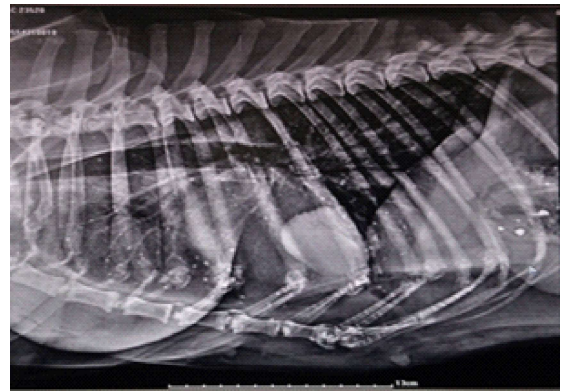
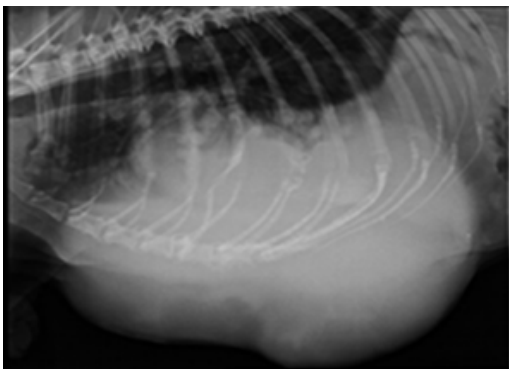
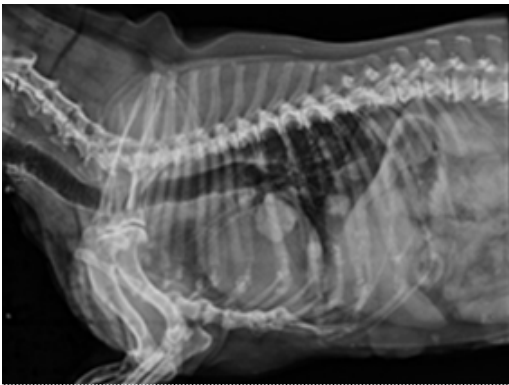
Surgeons from Veterinary Surgery and Radiology department, COVAS, Mannuthy evaluated clinical efficacy of midazolam - ketamine - isoflurane anaesthesia in butorphanol – xylazine premedicated bovines. Six crossbred cattle were premedicated with IV administration of butorphanol and xylazine at the dose rate of 0.05 and 0.02 mg/kg body weight respectively. On achieving sedation anaesthesia was induced by IV administration of ketamine and midazolam at the dose rate of 4.0 and 0.2 mg/kg body weight respectively. Anaesthesia was maintained through cuffed endotracheal tube connected with semiclosed system in large animal inhalant anaesthesia machine, using isoflurane 2.30 + 0.21% in 100% oxygen. The degree of sedation was moderate and time for sedation was 8.67 + 1.48 minutes. Induction time for anaesthesia was 4.33 + 0.61 minutes. The quality of induction was excellent with complete abolishment of the pain reflexes and excellent muscle relaxation. Recovery from anaesthesia was smooth in all the animals, with an overall time of 34.33 + 5.78 min. The results showed that the protocol adopted was suitable safe anaesthesia in cattle, with adequate unconsciousness, analgesia and muscle relaxation to carry out major surgical procedures.





**A protocol for TNM (Tumour – Node - Metastasis) staging of superficial and mammary neoplasms in dogs was developed using radiography**

Surgeons from Veterinary Surgery and Radiology department, COVAS, Mannuthy evaluated three way thoracic radiographs of twenty- four dogs with malignant superficial or mammary neoplasms to detect radiographically evident cardio-pulmonary, metastatic and paraneoplastic changes. The most evident metastatic lesions in malignant mammary neoplasm were pulmonary nodules and pulmonary masses whereas the miliary nodules and pulmonary micronodules were mostly appreciated in superficial neoplasms. The atypical metastatic lesions such as calcification, secondary pneumothorax, cavitation signs and feeding vessel sign were prominent in advanced stages of malignant neoplasms. The FNAC of primary tumour mass and histopathology were correlated with the radiographic findings. The detectability of the cardiopulmonary lesions were studied to develop a protocol for TNM (Tumour – Node - Metastasis) staging of superficial and mammary neoplasms in dogs.



**B-mode Ultrasonic ocular biometry is a non-invasive and versatile diagnostic modality for diagnosing ophthalmic disorders in dogs**

Department of Veterinary Surgery and Radiology, COVAS, Mannuthy conducted this study in 55 dogs presented with various ocular affections of various breeds between three months to 15 years of age presented with various ocular affections. Of the 55 dogs, biometry for various ocular parameters was recorded in 50 dogs. B-mode ultrasound scan was performed with manual restraint. Corneal desensitization was achieved by using topical anesthetic, 0.5% proparacaine hydrochloride. Biometry for ocular parameters like axial length of the eye (D1), vitreous chamber depth (D2), lens diameter (D3) and lens depth (D4) were recorded in all the dogs which differed significantly within the same group and between groups and between normal eyes and affected eyes. Various pathological lesions were diagnosed and imaged in rest of the five animals whose ocular biometry was not obtained in the current study. B-mode ultrasound scan is a non-invasive and versatile diagnostic modality which was helpful in diagnosing various disease conditions which otherwise would have been missed with conventional techniques.

**Propofol induced isoflurane anaesthesia is safe in geriatric dogs premedicated with diazepam and butorphanol**

Surgeons from Veterinary Surgery and Radiology department, COVAS, Mannuthy evaluated the efficacy of propofol-isoflurane anaesthesia with diazepam-butorphanol premedication for surgical procedures in geriatric dogs. Preanaesthesia was carried out in all the animals under study



by administering butorphanol at the dose rate of 0.2 mg per kg body weight and diazepam at the dose rate of 0.25 mg per kg body weight, intravenously with one minute gap between the administrations. Propofol (1% w/v) was administered slowly as a bolus, intravenously for induction of anaesthesia. Maintenance of surgical plane of anaesthesia was carried out with isoflurane in oxygen at the rate of 100 mL per kg body weight per minute by using Bain's circuit system incorporated with isoflurane vapourizer. The quality of sedation, induction, maintenance and recovery from general anaesthesia were good without any complication. Electrocardiographic, blood pressure and blood gas parameters were within the regular standard range in all geriatric animals and thus the anaesthetic protocol had minimum adverse effects on cardio-pulmonary system.

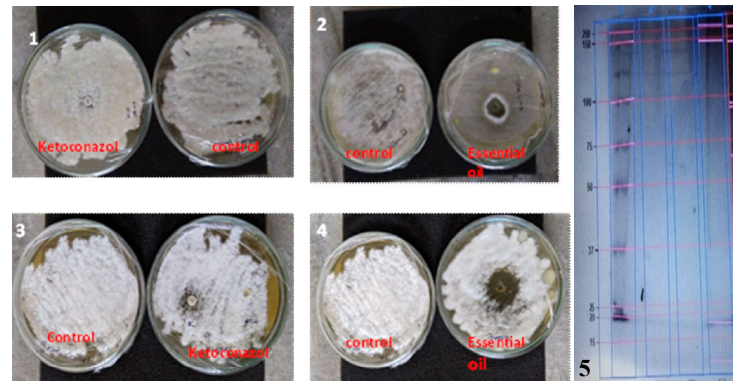
### String of pearls plating technique is an effective method for managing fracture of long bones in dogs

Department of Veterinary Surgery and Radiology, COVAS, Mannuthy conducted this study in nine dogs presented with fractures of long bones. Fracture stabilization by open reduction and internal fixation was done following standard AO principles using 3.5mm string of pearls plates and cortical screws. Radiographic examination revealed periosteal and endosteal callus formation from second postoperative week onwards and obliteration of fracture line and radiographic union of fracture fragments on sixth postoperative week. Complete radiographic union with minimal callus formation was observed by eighth post operative week in six dogs and implant failure due to screw loosening and plate instability was observed in three dogs. The SOP plate application was found to be effective in providing early pain free ambulation and also in managing fractures of long bones in dogs.



### Centrosema molle aqueous extract and protein from seed powder are having antifungal activity against fungal strains of Trichophyton rubrum and Microsporium gypseum

Studies on ethnoveterinary medicine conducted at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy, did the molecular weight identification of protein extracted from *Centrosema molle* seed powder. New fungal strains were used for the study of antifungal activity of essential oil of *Artemisia japonica* and *Centrosema molle* aqueous extract and protein from seed powder against fungal strains of *Trichophyton rubrum* and *Microsporium gypseum* were established. And in vitro antifungal activity of methanolic extract of new plant *Achalypha indica* and essential oil of *Artemisia japonica* were completed.



1. *Trichophyton rubrum* control plate and Ketoconazole treated plate.
2. *Trichophyton rubrum* control plate and essential oil treated plate.
3. *Microsporium gypseum* control plate and Ketoconazole treated plate.
4. *Microsporium gypseum* control plate and essential oil treated plate.
5. gel loaded with 5µl ladder at well 1 and 10µl protein at well 3.

### Selamectin spot on an effective therapy for flea bite dermatitis in cats

A study on flea allergy dermatitis in cats at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy, found out that flea bite dermatitis in cats could be successfully managed using topical Selamectin spot on @6mg/



kg every twenty four hours for seven days and Chlorpheniramine maleate @ 2mg orally every twelve hours for seven days.

