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Lysosomal storage diseases

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The University of Melbourne, Victoria 3030 AustraliaCitation: Georgy, S.R. 2021. Lysosomal storage diseases. *J. Vet. Anim. Sci.* **52**(1): 1-6.DOI: <https://doi.org/10.51966/jvas.2021.52.1.1-6>

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Abstract

Lysosomes play a pivotal role in cellular processes through an active interplay of enzymes, lysosomal membrane proteins, and cytosolic proteins. Lysosomal storage diseases are a group of inherited and acquired disorders. Clinically affected animals are presented with developmental and neurological symptoms. This review aims to discuss the function of lysosomes, the pathogenesis of lysosomal storage disease, and its diagnosis.

Lysosomal structure and function

Lysosomes are membrane-bound cytoplasmic organelles present in most vertebrate cells, invertebrates, and unicellular organisms. Its primary function is to dispose of and recycle cellular contents. Lysosomes function as the “intracellular digestive tract” and contain a battery of hydrolytic enzymes that aids in digestion and a special category of secretory proteins that are destined for intracellular organelles (Kumar *et al.*, 2015).

Lysosomes contain approximately 50 different acid hydrolases including proteases, nucleases, lipases, glycosidases, phosphatases, and sulfatases that are active in an acidic pH in the 4.5 to 5 range, but not at the neutral pH (about 7.2) characteristic of the rest of the cytoplasm (Saftig and Klumperman, 2009). These enzymes are synthesized in the endoplasmic reticulum and transported to the Golgi apparatus. These enzymes undergo a variety of post-translational modifications within the Golgi complex, including attachment of phosphorylated mannose residues, that serves as an address label and are segregated as lysosomal enzymes. Subsequently, small transport vesicles containing lysosomal enzymes are pinched off from the Golgi and proceed to fuse with the lysosomes (Luzio *et al.*, 2007). Genetically determined errors in this sorting mechanism may give rise to one form of lysosomal storage disease.

Lysosomal storage disease - pathogenesis

A variety of complex macromolecules are broken down by lysosomal enzymes. How cells accumulate these macromolecules? There are two processes from which these macromolecules are derived within the cell namely autophagy and heterophagy (Marques and Saftig, 2019). Autophagy is a cell survival mechanism, in which a cell eats its contents. The cytoplasmic materials

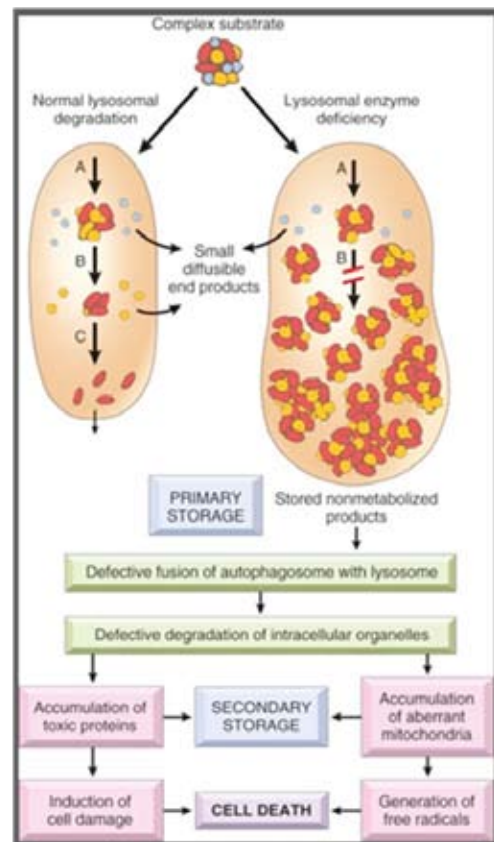
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to be recycled are delivered to the lysosome for degradation. Whereas in heterophagy, the lysosomes fuse with endosomes or phagosomes to facilitate the degradation of internalized contents. If there is any deficiency of the lysosomal enzymes, it gives rise to two pathological consequences. 1) accumulation of partially degraded insoluble metabolite within the lysosomes, called primary accumulation, 2) accumulation of autophagic substrates because of impaired lysosomal function within the cytoplasm, called secondary accumulation (Kumar *et al.*, 2015) (Figure 1). This will eventually lead to the accumulation of toxic proteins, generation of free radicals, damage to the plasma membrane leading to cell death. Any dysfunction of the lysosomal catabolic pathway results in the accumulation of macromolecules within the cells leading to lysosomal storage disease (Ballabio and Gieselmann, 2009). The severity of the disease is partially depending on the type of accumulating waste product. Approximately 51 lysosomal storage diseases are reported in humans and many of them are also reported in animals. Many different cell types and tissues are affected by lysosomal storage disorders and the involvement of the tissue varies at different stages of the disease process. The tissues commonly affected include the liver, spleen, bone and joint, eye, adrenal gland, cardiac and skeletal muscle, and brain (Ferreira and Gahl, 2017). In affected animals, brain lesions are particularly prevalent, and more than two-thirds of lysosomal storage disease exhibit neurological signs (Walkley, 2009).

Classification of lysosomal storage disease

Lysosomal storage disease can be categorized based upon the macromolecule that is abnormally stored in the cells and examples include carbohydrates (Pompe disease), neutral lipids (Wolman disease), and sphingolipids (Niemann- Pick disease) (Ferreira and Gahl, 2017). Inherited defects in the degradation of sphingolipids are one of the major categories of lysosomal disease and it particularly affects the nervous system (Jolly and Walkley, 1997). One of the byproducts of sphingolipid catabolism is ganglioside and it

Fig 1: Pathogenesis of lysosomal storage disease (Kumar *et al.*, 2015)



accumulates when there is a deficiency of the enzyme β galactosidase leading to generalized gangliosidosis. This disorder is described in Siamese cats, English springer spaniel, and rarely in German short-haired pointer. Clinical signs are exhibited by 2 to 4 months of age and include vision problems, lethargy, difficulty in walking and death occurs by 8 months (Skelly and Franklin, 2002).

Lysosomal storage disease can be categorized based on the cause as inherited or acquired. The deficiency of lysosomal enzymes is inherited in an autosomal recessive pattern and usually involves a single enzyme deficiency. Affected animals are usually normal at birth, and in most cases, the symptoms develop in the first year of life. The severity of the disease is determined by the level of the enzyme. When enzyme activity is less than 15% of the normal level, the accumulation of undegraded substrate occurs. Selected inherited lysosomal

storage diseases are given in table 1.

Acquired disorders could result from inhibition of α -mannosidase II by ingestion of plant materials or treatment with certain drugs. Chronic ingestion of legumes of the genera *Astragalus* and *Oxytropis* inhibit α -mannosidase II causing cells to accumulate oligosaccharides. This results in systemic lysosomal storage disease manifesting microscopically as clear cytoplasmic vacuoles in multiple organs including neurons of the brain (Alroy and Lyons, 2014). Neuronal vacuolation is seen in approximately two-thirds of all lysosomal storage diseases. The drugs leading to the acquired disease include amiodarone and chloroquine.

Diagnosis

Lysosomal storage disease affects males and females with equal frequency. Accumulation of the substrate within cells leads to cellular dysfunction either directly or indirectly. The clinical signs reflect the abundance of the substrate within the cells and the link between storage and clinical signs is poorly understood. Lysosomal storage disease should be among the differential diagnoses when an animal is presented with multifocal neurological disease. Animals may show a change in behavior, ataxia, proprioceptive deficits, apparent blindness, deafness, and seizures. These signs are prevalent in the early stages of gangliosidosis in cats and dogs (Alroy et al., 1985; De Maria et

Table1: Selected inherited lysosomal storage disease

Condition	Enzyme Defect	Storage Material	Species
G _{M1} gangliosidosis	β -galactosidase	G _{M1} ganglioside in neurons, glial cells, macrophages	Dogs Cats Friesian cattle Suffolk sheep
G _{M2} gangliosidosis	Hexaminidase	G _{M2} ganglioside in neurons, glial cells, macrophages	Cats German shorthaired pointer Golden retriever Japanese Spaniel Yorkshire pigs
Sphingomyelinosis	Sphingomyelinase	Sphingomyelin in neurons and macrophages	Dogs Cats
Globoid cell leukodystrophy	Galactocerebrosidase	Galactocerebrosides in oligodendrocytes, Schwann cells, macrophages	Dogs Cats Polled Dorset Sheep
Glucocerebrosidosis	Glucocerebrosidase	Glucosylceramide in kupffer cells and neurons	Dogs- Silky terrier
α Mannosidosis	α Mannosidase	Mannose oligosaccharide in neurons, macrophages, and secretory epithelial cells	Cattle
β Mannosidosis	β -mannosidase	Oligosaccharide in neurons, macrophages, and secretory epithelial cells	Cattle Goat
Mucopolysaccharidosis	Iduronidase Sulfaminidase	Dermatan sulfate Heparan sulfate	Cats Dogs Goats

al., 1998], globoid cell leukodystrophy (Wenger et al., 1999), and in feline α -mannosidosis (Jezyk et al., 1986).

Clinical pathology

In most cases the haematological and routine biochemical assessment is unremarkable. The presence of storage vacuoles within leukocytes during blood smear evaluation can be informative in some cases. However, examination of cells from several different tissues may be required to demonstrate the presence of the storage material. If a storage disease is suspected, and if the peripheral blood smear is unremarkable, lymphoid tissues including spleen can show evidence of vacuolation. Hence lymph node biopsies and aspirates can be diagnostically helpful. In animals showing hepatomegaly, liver aspirates or needle biopsies can be useful in identifying the storage material.

Radiography

A radiographic examination can reveal bony malformations in lysosomal storage disease. Animals with mucopolysaccharidosis do exhibit bone and connective tissue abnormalities and include facial deformities such as frontal bossing of the skull and facial deformity. Pain, gait abnormalities, and a history of fracture are reported in cases of lysosomal storage disease.

Cerebrospinal fluid analysis

Cerebrospinal fluid examination of patients with altered neurological function is part of their normal investigation. In cases of globoid cell leukodystrophy and fucosidosis, cerebrospinal fluid analysis may display vacuolated macrophages or lymphocytes containing storage materials (Skelly and Franklin, 2002).

Biopsy

Muscle biopsy, peripheral nerve biopsy, and electron microscopy of the skin can add to the diagnosis [Skelly and Franklin, 2002; Alroy and Ucci, 2006]. Peripheral nerve biopsy is used frequently to diagnose globoid cell

leukodystrophy (Vicini et al., 1988) and may provide a reliable antemortem method of diagnosis. Muscle biopsies are preferable for detecting glycogen storage disease, even though all glycogen storage diseases are not lysosomal storage diseases. In non-lysosomal storage disease, the glycogen accumulates in the cytosol and not within lysosomal compartments.

Lysosomal enzyme analysis

Lysosomal storage diseases result from specific defects in enzymes and hence enzyme analysis is required for confirming the diagnosis. In human medicine, enzyme panels are established for rapid diagnosis and include enzymes required for digestion of glycosphingolipids and oligosaccharides. Severe depletion of enzyme activity is reported in homozygotes up to 5% of normal. Ideally, age-matched control should be assayed in parallel to provide a normal set of control values. The samples for enzyme analysis include whole-blood leukocytes, biopsy samples from liver and kidney, and cultured skin fibroblasts. The techniques used for detecting enzyme activities in humans and dogs are the same (Meikle et al., 2004).

Molecular genetic testing

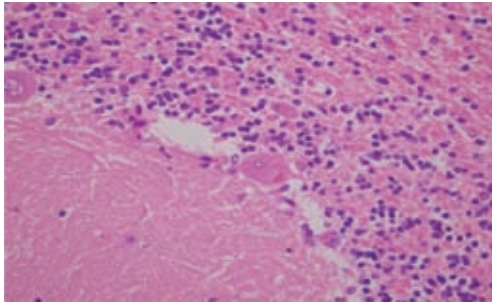
Identification of the genetic defect is the second definitive diagnostic method for lysosomal storage disease. With the advancement in molecular genetic testing, single-gene defects are identified in various storage diseases. Molecular genetic tests are available for canine fucosidosis, globoid cell leukodystrophy, and mucopolysaccharidosis (Skelly and Franklin, 2002).

Pathology

Affected animals are usually euthanized due to deterioration and debilitating consequences and are examined post-mortem. Typical gross findings include firm brain, hepatomegaly, splenomegaly, dwarfism, and lumbar bony dysplasia. Histologically prominent and widespread neuronal vacuolation, secondary axonal degeneration, storage vacuoles containing granular materials,

vacuolation in hepatocytes, leukocytes, renal tubular epithelium, and retinal neurons are present. The presence of brightly eosinophilic granular accumulation within the cytoplasm of neurons of an eleven-month-old English Springer Spaniel who suffered from lysosomal storage disease is given in Figure 2. Diagnosis of lysosomal storage disease involves a sequential series of steps requiring specialized techniques and expertise.

Fig 2: Accumulation of storage material in cerebellar neuron



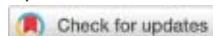
Conclusion

Lysosomal storage diseases are rare disorders and it is probable that many veterinarians are often unable to reach a specific diagnosis. Lack of testing techniques and a lack of understanding of this disease makes diagnosis difficult in veterinary practice. Awareness of this disease entity and its pathogenesis allows lysosomal storage disease to be included in the differential diagnoses in animals presenting with neurological disorders. Both acquired and inherited forms of the disease are uncommonly reported in domestic animals.

Reference

- Alroy, J. and Lyons, J.A. 2014. Lysosomal storage disease. *J. Inborn Errors Met. & Screening*. **2**: 1-20.
- Alroy, J., Orgad, U., Ucci, A. A., Schelling, S. H., Schunk, K. L., Warren, C. D., Raghavan, S. S. and Kolodny, E. H. 1985. Neurovisceral and skeletal GM1-gangliosidosis in dogs with beta-galactosidase deficiency. *Science*, **229**(4712): 470-472.
- Alroy, J. and Ucci A.A. 2006. Skin biopsy: a useful tool in the diagnosis of lysosomal storage diseases. *Ultrastruct. Pathol.* **30**(6): 489-503.
- Ballabio, A. and Gieselmann, V. 2009. Lysosomal disorders: from storage to cellular damage. *Biochimica et Biophysica Acta (BBA)-Mol. Cell Res.* **1793**(4), 684-696.
- De Maria, R., Divari, S., Bo, S., Sonnino, S., Lotti, D., Capucchio, M. T. and Castagnaro, M. 1998. Beta-galactosidase deficiency in a Korat cat: a new form of feline GM1-gangliosidosis. *Acta Neuropathol.* **96**(3): 307-314.
- Ferreira, C. R. and Gahl, W. A. 2017. Lysosomal storage diseases. *Transl. Sci. Rare Dis.* **2**(1-2): 1-71.
- Jezyk, P.F., Haskins, M.E. and Newman, L.R. 1986. Alpha-mannosidosis in a Persian cat. *J. Am. Vet. Med. Assoc.*, **189**(11): 1483-1485.
- Jolly, R. D. and Walkley, S. U. 1997. Lysosomal storage diseases of animals: an essay in comparative pathology. *Vet. Pathol.* **34**(6): 527-48.
- Kumar V., Abbas A.K., Aster J.C. and Fausto N. 2010. Robins and Cotran (editors). *Pathologic Basis of Disease*. 8th Ed. Saunders, Philadelphia, 1464p.
- Luzio, J.P., Pryor, P.R. and Bright, N.A. 2007. Lysosomes: fusion and function. *Nat. Rev. Mol. Cell Biol.* **8**(8): 622-32.
- Marques, A.R.A. and Saftig P. 2019. Lysosomal storage disorders - challenges, concepts and avenues for therapy: beyond rare diseases. *J. Cell Sci.* **132**(2).
- Meikle, P.J., Fietz, M.J. and Hopwood, J.J. 2004. Diagnosis of lysosomal storage disorders: current techniques and future directions. *Expert Rev. Mol. Diagn.* **4**(5): 677-691.
- Saftig, P. and Klumperman, J. 2009. Lysosome biogenesis and lysosomal membrane

- proteins: trafficking meets function. *Nat. Rev. Mol. Cell Biol.* **10**(9): 623-635.
- Skelly, B. J. and Franklin, R. J. 2002. Recognition and diagnosis of lysosomal storage diseases in the cat and dog. *J. Vet. Intern. Med.* **16**(2): 133-41.
- Vicini, D. S., Wheaton, L. G., Zachary, J. F. and Parker, A. J. 1988. Peripheral nerve biopsy for diagnosis of globoid cell leukodystrophy in a dog. *J. Am. Vet. Med. Assoc.* **192**(8): 1087-1090.
- Wenger, D. A., Victoria, T., Rafi, M. A., Luzi, P., Vanier, M. T., Vite, C., Patterson, D. F. and Haskins, M. H. 1999. Globoid cell leukodystrophy in cairn and West Highland white terriers. *J. Hered.* **90**(1): 138- 142.
- Walkley, S.U. 2009. Pathogenic cascades in lysosomal disease-Why so complex? *J. Inherit. Metab. Dis.* **32**: 181-189. ■



Alternate feed resources for safe usage in feeding practices



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Abstract

Several newer feed resources have been evaluated and found useful for livestock feeding. There is a need to upscale these technologies for wider application. Of late certain local feed resources like azolla, areca sheath, maize kadbi, fruit residues, ayurvedic and brewery residues, have been found useful and farmers have started using some of them in the livestock ration. Use of these alternative feed resources replacing part of conventional feed ingredients is wise way for sustainable livestock production. Some of such potential feed stuffs are discussed in this article.

Fodder resources

Areca sheath

In some regions of Karnataka, Kerala and Assam, the areca (*Areca catechu*) cultivation as a commercial crop has partly replaced the traditional cereal crops due to higher economic returns. This has resulted in deficit of dry fodder, especially in the coastal zones of Karnataka and livestock farmers are procuring paddy straw from adjoining districts at higher cost. The analysis of areca sheath for its nutritional composition has showed almost similar composition to paddy straw. Research at ICAR-NIANP has shown no untoward effect due to feeding of dried areca sheath to sheep and cows. For efficient utilization of dried and shredded areca sheath in the form of total mixed ration along with suitable proportion of concentrate feed is recommended (Gowda, 2016). Suitable machine to shredd the dried areca sheath is commercially available. In Andaman islands also areca sheath as dry fodder is becoming popular. In India, the potential availability of areca sheath is about 1.20 million tones, annually and can be a valuable resource. Apart from the whole sheath, the residue left after making plates also can be used as dry fodder.

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Sugarcane trash

Sugar cane dry trash and is a part of sugar cane tops and is a major by-product of the sugarcane industry which is left in the field after sugar cane harvest. The major states producing sugarcane are UP, Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Telangana, Bihar, Gujarat, Haryana, Uttarakhand and Punjab. With a cane to dry leaf ratio of 2-3 %, about 6.8-10.3 million tonnes of sugar cane trash is expected to be available at the time of harvest in India. The trash can be fed to livestock as a replacement of dry fodder like paddy straw or finger millet straw in situations of drought. Sugarcane trash is very bulky material and occupies lot of space. This can be a major drawback during transportation. This can be avoided if trash is chaffed to small pieces with the help of mechanical chaff cutter (4-6 inches). Research conducted at ICAR-NIANP has shown that sugarcane trash is good source of fiber (NDF:32.3%), and hemi-cellulose (26.9%). However, contains less protein (3.54%), similar to paddy/finger millet straw (3.65%) and can replace cereal dry fodders for livestock feeding (Rao *et al.*, 2019a).

Maize stover and cobs

Maize crop is being cultivated on a large scale due to the high demand for maize grains for feed, starch industry and distilleries. Maize stovers and cobs are the by-products that are currently being either not utilized or underutilized. Stover and cobs can be used as roughage source in diets of cattle, buffalo, sheep and goats. Simple interventions like chaffing of maize stovers and cobs will facilitate the effective utilization of untapped feed resources. Maize stovers in general after harvesting the cobs are either left in the field itself or sometimes burnt in the field to make way for the next crop. The nutritive value is almost similar to any cereal dry fodder. The cobs can be shredded and compressed into blocks for easy transportation (Rao *et al.*, 2019).

Sunflower heads

Sunflower head is a residue after extraction of seeds. Crushed sunflower head has a crude protein content of 7-10%, fibre

content of 45-50% and dry matter digestibility of 50-55%. It can be included in total mixed ration upto 40% as roughage source (Gowda and Anandan, 2019). Research conducted at ICAR-NIANP has shown no adverse effects after feeding sunflower head based total mixed ration.

Haulms of Groundnut and horsegram

The leafy residues of ground nut and horse gram (top feeds) are leguminous in nature and has better protein and digestible fibre value as compared to cereal dry fodders (Gowda and Anandan, 2019). Ruminant animals, particularly sheep and goat relish them very much. These top feeds can be dried and stacked to use as dry fodder.

Sugar cane crop

Some time due to surplus sugar cane production, lock down situations, labor shortage and closure of sugar factories, whole sugar cane plant is available to use as fodder resource. The whole plant should be chaffed and use along with other dry fodder. Its nutritive value is similar to maize green fodder. Care should be taken to avoid rumen acidosis and it should not be fed as a sole fodder (Rao *et al.*, 2019a).

Fruit and vegetable residues

In many Asian countries, there is a gradual shift in the cropping pattern from cereals to more remunerative fruit and horticultural crops. This results in generation of huge quantity of fruit and vegetable residues. Presently such residues are not effectively used and dumped in landfills causing environmental pollution. There is a need to develop suitable methods to convert waste to wealth and contribute to value added feed resources (Bakshi and Wadhwa, 2013). Some of the potential fruit residues that can be used in feeding are reviewed (Wadhwa and Bakshi, 2013). Available literature on some of the fruit residues as feed has been reviewed by Gowda and Vijay Bhasker (2017) and presented as below.

Apple: In India annually about 1.74 million tonne of apple is produced and waste

consists of peels, seed and pulp, which represents 25-35% of fresh apple. The apple pomace on dry matter basis consist of 4.72% crude protein and 48-60% total sugar. It is a good source of energy (75% TDN) and can replace 30% maize grain in ruminant ration. The residue contains high moisture and can be dried in sunlight or at 65% moisture level can be made as silage for preservation to use as feed.

Grape: Grape pomace is a by-product of wine industry comprising grape pulp, skin, stem and seeds accounting about 20% of the grape fruit. The annual production is about 1.6 million tonnes and limited quantity is processed for wine. On dry matter basis, grape pomace contains 11% crude protein, 27% crude fiber and 5% lignin. Due to lignified fiber, the digestibility and energy value is low. The dried pomace has phenolic anti-oxidant compounds. Due to lower nutritive value grape pomace can be used in smaller quantity in ruminants feeding.

Mango: Majority of the mango is consumed freshly and only about 2% is processed and generates 40-50% waste. The waste includes peels, juice extraction waste, seeds and kernel. The waste has more sugar and moisture and hence need to be dried and made silage for preservation. The silage of peels and juice extraction waste is a good source of energy (70% TDN). The seed kernel represents about 50% of the whole seed and contains 7-12% oil and 40% starch on dry matter basis. Boiling and autoclaving will remove certain anti-nutritional factors like cyanogenic glycosides, oxalate and trypsin inhibitors. Though protein content of mango seed kernel is low and quality in terms of amino acids is good.

Citrus fruit by-products: The by-products of orange and lemon are peel and rag. About 40-50% waste is generated from citrus juice industry and contains soluble sugars. It can be made as silage along with dry fodder and used as cattle feed.

Banana fruit by-product: The banana fruit waste like peels contains more moisture, low protein and more soluble sugars. This can be preserved as silage along with dry fodder and fed to cattle with other feeds.

Pineapple fruit residue: The residue includes leafy crown, peels and the pomace of juice extraction. Less than 30% of the pineapple fruit produced is processed in industry and more than 60% of the whole fruit is not edible for human consumption. The pineapple fruit residue on dry matter basis contains 6-7% crude protein 50% total sugar, and 70% energy(TDN). The residue can be chaffed and dried to less than 10% moisture and use as hay. Otherwise, after draining the excess moisture, the waste can be made as silage and can be used as a fodder source for cattle / sheep. Study at ICAR-NIANP has shown that nutritive value is better than maize green fodder. Ideal proportion of leafy crown and peel/pomace for silage making is 4:1(w/w) (Gowda *et al.*, 2015a).

Jack fruit residue: Jack fruit is a common horticultural crop in many Asian and African regions. In India about 1.2 million tonnes of jack fruit is produced leaving a residue of 0.8 million tonnes per year. This residue (skin, aerial parts) is inedible for human consumption and quickly putrefies due to high sugar and moisture content. Study at ICAR-NIANP has shown that jack fruit residue (JFR) contain crude protein (6-7% on DM basis), total sugars (60-65% on DM basis) and higher organic matter digestibility (70-78%). It is palatable and can be fed to ruminants as fodder source along with supplementary nitrogen(urea/tree leaves/oil cake) (Kusmartono, 2007) . The chaffed fruit residue can be ensiled for preservation or dried to less than 10% moisture content and ground to use as jack bran. Nutritive value of jack bran is better than any dry fodder and almost similar to medium quality green fodder.

Tomato pomace: India ranks second in the world in tomato production contributing 10.82% of world production. Tomato pomace is major by-product of tomato processing industries. The production of tomato pomace is seasonal and linked to harvest period. Most of the product is available during late warm-season and drying is necessary for storage. Sun-drying or artificial drying is the preferred method and resultant product is crispy and it can be mixed along with other feeds and use as roughage source. Use of tomato pomace as feed has been reported by Abdollahzadeh *et al.* (2010) and Ventura *et al.* (2009).

Hydroponic grain sprouts

Producing the green feed through germination of grains like maize, barley, oat or wheat produces consistent quantities of green feed throughout the year at the rate of about 4 to 6 kg per kg of seed within 10 days period. It is essentially a hydroponic technique, where the sprouted seeds spread over the trays grow into green feed in a 10 day cycle (Muthuramalingam *et al.*, 2015). There is about 18-20% dry matter loss and often mold growth is a problem. Soaking the seeds with 4% vinegar solution is quite effective in preventing mold growth (Giridhar *et al.*, 2019). Quality of seeds, moisture management and control of predators (squirrel, rodents) are some of the issues in grain sprout production. Improved method of grain sprouts cultivation with straw bedding has been developed by scientists of ICAR-NIAP. It can be considered as a contingency strategic green feed supplement along with dry fodder rather than alternative to conventional green fodder source (Chethan *et al.*, 2019). On dry matter basis, the nutritive value of grain sprout in terms of protein, energy and fibre is almost similar to good quality wheat bran.

Cactus

Spineless cactus is climate resilient and can grow on low fertile rocky soil and with less water. It will suit most for arid and semi-arid regions. The blades (cladodes) of cactus contain high moisture (>85%) and less protein (4-5%). This can be used as a supplementary fodder for livestock along with other cultivated fodders (Gowda and Anandan, 2019).

Azolla

Azolla (*A. Pinnata*, *A. nilotica*), a water floating fern and triangular in appearance, consisting of roots, stems (rhizomes) and leaves. Azolla grows in symbiotic association with a blue-green alga *Anabaena azollae*, a nitrogen fixing organism. Azolla is a promising supplementary green feed from the point of ease in cultivation, productivity and its nutritive value. Azolla is a protein rich green feed, leucine, lysine, arginine and valine are the predominant amino acids in Azolla, while tryptophan and sulfur containing amino acids are deficient.

Azolla is also rich in vitamin A, vitamin B₁₂, Beta-carotene, growth promoting intermediaries, bioactive compounds and bio-polymers and azolla is well digested by livestock (Indira *et al.*, 2009). Azolla generally grow best in less than full sun light. Optimum relative humidity for Azolla growth is 60-75% and pH range of water is 5.5-7. The use of Azolla as a green feed for fish, swine, poultry and cattle has been tested with favourable results. The chemical analysis of Azolla showed that it is a good source of protein (20-25%) and most of the minerals. Azolla can be used as a valuable green feed protein supplement mixed with crushed maize grain or wheat / rice bran. This is very useful under low input livestock production system (Gowda *et al.*, 2015).

Fodder trees

Providing top feeds from the trees will help to bridge the deficit of green fodder. In dry regions, their utility is much more realized. Trees like Sesbania, Subabul, Gliricidia, Melia etc. perform well even in dry lands (Garg and Digvijay Singh, 2011). The normal farmers' practice of lopping only the side branches and allowing the uninterrupted growth of main stem reduces the yield. Instead, main stem is to be pruned to a height of 5 feet when the trunks of fodder trees are about 1.5 inches in diameter. Normally, it takes about 8 to 10 months to reach this stage. After the first lopping, subsequent harvests can be done at an interval of around 60 days. Prakash Kumar and Dixit (2019) have reviewed the prospects of fodder trees as feed. Most of the tree leaves have a protein content of 18-20%, fibre content of 30-35% and calcium content of 1-2%. Tree leaves can be fed to meet 10-15% of total protein requirement. Besides, most tree leaves possess tannins and can be a good source for methane mitigation in ruminant diets (Sharma *et al.*, 2016).

Miscellaneous ingredients

Neem seed cake

Neem seed kernel meal, a by-product after neem oil extraction, is high in crude protein (36-38%) and as such, found unsuitable feeding due to presence of bitter and toxic triterpenoids (azadirachtin, salannin, nimbin, nimbiol etc.).

Detoxification can be done by alkali soaking with 1.5% NaOH. Studies have indicated that detoxified neem cake can replace 50% soybean protein in total mixed rations of cattle (Gowda and Sastry, 2000; Rao *et al.*, 2019).

Karanj seed cake

Karanja is a forest tree belonging to the family Leguminosae, grown in all parts of India, particularly in Tamil Nadu, Andhra Pradesh and Karnataka, for its ecological advantages. Karanj seed cake, a by-product of karanj oil extraction is rich in protein, but unpalatable and toxic due to the presence of karanjin, pongamol and an unusual amino acid, glabrin. Detoxification of karanja cake can be done by complete removal of oil and refluxing with solvent. Detoxified material could replace 50% of soybean meal in total mixed rations dairy cattle (Rao *et al.*, 2019).

Castor seed cake

Castor seed cake is a by-product of the castor seed industry, contains fairly good amounts of protein (CP: 35%). It can be a substitute of conventional oil cakes like soybean meal in livestock diets but for the presence of a toxic glycoprotein, ricin. Lime treatment (4.0%, w/w) followed by extrusion cooking results in ricin free castor seed cake. Such treated cake could be a protein supplement upto 10% level in the total mixed ration of cattle (Gowda *et al.*, 2009).

Rain tree pods

Rain tree is widely distributed in the tropics. A mature tree can yield 500-600 kg green forage foliage and 250-300 kg pods. Ripened pods are fallen from tree from February to April months. Rain tree pods are a good source of crude protein (15.3%), sugars (69.9%) and low crude fiber (10.0%) and can be a substitute for good quality rice bran (Hosamani *et al.*, 2005). Small quantity of crushed pods can be fed to cattle mixed with urea as per recommended level.

Hotel food and kitchen waste

Wadhwa and Bakshi (2013) have

reviewed the prospects of using vegetable waste as animal feed. Disposal of food waste generated by hotel industries is an enormous challenge. There is enormous opportunity for utilizing these wastes as feed for livestock. There should be a mechanism to collect food wastes from the hotels and transporting to livestock farms. The vegetable and vegetarian food wastes can be fed to dairy animals along with other concentrates and roughages. However, the wastes need to be cooked or heat treated or microbiologically treated before offering to cattle to ensure that they are bacteria free and edible. It is a rural house hold practice to feed left over edible food and vegetable in domestic kitchen to their cattle.

Brewer's grains

Brewer's grains are materials remaining after fermentation of grains during the liquor / beer making process. These materials can be fed in the wet form (wet brewer's grain) or dried form (dried brewer's grain). The nutritional content varies depending on the type of grain used (barley, wheat, corn, sorghum). Dried brewer's grain contains 22-25% protein on dry matter basis. It is a good source of high quality bypass protein and digestible fiber. It is usually recommended to include dried brewer's grains up to 20-25% of the concentrate mixture and up to 15% of the total dietary dry matter of adult ruminant diet. Wet Brewer's grains have limitation of low shelf life (less than 2 days) and hence to be used without spoilage. Wet edible Brewer's grains can be mixed with crushed maize grain or wheat bran at 5 : 1 ratio and feed to cattle to balance energy and protein (Rao *et al.*, 2019).

Ayurvedic medicinal residues

Indian ayurvedic industry has a turnover of around Rs. 3500 crores with 7-10 per cent annual growth rate. It is estimated that there are over 7800 medicinal drug- manufacturing units in India, includes 14 well-recognized and 86 medium scale manufactures of herbal drugs, producing ayurvedic preparations employing thousands of tons of herbs. Thailam (oil based) and Kasayam (decoction) are the major available Ayurvedic medicinal residues. The thailam residues have higher crude

protein and oil content ranged from 21-27% and 11-23% respectively, whereas Kasayam residues were mostly fibrous with low protein content ranges between 5.5 to 6.5% on DM basis. The potential of Ayurvedic medicinal residues as livestock feed has not been fully explored. Study conducted in goat at ICAR-NIANP (unpublished data) using Thailam Ayurvedic medicinal residues viz. Ksheerabala (K) and Dhanwantharam (D) has shown that 40% of Soybean and groundnut cake protein can be replaced with the above ayurvedic residues without adverse effects on health and production (22.7 kg Ksheerabala and 25.4 kg Dhanwantharam per 100kg concentrate mixture). The cost of feeding was also reduced by 18-20%.

Conclusion

Strategies for strengthening the feed / fodder base should focus on regional availability and suitability of potential resources. Use of certain non-traditional feed stuffs lessen the dependency on conventional ingredients. A major drawback in the use of the non-traditional oil seeds is lack of appropriate processing methods that are not only effective to neutralizing the toxins but are economical and simple enough to be taken up by the processing industries. Perception of end users about the technology and their involvement in technology validation is a key to its successful adoption. Local milk unions, krishi vigyana kendras, organised livestock farms and village level self-help groups should act as sub-centres of technology transfer and harness benefits of these innovations.