### Arterial blood gas analysis - A valuable tool for assessing the severity of respiratory tract disorders in dogs

Pulmonary functions of dogs having respiratory and cardiac disorders were assessed in a study conducted at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy. Radiographic evaluation along with assessment of pulmonary function test helped to evaluate respiratory tract disorders both subjectively and objectively. Arterial blood gas analysis is a valuable tool that provides insight into the severity of respiratory tract diseases in dogs with respiratory tract dysfunction.

### Xenotransfusion in anemic cats

Xenotransfusion studies conducted at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy found out that there is a high prevalence of Type A blood group in cats in Kerala. All Persian cats were of Type A. Xenotransfusion of cats with canine blood can be advocated in emergency situations as a life saving measure.

### Vitamin E and selenium supplementation along with oxytetracycline therapy is effective to correct oxidative stress in caprine anaplasmosis

In a study conducted on oxidative stress in caprine anaplasmosis at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy, concluded that oxidative stress contributes to the severity of anemia in animals affected with anaplasmosis. Parasitemia and oxidative stress are positively correlated while parasitemia and antioxidants level are negatively correlated. Oxidative stress in anaplasmosis in goats can be corrected with vitamin E and selenium supplementation along with oxytetracycline therapy.

### Marked increase in urinary immunoglobulin G and urinary retinol binding protein in Babesia infected animals suggesting renal damage

A study on evaluation of renal function and therapeutic management of *Babesia gibsoni* infection in dogs were done at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy. There is a marked increase in urinary immunoglobulin G and urinary retinol binding protein in *Babesia* infected animals suggesting renal damage in glomerular and tubular level. *Babesia gibsoni* infection in dogs can be successfully treated with two injections of Bubarvaquone @ 5mg/kg body weight intramuscularly at 48 hour interval along with Azithromycin @ 10mg/kg body weight orally once daily. Ten days therapy was inadequate for complete elimination of parasite in clinically infected dog.

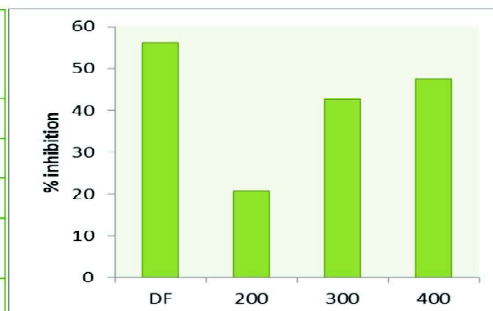
### Methanolic extract of *Acalypha indica* and essential oil of *Artemisia japonica* exhibited *in-vitro* anti-inflammatory activity

Studies on Ethnoveterinary medicine at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy, Evaluated anti-inflammatory activity of methanolic extract of *Acalypha indica* and essential oil of *Artemisia japonica* by Inhibition of protein denaturation. Methanolic extract of *Acalypha indica* and essential oil of *Artemisia japonica* showed *in vitro* anti-inflammatory activity by membrane stabilization method. Methanolic extract of *Acalypha indica* and essential oil of *Artemisia japonica* exhibited *in vitro* anti-inflammatory activity by heat induced haemolysis.



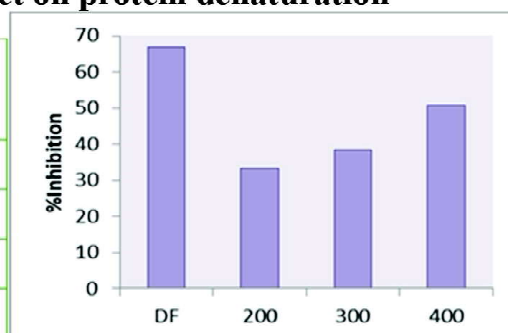
### Effect of *A. japonica* essential oil on protein denaturation

Sample	Concentration $\mu\text{g/ml}$	Percentage inhibition of Protein denaturation	Viscosity
Essential oil	200	20.73	$0.005 \pm 0.001$
Essential oil	300	42.68	$0.011 \pm 0.0005$
Essential oil	400	47.56	$0.013 \pm 0$
Distilled water Control	-	0	$0.027 \pm 0.0005$
Diclofenac sodium	100	56.09	$0.015 \pm 0.001$



### Effect of *Acalypha indica* methanolic extract on protein denaturation

Sample	Concentration $\mu\text{g/mL}$	Percentage inhibition of protein denaturation	Viscosity
Methanol extract	200	33.33	$0.009 \pm 0.001$
Methanol extract	300	38.27	$0.0103 \pm 0.001$
Methanol extract	400	50.61	$0.0136 \pm 0.0005$
Diclofenac sodium	100	66.66	$0.018 \pm 0.001$



### Heat induced haemolysis

Percentage inhibition of haemolysis by acetyl salicylic acid

Con [ $\mu\text{g/mL}$ ]	% Inhibition of haemolysis
0.976	$45.105 \pm 4.5$
1.953	$46.705 \pm 2.5$
3.9	$47.27 \pm 1.8$
7.81	$47.705 \pm 1.2$
15.6	$48.426 \pm 0.57$
31.25	$49.075 \pm 0.7$
62.5	$49.965 \pm 1.4$

Percentage inhibition of haemolysis by methanolic extract of *Acalypha indica*

Con [ $\mu\text{g/mL}$ ]	% Inhibition of haemolysis
0.976	$17.9 \pm 2.46$
1.953	$20.58 \pm 1.45$
3.9	$21.46 \pm 1.52$
7.81	$22.255 \pm 1.40$
15.6	$24.235 \pm 0.51$
31.25	$24.69 \pm 0.53$
62.5	$26.47 \pm 0.07$

Percentage inhibition of haemolysis by essential oil of *Artemisia japonica*

Con [ $\mu\text{g/mL}$ ]	% Inhibition of haemolysis
31.25	$1.45 \pm 0.67$
62.5	$3.435 \pm 3.27$
125	$5.54 \pm 0.66$
250	$6.02 \pm 0.50$
500	$6.62 \pm 0.53$

### Hypotonic solution induced haemolysis

Percentage protection by Diclofenac sodium

Con ( $\mu\text{g/ml}$ )	% protection
31.125	$31.725 \pm 4.080$
62.25	$35.575 \pm 12.24$

Percentage protection by Methanolic extract of *Acalypha indica*

Con ( $\mu\text{g/ml}$ )	% protection
31.125	$18.415 \pm 18.6$
62.25	$47.365 \pm 9.92$

Percentage protection by essential oil of *Artemisia japonica*

Con ( $\mu\text{g/ml}$ )	% protection
31.125	$80.765 \pm 5.43$
62.25	$90.38 \pm 2.71$



### Healthcare of Farm Animals and Pets

Blood transfusion facility for farm and pet animals are established in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy. Blood transfusion were carried out on seven anemic goats and twenty anemic dogs affected with blood parasites. Haemato -biochemical changes with special reference to iron status in anaemic goats are under progress.

state in thrombocytopenic dogs. Hypoantithrombinemia with severely prolonged coagulation parameters indicated hypocoagulable state in thrombocytopenic dogs.

### CPDA-SAGM is a suitable storage media for caprine packed RBCs

In a study conducted at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy, found out that Saline Adenine Glucose Mannitol additive in citrate phosphate dextrose adenine is a suitable storage media for storing packed red blood cells of goats.

### Establishment of training hall for livestock farmers, entrepreneurs, veterinarians and paraveterinarians

A Well-equipped conference hall with all digital facilities to accommodate thirty participants was established at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy.

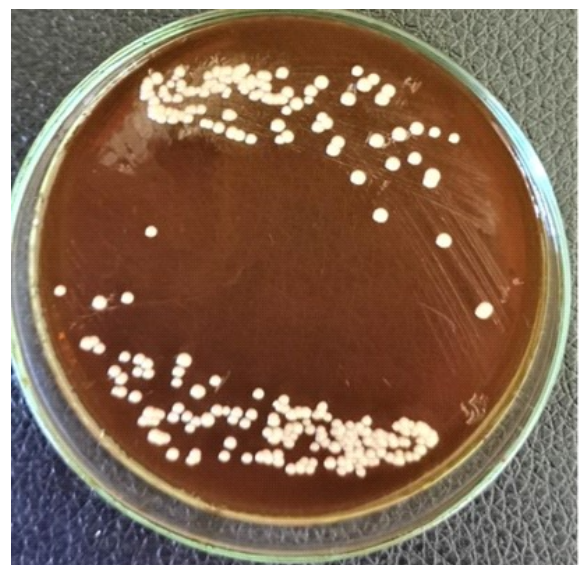


### *Malassezia pachydermatis* and *Malassezia japonica* were identified using nucleic acid sequencing from dog skin lesions

In a study conducted at Department of Veterinary Clinical medicine, Ethics and Jurisprudence, Pookode, the prevalence of malasseziosis among dogs with dermatological problems was found to be 14.7 per cent. The isolation rate of malassezia yeasts was found to be highest on MDA with 76.7 per cent. On PCR targeting the LSU rRNA gene of *Malassezia* spp., twelve sequences showed similarity to sequences of *Malassezia pachydermatis* and one sequence to *Malassezia japonica*. On clinical and cytological examinations, orally itraconazole and topically neem oil were found to be very effective by 28th day of treatment.

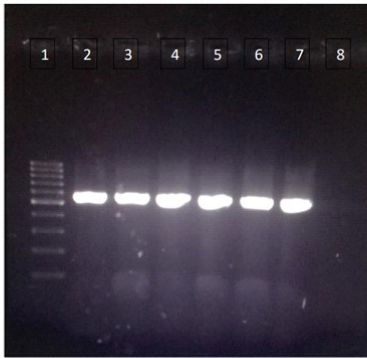
### Hypoantithrombinemia and elevated D Dimer as markers for early identification of hypercoagulable state in thrombocytopenic dogs

In a study conducted at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy on thrombocytopenic dogs showed that major causes of thrombocytopenia include infection, inflammation, neoplasia, disseminated intravascular coagulation and other miscellaneous diseases. Hypoantithrombinemia and elevated D Dimer with normal to mildly elevated coagulation parameters could be used as markers for early identification of hypercoagulable

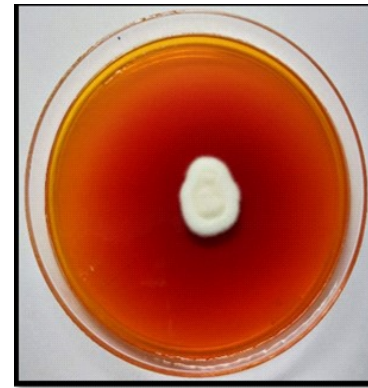


Colonies of *Malassezia pachydermatis* on MDA





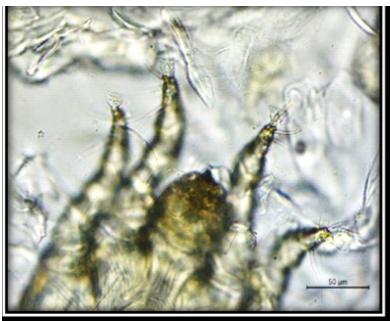
Molecular detection of *Malassezia* spp.  
(Lane 1: 100 bp ladder, Lanes 2 to 7: *Malassezia* isolates, Lane 8: No template control)



White coloured granular colony  
produced by *Trichophyton*

***Chorioptes texanus* was identified as the main organism causing mange in goats**

In a study conducted on dermatological disorders in goats at the Department of Veterinary Clinical medicine, Ethics and Jurisprudence, Pookode, 50 per cent parasitic, 47.06 per cent fungal and 2.94 per cent bacterial dermatitis were observed. The prevalence of mange and dermatophytosis was same and it was 38.24 per cent. *Chorioptes texanus* was identified as the organism causing mange in goats and alopecia with crusts, thickening, wrinkling, hyperpigmentation and fissure formation on the skin at legs, axillae, inguinal region, perineal region and ears were the major clinical findings. It was effectively treated with ivermectin @ 200 µg/kg BW S/C at 10 days interval till two consecutive skin scrapings were negative for mites. Topical application of Permethrin 0.05 per cent and lime sulphur dip were also found to be effective. *Trichophyton mentagrophyte* was found out as the major organism causing dermatophytosis in goats. Topical application of two per cent miconazole and Tincture Iodine: Glycerin (1:1) for 15 days along with Vitamin A supplementation were found to be effective.



*C. texanus* with cup shaped suckers on unsegmented pedicel of first and second pairs of legs

**Estimation of Alpha Glutathione S transferase for early diagnosis of hepatic disorders in dogs**

In a study conducted at the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Pookode, the scientists found out that there is significant increase in the level of mean Alpha Glutathione S Transferase level in the serum of hepatic disorders in dogs and can be used for early diagnosis of the hepatic disorders in dogs. Eighty five percent of the animals treated with silibin-phosphatidylcholine showed good therapeutic response. The study concluded that the estimation of Alpha GST was more efficacious in diagnosing liver diseases.

**Qualitative analysis of the dog urinary calculi using Fourier transform infrared spectrophotometer**

A study on urolithiasis in dogs at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Pookode, identified *staphylococcus spp* as the predominant organism causing urolithiasis in dogs. Amoxicillin- Clavulanic acid was the most sensitive antibiotic for treating urolithiasis. On qualitative analysis of the urinary calculi using Fourier transform infrared spectrophotometer, the presence of calcium oxalate and calcium phosphate stones were detected in all the cases.

**Two species of *Demodex* mites *D. canis* and *D. cornei* were identified in dogs**

In a study conducted at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Pookode, on demodicosis in dogs, noted that alopecia, papules, pustules,



scales and hyperpigmentation were the predominant skin lesions in canine demodicosis. Tape impression smear method is found to be a sensitive diagnostic technique for generalised demodicosis. When conventional diagnostic methods fail, PCR can be recommended as a test of higher sensitivity for detecting *Demodex* spp. mites. Two species of *Demodex* mites ie. *D. canis* and *D. cornei* were identified using morphometry and nucleic acid sequencing. On phylogenetic analysis it was found that *Demodex cornei* is a morphological variant of *D. canis*. Treatment with doramectin injections offered a faster recovery when compared with ivermectin therapy against demodicosis.

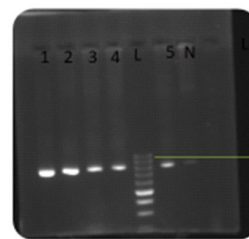
### BLAST analysis showed presence of non-tuberculous mycobacteria in the free ranging wild animals

Screening of selected species of free ranging wild animals of Kerala for *Mycobacterium tuberculosis* complex by Real-Time Polymerase Chain Reaction was done at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Pookode. All the samples of wild boars, deer and monkeys were negative for *Mycobacterium tuberculosis* complex. A total of four elephant samples shown positive result by IS6110 gene targeted real-time PCR and three were positive for conventional PCR targeting same gene. All the samples shown negative result in senX3-regX3 intergenic region targeted real-time PCR. A total of three elephant samples shown positive result by 16SrRNA targeted PCR and among them one sample shown positive result by hsp65 gene targeted PCR also. All samples were negative for plcA and recA intein gene. Though IS6110 has been detected in the samples, none of the other tests conducted were positive for MTBC. The BLAST analysis of positive isolates points towards presence of non-tuberculous mycobacterium in these samples. Hence it is concluded that the lesions observed in these animals could be due to non-tuberculous mycobacteria.

### Enrofloxacin or oxytetracycline were equally effective in treating caprine anaplasmosis caused by *A. bovis*

A study was conducted on anaplasmosis in goats at Department of Veterinary Clinical medicine, Ethics and Jurisprudence, Pookode, found out the main clinical signs exhibited by goats with *A. bovis* infection were anaemia,

anorexia, fever, debility, loss of appetite, jaundice and swollen pre-scapular lymph nodes. The overall incidence of anaplasmosis was 18.33 per cent. Oxytetracycline @ 20mg/kg body weight intravenously for three days and long acting enrofloxacin @ 7.5 mg/kg body weight intramuscularly was found to be equally effective for treating caprine anaplasmosis caused by *Anaplasma bovis*. Polymerase chain reaction was highly sensitive compared to blood smear examination in detecting *A. bovis*.



#### Legends

- L – 100 bp ladder
- 1 – 05-Positive Samples
- N – Negative control

Amplicons at 781 bp

Sequencing – 99 per cent identity

### Oxytetracycline is the more efficient drug in treating caprine respiratory mycoplasmosis

Scientists of Veterinary Clinical Medicine, Ethics and Jurisprudence Department, COVAS, Pookode studied respiratory mycoplasmosis in goats of Wayanad district and found higher prevalence of caprine respiratory mycoplasmosis especially in young ones and in winter season. Cough, dyspnea, nasal discharge, lethargy, ocular discharge, anorexia and fever were the major clinical signs. Amplicons from genus specific PCR of *Mycoplasma* DNA from nasal swabs of affected goats upon sequencing were identified as *Mycoplasma ovipneumoniae*. Higher rates of complete recovery in the affected goats after five days was seen in goats treated with Oxytetracycline using clinical score card method.

### Drop in serum progesterone level is not a mandate for elective caesarean section in high-risk pregnant dogs

In high-risk pregnant dogs, elective caesarean is performed once the serum progesterone levels fall below 2 ng/mL. A series of progesterone assays are required to confirm this drop and this voluntary waiting period for the serum progesterone to decline may often result in foetal loss. Scientists of Veterinary



Genecology and Obstetrics department, COVAS, Mannuthy conducted a study to assess the feasibility of elective CS in dogs at higher levels of before the pre-partum decline of serum progesterone concentration to  $> 2$  ng/mL and without administration of any priming agents. They concluded that drop in serum progesterone level to less than 2 ng/ml is not a mandate in dogs for elective CS and surgeries could be safely performed up to a level of 4 ng/mL of serum progesterone.

### F1 fraction of sperm rich fraction of boar ejaculate offers better cryopreservability

Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy evaluated a total of 66 ejaculates from four adult healthy Large White Yorkshire boars for assessing the difference in semen qualities among the fractions. Significant differences were noted in volume, pH, sperm concentration and sperm membrane cholesterol between F1 and F2 of the ejaculate. On analyzing sperm preservability in liquid state at 15°C, it was apparent that the initial 10 mL of sperm rich fraction (SRF) (F1) had better preservability than rest of SRF (F2). The parameters of sperm progressive motility, viability, abnormality, acrosome integrity, plasma membrane integrity, functional membrane integrity and sperm membrane cholesterol content were assessed and these varied significantly between fractions. It was concluded that F1 fraction of LWY boar semen with supplementation of Sodium dodecyl sulphate (SDS, as a detergent) could yield better cryopreservability.