References

- Abdollahzadeh, F., Pirmohammadi, R., Fatehi, F. and Bernousi, I. 2010. Effect of feeding ensiled mixed tomato and apple pomace on performance of holstein dairy cows. *Slovak J. Ani. Sci.* **43**(1):31-35.
- Bakshi, M.P.S. and Wadhwa, M. 2013. Nutritional evaluation of cannery and fruit wastes as livestock feed. *Indian J. Ani. Sci.* **83**(11): 1198-1202.
- Chethan, K.P., Gowda, N.K.S., Prabhu T.M. and Debpriyo Kumar Dey. 2019. Comparative evaluation of mineral profile of maize grain, hydroponic maize sprout and conventional maize fodder. In : International Conference of Animal Nutrition (INCON 2019), 17-19, December 2019, Kolkata, Abst No. APQ 015.
- Garg, A.K. and Digvijay Singh 2011. Increasing availability of green fodder through a sustainable approach for fodder seed production. *Indian Dairyman*. **11**:54-60.
- Giridhar, K., Gowda, N.K.S. and Anandan, S. 2019. Farmer friendly green fodder production technologies. In : Compendium of Regional workshop on "Sustainable livestock production in doubling the farmers income" 11-12, July 2019 organised by ANSI at SVVU, Tirupati, PP 67-72.
- Gowda, N.K.S. and Anandan, S. 2019. Yelgod S G (Edited) Approaches to improve fodder availability for livestock. In : Compendium of Technical seminar of KVA at GKVK, UAS, Bangalore, 20-21 July 2019. 24-30.
- Gowda, N.K.S. 2016. Areca sheath as an alternate dry fodder for livestock. In: Broadening Horizons, FAO, Feedipedia, 29 May 2016, www.feedipedia.org, 1-2.
- Gowda, N.K.S. and Sastry, V.R.B. 2000. Neem (*Azadirachta indica*) seed cake in animal feeding - Scope and limitations. A review. *Asian -Australasian J. Ani. Sci.*, **13**(5) : 1-8.
- Gowda, N.K.S. and Vijay Bhasker, T. 2017. Fruit residues as alternate forage resources for livestock. In: Approaches towards fodder scarcity in India. (Eds.) Ghosh, P.K., Mohanta, S.K. *et al.*, Studera Press, New Delhi, PP. 534-550, ISBN 978-93-85883-43-9.
- Gowda, N.K.S., Manegar, A, Verma S, Vallesha, N.C. Maya, G., Pal, D.T. and Suresh, K.P. 2015. Azolla (*Azolla pinnata*) as a green feed supplement for dairy cattle – An

- of farm study. *Ani. Nutri. Feed Tech.* **15**: 283-287.
- Gowda, N.K.S., Pal, D.T., Bellur, S.R., Bharadwaj, U. Sridhar, M., Satyanarayana, M.L., Prasad, C.S., Ramachandra, K.S. and Sampath, K.T. 2009. Evaluation of castor (*Ricinus communis*) seed cake in the total mixed ration for sheep. *J. Sci. Food and Agri.* **89** : 216-220.
- Gowda, N.K.S., Vallesha. N.C., Awachat, V.B., Anandan, S., Pal, D.T. and Prasad, C.S. 2015a. Study on evaluation of silage from pineapple (*Ananos comosus*) fruit residue as livestock feed. *Trop. Ani. Health Prod.* **47**: 557-561.
- Hosamani, S.V., Gowda, N.K.S. and Kolalgi, S.D. 2005. Evaluation of chemical, nutritive and feeding value of rain tree pods. *Karnataka J. Agri. Sci.* **18** (1): 110-113.
- Indira, D., Sarjan Rao, K., Suresh, J., Venugopal Naidu, K and Ravi, A. 2009. Azolla (*A. pinnata*) as feed supplement in buffalo calves on growth performance. *Indian J. Ani. Nutri.* **26**(4): 345-348.
- Kusmartono. 2007. Effects of supplementing jack fruit wastes with urea or gliricidia / cassava leaves on growth, rumen digestion and feed degradability of sheep fed on rice straw basal diet. *Livestock Res. Rural Development.* **19**(2): 1-11.
- Muthuramalingam, T., Pothiappan, P., Tensingh Gnanaraj, P., Meenakshi Sundaram, S. and Pugazhenth, T.R. 2015. Studies on growth performance of goats fed hydroponic maize fodder. *Indian Vet. J.* **92**(4):94-96.
- Prakash Kumar R and Dixit S. 2019. Green fodder production : A manual for field functionaries. Publication of ICRISAT, Patancheru, Telangana, 56 pp.
- Rao, S.B.N. Gowda, N.K.S., Soren, N.M., Pavan Kumar, M.A., Awachat, V.B., Karthik Bhat, S., Prasad, K.S. and Ramachandra, K.S. 2019a. Nutritional evaluation of sugar cane trash as dry fodder source for cattle. *Indian J. Ani. Sci.* **89**(6): 667-670.
- Rao, S.B.N., Gowda, N.K.S., Anandan, S. and Prasad, K.S. 2019. Potential of unconventional feed resources for livestock feeding. In : Compendium of Regional workshop on " Sustainable livestock production in doubling the farmers income" 11-12, July 2019 organised by ANSI at SVVU, Tirupati, pp.56-62.
- Sharma, R.K., Rastogi, A. and Haq, Z. 2016. Top feeds for sustainable and ecofriendly small ruminant production. In: Dutta, N., Jadhav, S.E., Kala, A., Gopi, M., & Ramana, J.V. (Eds.), *Newer perspectives in Animal Nutrition Research for Augmenting Animal Productivity*, pp 22-35, Proceedings of X Biennial Animal Nutrition Association Conference, 9-11 November, 2018, Tirupati.
- Ventura, M.R., Pieltin, M.C. and Castanon, J.I.R. 2009. Evaluation of tomato crop by-products as feed for goats. *Ani. Feed Sci. Tech.* **154** : 271-275.
- Wadhwa, M. and Bakshi, M.P.S. 2013. Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products. RAP Publication 2013/04, FAO 2013, (Ed), H.P.S. Makkar. ■



Linkage disequilibrium over short physical genomic distances measured using medium density SNP beadchip in native goat breeds of Kerala

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Abstract

The extent of linkage disequilibrium (LD) at genome wide level is crucial in determining the effectiveness of genomics tools in livestock breeding. The present population genomic study was conducted in native goat breeds of Kerala namely; Attapady Black goats (n=24) and Malabari goats (n=24) to characterise extent of LD within 40kbp marker interval using genome wide single nucleotide polymorphism (SNP) marker data obtained by SNP50 BeadChip genotyping. Extent of LD between bi allelic markers was measured using correlation coefficient (r^2). Mean r^2 between adjacent SNP pairs across all autosomes within 40Kbp marker interval was low (Attapady Black: 0.1336; Malabari: 0.1284). The LD varied across autosomes in native goats. It was the highest for SNP pairs on *Capra hircus* autosome 6 (CHI 6) and the lowest for SNP pairs harboured in CHI 28 in Attapady Black goats and for SNP pairs in CHI 29 in Malabari goats. The low LD estimates indicate the genetically diverse nature of native goats. Current results also imply that denser SNP beadchip array with inter marker interval of below 40kbp would be desirable for effective genome wide association study (GWAS) and genomic selection in native goats.

Keywords: Linkage disequilibrium, goat, SNP BeadChip

The goat is an important farm animal genetic resource of Kerala, constituting 45.56 per cent of its total livestock population (Government of India, 2014). Recently, the advent of draft reference goat genome, coupled with the introduction of single nucleotide polymorphism (SNP)

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array viz., Illumina caprine 50K SNP BeadChip (Dong *et al.*, 2013; Tosser-Klopp *et al.*, 2014) offers unprecedented opportunities for genomic applications in goat production. The medium density caprine SNP50 BeadChip enabling cheap and fast high throughput genotyping was designed by International Goat Genome Consortium (IGGC) for global use in a wide variety of goat breeds with 53,347 SNPs distributed across genome with a median spacing of 40 Kb (Tosser-Klopp *et al.*, 2014).

Linkage disequilibrium (LD) is defined as the non-random association of alleles between two loci. The utility of medium density SNP bead chip currently available for genomic applications such as genomic selection and genome wide association study (GWAS) in a population depends on the extent of LD between SNP markers in the respective population (Kijas *et al.*, 2015). The genomic LD of native goats of Kerala was not reported earlier.

The present population genomics study was carried out in two native goat breeds of Kerala namely, Attapady Black and Malabari goats to characterise the extent of LD between SNP markers over short physical distances up to 40 Kb using genome wide SNP marker data generated by SNP50 BeadChip genotyping. The results of the present study could provide insights with respect to the usefulness of SNP50 BeadChip with median inter marker interval of 40kb for practical application in genomic predictions among native goat population of Kerala.

Materials and methods

The study population comprised of 24 each of Attapady Black and Malabari goats sampled from farmers' herds in their breeding tracts. Genomic DNA was extracted from blood samples of goats using standard phenol chloroform method (Sambrook and Russel, 2001). The DNA of 48 samples were genotyped by Illumina goat SNP50 BeadChip. Quality control of whole genome raw SNP genotype data was performed for individuals and SNPs using the Plink V.1.9 software (Purcell *et al.*, 2007). Individuals missing more than 10 per cent of genotypic data were removed. The SNPs that did not map to any autosomes of ARS1 caprine

genome assembly (<https://www.ncbi.nlm.nih.gov/assembly>) and those that belonged to sex chromosomes were discarded. The SNPs of minor allele frequency of above 0.05, SNP call rate of ≥ 0.90 and Hardy Weinberg equilibrium exact test *P*-value above the threshold level of 0.001 were only retained for downstream analysis.

Extent of LD between bi allelic markers was measured using correlation coefficient (r^2). For a single pair of loci, *A* and *B* with a pair allele *A1* and *A2* at locus *A* and *B1* and *B2* at locus *B*, correlation coefficient (r^2) was computed as proposed by Hill and Robertson (1968)

$$r^2 = \frac{D^2}{f(A1)f(A2)f(B1)f(B2)}$$

Where $D = f(A1B1)f(A2B2) - f(A1B2)f(A2B1)$ and $f(A1B1)$, $f(A2B2)$, $f(A1B2)$ and $f(A2B1)$ are haplotype frequencies of *A1B1*, *A2B2*, *A1B2* and *A2B1* respectively; $f(A1)$, $f(A2)$, $f(B1)$, $f(B2)$ are allele frequencies of *A1*, *A2*, *B1* and *B2* respectively.

For each breed, extent of LD (r^2) for each pair of adjacent SNPs within 40 Kb marker intervals was calculated. Analysis was done in Plink V1.9 (Purcell *et al.*, 2007).

Results and discussion

Overall LD and autosome wise LD estimate for SNPs within 40 kb interval in Attapady Black and Malabari goats are given in Table 1. Mean r^2 between adjacent SNP across all autosomes within 40Kb marker interval was 0.1336 and 0.1284 for Attapady Black and Malabari goats respectively.

The present study quantified LD of native goat breeds of Kerala utilising r^2 measure. It is a more robust LD measure when compared to the other LD measures (Ardlie *et al.*, 2002; Kijas *et al.*, 2014). At 40 Kb marker interval, the LD estimate of the native goats of Kerala was lower than that reported for important international trans-boundary goat breeds like Alpine, Boer, Nubian, Saanen and Toggenburg but comparable to that of regional goat breeds like Australian Rangeland (Brito *et al.*, 2015). Finite population size and intense

Table 1. Average linkage disequilibrium (LD, r^2) in different autosomal chromosomes for Single nucleotide polymorphisms (SNP) pairs within 40 kb interval in Attapady Black and Malabari goats of Kerala.

Chromosome	Attapady Black		Malabari	
	Number of SNP pairs	r^2	Number of SNP pairs	r^2
1	1137	0.1320	1190	0.1309
2	1022	0.1373	1045	0.1136
3	758	0.1403	799	0.1434
4	906	0.1304	957	0.1287
5	701	0.1307	738	0.1246
6	952	0.2004	1034	0.1946
7	743	0.1473	782	0.1370
8	834	0.1301	866	0.1309
9	619	0.1147	638	0.1071
10	758	0.1205	759	0.1142
11	674	0.1249	727	0.1231
12	597	0.1474	661	0.1386
13	604	0.1501	627	0.1473
14	657	0.1485	690	0.1409
15	556	0.1534	604	0.1380
16	533	0.1405	559	0.1307
17	494	0.1306	521	0.1197
18	432	0.1091	445	0.1085
19	376	0.1115	395	0.1087
20	526	0.1070	538	0.1090
21	519	0.1213	540	0.1120
22	386	0.1182	414	0.1213
23	366	0.1196	378	0.1202
24	486	0.1149	508	0.1208
25	287	0.1250	287	0.1064
26	334	0.1330	359	0.1296
27	347	0.1157	354	0.1097
28	339	0.1003	365	0.1053
29	314	0.1133	314	0.0991
All	17257	0.1336	18094	0.1284

selection undergone by improved breeds during breed formation and improvement are implicated for high genomic LD in most of the modern-day livestock breeds (Hayes, 2007). The low genomic LD in native goats indicates the diverse nature of these goat breeds.

The information about the LD among adjacent markers in the genome of native goats has practical consequences for genomic applications and predictions. The r^2 values of 0.2 and 0.3 are important indicators determining effectiveness of accuracy of genomic selection and effectiveness of association studies respectively (Meuwissen *et al.*, 2001; Ardlie *et al.*, 2002). The r^2 of 0.13 even at a short chromosomal distance of 40 kb in native goats

of Kerala clearly indicates limited application of current 50K SNP panel available, for genomic selection and GWAS in native breeds of Kerala. Alternatively, more denser SNP bead array with inter marker interval of below 40 kb would be required to capture LD information needed for implementing genomic selection with reasonable accuracy in native goat breeds of Kerala.

The LD varied across autosomes in native goats (Table 1). The r^2 estimate was the highest for *Capra hircus* autosome 6 (CHI 6) (Attapady Black: 0.20; Malabari: 0.19). Nevertheless, it was the lowest for SNP pairs harboured in CHI 28 in Attapady Black goats and for SNP pairs in CHI 29 in Malabari goats.

Marked inter chromosomal heterogeneity in LD detected in the present study is in support of the observations of Mdladla *et al.* (2016) in South African goats. Selection could result in inter chromosomal heterogeneity in LD (Biegelmeyer *et al.*, 2016). Hence, higher LD estimates detected in CHI 6 of native goat breeds compared to other autosomes could be suggestive of the presence of common genomic region influencing traits that have been under selection in both breeds. Evidence of genomic regions spanning in CHI6 influencing adaptation to hot arid environment has been reported in goats (Kim *et al.*, 2015). Both native goat breeds of Kerala are well known for their adaptation to humid tropical stressors of Kerala.

Conclusion

This population genomics study characterised the extent of LD between genetic markers at short marker intervals among native goat breeds of Kerala using high throughput genomic data obtained by goat SNP50 BeadChip genotyping. The LD (r^2) between adjacent SNPs across all autosomes was low and this result was contrary to the findings in modern breeds of livestock that displayed high LD due to factors like low N_e and intense selection.

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References

- Ardlie, K.G., Kruglyak, L. and Seielstad M. 2002. Patterns of linkage disequilibrium in the human genome. *Nat. Rev. Genet.* **3**:299-309.
- Biegelmeyer, P., Gulas-Gomes, C. C., Caetano, A. R., Steibel, J. P. and Cardoso, F. F. 2016. Linkage disequilibrium, persistence of phase and effective population size estimates in Hereford and Braford cattle. *BMC Genet.* **17**:32
- Brito, L.F., Jafarikia, M., Grossi, D.A., Kijas, J.W., Porto-Neto, L.R., Ventura, R.V., Salgorzaei, M. and Schenkel, F.S. 2015. Characterization of linkage disequilibrium, consistency of gametic phase and admixture in Australian and Canadian goats. *BMC Genet.* **16**:1.
- Dong, Y., Xie, M., Jiang, Y., Xiao, N., Du, X., Zhang, W., Tosser-Klopp G., Wang, J., Yang, S., Liang, J., Chen, J., Zeng, P., Hou, Y., Bian, C., Pan, S., Li, Y., Liu, X., Wang, W., Servin, B., Sayre, B., Zhu, B., Sweeney, D., Moore, R., Nie, W., Shen, Y., Zhao, R., Zhang, R., Li, J., Faraut, T., Womack, J., Zhang, Y., Kijas, J., Cockett, N., Xu, X., Zhao, S., Wang, J. and Wang, W. 2013. Sequencing and automated whole genome optical mapping of the genome of a domestic goat (*Capra hircus*). *Nat. Biotechnol.* **31**:135-141.
- Government of India. 2014. 19th livestock census-2012. All India report. pp.78.
- Hill, W. G. and Robertson, A. 1968. Linkage disequilibrium in finite populations. *Theor. Appl. Genet.* **38**:226-231.
- Hayes, B. 2007. *QTL mapping, MAS and genomic selection*. A short-course organized by Animal Breeding and Genetics. Department of Animal Science, Iowa State University. 179pp.
- Kijas, J. W., Porto-Neto, L., Dominik, S., Reverter, A., Bunch, R., McCulloch, R., Hayes, B.J., Brauning, R., McEwan, J. and the International Sheep Genomics Consortium. 2014. Linkage disequilibrium over short physical distances measured in sheep using a high-density SNP chip. *Anim. Genet.* **45**: 754-757.
- Kim, E.S., Elbeltagy, A.R., Aboul-Naga, A.M., Rischkowsky, B., Sayre, B., Mwacharo, J.M. and Rothschild, M. F. 2015. Multiple genomic signatures of selection in goats and sheep indigenous to a hot arid environment. *Heredity*. **10**:1-10.
- Meuwissen, T. H., Hayes, B. J. and Goddard, M. E. 2001. Prediction of total genetic

- value using genome-wide dense marker maps. *Genetics* **157**:1819–1829.
- Mdladla, K., Dzomba, E.F., Huson, H.J. and Muchadeyi, F.C. 2016. Population genomic structure and linkage disequilibrium analysis of South African goat breeds using genome-wide SNP data. *Anim. Genet.* **47**:471–482.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J. and Sham, P.C. 2007. PLINK: a toolset for whole-genome association and population-based linkage analysis. *Am. J. Hum. Genet.* **81**(3):559–575.
- Sambrook, J. and Russell, D.W. 2001. *Molecular Cloning: A laboratory manual*. 3rd Ed, Cold Spring Harbor Laboratory Press, New York. 2100.
- Tosser-Klopp, G., Bardou, P., Bouchez, O., Cabau, C., Crooijmans, R., Dong, Y., Donnadieu-Tonon, C., Eggen, A., Heuven, H.C., Jamli, S., Jiken, A. J., Klopp, C., Lawlet, C.T., McEwan, K., Martin, P., Moreni, C.R., Mulsant, P., Nabihoudine, I., Pailhoux, E., Palhière, I., Rupp, R., Sarry, J., Sayre, B.L., Tircazes, A., Wang, J., Wang, W., Zhang, W. and International Goat Genome Consortium. 2014. Design and characterization of a 52 K SNP chip for goats. *PLoS one*. **9**: e86227.





Deltamethrin resistance in *Rhipicephalus sanguineus* and *Rhipicephalus (Boophilus) microplus* tick population in Kerala

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Abstract

The study evaluated deltamethrin resistance in *Rhipicephalus sanguineus* and *Rhipicephalus (Boophilus) microplus* tick populations of Kerala in India, using larval packet test (LPT). Dose response data were analysed by the probit method, the LC_{50} and LC_{95} of deltamethrin against ticks were determined by applying regression equation analysis to the probit-transformed data of mortality. In *R. sanguineus*, 50 per cent of isolates were found resistant at discriminating dose (600 ppm) by larval packet test. The p value obtained upon regression analysis was < 0.05 and was considered as significant. A majority of *R. (B.) microplus* were found to be susceptible to deltamethrin. However, these susceptible isolates survived doses which were twice the recommended doses (1.25 ppm – 100 ppm). The p value of isolates except isolate 1 and 5 were < 0.05 and statistically significant. The results highlight acaricide resistance to be one of the reasons for the alarming prevalence of tick-borne haemoparasites in Kerala and demand urgent interventions to ameliorate the resistance by alternate control strategies.

Keywords

Rhipicephalus sanguineus, *Rhipicephalus (Boophilus) microplus*, Larval Packet test

Ticks are considered harmful obligate blood-sucking ectoparasites of medical and veterinary importance, as they not only transmit economically important infections to animals but also play a major role in zoonotic pathogen transmission to humans (Balasubramanian *et al.*, 2019).

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In India, the warm humid climate favours the propagation and perpetuation of ticks (Haque *et al.*, 2014; Rani *et al.*, 2018).

Rhipicephalus (Boophilus) microplus, one host cattle tick, is the most prevalent tick causing considerable economic loss in dairy cattle worldwide (Guerrero *et al.*, 2001). They transmit rickettsial diseases such as ehrlichiosis and anaplasmosis and protozoal diseases such as babesiosis, besides inflicting direct effects on the animal *per se* through blood loss. *Rhipicephalus sanguineus*, a three-host brown dog tick, transmits several protozoan parasites like *Babesia canis*, *Ehrlichia canis* and *Leishmania infantum* (Dantas-Torres, 2008). Ticks and tick-borne diseases (TBDs) are a major constraint in the development and improvement of the livestock industry (Ahamed *et al.*, 2007). Chemical acaricides play a vital role in tick control. However, the indiscriminate and frequent use of acaricides has led to drug and multidrug resistance against almost all commercially accessible acaricides. Monitoring of ticks and drug efficacy are important to detect resistance at an early stage to help minimize the spread of resistance and to document the distribution pattern of acaricide resistance (Lovis *et al.*, 2011).

The common acaricides in use include synthetic pyrethroids, organophosphates, organochlorines, carbamates, amidines, macrocyclic lactones and formamidines. Synthetic pyrethroids (SP) are highly biodegradable and not very toxic to mammals and had been introduced in the 1960s and 1970s. Already in the late 1980s, resistance to synthetic pyrethroids (SP) had been reported in Brazil and Australia, and it was reported in Mexico in 1994. Nowadays SP resistance is extremely common and prevalent world over (Yessino *et al.*, 2016). In South India, deltamethrin resistance to cattle ticks, *R. (Boophilus) annulatus* and *R. (Boophilus) microplus* has been reported (Jyothimol *et al.*, 2014; Lenka *et al.*, 2016). While, in North India, acaricide resistance has been widespread against flumethrin, fipronil (Shyma *et al.*, 2015) and deltamethrin (Abdullah *et al.*, 2012; Shyma *et al.*, 2015) in different tick species infesting large ruminants. In India, there have not been many studies on drug resistance in *R.*

sanguineus. Mathivathani *et al.* (2011) reported 64.72 per cent resistance against flumethrin and 59.35 per cent resistance against deltamethrin in *R. sanguineus* in Chennai by using adult immersion tests.

Among the various bioassay techniques developed for recognizing acaricide resistance in ticks, larval packet technique (LPT) is a reliable method (Stone and Haydock, 1962). The Food and Agriculture Organization of the United Nations (FAO) had adopted this method as the standard method for the detection and measurement of acaricide resistance (Morgan *et al.*, 2009). Singh *et al.* (2015) reported that the acaricide resistance frequency is higher in one host ticks, since a much larger portion of the entire population of such species are exposed to chemical encounter at any one time than multi host ticks.

In the light of high prevalence of tick-borne pathogens among domestic animals in Kerala and scanty reports of acaricide resistance in ticks, this study particularly focused upon the assessment of deltamethrin resistance in cattle and dog ticks by bioassay.

Materials and methods

Collection of ticks

Fully engorged adult female *R. sanguineus* and *R. (B.) microplus* ticks were collected from animals presented to veterinary hospitals, as well as from private kennels and farms in Thrissur district of Kerala. Kerala is a southern state of India, spanning an area of approximately 3,032 km², characterised with a tropical climate with an average annual temperature of 27.6°C and the relative humidity being generally over 70 per cent. The collected ticks were cleaned with distilled water, dried on an absorbent paper, identified by morphological keys (Sen and Fletcher, 1962) and sampled in separate bottles. Each bottle containing around three to five engorged female ticks were labelled and closed with a muslin cloth to maintain aeration and moisture. For egg laying and hatching, the bottles were placed in a desiccator maintained with a temperature of 28°C and relative humidity of 85 per cent. Nearly 2-10 days were

taken for egg laying (Fig. 1), while 15-21 days were required for the eggs to hatch into larvae. The hatched out tick larvae were maintained for 14-21 days in desiccators for performing larval packet test (LPT).

Fig. 1 Egg mass of *R. (B.) microplus*



Protocol of LPT

The LPT was performed as per the recommendations of FAO (2004) with minor modifications. Technical grade 100 per cent pure deltamethrin (PESTANAL, Sigma- Aldrich, USA) was used for the bioassay. Working concentrations of 240 ppm, 120 ppm, 60 ppm, 30 ppm of deltamethrin were prepared by serially diluting the stock concentration with distilled water. Bioassay was also performed with discriminating dose (DD) of deltamethrin (600 ppm). Rectangular packets (8 cm x 4.2 cm) were prepared using Whatman filter paper No.1. These packets were carefully impregnated with 500 μ L of the above solutions and allowed to dry at room temperature. Distilled water was used as the control. Three replicates of each treatment were tested. Approximately, 100 numbers of 14-21 day old live larvae were deposited into each dried packet. The open end of these packets were sealed with bulldog clips and kept in desiccators at room temperature for 24 h. After the incubation period, the packets were opened and the number of dead and live larvae were counted manually to estimate the per cent mortality.

Statistical analysis

Dose response data were analysed by the probit method (Finney, 1952). The 50 per cent (LC_{50}) and 95 per cent (LC_{95}) lethal concentrations of deltamethrin against *R. sanguineus* and *R. (B.) microplus* were determined by applying regression equation analysis to the probit-transformed data of mortality with regression analysis. A value of $p < 0.05$ was considered as statistically significant.

Results and Discussion

The study was conducted to evaluate deltamethrin resistance in *R. sanguineus* and *R. (B.) microplus*, the most economically important ectoparasites of dogs and cattle, respectively. Per cent mortality of larvae was assessed at different deltamethrin concentrations. The isolates were considered resistant, if mortality of *R. sanguineus* and *R. (B.) microplus* treated with discriminating dose (DD: 600 ppm), were below 90 per cent.

Analysis of probit transformed mortality of *R. sanguineus* against log concentration of deltamethrin is given in Table 1. Isolate 5 and 6 of *R. sanguineus* showed 100 per cent mortality even at the lowest concentration of deltamethrin and hence LC_{50} and LC_{95} were not derived. Isolate 4 had the least LC_{50} (8.71 ppm) and LC_{95} (2089.29 ppm). Isolates 1, 2 and 3 were shown to be resistant at DD. Isolate 4, 5 and 6 were considered susceptible and mortality at DD was > 90 per cent. The LC_{95} of all the resistant isolates were higher than the recommended acaricidal dose of deltamethrin (1.25 ppm – 100 ppm). The p value obtained upon regression analysis with *R. sanguineus* isolates was < 0.05 and was considered significant.

The LC_{50} and LC_{95} values of different isolates of *R. (B.) microplus* (Table 2) showed that isolate 1 had the lowest LC_{50} (16.98 ppm) and LC_{95} (131.82 ppm), while the highest LC_{50} (223.87 ppm) and LC_{95} (602.56 ppm) were observed for isolate 5. Except for isolate 5, all other *R. (B.) microplus* isolates were found to be susceptible to deltamethrin. However, the LC_{50} and LC_{95} of all isolates were higher than the recommended dose. In susceptible isolates the least LC_{50} observed was 16.98 and LC_{95}

Table 1 Probit analysis of LPT with *R. sanguineus*

Tick	Slope \pm SE	R ² value	LC ₅₀ (in ppm)	LC ₉₅ (in ppm)	P value	Mortality % at DD
Isolate 1	0.6742 \pm 0.0755	0.9648	107.15	26302.68	0.002	69.86%
Isolate 2	0.7624 \pm 0.0615	0.9814	467.74	67608.29	0.001	54.80%
Isolate 3	0.4846 \pm 0.0697	0.9430	26.30	67608.29	0.005	76.60%
Isolate 4	0.6955 \pm 0.1548	0.8738	8.71	2089.29	0.019	91.02%
Isolate 5	Not calculated since 100 % mortality was observed with lowest concentration					100%
Isolate 6						100%

Table 2 Probit analysis of LPT with *R. (B.) microplus*

Tick	Slope \pm SE	R ² value	LC ₅₀ (in ppm)	LC ₉₅ (in ppm)	P value	Mortality % at DD
Isolate 1	1.8852 \pm 0.8352	0.6363	16.98	131.82	0.105	100 %
Isolate 2	2.0819 \pm 0.2085	0.9716	51.29	309.03	0.002	99.11%
Isolate 3	2.0801 \pm 0.5285	0.8418	26.92	165.95	0.028	98.69%
Isolate 4	1.6788 \pm 0.4772	0.8095	24.55	229.09	0.037	98.28%
Isolate 5	3.8499 \pm 1.4578	0.7054	223.87	602.56	0.075	81.58%
Isolate 6	5.4367 \pm 1.1416	0.8862	151.36	301.99	0.016	100%

was 131.82. The *p* value of analysis with all *R. (B.) microplus* isolates except isolate 1 and 5 were less than 0.05 and statistically significant.

Resistance of ticks against acaricides could be monitored by using various bioassays, such as larval packet test (LPT), adult immersion test (AIT), larval tarsal test (LTT) and larval immersion test (LIT) (FAO, 2004). Acaricide resistance in ticks is an inherited phenomenon, defined as a reduction in susceptibility of ticks to the acaricide when it is used at the recommended dose (FAO, 2004). Fular *et al.* (2018) observed that the resistance level and mechanisms of resistance development varied in different parts of the world. Hence, regular monitoring of resistance is an inevitable control strategy in areas where tick and tick-borne parasites continue to pose a significant threat to livestock population.

Our study established that deltamethrin resistant phenotypes persisted among tick populations infesting cattle and dogs of Kerala. Bioassay using LPT revealed that at discriminating dose, 50 per cent of *R. sanguineus* were resistant to deltamethrin, while 83 per cent of *R. (B.) microplus* were susceptible. However, these susceptible isolates survived doses that were twice the recommended doses of 1.25 ppm to 100 ppm (Sandhu, 2006).

Resistance status of *R. (B.) microplus* ticks against synthetic pyrethroids was globally studied using the FAO recommended larval packet test (Rosario-Cruz *et al.*, 2009; Abdullah *et al.*, 2012; Domingues *et al.*, 2012; Shyma *et al.*, 2013; Shyma *et al.*, 2015). Lovis *et al.* (2012) assigned phenotype resistance to tick population based on three criteria: Survival rate at DD, resistance ratio (RR) 50 and RR90. The population was designated resistant when their survival rate at DD was greater than 10 per cent or when the RR50 and RR90 were above four. In the present study, all *R. (B.) microplus* isolates, except isolate 5 were found susceptible, since mortality per cent was above 90 per cent at DD. The observed LC₅₀ and LC₉₅ of deltamethrin in susceptible tick population were much lower than that reported for resistant isolates (Shyma *et al.*, 2012; Aboelhadid *et al.*, 2018).

In Kerala, Jyothimol *et al.* (2014) had reported a low level of deltamethrin resistance in cattle ticks with LC₅₀ and LC₉₅ values being 2.15 ppm and 52.24 ppm, respectively in susceptible isolates of *R. (B.) microplus*, while those for *R. (B.) annulatus* were 2.11 ppm and 34.75 ppm, respectively. In Uttar Pradesh, a north Indian state, LPT to detect acaricide resistance revealed that the LC₉₉ values of fenvalerate and fipronil were 2007.0 ppm and 4.8 ppm, respectively, while that of coumaphos

and malathion, that were highly toxic to larvae, yielded LC_{99} values as low as 28.4 ppm and 55.9 ppm, respectively (Kumar *et al.*, 2015). The LC_{50} and LC_{95} values of deltamethrin acaricide against susceptible lines of *Hyalomma anatolicum* in the Indian cattle population were 11.7 ppm and 34.9 ppm, respectively by LPT, as reported by Shyma *et al.* (2012). Shyma *et al.* (2015) reported LC_{50} and LC_{95} values of deltamethrin against resistant *R. (B.) microplus* isolates in North Gujarat to be 75.24 ppm and 367.74 ppm, respectively. Lenka *et al.* (2016) observed a wide variation (2.4 ppm-13.8 ppm) in the LC_{50} values in *R. (B.) microplus* isolate collected from South Indian states which was lower than that for North Indian isolates. Perusal of literature suggests that cattle ticks are resistant to almost all commercially available acaricides in India (Sharma *et al.*, 2012; Shyma *et al.*, 2013; Kumar *et al.*, 2014; Singh *et al.*, 2015; Lenka *et al.*, 2016). The global situation is not different. The resistant Ehanasia population of *R. annulatus* showed a high LC_{50} value of 100 ppm (Aboelhadid *et al.*, 2018) against deltamethrin. Domingues *et al.* (2012) reported resistance to cypermethrin by LPT, since resistance ratio (RR) between 16.0 to 25.0 and 85.7 per cent were resistant to chlorpyrifos because RR value ranged from 2.2 to 15.6. Santos *et al.* (2013) observed wide variation of LC_{50} values (264.9 μ g/mL - 9923.9 μ g/mL) in resistant field isolates in Brazil.

In South India, apart from the report of Mathivathani *et al.* (2011) in Tamil Nadu, deltamethrin resistance has not been studied in *R. sanguineus* isolates. The present study is the first report of acaricide resistance in dog ticks in Kerala state and it is identified that 50 per cent of *R. sanguineus* population in the state, selected for study, exhibited a mortality ranging from 53 to 77 per cent, at DD and were resistant to deltamethrin. The LC_{50} value of deltamethrin against susceptible *R. sanguineus* isolate was 8.71 ppm. Studies by Miller *et al.* (2002) revealed a resistance ratio of 7.3 against amitraz in a Panamanian strain of *R. sanguineus* by using LPT bioassay (Miller *et al.*, 2002).

The current investigation showed that deltamethrin resistance in Kerala is comparatively higher in the three host ticks

(*R. sanguineus*) than among one host ticks (*R. (B.) microplus*). The high prevalence of tick-borne canine haemoparasites in this part of South India (Jain *et al.*, 2017; Wahlang *et al.*, 2019) despite the intensive use of deltamethrin, could be due to widespread drug resistance in the tick population. This necessitates studies on alternate control strategies, incessant monitoring and strategic application of other acaricides to control the tick population in this geographical area.

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References

- Abdullah, S., Yadav, C.L. and Vatsya, S. 2012. Esterase profile of *Rhipicephalus (Boophilus) microplus* populations collected from Northern India exhibiting varied susceptibility to deltamethrin. *Exp. Appl. Acarol.* **58**(3): 315-325.
- Aboelhadid, S.M., Arafa, W.M., Mahrous, L.N., Fahmy, M.M. and Kamel, A.A. 2018. Molecular detection of *Rhipicephalus (Boophilus) annulatus* resistance against deltamethrin in middle Egypt. *Vet Parasitol. Reg. Stud. Reports.* **13**: 198-204.
- Ahmed, J., Alp, H., Aksin, M. and Seitzer, U. 2007. Current status of ticks in Asia. *Parasitol. Res.* **101**(2): 159-162.
- Balasubramanian, R., Yadav, P.D., Sahina, S. and Nadh, V.A. 2019. Distribution and prevalence of ticks on livestock population in endemic area of Kysanur forest disease in Western Ghats of Kerala, South India. *J. Parasit. Dis.* **43**(2): 256-262.
- Dantas-Torres, F. 2008. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. *Vet. Parasitol.* **152**(3-4):173-185.

- Domingues, L.N., Brasil, B.D.S.A.F., de Paiva Bello, A.C.P., da Cunha, A.P., de Barros, A.T.M., Leite, R.C., Silaghi, C., Pfister, K. and Passos, L.M.F. 2012. Survey of pyrethroid and organophosphate resistance in Brazilian field populations of *Rhipicephalus (Boophilus) microplus*: Detection of C190A mutation in domain II of the para-type sodium channel gene. *Vet. Parasitol.* **189**(2-4): 327-332.
- FAO (2004) Guidelines resistance management and integrated parasite control in ruminants, Rome.
- Finney DJ (1952) Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge university press, Cambridge.
- Fular, A., Sharma, A.K., Kumar, S., Nagar, G., Chigure, G., Ray, D.D. and Ghosh, S. 2018. Establishment of a multi-acaricide resistant reference tick strain (IVRI-V) of *Rhipicephalus microplus*. *Ticks Tick Borne Dis.* **9**(5): 1184-1191.
- Guerrero, F.D., Davey, R.B. and Miller, R.J. 2001. Use of an allele-specific polymerase chain reaction assay to genotype pyrethroid resistant strains of *Boophilus microplus* (Acari: Ixodidae). *J. Med. Entomol.* **38**(1): 44-50.
- Haque, M., Singh, N.K. and Rath, S.S. 2014. Effect of various acaricides on hatchability of eggs of *Rhipicephalus (Boophilus) microplus*. *Biomed Res. Int.* 5p.
- Jain, K.J., Lakshmanan, B., Syamala, K., Praveena, J.E. and Aravindakshan, T. 2017. High prevalence of small *Babesia* species in canines of Kerala, South India. *Vet. World.* **10** (11): 1319-1323.
- Jyothimol, G., Ravindran, R., Juliet, S., Ajithkumar, K.G., Suresh, N.N., Vimalkumar, M.B., Lenka, D.R., Varghese, S. and Ghosh, S. 2014. Low level deltamethrin resistance in ticks from cattle of Kerala, a South Indian state. *Vet. Parasitol.* **204**(3-4): 433-438.
- Kumar, S., Sharma, A.K., Nagar, G. and Ghosh, S. 2015. Determination and establishment of discriminating concentrations of malathion, coumaphos, fenvalerate and fipronil for monitoring acaricide resistance in ticks infesting animals. *Ticks Tick Borne Dis.* **6**(3): 383-387.
- Kumar, S., Sharma, A.K., Ray, D.D. and Ghosh, S. 2014. Determination of discriminating dose and evaluation of amitraz resistance status in different field isolates of *Rhipicephalus (Boophilus) microplus* in India. *Exp. Appl. Acarol.* **63**(3): 413-422.
- Lenka, D.R., Ravindran, R., Jyothimol, G., Udaykumar, M., Reddy, P.M.T., Sathish, N., Palla, I., Chandramohan, B., Ajithkumar, K.G., Nair, S.N. and Chandrasekhar, L. 2016. Deltamethrin resistance in South Indian isolates of *Rhipicephalus (Boophilus) microplus*. *Vet. Parasitol. Reg. Stud. Rep.* **5**: 37-41.
- Lovis, L., Guerrero, F.D., Miller, R.J., Bodine, D.M., Betschart, B. and Sager, H. 2012. Distribution patterns of three sodium channel mutations associated with pyrethroid resistance in *Rhipicephalus (Boophilus) microplus* populations from North and South America, South Africa and Australia. *Int. J. Parasitol.: Drugs Drug Res.* **2**: 216-224.
- Lovis, L., Perret, J.L., Bouvier, J., Fellay, J.M., Kaminsky, R., Betschart, B. and Sager, H. 2011. A new in vitro test to evaluate the resistance level against acaricides of the cattle tick, *Rhipicephalus (Boophilus) microplus*. *Vet. Parasitol.* **182**(2-4): 269-280.
- Mathivathani, C., Basith, S.A., Latha, B.R. and Raj, G.D. 2011. In vitro evaluation of synthetic pyrethroid resistance in *Rhipicephalus sanguineus* ticks of Chennai. *J. Vet. Parasitol.* **25**: 56-58.
- Miller, R.J., Davey, R.B. and George, J.E. 2002. Modification of the food and agriculture organization larval packet test to

- measure amitraz-susceptibility against Ixodidae. *J. Med. Entomol.* **39**: 645-651.
- Morgan, J.A., Corley, S.W., Jackson, L.A., Lew-Tabor, A.E., Moolhuijzen, P.M. and Jonsson, N.N. 2009. Identification of a mutation in the para-sodium channel gene of the cattle tick *Rhipicephalus (Boophilus) microplus* associated with resistance to synthetic pyrethroid acaricides. *Int. J. Parasitol.* **39**: 775-779.
- Rani, S., D'Souza, P.E., Byregowda, S.M., Veeregowda, B.M., Sengupta, P.P., Chandranai, B.M. and Thimmareddy, P.M. 2018. In vitro acaricidal efficacy of deltamethrin, cypermethrin and amitraz against sheep ticks in Karnataka. *Exp. Parasitol.* **201**: 42-48.
- Rosario-Cruz, R., Guerrero, F.D., Miller, R.J., Rodriguez-Vivas, R.I., Tijerina, M., Dominguez-Garcia, D.I., Hernandez-Ortiz, R., Cornel, A.J., McAbee, R.D. and Alonso-Diaz, M.A. 2009. Molecular survey of pyrethroid resistance mechanisms in Mexican field populations of *Rhipicephalus (Boophilus) microplus*. *Parasitol. Res.* **105**: 1145-1153.
- Sandhu, H.S. 2006. Veterinary Pharmacology and Therapeutics. (2nd Ed) Kalyani publisher, New Delhi, 1283p.
- Santos, T.R.B., Klafke, G.M., Pappen, F.G., Nizoli, L.Q., Biegelmeyer, P. and Farias, N.A.R. 2013. Comparison of three larval bioassays to evaluate susceptibility of *Rhipicephalus (Boophilus) microplus* to amitraz. *Rev. Bras. Parasitol. Vet.* **22**: 495-501.
- Sen, S.K. and Fletcher, T.B. (1962) Veterinary Entomology and Acarology in India, Indian Council of Agricultural Research, New Delhi.
- Sharma, A.K., Kumar, R., Kumar, S., Nagar, G., Singh, N.K., Rawat, S.S., Dhakad, M.L., Rawat, A.K.S. and Ray, D.D. 2012. Deltamethrin and cypermethrin resistance status of *Rhipicephalus (Boophilus) microplus* collected from six agro-climatic regions of India. *Vet. Parasitol.* **188**: 337-345.
- Shyma, K.P., Gupta, J.P., Singh, V. and Patel, K.K. 2015. In vitro detection of acaricidal resistance status of *Rhipicephalus (Boophilus) microplus* against commercial preparation of deltamethrin, flumethrin, and fipronil from North Gujarat, India. *J. Parasitol. Res.* 1-7p.
- Shyma, K.P., Kumar, S., Sangwan, A.K., Sharma, A.K., Nagar, G., Ray, D.D. and Ghosh, S. 2013. Acaricide resistance status of *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Haryana. *Indian J. Anim. Sci.* **83**: 591-594.
- Shyma, K.P., Kumar, S., Sharma, A.K., Ray, D.D. and Ghosh, S. 2012. Acaricide resistance status in Indian isolates of *Hyalomma anatolicum*. *Exp. Appl. Acarol.* **58**: 471-481.
- Singh, J., Singh, N.K., Singh, H., Mehta, N. and Rath, S.S. 2019. In vitro assessment of synergistic combinations of essential oils against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Exp. Parasitol.* **201**: 42-48.
- Stone, B.F. and Haydock, K.P. 1962. A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Can.). *Bull. Entomol. Res.* **53**(3): 563-578.
- Wahlang, L., Lakshmanan, B., Thomas, N., Bosewell, A., Jose, J., Chulliyil, S. and Thazhathuveetil, A. 2019. SYBR green-based real-time PCR detection of canine *Babesia* spp. in ixodid ticks infesting dogs in Kerala, South India. *Turkish J. Vet. Anim. Sci.* **43**(3): 427-431.
- Yessinou, R.E., Akpo, Y., Adoligbe, C., Adinci, J., Assogba, M.N., Koutinhouin, B., Karim, I.Y. and Farougou, S. 2016. Resistance of tick *Rhipicephalus microplus* to acaricides and control strategies. *J. Entomol. Zool. Stud.* **4**(6): 408-414. ■



Annual temperature profile of Thrissur: a climate change perspective*

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Abstract

Climate change is one of the most serious issues faced by the global community of the present era. Hence, maximum, minimum and average temperatures for the period 1986 to 2016 collected from the Department of Agricultural Meteorology, Kerala Agricultural University (KAU), Vellanikkara were analysed to study the change in climate over years of Thrissur district. The present study showed no significant trend with respect to the annual mean maximum temperature from 1986 to 2016 as the temperature was not continuously progressive. But during 2011- 2016, for the annual mean maximum temperature, increasing trend was found and it was significantly increasing by 0.1495°C per year. The annual mean minimum and average temperatures also showed no significant trend from 1986-2016.

Keywords: Maximum temperature, Minimum temperature, Average temperature, Temperature trend

Global warming is one of the major factors that cause climate change (Webster *et al.*, 2008). Temperature and humidity changes have a profound impact on agricultural and animal husbandry systems when compared to other climatic variables. As climate is the long-term average of the meteorological variables, data for 30 years were collected and analysed. This study investigated the trends in annual mean maximum, minimum and average temperatures of Thrissur district over a year, each year from 1986 to 2016.

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Materials and Methods

Meteorological data of 30 years from 1986 to 2016 regarding maximum, minimum and average temperatures were collected for the present study from the Department of Agricultural Meteorology, Kerala Agricultural University (KAU), Vellanikkara, to analyse the trends in annual mean maximum, mean minimum and average temperatures of Thrissur. The total period was also classified decennially. The annual and decadal trends were found using regression methods. Statistical analysis was done by using the software Statistical Products and Service Solutions (SPSS), Version 24.0

Results and discussion

Trends in annual mean maximum temperature from 1986 to 2016

During the period from 1986 to 1990, the highest mean maximum temperature was recorded in 1987 (32.86°C) while the lowest was in 1986 (31.72°C). An increase of 0.11°C over a period of five years was observed (Fig.1a) but the trend was not significant ($P>0.05$). During the period from 1991-2000, the highest mean maximum temperature was recorded in 1995 (33.00°C) while the lowest was recorded in 1999 (31.57°C). A decrease of 0.64°C was observed during these ten years (Fig.1b) but the trend was not significant ($P>0.05$). During the period from 2001-2010, the highest mean maximum temperature was recorded in 2003 (32.22°C) while lowest in 2006 (31.62°C). There was a decrease of 0.16°C during these ten years (Fig.1c) but there was no significant trend ($P>0.05$) was observed. During the period from 2011-2016, the highest mean maximum temperature was recorded in 2016 (32.64°C) while the lowest was recorded in 2011 (31.78°C). There was an increase of 0.89°C in the above six years in mean maximum temperature (Fig.1d). A significant increase of 0.1495°C per year ($P<0.05$) was observed over a period of six years (2011-2016). When the temperature trend of 30 years (1986-2016) was analysed, the highest mean maximum temperature was recorded in 1995 (33°C) while the lowest was recorded in 1999 (31.57°C). A decrease of 0.16°C was recorded

during these 30 years (Fig.1e) but the trend was not significant ($P>0.05$). The present study showed no significant trend for the annual mean maximum temperature from 1986 – 2016 as the temperature was not continuously progressive.

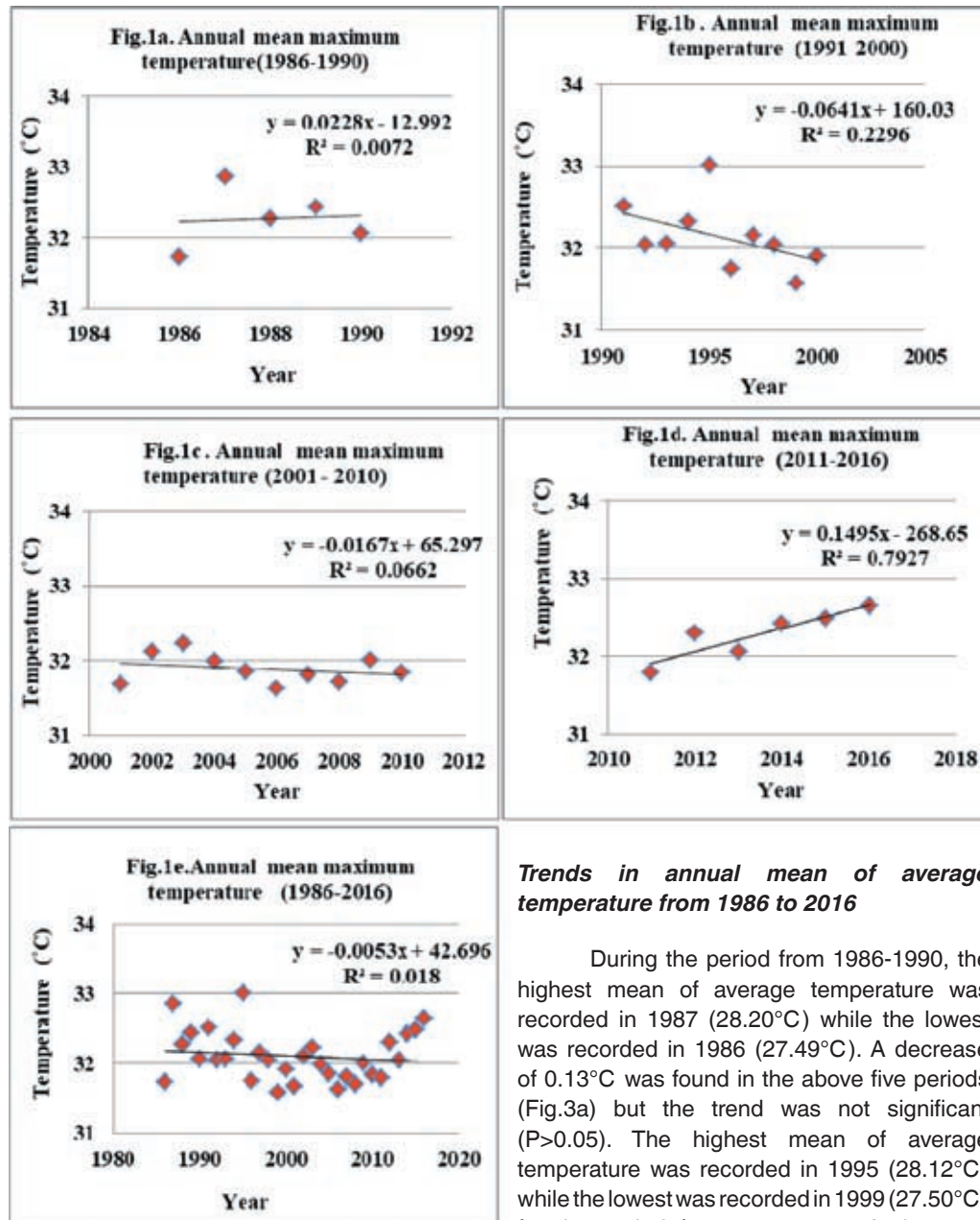
According to Rao (2016), the temperature in Kerala has shown a significantly increasing trend since 1980. The annual mean maximum temperature across the state of Kerala, varied from 30.4°C to 32.4°C with an average of 30.5°C.

Trends in annual mean minimum temperature from 1986 to 2016.

During the period from 1986 to 1990, the highest mean minimum temperature was recorded in 1987 (23.54°C) while the lowest in 1990 (23.10°C). A decrease of 0.36°C was observed during these five years (Fig.2a) but the trend was not significant ($P>0.05$). During 1991-2000, the highest mean minimum temperature was recorded in 1998 (23.70°C) and lowest in 1992 (22.99°C). An increase of 0.5°C was also observed in the same period but it was not a significant trend ($P>0.05$). (Fig.2b). During the period from 2001-2010, the highest mean minimum temperature was recorded in 2003 (23.56°C) and lowest in 2007 (23.10°C). An increase of 0.07°C was noticed during these ten years (Fig.2c) but it was not a significant trend ($P>0.05$).

During the period from 2011-2016, the highest mean minimum temperature was recorded in 2015 (23.79°C) and lowest in 2011 (23.24°C). An increase of 0.32°C was observed in this six-year period (Fig.2d) but no significant trend ($P>0.05$) was observed. While analysing the data of the 30-year period under consideration, from 1986 to 2016, the highest mean minimum temperature was recorded in 2015 (23.75°C) while the lowest was recorded in 1992 (22.99°C). An increase of 0.22°C was recorded in this period (Fig.2e) but it was not a significant trend ($P>0.05$).

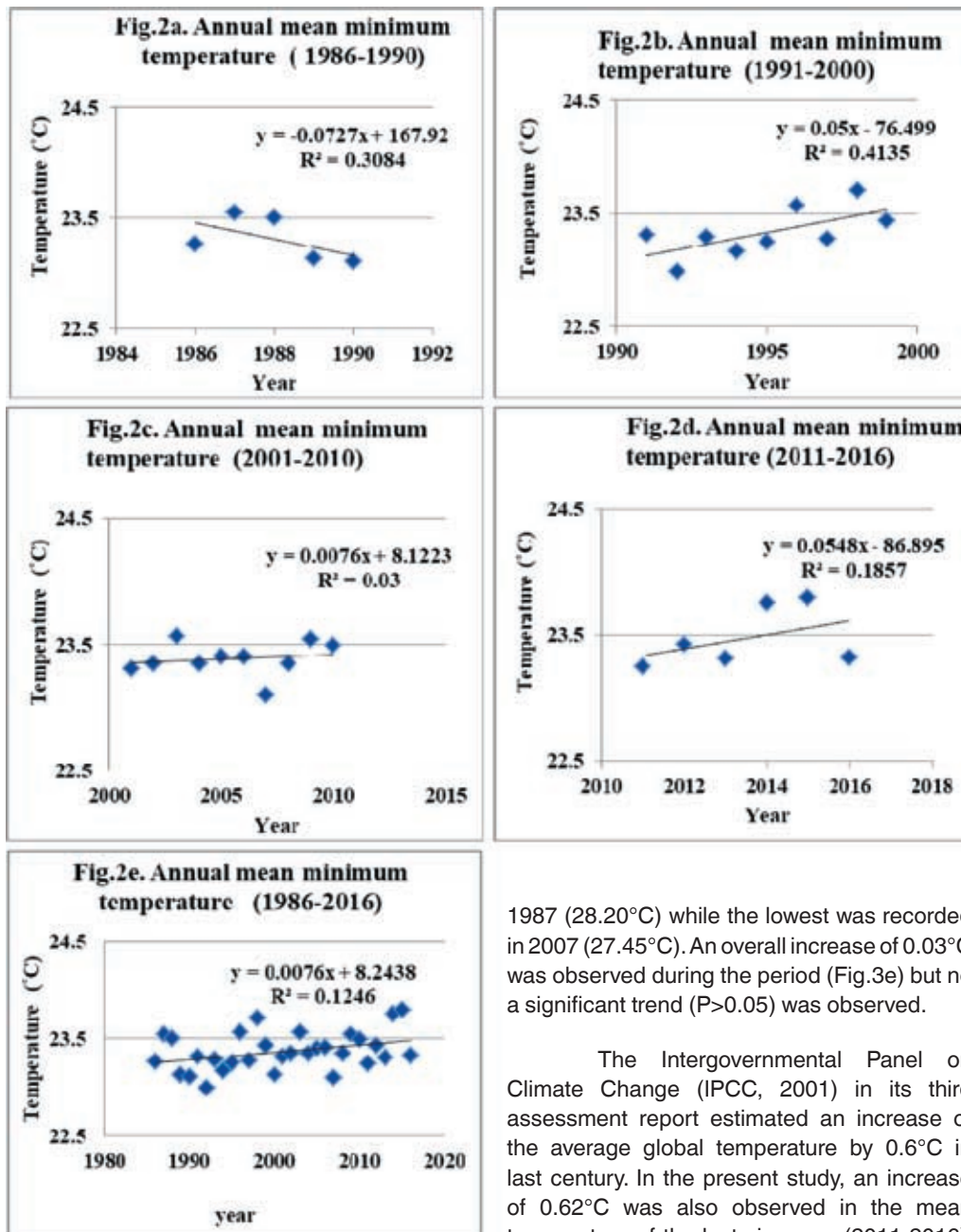
Sahai (1998) observed an increasing tendency in the mean temperature in some cities of India. Rao (2016) reported that the annual minimum temperature varied from 22.3°C to 24.1°C across the state with an average of

Fig1. Trends in annual mean maximum temperature from 1986 to 2016

Trends in annual mean of average temperature from 1986 to 2016

During the period from 1986-1990, the highest mean of average temperature was recorded in 1987 (28.20°C) while the lowest was recorded in 1986 (27.49°C). A decrease of 0.13°C was found in the above five periods (Fig.3a) but the trend was not significant ($P>0.05$). The highest mean of average temperature was recorded in 1995 (28.12°C) while the lowest was recorded in 1999 (27.50°C) for the period from 1991-2000. A decrease of 0.2°C over ten years was noticed (Fig.3b) but it was not a significant trend ($P>0.05$). The highest mean average temperature was recorded in 2003 (27.89°C) while the lowest was recorded in 2006 (27.45°C) in the ten-year period from 2001-2010. A decrease of 0.04°C was observed during this period (Fig.3c) but no significant trend ($P>0.05$) was observed. The highest mean average temperature was

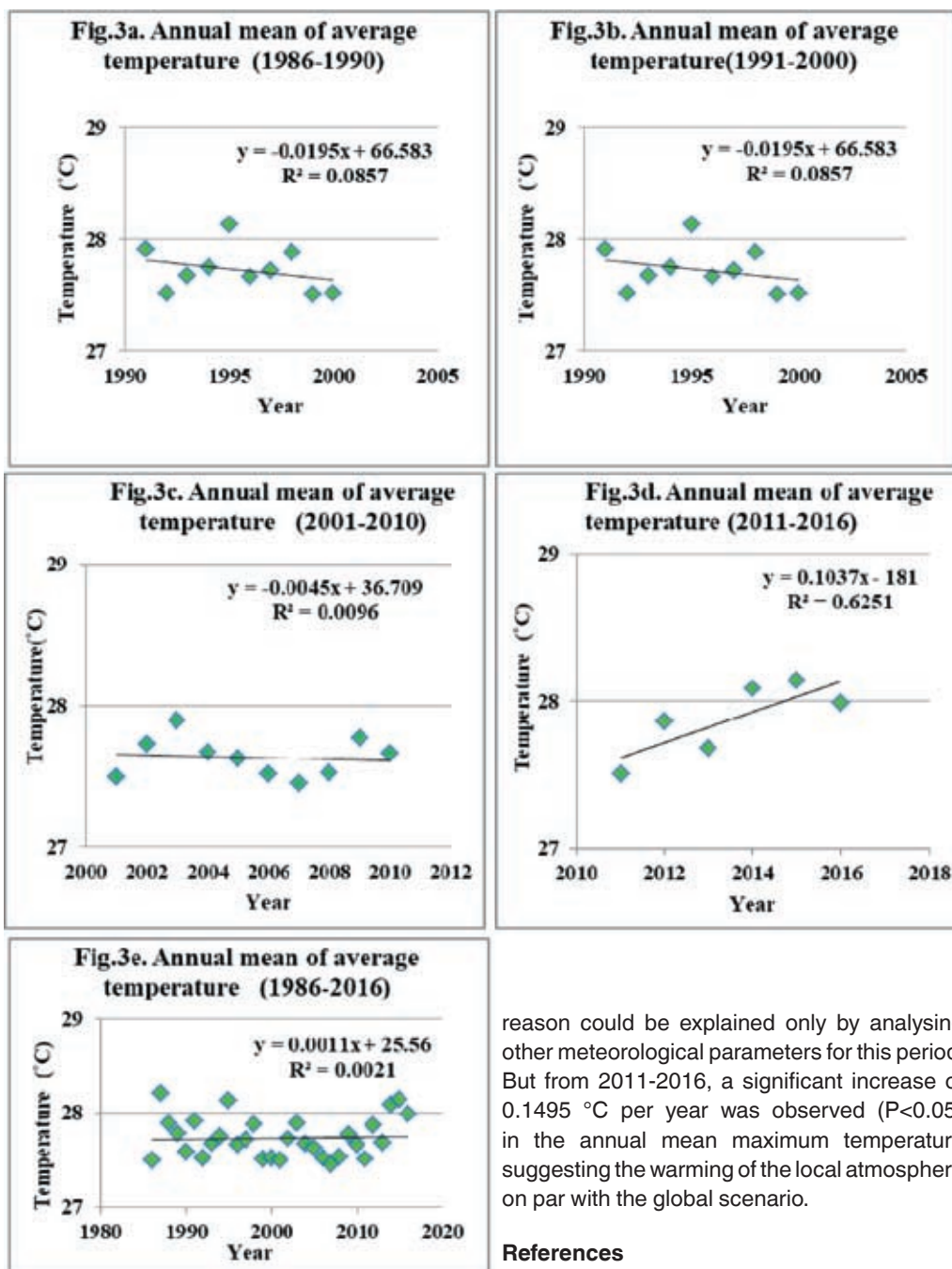
22.1°C. Anie (2018) reported that an increasing trend was shown in the minimum temperature, monthly average temperature and annual average temperature of Thiruvananthapuram, Ernakulam and Kannur districts of Kerala and observed these changes as the signs of climate change and warming nature of Kerala.

Fig.2. Trends in annual mean minimum temperature from 1986 to 2016

recorded in 2015 (28.14°C) while the lowest was recorded in 2011 (27.50°C) in a period of recent 6 years from 2011-2016. There was an increase of 0.62°C over the period of six years (Fig.3d) but it was not a significant trend ($P > 0.05$). When the data of the 30-year period under consideration were analysed, the highest mean average temperature was recorded in

1987 (28.20°C) while the lowest was recorded in 2007 (27.45°C). An overall increase of 0.03°C was observed during the period (Fig.3e) but no a significant trend ($P > 0.05$) was observed.

The Intergovernmental Panel on Climate Change (IPCC, 2001) in its third assessment report estimated an increase of the average global temperature by 0.6°C in last century. In the present study, an increase of 0.62°C was also observed in the mean temperature of the last six years (2011-2016). However, when the data for the 30-year period under consideration was analysed, no significant increase in temperature could be observed. This difference could be due to the local variability in meteorological parameters. Das (2008) reported that in Pune the incidence of slight decrease of maximum, minimum, and mean annual temperature had occurred in the last decades of the twentieth century.

Fig.3. Trends in annual mean of average temperature from 1986 to 2016

reason could be explained only by analysing other meteorological parameters for this period. But from 2011-2016, a significant increase of 0.1495 °C per year was observed ($P < 0.05$) in the annual mean maximum temperature suggesting the warming of the local atmosphere on par with the global scenario.

References

- Anie, S.S., 2018. Long term temperature variability and trend over Kerala, *Int. J. Appl. Sci. Engng. Technol.* **6**(3):164-167.
- Das, H.P., Dhotre, A.K. and Rase, D.M. 2008. Temperature variability and trends over Pune. *Mausam*, **59**: 291-296.

Conclusion

In the present study the annual mean maximum temperature, minimum temperature and average temperature did not show any increase when the data pertaining to the 30-year period under consideration were analysed. The

- IPCC.2001. Climate change 2001: Impacts, adaptation and vulnerability. McCarthy, J.J., Canziani, O.F., Leary, N.A., Dokken, D.J. and White, K.S., (eds.), Cambridge: Cambridge University Press.
- Rao, G.P. 2016. Weather extremes and plantation crops in the humid tropics. *Weather*, **631**(540): 251-258.
- Sahai, A.K. 1998. Climate change: A case study over India. *Theor. Appl. Climatology*, **61**(1): 9-18.
- Webster, P.J., Holland, G.J., Curry, J.A. and Chang, H.R. 2005. Changes in tropical cyclone number, duration, and intensity in a warming environment. *Science*, **309**(5742): 1844-1846. ■



Nutrient evaluation of *Couroupita guianensis* fruits and flowers and effect of feeding *Couroupita guianensis* flowers on growth and haemato - biochemical parameters in Wistar rats



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Abstract

A study was undertaken to access the nutritive value of fruits and flowers of cannon ball tree (*Couroupita guianensis* Aubl.) harvested from different trees at the College of Veterinary and Animal Sciences, Mannuthy, Thrissur. The samples were oven dried at 100°C until constant weight was obtained for dry matter determination. The dried samples were ground and subjected to proximate analysis. Results showed that *Couroupita guianensis* fruit and flowers contain moderate crude protein, nitrogen free extract and total ash. For animal experimentation fifteen healthy Wistar rats of three weeks of age were selected and divided into three groups of five each and allotted randomly to three experimental treatments T_1 (control), T_2 ($T_1 + 1\%$ *Couroupita guianensis* flower (CGF) on dry matter basis), T_3 ($T_1 + 2\%$ CGF on dry matter basis). All the experimental animals were fed with feed containing 24% CP and 3000 kcal/kg energy and were fed as per NRC standard. Feeding trial was conducted for a period of 30 days. The results showed that dry matter intake and body weight of animals were similar among the groups. Haemato-biochemical parameters such as haemoglobin, total protein and plasma glucose were similar among the groups whereas the triglycerides and total cholesterol were significantly lower in CGF supplemented group.

Key words: *Couroupita guianensis* flower, Wistar rats, Growth, Blood

Couroupita guianensis Aubl. (Cannon ball) is a large deciduous tropical tree 20-30 metres in height commonly called "Kailashpati". The leaves, up to 15 cm long, are simple with serrate margin; the flowers are yellow, reddish and pink with a stunning fragrance. The flowers which grow on the trunk are aromatic, large and waxy and look like a Shivalinga and it's pollen is snake shaped. Fruits are large and globose with a woody look like big rusty cannonballs hanging in clusters or like balls on a string. The fruit contains small seeds in a white, unpleasant smelling edible jelly (Kumar *et al.*, 2017). The plant is indigenous to rainforest of the Guianas in North-eastern South America.

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It is a popular ornamental in Caribbean and South East Asian botanical gardens and listed as a rare tree and flower in India.

The components of this tree have many medicinal properties such as antioxidant (Pinheiro *et al.*, 2010), anthelmintic (Regina and Uma Rajan, 2012), anti-inflammatory (Gupta *et al.*, 2012), antiulcer (Elumalai *et al.*, 2012) and antibacterial (Sivakumar *et al.*, 2015). Researches to evaluate the nutritive value of *Couroupita guianensis* fruit and flower and feeding trials as feed additive are very scanty. Hence this research work was done to evaluate the nutritive value of the *Couroupita guianensis* fruit and flower and to study the effect of supplementation of CGF on growth performance in Wistar rats.

Materials and Methods

Fruits and flowers of cannon ball tree were harvested from different trees from the College of Veterinary and Animal Sciences, Mannuthy (geographical coordinates 10.5332° N, 76.2641° E). The samples collected were sliced and oven dried at 100 °C until constant weight was obtained for dry matter determination. The dried samples were grinded and subjected to proximate analysis as per AOAC 2016. The samples then analysed for nutrient content viz. Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE), Total Ash (TA), Acid insoluble ash (AIA) and Nitrogen free extract (NFE).

The experimental animals of this study were fifteen male Wistar rats with good health selected from Small animal breeding station (SABS), Mannuthy. The rats were allotted randomly to three experimental treatments T₁ (control), T₂ (T₁ + 1% *Couroupita guianensis* flower (CGF) on dry matter basis) and T₃ (T₁ + 2% CGF on dry matter basis) uniformly. All the experimental animals were fed with feed containing 24% CP and 3000 kcal/kg energy.

The experimental animals were kept individually in clean and dry cage with good air circulation with amenities for feeding and watering. The feed was given in the morning after weighing the quantity. Potable water was given *ad libitum* to all the animals. Ambient

conditions were maintained in the experimental house. Daily recording of the dry matter intake was performed. Also, the body weights of the rats were taken weekly and based on this data, the feed offered was revised.

Blood samples were collected from all the animals at the end of the experiment to estimate haemoglobin (cyanmetahemoglobin method), total protein (Biuret method), glucose (GOP-PAP methodology), total cholesterol and triglycerides (enzymatic colorimetric methods) using standard kits supplied by Agappe diagnostics Ltd, Ernakulam, India. Feed samples were analysed for proximate principles (AOAC, 2016). Analysis of Variance (Snedecor and Cochran, 1994) method was used for the statistical analysis.

Results and Discussion

Chemical composition of *Couroupita guianensis* fruit and flower (DM basis) is presented in Table 1. *Couroupita guianensis* fruit and flower contain moderate CP, NFE and TA. Shah *et al.*, 2012 observed comparable TA value in the fruit of *Couroupita guianensis*.

Initial body weight of rats in treatments T₁, T₂ and T₃ were 149.60, 148.0 and 150.6 g respectively. Body weight of rats recorded at weekly intervals and final body weight was 208.6, 214.2 and 209.0 g, respectively for T₁, T₂ and T₃ at the end of experiment. There was no significant difference ($P > 0.05$) in body weight between the dietary treatments. Total weight gain and average dry matter intake of animals were similar in all the dietary treatments and presented in Table 3. James *et al.* (2002) and Manjula *et al.* (2016) observed similar total dry matter intake in rats.

Data on haemato-biochemical studies have been given in Table 4. The haemoglobin concentrations at the end of the experiment for group T₁, T₂ and T₃ were 13.72, 13.78 and 14.02 g/dL respectively and were similar in all the groups indicating that dietary incorporation of CGF powder did not affect these parameters to any significant effect. The average plasma protein concentrations at the end of the experiment for group T₁, T₂ and T₃ were 6.31, 6.20 and 6.24 g/dL, respectively. The average

Table 1. Chemical composition of experimental feed % (DM basis)

Parameters	T ₁	T ₂	T ₃
Dry matter (DM)	91.66	91.05	91.25
Crude Protein (CP)	24.04	24.56	24.61
Ether extract (EE)	5.12	5.64	5.81
Crude fibre (CF)	4.99	5.08	5.14
Total Ash (TA)	6.12	6.44	6.72
Nitrogen free extract (NFE)	59.73	58.28	57.72
Acid insoluble ash (AIA)	0.84	0.79	0.82

Table 2. Chemical composition of *Couroupita guianensis* fruit and flower % (DM basis)

Parameters	Fruit	Flower
Dry matter (DM)	14.13	7.82
Crude protein (CP)	7.60	11.23
Ether extract (EE)	9.13	4.06
Crude fibre (CF)	13.58	6.78
Total Ash (TA)	11.43	8.94
Nitrogen free extract (NFE)	58.26	68.99

Table 3: Body weight (g) and dry matter intake (g) of experimental rats

Parameters	T ₁	T ₂	T ₃
Initial body weight (g)	149.60 ± 3.23	148.00 ± 2.92	150.60 ± 3.08
Final body weight (g)	208.60 ± 5.90	214.2 ± 5.24	209.00 ± 6.50
Total weight gain (g)	59.00 ± 8.75	66.20 ± 3.92	58.40 ± 6.14
Total dry matter intake (g/animal)	332.60 ± 9.21	337.60 ± 11.66	322.20 ± 9.64

Table 4. Haemato- biochemical parameters of experimental rats

Parameters	T1	T2	T3
Haemoglobin (g/dL)	13.72 ± 2.62	13.78 ± 2.28	14.02 ± 1.53
Total Protein (g/dL)	6.31 ± 0.05	6.20 ± 0.07	6.24 ± 0.05
Glucose (mg/dL)	88.73 ± 0.36	88.77 ± 0.34	89.38 ± 0.25
Total Cholesterol (mg/dL)	78.42 ± 0.33 ^a	77.01 ± 0.31 ^b	76.23 ± 0.26 ^b
Triglycerides (mg/dL)	44.56 ± 0.28 ^a	41.77 ± 0.36 ^b	40.40 ± 0.31 ^b

plasma glucose values were 88.73, 88.77 and 89.38 mg/dL for group T₁, T₂ and T₃ respectively. The average serum cholesterol and triglyceride values were 78.42, 44.56, 77.01, 41.77 and 76.23, 40.40 mg/dL, respectively for group T₁, T₂ and T₃.

The results revealed that the serum biochemical values are within the normal range reported to the species. There was no significant difference in the values of serum total protein and glucose. In contrary to this Morankar *et al.* (2013) reported that supplementation of the aqueous and methanolic extracts of *Couroupita guianensis* flower (100 mg/kg) significantly

(p<0.05) reduced the blood glucose level in alloxan induced diabetic mice and has antidiabetic activity.

The values of total cholesterol and triglycerides were significantly lower (p < 0.05) in CGF supplemented group compared to the control group. The hypercholesterolaemic effects may be due to the polyphenolics and flavonoids present in the CGF which positively modify lipid profile. In agreement with present results Ramyasai *et al.* (2013) also reported that treatment with methanolic extract of *Couroupita guianensis* Aubl. flowers significantly decreased the total serum cholesterol, triglycerides,

low density lipoproteins, very low-density lipoproteins and increased the high-density lipoproteins in obese rats and was comparable with that of standard Atorvastatin. Hassan *et al.* (2018) also reported that supplementation of extracts of *Couroupita guianensis* leaves to rats at a dose rate of 1.25 g/kg showed significant reduction in the serum cholesterol level.

Conclusion

It can be concluded that *Couroupita guianensis* flower has anti-hyperlipidemic and atherosclerotic properties and that it can be used as a feed additive with anti-atherogenic property without any adverse effect on their growth in Wistar rats. Further research is needed to find the phytochemical profile and to detect the active principles for the beneficial health effects.

References

- AOAC [Association of Official Analytical Chemists]. 2016. *Official Methods of Analysis*. (20th Ed.). Association of Official Analytical Chemists, Washington DC, 1885p.
- Elumalai, A., Naresh, V., Eswaraiyah, M.C., Narendar, R. and Kumar, R. 2012. Evaluation of Antiulcer Activity of *Couroupita guianensis* Aubl. leaves. *Asian J. Pharmaceut.Sci.* **2**: 64-66.
- Gupta, V. H., Gunjal, M. A., Wankhede, S. S. and Deshmukh, V.S. 2012. Neuropharmacological Evaluation of the Methanolic Extract of *Couroupita guianensis* Aubl. Flower in Mice. *Int. J. Pharmaceut. Pharmacol. Res.* **1**: 242-246.
- Hassan, M. M., Islam, M. M., Uddin, S., Bhowmik, A. and Rokeya, B., 2018. Antihyperglycemic potential of ethanolic extract of *Couroupita guianensis* on Streptozocin induced experimental diabetic rat model. *Asian J. Res. Med. Pharm. Sci.* **5**(3): 1-10.
- James, K. A. C., Butts, C. A., Koolaard, J. P., Donaldson, H. E., Scott, M. F. and Moughan, P. J. The effect of food dry matter intake on the flow of amino acids at the terminal ileum for rats fed an enzyme-hydrolysed casein-based diet. *J. Sci. Food. Agric.* **82**: 1128–1135.
- Kumar, V., Tiwari, A. and Ashwin, S. 2017. *Couroupita guianensis* : A potential medicinal tree. *Van Sangyan.* **4**: 30-34.
- Manjula, K. and Krishna, R. 2016. Feed efficiency and sero biochemical profile of Wistar rat fed with spirulina as functional food. *Curr. Res. Nutr. Food. Sci.* **4**: 2754.
- Morankar, P. G., Dhake, A.S., Kumbhare, M. R., Ushir, Y.V., Surana, A. R. and Patil, S. D. 2013. An evaluation of the antidiabetic effects of *Couroupita guianensis* aubl. flowers in experimental animals. *Indo Am. J. Pharm. Res.* **3**: 3114-3122.
- Pinheiro, M. M. G., Bessa, S. O., Fingolo, C. E., Kuster, R. M., Matheus, M. E., Menezes, F. S. and Fernandes, P.S. 2010. Antinociceptive activity of fractions from *Couroupita guianensis* Aubl. leaves. *J. Ethnopharmacol.* **127**: 407-413.
- Ramayasai, K., Manohar babu S., Vadivel, K. 2013. Anti-obesity and atherosclerotic activity of methanolic extract of *Couroupita guianensis* Aubl. flowers in rats fed with high fat diets, *Int. J. Universal Pharm. Bio Sci.* **2**(6): 288-300.
- Regina, V. and Uma Rajan, K.M. 2012. Phytochemical analysis, antioxidant and antimicrobial studies of fruit rind of *Couroupita guianensis* (AUBL). *Int. J. Curr. Sci.* 262-267.
- Shah, G. N., Shete, S. A., Patil, V. S., Patil, K. D., Killedar, S. G. 2012. Standardization and anti-bacterial activity of *Couroupita guianensis* fruit pulp extract. *Int. J. Pharmacogn. Phytochem. Res.* **4**: 185-189.
- Sivakumar, T., Rathimeena, T., Shankar, and Ponmanickam, P. 2015. Production of silver nanoparticles synthesis of *Couroupita guianensis* plant extract against human pathogen and evaluations of antioxidant properties. *Int. J. Life Sci.* **3**: 333-340.
- Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*. (8th Ed.). The Iowa State University press, Ames, 503p.