Fig 1: Semen collection as fractions from LWY boar with gloved hand technique.

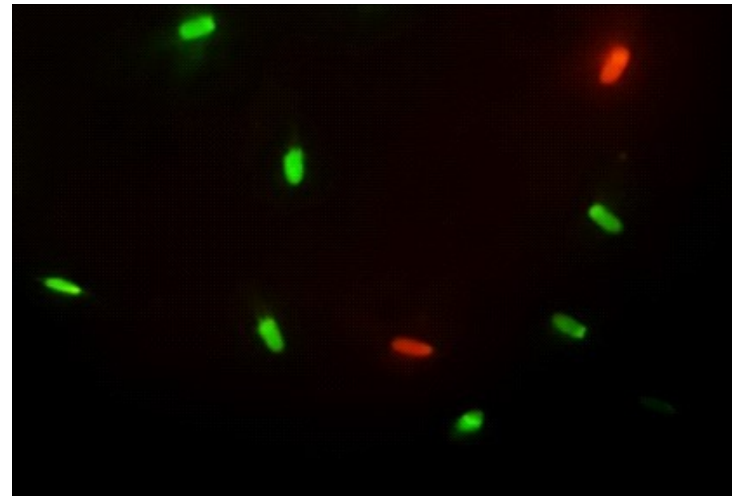


Fig 2: Sperm plasma membrane integrity assessed with SYBR-PI fluorescent probes. The sperm heads fluorescing green are having an intact plasma membrane and those with red fluorescence are damaged.

### Dexamethasone and epidermal growth factor will significantly enhance embryo yield during *in vitro* production (IVP) of bovine embryos

In recent years *in vitro* production (IVP) of embryos is increasingly overtaking multi-ovulation embryo transfer technology. But due to suboptimal support provided by the *in vitro* culture medium to embryo subjected to IVP, it remains a relatively inefficient process. Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy assessed the individual and combined effect of dexamethasone and epidermal growth factor in synthetic oviduct fluid on blastocyst production. Presumptive zygotes obtained by *in vitro* maturation and fertilisation of abattoir origin oocytes were treated in four groups with dexamethasone, Epidermal Growth Factor (EGF), combination of dexamethasone and EGF and without any supplementation. The study concluded that a significantly higher embryo yield and developmental competence for EGF alone and combination of EGF and dexamethasone. This research opens up the possibility of modifying the embryo culture with dexamethasone and EGF.

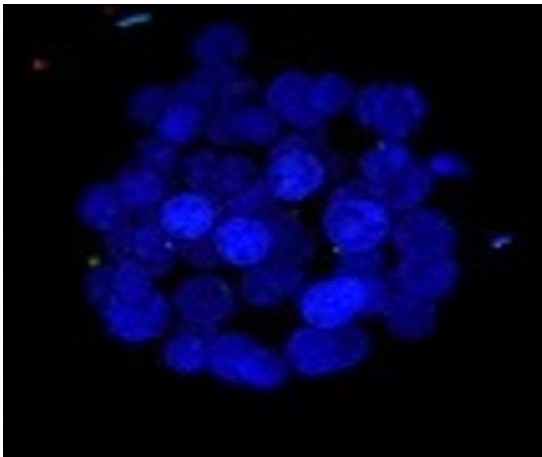


Fig 1 Embryo viability by Hoechst 33342 staining



Fig1 Four cell stage embryo

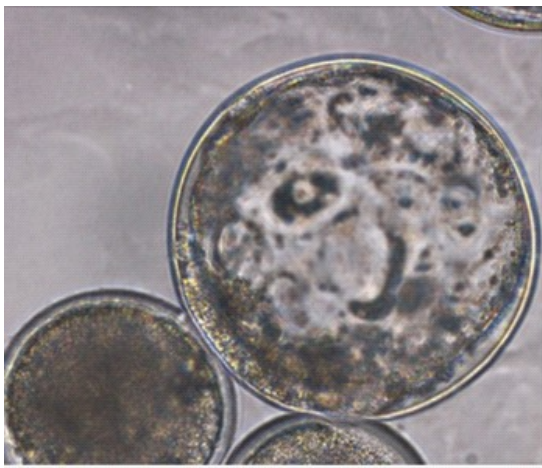


Fig 2: Blastocyst

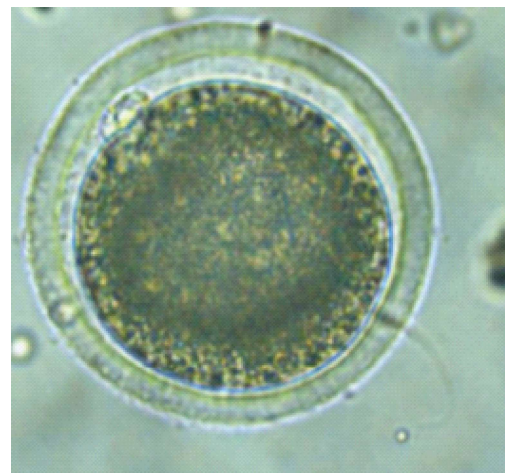


Fig 2 Sperm penetration into ooplasm

**In Kerala, rainy season is the most favourable season for good quality semen production**

Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy analysed fertilization capacity of frozen crossbred bull semen produced during different seasons, *in vitro*. Aspirated culture grade oocytes from slaughter ovaries were subjected to *in vitro* maturation and randomly divided into three groups. In group I oocytes were allowed to fertilize with the semen from six crossbred Holstein Friesian bulls having same exotic inheritance cryopreserved during rainy season (June- September), in group II with post monsoon season (October- January) and group III with summer season (February- May). The present study concluded that hot dry summer season in Kerala will adversely affect the fertilization capacity of spermatozoa. Rainy season in Kerala is the most favourable season for good quality semen production and post monsoon season is the intermediate between summer and rainy season.

**Pre-maturation with cyclic adenosine monophosphate modulators enhances the developmental competence of bovine oocytes**

The mammalian oocyte is arrested at prophase I of cell cycle and its meiotic arrest is maintained by its higher intracellular cyclic AMP levels. Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy conducted a study to assess the effect of pre-maturation with cAMP modulators on *in vitro* maturation (IVM), fertilisation and cleavage of bovine oocytes and to determine the effect of different duration of pre-maturation on oocyte developmental competence. Ovaries from crossbred cattle of unknown reproductive status were collected and culture grade oocytes were divided into four groups with one, two, four hours



prematuration respectively in group I, II and III, and without prematuration in group IV. In all three treatment groups cyclic adenosine monophosphate modulators (Forskolin and IBMX) were added right from the time of aspiration of follicles itself. The research concluded that prematuration with cAMP modulators and increasing the duration of prematuration have a direct positive correlation with the developmental competence of bovine oocytes.

+ 25.9 and in mid to late pregnancy,  $GA = (13.28 \times BPD) + 27.58$  where, ICC and BPD is in cm. The prediction accuracy of whelping date within  $\pm 3$  days for new and existing model based on ICC was 100 and 85 per cent, while for BPD, it was 100 and 17 per cent respectively. So present study concluded that existing model overestimated the prediction of parturition date, while the new model increased accuracy of prediction of whelping dates in small sized breeds.

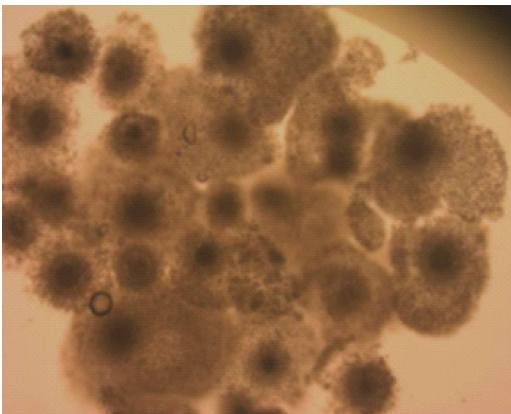


Fig.1. Oocytes after maturation

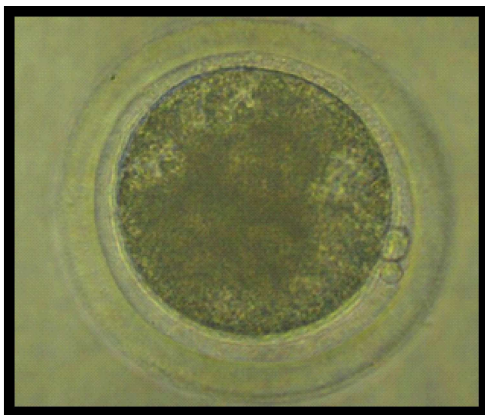


Fig 2 Second polar body extrusion



Fig 1 USG image showing head of foetus

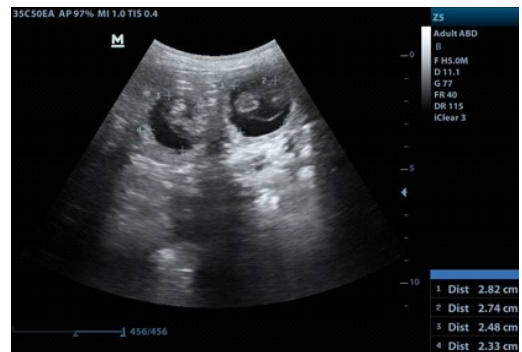


Fig 2 ICC measurement of 54 days pregnant dog

**Brilliant cresyl blue (BCB) staining could be used as a non-invasive selection tool for competent goat oocytes**

**A more accurate model for prediction of whelping date in small breeds of dogs**

In the first phase of this study, scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy developed a new model based on ultrasonographic measurements like inner chorionic cavity diameter (ICC) and biparietal diameter (BPD) of early and mid to late pregnancy small sized breeds. In second phase of the study, they validated the newly developed model in comparison with the existing model. The equation developed for prediction of parturition in early pregnancy was  $Gestational\ age\ (GA) = (4.2 \times ICC)$

Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy designed a study to assess the developmental competence of goat oocytes selected using brilliant cresyl blue (BCB) staining and to evaluate the individual and combined effect of foetal bovine serum (FBS) and oestrus goat serum (OGS) on *in vitro* maturation (IVM). Goat oocytes selected using BCB (BCB+) stain showed significantly higher maturation and fertilisation rate. No significant difference with respect to maturation and fertilization rate could be observed between the media. The study revealed that BCB staining could be used as a non-invasive selection tool for competent goat oocytes in *in-vitro* embryo production.



### **Cytology and biopsy could be valuable diagnostic techniques for detecting subclinical endometritis in postpartum dairy cows**

Different diagnostic techniques like endometrial cytology, biopsy and ultrasonography on identifying subclinical endometritis in cross bred postpartum dairy cows were analysed by Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy in 24 apparently normal cows without any periparturient complications. It was concluded from the study that ultrasound findings alone have poor efficacy in diagnosing subclinical endometritis but it could be used as a supporting tool and cytology and biopsy could be used as valuable diagnostic techniques in detecting subclinical endometritis with biopsy provide more detailed information about the deeper layers of uterus.

### **Echobiometric and doppler ultrasound studies of the prostate and testes could be useful in subfertility and designing treatment in dogs**

Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy evaluated B-mode and Doppler ultrasonography of testes and prostate in male dogs presented for breeding soundness evaluation (BSE). The peak systolic volume (PSV) of cranial prostatic artery was positively correlated with sperm concentration and volume, while pulsatility index (PI) of the same artery was negatively correlated with concentration. The PSV of testicular artery was significantly lower in subfertile dogs, while PI of the same artery was negatively correlated with sperm concentration. PSV and end diastolic volume (EDV) of testicular artery were positively correlated with sperm motility, viability and concentration. The study suggested use of echobiometric and Doppler studies of the canine prostate and testes in the critical evaluation of dogs with subfertility and the use of such tools in the design of relevant treatment protocols.

### **Ultrasonography and haematobiochemical evaluations are significant in the diagnosis and prognosis of uterine torsion in does**

Studies conducted at Veterinary Genecology and Obstetrics department, COVAS, Mannuthy suggested

parameters like serum alkaline phosphatase, creatinine and cortisol assay could be used for diagnostic and prognostic evaluation of caprine uterine torsion. Changes in the placentome dimensions and uterine wall thickness, measured by B-mode ultrasonography could be used for diagnosis; echogenicity of foetal fluids could act as prognostic indicator of foetal viability. Doppler blood flow velocimetric studies could also be used for diagnostic and prognostic evaluation of uterine torsion, especially of pre-cervical nature, where per vaginal examination would not bring about any elucidation.

### **Elective caesarean section can be safely done in dogs with high-risk pregnancy after induction of foetal maturation**

The study was carried out by the scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy to assess the maternal and neonatal outcome in dogs with high-risk pregnancy, subjected to elective caesarean section after induction of foetal maturation. The study concluded that Elective caesarean section prior to serum progesterone drop may be adopted in high-risk pregnant bitches, by using mifepristone as an agent to induce foetal maturity, without any adverse effects to the dam or neonates.

### **Proteinuria might be a better choice in the diagnostic and prognostic evaluation of canine pyometra**

Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy evaluated serum prostaglandin metabolite concentration and haematological changes in canine pyometra and suggested that changes in total leucocyte count, band cell per cent, total erythrocyte count, haemoglobin concentration and volume of packed red cells could be useful diagnostic and prognostic markers in canine pyometra. They also reported proteinuria as a better choice than BUN and creatinine in evaluating diagnosis and prognosis of canine pyometra. Feasibility of serum prostaglandin metabolite measurement as a tool for diagnostic and prognostic evaluation of canine pyometra needs further studies.



### Shortening of inter-oestrous interval in dogs can be done using GnRH agonist and PGF<sub>2A</sub>

Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy observed that shortening of inter-oestrus interval in dogs through manipulation of diestrus can be achieved by combination therapy using PGF<sub>2</sub> analogue (Cloprostenol) and GnRH agonist (leuprolide acetate) but, further investigations are warranted to elucidate on conception and pregnancy after oestrus induction.

### Labrador retrievers showed more occurrence of vaginal hyperplasia

Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy studied various clinicopathological changes associated with vaginal hyperplasia in dogs. They found it more common in one to two years of age, nulliparous intact dogs during the follicular phase of the oestrous cycle and in Labrador Retrievers. Type II vaginal hyperplasia with normal follicular characteristics and vaginal cytological findings of oestrus period were observed. The serum oestradiol levels were similar to an oestrus dog. Histopathology revealed more oestrogenic response in stratum germinativum of vaginal mucosa. Immunohistochemistry revealed significantly high proportional score of ER- $\alpha$  receptors in the vaginal epithelium of vaginal hyperplasia affected dogs.

### GnRH and PGF<sub>2a</sub> treatment improved conception rate in crossbred cattle exhibiting prolonged oestrus

Suprabasal levels of progesterone during oestrus and luteal deficiency during midluteal phase were observed in animals exhibiting prolonged oestrus. Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy found that treatment using GnRH in early luteal phase or GnRH in early luteal phase with PGF<sub>2</sub> in midluteal phase lowered the suprabasal levels of progesterone and corrected the luteal deficiency in subsequent oestrous cycle. They concluded study by suggesting this treatment followed by timely insemination for improved conception rate among crossbred cattle exhibiting prolonged oestrus.

prolificacy in goats after confirming the influence by extensive association studies.

### Serum LPS could be used as a better diagnostic marker in pyometra of dogs

Scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy studied various clinicopathological and therapeutic aspects of open and closed pyometra in dogs. Ultrasonographic examination revealed uterine enlargement with accumulation of fluid of varying echogenicity, uterine wall thickening and cystic endometrial changes. High levels of toxic neutrophils and eight mixed isolates and four pure isolates of bacteria with predominance of Gram-negative bacilli were observed. Antibiogram showed maximum sensitivity to ceftriaxone-sulbactam in open cervix pyometra dogs and amoxicillin-clavulanic acid in closed-cervix pyometra dogs. Serum LPS levels were elevated during all days of observation and because of its early appearance and stable nature, it could be used as a better diagnostic marker in canine pyometra. Total erythrocyte and leucocyte counts, neutrophil and band cell per cent, toxic neutrophil scoring and uterine ultrasonography could be used as prognostic indicators in monitoring treatment response to pyometra. Medical management using mifepristone and cloprostenol were found to be successful in both open and closed cervix pyometra affected dogs.

### Single nucleotide polymorphisms (SNP) of GUCY1B3 gene that can be recommended as molecular markers for improving prolificacy in goats identified

In this study, scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy evaluated uterine artery haemodynamics in singleton and multiple foetal gestations in malabari goats. It was observed that the birth weight of kids and gestation length decreased with increase in litter size. Equation formulated to estimate gestational age by using FOD, PLD, UCD and FHR are the first equations in Malabari breed as well as in Indian goat breeds. Doppler indices (PI, RI and S/D ratio) of both Uterine artery and umbilical artery standardized in singleton and multiple foetal gestations, which can be used for identifying pathological conditions in pregnancy. Two novel single nucleotide polymorphisms (SNP) identified in the *Guanylate cyclase-1 soluble subunit beta (GUCY1B3)* gene could be recommended as molecular markers for improving



Fig 1. Pulse wave Doppler ultrasonography of uterine artery

### Combination treatment using mifepristone and dinoprost is superior for therapeutic management of canine pyometra

Study was undertaken by the scientists of Veterinary Genecology and Obstetrics department, COVAS, Mannuthy to evaluate the efficacy of different therapeutic protocols in the treatment of canine pyometra, based on clinical, haemato-biochemical, B-mode ultrasonographic and Doppler ultrasonographic evaluation. Combination treatment using mifepristone @ 2.5mg/kg b wt. twice daily orally for five days, along with incremental doses of dinoprost from 10 to 50 µg/kg b. wt. thrice daily; 48 h after initiation of mifepristone, till complete uterine evacuation was found to be superior for therapeutic management of canine pyometra in terms of clinical recovery and future fertility. Serum progesterone concentration were not abnormally high among the affected bitches. Recurrence among considerable proportion of bitches were noticed after medical management of canine pyometra.