Molecular characterization of exon region of type 2 diabetes associated gene (*KCNJ11*) in Labrador retriever dogs



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Abstract

The study was undertaken with the objective of characterization of the single exon region of potassium inwardly rectifying channel, subfamily J, member 11 (*KCNJ11*) gene in Labrador retriever breeds of dogs. The gene encodes the islet ATP-sensitive potassium channel Kir6.2 which plays a major role in insulin secretion and is of substantial interest as a candidate gene for type 2 diabetes. The genomic DNA was isolated from six pedigreed Labrador retriever breeds of dogs and a 1173 bp fragment of the coding region of *KCNJ11* gene was amplified by PCR technique using synthetic oligonucleotide primer pairs and the confirmation of PCR product was done by agarose gel electrophoresis. The PCR amplified products were purified and sequenced commercially. The sequenced data was compared with nucleotide sequences available in the data bank using suitable software. The nucleotide sequence showed 99 per cent homology between Labrador and Boxer breeds of dogs with two bases found to be replaced among the two sequences whereas, 100 per cent homology was observed between sequences of Labrador, Basenji and Great Dane dogs. Predicted amino acid sequence and the secondary protein structure revealed no characteristic change between the dog breeds suggestive of the highly conserved nature of the gene studied. The study can be extended in larger dog population to find out any polymorphism in the respective gene studied which might serve as a breed specific marker for screening diabetic dog population in future.

Keywords: Diabetes mellitus, Labrador retriever, *KCNJ11* gene, kir 6.2 subunits, ATP-sensitive potassium channel

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Diabetes mellitus is a common endocrinopathy in companion animals, characterized by hyperglycemia and glycosuria, resulting from an absolute or relative deficiency or dysfunction of the pancreatic hormone insulin. It forms a major global public health problem in humans, and is also of great concern in dogs currently. Canine diabetes is a heterogeneous disease and, in majority of the cases, the underlying pathogenesis of the disease is not well established, although insulin deficiency is a consistent feature. The presence of certain diabetic phenotypes, along with specific breed predisposition to these different types of diabetes mellitus, suggests an underlying genetic basis for the disease susceptibility. This might vary from breed to breed with some breeds exhibiting greater susceptibility (Labrador retrievers, Dachshund etc.) while a few showing relative resistance (Boxer). Even though, canine diabetes mostly resembles type 1 diabetes, the involvement of type 2 diabetes is also reported particularly when the animals are overweight or when they are in dioestrous stage (Fall *et al.*, 2007).

Many genes like *KCNJ11*, *TCF7L2*, *GCK* and *SLC30A8* are associated with type 2 diabetes susceptibility but have received little attention to date with respect to canine diabetes (Catchpole *et al.*, 2013). Among the different type 2 diabetes linked genes, *KCNJ11* gene encodes the ATP-sensitive potassium (KATP) channel subunit Kir6.2, which play a major role in insulin secretion and is of substantial interest as a candidate gene for type 2 diabetes (Ashcroft, 2005). Mutations in *KCNJ11* gene is associated with increased activity of the β cell ATP-sensitive potassium channel, which account for the failure of glycaemic control during periods of hormone-induced peripheral insulin resistance, particularly when animals are overweight (Bonfanti *et al.*, 2015). But the information regarding the involvement of this particular gene in the occurrence of canine diabetes is meager. Therefore, the study was undertaken with the objective of molecular characterization of single coding region of *KCNJ11* gene in Labrador retriever breed of dogs, a breed more susceptible to type 2 diabetes; using PCR technique and its comparison with the nucleotide sequences

available in the data bank. The present study also aims to analyze the predicted amino acid sequence and secondary protein structure of the exon region of the candidate gene with suitable online sequence analysis tools.

Materials and Methods

Blood samples were collected from pedigree dogs of Labrador retriever breed. Genomic DNA was isolated as per the standard protocols (Sambrook and Russel, 2001).

The primers targeting the 1173 bp of the *KCNJ11* gene of Labrador breed of dogs were designed using the Primer 3 software (www.bioinfo.ut.ee/primer3-0.4.0). The sequence of the *KCNJ11* gene of Boxer breed of dogs available in the ensembl (Accession No. ENSCAFT00000013869) was utilized for designing primers. The suitability was checked with sequence manipulation suite software (www.bioinformatics.org/sms2) and specificity confirmed by blast analysis (BLASTn tool: www.ncbi.nlm.nih.gov).

The primer sequences used to amplify the coding region of *KCNJ11* gene were

Forward (F1) – 5' TGCTGTCCCGAAAAGGCAT 3'

Reverse (R1) – 5' AGGGAATCCGGAGAGATGCT 3'

PCR conditions were standardized to minimize nonspecific amplification and to get maximum amplification of the desired product. Annealing temperature was standardized by performing gradient PCR. The optimum concentrations of various components obtained after standardization is shown below (Table 1.):

Table 1. Composition of PCR mix

Components	Volume
10X buffer with MgCl ₂ (15mM)	2.5μL
dNTP (2Mm each)	3.0 μL
Forward primer (20 pM/μl)	0.5μL
Reverse primer (20 pM/μl)	0.5μL
Accu Taq DNA polymerase (5 U/μl)	0.5μL
Template DNA (255.0 ng/μL)	2.0μL
Nuclease free water	16.0μL
Total volume	25μL

The reaction was carried out in a Bio-Rad T100 Thermal Cycler using the following cycle parameters which was found to be optimal for the amplification of expected size of 1173 bp shown in figure 1. A standard PCR protocol was used for amplifications, which consisted of an initial denaturation 95°C for 10 min, 34 cycles for denaturation at 95°C for 1 min, annealing at 55.5°C for 40 sec, extension at 72°C for 1 min and final extension at 72°C for 10 min. The amplified products were electrophoresed at 70 V for 40 min in 0.8 % agarose gel and visualized under UV light in a gel documentation system (Chemi doc MP). PCR products were purified and sequenced by Sanger's enzymatic DNA sequencing technique. The complete single coding sequence of *KCNJ11* gene was assembled by joining the corresponding forward and reverse sequence fragments.

Various online and offline sequence analysis software and data bases were used to analyze the sequence obtained. The sequences were aligned using EMBOSS (www.bioinformatics.nl/cgi-bin/merger) and blasted using NCBI BLAST tool (www.blast.ncbi.nlm.nih.gov/bblast) to analyze their similarity with other published sequences available in online databases. The obtained sequence were compared with the corresponding nucleotide sequence of different dog breeds available in the

data bank by the multiple sequence alignment programme of ClustalW available at <http://www2.ebi.ac.uk/clustalw/>. The aligned nucleotide sequences were subjected to phylogenetic analysis using the neighbor joining method of Clustal W to derive the ancestral relationship between different breeds. The translate tool of the sequence manipulation suite available at <http://www.bioinformatics.org/sms2/translate.html>, was used to translate the nucleotide sequence with a proper reading frame to the corresponding amino acid sequence. The secondary structure of the region containing the coding region was predicted using the Scratch protein predictor system (http://molbioltools.ca/Protein_secondary_structure.html) of the institute for Genomics and Bioinformatics, University of California, Irvine, U.S.A. The amino acid sequences in one letter code were submitted for the structure prediction.

Results and Discussion

The *KCNJ11* gene encodes Kir6.2 which is the pore forming subunit of the β -cell ATP-dependent potassium (KATP) channel which has attracted considerable attention as a promising candidate for type 2 diabetes because of its function as a key factor in the regulation of glucose-induced insulin secretion (Gloyn *et al.*, 2004).

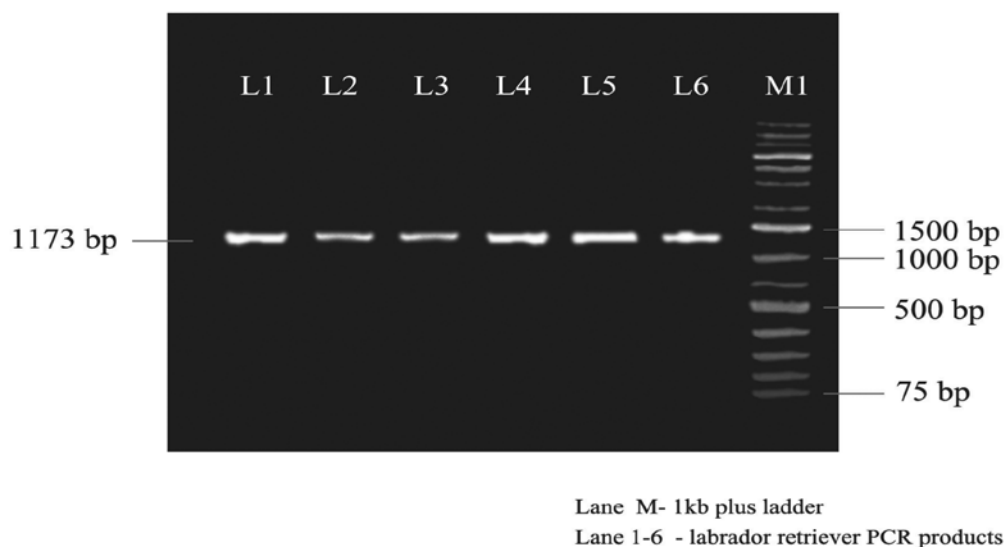


Plate 1. PCR amplification of exon region of *KCNJ11* gene of labrador retriever

Gr.Dane	GCCCCGGTGAGGGCACTGCGGTGCCCCGTGTGTACCAGCATCCACTCTTTTTCATC	360
Basenji	GCCCCGGTGAGGGCACTGCGGTGCCCCGTGTGTACCAGCATCCACTCTTTTTCATC	360
Boxer	GCCCCGGTGAGGGCACTGCGGTGCCCCGTGTGTACCAGCATCCACTCTTTTTCATC	132
Labrador	GCCCCGGTGAGGGCACTGCGGTGCCCCGTGTGTACCAGCATCCACTCTTTTTCATC	79

Gr.Dane	TTCCTTTTCTCCATTGAGGTCCAGGTGACCAATTGGCTTTGGCGGGCGCATGGTGACCGAG	420
Basenji	TTCCTTTTCTCCATTGAGGTCCAGGTGACCAATTGGCTTTGGCGGGCGCATGGTGACCGAG	420
Boxer	TTCCTTTTCTCCATTGAGGTCCAGGTGACCAATTGGCTTTGGCGGGCGCATGGTGACCGAG	192
Labrador	TTCCTTTTCTCCATTGAGGTCCAGGTGACCAATTGGCTTTGGCGGGCGCATGGTGACCGAG	139

Gr.Dane	GAGTGGCCGCTGGCCATCTTGATCCTCATTGTGCAGAACATCGTGGGGCTCATGATCAAT	480
Basenji	GAGTGGCCGCTGGCCATCTTGATCCTCATTGTGCAGAACATCGTGGGGCTCATGATCAAT	480
Boxer	GAGTGGCCGCTGGCCATCTTGATCCTCATTGTGCAGAACATCGTGGGGCTCATGATCAAT	252
Labrador	GAGTGGCCGCTGGCCATCTTGATCCTCATTGTGCAGAACATCGTGGGGCTCATGATCAAT	199

Gr.Dane	GCCATTATGCTGGGCTGCATCTTCATGAAGACGGCCAGGCCCATCGGCGGGCCGAGACC	540
Basenji	GCCATTATGCTGGGCTGCATCTTCATGAAGACGGCCAGGCCCATCGGCGGGCCGAGACC	540
Boxer	GCCATTATGCTGGGCTGCATCTTCATGAAGACGGCCAGGCCCATCGGCGGGCCGAGACC	312
Labrador	GCCATTATGCTGGGCTGCATCTTCATGAAGACGGCCAGGCCCATCGGCGGGCCGAGACC	259

Figure 1: Multiple sequence analysis of exon region of *KCNJ11* gene

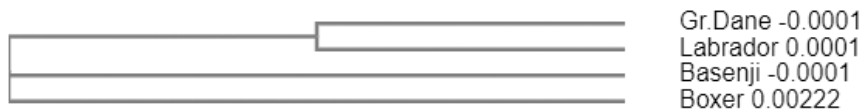


Figure 2. Phylogenetic analysis of nucleotide sequence of exon region of *KCNJ11* gene

Predicted amino acid sequence	SPTVTWAPGEGTAVPCVTSIHSFSSAFLFSIEVQVTIGFGGRMVTE ECPLAILILIVQNIVGLMINAIMLGICIFMKTAQAHRRATLIFS KHAVIAVRHGRCLFMLRVGDLRKSMIISATIHMQVVRKTTSP EGEVVPLHQVDIPMENGVGNSIFLVAPLIHYHVIDANSPLYDL APSDLHHHQDLEIIVILEGVVETTGITTQARTSYLADEILWGQ RFVPIVAEEDGRYSVDYSKFGNTIKVPTPLCTARQLDEDRSLD
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Figure.3. Predicted amino acid sequence of exon region of *KCNJ 11* gene

The *KCNJ11* gene has been shown to be highly polymorphic and is associated with permanent and neonatal diabetes (Vaxillaire *et al*, 2004).

The present study has been undertaken to analyze the *KCNJ11* gene in Labrador retriever breed of dogs which are more prone to diabetes.

The single coding region of *KCNJ11* gene was amplified using gradient thermal cyclers with an optimum annealing at 55.5°C. The size of the amplified products was confirmed by agarose gel electrophoresis (Thermo Scientific GeneRuler 1 kb plus DNA Ladder). Expected size of coding region of *KCNJ11* gene was 1173 bp and the sequencing result revealed a size of 793 bp. The amplicons obtained had a size of 1173 bp as shown in plate 1. All the amplicons were purified and sequenced commercially at

AgriGenome Labs Private Limited, Kakkannadu, Ernakulam using Sanger's dideoxy nucleotide chain termination method.

The sequenced data was compared with the sequence available in the data bank using suitable software like multiple sequence alignment programme and phylogenetic analysis of Clustal W to find regions of local similarity between sequences. Translate tool of the sequence manipulation suite and Scratch protein predictor system were used to study the deduced amino acid sequences.

Multiple Sequence Analysis

To study sequence similarities, multiple sequence analysis was carried out between the sequences of Labrador retriever with nucleotide sequences of different dog breeds available in the data bank (Boxer-Access

on # ENSCAFT00000013869, Basenji Access on # ENSCAFG00030024886 and Great Dane Access on # ENSCAFG00040022090) downloaded from ensembl database. Nucleotide sequences of exon region of *KCNJ11* gene exhibited 100 per cent identity between Labrador retriever, Basenji and Great Dane breeds of dogs whereas, only 99 % identity was showed for boxer breed. Thymine (T) was replaced by Cytosine(C) in Boxer at two different positions when compared to that for Labrador, Basenji and Great Dane (Fig.1)

Phylogenetic Analysis

Phylogenetic analysis of the nucleotide sequence of exon region of *KCNJ11* gene in different dog breeds revealed clustering of Labrador and Great Dane breeds with Basenji and Boxer breed delineated themselves from other breeds by clustering out on a separate single branch from the main. Phylogenetic analysis of different dog breeds is shown in figure 2.

Amino Acid Sequence Analysis

Even though, variations were noticed in the nucleotide sequence of exon region of *KCNJ11* gene between Boxer breed and other three dog breeds including that for Labrador retriever breed of present study, the deduced amino acid sequences couldn't reveal any appreciable variation (Fig.3). The results suggest that the nucleotide sequence of *KCNJ11* gene is highly conserved within a species (Sagen *et al.*, 2004). Thus the present study, point out the significant role of the particular gene in regulation of insulin secretion from pancreatic β cells thereby controlling blood glucose level. Differences in the breed prevalence of diabetes mellitus suggest that there are genetic factors that are involved in determining susceptibility to the disease (Andrea *et al.* 2007).

Some specific types of diabetes mellitus also seem to be breed related (Catchpole *et al.*, 2013), there might be differences in the specific susceptibility genes that contribute to the overall genetic risk. As there are no previous reports on the molecular evidence of the role of *KCNJ11* gene in canine diabetes, the study on nucleotide and amino

acid sequence analysis would throw light on canine *KCNJ11* gene architecture and the extent of functional and structural similarity with that of other dog breeds.

Conclusion

As type 2 diabetes is a multifactorial disorder where obesity plays a key role in its occurrence, the analysis of type 2 diabetes associated genes like *KCNJ11* will pave the way in understanding this particular diabetic form. Considering the highly conserved nature of the *KCNJ11* gene, polymorphism studies on this gene will shed light in screening the diabetic dog population in future. This would help to bridge the knowledge gap between the underlying pathophysiology and genetic basis of the disease and will help in development of interventional therapies.

References

- Andrea, D. S., Catchpole, B., Kennedy, L. J., Barnes, A., Fretwell, N., Jones, C., Thomson, W. and Ollier, W.E.R. 2007. Analysis of Candidate Susceptibility Genes in Canine Diabetes. *J. Hered.* **98**(5): 518-525.
- Ashcroft, F. M., 2005. ATP-sensitive potassium channelopathies: focus on insulin Secretion. *J. Clin. Invest.* **115**(8): 2047-2058.
- Bonfanti, D. H., Alcazar, L. P., Arakaki, P. A., Martins, L. T., Agustin, B. C., DeMoraes Rego, F. G. and Friger, H. R. 2015. ATP-dependent potassium channels and type 2 diabetes mellitus. *Clin. Biochem.* **48**(7): 476-482.
- Catchpole, B., Adams, J. P., Holder, A. L., Short, A. D., Ollier, W. E. and Kennedy, L. J. 2013. Genetics of canine diabetes mellitus: are the diabetes susceptibility genes identified in humans involved in breed susceptibility to diabetes mellitus in dogs? *Vet. J.* **195**(2): 139-147.
- Fall, T., Hamlin, H.H., Hedhammar, A., Kämpe, O., Egenvall, A., 2007. Diabetes mellitus in a population of 180,000 insured

- dogs: Incidence, survival, and breed distribution. *J. Vet. Intern. Med.* **21**: 1209–1216.
- Gloyn, A. L., Pearson, E. R., Antcliff, J. F., Proks, P., Bruining, G. J., Slingerland, A.S., Howard, N., Srinivasan, S., Silva, J.M., Molnes, J. and Edghill, E.L., 2004. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6. 2 and permanent neonatal diabetes. *N. Engl. J. Med.* **350**(18): 1838-1849.
- Sagen, J.V., Reder, H., Hathout, E., Shehadeh, N., Gudmundsson, K., Bævre, H., Abuelo, D., Phornphutkul, C., Molnes, J., Bell, G. I. and Gloyn, A. L. 2004. Permanent neonatal diabetes due to mutations in KCNJ11 encoding Kir 6. 2. *Diabetes.* **53**(10): 2713-2718.
- Sambrook, J. and Russel, D.W. 2001. *Molecular Cloning: A Laboratory Manual*. Third edition. Cold Spring Harbor Laboratory Press, New York. 1886p.
- Vaxillaire, M., Populaire, C., Busiah, K., Cavé, H., Gloyn, A.L., Hattersley, A.T., Czernichow, P., Froguel, P. and Polak, M. 2004. Kir6. 2 mutations are a common cause of permanent neonatal diabetes in a large cohort of French patients. *Diabetes.* **53**(10): 2719-2722. ■



Haemato-biochemical studies of *Theileria orientalis* infection in cross bred dairy cattle



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Abstract

The present work has been carried out to study the haemato-biochemical profiles of cattle infected with oriental theileriosis. Theileriosis was diagnosed in 30 cross bred cattle by blood smear examination and confirmed by PCR. Whole blood samples were collected from positive animals and were subjected to estimation of haemato-biochemical parameters. Haematological analysis revealed significant decrease in total erythrocyte count (TEC), haemoglobin, volume of packed red cells (VPRC) and granulocyte count, significant increase in total leucocyte count (TLC), lymphocyte count, monocyte count and granulocyte count in *T. orientalis* infected animals. Non-significant changes were noticed in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Biochemical analysis revealed significant decrease in albumin concentration and significant increase in blood urea nitrogen level in *T. orientalis* infected animals. The knowledge on alterations in haemato-biochemical profiles of affected animals will help to assess the severity of infection and to make a tentative diagnosis of the condition.

Key words: Anaemia, Cattle, Theileriosis

Theileria are protozoan parasites that belong to phylum Apicomplexa and infect both domestic and wild animals. Seven different *Theileria* spp. are known to infect cattle among them *Theileria parva* (causative agent of East coast fever) and *T. annulata* (responsible for

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Tropical theileriosis) are considered as most pathogenic species due to their ability to transform host lymphocytes. For many years *Theileria orientalis*, causative agent of oriental theileriosis was considered as benign parasite and caused mild anaemia in infected animals (Kamau *et al.*, 2011). Recently outbreaks of oriental theileriosis were reported from various parts of the world leading to significant production losses, due to high morbidity and death in severe cases (Ota *et al.*, 2009; Aparna *et al.*, 2011, McFadden *et al.*, 2011; Perera *et al.*, 2014; Kim *et al.*, 2017). *Theileria orientalis* exerts its main effects during piroplasm stage by causing destruction of erythrocytes leading to anaemia in infected animals. Major clinical signs associated with these organisms include fever, inappetence, lethargy, pale mucous membrane, decreased milk production and abortions in pregnant animals (Eamens *et al.*, 2013). The exact pathogenesis of anaemia resulting from *T. orientalis* infection was not clearly understood.

The aim of the present study is to determine the alterations in haematological and biochemical parameters in *T. orientalis* infected cross bred cattle.

Materials and methods

Cross bred dairy cattle presented with clinical signs suggestive of theileriosis such as inappetence, lethargy, pale mucous membrane, fever and decreased milk production were included in the study. The infected animals were presented to local veterinary hospitals for treatment.

The animals were screened for theileriosis by microscopic examination of Giemsa stained blood smears. The positive cases were confirmed by species specific PCR as per Tanaka *et al.* (1993) with slight modification. Thirty animals which were positive for *T. orientalis* were selected for further study. Six millilitres of blood was collected aseptically from jugular vein from infected animals and control group and 2 ml was transferred to EDTA-coated plastic vials and 4 ml was added into the clot activator vials. The coagulated blood samples were centrifuged at 4000 rpm for 15

min and supernatant (serum) was collected for biochemical estimations.

Haemato-biochemical analysis

The whole blood samples were analysed for haematological parameters including total erythrocyte count, haemoglobin, volume of packed red cells, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), thrombocyte count, total leukocyte count (TLC) and differential leukocyte count by automatic haematological analyser (Orphee Mythic Vet 18). The serum total protein, albumin, aspartate aminotransferase (AST), creatinine and blood urea nitrogen (BUN) were estimated by semi auto analyser (Erba Chem 5 plus V2, India) using commercial kits (Erba Mannheim, India).

Statistical analysis

The IBM-SPSS software version 24 was used to analyse the data. The differences of mean values of haematological and biochemical parameters were compared by independent sample t-test.

Results and Discussion

Examination of Giemsa stained blood smear revealed presence of *Theileria* piroplasms in all the 30 cattle selected for the study. The piroplasm appeared as rod shaped with trailing cytoplasm (Fig. 1).

The DNA extracted from all the infected cattle was subjected to species specific PCR for molecular identification. The species specific PCR with MPSP-F and MPSP-R primer pair yielded 875bp specific for *T. orientalis*.

Haematological findings

The haematological values of *T. orientalis* infected animals and control group were shown in Table 1. *Theileria orientalis* infected animals had significantly decreased TEC, haemoglobin and VPRC compared to control group. These findings were in agreement with Sivakumar *et al.* (2017). The decrease in TEC, haemoglobin and VPRC values

Table 1. Haematological parameters in control group and *T. orientalis* infected cattle

Haematological parameter	Control group (n=6)	<i>T. orientalis</i> infected cattle (n=30)	p-value
Total Erythrocyte count ($\times 10^6/\mu\text{L}$)	8.22 \pm 0.64	4.06 \pm 0.19	0.00**
Haemoglobin (g/dl)	10.85 \pm 1.13	6.15 \pm 0.26	0.00**
VPRC (per cent)	35.88 \pm 3.99	19.30 \pm 0.82	0.00**
MCV (fl)	43.88 \pm 3.39	48.63 \pm 1.39	0.17 ^{ns}
MCH (pg)	13.22 \pm 0.92	15.48 \pm 0.53	0.07 ^{ns}
MCHC (per cent)	30.33 \pm 0.51	31.98 \pm 0.68	0.06 ^{ns}
Platelet count ($\times 10^3/\mu\text{L}$)	280.17 \pm 42.63	237.67 \pm 25.54	0.47 ^{ns}
Total leucocyte count ($\times 10^3/\mu\text{L}$)	9.93 \pm 0.98	14.41 \pm 1.28	0.01**
Lymphocytes ($\times 10^3/\mu\text{L}$)	2.95 \pm .54	10.00 \pm 1.21	0.01**
Monocytes ($\times 10^3/\mu\text{L}$)	0.82 \pm 0.87	0.53 \pm 0.04	0.001**
Granulocytes ($\times 10^3/\mu\text{L}$)	6.17 \pm 0.14	3.89 \pm 0.42	0.05*

** Highly significant ($p < 0.01$), * significant at ($p < 0.05$) and ^{ns}: Non-Significant

Table 2. Comparison of biochemical parameters between control animals and *T. orientalis* infected cattle

Biochemical parameter	Control animals (n=6)	<i>T. orientalis</i> infected cattle (n=30)	p-value
Total Proteins (g/dL)	7.91 \pm 0.34	7.25 \pm 0.29	0.33 ^{ns}
Albumin (g/dL)	3.19 \pm 0.32	2.19 \pm 0.08	0.00**
Globulin (g/dL)	4.72 \pm 0.45	5.05 \pm 0.25	0.59 ^{ns}
Albumin: globulin ratio	0.73 \pm 0.13	0.46 \pm 0.02	0.09 ^{ns}
Aspartate aminotransferase (U/L)	62.23 \pm 3.54	60.75 \pm 3.19	0.84 ^{ns}
Creatinine (mg/dL)	1.38 \pm 0.06	1.24 \pm 0.07	0.37 ^{ns}
Blood urea nitrogen (mg/dL)	13.72 \pm 2.48	21.89 \pm 1.46	0.02*

** Highly significant ($p < 0.01$), * Significant at ($p < 0.05$) and ^{ns}: Non-Significant

resulted due to development of anaemia in *T. orientalis* infected animals. The pathogenesis of anaemia in *T. orientalis* infected animals was not clearly established and it may be multifaceted. Increased osmotic fragility of erythrocytes leading to abnormal morphology (Yagi *et al.*, 1989), reduced survival rate of both infected and uninfected erythrocytes (Yagi *et al.*, 1991) and oxidative damage and lipid peroxidation of erythrocytes (Yagi *et al.*, 2002) were considered to play important role in the development of anaemia in *T. orientalis* infected animals. Shiono *et al.* (2003) reported increased methaemoglobin concentration leading to production of free radicals and resulting in oxidative damage to RBC membranes in *T. orientalis* infected animals.

The infected animals had increase

in MCV, MCH and MCHC values compared to control group, but the increase was not statistically significant. Kim *et al.* (2017) and Jackson (2018) reported significantly increased MCV, MCH and MCHC values in *T. orientalis* infected animals. This might be due to host specific responses to haemolysis that determine the development of anaemia in infected animals (Sivakumar *et al.*, 2017). No significant difference was observed in the platelet values between infected animals and control group, indicating that thrombocytopenia was not a feature of *T. orientalis* infection in cattle (Lawrence *et al.*, 2018).

A significant increase in TLC, lymphocyte count and significant decrease in monocyte count and granulocyte count was observed in *T. orientalis* infected animals

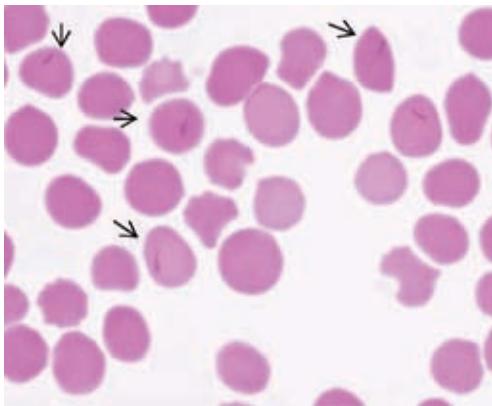
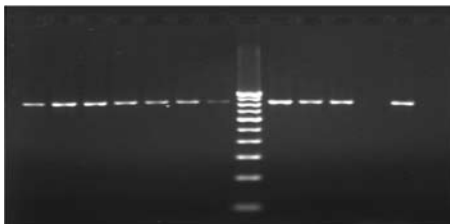


Fig. 1. Giemsa stained blood smear showing rod shaped *T. orientalis* piroplasm

1 2 3 4 5 6 7 8 9 10 11 12



875bp

Lane M: 100 bp ladder

Lane 1 to 10 samples positive for *T. orientalis*

Lane 11: Negative control ; Lane 12: Positive control

Fig. 2. Agarose gel electrophoresis of PCR amplified products of *T. orientalis*

compared to control group. Marked leucocytosis was observed in *T. orientalis* infected animals this was in agreement with Kim *et al.* (2017). Shimizu *et al.* (1990) reported that *T. sergenti* piroplasms contain an adjuvant substance that activates the bovine lymphoid or myeloid system leading to changes in peripheral lymphocyte and leucocyte count during infection.

The morphological changes observed in erythrocytes of *T. orientalis* infected cattle include anisocytosis, basophilic stippling, poikilocytosis. The haematological changes revealed macrocytic and normochromic anaemia. Macrocytosis, polychromacia and basophilic stippling indicated regenerative response in *T. orientalis* infected animals which was consistent with that of haemolytic diseases.

Biochemical findings

The mean serum biochemical values of *T. orientalis* infected animals and control group were shown in Table 2. Hypoproteinaemia was observed in *T. orientalis* infected animals but it was not statistically significant when compared with control group. The finding of significant decrease in mean albumin concentration in *T. orientalis* infected animals was in agreement with Stockham *et al.* (2000). Hypoalbuminaemia might be due to impairment in synthesis, extensive protein degradation and increased albumin excretion due to renal damage, in infected animals. Hypoproteinaemia and hypoalbuminaemia might be due to extravascular accumulation of proteinaceous fluid in body cavities (Stockham *et al.*, 2000).

Theileria orientalis infected animals had increased mean globulins compared to control group. This might be due to immune response to the *T. orientalis* infection as suggested by Izzo *et al.* (2010).

In the present study, BUN concentration but not creatinine was significantly increased in *T. orientalis* infected animals. Similar findings were reported in *T. annulata* infected animals by Dede *et al.* (2014), which they suggested might be due to increased turnover of proteins and renal damage associated with increased catabolism of haemoglobin in infected animals.

There was no significant difference in the mean AST activity, as expected to raise in response to extravascular haemolysis in *T. orientalis* infected animals, which was in agreement with Lawrence *et al.*, (2018). No significant difference was observed in the mean serum creatinine, total proteins, globulins and aspartate aminotransferase (AST) activity between infected animals and control group.

Ethical approval and consent statement

Oral consent was taken from the owner of the animals before drawing blood from animals. There is no specific law in India that requires permission from the ethics committee for collecting less than 5 ml of blood and further

blood samples were collected from the infected animals presented to veterinary hospitals as a part of clinical diagnosis by qualified veterinarians.

Acknowledgement

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References

- Aparna, M., Ravindran, R., Vimalkumar, M.B., Lakshmanan, B., Rameshkumar, P., Kumar, K.A., Promod, K., Ajithkumar, S., Ravishankar, C., Devada, K. and Subramanian, H. 2011. Molecular characterization of *Theileria orientalis* causing fatal infection in crossbred adult bovines of South India. *Parasitol. Int.* **60**: 524-529.
- Dede, S., Altug, N., Deger, Y., Ozdal, N. and Ceylan, E. 2014. Serum biochemical profile and protein fractions in cattle with Theileriosis. *Revue. Med. Vet.* **165**: 137-143.
- Eamens, G.J., Gonsalves, J.R., Jenkins, C., Collins, D. and Bailey, G. 2013. *Theileria orientalis* MPSP types in Australian cattle herds associated with outbreaks of clinical disease and their association with clinical pathology findings. *Vet. Parasitol.* **191**: 209-217.
- Izzo, M.M., Poe, I., Horadagoda, N., De Vos, A.J. and House, J.K. 2010. Haemolytic anaemia in cattle in NSW associated with *Theileria* infections. *Aus. Vet. J.* **88**: 45-51.
- Jackson, B. 2018. Clinico-therapeutic studies on bovine theileriosis. *M.V.Sc thesis*, Kerala Veterinary and Animal Sciences University, Pookode, 123p.
- Kamau, J., de Vos, A.J., Playford, M., Salim, B., Kinyanjui, P. and Sugimoto, C. 2011. Emergence of new types of *Theileria orientalis* in Australian cattle and possible cause of theileriosis outbreaks. *Parasit. Vectors* **4**: 22-31.
- Kim, S., Yu, D.H., Chae, J.B., Choi, K.S., Kim, H.C., Park, B.K., Chae, J.S. and Park, J. 2017. Pathogenic genotype of major piroplasm surface protein associated with anemia in *Theileria orientalis* infection in cattle. *Acta. Vet. Scand.* **59**: 1-5.
- Lawrence, K.E., Forsyth, S.F., Vaatstra, B.L., McFadden, A.M.J., Pulford, D.J., Govindaraju, K. and Pomroy, W.E. 2018. Clinical haematology and biochemistry profiles of cattle naturally infected with *Theileria orientalis* Ikeda type in New Zealand. *N.Z. Vet. J.* **66**: 21-29.
- McFadden, A.M.J., Rawdon, T.G., Meyer, J., Makin, J., Morley, C.M., Clough, R.R., Tham, K., Müllner, P. and Geysen, D. 2011. An outbreak of haemolytic anaemia associated with infection of *Theileria orientalis* in naive cattle. *N.Z. Vet. J.* **59**: 79-85.
- Ota, N., Mizuno, D., Kuboki, N., Igarashi, I., Nakamura, Y., Yamashina, H., Hanzaike, T., Fujii, K., Onoe, S., Hata, H. and Kondo, S. 2009. Epidemiological survey of *Theileria orientalis* infection in grazing cattle in the eastern part of Hokkaido, Japan. *J. Vet. Med. Sci.* **71**: 937-944.
- Perera, P.K., Gasser, R.B., Firestone, S.M., Anderson, G.A., Malmo, J., Davis, G., Beggs, D.S. and Jabbar, A. 2014. Oriental theileriosis in dairy cows causes a significant milk production loss. *Parasit. Vectors* **7**: 73-81.
- Shimizu, S., Yagi, Y., Nakamura, Y., Shimura, K., Fujisaki, K., Onodera, T., Minami, T. and Ito, S. 1990. Clinico-hematological observation of calves experimentally infected with *Theileria sergenti*. *Nippon Juigaki Zasshi* **52**: 1337-1339.
- Shiono, H., Yagi, Y., Chikayama, Y., Miyazaki, S. and Nakamura, I. 2003. Oxidative damage and phosphatidylserine expression of red blood cells in cattle experimentally infected with *Theileria sergenti*. *Parasitol. Res.* **89**: 228-234.