### Nutritional deficiency is the main reason behind the delayed attainment of puberty in heifers

This study was conducted at Veterinary Genecology and Obstetrics department, COVAS, Mannuthy for enhancing reproductive efficiency in ruminants. They found that nutritional deficiency is the main reason behind the delayed attainment of puberty in heifers and delayed ovulation is one among the prime reasons of conception failure in cows. Ultrasonography is found to be an effective diagnostic aid in infertility management in cattle and goats. Supplementation of minerals, vitamins and various hormonal preparations could be successfully used to improve the conception rate in ruminants.

### Detection of *Leptospira* serovar infecting symptomatic dogs in Wayanad District

In a study conducted at KVASU, predominant leptospiral serovar among dogs in Wayanad district identified by Microscopic agglutination test were Pyrogens (35.71%), followed by Javanica and Icterohaemorrhagiae (21.42% each) Australis, Bataviae, Grippotyphosa and Tarasovi (12.28 per cent each) and Canicola (7.14 per cent). Major clinical manifestations of affected dogs were pyrexia, anorexia, vomiting, jaundice, haematuria and melena. Mostly, males, working breeds (Rottweilers and Labrador retrievers) and middle-aged dogs (2 to 3 years of age) were found to be affected. Major clinic-pathological evident in positive dogs were significant leucocytosis with granulocytosis, thrombocytopenia, decrease in lymphocyte and monocyte counts, anaemia, azotemia, elevated ALT and bilirubin levels, with increased serum total protein, globulin and A:G ratio, proteinuria and haematuria. Post-mortem findings were icterus on gingiva, sclera and subcutaneous tissues, petechial haemorrhages on liver, lungs, kidney, gastrointestinal tract and urinary bladder.

### Tuberculosis in captive sloth bears

Sloth bears (*Melursus ursinus*) are highly vulnerable species as per IUCN and are possible spill over hosts for tuberculosis due to their prolonged cohabitation with human beings under highly compromised situations. A study was conducted at KVASU for ascertaining the seroprevalence and molecular identification of TB in 37 rescued bears from Bannerghatta Bear Rescue Centre, Bengaluru. The study showed a total sero-positivity of 37.84 per cent using a lateral flow antibody detection kit. Majority of positive animals were belonging to 10 to 20 years of age (64.28%), females (57.15%) and with normal body condition (64.29%). A total of 4 (12.9%) live animals and 2 (66.67%) dead animals were confirmed positive for TB by PCR. Major clinical signs showed by animals died of TB were anorexia, dullness, isolation from the group and respiratory signs for few weeks before death. On thoracic radiography, 78.57 per cent seropositive animals had miliary soft tissue opacities in their lungs and major clinic-pathological findings were oesinopenia, basophilia, increased serum cholesterol levels.





### Multidrug resistant Staphylococcal pyoderma were identified in dogs

Clinical samples of canine pyoderma were subjected for culture examination and antibiogram studies. *Staphylococcus* spp. was identified as the commonest organism (84.51 per cent). Among the 60 *Staphylococcus* spp., 53.33 per cent were identified as *S. epidermidis* followed by *S. pseudintermedius* (16.67 per cent), *S. lugdenensis* (13.33 per cent), *S. hyicus* (5 per cent), *S. schleiferi* (5 per cent), *S. aureus* (3.33 per cent) and *S. delphini* (3.33 per cent). On antibiogram analysis of the isolates majority showed multidrug resistance. Penicillin resistant staphylococci were confirmed the most (62 per cent) in the study followed by tetracycline resistance (50 per cent), fluoroquinolone resistance (35 per cent) and Macrolide resistance (20 per cent).

### Infectious etiologies of respiratory diseases in psittacines were identified

A total of 30 aviaries from seven districts namely Thiruvananthapuram, Alappuzha, Ernakulam, Thrissur, Kozhikode, Wayanad and Kannur were visited and 190 birds with respiratory signs like tail bobbing, dyspnoea, nasal discharge, conjunctivitis, etc. were sampled. Out of 190 birds, 71 (37.37 per cent) were positive for *Chlamydia psittaci*. *M. gallisepticum* was positive in 60 birds with a prevalence of 31.58 per cent. Least occurrence was for Paramyxoviral infection with 5.79 per cent and *Mycobacterium* spp. was not identified. Major clinical signs seen in Chlamydia infection were conjunctivitis and greenish diarrhoea. Birds positive for Mycoplasmal infection had tail-bobbing and conjunctivitis and in Paramyxoviral infection dyspnoea and neurological signs were seen. Budgerigars were most commonly affected with Chlamydial infection whereas small psittacines and finches were susceptible for Mycoplasmal infection. Cockatiels and alexandrine parakeets were positive for Paramyxoviral infection. Source of the bird and poor biosecurity practices was found to play a major role in spread of infections among psittacine birds in Kerala.

### A new urine biomarker, Galactosylgalactosyl-xylosylprotein 3-beta-glucuronosyltransferase identified for diagnosis of tuberculosis in elephants

The study was envisaged by KVASU on utilization of serological, molecular and proteomic diagnostic assays for tuberculosis diagnosis. The use of urine as a biological sample for the identification of biomarkers for tuberculosis forms the prime focus of the study. Seroprevalence of tuberculosis in elephants were evaluated in 86 elephants from Kerala using Chembio DPP VetTB, which revealed 37.2 per cent seroprevalence. The urine of six TB confirmed elephants (positive for DPP VetTB assay, LF-LAM (Lateral flow test for LAM antigen), AFB (acid fast bacilli) and PCR) and six healthy elephants (negative for DPP VetTB assay, LF-LAM, AFB and PCR) were selected for proteomic analysis. Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase was identified as a potent biomarker for tuberculosis. The present study is the first report of the presence of Galactosylgalactosylxylosyl protein 3-beta-glucuronosyltransferase protein in urine of elephant.

### Clinico- pathological and therapeutic studies on colibacillosis in neonatal calves

Neonatal diarrhoea caused by pathogenic strains of *Esherichia coli* is very important among the economically important diseases of bovine calves and is associated with morbidity, retarded growth and mortality. A total of thirty neonatal calves affected with diarrhea were randomly selected from those dairy farms and rural households in Wayanad district. Different strains of *E. coli* associated with neonatal calf diarrhoea were characterized based on bacterial isolation and identification of virulence factors by polymerase chain reaction (PCR). Pathogenic *E. coli* isolates from neonatal calves were compared with those from drinking water and milk samples of the dam. Affected calves were treated based on result of antibiogram of the isolates and the pathological changes noticed in colibacillosis. Sulphamethoxazole/ trimethoprim gave sensitivity to 18 isolates which was similar to clinical response. Calves gave good response to fluid therapy and sodium bicarbonate administration was very helpful to control metabolic acidosis. Timely intervention is mandatory to save calves as dehydration manifest in a short time.



### Loop mediated isothermal amplification (LAMP) for detection of panleukopenia virus in cats

Cat population in Kerala is increasing, and so is the number of diseases of cats. Feline panleukopenia (FP) is one of the most important disease of the cats. The study aimed in determining the seroprevalence of feline panleukopenia in cats of northern districts of Kerala. It was observed that 29.11 per cent of 79 unvaccinated cats selected for the study were seropositive for FP. Clinical examination of the infected cats revealed Pyrexia, dysentery, vomiting, dehydration and respiratory distress were predominant clinical findings. The affected cats were found to be suffering from anaemia, thrombocytopenia, leucopenia and elevated liver enzymes. Ultrasonography revealed gas and fluid filled intestinal loops. A LAMP was standardised and successfully used for diagnosis of FP in cats. The assay eliminates the need for costly instrumentation for molecular diagnosis of FP, which traditionally relies on PCR.

### Salmonellosis in exotic pigeons from Northern districts of Kerala

Cloacal swabs from a total of 200 exotic pigeons belonging to 24 lofts from Northern districts of Kerala were collected and attempted to isolate Salmonella and understand its antimicrobial resistance profile. A percentage prevalence of 2.5 per cent was identified for salmonellosis with 16.67 per cent of the lofts affected. The isolates were resistant to tetracycline and enrofloxacin and most of the isolates were sensitive to amoxicillin clavulanate, chloramphenicol and ampicillin. Genes coding for the resistance towards tetracycline, tetracycline and chloramphenicol were detected in the isolates studied. Two isolates were serotyped and were found to be *Salmonella enterica* subsp. *enterica* serovar Kentucky.

### Surveillance of infectious diseases and seromonitoring of vaccination in farm animals

Infectious diseases of livestock are major threats to the economic sustainability of any livestock enterprise. KVASU

scientists has been involved in surveillance and monitoring of infectious diseases in various farms of KVASU, Animal Husbandry Department, KLDB and under private ownership. Under the project, intradermal tuberculin and Johnin test in various university farms, screening of cattle for sub clinical mastitis in university farms, blood smear and fecal sample examination of animals in various university as well as other farms etc. were done. Based on the results, customized necessary instructions for therapeutic management, prevention and control of infectious diseases were given to the management of each farm. Vaccinations against prevalent infectious diseases were also carried out in the farms of KVASU at regular intervals.

### Predominant prevalence of fatal oriental theileriosis detected in cattle population of Kerala

The genotypes of *T. orientalis* and the clinico-therapeutic aspects of *T. orientalis* infection in dairy cattle were studied by the scientists of KVASU. In species specific PCR, out of the 189 clinically infected cattle, 186 were found positive for *T. orientalis* and the remaining three cattle were positive for *T. annulata*. Among asymptomatic cattle, all 40 cases were positive for *T. orientalis*. Genotype specific PCR identified presence of Chitose and Buffeli genotypes of *T. orientalis* in both clinically infected and asymptomatic cattle. Majority of the dairy cattle positive for *T. orientalis* infection harbored mixed parasite populations. The occurrence of *T. orientalis* infection was highest (44.09 per cent) during monsoon. Most common clinical signs recorded in *T. orientalis* infected cattle were inappetance, pale mucous membrane, fever, lethargy and decreased milk production. Among the different treatment regimens used, treatment with combination of buparvaquone and oxytetracycline LA was found to be effective for *T. orientalis* infected animals in terms of remission of clinical signs, but complete elimination of the organism was not observed in all the three treatment groups tested.



### Methicillin resistant *Staphylococcus aureus* identified in healthy pigs

A study was conducted by scientists of KVASU to evaluate the occurrence and molecular characteristics of MRSA among healthy pigs. The prevalence of *S. aureus* was 42.86 per cent by Polymerase Chain Reaction (PCR). Methicillin resistance in *S. aureus* isolates were identified genotypically by molecular characterization of *mecA* gene and the prevalence was 5.89 per cent. The high Multiple Antibiotic Resistance (MAR) indices obtained in the study indicated a remarkable spread of resistance among the isolates due to frequent exposure to the antibiotics. This study confirmed the occurrence of nasal colonization of MRSA among healthy pigs, which is a major threat to public health and should be viewed seriously.

### Periodic screening and appropriate deworming of exotic birds can prevent parasitic infestations in birds

A study was undertaken to determine the prevalence of parasitic infestation among exotic and zoo birds. Only *Haemoproteus columbae* was identified by species specific PCR and sequencing of genus specific PCR product from pigeons, budgerigars, parrots and cockateils. Plasmodium spp. and Leucocytozoan spp. could not be detected among exotic pet birds. Ascaridia, Capillaria spp. Eimeria and tapeworm ova were the common parasites. Mixed infections were noticed in 20.5 per cent pet birds. Based on micrometry, the parasites identified from exotic pet birds were *Ascaridia galli*, *Capillaria obsignata* and *Eimeria labbeana* and those from zoo birds were *Ascaridia galli* and *Capillaria caudinflata*. None of the samples from pet and zoo birds revealed *Cryptosporidium* spp. and *Giardia* spp. either by microscopy or PCR. The findings of this study stress the need for periodic screening and appropriate deworming of exotic birds against parasitic infestations.

### Therapeutic regimen for treatment of *Babesia gibsoni* infections in dogs was studied

A study was carried out in 45 *B. gibsoni* infected dogs with an objective of assessing the haemato-biochemical alterations of the affected dogs and to evaluate and compare the efficacy of three treatment groups diminazine aceturate, imidocarb dipropionate and buparvaquone in combination with clindamycin. Incidence of *B. gibsoni* infection was more in animals below 2 years of age and Rottweiler was the most affected breed in the study while the disease was equally distributed among both male and female animals. The three treatment groups showed an equal and comparable efficacy in terms of remission of clinical signs and haematological improvement. However, remission of clinical signs was noticed in all the treatment groups post treatment. Relapse of parasitaemia was more in group treated with diminazine aceturate and clindamycin. The results of this study are important for clinicians in selecting a therapeutic regimen for treatment of *Babesia* infections in dogs.

### Virulent *Babesia* species causing multiple organ dysfunction identified in dogs of Kerala

Molecular level identification of the aetiological agents of complicated canine babesiosis cases, their clinical progression and response to standard protocols of therapy were studied by the scientists of Kerala. Peripheral blood smears were subjected for microscopical examination and revealed intra-erythrocytic signet ring shaped piroplasm, which were later confirmed to be *B. gibsoni* and *B. c. Vogeli* by PCR. Vital signs, clinical signs, haematological, biochemical, blood gas analysis, ultrasonographic and radiographic findings were suggestive of organ derangement. The alterations in total leukocyte counts, temperature, heart rate and respiratory rates in thirteen animals affected with *B. gibsoni* and two affected with *B. canis* were significant enough to be classi-



fied as SIRS positive. Eight animals affected with *B. gibsoni* had multiple organ dysfunction. But the alterations in the haematobiochemical parameters of the dogs affected with *B. c. vogeli* were not as significant as those reported for *B. rossi* infection. Detection of SIRS and MODS in canine babesiosis helps in the prediction of probable prognosis of the disease. The *B. gibsoni* and *B. c. vogeli* organisms which were considered as less virulent resulted in SIRS and further progression to MODS and death of the animals. This study has confirmed the virulent nature of two species of organisms previously considered benign in canines, and has elucidated parameters for accurate prognosis.

### High level of antimicrobial resistance among the *Staphylococcus aureus* strains identified from bovine mastitis

Identification of different bacteria causing mastitis, typing of *Staphylococcus* spp., their antibiogram and response to therapy with three antimicrobials were analysed in this study. Genotypic methicillin resistance was confirmed in 18.18 per cent of isolates and betalactamase resistance was confirmed in all the isolates. Antibiotic sensitivity test of all the clinical *S. aureus* isolates by disc diffusion method revealed a maximum resistance for beta lactam antibiotics. All the 22 isolates of *S. aureus* were confirmed as MDSA with 100 per cent phenotypic resistance to penicillin, methicillin and oxacillin with co-resistance to many other classes of antimicrobials. Multiple antibiotic resistance index (MAR) calculated for each antibiotic had good agreement with the results of antibiotic sensitivity test and a remarkable resistance to many antibiotic was confirmed. Treatment trial using three different antibiotics revealed that all the three had good therapeutic efficacy against contagious mastitis with no significant difference. Screening for subclinical mastitis indicated a high prevalence (54 per cent) of SCM in organised farms. This study has categorized *S. aureus* and detected high level of antimicrobial resistance among the different strains, which

have got high significance for both human and veterinary medicine.

### Neonatal calf diarrhoea among calves reared in and around Thrissur district

Neonatal calf diarrhea has tremendous economic impact for dairy farming because of the high fatality rates observed. The risk factors, etiological agents responsible and the haemato-biochemical changes in neonatal calf diarrhoea among calves reared in and around Thrissur district was studied. Among 50 diarrhoeic faecal sample, five were positive for *Cryptosporidium bovis* was detected in five calves. A multi-purpose commercial sandwich antigen ELISA gave positive result for rota virus in a single sample and negative results for corona, *Cryptosporidium parvum* and *E. coli* K99 in all. Microscopic examination of faecal sample by concentration method detected the presence of ova of *Strongyloid*, *Toxocara* and unidentified nematode larvae in one sample each. Haemato-biochemical values of diarrhoeic animals when compared with normal, showed significant difference in WBC, lymphocyte count, monocyte count, serum albumin level and serum globulin levels. This study the predominant pathogens for neonatal calf diarrhoea, and recommended measures for effective control.

### Feline herpes virus among cats was detected for the first time in India

Felines are fast becoming the favored companion animals in the world. Presence of most common pathogens such as Feline Herpes virus-1 (FHV-1), *Chlamydia felis* (*C. felis*) and *Mycoplasma felis* (*C. felis*) in upper respiratory infections of cats was studied. All 25 samples were positive for FHV-1; however, none of the samples tested were positive for *C. felis* and *M. felis* using PCR method. Haematological results were suggesting leucocytosis, monocytosis, granulocytopenia, normocytic normochromic hypochromic anaemia and thrombocytopenia. Risk factors were mainly



associated with young age, improper vaccination, inadequate hygiene measures, and overcrowding in catteries. This study detected Feline herpes virus among cats for the first time in India.

### High prevalence of antimicrobial resistance in bacteria associated with bovine mastitis

Antimicrobial resistance among bacteria is recognized as the most significant threat to the human race. The results of the study indicated a higher prevalence of staphylococcal udder infections among the cattle population of the study area. The coagulase negative staphylococci (CNS) dominated in both subclinical and clinical mastitis. Treatment trial using three different antibacterials i.e, ceftriaxone, ceftriaxone-sulbactam and sulphadiazine trimethoprim revealed that all the three protocols were equally effective against contagious mastitis with no significant difference in their clinico-therapeutic efficacy. This study brought to light the high prevalence of AMR in bacteria associated with bovine mastitis.

### Dreaded elephant endotheliotropic herpesvirus infection among the captive elephant population of Kerala

Elephant endotheliotropic herpesvirus (EEHV) has emerged as a major threat to the survival of endangered Asian elephant population. Elephant endotheliotropic herpesvirus (EEHV) infection in Asian elephants is an acute fatal haemorrhagic disease primarily affecting juvenile Asian elephants. The present study was proposed to confirm the presence of EEHV infection in Asian elephants and identification of the chronically infected shedders, which will help in controlling the spread of disease among elephant population. A total of 31 elephants presented to Teaching Veterinary Clinical Complex, Mannuthy, and those under private ownership (Thrissur districts), temples and Department of Forests and Wildlife, Kerala from Thiruvananthapuram, Pathanamthitta and Ernakulam during 2019-2020 were in-

cluded in this study. The faecal examination revealed strongyle ova in 22.7 per cent cases and strongyloides ova and larva in 9.7 per cent cases. Out of 31 samples, one yielded positive for EEHV and EEHV1 genotype in PCR of *trunk* wash samples. Haemato-biochemical studies revealed leukopenia, lymphocytosis and hypoalbuminemia in the affected animal. This study has proved the presence of the dreaded EEHV infection among the captive elephant population of Kerala.