- Sivakumar, T., Ikehara, Y., Igarashi, I., Inokuma, H. and Yokoyama, N. 2017. Dynamics of erythrocyte indices in relation to anemia development in *Theileria orientalis*-infected cattle. *J. Protozool. Res.* **27**: 23-33.
- Stockham, S.L., Kjemtrup, A.M., Conrad, P.A., Schmidt, D.A., Scott, M.A., Robinson, T.W., Tyler, J.W., Johnson, G.C., Carson, C.A. and Cuddihee, P. 2000. Theileriosis in a Missouri beef herd caused by *Theileria buffeli*: case report, herd investigation, ultrastructure, phylogenetic analysis, and experimental transmission. *Vet. Pathol.* **37**: 11-21.
- Tanaka, M., Onoe, S., Matsuba, T., Katayama, S., Yamanaka, M., Yonemichi, H., Hiramatsu, K., Baek, B.K., Sugimoto, C. and Onuma, M. 1993. Detection of *Theileria sergenti* infection in cattle by polymerase chain reaction amplification of parasite-specific DNA. *J. Clin. Microbiol.* **31**: 2565-2569.
- Yagi, Y., Furuuchi, S., Takahashi, H. and Koyama, H. 1989. Abnormality of osmotic fragility and morphological disorder of bovine erythrocytes infected with *Theileria sergenti*. *Jpn. J. Vet. Sci.* **51**: 389-395.
- Yagi, Y., Ito, N. and Kunugiyama, I. 1991. Decrease in erythrocyte survival in *Theileria sergenti*-infected calves determined by non-radioactive chromium labelling method. *J. Vet. Med. Sci.* **53**: 391-394.
- Yagi, Y., Thongnoon, P., Shiono, H. and Chikayama, Y. 2002. Increase in oxidized proteins in *Theileria sergenti*-infected erythrocyte membrane. *J. Vet. Med. Sci.* **64**: 623-625. ■



Uniqueness of seasons in Kerala – Implications on thermal stress and productivity of animals



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Abstract

Pattern of seasons in Kerala, with predominantly hot humid climate, has been found to be hostile for the enhancement of animal productivity. In the context of climate change, alterations and inconsistencies of the seasons has been reported worldwide. Hence, weather parameters of Kerala were studied over a period of six years with emphasis on the seasonality and causation of thermal stress in animals. The study was carried out at Livestock Research Station, Thiruvazhamkunnu. Ambient temperature (AbT) and humidity of six years were collected from Automatic weather stations (AWS) and Temperature Humidity Index (THI) was calculated using formula. The weather parameters were analysed for monthly, seasonal and yearly pattern and the findings are discussed. It is evident that summer season in Kerala (March to May) does not correspond to the entire long day period. Even though AbT and THI were increasing from January onwards with increasing day length, onset of raining in June causes sudden drop in AbT responsible for the peculiar Kerala climate. Climatic parameters in September were more similar to October-November (North-east monsoon) than June to August (South-west monsoon). Even though December to February (post monsoon) forms winter season in rest of the country, the lowest temperature recorded in the study was 18.3 °C, making the term “winter” a misfit except for high ranges. Thus it appears that four quarters of three months each, designated as South-west monsoon, North-east monsoon, Post monsoon and Summer forms better classification of seasons in Kerala and THI was high enough to cause thermal stress throughout the year.

Keywords: Season; Kerala; Climate; THI; Stress; Weather

Climatic factors have been found to play a crucial role in the regulation of physiological processes in animals. The potential impact of seasons on a particular animal species is reflected by the seasonal pattern of reproduction (Hansen, 2009; Kutty and Mathew, 2000). While photoperiod formed the major regulator of circannual rhythm of breeding activity, ambient temperature (AbT) regulated the circadian and seasonal rhythms through the influence on endocrine and molecular mechanisms of reproductive events (Marai *et al.*, 2008). In countries of arid and semi-arid regions, high AbT during the summer months, and moderate to high AbT together with high humidity,

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contributed by intermittent rainfall of the months adjoining summer, have been found to be the potential regulators of seasonality in animals (Macias-Cruz *et al.*, 2016).

Hot humid climate prevailing in Kerala has already been found to be hostile to enhancement of the productivity of dairy cattle. Prevalence of high atmospheric humidity (RH) during most part of the year contributed by extended rainy season and stretched out sea shore, together with moderately high AbT caused by geographical proximity to the equator makes the climate of Kerala highly adverse for animal welfare with a distinct pattern of seasonality, compared to other regions of the country. Different classifications of seasons in Kerala are being used in various studies and the classification most often used is with four months under Southwest monsoon (June to September), two months under North East monsoon (October and November) and three months each under winter (December to February) and summer (March to May). This classification is mainly based on the occurrence of monsoon rain fall and direction of wind causing the rain (Kumar, 2013; Rao, 2013).

The climate change phenomena has been found to cause major threats for enhancement of animal productivity especially at the tropical regions contributed by increasing atmospheric temperature (AtT) and is being projected upto 0.2 degree Celsius (°C) per decade, reaching 1.5°C above the level of pre-industrial period, between 2030 and 2050 (IPCC, 2018). Alterations and inconsistencies of the seasons have also been reported in various parts of country and were found to be seriously affecting crop and animal production. In this context, weather parameters of Kerala already having peculiar climate and seasonal pattern were studied over a period of six years with emphasis on the seasonality and causation of thermal stress in animals.

Materials and methods

The study was carried out at the dairy farm of Livestock Research Station (LRS), Thiruvazhamkunnu in Palakkad district, under Kerala Veterinary and Animal Sciences

University. Weather parameters in the past six years starting from September 2013 to August 2019 were collected from the Automatic Weather Station (Campbell Scientific, CR 800 series data logger) situated at the station and two other weather stations located at VFPCCK Karimpuzha and Elavanchery, situated within 25 kilometers from the station.

Weather parameters such as daily average temperature (AvT), average relative humidity (AvRH), maximum temperature (MxT), minimum temperature (MnT), maximum relative humidity (MxRH) and minimum relative humidity (MnRH) were collected from the hourly recordings of AbT and RH. Temperature Humidity Index (THI) values were calculated from AvT and AvRH using the formula for livestock and poultry heat stress index (LPHSI, 1990) given by

$$THI (LPHSI) = T - \left(\left(0.55 - \frac{0.55 \times RH}{100} \right) \times (T - 58) \right)$$

Where T - Average temperature
(in Degree Fahrenheit)

RH - Per cent relative humidity

Derived data were analysed using statistical software SPSS V. 24.0 for monthly, quarterly, half yearly and annual patterns with emphasis on the chances for the occurrence of thermal stress in dairy cattle. Weather parameters were compared between already established seasons and operational classification of seasons corresponding to four quarters adopted from an earlier study (Kutty, 2013) in order to assess their suitability for interpreting the possibility of climatic stress affecting the animal productivity and the observations are described.

Results and discussion

Monthly average of daily AvT (in °C) was lowest during January (25.06) and highest in April (28.99). MnT also had the same pattern (18.32 versus 24.16). However, monthly average of MxT had different pattern with the highest during March (37.04) and the lowest (29.70) in July. Monthly mean of daily AvRH during the 6 years was highest (97.75 %) in July and lowest (77.37 %) in February. The THI calculated from

daily AvT and AvRH showed highest monthly mean during April (82.28) as against the lowest of 75.10 during January. Even though THI 72 is often considered as the demarcation for the zone of thermal comfort for dairy animals (Dash *et al.* 2016), none of the months had THI below 75.

Quarterly mean values of major environmental variables recorded during the study period such as maximum, minimum and daily average of AbT and RH are shown in Table 1. All of these climatic parameters varied significantly ($P < 0.001$) between the seasons with highest daily mean AbT during MAM and the minimum during DJF. Quarterly means of THI calculated from daily mean values of AbT and RH are compared in Table 2. THI ranged from the lowest in DJF to highest during MAM indicating exposure of the animals to varying levels of mild to moderate stress throughout the year.

Other Climatic Stress Factors

Quarterly means of MxT, MnT, MxRH and MnRH are shown in Table 3. The extent of daily variation of these variables determines the severity of TS exposed. Hence, the variation between maximum and minimum of AbT and RH are also included in Table 3.

While MnT and MxT attained highest during MAM, lowest of these two variables were during DJF and JJA, respectively. Similarly, highest means of MnRH and MxRH were during JJA, while the lowest were during DJF and MAM, respectively. The differences between MnT and MxT had similar seasonal pattern as that of MnRH and MxRH with the lowest and highest variations during JJA and DJF respectively; also showing inverse relationship with day length pattern. However, the variations of MxT and the difference between MnT and MxT were not significant between quarters corresponding to four seasons of the region indicating persistence of high AbT across all the seasons.

Seasons in Kerala

Based on the occurrence of monsoon rainfall and direction of wind causing the

rain (Kumar, 2013; Rao, 2013), seasons in Kerala are classified with four months under Southwest monsoon (June to September), two months under North East monsoon (October and November) and three months each under winter (December to February) and summer (March to May). However, comparison of weather parameters together with day length and raining pattern showed that four quarters of three months each better represent seasons in Kerala as reported in earlier study (Kutty, 1995; Kutty, 2013).

September is the month of equinox, with equal duration of day and night at the equator. In the present study, weather

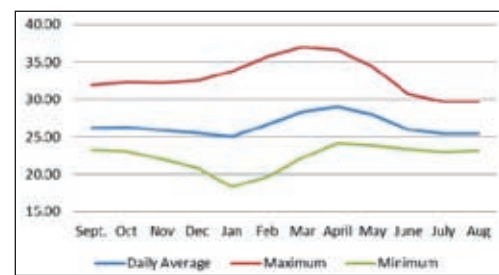


Fig. 1. Monthly mean ambient temperature of six years in °C

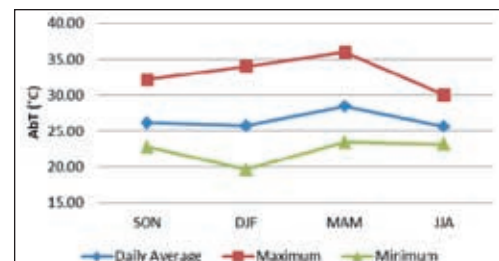


Fig. 2. Quarterly means of ambient temperature for six years

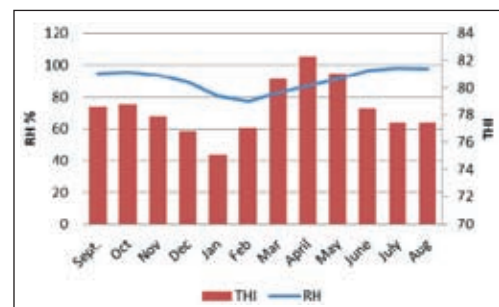


Fig. 3. Monthly means of RH and THI

Table 1. Maximum, minimum and daily mean values of ambient temperature and relative humidity (Mean \pm SE) of the study area during the four seasons

Variable	SON	DJF	MAM	JJA	Mean	F-value
MxT (°C)	32.17 \pm 0.28 ^b	34.01 \pm 0.19 ^c	36.00 \pm 0.33 ^d	30.08 \pm 0.28 ^a	33.06 \pm 0.47	84.59
MnT (°C)	22.79 \pm 0.28 ^b	19.62 \pm 0.31 ^a	23.41 \pm 0.23 ^b	23.19 \pm 0.09 ^b	22.25 \pm 0.34	52.34
AvT(°C)	26.15 \pm 0.08 ^a	25.77 \pm 0.14 ^a	28.43 \pm 0.28 ^b	25.62 \pm 0.14 ^a	26.49 \pm 0.25	56.35
MxRH (%)	98.03 \pm 0.60 ^{bc}	93.61 \pm 1.41 ^a	95.20 \pm 1.14 ^{a b}	99.00 \pm 0.24 ^c	96.46 \pm 0.64	6.70
MnRH (%)	71.82 \pm 3.99 ^b	45.10 \pm 1.78 ^a	53.51 \pm 3.27 ^a	86.14 \pm 2.26 ^c	64.14 \pm 3.60	38.87
AvRH (%)	94.54 \pm 0.95 ^b	82.61 \pm 2.02 ^a	86.98 \pm 1.96 ^a	97.32 \pm 1.05 ^b	90.36 \pm 1.43	18.49

Values with different superscripts in each row varied significantly (P<0.001)

Table 2. Maximum, minimum and daily mean THI values across quarters

Quarters	THI		
	Min	Max	Mean \pm SE
SON	77.92	79.15	78.43 \pm 0.17 ^b
DJF	75.87	77.11	76.42 \pm 0.22 ^a
MAM	80.52	82.51	81.36 \pm 0.33 ^c
JJA	76.86	78.31	77.82 \pm 0.21 ^b

Values with different superscript varies significantly (P<0.001) between seasons

Table 3. Quarterly mean \pm SE of maximum and minimum temperature and relative humidity and the extent of variations between seasons

Weather parameters	Season					P value	P value
	SON	DJF	MAM	JJA	Total		
Min. AbT (°C)	24.25 \pm 0.34 ^{ab}	22.09 \pm 1.19 ^a	26.30 \pm 0.66 ^b	24.75 \pm 0.38 ^b	24.35 \pm 0.55	5.754*	0.021
Max. AbT (°C)	33.08 \pm 0.70	34.72 \pm 0.86	35.93 \pm 1.84	30.82 \pm 0.98	33.64 \pm 0.77	3.527 ^{ns}	0.068
Min RH (%)	63.17 \pm 5.45 ^{bc}	41.55 \pm 5.11 ^a	52.63 \pm 9.43 ^{ab}	75.67 \pm 3.46 ^c	58.25 \pm 4.65	5.431*	0.025
Max RH (%)	94.62 \pm 0.50 ^b	90.89 \pm 1.08 ^a	89.72 \pm 0.63 ^a	94.92 \pm 0.32 ^b	92.54 \pm 0.75	14.414**	0.001
Max-Min AbT	8.84 \pm 1.04	12.62 \pm 1.03	9.62 \pm 2.44	6.06 \pm 0.59	9.28 \pm 0.94	3.459 ^{ns}	0.071
Max- Min RH	31.45 \pm 5.11 ^{ab}	49.34 \pm 4.05 ^b	37.09 \pm 9.78 ^{ab}	19.25 \pm 3.17 ^a	34.28 \pm 4.17	4.221*	0.046

* Significant(P<0.05), ** Significant (P<0.01), ^{ns} Non-significant

Means with different superscripts vary significantly within a column

parameters of September was found to have more similarity to that of October-November period with respect to AbT, RH and THI than the previous period of June to August. Similar pattern was also evidenced with respect to amount of rainfall, day length and hours of sunshine. Since all these weather parameters are more influential on thermal comfort as well as reproductive processes of the animals, the month of September was considered together with October and November, to form the operational classification of seasons in this

study as four quarters of three months each.

The period of December to February forms the winter season in rest of the country (Kumar, 2013). However, the lowest temperature recorded in the study location across the year was 18.3 °C indicating lack of winter climate. Hence the period of DJF was designated as post monsoon to denote the climate that prevails in most parts of the state. Thus it can be inferred that Kerala is lacking a winter season except in high ranges.

Monthly trends of mean AbT in °C of the six years are shown in Fig. 1. Both AvT and MnT showed a steep rise from the lowest to highest in a matter of three months. MxT had its lowest monthly average in July (29.70) and attained the highest average during March (37.04). Even though day length is the primary regulator of MnT and MxT as seen in other parts of the country, early occurrence of rain fall in Kerala (Rao, 2013) caused a major drop in AbT starting from April, changing the pattern of MxT irrespective of the day length and the same resulted in the lowest MxT and AvT respectively during June and July.

As shown in Fig 2. quarterly means of daily AvT and MnT had almost the same pattern of variations, whereas MxT showed a consistent increase from JJA to MAM and a subsequent drop to the lowest in JJA, attributable to the effect of monsoon rainfall. Thus, even though the highest averages of all the types of temperatures were in summer (MAM), the lowest of MxT and AvT was in JJA and only MnT had the lowest mean value in DJF. This in turn makes it very clear that there is no winter season in the study locality. Hence, the usage of the term post monsoon is well substantiated, deviating from the usual description of DJF as winter in other parts of the country with clear cut climatic features, especially the lowest of AbT (Kumar, 2013).

Even though climatic categorization necessitate at least 30 years weather data, similarity of the pattern between the two studies 25 years apart (Kutty 1995) and striking deviation from the existing categorization of seasons, formed the basis for proposing the revised categorization, even though further studies are indicated to establish the same.

The pattern of MnT and MxT as shown in Fig. 1 showed increasing difference between the two starting from December, attaining maximum in January, maintains almost same level until March and thereafter declined. Comparing the two half years of rainy and non-rainy seasons, there is significant difference ($P < 0.001$) between MnT and MxT, with more difference during non-rainy than rainy season. The range of variation between MnT and MxT

appears to have direct association with the day length since shortest day length is during December (Winter solstice) and increases thereafter to equalize the duration of day and night hours during March (Spring equinox). Even though the difference between MnT and MxT started to reduce from March, the rate of reduction was very slow until June - the period of longest days (Summer Solstice) and there after declines faster until September (Autumn equinox) and start to increase again thereafter.

The trend of mean RH for the six years (Fig.1) showed significant variation between months with the lowest mean value (77.37 %) during February and the highest (97.75 %) in July. It is evident that the RH is associated with rainfall, since the two months of lowest and highest RH corresponds respectively the periods of lowest and highest rainfall in the region. Correspondingly, quarterly means of all the three measurements of RH (Table 1) showed similar pattern of variation across seasons with the lowest in DJF, increased thereafter to reach the highest in JJA and decreased again towards the lowest in DJF.

Implications on thermal stress

AbT forms the main contributor of TS and is precipitated by elevation of RH as it interfere evaporative cooling mechanisms of the body (Polsky and Keyserlingk, 2017). Hence, simultaneous elevation of both becomes more stressful to the animals. However, it is less likely under climatic situation of Kerala since rainfall regulates both these variables in an opposite manner. This is evident not only in this study but the earlier study as well (Kutty, 1995). The period of highest RH (97.75 %) happened to be during July which is the period of lowest mean for MxT (29.70 °C). Likewise, RH is lowest in February while highest mean of MxT is during the adjoining months such as March (37.04 °C) followed by April (36.57°C) and February (35.74 °C). Thus, besides extremes of AbT and RH, moderate elevation of both these variables and their different combinations also produces more stress in animals. Thus a composite measurement of AbT and RH becomes more meaningful in the context of TS.

Monthly trend of mean THI (LPHSI) for the six years is shown in Fig. 3. The pattern of THI variation across months had more similarity with that of AvT than RH, indicating more influence of AvT to the THI than RH. Across seasons, highest mean value for daily AvRH was during JJA and the lowest during DJF. As shown in Table 2, THI value was highest during MAM and lowest during DJF. Thus, quarterly averages of THI and AbT followed same pattern, while the pattern was different for RH.

Highest mean value for THI was observed during MAM – the quarter of high AbT and moderate RH, while lowest of mean THI was during DJF characterized by lowest RH and low AbT. THI was moderate during JJA (the quarter of highest RH and lowest AbT) as well as SON (with moderate values for RH and AbT) as shown in Tables 1 and 2. Thus, combinations of moderate AbT and RH contributes TS more often than higher values of AbT and RH, since occurrence of both together is less likely in Kerala climate (Kutty, 2013; Rao, 2013).

Lowest of monthly mean THI (75.87) exceeded the THI level prescribed for thermal comfort of dairy cattle (68 to 72) (Schuller *et al.*, 2014), indicating that the animals are exposed to mild to moderate TS throughout the year, irrespective of the months or seasons. THI remained within the level for mild stress (72 to 79) during most of the seasons and even elevated to the level for moderate stress (80 to 89) during summer (MAM). However, THI of the study locality in any of the seasons did not reach the level for severe stress (90 or more) during the period of study.

Even though well defined seasons comparable to temperate region is lacking in the tropical climate, seasonal pattern of reproductive performance has been well established among farm animals (Kutty, 2005; Sonmez *et al.*, 2005). Various factors have been reported as determinants of the seasonal variations (Bouhroum *et al.*, 2014), major one being TS (Wolfenson *et al.*, 2000; Collier *et al.*, 2017) influenced by AbT and RH (Kutty, 2005; De-Rensis, *et al.*, 2017).

Photoperiodicity also forms major regulator of seasonality and TS (Kilgoura *et*

al., 2012). Besides direct influence through pineal gland secretion, photoperiodicity influences reproduction in animals indirectly by regulating AbT and RH (Orihuela, 2000). Hence classification of the seasons as four quarters of three months each appears better for studies on animal productivity as it considered not only AbT and raining but day length as well, as the main regulators of season and thermal stress affecting performance of the animals.

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References

- Bouhroum, N., Bensahli, B. and Niar, A. 2014. Effect of season on artificial insemination in Holstein dairy cows. *J. Exp. Biol. Agri. Sci.* **2** : 178-181.
- Collier, R.J. Renquist, B.J. and Xiao, Y.A. 2017. 100-Year Review: Stress physiology including heat stress, *J. Dairy Sci.* **100** (12): 10367-10380.
- Dash, S., Chakravarty, A.K., Singh, A., Upadhyay, A., Singh, M. and Yousuf, S. 2016. Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. *Vet. World.* **9** (3): 235 - 244.
- De-Rensis, F., Lopez-Gatius, F., Isperto, I.G., Morini, G. and Scaramuzzi, R.J. 2017. Causes of declining fertility in dairy cows during the warm season. *Theriogenology* **91**: 145 - 153.
- Hansen, P.J. 2009. Effects of heat stress on mammalian reproduction. *Philosophical Transactions Royal Soc. Bio. Sci.* **364** (1534): 3341 - 3350.
- IPCC. 2018. Summary for policymakers. In: Global warming of 1.5°C. An IPCC special report on the impacts of global warming of 1.5°C above pre-industrial levels.

Electronic copies available from the IPCC website www.ipcc.ch

- Kilgoura, R.J., Katsuji, U., Ishiwatab, T. and Gavin J.M. 2012. The behaviour of beef cattle at pasture. *Appl. Anim. Beh. Sci.* **138**: 12 - 17.
- Kumar, M.S. 2013. Indian climatology; In Rao, G.S.L.H.V.P. and Varma, G.G. 2013. *Fundamentals of Livestock Meteorology*, Vol 1., Centre for Animal Adaptation to Environment and Climate Change Studies, Kerala Veterinary and Animal Sciences University. pp.72-98.
- Kutty, C.I. and Mathew, S. 2000. Seasonal variation in morphometry of male goats in Kerala. *Intas-Polivet* **1**: 299-304.
- Kutty, C.I. 1995. Seasonal fertility of billy goats, *M. V. Sc. Thesis*, Kerala Agricultural University, Thrissur. 100 p.
- Kutty, C.I. 2005. Fertility of female goats across the seasons in Kerala. *Indian J. Anim. Reprod.* **26**(2): 113-116.
- Kutty, C.I. 2013. Role of climate in reproductive pattern of small ruminants in humid tropics; Rao, G.S.L.H.V.P. and Varma, G.G., *Fundamentals of Livestock Meteorology*, Vol. II., Centre for Animal Adaptation to Environment and Climate Change Studies, Kerala Veterinary and Animal Sciences University, Pookode. pp. 194-202.
- LPHSI. 1990. The livestock and poultry heat stress indices for cattle, sheep and goats. In: *The agriculture engineering technology guide*. Clemson University, Clemson, USA.
- Macias-Cruz, U., Gastelum, M.A., Alvarez, F.D., Correa, A., Diaz, R., Meza-Herrera, C.A. and Avendano-Reyes, L. 2016. Effects of summer heat stress on physiological variables, ovulation and progesterone secretion in Pelibuey ewes under natural outdoor conditions in an arid region. *Anim. Sci. J.* **87**(3): 354 - 360.
- Marai, I.F.M., El-Darawany, A.A., Fadiel, A. and Abdel-Hafez, M.A.M. 2008. Reproductive performance traits as affected by heat stress and its alleviation in sheep - a review. *Trop. Sub-trop. Agroecosystems* **8**: 209-234.
- Orihuela, A. 2000. Some factors affecting the behavioural manifestation of oestrus in cattle: a review. *Appl. Anim. Beh. Sci.* **70**: 1 -16.
- Polsky, L. and Von-Keyserlingk, M.A.G. 2017. Invited review: Effects of heat stress on dairy cattle welfare. *J. Dairy Sci.* **100**(11): 8645 - 8657.
- Rao, G.S.L.H.V.P. 2013. Introduction to Livestock Meteorology; In Rao, G.S.L.H.V.P. and Varma, G. G. 2013. *Fundamentals of Livestock Meteorology*. Vol 1., Centre for Animal Adaptation to Environment and Climate Change Studies, Kerala Veterinary and Animal Sciences University, Pookode. pp. 1-11.
- Schuller, L.K., Burfeind, O. and Heuwieser, W. 2014. Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature-humidity index thresholds, periods relative to breeding and heat load indices. *Theriogenology* **81**: 1050 - 1057.
- Sonmez, M., Demirci, E., Turk, G. and Gur, S. 2005. Effect of season on some fertility parameters of dairy and beef cows in Elazig province. *Turkish J. Vet. Anim. Sci.* **29**(3): 821 - 828.
- Wolfenson, D., Roth, Z. and Meidan, R. 2000. Impaired reproduction in heat- stressed cattle: basic and applied aspects. *Anim. Reprod. Sci.* **60**: 535 - 547. ■



Comparative efficacy of different treatment regimens against bovine mastitis caused by *Staphylococcus aureus*

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Abstract

Bovine mastitis is an endemic disease among dairy cattle all over the world and antimicrobial therapy is one of the prime therapeutic and prophylactic tool against mastitis. In the present scenario, the efficacy of antimicrobial chemotherapy is being threatened by the rising tide of antimicrobial resistance. A study was conducted to evaluate the comparative clinico-therapeutic efficacy of three selected antimicrobial protocols among 21 animals with clinical mastitis, from which *S. aureus* could be isolated. Therapeutic trials were conducted with Inj. Ceftriaxone (10 mg/kg body weight BID) for five days in group I, Inj. Ceftriaxone-sulbactam (6 mg/kg IV BID for five days) in group II and Inj. Sulphadiazine-trimethoprim (15 mg/kg IV BID for five days) in group III. The bacteriological cure was assessed by streaking the milk samples collected 24 h after termination of the treatment on to a bacteriological medium and the clinical cure was assessed by the return to normal colour and consistency of milk and remission of the pathological manifestations in udder. All the treatment groups had exhibited an excellent bacteriological cure rate as evident from the 100 per cent post treatment culture negative samples. The group I exhibited 100 per cent clinical cure whereas, in group II and group III, the clinical cure was 87.5 per cent and 75 per cent respectively. Statistical analysis using Fisher's exact test revealed that there is no significant difference among the three treatment groups and all the treatment regimens are equally effective.

Keywords: Mastitis, ceftriaxone, ceftriaxone sulbactam, sulphadiazine trimethoprim, clinico-therapeutic efficacy

Mastitis, defined as the inflammation of the mammary gland parenchyma, continues to be a persistent problem in the dairy industry all over the globe, despite decades of novel treatment and

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control programmes. It engenders a negative impact on the economic status of farmers and ultimately jeopardises the national economy. In India the annual economic loss due to bovine mastitis had increased 135 folds in about almost 5 decades from INR 529 million/annum in 1962 (Dhanda and Sethi, 1962) to INR 71655.1 million/annum in 2009 (Bansal and Gupta, 2009). In addition, it also poses public health threat due to its potential for transmission of many zoonotic milk borne diseases, antibiotic residues, bacterial toxins as well as the organisms carrying numerous virulent and antimicrobial resistant genes. Targeted antimicrobial therapy plays an important role in mastitis control by reducing the level of herd infection and preventing new infections. Hence the present study was envisaged to detect the clinico-therapeutic efficacy of three selected antimicrobial protocols.

Materials and methods

The present study was conducted in 83 lactating dairy cows presented with clinical signs suggestive of mastitis. Milk samples were collected aseptically from each animal using separate sterile screw capped vials. Isolation of bacteria was attempted from individual quarter samples by direct streaking of the milk on to brain heart infusion agar (BHIA; M211, Himedia) followed by incubation of the plates at 37°C for

24 h. The isolates were identified based on morphological characterisation using Gram's staining, cultural characteristics on selective media and biochemical characterisation as per Barrow and Feltham (1993) and Quinn *et al.* (2013).

The isolates were then subjected to *in vitro* antibiotic susceptibility testing by Kirby Bauer disc diffusion assay (Bauer *et al.*, 1966) as per the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). Antibiotic discs impregnated with known concentration of nine antimicrobial agents viz., amoxicillin-sulbactam, ceftriaxone, ceftriaxone-sulbactam, cotrimoxazole, enrofloxacin, gentamicin, methicillin, penicillin G and tetracycline were used.

Twenty one animals with CM from which *S. aureus* isolates were obtained was divided into three different groups with a minimum of six animals in each group using random number generation. Each group was administered three different antimicrobial protocols as shown in Table. 1 based on the results of *in vitro* drug sensitivity by disc diffusion method.

The rate of recovery was assessed based on clinical cure and bacteriological cure. The clinical manifestation of animal before and after treatment are shown in fig. For the

Table 1. Antimicrobial protocol used for treatment trial

Treatment groups	Drug used	Dose, route and duration of treatment
Group I	Inj. Ceftriaxone (Inj. Cefstan 3 g, Zoetis, India Ltd.)	10 mg/kg body weight IV, twice daily for five days
Group II	Inj. Ceftriaxone-sulbactam (Inj. Safevet Forte 4.5 g, Boehringer Ingelheim, India Pvt. Ltd.)	6 mg/kg body weight IV, twice daily for a period of five days
Group III	Sulphadiazine-trimethoprim (Inj. Biotrim, Zydus AHL, India)	15 mg/kg body weight, IV, twice daily for five days

Table No 2. Statistical analysis of the three treatment trials

Groups	Antibiotic used	Number of animals	Clinical cure		Bacteriological cure	
			Number	Per cent	Number	Per cent
1	Ceftriaxone	6	6	100	6	100
11	Ceftriaxone-sulbactam	7	6	85.7	7	100
111	Sulphadiazine-trimethoprim	8	6	75	8	100
Fisher's exact test: Exact two sided significance p value: 0.747						



Fig 1. Changes in clinical manifestation of animals before and after treatment

determination of the bacteriological cure, the quarter samples were collected aseptically in sterile vials 24h after the completion of antibiotic therapy and was subjected to isolation to see the presence of any bacteria. The results of the clinico - therapeutic efficiency between three treatment groups were compared using by Fisher's exact test with the help of IBM – SPSS version 24.0.

Results and discussion

Out of the 83 quarter samples examined, 57 samples were positive in culture, of which five had mixed infection with two different bacterial isolates there by accounting for a total of 67 bacterial isolates. Of these isolates, the coagulase negative Staphylococci (CNS) predominated (40.29 per cent) followed by *S. aureus* (32.84 per cent), Micrococci (11.94 per cent) *Klebsiella* spp. (7.40 per cent), *E. coli* (4.47 per cent) and Streptococci (2.9 per cent). This was in accordance with previous studies by Kulangara *et al.* (2017) who reported 50 per cent prevalence of CNS from dry bovine udder whereas it was substantially higher when compared to Sebastian (2001) and Rathish (2014) who isolated 6.4 per cent and 23.81 per cent of CNS from all bacterial isolates.

The *in vitro* antimicrobial susceptibility testing by disc diffusion assay revealed that irrespective of the genus, majority of the isolates were found resistant to penicillin and methicillin whereas ceftriaxone and ceftriaxone-sulbactam were reported to be sensitive towards majority of the isolates. This was in concordance with

the previous findings by Amrithapriya (2019) and Rathish (2014) who analysed the *in vitro* antibiogram of bacterial isolates from bovine clinical mastitis in and around Thrissur district.

Among the six animals in group 1 which were treated Ceftriaxone, a bactericidal third generation cephalosporin, all the animals (100 per cent) exhibited clinical cure and bacteriological cure following a treatment period of five days. This was in accordance with Dasohari *et al.* (2017) who proved 100 per cent animal wise and quarter wise cure rate for bovine SCM following intramuscular administration of ceftriaxone. In contrast to these reports, a comparatively low clinical and bacteriological cure rate of 82.35 per cent and 66.66 per cent was reported by Charaya *et al.* (2015) among buffaloes treated with ceftriaxone. This may be due to the disparity in innate immunity between different species of animals, which in turn influences the invasiveness, intracellular penetration and colonisation of mastitis pathogens.

The development and prevalence of bacterial resistance to ceftriaxone, one of the most widely used broad spectrum third generation cephalosporin, has been documented in recent years due to the production of beta lactamases (Xin *et al.*, 2013). In order to combat these beta lactamase producing bacterial strains, ceftriaxone sulbactam was selected, in which cephalosporins are combined with the beta lactamase inhibitor sulbactam, which could irreversibly inhibit the hydrolytic action of beta lactamases and restore the activity of ceftriaxone

against beta lactamase-producing bacteria. Seven cases of staphylococcal mastitis were treated with ceftriaxone – sulbactam at a dose rate of six milligram per kilogram body weight IV twice daily for a period of five days. The clinical cure was 85.7 per cent whereas all the animals had a bacteriological cure. The results were similar to Singh *et al.*, 2015 who reported 84.61 per cent recovery rate from mastitis following therapy with ceftriaxone-tazobactam. However, Akova (2008) analyzed the *in vitro* and clinical efficacy of different beta lactamase inhibitors and concluded that sulbactam is the least potent inhibitor of this class compared to tazobactam and clavulanate.

Sulphadiazine-trimethoprim, a potentiated folate inhibitor was used to treat eight cases of CM in group III animals and it was found that all the cases had a bacteriological cure following the treatment period of five days whereas, only 75 per cent had clinical cure with the oedema of udder and reduction in milk yield being persisting even after the treatment course for two out of the eight cases treated. This was in accordance with Kalmus *et al.* (2011), who reported that 96.6 per cent susceptibility of *S. aureus* isolates to sulphadiazine-trimethoprim based on *in vitro* antibiogram. This higher per cent of sensitivity could be attributed to the mutual potentiation of activity between each drug that enhances its therapeutic efficacy (Mac Diarmid, 1978). However, based on *in vivo* pharmacokinetic studies in mid or late lactation dairy cows, Kaartinen *et al.* (1999), reported that the effectiveness of sulphadiazine-trimethoprim in maintaining adequate therapeutic concentrations in milk against mastitis pathogens appears to be unsatisfactory. This incongruity might be due to qualitative nature of *in vitro* assay that does not consider the host factors that contributes to the clinical cure or lack of *in vitro* susceptibility breakpoint data specific for mastitis (Kasravi *et al.* 2010).

A statistical analysis of the clinico-therapeutic efficacy of the three selected antimicrobial protocols using Fischer's exact test revealed that all three protocols were equally effective against contagious mastitis with no significant difference in their clinico-therapeutic efficacy (p value 0.747). In the

present study, prompt treatment based on the findings of *in vitro* antibiogram and proper management of the underlying factors together with the efficacy of the antibacterial may have enhanced the clinical and bacteriological cure. The figure 1. shows the variation of clinical manifestations in the udder of animals before and after treatment. However, the absence of clinical cure even in culture negative samples of group II and group III could be attributed to the highly invasive and adaptable nature of *S. aureus* that enables them to survive and colonise deep inside the tissue.

Conclusion

The effectiveness of mastitis therapy depends on the nature of the aetiological agent, pathological changes in udder parenchyma, pharmacokinetics of antimicrobial agents, animal husbandry activities and timely veterinary intervention. Therefore, further scientific studies on the pharmacological effectiveness of various therapeutic regimens targeting a larger group of animals is warranted to develop an on farm herd level mastitis protocol that promotes judicious use of antimicrobials.

Ethical approval and consent statement

Oral consent was taken from the owner of the animals before treating them. There is no specific law in India that requires permission from ethics committee for collecting milk samples and treating the animals presented to veterinary hospitals by a qualified veterinarian.

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References

- Akova, M., 2008. Sulbactam-containing β -lactamase inhibitor combinations. *Clin. Microbiol. Infect.* **14**: 185-188.
- Amrithapriya M. G. 2019. Clinico-therapeutic studies of bovine mastitis caused by *Staphylococcus aureus* and its molecular typing. MVSc thesis, Kerala Veterinary and Animal Sciences University, Pookode, 154p.

- Bansal, B. K. and Gupta, D. K. 2009. Economic analysis of bovine mastitis in India and Punjab- A review. *Indian J. Dairy sci.* **62**: 337-345.
- Barrow, C. I. and Feltham, R. K. A. 1993. *Cowan and Steel's Manual for the Identification of Medical Bacteria*. (3rd Ed.). Cambridge University Press, Great Britain, 331p.
- Bauer, A. W., Kirby, M. M., Sherris, J. S. and Tenckhoff, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**: 493-496.
- Charaya G., Kumar A., Sharma A., Singh M. and Goel P. Comparative efficacy of Tylosin, Enrofloxacin and Ceftriaxone in treatment of buffaloes suffering from mastitis. *Haryana Vet.* **54**: 154- 156.
- Clinical and Laboratory Standards Institute. 2017. Performance standards for Antimicrobial Susceptibility testing (25th Ed.). Clinical and Laboratory Standards Institute, Wayne, 15p.
- Dasohari, A., Somasani, A. and Nagaraj, P. 2017. Therapeutic management of sub clinical mastitis in cows. *The Pharma Innovation.* **6**: 198.
- Dhanda, M.R. and Sethi, M.S. (1962). Investigation of mastitis in India. ICAR Res. Series No. 35. New Delhi, India.
- Kaartinen, L., Löfhöner, K., Wiese, B., Franklin, A. and Pyörälä, S. 1999. Pharmacokinetics of sulphadiazine-trimethoprim in lactating dairy cows. *Acta. Vet. Scand.* **40**: 271-278.
- Kalmus, P., Aasmäe, B., Kärssin, A., Orro, T. and Kask, K., 2011. Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. *Acta. Vet. Scand.* **53**: 1-4.
- Kasravi, R., Bolourchi, M., Farzaneh, N., Seifi, H.A., Barin, A., Hovareshti, P. and Gharagozlou, F. 2010. Relationship between *in vitro* antimicrobial sensitivity of bovine subclinical mastitis isolates and treatment outcome in lactating dairy cows. *Iranian J. Vet. Res.* **11**: 249-254.
- Kulangara, V., Nair, N., Sivasailam, A., Sasidharan, S., Kollannur, J. D. and Syam, R. 2017. Genotypic and phenotypic β -lactam resistance and presence of PVL gene in Staphylococci from dry bovine udder. *PLoS one.* **12**: p.e0187277.
- MacDiarmid, S.C., 1978. Antibacterial drugs used against mastitis in cattle by the systemic route. *N. Z. Vet. J.* **26**: 290-295.
- Quinn, P., Markey, B., Carter, M. and Carter, G. R. 2013. In: *Clinical Veterinary Microbiology*. (2nd Ed) Mosby, St. Louis, 514p.
- Rathish, R. L. 2014. Clinico- therapeutic studies and experimental evaluation of a bacterin against common bacterial isolate of bovine mastitis. PhD thesis, Kerala Veterinary and Animal Sciences University, Pookode, 177p.
- Sebastian, S, R. (2001). Clinico-therapeutic studies on bacterial etiology of bovine mastitis. M.V.Sc thesis, Kerala Veterinary and Animal Sciences University, Pookode, 57p.
- Singh, K.P., Singh, R.V., Singh, P., Singh, S.K. and Singh, J.P. 2015. Comparative evaluation of antimicrobials for therapeutic management of bovine mastitis. *Intas Polivet.* **16**: 261-264.
- Xin, X., Jian, L., Xia, X., Jia, B., Huang, W., Li, C., Wang, C., Zhou, L., Sun, X., Tang, X. and Huang, Y. 2013. A multicentre clinical study on the injection of ceftriaxone/sulbactam compared with cefoperazone/sulbactam in the treatment of respiratory and urinary tract infections. *Ann. Clin. Microbiol. Antimicrob.* **12**: 38-47. ■



Effect of feeding Ksheerabala residue on growth and economics of production in Malabari kids

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Abstract

A feeding trial was done in 18 Malabari kids of 3 months of age (average body weight of 8.29 ± 0.81 kg) for a period of 90 days to assess the effect of dietary incorporation of Ksheerabala residue on growth performance and economics of production. Kids were randomly allotted into three dietary treatments (T_1 , T_2 and T_3) and were fed with kid starter containing Ksheerabala residue at 0, 10 and 20 per cent, respectively. Kid starters were made isonitrogenous and isocaloric (24 % CP and 70% TDN) and were fed as per ICAR standards (ICAR, 2013). Green grass (Hybrid Napier) was fed as source of roughage. Data on body weight gain, daily dry matter consumption, feed conversion efficiency and economics of production of the experimental kids were recorded. Average daily gain (82 and 73 g) and feed conversion efficiency (4.49 and 4.80) were found to be similar in up T_1 and T_3 groups, however in T_2 group the gain (54 g) and FCR (6.19) was significantly lower than T_1 and T_3 . Feed cost per kg gain was found to be lowest (Rs. 80.85) for group T_3 . It could be concluded that Ksheerabala residue can be incorporated in kid starter at 20 per cent level without affecting the growth performance of kids and it is more profitable.

Key words: Ksheerabala residue, kid starter, growth, economics

Increase in feed ingredient prices and the scarcity of grains are the most important constraints hindering animal production sector in India. Moreover, insufficient production of farm crops to meet the needs of both humans and their domestic animals leads to competition between man and animals for these feed ingredients. This has enforced animal nutrition researchers to

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intensify their research into the feeding values of potentially useful and easily available non-conventional feed resources. Though various agro industrial by-products and crop residues are being used in animal feeds to reduce feed cost, the potential of by-products from ayurvedic pharmaceuticals has not been explored so far. Ksheerabala residue is a by-product obtained during the manufacturing of Ksheerabala oil which is prepared by combining *Sida cordifolia*, cow milk and gingelly oil. This residue is available in plenty, free of cost, but the level of inclusion and the effect of Ksheerabala residue on growth in kids are not yet studied. Hence the present study was done to evaluate the effect of dietary incorporation of Ksheerabala residue as a nonconventional feed in the ration of kids on their performance.

Materials and methods

Eighteen healthy kids of three months of age were selected from University Goat and Sheep farm, College of Veterinary and Animal Sciences, Mannuthy as the experimental animals and were housed individually in well - aired, hygienic and dry shed with amenities for feeding and watering. The kids were allotted randomly in to three dietary treatments, T₁ (kid starter), T₂ (kid starter containing 10 per cent ksheerabala residue) and T₃ (kid starter containing 20 per cent ksheerabala residue). All the rations were made isonitrogenous and isocaloric (24 % CP and 70 % TDN).

Kid starter was offered in the forenoon and green grass (Hybrid Napier) was fed in the afternoon after weighing. Daily feed offered and left-over portion of the kid starter and green grass were recorded and moisture content was evaluated to calculate the dry matter intake. Body weights of animals were recorded at fortnightly intervals. Kids were fed as per ICAR standard (ICAR, 2013) and kept on their respective feeding regime for three months.

Kid starter, fodder and dung samples were evaluated for proximate principles (AOAC, 2012). The acid detergent fiber (ADF) was determined by the method suggested by Van Soest (1963) and neutral detergent fiber (NDF) by the method suggested by Van Soest and Whine (1967). Data collected on various

parameters were statistically analysed using Analysis of Variance (Snedecor and Cochran, 1994). Cost of feeding per kg body weight gain was worked out. Proximate composition of Ksheerabala residue is depicted in Table 1. The ingredient and chemical composition of rations are depicted in Table 2.

Results and discussion

Growth

Data on the average body weight of kids belonging to groups T₁, T₂ and T₃ recorded at fortnight intervals (Table 2) revealed that the kids of all the three groups recorded similar pattern of gain, without any significant difference ($P > 0.05$). Kids of T₁, T₂ and T₃ had a cumulative weight gain of 6.39, 4.23 and 5.67 kg and an average daily body weight gain (ADG) of 82, 54 and 73g/d, respectively (Table 4). Statistical analysis of the data revealed that T2 group had significantly ($P < 0.01$) lower total gain and average daily gain compared to T₁ and T₃. The result in this study indicates that inclusion of Ksheerabala residue at 10 per cent level in kid starter ration reduced the growth rate of kids but at 20 per cent inclusion was comparable with that of control group.

Rani *et al.* (2016) observed similar daily gain in crossbred calves fed starter diets with dietary inclusion of Ksheerabala residue at 40 per cent level and control group. In contrary to the results, Obeidat and Gharaybeh (2011) observed higher weight gain in kids fed diet containing 10 per cent sesame hull compared to control diet with zero per cent sesame hull.

Table 1. Proximate composition of Ksheerabala residue

Nutrients	%
Dry matter (DM)	92.55
Crude protein (CP)	29.52
Ether extract (EE)	13.26
Crude fibre (CF)	6.39
Total ash (TA)	8.42
Nitrogen Free Extract (NFE)	42.41
Acid insoluble ash (AIA)	0.06

Table 2. Ingredient and chemical composition of kid starters, with dietary incorporation of ksheerabala residue at different levels

Ingredients, %	Percentage composition of kid starter		
	T ₁	T ₂	T ₃
Maize	35	29	23
Wheat bran	25	26	28
Soya bean meal	28	23	17
Dried fish	9	9	9
Ksheerabala residue	0	10	20
Salt	1	1	1
Calcite	2	2	2
Total	100.00	100.00	100.00
Chemical composition			
Dry matter (DM)	92.19	91.73	91.93
Crude protein (CP)	23.74	23.99	24.51
Ether extract (EE)	4.95	5.38	6.04
Crude fibre (CF)	5.43	5.71	6.08
Total ash (TA)	9.72	10.07	10.41
Nitrogen free extract (NFE)	55.65	54.84	52.87
Acid insoluble ash (AIA)	1.11	1.23	1.28
Neutral detergent fibre (NDF)	22.34	22.83	25.84
Acid detergent fibre (ADF)	6.61	7.81	9.48
Calcium	1.03	1.12	1.18
Phosphorus	0.65	0.70	0.86

Table 3. Fortnightly average body weight of experimental kids fed diets containing ksheerabala residue

Fortnights	Dietary treatments		
	T ₁	T ₂	T ₃
0	8.27 ± 0.58	8.39 ± 0.66	8.23 ± 1.25
1	9.31 ± 0.64	9.24 ± 0.68	9.01 ± 1.44
2	10.37 ± 0.59	9.83 ± 0.68	10.05 ± 1.59
3	11.00 ± 0.53	10.36 ± 0.63	11.19 ± 1.75
4	12.42 ± 0.53	11.42 ± 0.69	12.26 ± 1.83
5	13.34 ± 0.5	11.89 ± 0.73	13.29 ± 1.72
6	14.66 ± 0.63	12.63 ± 0.77	13.91 ± 1.72

Table 4. Growth rate, feed efficiency and cost of feeding per kg gain of kids fed with ksheerabala residue

Parameters	T ₁	T ₂	T ₃
Initial body weight (kg)	8.27 ± 0.58	8.39 ± 0.66	8.23 ± 1.25
Final body weight (kg)	14.66 ± 0.63	12.63 ± 0.77	13.91 ± 1.72
Total weight gain (kg)	6.39 ± 0.13 ^a	4.23 ± 0.23 ^b	5.67 ± 0.54 ^a
Average daily gain (g)	82 ± 0.002 ^a	54 ± 0.003 ^b	73 ± 0.01 ^a
Total dry matter consumed (kg/calf)	28.7 ± 0.89	26.17 ± 1.58	27.26 ± 2.94
Average daily dry matter consumed (kg/calf)	0.37 ± 0.01	0.34 ± 0.02	0.35 ± 0.04
Feed efficiency	4.49 ± 0.13 ^a	6.19 ± 0.21 ^b	4.8 ± 0.26 ^a
Cost per kg gain (Rs.)	91.97	115.92	80.85

Dry matter intake

There was no significant difference in the average daily dry matter intake (DMI) and total DMI and the values were 0.37, 0.34 and 0.35 kg and 28.70, 26.17 and 27.26 kg respectively for T_1 , T_2 and T_3 (Table 4). The result of present study is in agreement with Bambidis *et al.* (2005) who observed no significant difference in DMI of lambs which were fed with diet containing garlic bulb at zero, three and six per cent level. The results are in contrast with that of Rani *et al.* (2016) and Sheethal *et al.* (2016) who reported higher DMI in growing kids fed Ksheerabala residue at 40 per cent level inclusion.

Feed conversion efficiency

Feed conversion efficiency of experimental kids was 4.49, 6.19 and 4.80, respectively for T_1 , T_2 and T_3 (Table 4) and statistical analysis of the data revealed that kids of T_3 had significantly lesser feed conversion efficiency ($P < 0.01$) compared to T_1 and T_2 . From the results it can be inferred that inclusion of Ksheerabala at 10 per cent level in kid starter ration reduced the feed conversion efficiency but at 20 per cent level, feed conversion efficiency was similar to that of control group. In contrast to this findings, Jasmine *et al.* (2017) reported higher FCR (9.2) in kids fed starter diets containing rosemary residue.

Economics of gain

The cost of feed per kilogram body weight gain of kids maintained on dietary treatments T_1 , T_2 and T_3 were Rs. 91.97, 115.92 and 80.85, respectively (Table 4). This result indicates that, kid starter containing 20 per cent Ksheerabala residue supported growth of kids similar to that of control ration and it is more economical.

Conclusion

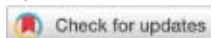
Critical assessment of the results attained in the present study revealed that dietary incorporation of Ksheerabala residue at 10 per cent level in kid starter ration significantly reduced growth rate and feed conversion efficiency but at 20 per cent level, kids had similar growth rate as that of control group. Also, economics of production

was lowest for kids maintained on kid starter containing 20 per cent Ksheerabala residue. From the overall results it can be concluded that inclusion of Ksheerabala residue at 20 per cent level in kid starter ration provided satisfactory growth rate and better profit.

References

- AOAC. 2012. *Official Methods of Analysis*, (15th Ed.). Association of official analytical chemists. Washington, D.C, 587p.
- Bampidis, V. A., Christodoulou, V., Paneri, F. P., Christaki, E., Spais, A.B. and Chatzopoulou, P.S. 2005a. Effect of dried oregano leaves supplementation on performance and carcass characteristics of growing lambs. *Anim. Feed Sci. Technol.* **121**: 275
- BIS. 2012. Bureau of Indian Standards. Specification for compounded feeds for young stock: IS: 5560-(1970). Manak Bhavan, 9, Bhahaadur Shah Zafar Marg, New Delhi. 15p.
- ICAR. 2013. *Nutrient requirement of cattle and buffalo*. Indian Council of Agriculture and Research, New Delhi, 30-34p.
- Jasmine S M S., Shyama K., Gangadevi P., Ally K and Thirupathy Venkatachalapathy R. Effect of incorporation of spent rosemary leaves on serum biochemical parameters of Malabary kids. *J. Vet. Anim. Sci.* 2018. **49**(7): 67-70
- Obeidat, B.S. and Gharaybeh, F.F. 2011. Effect of feeding sesame hull on growth performance, nutrient digestibility, and carcass characteristics of Black goat kids. *Asian-Aust. J. Anim. Sci.* **24**(2): 206
- Rani, K. J., Ally K., Sheethal, C. R., Surej Joseph Banglavan, Gangadevi, P. and Shyama, K. 2016. Dietary incorporation of Ksheerabala residue and its effect on nutrient utilisation, growth performance and blood biochemical parameters in crossbred calves. *Ind. J. Anim. Nutri.* **33**(4): 383-388.

- Sheethal C R, K.Jasmine Rani Surej Joseph Banglavan and Ally.K. Effect of incorporation of
- Ksheerabala residue on dry matter intake and nutrient digestibility in crossbred calves. 2016. *Int. J. Food Sci. Tech.* **7**(1): 7-11.
- Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* (8th Ed.). The Iowa State University press, Ames, IA. 314p.
- Van Soest, P.J. 1963. Use of detergents in analysis of fibrous feeds. II. A rapid method for the determination of fibre and lignin. *J. Assoc. Off. Analyt. Chem.* **46**: 829.
- Van Soest, P.J. and Whine, R.H. 1967. Use of detergents in the analysis of fibrous feeds. IV. The determination of plant cell wall constituents. *J. Assoc. Off. Analyt. Chem.* **50**: 50. ■



Toxicity of ethylene glycol monomethyl ether on reproduction parameters and histomorphological changes in Wistar rats*



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Abstract

The aim of this study was to evaluate the toxicity of Ethylene Glycol Monomethyl Ether (EGME) on fertility and early embryonic development to implantation, following oral gavage to Wistar rats. EGME, which is a known testicular toxicant, was administered to male rats for four weeks and to female rats for two weeks prior to mating at dose levels of 20, 40 and 80mg/kg orally, once daily. Dosing was continued in males until sacrifice (Day 43) and in females until day six of gestation. Females were sacrificed on Day 15 of gestation and examined for implantation sites, viable fetuses and ovarian corpora lutea. Males were evaluated for sperm parameters as well as organ weight and histopathology of the reproductive tissues. At the end of dosing, the 80 mg/kg/day males had decreased weight and size of testes and epididymides which correlated with tubular atrophy of the testes and ductal atrophy plus reduced sperm in the epididymides. Testicular changes were less severe in the 40 mg/kg/day group, comprising Sertoli cell vacuolation, with degeneration and depletion of elongating spermatids and spermatid retention in the testes and luminal cell debris in the epididymides. No testicular abnormalities were observed in the 20 mg/kg/day males but cell debris was present in the epididymal lumen. There was a dose related decrease in total sperm count and sperm viability (≥ 20 mg/kg/day) and sperm motility (≥ 40 mg/kg/day). The fertility index in the EGME dosed groups showed a dose-related decline and the time taken for females to conceive was increased.

Key words: EGME, testis, implantation, rats

Ethylene glycol monomethyl ether (EGME) and its acetate ester (MEA) are highly volatile liquids used in paints, lacquers, stains, inks and surface coatings, silk-screen printing,

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photographic and photo lithographic processes and as an anti-icing additive in hydraulic fluids and jet fuel (Johanson, 2000; Starek and Szabla, 2008). These compounds may be ingested, inhaled or absorbed into the systemic circulation via the dermal route after contact on the skin (Johanson, 2000). EGME and MEA both are listed under Proposition 65 of the Safe Drinking Water and Toxic Enforcement Act of 1986 as known agents causing developmental toxicity and reproductive toxicity ("Ethylene Glycol Monomethyl Ether I OEHHA," 1989). Reports suggest presence of oligospermia and azoospermia and the odds ratio for lower sperm counts per ejaculate were found increased in workers as compared to those workers who were not exposed to glycol ethers, in a study conducted on shipyard painters occupationally exposed to EGME and MEA (Welch *et al.*, 1988). Other cross-sectional studies were also conducted to relate occupational exposure of EGME and male infertility and showed negative effects of the ethers (Cherry *et al.*, 2008; Multigner *et al.*, 2007). In women, disturbance in the corpus luteum function and inhibition of ovulation as well as disturbance of the menstrual cycle have been observed (Davis *et al.*, 1997; Gold *et al.*, 1995). In this study, we have examined the toxicity of EGME on fertility and early embryonic development to implantation following exposure by oral route in male and female Wistar rats at three different dose levels of EGME.

Material and Methods

Experimental Protocol:

The study was carried out as per the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The project was approved by Institutional Animal Ethics Committee. The test facility is accredited with GLP certificate from National GLP Compliance Monitoring Authority, Govt. of India and additionally accredited by AAALAC International for animal ethics. Wistar rats (50 male and 50 female, of 150-250 g) were received from Laboratory Animal Resources, Sun Pharma Advanced Research Co. Ltd., Vadodara and were housed in Individually

Ventilated Caging system. Sterilized corncob was provided as bedding material. Controlled environment was maintained (light: 12 h light/dark; Temperature: 20-26°C; RH: 30-70%). Animals were provided with Irradiated Certified rodent diet (Envigo, 2018C) and autoclaved R.O. water *ad libitum*. Females were virgin. Animals were of 200-300g at the initiation of dosing and acclimatized for 7 to 10 days. Veterinary health check and randomization was performed during acclimation period to select 48 males and 48 females for study. Animals were divided into 4 groups with 12 animals/sex/group. EGME was dissolved in de-ionized water and to a final concentration of 2, 4, 8 mg/ml; rats were given 20 (G2, low dose), 40 (G3, mid dose) and 80 (G4, high dose) mg EGME per kg per day orally. Dose volume of each animal was calculated based on the recently recorded body weights. Control animals (G1) received de-ionized water. Dosing in females of the respective group was initiated on Day 15 of male dosing. Dosing was continued through the mating period also. Dosing in mated females was continued up to Day 6 of gestation and in males until terminal sacrifice (total 42 days of dosing in males).

Mating Protocol and Justification of Doses and Route Selection:

On last day of pre-mating dosing (i.e. Day 28 in male and Day 14 in female), males and females from the same group were co-habited (1M:1F) for mating. Mating period was up to 10 days or if the female was found to be mated (presence of vaginal plug or presence of sperms in vaginal smear), whichever was earlier. During the mating period vaginal smears were obtained daily in the morning and examined under the microscope for presence of spermatozoa. Vaginal canal was lavaged with normal saline (0.9%) and the contents were delivered onto a glass slide. Smears were observed under a microscope and presence of sperms was considered as positive sign of mating i.e. Day 0 of pregnancy. If any female failed to mate during mating period of 10 days; Day 11 was considered as Day '0' of gestation for that female/s. By exposing to Isoflurane (anesthesia) and CO₂ (euthanasia), females were sacrificed on gestation Day 15 and males

were sacrificed on Day 43 and observed for gross pathology.

The doses were selected on the basis of 2 and 4 week repeated dose oral toxicity study of EGME in rats (Dodo *et al.*, 2009, Watanabe *et al.*, 2000). Doses of EGME employed in these studies were 0/30/100/300 mg/kg/day for females (Dodo *et al.*, 2009) whereas males were dosed for 100/200 mg/kg/day for 2 weeks and 100 mg/kg/day for 4 weeks (Watanabe *et al.*, 2000).

Chemicals:

Ethylene Glycol Monomethyl Ether (CAS Number: 109-86-4) was purchased from MP Biomedical, LLC, 29525 Fountain Parkway, Solon, OH 44139, United States.

Body weight, Food consumption and Clinical Sign:

Males were weighed on Day1 of pre-mating dosing and weekly thereafter, and on day of sacrifice. Female body weights were recorded on Day1 of pre-mating dosing and thereafter weekly until found mated. During pregnancy, body weight was recorded on Days 0, 3, 7, 10 and on day of sacrifice. Feed weights were recorded weekly during pre-mating dosing and until cohabited for mating and in females on gestation Days 0, 3, 7, 10 and on the day of sacrifice.

Cage side observations were done, once daily, for all rats during the acclimation period and twice daily for all rats, once before dosing and post-dosing until rats were sacrificed.

Necropsy, Histopathology, Sperm Evaluation and Reproductive Performance/Parameters:

Females were sacrificed on Gestation Day (GD) 15 and males were sacrificed on Day 43 of the treatment period using isoflurane (for anesthesia) and CO₂ (for euthanasia) and observed for gross pathology. 10% ammonium sulphide solution was used to identify post implantation loss (early death). Number of live and dead conceptus and number of corpora lutea were recorded. The following tissues were

removed, blotted, and weighed: epididymis, kidneys, spleen, thymus, lungs, liver, pituitary, right and left testis, brain, adrenal, ovaries and uterus-cervix. The tissues were then stored in 10% neutral buffered formalin until processed for sectioning except testes and epididymis, which were preserved in Modified Davidson Fixative for 24 h and then transferred to 10% neutral buffered formalin for storage. Sections of testes and left epididymis of animals from all groups were taken at four to six microns and stained with periodic acid schiff (PAS) and haematoxylin-eosin (H&E). Measurement of sperm motility, viability, total sperm count and morphology from all animals was performed manually (Hood, 2005) as per below procedures. Cauda of right epididymis was used from all animals.

Sperm motility:

A small piece of right cauda was collected in 5 ml of 0.9% normal saline in petri-plate kept at room temperature (23-28 °C). Sperms were allowed to ooze out in saline for approximately 5-10 minutes. A small drop of saline contained semen from junction of cauda was taken with pipette and loaded the haemocytometer chamber. WBC counting squares in haemocytometer were observed under 10x objective and counting of non-motile sperms followed by counting of total sperms was done. Percentage motility was determined as: Motile spermatozoa = Total spermatozoa count – Non-motile spermatozoa

Motility (%) = Motile spermatozoa/Total Spermatozoa X 100

Total sperm count:

The remaining part of cauda was weighed (mg) and crushed with BP blade and forceps in 5 ml of normal saline in a petri-plate kept at room temperature (22-26°C). Sperms were allowed to ooze out into saline for approximately 5-10 minutes. A homogenate was obtained by swirl action of contents in petri-plate to get maximum free spermatozoa. The sample was sucked in WBC diluting pipette up to the 0.5 marks and then normal saline up to 11 marks on WBC pipette (20 times dilution). It was

mixed well by rotating the pipette between two palms. First few drops were discarded and then haemocytometer was charged with the diluted sample. Total spermatozoa were counted from WBC counting squares under 10X objective. Total sperm count was determined as follows:

Total sperm count = $\{(N \times 20 \times 1000 \times \text{Dilution Volume}) / 0.4\}$ in weighed cauda

N = Total number of spermatozoa in four WBC counting squares

Total sperm count was expressed as number of spermatozoa per gram of cauda.

Sperm morphology:

Sperm morphology was determined from sample prepared as above (followed for Total sperm count). 100 μL of the sample was added in 500 μL of 10% neutral buffered formalin in micro centrifuge tube. After proper mixing, smear was prepared, in duplicates, on clean and grease free slides. Slides of each animal were identified with animal ID number followed by set A or set B. First set of slide (i.e. A) was stained for evaluation. Other set (i.e. B) was not used, as slide from set A was satisfactory. Slide was stained with 1% eosin for 30 minutes, air dried and observed under microscope (10-40X objective) on next day after mounting with DPX mountant and coverslip. At least 200 spermatozoa was observed for morphology and any abnormality such as tailless sperm, headless sperm, no hook head sperm, neck abnormality, double head, double tail, coiled tail, bent tail were recorded. At the time of archival slides will be identified with study number and animal ID. Percentage of abnormal spermatozoa was determined as below:

Abnormal spermatozoa (%) = $(\text{Total no. of abnormal spermatozoa} / \text{Total sperm observed}) \times 100$

Sperm viability:

Sperm viability was determined from sample prepared as above (Sperm Motility). About 2-5 minutes after cauda collection, 5 μL of the sample was taken on glass slide and mixed with 5 μL of normal saline and 5 μL of 0.5%

Eosin (in normal saline) each by swirling action using micropipette tip. Slide was observed under microscope at 40X for counting viable and non-viable sperms. Viable sperms remain colourless while nonviable sperms remain red colour. Total 200 sperms (viable plus non-viable) were counted. Viability was calculated as below.

Sperm Viability (%) = $(\text{Viable Sperm} / \text{Total Sperm}) \times 100$

Male mating index, male fertility index, females mating index, female fertility index, implantation index, pre-implantation loss, post-implantation loss, fecundity index and live/dead conceptus were calculated. Formulas for calculations are as follows:

Male mating index (%) = $(\text{No. of males with confirmed mating} / \text{No. of males cohabitated}) \times 100$

Male fertility index (%) = $(\text{No. of males impregnating a female} / \text{No. of males cohabitated}) \times 100$

Female Mating Index (%) = $(\text{No. of females mated} / \text{No. of cohabitated females}) \times 100$

Female Fertility Index (%) = $(\text{No. of pregnant females} / \text{No. of females with cohabitated}) \times 100$

Implantation Index (%) = $(\text{No. of implants} / \text{No. of corpora lutea}) \times 100$

Pre – Implantation loss (%) = $(\text{No. of implants} / \text{No. of corpora lutea} - \text{No. of implants} / \text{No. of corpora lutea}) \times 100$

Post – Implantation loss (%) = $(\text{No. of implants} / \text{No. of implants} - \text{No. of viable fetuses} / \text{No. of implants}) \times 100$

Fecundity Index (%) = $(\text{No. of pregnant females} / \text{No. of females with confirmed mating}) \times 100$

Statistical analysis:

One-way analysis of variance ($p < 0.05$) and Dunnett's test was done.

Results and Discussion

Body Weight and Food Consumption:

Compared with controls (11.58%

gain at week 6), treatment related significant decrease in percent body weight changes was observed in G4 group males (1.46% gain at week 6) throughout the experimental period. Compared with control (22.1g/day/animal at week 2 and 19.29g/day/animal at week 4), treatment related statistically significant decrease was observed in the feed consumption in Week 2 (16.68g/day/animal) and Week 4 (14.72g/day/animal) for G4 males, while females from G2 (10.4g/day/animal), G3 (10.63g/day/animal) and G4 (10.28g/day/animal) groups showed treatment related significant decrease in feed consumption in Week 2 in comparisons to control (12.44g/day/animal).

Necropsy and Histopathology

Organ Weights:

In G4 males, statistically significant decrease in absolute and relative weights were seen in epididymides and testes (Table 1).

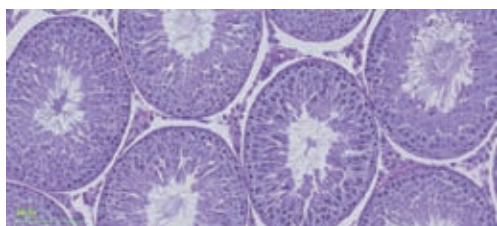


Fig 1a Control Group - 102 - 20x Normal Testis

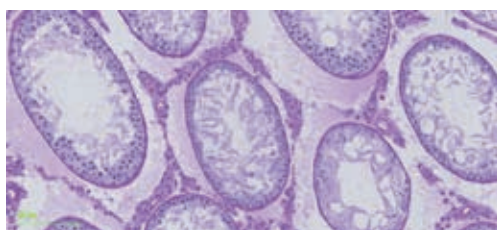


Fig 1b High dose - 20x

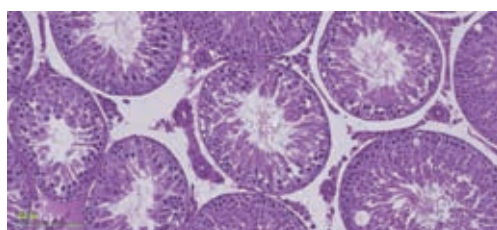


Fig 2 - 20x Sertoli Cell Vacuolation and Partial Germ Cell Loss

Macroscopic Observation:

In G4 males, gross findings included small testes (11/12), epididymides (8/12), adrenals (4/12) and thymus (2/12) which correlated with decreased organ weights.

Microscopic Observation:

Testes: Tubular degeneration/atrophy was present in the testes of all rats in G4 (12/12) and in most rats (7/12) rats from G3. The lesion was severe in G4 and minimal to slight in G3. No abnormalities were identified in the testes of G2. In G4 rats, most tubules had depletion of elongating spermatids, round spermatids and pachytene spermatocytes, leaving tubules lined by Sertoli cells, spermatogonia and prepachytene spermatocytes (Fig 1b). Partial germ cell depletion and actively degenerating (apoptotic) germ cells were observed in some tubules with disorganisation of the remaining germ cells. The affected tubules were reduced in diameter, resulting in expansion of the

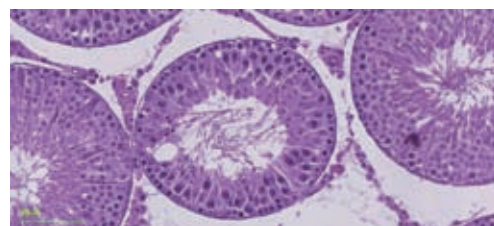


Fig 3 Mid Dose - 30x Spermatid Retention

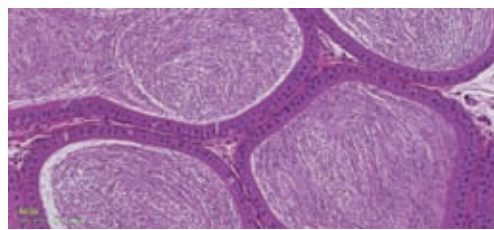


Fig 4a Control - 20x caput

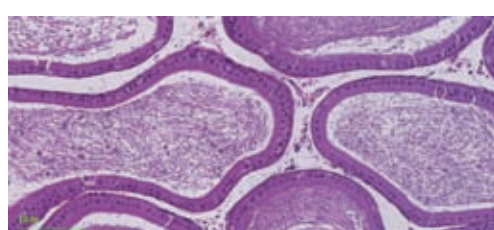


Fig 4b Low Dose -20x Cell debris in caput

Table 1: Major findings on male and female treated with EGME

EXPOSURE LEVEL (mg/kg/day)		Deionized water (G1)	20 (G2)	40 (G3)	80 (G4)
Male	Total Sperm Count (X 10 ⁶) / gm of Cauda	644.85±138.095	549.89±200.325	304.46±137.366 *	104.02±68.261 *
	Sperm Motility (%)	76.24±4.845	51.69±17.703	50.65±14.585 *	9.58±9.356 *
	Sperm Viability (%)	68.92±5.961	51.71±23.506	48.64±21.620	27.35±15.202 *
	Sperm Abnormality (%)	2.71±0.450	3.00±0.477 *	3.13±0.528 *	3.04±0.450 *
	Left epididymis (Weight, g)	0.7641±0.07441	0.7549±0.11608	0.6977±0.07539	0.5109±0.06829 *
	Right epididymis (Weight, g)	0.73623±0.087157	0.71480±0.083899	0.67494±0.058589	0.53153±0.113885 *
	Testes (Weight, g)	4.07638±0.351035	3.87877±0.500557	3.94159±0.379449	2.01477±0.401314 *
	Left epididymis (%)	0.17237±0.019665	0.17692±0.024427	0.15914±0.019426	0.12614±0.012900 *
	Right epididymis (%)	0.16633±0.022686	0.16751±0.015280	0.15438±0.020742	0.13233±0.031785 *
	Testes (%)	0.92103±0.115062	0.90775±0.084522	0.90275±0.132032	0.49388±0.066198 *
Female	Fertility Index (%)	11/12 (91.7%)	8/12 (66.6.0%) *	7/12 (58.3%) *	3/12 (25.0%) *
	Time to Conception (days)	1.9	5.4	2.9	4.7
	Total Corpora Lutea (Mean CL) from pregnant females (nos.)	148 (13)	103 (12)	85 (13)	33 (11)
	Live Foetus (Mean Live Foetus/litter) (nos.)	98 (8.9)	79 (9.9)	36 (5.1 *)	14 (4.7 *)
	Total Implantation Sites (nos.), (%)	110 (75.90 %)	86 (82.23%)	57 (67.85%)	18 (49.76%)
	Mean Implantation Sites (nos.)	9.2	8.6	5.1	1.5 *
	% Implantation Loss: Pre and post	24.10 and 19.25	17.77 and 7.26	32.15 and 27.08	50.24 and 27.35

Note: n=12, * : significantly different from control group (p<0.05)

interstitial space but there was no apparent increase in the number or size of Leydig cells. In G3, fewer testes were affected (7/12), and the severity of the changes was much less (predominantly minimal). Only a small proportion of tubules were affected, and the changes were characterised by focal Sertoli cell vacuolation, degeneration and depletion of elongating spermatids (Fig 2) and spermatid retention (Fig. 3). In one rat (graded slight severity) the germ cell degeneration and depletion affected spermatids and spermatocytes and was present in more tubules than the remainder of the group.

Epididymides: In G4, most epididymides had severely reduced, or absent sperm content as well as the presence of cell debris, that had originated from the testes. In most cases, the ducts were atrophic due to the reduction/absence of sperm. When sperm was present, it was located in the cauda epididymis, leaving the corpus and caput empty. The findings may be secondary to the tubular degeneration/atrophy present in the testes of these animals. In G3, 10/12 epididymides contained cell debris (Fig 4b). All of the animals with testicular changes contained cell debris, reflecting minor sloughing of germ cells from affected seminiferous tubules, but there were also a few G3 rats that had minimal cell debris in the

epididymides but had normal appearing testes. This is explained by the fact that epididymal cell debris is generally considered a more sensitive indicator of testicular germ cell sloughing than testicular histopathology. In G2, 5/12 rats had epididymal cell debris but had normal appearing testes.

Histopathological changes in the testes are consistent with the known effects of EGME on spermatogenesis. EGME is known to target the spermatocyte within 24 h of dosing (Foster *et al.*, 1984, Chapin *et al.*, 1985), but with continued dosing, progressive maturation depletion and/or degeneration of subsequent cell types (round and elongating spermatids) were observed.

As per earlier report, EGME causes its maximal effect on fertility parameters after at least 6 weeks of dosing (Anderson *et al.*, 1987). Similarly, in current study, in male rats dosed only for 28 days before mating, the changes in fertility parameters were evident. Males necropsied after 42 days of dosing showed severe testicular toxicity and decreased sperm parameters in the high dose group and less severe changes down to the low dose group. The severity of the testicular toxicity in the high dose group reflects the effects of the long duration dosing, which resulted in an end

stage lesion comprising depletion of most germ cells except prepachytene spermatocytes and spermatogonia, as well as severe depletion of epididymal sperm.

Sperm Evaluation and Reproductive Performance/Parameters:

Fertility index (%) in the EGME dosed groups declined compared to the vehicle control group (G1), and more time (days) was required for females to conceive. Statistically significant ($p < 0.05$) decreases were observed in the number of corpora lutea and % implantation sites in G4 group females. Dose related, statistically significant ($p < 0.05$) decreases were observed in total sperm count, viability and sperm motility in G3 and G4 males, while in G2 males, a significant ($p < 0.05$) decrease in sperm motility was observed. Sperm abnormality was seen more in EGME dosed groups as compared to the control males. The presence of sperm changes in EGME treated groups correlates with the histopathological abnormalities in the testes and epididymides.

In this study, the low dose was expected to be a NOEL, however, there was a slight diminution in total sperm count, sperm motility and sperm viability and an increase in abnormal sperm. Although not statistically significant, they correlated with a significant decrease in fertility index for this group and are considered to be related to EGME treatment. Histologically, there were no detectable changes in the testes although there was an increased incidence of minimal cell debris in the epididymis, indicating some germ cell sloughing from the testis.