### Virulence factors and antimicrobial susceptibility of *Malassezia pachydermatis* from dogs was investigated

Malasseziosis is a major dermatological disease in pet animals. A study was aimed at determining the occurrence of malasseziosis in dogs, and to study the virulence factors and susceptibility to antifungal agents for the etiological agent. Fifteen dogs with dermatitis, ten dogs with otitis, both of which were positive for the presence of organism by culture and twenty healthy dogs without any clinical signs of dermatitis were included in the study. The virulence determinants of *M. pachydermatis* investigated were adherence, cellular surface hydrophobicity and biofilm formation. The high susceptibility of *M. pachydermatis* isolates from control group was statistically significant in comparison with those from the diseased group for ketoconazole and itraconazole, but not for miconazole and fluconazole. The presence of three virulence factors investigated from both diseased and control group warrants their consideration in further studies for pathogenicity of *malassezia* dermatitis in dogs. Evidence of resistance to the commonly used azole antifungal drugs among majority of the isolates points to the necessity of regulating their use in therapeutic management.

### Caprine theileriosis and its clinico-therapeutic aspects in Kerala was studied

Goat farming is a major source of income to the marginal farmers in Kerala. Theileriosis is an important haemoparasitic disease of goats, which causes serious economic losses to



farmers. There is a paucity of information on caprine theileriosis and its clinico-therapeutic aspects in Kerala. The present study envisages the identification of aetiological agent, clinical, haemato-biochemical alterations associated with the disease and assessment of the efficacy of the therapeutic protocols adopted. Out of the one hundred goats studied, 62 per cent were positive for theileriosis, 15 per cent for anaplasmosis and three per cent with combined infection of theileria and anaplasma. *T. ovis* in nine goats (36 per cent) out of 25 goats. Mixed infection of *T. ovis* and *T. luwenshuni* could be detected in four goats (16 per cent) were seen among 25 goats. Major clinical signs observed were anaemia, fever, lymph node enlargement, anorexia and respiratory distress. The treatment with buparvaquone and oxytetracycline dihydrate was found to be the treatment of choice for caprine theileriosis

### **Bacterial etiology of infertility in dog with special reference to brucellosis**

Reduction in fertility and fecundity in dogs has emerged as an important problem in breeding kennels for the last few years. Infertility due to brucella infection carries specific relevance considering its zoonotic potential and endemic status in India. A total of 60 dogs including 10 control group dogs with a normal breeding history and the remaining 50 dogs with various reproductive problems were selected. The main clinical signs noticed were persistent greenish grey vaginal discharge, mid to late term (45 to 60 days) abortion, orchitis/epididymitis, scrotal oedema and azoospermia. Out of 60 sera samples screened for brucellosis with Rose Bengal Plate Test (RBPT), 26 (43.33 per cent) dogs from the test group were detected as reactors. Stamp stained smears prepared from aborted foetal stomach contents revealed red coccobacillary organisms against blue background in 10 (20 per cent) positive cases, suggestive of *Brucella* organism. Out of twelve *Brucella* spp. positive samples, eleven samples (91.67 per cent) yielded amplicons with a 100 per cent homology to *B. abortus* on sequencing. This

study recommends treating all urogenital infections in with antibiotics only if required, and if done, to be in accordance with the culture and sensitivity pattern of antibiotics. Dogs of reproductive age should be regularly screened for brucellosis and animals tested positive should not be used for further breeding purposes.

### **Screening for methicillin resistant *Staphylococcus aureus* associated with caprine mastitis**

Mastitis is one of the costliest diseases affecting dairy animals, worldwide. The increased incidence of mastitis along with emergence of antimicrobial resistant bacteria associated with mastitis is a major concern in the health sector. The study was conducted to identify the etiological agents associated with caprine mastitis, their antibiogram, identification of risk factors associated with occurrence of mastitis and to screen the presence of methicillin resistant *Staphylococcus aureus*. The screening for subclinical mastitis in 150 apparently healthy goats revealed occurrence of 61.59 per cent subclinical mastitis by California mastitis test. On bacteriological examination, milk samples from 26 animals yielded bacterial isolates. The isolates obtained were identified as coagulase negative staphylococci *S. hominis*, *S. lugdunensis*, *S. xylosus* and *S. chromogenes* using cultural and biochemical examination. Milk samples from sixty-six lactating goats suffering from mastitis were collected and subjected to bacterial isolation and identification. Sixty-six isolates obtained constituted 34 coagulase negative staphylococci, 10 *S. aureus*, 13 coliforms, six streptococci and three micrococci. The invitro antibiotic sensitivity test was conducted against eight antibiotics revealed higher susceptibility to tetracycline (53) followed by levofloxacin, ceftriaxone-tazobactam, enrofloxacin, amoxicillin-sulbactam and cotrimoxazole. The two least sensitive antibiotics were methicillin followed by gentamicin. Among 10 *S. aureus* isolates, only two isolates yielded amplicons of *mecA* gene suggestive of methicillin resistance. None of the isolates yielded



amplicons for PVL gene. Treatment trial was conducted to identify the efficacy of four antibiotics namely, enrofloxacin, levofloxacin, gentamicin and sulpha-trimethoprim against mastitis and identified levofloxacin (as the most effective antibiotic in terms of clinical cure. The occurrence of MRSA in the present study was 20 per cent (2/10). Detection of resistance gene even though at a lower rate warrants constant monitoring for presence of antimicrobial resistance in goats.

### Complete rations incorporating unconventional feeds developed

Grass based complete feeds for lactating dairy cows, with the optimum level of NDF viz., 35 per cent, containing the unconventional protein rich feed ingredient coffee husk, was successfully developed for the first time in Kerala, with economic benefits, by scientists of Department of Animal Nutrition, CVAS, Pookode

### Ayurvedic medicinal residues safely used as unconventional feeds for Malabari kids

Studies on effect of dietary incorporation of ayurvedic pharmaceutical gritham residues, which are rich in energy, protein and fibre as replacement for costly energy feeds like maize and protein supplements like soybean meal were carried out and it was established that Panchagavya Gritham residue and Brahmi gritham residue could safely and economically be incorporated in the rations of Malabari kids, with beneficial effects on their growth performance.

### Influence of dietary substitution of palm oil by rapeseed oil in broilers

Studies on dietary substitution of costly palm oil with cheaper rape seed oil carried out as part of the Master's Programme showed that substitution at different levels had no adverse on growth performance and economics of broilers.

### First report of *Anaplasma platys* in dogs of Kerala

About 60 dogs with clinical signs suggestive of blood parasite infestation were screened for *Ehrlichia canis* and *Anaplasma platys* by molecular methods. Out of the 60 animals, 5 dogs were found positive for *A. platys* by PCR even though their blood smear was negative for these pathogens.

### Occurrence of ESBL *E.coli* detected among broiler chicken

Extended spectrum  $\beta$  lactamase (ESBL) producing *Escherichia coli* was found to be present in the broiler shops in and around Wayanad district using molecular methods. This confirms the presence of this bacterium in the food chain.

### Antibacterial and bio-preservative potentials of lactoferrin from Vechur cow milk

The investigation of properties of lactoferrin isolated from Vechur cow milk using cation exchange chromatography revealed that it possesses significantly higher antibacterial activity against Gram positive and negative organisms compared to lactoferrin from crossbred cattle. The bio-preservative potential of the protein was evaluated using lactoferrin treated and non-treated paneer samples. Lactoferrin treatment significantly extended the shelf life of paneer up to 12 days under refrigeration condition when compared to non-treated paneer samples which was consumable only for 2 days.

### Development of biomass briquettes using slaughterhouse waste

Technology for optimum drying of rumen contents-blood mixture has been standardised and biomass briquettes which could be transported and stored easily were developed at Meat Technology Unit, KVASU. Blood-rumen contents-coir pith mixture was briquetted using the hydraulic briquetting machine.



### Development and evaluation of functional chicken noodles and ready to eat chicken snacks

Department of Livestock Products Technology developed functional instant chicken noodles with spent chicken (70%), natural antioxidants Aloe vera (0.3%) and natural colour Paprika Oleoresin (0.5%) which could be marketed at ambient temperature in laminated pouches for 60 days without spoilage with better nutritional and sensory properties. Nutrient rich ready-to-eat snacks which are stable at room temperature for 90 days was also developed by incorporating chicken upto 73.72% and different flours viz. rice, bengal gram and finger millet. The snacks were high in dietary fibre and had good antioxidant properties.

### Pet treat incorporating buffalo by products developed

Shelf stable pet pasta with good nutritive value and palatability containing cereal flour mix (39%), offal powder mix (30%), egg (15%), tripe powder (5%), tallow (3%), plasma (2.5%), gelatin (2%), dicalcium phosphate (1%), paprika oleoresin (0.9%), salt (0.5%), guar gum (0.5%), wheat gluten (0.4%) and potassium sorbate (0.2%) was developed. It could be fed as dry or moist food as per the preference of the dog and convenience of the owner.

□



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