In the mid dose group (40mg/kg/day), there were statistically significant ($p < 0.05$) decreases in total sperm count, sperm motility and in the number of abnormal sperm. These abnormalities were consistent with microscopic evidence of germ cell depletion and degeneration in the testis, which mostly affected the spermatid population, as well as sloughed germ cells (luminal cell debris) in the epididymis. The decreased sperm parameters and histopathological changes correlated with a significantly decreased fertility index in this group. These effects in the high dose group (80mg/kg/day) were more marked than those

seen in the mid dose group. All animals in the group showed severe tubular degeneration/atrophy in the testes and reduced sperm with ductal atrophy in the epididymides. The findings correlated with marked decreases in total sperm count, sperm motility and viability and increased abnormal sperm. The fertility index in this group was only 25%. In addition, the time taken for females to conceive was increased.

In this study females and males were dosed and therefore the possibility exists that the affected fertility parameters (fertility index, time to conception, number of corpora lutea, number of live fetuses and number of implantation sites) could be the result of female mediated toxicity rather than male-mediated toxicity, or could be a mixture of both. However, based on the results of previous studies (Davis *et al.*, 1997, Dodo *et al.*, 2009), female reproductive toxicity of EGME was only seen at doses ≥ 100 mg/kg/day. In those studies, effects included suppression of cyclicity and hypertrophy of the corpora lutea. Contrastingly, in the current study, females were dosed at < 100 mg/kg/day, so the changes in fertility parameters are considered to be male-mediated. The EGME-related changes in fertility parameters included a dose-related decrease in the number of corpora lutea, live fetuses, and implantation sites. Most of the implantation loss might be due to preimplantation failure, which is consistent with the decreased numbers and motility of sperm. The small increase in post implantation loss was not dose related and is considered incidental. This is consistent with the study reported by Anderson *et al.*, (1987).

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References

- Anderson, D., Brinkworth, M.H., Jenkinson, P.C., Clode, S.A., Creasy, D.M., Gangolli, S.D. (1987). Effect of ethylene glycol monomethyl ether on spermatogenesis, dominant lethality, and F1 abnormalities in the rat and the mouse after

- treatment of F0 males. *Teratogenesis, Carcinogenesis, and Mutagenesis*, 7(2):141–158.
- Chapin, R.E., Dutton, S.L., Ross, M.D., Lamb, J.C. 1985. Effects of ethylene glycol monomethyl ether (EGME) on mating performance and epididymal sperm parameters in F344 rats. *Fundam. Appl. Toxicol.*, 5: 182–189. [https://doi.org/10.1016/02720590\(85\)90063-6](https://doi.org/10.1016/02720590(85)90063-6)
- Cherry, N., Moore, H., McNamee, R., Pacey, A., Burgess, G., Clyma, J.-A., Dipnall, M., Baillie, H., Povey, A., participating centres of Chaps-UK. 2008. Occupation and male infertility: glycol ethers and other exposures. *Occup. Environ. Med.* 65, 708–714. <https://doi.org/10.1136/oem.2007.035824>
- Dodo, T., Taketa, Y., Sugiyama, M., Inomata, A., Sonoda, J., Okuda, Y., Mineshima, H., Hosokawa, S., Aoki, T. 2009. Collaboration work on evaluation of ovarian toxicity 11) Two- or four-week repeated-dose studies and fertility study of ethylene glycol monomethyl ether in female rats. *J. Toxicol. Sci.*, 34: Suppl. 1: SP 121-SP128.
- Ethylene Glycol Monoethyl Ether | OEHA [WWW Document]. 1989. URL <https://oeha.ca.gov/proposition-65/chemicals/ethylene-glycol-monoethyl-ether> (accessed 5.3.19).
- Foster, P.M., Creasy, D.M., Foster, J.R., Gray, T.J., 1984. Testicular toxicity produced by ethylene glycol monomethyl and monoethyl ethers in the rat. *Environ. Health Perspect.* 57:207–217. <https://doi.org/10.1289/ehp.8457207>
- Gold, E.B., Eskenazi, B., Hammond, S.K., Lasley, B.L., Samuels, S.J., O'Neill, R., Rasor, M., Hines, C.J., Overstreet, J.W., Schenker, M.B. 1995. Prospectively assessed menstrual cycle characteristics in female wafer-fabrication and nonfabrication semiconductor employees. *Am. J. Ind. Med.*, 28: 799–815.
- Hood, R. D., and Hood, R. D. 2005. Developmental and Reproductive Toxicology: A Practical Approach. Page: 444-458. CRC Press. Retrieved from <https://books.google.co.in/books?id=8X7LBQAAQBAJ>,
- Johanson, G., 2000. Toxicity Review of Ethylene Glycol Monomethyl Ether and its Acetate Ester. *Crit. Rev. Toxicol.*, 30: 307–345. <https://doi.org/10.1080/10408440091159220>
- Multigner, L., Ben Brik, E., Arnaud, I., Haguenoer, J.M., Jouannet, P., Auger, J., Eustache, F. 2007. Glycol ethers and semen quality: a cross-sectional study among male workers in the Paris Municipality. *Occup. Environ. Med.*, 64: 467–473. <https://doi.org/10.1136/oem.2005.023952>
- Watanabe, A., Nakano, Y., Endo, T., Sato, N., Kai, K., Shiraiwa, K. 2000. Collaborative work to evaluate toxicity on male reproductive organs by repeated dose studies in rats 27). Repeated toxicity study on ethylene glycol monomethyl ether for 2 and 4 weeks to detect effects on male reproductive organs in rats. *J. Toxicol. Sci.*, 25: 259-266.
- Welch, L.S., Schrader, S.M., Turner, T.W., Cullen, M.R. 1988. Effects of exposure to ethylene glycol ethers on shipyard painters: II. Male reproduction. *Am. J. Ind. Med.*, 14:509–526. ■



Screening of sub-clinical mastitis in dairy cows of organised farms using three diagnostic tests



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Abstract

The present study was envisaged for the detection of subclinical mastitis in 108 apparently healthy dairy cows of University Livestock Farm, Mannuthy and Livestock Research Station, Thiruvazhamkunnu. The quarter milk samples collected under sterile conditions were screened using California mastitis test, somatic cell counting and electrical conductivity. A high prevalence of 54 per cent was recorded in the present study. The results indicated the importance of maintaining proper hygienic practices inside farm and good udder health management to enhance quality milk production.

Keywords: Subclinical mastitis, Screening tests, Prevalence study, Thrissur district.

Subclinical mastitis (SCM) is the inflammation of the mammary gland that does not create any visible changes in the milk or of the udder (Langer *et al.* 2014). Even though the milk appears normal, SCM can result in 10-20 per cent loss in total yield and causes deterioration in quality and nutritional value of milk produced. Hence, early detection of subclinical mastitis for proper management is of great importance to reduce its undesirable effects. Absence of any visible abnormalities in milk demands the requirement of special diagnostic aids for early detection of SCM. Several cow side tests such as California Mastitis Test (CMT), modified white side test (MWT), bromothymol blue card test, determination of electrical conductivity, chloride estimation test, modified Aulendorfer mastitis probe test (MAMP), inline monitoring of SCC and infrared thermography have been used for SCM screening at field level (Kamphuis *et al.*, 2008). Laboratory tests used for screening include N-acetyl- β -D-glucosaminidase (NAGase), enzyme-linked immunosorbent assay

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(ELISA) (Polat *et al.*, 2010) and acute phase protein determination in milk and in serum (More, 2009). The microbiological status of the quarter and the somatic cell count (SCC) are the most common tests to detect changes in the milk because of an inflammatory process.

Materials and methods

The study was conducted apparently healthy dairy cows of University Livestock Farm, Mannuthy and Livestock Research Station, Thiruvizhamkunnu during the period October 2017 to January 2018.

A total of 420 quarter milk samples from 108 apparently healthy cows were aseptically collected for screening of SCM using CMT, SCC and EC score. Twelve quarters were found to lack any kind of secretions. About 5 mL of the milk was collected into a sterile sample collection vial after discarding initial squirts of milk for bacterial isolation.

When all the three diagnostic tests are considered together, the animals having minimum of two quarter CMT grade above 2, SCC value above 200000 cells/ml and EC score below 300 were considered positive for SCM. The criteria to classify the affected and healthy quarters were followed as described by Raj (2017).

California mastitis test (CMT)

The CMT was performed using reagents obtained from Nice Chemicals Pvt. Ltd. Kochi, Kerala. A plastic paddle with four chambers was used to perform the test. Milk from each quarter was drawn to separate chambers. An equal quantity of milk was mixed with the mastitis reagent and mixed well. The paddle was shaken gently in a rotating manner in a horizontal plane and the reaction was scored within 15 sec.

Table 1. Interpretation of CMT results

Sl. No.	CMT score	Interpretation
1	0	No reaction or trace
2	1	Weak positive
3	2	Moderate positive
4	3	Strong positive

The samples with CMT scores above two for minimum two quarters were considered positive for SCM

Somatic cell count (SCC)

The milk samples were mixed well by shaking the vials and 10 microliters of milk was drawn using a micropipette into the pre-drawn one cm² marked glass slides. Milk was spread uniformly over the pre-drawn area using a standard sterilized bacteriological platinum loop and the smear was dried at room temperature. The dried smears were stained by Modified Newman's stain by keeping the smear in the staining solution for 5 min. The smears were gently washed in tap water, dried and examined under oil immersion objective of microscope to count cells in ten fields. The average of the cells per field was multiplied by the microscopic factor (4500).

The total number of cells per millilitre of milk was calculated by the formula:

Cell count per millilitre of milk = Average number of cells per field × 4500 × 100

The quarters with an SCC value of more than 200000 cells/ml of milk were considered positive for SCM.

Electrical conductivity score (EC)

A commercial multi quarter hand held electrical conductivity meter (Draminski, Poland) was used for the detection of EC. The procedure was followed under the manufacturer's instructions. Based on the EC, values were assigned to the samples by the machine. The values below 300 were taken as positive for subclinical mastitis. The quarters with an EC score below 300 were taken as positive for SCM.

Results and Discussion

The results of three screening tests for detection of SCM are depicted in the Fig (1). Out of 420 quarter samples screened 195 samples were categorised as grade '0', 62 were grade '1', 108 samples were grade '2' and 55 samples were grade '3'. The results indicated that 163 (39 per cent) animals had a CMT score above grade 2 and were considered positive by CMT.

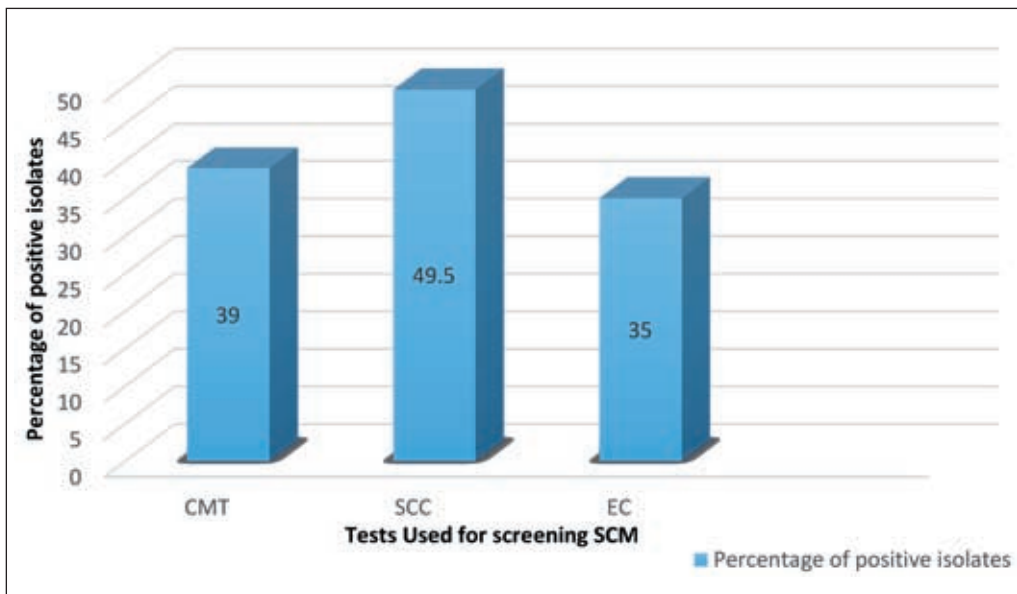


Fig. 1 Results of three diagnostic tests used for screening subclinical mastitis

The results were agreement with the results obtained by Islam *et al.* (2010) with 37.58 per cent positive cases. The variation in CMT value in different studies may be attributed to the differences in management practices, breed of the animal and geographical locations.

Among 420 samples, 72 samples had SCC score below 100000 cells/ml, 140 samples had SCC value between 100000 and 200000 cells/ml, 128 samples had value between 200000 and 500000 cells/ml and 80 samples had SCC value above 500000 cells/ml of milk. Out of total quarters tested, 49.5 per cent of quarters had an SCC value above 200000 cells/ml and were considered positive by SCC value. A much higher prevalence of 71.9 per cent positive cases of SCM was observed in a study conducted in Bangladesh by Hoque *et al* (2015). Apart from IMI, SCC of bovine milk can show variations depending upon the lactation period of cow, age, breed, stress level and management practices adopted (Sargeant *et al.*, 2001 and Pyorala, 2003).

The EC values interpreted using Draminski EC meter grouped 273 quarter samples as >300 score, 107 samples between 250 and 300 and 40 samples below 250.

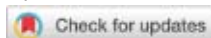
Out of total quarters tested, 35 per cent of quarters had an EC value below 300 and were considered positive by EC value. The results were contradictory to that obtained by Langer *et al.* (2014) and Raj *et al.* (2017) which were 11.3 per cent and 14 per cent respectively. Many other factors can contribute to variations in conductivity of milk such as temperature, diseases other than mastitis, breed, environmental conditions, milk fat and stage of lactation (Panchal *et al.*, 2016).

The study indicated variations in test results for the same animal in detection of true positive animals having SCM infections when a single test is used. A combination of two or more tests should be hence employed for accurate screening of SCM. In the present study, from among 108 cows, 58 animals (54 per cent) which had at least two quarters CMT grade above 2, EC value below 300 and SCC value above 200000 cells/ml were considered positive for SCM. The results were similar to the findings obtained by Sharma *et al.* (2010) who got an overall prevalence of SCM at cow level as 54 per cent. Even though, pathogen identification by culture technique is considered as gold standard diagnostic test, the time and labour consumption makes it less applicable for field level diagnosis.

References

- Hoque, M.N., Das, Z.C., Talukder, A.K., Alam, M.S. and Rahman, A.N.M.A. 2015. Different screening tests and milk somatic cell count for the prevalence of subclinical bovine mastitis in Bangladesh. *Trop. Anim. Hlth. prod.* **47**:79-86.
- Islam, M.A., Rahman, A.K.M.A., Rony, S.A. and Islam, M.S. 2010. Prevalence and risk factors of mastitis in lactating dairy cows at Baghabari milk shed area of Sirajganj. *Bangladesh J. Vet. Med.* **8**:157-162.
- Kamphuis, C., Sherlock, R., Jago, J., Mein, G. and Hogeveen, H. 2008. Automatic detection of clinical mastitis is improved by in-line monitoring of somatic cell count. *J. Dairy Sci.* **91**:4560-4570.
- Langer, A., Sharma, S., Sharma, N.K. and Nauriyal, D.S. 2014. Comparative efficacy of different mastitis markers for diagnosis of sub-clinical mastitis in cows. *Int. J. Appl. Sci. Biotechnol.* **2**:121-125.
- More, S.J., 2009. Global trends in milk quality: implications for the Irish dairy industry. *Irish Vet. J.* **62**:S4-S5.
- Panchal, I., Sawhney, I.K. and Dang, A.K. 2016. Relation between electrical conductivity, dielectric constant, somatic cell count and some other milk quality parameters in diagnosis of subclinical mastitis in Murrah buffaloes. *Indian J. Dairy Sci.* **69**:453-462.
- Polat, B., Colak, A., Cengiz, M., Yanmaz, L.E., Oral, H., Bastan, A., Kaya, S. and Hayirli, A. 2010. Sensitivity and specificity of infrared thermography in detection of subclinical mastitis in dairy cows. *J. Dairy Sci.* **93**:3525-3532.
- Pyorala, S. 2003. Indicators of inflammation in the diagnosis of mastitis. *Vet. Res.* **34**:565-578.
- Sargeant, J.M., Leslie, K.E., Shirley, J.E., Pulkabek, B.J. and Lim, G.H. 2001. Sensitivity and specificity of somatic cell count and California Mastitis Test for identifying intramammary infection in early lactation. *J. Dairy Sci.* **84**:2018-2024.
- Raj, 2017. Acute phase proteins in serum and milk as diagnostic tools in bovine subclinical mastitis. M.V.Sc thesis, Kerala Veterinary and Animal Sciences University, Pookode, 91p.
- Sharma, N., Pandey, V. and Sudhan, N.A. 2010. Comparison of some indirect screening tests for detection of subclinical mastitis in dairy cows. *Bulgarian J. Vet. Med.* **13**:43-54.
- Smith, K.L., 1999. Suggested interpretation of mastitis terminology. *Bull. Int. Dairy Fed.* **338**:3-26.

■



Production of zinc enriched designer eggs through dietary supplementation



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Abstract

Designer eggs have high market demand because of the consumers' willingness to purchase them owing to its' nutritional qualities additional to regular eggs. The current study was conducted to produce zinc enriched eggs by supplementing laying hens' diet with required levels of zinc as inorganic zinc sulphate (75mg/kg). Thirty-two number of crossbred (White Leghorn N strain and Desi) layer birds were given zinc enriched diet from 29 weeks of age for 12 weeks. The concentration of zinc in egg of birds supplemented with zinc diet was significantly higher ($p < 0.01$) than that of unsupplemented group. The egg zinc concentration of supplemented birds was elevated from 33.36 ± 0.89 ppm (control group) to 40.85 ± 0.47 ppm (supplemented group)

Keywords: Designer eggs, Zinc sulphate

Zinc is an essential trace micronutrient mineral which is an integral part of over 300 enzymes involved in carbohydrate, nucleic acid and protein metabolism. Zinc has a systemic role in fertility, antioxidation, skeletal and neurobehavioural functions. In addition, zinc plays a key role in the immune system, transport and the use of vitamin A. Many plant foods have high zinc content particularly cereal grains and legumes, but bioavailability is less due to presence of phytates. Though the richest zinc sources are animal products such as beef, pork and shellfish, they are expensive. Therefore, zinc deficiency is widely felt in human population. The zinc content of hen's eggs can be upgraded by relatively simple and economical dietary approaches and can be marketed at reasonable price.

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Materials and Methods

Experimental layout

The study was conducted using thirty-two numbers of 28 weeks old crossbred (White Leghorn N strain X Desi) layer birds procured from All India Coordinated Research Projects (AICRP) on Poultry for Eggs, Mannuthy, KVASU. The birds were distributed in a completely randomized experimental design and they were placed into two treatment groups, each with four replicates having four birds in each replicate. All birds were fed with a standard layer diet and raised under standard managemental conditions up to 28 weeks of age at AICRP on Poultry for Eggs Mannuthy, KVASU. The birds were immunized against diseases as per standard protocol followed in the farm. On completion of 28 weeks of age, the birds were brought and housed in well ventilated cages of animal house attached to Department of Veterinary Physiology. They were kept at $24.5 \pm 0.5^\circ\text{C}$ ambient temperature and at relative humidity ranging from 60-80 per cent. A photoperiod of 16 h per day was ensured throughout the period.

Feeding of birds with zinc enriched diet

Zinc level in control diet was formulated according to BIS 2007 standards. Treatment group was provided with a diet incorporating inorganic zinc sulphate @ 75 mg/kg mash diet. The total ration was provided as a single lot in the morning. Water was provided *ad libitum* throughout the experimental period of 84 days.

Estimation of zinc content of egg

One egg was randomly selected from each treatment group at weekly interval and the contents of raw egg were subjected for microwave acid digestion to estimate Zn content. Three mL of pooled whole egg sample

was subjected for digestion using 6.0 mL of HNO_3 and 1.0 mL of H_2O_2 in teflon digestion vessel. The mixture was carefully stirred with a clean glass rod for 10 min before closing the vessel. Microwave digestion was set with the following program (Table 1).

The digested samples were analysed for Zn by Atomic Absorption Spectrometry standard method, (AOAC., 2016). The PinAAcle 900H Series AAS enabled a simple automated measurement of Zn using hollow cathode lamp of wavelength 213.86 nm and lamp current of 15 mA. Calibration curve was plotted with standards ranging from 0 to 6 ppm with a calibration equation non-linear through zero. Slit width of 0.7nm ensured accurate analysis. The flame atomizer with a temperature of 2400°C , was ideal for the measurement. Fuel and oxidant gases were acetylene (2.5 L/min) and air (10 L/min) respectively.

Statistical analysis

Results were expressed as means ($\pm\text{SE}$). The statistical significance of difference or relation between the two treatments were analysed by CRD using the software Statistical Product and Services (SPSS) version 24.0 and the differences were considered statistically significant at 5% level ($p < 0.05$) (Snedecor and Cochran, 1994).

Results and Discussion

Zinc content in egg sample

Consumers are always driving the market for a new category of food with health benefits beyond the traditionally value. Zinc enriched eggs in the market have a potential demand because of its systemic role in immunocompetence, skeletal and neurobehavioural development.

Table 1: Microwave digestion programme

Step	Target temperature ($^\circ\text{C}$)	Pressure (max) (bar)	Ramp time (min)	Hold time (min)	Power (%)
1	165	30	5	10	50
2	190	30	5	20	80
3	50	30	1	10	0

Table 2: Mean value of zinc concentration (mg/L (ppm)) in eggs of different treatment groups

Treatment group	No of samples	Mean value of amount (ppm)	p-value	F value
		Mean±S.E.		
T1	12	33.36 ^a ±0.89	0.000**	31.798
T2	12	40.85 ^b ±0.47		

Means bearing same superscript within a column do not differ significantly ($p < 0.05$)

** Highly significant ($p < 0.01$)

Samples subjected for microwave digestion were analyzed for zinc by AAS and zinc concentration in egg yolk, when hens were supplemented with inorganic zinc sulphate at a level of 75 mg / kg mash diet are presented in the table 2

The effect of zinc feeding was highly significant ($p < 0.01$) for zinc concentration range in eggs. It was noticed that hens fed the diet enriched with inorganic zinc sulphate exhibited higher zinc level in eggs compared to other group under conventional feed. Supplemented zinc resulted in significant difference in zinc content of eggs. It was clearly noticed that egg zinc content increased significantly ($p < 0.01$) by increasing the zinc level to 75 mg/Kg diet.

In the present study, the mean zinc content in diet supplemented group (T2) was significantly ($p < 0.01$) greater than basal diet fed group (T1) as represented in table 2. These findings were completely agreed with the data of Kim and Patterson (2005) who confirmed that, linear increase in zinc supplementation through diet could result more pronounced incorporation of zinc in eggs. The present study findings are also supported by Stahl *et al.* (1988) who found an increase of 57-95 % zinc in eggs while feeding hens with elevated levels of zinc at the rate of 1861 mg/kg diet. This linear increment in zinc content of eggs through dietary supplementation could be attributed to the production of a trace mineral transporting protein called vitellogenin. Vitellogenin transport zinc from liver storage to ovaries (Richards, 1997).

Cousins (1985) reported that excess supplementation of zinc through diet would make the absorptive membrane more leaky,

which allowed zinc to enter the cell and bind non-specifically to cell proteins and other ligands. Hence an optimum level of dietary supplementation (75mg/kg) was selected for the present study, which had yielded Zn enriched eggs. As per BIS 2007, the recommended zinc requirement of layer birds (21-45 weeks) was 60 mg/kg.

Reports of Williams *et al.* (1989), Plaimast *et al.* (2008), Bahakaim *et al.* (2014) and Aghaei *et al.* (2017) indicated a strong positive correlation between dietary as well as yolk content of zinc. The zinc content of enriched eggs obtained in the current study, 40.85±0.47 ppm (T2) was comparable with the results Kaya *et al.* (2001) who reported a zinc level of 27.62 to 36.69 µg/g egg.

On contrary, when 80 mg Zn was introduced to the hens' basal diet, Skrivan *et al.* (2005) found no major variations in egg yolk, white and shell zinc content and they attributed the antagonistic effects of Zn and Cu as one of the reasons for non enrichment of eggs.

Conclusion

The dietary supplementation of hens with zinc is an effective approach to supply consumers with foods from animal sources that are enriched with zinc. Current findings showed that feeding of zinc supplemented diet to chickens offered a promising measure to enrich egg with zinc.

Ethical approval and consent statement

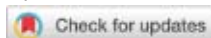
The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Mannuthy.

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References

- Aghai, A., Khosravinia, H., Mamuoiei, M., Azarfar, A. and Shahriari, A. 2017. Effects of dietary supplementation of zinc and α -tocopheryl acetate on performance and zinc concentrations in egg and tissues of Japanese quails. *Poult. Sci. J.* **5**: 57-64.
- Bahakaim, A., Abdel Magied, H., Osman, S., Omar, A., Abdel Malak, N. Y. and Ramadan, N. 2014. Effect of using different levels and sources of zinc in layer's diets on egg zinc enrichment. *Egypt. Poult. Sci. J.* **34**: 39-56.
- Cousins, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol. Rev.* **65**: 238-309.
- Kaya, S., Umucalilar, H. D., Haliloglu, S. and Ipek, H. 2001. Effect of dietary vitamin A and zinc on egg yield and some blood parameters of laying hens. *Turk. J. Vet. Anim. Sci.* **25**: 763-769.
- Kim, W. K. and Patterson, P. H. 2005. Effects of dietary zinc supplementation on hen performance, ammonia volatilization, and nitrogen retention in manure. *J. Environ. Sci. Health B.* **40**: 675-686.
- Plaimast, H., Sirichakwal, P., Puwastien, P. and Kijparkorn, S. 2008. Effect of supplementary zinc from organic and inorganic sources on laying performance and zinc deposition in eggs. *Thai. J. Vet. Med.* **38**: 47-53.
- Richards, M. P. 1997. Trace mineral metabolism in the avian embryo. *Poult. Sci.* **76**: 152-164.
- Skrivan, M., Skrivanova, V. and Marounek, M. 2005. Effects of dietary zinc, iron, and copper in layer feed on distribution of these elements in eggs, liver, excreta, soil, and herbage. *Poult. Sci.* **84**: 1570-1575.
- Snedecor, G. W. and Cochran, W. G. 1994. *Statistical Methods.* (8th Ed.). Oxford and IBM Publishing Company, New Delhi, 313p.
- Stahl, J. L., Cook, M. E. and Greger, J. L. 1988. Zinc, iron, and copper contents of eggs from hens fed varying levels of zinc. *J. Food Compos. Anal.* **1**: 309-315.
- Williams, S. N., Miles, R. D., Quart, M. D. and Campbell, D. R. 1989. Short-term high level zinc feeding and tissue zinc concentration in mature laying hens. ■



Evaluation of haematological and serum biochemical profile of propofol induced isoflurane anaesthesia in geriatric dogs premedicated with diazepam and butorphanol



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Abstract

Six geriatric dogs of various breeds belonging to either sex presented for various surgical procedures to Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kottalai, were selected for the study. On the day of surgery, preanaesthesia was performed with diazepam and butorphanol. After ten minutes of premedication, preoxygenation was carried out for three minutes. Propofol (1 per cent w/v) was administered as a slow bolus, intravenous injection for induction of anaesthesia. Maintenance of surgical plane of anaesthesia was carried out with isoflurane in oxygen using Bain's circuit system incorporated with isoflurane vapourizer. Haematological and serum biochemical parameters, which were recorded before premedication, ten minutes after commencement of isoflurane and after recovery from general anaesthesia are described here. The present study concluded that the anaesthetic protocol is safe in geriatric animals with regard to haematological and serum biochemical parameters.

Keywords: geriatric dogs, anaesthesia, haematological and serum parameters

Geriatric patients are defined as those individuals that have completed 75 to 80 per cent of their expected life span (Baetge and Matthews, 2012). According to Joubert (2007) it is necessary to evaluate the geriatric patient before anaesthesia to know the underlying disease conditions. Hence the drugs used for anaesthesia in these animals must be titrated in their dose

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and administered according to the health of the animal. This paper places on record of haematological and serum biochemical profile of propofol induced isoflurane anaesthesia in geriatric dogs premedicated with diazepam and butorphanol.

Materials and Methods

Six geriatric dogs aged from ten to nineteen years of various breeds of either sex presented to the University Veterinary Hospitals, at Mannuthy and Kokkalai of Kerala Veterinary and Animal Sciences University for various surgical procedures were selected for the study. All the animals were subjected for thorough preanaesthetic evaluation before surgery. Food was withheld for 12 hours and water for six hours before administration of the anaesthesia. Diazepam at the dose rate of 0.25mg per kg body weight and butorphanol at the dose rate of 0.2mg per kg body weight were administered intravenously as pre-anaesthetic medication at one minute interval. After ten minutes of premedication, pre-oxygenation was carried out in all animals for three minutes. After pre-oxygenation, propofol (1% w/v) emulsion was administered as slow intravenous bolus injection, to effect general anaesthesia. Soon after induction animals were intubated with appropriate sized endotracheal tube. Anaesthesia was maintained with isoflurane in oxygen at the rate of 100 mL/kg body weight, by using Bain's circuit system incorporated with isoflurane vapourizer.

All the vital parameters were monitored during preanaesthesia, maintenance and recovery period. Blood sample for haematological and serum biochemical studies was collected and analysed before premedication, ten minutes after commencement of isoflurane administration and after recovery from general anaesthesia. The parameters like rectal temperature, pulse rate, respiration rate, heart rate, capillary refilling time and colour of mucus membrane were observed after administration of anaesthesia in every five minutes were recorded. The animals were prepared for the relevant surgical procedures, under strict aseptic conditions and surgery was performed as per standard procedures.

Amoxicillin at the dose rate of 10 mg per kg body weight IV and meloxicam at the dose rate of 0.2 mg per kg body weight IM was administered before the surgery to all the animals. Postoperatively, animals were maintained with antibiotic and anti-inflammatory drugs orally for five consecutive days.

Results and Discussion

The mean age of geriatric dogs selected for the study was 13.33 ± 1.33 years and the mean body weight was 15.8 ± 2.40 kg. The mean values of the haematological and biochemical parameters are expressed in Table 1 and 2.

The mean value of total erythrocyte count and the mean total leucocyte count decreased after ten minutes of isoflurane commencement with non-significant and significant changes respectively and returned to baseline values after recovery from surgical plane of general anaesthesia with significant and non-significant changes respectively. Similar results were obtained with propofol-isoflurane anaesthesia in dogs by Kavechiya (2010). The reason for decreased total erythrocyte count might be the pooling of red blood cells in the spleen because of stimulated adrenocortical area and the interstitial fluid migrating in to the circulating compartment (Sankar *et al.*, 2011). Isoflurane is more immunosuppressive compared to propofol and thus might have resulted in the decreased leucocyte count due to changes in the anti-inflammatory cytokines during trans-anaesthetic period (Tomihari *et al.*, 2015).

The mean lymphocyte count increased non-significantly while mean monocytes per cent increased significantly after ten minutes of isoflurane commencement which returned to baseline values after recovery from anaesthesia without any significant change. The mean granulocytes per cent decreased after ten minutes of isoflurane commencement which returned to baseline values after recovery from anaesthesia without any significant change. The results are in accordance with the observations made by Kavechiya (2010), Ramankutty (2008) and Zlateva and Marinov (2015) respectively

Table 1. Observations on haematological parameters (Mean \pm SE) n=6

Parameters	Before premedication	Ten minutes after commencement of Isoflurane administration	After recovery from general anaesthesia.
Total erythrocyte count ($10^6/\mu\text{L}$)	4.62 \pm 0.2	3.47 \pm 0.22	4.59 \pm 0.36*
Total leucocyte count ($10^3/\mu\text{L}$)	14.92 \pm 2.04	10.07 \pm 1.84*	12.97 \pm 2.07
Lymphocyte (%)	22.5 \pm 2.63	26.93 \pm 5.69	24.57 \pm 3.27
Monocytes (%)	6.27 \pm 0.4	7.53 \pm 0.42*	6.92 \pm 0.57
Granulocytes (%)	71.23 \pm 2.84	65.62 \pm 5.95	69.52 \pm 3.38
Haemoglobin (g/dL)	9.83 \pm 1.20	7.78 \pm 0.91	9.57 \pm 1.3
Volume of packed red cells (%)	26.27 \pm 3.17	20.52 \pm 1.78	23.77 \pm 3.27

Means with ** as superscript within a row differ significantly at 5% significance level

Table 2. Observations on serum biochemistry profile (Mean \pm SE) n=6

Parameters	Before premedication	Ten minutes after commencement of Isoflurane administration	After recovery from general anaesthesia.
Alanine aminotransferase (IU/L)	46.26 \pm 10.9	39.43 \pm 7.76	33.42 \pm 6.88
Aspartate aminotransferase (IU/L)	32.28 \pm 6.07	26.3 \pm 2.15	32.29 \pm 4.0
Blood Urea Nitrogen (mg/dL)	13.59 \pm 3.34	9.07 \pm 1.17	8.1 \pm 1.34
Creatinine (mg/dL)	1.11 \pm 0.15	1.01 \pm 0.03	1.01 \pm 0.04

Means with ** as superscript within a row differ significantly at 5% significance level

for changes of lymphocyte, monocyte and granulocyte per cent during propofol-isoflurane anaesthesia. The reason could be stimulation of lymphocytes and neutrophils by glucocorticoids due to stimulation at adrenocortical region during general anaesthesia (Brand *et al.*, 2003).

The mean haemoglobin, volume of packed red cells, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen and creatinine decreased after ten minutes of isoflurane commencement and returned to baseline values after recovery from anaesthesia without any significance. The results are in agreement with the observations made by Sen and Kilic (2018) with propofol-isoflurane anaesthesia in geriatric dogs. According to Sankar *et al.* (2011) the reason for the decrease in haemoglobin concentration could be due to pooling of erythrocytes in to the spleen. Decreased total erythrocyte count might have also attributed to the decrease in the

haemoglobin level during general anaesthesia. During general anaesthesia the interstitial fluid migrating in to the circulating compartment (Sankar *et al.*, 2011), might also have led to haemo dilution and subsequent reduction in volume of packed red cells values (Kavechiya, 2010). The reason for the reduction in hepatic function parameters during anaesthetic period could be due to hepatic hypoperfusion with anaesthetic agents (Meierhenrich *et al.* 2009). Since liver and kidney functions will not be altered by isoflurane anaesthesia (Sen and Kilic, 2018), the values like creatinine and blood urea nitrogen were within the normal range in all the animals under study.

Conclusion

The quality of sedation, induction, maintenance and recovery from general anaesthesia were good without any complications. The present anaesthetic protocol was found be safe in the geriatric

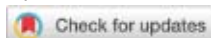
animals with regard to haematological and serum biochemical parameters as the variations remained within the normal acceptable range.

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References

- Baetge, C.L. and Matthews, N.S. 2012. Anesthesia and analgesia for geriatric veterinary patients. *Vet. Clin. N. Am. Small Anim. Pract.* **42**: 643-653.
- Brand, J.M., Frohn, C., Luhm, J., Kirchner, H. and Schmucker, P. 2003. Early alterations in the number of circulating lymphocyte subpopulations and enhanced proinflammatory immune response during opioid-based general anesthesia. *Shock*. **20**: 213-217.
- Joubert, K.E. 2007. Pre-anaesthetic screening of geriatric dogs. *J. S. Afri. Vet. Ass.* **78**: 31-35.
- Kavechiya, V.P. 2010. Studies on balanced anaesthesia using butorphanol-acepromazine-glycopyrrolate (BAG) as preanaesthetic to ketamine-diazepam, ketamine-midazolam, propofol and isoflurane maintenance in canines. *M.V.Sc. thesis*, Anand Agricultural University, Anand, 137p.
- Meierhenrich, R., Wagner, F., Schutz, W., Rockemann, M., Steffen, P., Senftleben, U. and Gauss, A. 2009. The effects of thoracic epidural anesthesia on hepatic blood flow in patients under general anesthesia. *Anaesth. Analg.* **108**: 1331-1337.
- Ramankutty, S. 2008. Clinical evaluation of propofol-isoflurane anaesthesia with xylazine premedication in dogs. *M.V.Sc. thesis*, Kerala Agricultural University, Thrissur, 137p.
- Sankar, P., William, B.J., Rao, G.D., Prathaban, S., Kumar, R.S. and Leela, V. 2011. Cardiopulmonary and haematobiochemical alterations during ketamine or propofol anaesthesia in acepromazine-xylazine premedicated horses. *Indian J. Vet. Surg.* **32**: 23-26.
- Sen, Z.B. and Kilic, N. 2018. General anesthesia in geriatric dogs with propofol-isoflurane, propofol-sevoflurane, alphaxalone-isoflurane, alphaxalone-sevoflurane and their comparison of biochemical, hemodynamic and cardiopulmonary effects. *Acta. Sci. Vet.* **46**: 1-9.
- Tomihari, M., Nishihara, A., Shimada, T., Yanagawa, M., Miyoshi, M., Miyahara, K. and Oishi, A. 2015. A comparison of the immunological effects of propofol and isoflurane for maintenance of anesthesia in healthy dogs. *J. Vet. Med. Sci.* **77**: 1227-1233.
- Zlateva, N. and Marinov, G. 2015. Effect of three anesthetic protocols on the haematological indices in cats during ovariohysterectomy. *Med. Info.* **2**: 184-193.



Identification of deer and goat species from skin samples-a DNA barcoding approach

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Abstract

DNA barcoding is a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed-upon position in the genome. In the present study, DNA barcoding was used as a technology to differentiate deer and goat species from the skin samples. The samples were collected from different regions of the skin, viz. ear pinna, dorsal abdomen, muzzle and interdigital space of forelimb of deer brought for post mortem at College of Veterinary and Animal Sciences, from Thrissur zoo and forest department and of goat freshly slaughtered in Meat Technology Unit, Mannuthy. Samples were chopped and preserved in ethanol and processed. The mitochondrial sequence of Cytochrome C Oxidase subunit (COI) was deliberated as a barcode marker suited best for species identification of the animals and based on this the samples were identified as *Cervus unicolor*, *Rusa timorensis* and *Capra hircus*. The results recommended that unidentified specimens from wild animals can be recognized competently using DNA sequence-based analysis.

Keywords: Deer, DNA barcoding, goat, skin

DNA barcoding is a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed-upon position in genome. It is one among the most authoritative tools for discriminating species from minute pieces of biological specimens, as well as processed produces. It is a suitable tool for wild animal forensics (Ferri *et al.* 2009). Mitochondrial DNA (mtDNA) is seen in all eukaryote cells and has a relatively fast mutation rate. Eventhough significant variation occurs in mtDNA sequences between species, only small comparative variance is seen within species. All mtDNA genes are maternally inherited and hence more constant. A 658-bp region (**Folmer region**) of mitochondrial cytochrome c oxidase subunit I (COI) gene is being proposed as a potential 'barcode'. Mitochondrially encoded cytochrome c oxidase I (MT-CO1)- is a protein and that in humans has been encoded by MT-CO1 gene. In other eukaryotes, gene is called cox1 or COI and forms the main subunit of cytochrome c oxidase complex. The advantage of using COI is that it is short enough to be sequenced quickly and cheaply, yet long enough to identify

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variations among species. In the present study, DNA barcoding was used as a technology to differentiate deer and goat species from the skin samples.

MATERIALS AND METHODS

Very thin samples of skin were collected from ear pinna, dorsal abdomen, muzzle and interdigital space of forelimb of deer brought for post mortem at College of Veterinary and Animal Sciences, from Thrissur zoo and forest department and of goat freshly slaughtered in Meat Technology Unit, Mannuthy. Samples were chopped, preserved in absolute alcohol (ethanol). Samples were sent; processed for DNA barcoding to laboratory at Rajiv Gandhi Centre of Biotechnology, TVM, where reference barcode sequences were generated to identify species of wild animals in Kerala. DNA analysis was done to establish identity. Samples were cut into small pieces, powdered finely using liquid nitrogen. The material weighing upto 250 mg was added to 2 ml Eppendorf tubes having 500 µl of lysis buffer (100 mM Tris-HCl, pH 8; 5 mM EDTA, 50 mM NaCl, 20% (w/v) SDS and 25 ml of Proteinase K [50 mg/ml]), vortexed for 30s and incubated overnight at 37°C. DNA extraction (Sambrook *et al.* 1989) was done with the digested materials following phenol: chloroform method. GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare) was used to purify the extracted DNA. CO1 gene was subjected to PCR amplification in a volume of 25 µl containing 1 X PCR Gold Buffer, 2.5 mM MgCl₂, 0.2 mM of each of dNTPs, 20 pM each of forward and reverse primers LCO and HCO (Folmer *et al.* 1994), three units of AmpliTaq Gold polymerase (Applied Biosystems) and 20–40 ng of purified DNA.

Amplification was carried out for an initial 10 min at 94°C; followed by 40 cycles at 94°C, 45°C and 72°C, each for 1 min; and a final elongation step for 5 min at 72°C in a GeneAmp[®] PCR System 9700 (Applied Biosystems). PCR products were confirmed in 2% agarose gel stained with ethidium bromide. Sequencing reactions were carried out using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) using forward and reverse primers independently following manufacturer's instructions (www.ibt.lt/sc/files/BDT_v3.1_

Protocol_04337035.pdf). Sequencing products were purified and sequenced on an Applied Biosystems 3730 DNA Analyser.

Results and Discussion

The mitochondrial sequence of Cytochrome C Oxidase subunit (COI) is an ideal barcode marker suitable for species identification of the animals and based on this the samples were identified as *Cervus unicolor*, *Rusa timorensis* and *Capra hircus*.

Nucleotide sequence similarity search in NCBI (Accession No. KF317912.1) yielded most similarity with *Rusa unicolor* (identity 99%); *Cervus unicolor* (identity 98%) and *Rusa timorensis* (identity 98%) for COI with reference sequences of Deer species for deer specimens.

Nucleotide sequence similarity search in NCBI (Accession Nos. KF317913.1; AB736109.1; KC679017.1) yielded most similarity with *Capra hircus* (identity 99%) for COI with reference sequences of goat species for goat specimens.

The sequences of present study, were aligned along with sequences of closely related species obtained from GenBank and MEGA v3.1 software was used (Kumar *et al.* 2004) to create neighbor joining (NJ) tree of K2P distances. The samples from the present study were identified as *Cervus unicolor*, *Rusa timorensis* and *Capra hircus*. Samples confirmed as belonging to sambar deer and goat. Study supports that the mitochondrial sequence of Cytochrome C Oxidase subunit (COI) is an ideal barcode marker suitable for species identification of the animals. From this study it was evident that mitochondrial CO1 can differentiate sambar deer and goat.

The expanding use of COI as genetic marker for animal species identification (Linacre and Tobe, 2011) can be highlighted by DNA barcoding. Present study differentiated the skin samples of sambar deer and goat. It is found that the accuracy of DNA barcoding will be dependent on data quality of database. Erroneous identification of species may result in misinterpretation based on DNA sequences

present in database. Hence, updating the reference datasets (Parson *et al.* 2004) is of utmost importance. The present study elucidates that barcode sequences may be applied for identification of species from even small pieces of material brought as evidence and therefore, stress the need of localized reference datasets for application in wildlife forensics. Thus it is concluded that DNA sequence-based analysis will be of use the effective identification of even unrecognizable wildlife specimens.

Conclusion

DNA barcoding was used to differentiate deer and goat species from the skin samples in the present study, which suggested that grotesque wildlife specimens can be recognized effectively by DNA sequence-based analysis. The mitochondrial sequence of Cytochrome C Oxidase subunit (COI) is an ideal barcode marker suitable for species identification of the animals and based on this the samples were identified as *Cervus unicolor*, *Rusa timorensis* and *Capra hircus*. Nucleotide sequence similarity search in NCBI (Accession No. KF317912.1) yielded most similarity with *Rusa unicolor* (identity 99%); *Cervus unicolor* (identity 98%) & *Rusa timorensis* (identity 98%) for COI with reference sequences of Deer species for deer specimens. Nucleotide sequence similarity search in NCBI (Accession Nos. KF317913.1; AB736109.1; KC679017.1) yielded most similarity with *Capra hircus* (identity 99%) for COI with reference sequences of goat species for goat specimens.

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References

- Ferri G., Alu M., Corradini B., Licata M., Beduschi G. 2009. Species identification through DNA 'barcodes'. *Genet-Test. Mol Biomark.* 13: 421–426.
- Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol.* 3: 294–299.
- Kumar S., Tamura K., Nei M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform.* 5: 150–163.
- Linacre A., Tobe S.S. 2011. An overview to the investigative approach to species testing in wildlife forensic science. *Investig. Genet.* 2: 2. doi:10.1186/2041-2223-2-2.
- Parson W., Brandstaetter A., Alonso A., Brandt N., Brinkmann B., Carracedo A., Corach D., Froment O., Furac I., Grzybowski T., Hedberg K., Keyser-Tracqui C., Kupiec T., Lutz-Bonengel S., Mevag B., Ploski R., Schmitter H., Schneider P., Syndercombe-Court D., Sørensen E., Thew H., Tully G., Scheithauer R. 2004. The EDNAP mitochondrial DNA population database (EMPOP) collaborative exercises: organisation, results and perspectives. *Forensic. Sci. Int.* 139: 215–226.
- Sambrook J., Fritsch E.F., Maniatis T. 1989. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Press.



Effect of high-energy diets on live weight change in sow *

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Abstract

An experiment was carried out on Large White Yorkshire sows for a duration of 63 days at the Centre of Pig Production and Research, Mannuthy to elucidate the impact of higher feed energy levels through corn oil supplementation. Eighteen Large White Yorkshire pregnant sows were selected three weeks before the anticipated date of parturition and were divided into three uniform groups of six each and were allocated the treatments, T1{(Control ration (18% CP and 3280 kcal/kg ME as per ICAR, 2013)), T2{(Control ration +1 % Corn oil (w/w) (18 % CP and 3365 kcal ME/ kg feed)) and T3 {(Control ration + 2 % Corn oil (w/w) (18% CP and 3450 kcal ME/kg feed))}. The dry matter intake of the sows of the three groups was similar ($p>0.05$). At 42 days of lactation, the lactational loss in body weight of sows was statistically similar ($p>0.05$).

Keywords: pregnant sows, lactating sows, corn oil, dietary energy

In sows, dietary energy has a decisive role to play especially during gestation and lactation. It aids in foetal and maternal membrane development and is also deposited as lipids in maternal tissue, which will be mobilized later on during lactation. Adequate feed intake and body condition during gestation can reduce lactational body weight loss in sows (Schenkel *et al.*, 2010). Excessive mobilization of maternal tissue during lactation can result in extreme weight loss in sows and subsequent reproductive complications (Quensel *et al.*, 2008). Supplementation of extra energy during gestation has been reported to counter extreme lactational body weight loss and back fat reduction in sows (Wang *et al.*, 2016). Many lipid sources including vegetable oils and animal fats have been used in sows as feed supplements. Only few studies have been done with corn oil as an

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energy supplement in sow diet due to its high polyunsaturated fatty acid content. Taking into account, to its high digestibility and high content of antioxidants, carotenoids and conjugated linoleic acid, corn oil was studied with a view to evaluate its effect on feed intake and body weight of pregnant and lactating sows.

Eighteen Large White Yorkshire pregnant sows from the Centre for Pig Production and Research, Mannuthy were selected three weeks prior to their expected date of farrowing. The sows were divided into three groups of six each as uniformly as possible with regard to body weight (204.65 kg) and parity (first and second). They were dewormed with ivermectin suspension through feed before the start of the experiment. All the animals were housed under uniform management conditions and were fed twice daily. The animals were offered feed for one hour and the residual feed, if any, was collected and weighed daily and the moisture content was analysed to calculate the dry matter intake.

The feeding trial was done on pregnant sows selected three weeks prior to farrowing and extended till the 42nd day of weaning of piglets. The three dietary treatments were formulated as per ICAR, 2013, as follows:

T1- Control ration (Ingredient composition as per Table 1) (18% CP and 3280 kcal ME/kg feed)

T2- Control ration + 1 % corn oil (w/w) (18 % CP and 3365 kcal ME/kg feed)

T3- Control ration + 2 % corn oil (w/w) (18% CP and 3450 kcal ME/kg feed)

The sows of each group were fed with the corresponding treatment ration. A record of the daily feed intake of the animal was maintained throughout the experiment. Residue feed was collected, weighed and was analysed for moisture estimation. Body weights of the sows were recorded at the beginning of the experiment and thereafter at fortnightly intervals. Data collected on various parameters were analysed by Analysis of Variance (ANOVA) (Snedecor and Cochran, 1994).

The weekly feed intake and fortnightly body weight of the sows kept on the three diets are listed in Tables 2 and 3, respectively.

The initial and final dry matter intake (kg/d) of the sows kept on T1, T2 and T3 were 3.67, 3.57, 3.81 and 4.03, 4.12, 4.29, respectively. There was no significant difference ($p>0.05$) between sows of the three groups with respect to dry matter intake during the experimental period. Similar results were reported by Quiniou *et al.*, (2008), whereas the feed intake was lowered with higher energy concentrations as suggested by reports of Prunier *et al.* (2001) and Jin *et al.* (2016). Increased feed intake observed with higher energy concentration was reported by Rosero *et al.*, (2012). In the present study, the dry matter intake was not affected probably due to the restricted feeding practised. This agrees with results reported by Sulabo *et al.* (2010) who compared *ad libitum* and restricted feeding in sows fed on high-energy diets.

Table 1. Ingredient composition of control ration, %

Ingredients, %	Control ration
Yellow maize	71
Soya bean meal	25
Fat	2
Salt	0.5
Mineral mixture	1.5
Total	100
Nicomix AB2D3 ¹	25 gm
Nicomix BE ²	25 gm
Zinc oxide ³	45 gm
Oxylock antioxidant ⁴	10 gm
Nicomix AB2D3 ¹	25 gm

¹Nicomix A, B2, D3, (Nicholas Piramal India Ltd, Mumbai) containing Vitamin A- 82,500 IU, Vitamin B₂- 50 mg, Vitamin D₃- 12000 IU and Vitamin K-10 mg per gram

²Nicomix BE (Nicholas Piramal India Ltd, Mumbai) containing vitamin B₁-4 mg, Vitamin B₆-8 mg, Vitamin B₁₂- 40 mg, Niacin – 60 mg, Calcium pantothenate-40 mg and Vitamin E- 40 mg per gram.

³Zinc oxide (Nice Chemicals Pvt. Ltd., Kochi) containing 81.38 % Zn

⁴Oxylock antioxidant (Vetline Ltd., Indore) contains Ethoxyquin, Butylated Hydroxy Toluene (BHT), Chelators and Surfactant.

Table 2. Weekly average feed intake of sows maintained on the three experimental rations on dry matter basis, kg.

Weekly	Treatments ¹			SEM value	p-value
	T1	T2	T3		
1	3.67±0.21	3.57±0.18	3.81±0.22	0.115	0.703 ^{ns}
2	3.41±0.16	3.91±0.25	3.61±0.33	0.115	0.400 ^{ns}
3	3.09±0.26	3.12±0.51	3.37±0.32	0.208	0.856 ^{ns}
4	1.85±0.16	2.05±0.26	2.06±0.13	0.107	0.695 ^{ns}
5	2.56±0.27	2.88±0.22	2.50±0.30	0.151	0.572 ^{ns}
6	2.75±0.43	2.87±0.19	3.05±0.34	0.184	0.817 ^{ns}
7	3.25±0.49	3.29±0.32	3.69±0.31	0.214	0.680 ^{ns}
8	3.74±0.38	3.73±0.14	3.98±0.10	0.133	0.717 ^{ns}
9	4.03±0.24	4.12±0.17	4.29±0.19	0.114	0.650 ^{ns}

¹Mean of six values with SE

ns- Non significant (p>0.05)

Table 3. Fortnightly average body weight of sows maintained on three experimental rations, kg

Days	Treatments ¹			SEM value	p-value
	T1	T2	T3		
21 days before farrowing	202.33±12.08	202.51±10.99	209.12±16.86	7.388	0.922 ^{ns}
7 days before farrowing	216.38±13.33	231.52±10.78	229.7±16.58	7.644	0.703 ^{ns}
At farrowing	194.18±11.43	210.05±10.13	210.55±17.21	7.437	0.621 ^{ns}
14 days after farrowing	184.53±13.85	198.43±9.58	196.58±16.87	7.61	0.744 ^{ns}
28 days after farrowing	178.26±15.85	198.18±8.63	193.12±17.04	8.036	0.603 ^{ns}
42 days after farrowing	175.08±15.59	195.39±11.59	190.65±17.02	8.357	0.612 ^{ns}

¹Mean of six values with SE

ns- Non significant (p >0.05)

The initial and final average body weight (kg) of the sows fed T1, T2 and T3 were 202.33, 202.51, 209.12 and 175.08, 195.39, 190.65, respectively. There was no significant difference (p>0.05) among the sows of the three groups with respect to body weight during the experimental period. When compared with the body weight of sows at farrowing, the average loss of body weight ranged from 10-14 kg. But, there was no significant difference (p>0.05) among the groups at 14 days after farrowing. At 28 days of farrowing the average loss of body weight ranged from 12-17 kg. At 42 days after farrowing the loss of body weight ranged from 15-20 kg when compared with the farrowing body weight. Although there was no statistically significant difference (p>0.05) among the groups at 42 days of farrowing, an increasing trend in body weight was noticed in both energy

supplemented groups T2 and T3. The results during the lactation period were in agreement with the reports of Long *et al.*, (2010), Jin *et al.*, (2016) and Rosero *et al.*, (2012), who observed that sows fed with high-energy diet gained significantly more body weight and back fat during gestation whereas the body weight loss during suckling was not significantly affected.

Reports by Wang *et al.*, (2016) and Cabezon *et al.*, (2016) indicated that there were better gestational body weights and lesser lactational weight loss in sows supplemented with high-energy diets.

In the present study, the lactational weight loss was not different among the groups. Renaudeau and Noblet (2001), suggested that high-energy diets provide lipids to be transferred to lacteal glands and resulted in milk with a high lipid content and hence lactational loss was not affected.

The dry matter intake of sows of the three groups was similar which indicated that high-energy diets had no effect on feed intake. At 42 days of lactation, the body weight of sows in the high-energy supplemented groups was numerically better; however, the lactational loss in body weight of sows was statistically similar.

Reference

- Cabezón, F.A., Schinkel, A.P., PAS, Richert, B.T., Stewart K.R., Gandarillas, M. and Peralta, W.A. 2016. Analysis of lactation feed intakes for sows including data on environmental temperatures and humidity. *The Professional Anim. Scientist*. **32**: 333-345.
- ICAR [Indian Council of Agricultural Research]. 2013. *Nutrient requirements of animals – Pig (ICAR-NIAP)* (3rd Ed). Indian Council of Agricultural Research, New Delhi, 22p.
- Jin, S.S., Jung, S.W., Jang, J.C., Chung, W.L., Jeong, J.H. and Kim, Y.Y. 2016. Effects of dietary energy levels on the physiological parameters and reproductive performance of gestating gilts. *Asian Australas. J. Anim. Sci.* **29**: 1004-1012.
- Long, H.F., Ju, W.S., Piao, L.G. and Kim, Y.Y. 2010. Effect of dietary energy levels of gestating sows on physiological parameters and reproductive performance. *Asian-Aust. J. Anim. Sci.* **23**: 1080-1088.
- Prunier, A., Guardarrama, C.A. M., Mouro, J. and Quensel, H. 2001. Influence of feed intake during pregnancy and lactation on fat body reserve mobilization, plasma leptin and reproductive function of primiparous lactating sows. *Reprod. Nutr. Dev.* **41**: 333-347.
- Quensel H., Brossard L., Valacogne, A. and Quiniou, N. 2008. Influence of some sow characteristics on within-litter variation of piglet birth weight. *Anim.* **2**: 1842-1849.
- Quiniou N., Richard S., Mouro, J., and Etienne M. 2008. Effect of dietary fat or starch supply during gestation and/or lactation on the performance of sows, piglets' survival and on the performance of progeny after weaning. *Anim.* **2**: pp 1633-1644.
- Renaudeau, D. and Noblet, J. 2001. Effects of exposure to high ambient temperature and dietary protein level on sow milk production and performance of piglets. *J. Anim. Sci.* **79**: 1540-1548.
- Rosero, D.S., van Heugten, E., Odle, J., Cabrera, R., Arellano, C. and Boyd, R.D. 2012. Sow and litter response to supplemental dietary fat in lactation diets high ambient temperatures. *J. Anim. Sci.* **90**: 550-559.
- Schenkel, A.C., Bernadi, M.L., Bortolozzo, F. and Wentz, I. 2010. Body reserve mobilization during lactation in first parity sows and its effect on second litter size. *Livestock Sci.* **132** :165-172.
- Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods* (8th Ed.). The Iowa state university press, Ames, Iowa, USA, 314p.
- Sulabo, R.C., Jacela, J.Y., Tokach, M.D, Dritz, S.S., Goodband, R.D., DeRouchey, J.M. and Nelssen, J.L. 2010. Effects of lactation feed intake and creep feeding on sow and pig performance. *J. Anim. Sci.* **88**: 3145-3153
- Wang, J., Yang, M., Cao, M., Lin, Y., Che, L., Veeramuthu, D., Al-Dhabi, N. A., Fang, Z., Xu, S., Feng, B., Liu, G. and Wu, D. 2016. Moderately increased energy intake during gestation improves body condition of primiparous sows, piglet growth performance, milk fat and protein output. *Livestock Sci.* **194**: 23-30. ■



Unusual cytological finding of sheathed microfilariae in a fine needle aspirate: a case report



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Filariasis is a neglected mosquito-borne tropical parasitic disease with public health importance in tropical and subtropical countries. Canine filarial infections are caused by many nematode species such as *Dirofilaria immitis*, *D. repens*, *Brugia pahangi*, *B. malayi* and *Acanthocheilonema reconditum* (Satjawongvanit *et al.*, 2019). Microfilariae of *Brugia* spp. are sheathed while those of others are non-sheathed (Ravindran *et al.*, 2014). Canine filarial infections are highly endemic to the state of Kerala with the reports of prevalence of microfilaraemia ranging from 8.1 per cent in Thrissur district to very high prevalence of 42.68 per cent in Alappuzha district (Chirayath *et al.*, 2017; Ravindran *et al.*, 2014). The major clinical signs in dogs associated with circulating microfilariae are fever, anorexia, conjunctivitis, dermatitis, oedema of limb and scrotum (Tarello, 2011). The traditional methods for identification of the microfilariae are based on morphological microscopic observation of Giemsa stained peripheral blood smears. Despite the high prevalence of filarial infections in dogs, it is infrequent to find the microfilariae in fine needle aspiration cytology (FNAC) in suspected cases of limb oedema. The demonstration of microfilariae in the aspirate or body fluids plays a pivotal role in diagnosis of the condition and institution of specific treatment modalities, especially in case of lymphatic filariasis (Panicker *et al.*, 2012). In the present communication, a case of canine filarial infection with right forelimb oedema, chronic non-healing ulcers and localized alopecia diagnosed based on fine needle aspiration cytology (FNAC) is discussed.

A seven-year old female Labrador dog (Fig. 1A) was presented at the Out Patient Ward of the District Veterinary Centre, Kannur (Dist), Kerala with complaints of oedema and wounds on the right forelimb (Fig. 1B). Whole blood (2mL) was collected from the saphenous vein for complete blood count (CBC) using automated hematology analyzer (Exigo EOS, Sweden). Peripheral blood from the tip of the ear was collected for wet film examination and for making blood smears for microscopic examination after Giemsa staining. Skin scrapings were collected from the lesions in the oedematous area for parasitological examination. Fine needle aspirate and impression smears were also collected from the oedematous area of the limb for parasitological and cytological examinations. Hematological analysis revealed moderate regenerative anaemia with a haemoglobin level of 9.4g/dL, total RBC count of 4.5 million/cu.mm and haematocrit of 28 per cent.

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On wet film examination, numerous live and motile microfilariae were detected exhibiting snake like wriggling movement across the microscopic field (average of 6 organisms per 10X field). No ectoparasites were detected in the skin scrapings collected, while FNAC and impression smears revealed moderately cellular smears with neutrophils and multiple (sheathed) microfilaria on a haemorrhagic background (Fig. 2A). Giemsa stained blood smears on microscopical examination revealed numerous polychromatophils, a few toxic neutrophils and sheathed microfilariae. Microfilariae showed a bluish violet coloured body and a pink coloured sheath extending beyond the anterior and posterior ends. The anterior end was blunt with the cephalic space devoid of any nuclei, darkly stained tightly packed nuclear column and the posterior end with two distinctly separated terminal nuclei at the tail tip (Fig. 2B). Presence of sheath and two distinct widely separated terminal nuclei confirmed the *Brugia* spp.

Based on cytological and hematological evidences, levamisole @ 10mg/kg OD per orally for one week, doxycycline @ 10 mg/kg per orally for two weeks, pantoprazole @ 1 mg/kg per orally before food for two weeks were administered. In addition, liver tonics and an antiseptic wound spray were also prescribed for two weeks. Considerable improvement was noticed after two weeks of treatment. There was a marked reduction in oedema and swelling along with healing of the ulcerated wounds. No

microfilariae could be detected at this stage in FNAC. In addition, numerous degenerated neutrophils, plasma cells, eosinophils and neutrophils engulfing hemosiderin pigments were also noticed in FNAC. Panicker *et al.* (2012) stated that dead or fixed worms and microfilaria can excite severe reaction leading to eosinophilia, eosinophilic abscess, necrosis, fibrosis and epithelioid granuloma.

Previous studies had diagnosed most of the cutaneous and subcutaneous masses in dogs as being due to infection with *D. repens* (Abd Rani *et al.*, 2010). Thus from the present study, it could be concluded that the presence of sheathed microfilariae in the fine needle aspirate and impression smears can be used



Fig. 1A: The dog presented with limb oedema & localized alopecia; **1B:** The edematous right forelimb with ulcerated non-healing wounds could be noted

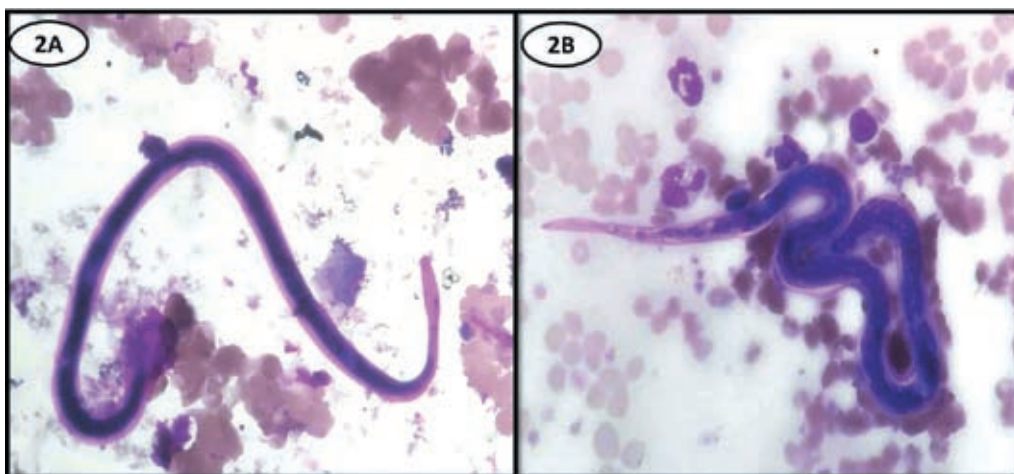


Fig 2A: Presence of sheathed microfilaria in the fine needle aspirate of edematous limb; **2B:** Presence of sheath and two distinct widely separated terminal nuclei of the microfilaria in the blood smear may be noted

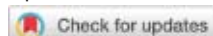
as a method for diagnosis of lymphatic filarial infections of dogs. For confirmation of the filarial species, molecular techniques are essential.

SUMMARY

Fine needle aspiration cytology was used to confirm the presence of *Brugia spp.* infection in dogs causing limboedema and dermatopathies. Clinicians must carefully consider this diagnostic possibility when dealing with similar cases.

REFERENCE

- Abd Rani, P.A.M., Irwin, P.J., Gatne, M., Coleman, G.T., McInnes, L.M. and Traub, R.J. 2010. A survey of canine filarial diseases of veterinary and public health significance in India. *Parasit. Vectors*. **3**(1): 30.
- Chirayath, D., Alex, P.C., Usha, N.P., George, S., Ajithkumar, S. and Panicker V.P. 2017. Identification of *Brugia malayi* in dogs in Kerala, India. *Trop. Biomed*. **34**: 804-814.
- Panicker, N.K., Buch, A.C., Vimal, S. and Dharwadkar, A.P. 2012. Cytological diagnosis of microfilariae in subcutaneous nodule. *Med. J. DY Patil University*. **5**(1):71.
- Ravindran, R., Varghese, S., Nair, S.N., Balan, V.M., Lakshmanan, B., Ashruf, R.M., Kumar, S.S., Gopalan, A.K.K., Nair, A.S., Malayil, A. and Chandrasekhar, L. 2014. Canine filarial infections in a human *Brugia malayi* endemic area of India. *BioMed Res. Int*. **2014**: 630160.
- Satjawongvanit, H., Phumee, A., Tiawsirisup, S., Sungpradit, S., Brownell, N., Siriyasatien, P. and Preativatanyou, K. 2019. Molecular analysis of canine filaria and its *Wolbachia* endosymbionts in domestic dogs collected from two animal university hospitals in Bangkok Metropolitan Region, Thailand. *Pathogens*. **8**(3): 114.
- Tarello, W. 2011. Clinical aspects of dermatitis associated with *Dirofilaria repens* in pets: a review of 100 canine and 31 feline cases (1990–2010) and a report of a new clinic case imported from Italy to Dubai. *J. Parasitol. Res*. **2011**:578385. ■



Partial genome analysis of cox1 subunit-I region in mitochondrial DNA of canine mammary tumours



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Abstract

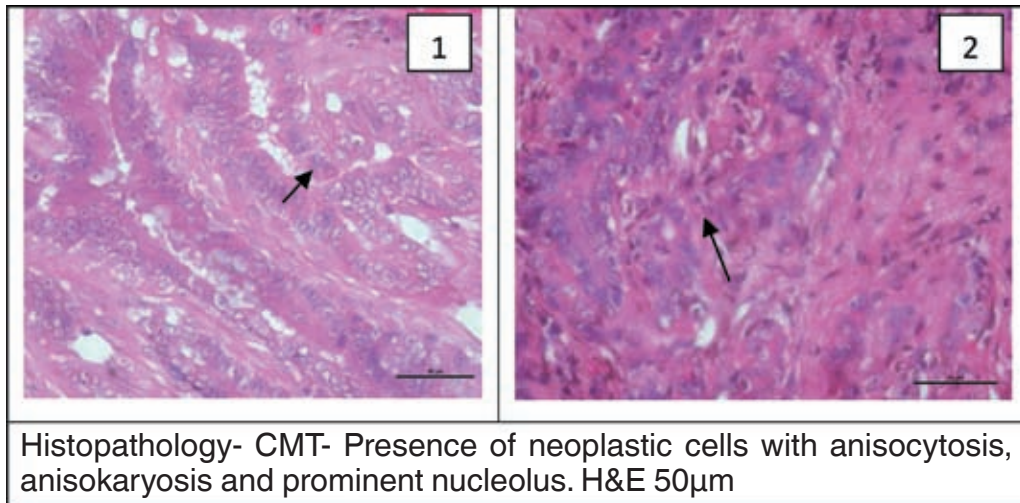
Oncogenesis is an area which continuously elicits research interest. There are innumerable factors which contribute to oncogenesis of which mitochondrial DNA (mtDNA) mutations play a major role. Though extensive studies have been conducted relating mtDNA mutations to human cancers, there is sparse information available on canine neoplasia. Cytochrome oxidase1 (COX1) is an important component of electron transport chain of mitochondria and any alteration in it would result in altered energy production which is very essential for proliferating neoplastic cells. Two samples of canine mammary carcinomas (CMT) were subjected to partial genome sequencing of COX1 subunit -I. There was no change in the gene sequence of COX1. Further studies in this aspect would pave the way for investigations on the role of mtDNA mutation in oncogenesis and could provide insights into how canines can serve as models for human cancers.

Keywords: Canine mammary tumour, mtDNA, mutation

The quest to unravel the secret of neoplastic transformation has not left any stone unturned. More than eighty years ago, Otto Warburg, in 1956, described the accelerated aerobic glycolysis in neoplastic cells despite the presence of oxygen (Warburg effect). This process helps neoplastic cells to maintain their vitality, ability to proliferate, invade and evade apoptosis. (Grzybowska-Szatowska and Slaska, 2012). Cytochrome c oxidase (COX) is one of the five-member protein complex involved in oxidative phosphorylation resulting in energy production and forms an integral part of mtDNA. COX1 upregulation has been reported in breast cancers when compared to normal tissues (Haakensen et al., 2011). The mtDNA are said to be more prone to mutations due to their lack

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Fig.1. Histopathological observations of the two samples of CMT

Histopathology- CMT- Presence of neoplastic cells with anisocytosis, anisokaryosis and prominent nucleolus. H&E 50µm

Fig.2. NCBI BLASTn analysis showing 100% identity between COX1 sequence from CMT case 1 and the reference sequence **MN542345.1** from *Canis lupus familiaris* isolate 38 cytochrome c oxidase subunit 1 (COI) gene, partial cds.

Score	Expect	Identity	Gaps	Strand
841 bits(932)	0.0	466/466(100%)	0/466(0%)	Plus/Minus
Query 1	GTTTTATGTTGTAATAGTAGTGAATTAATGCCCCAAAATAGAGAGACTCCGG	60		
Sbjct 478	GTTTTATGTTGTAATAGTAGTGAATTAATGCCCCAAAATAGAGAGACTCCGG	419		
Query 61	CTAAGTGTAAGGAGAAAATTTGAAGGTCAACGGATGCTCTCGCATGGGCGAGATTGCCAG	120		
Sbjct 418	CTAAGTGTAAGGAGAAAATTTGAAGGTCAACGGATGCTCTCGCATGGGCGAGATTGCCAG	359		
Query 121	CCAGTGGGGGGTATACGGTTCATCCGTTCTCGACCTGCTTCTACCATAGAAGATGCTA	180		
Sbjct 358	CCAGTGGGGGGTATACGGTTCATCCGTTCTCGACCTGCTTCTACCATAGAAGATGCTA	299		
Query 181	ATAGTAGAAGAAAGGATGGAGGAAGAGTCAGAAGCTCATGTTATTATTTCGGGGGAATG	240		
Sbjct 298	ATAGTAGAAGAAAGGATGGAGGAAGAGTCAGAAGCTCATGTTATTATTTCGGGGGAATG	239		
Query 241	CCATGTCGGGACCACTAATTATTAAAGGCACTAGTCAGTTTCAAAAGCCCCAATTATGA	300		
Sbjct 238	CCATGTCGGGACCACTAATTATTAAAGGCACTAGTCAGTTTCAAAAGCCCCAATTATGA	179		
Query 301	TGGGCATGACTATGAAGAAGATTATTACGAAGCATGGGGGTTACGATGACATTATAAA	360		
Sbjct 178	TGGGCATGACTATGAAGAAGATTATTACGAAGCATGGGGGTTACGATGACATTATAAA	119		
Query 361	TTTGATGTCACCTAGTAAGATACGGGCTGACCTAGTTTCGGCTCGGATGAGGAGGCTCA	420		
Sbjct 118	TTTGATGTCACCTAGTAAGATACGGGCTGACCTAGTTTCGGCTCGGATGAGGAGGCTCA	59		
Query 421	AGGCAGTGCCTACTATACCGGCTCATGCTCCAAATAGTAGTATAA	466		
Sbjct 58	AGGCAGTGCCTACTATACCGGCTCATGCTCCAAATAGTAGTATAA	13		

Score	Expect	Identity	Gaps	Strand
836 bits(926)	0.0	463/463(100%)	0/463(0%)	Plus/Plus
Query 1	CTTTGGAACCTGACTAGTGCCTTAATAATTGGTGCTCCGGACATGGCATTCCTCCCGAAT	60		
Sbjct 192	CTTTGGAACCTGACTAGTGCCTTAATAATTGGTGCTCCGGACATGGCATTCCTCCCGAAT	251		
Query 61	AAATAACATGAGCTTCTGACTCTCTCTCCATCTTCTTCTACTATTAGCATCTTCTAT	120		
Sbjct 252	AAATAACATGAGCTTCTGACTCTCTCTCCATCTTCTTCTACTATTAGCATCTTCTAT	311		
Query 121	GGTAGAAGCAGGTGACGAGAACGGATGACCCGATATACCCCACTGGCTGGCAATCTGGC	180		
Sbjct 312	GGTAGAAGCAGGTGACGAGAACGGATGACCCGATATACCCCACTGGCTGGCAATCTGGC	371		
Query 181	CCATGAGAGGATCCGTTGACCTTACAAATTTCTCTTACACTTACCGGAGCTCTTCTC	240		
Sbjct 372	CCATGAGAGGATCCGTTGACCTTACAAATTTCTCTTACACTTACCGGAGCTCTTCTC	431		
Query 241	TATTTTAGGGGCAATTAATTCATCACTACTATTATGACATAAAACCCCTCCCAATATC	300		
Sbjct 432	TATTTTAGGGGCAATTAATTCATCACTACTATTATGACATAAAACCCCTCCCAATATC	491		
Query 301	CCAGTATCAAACTCCCTGTTTATGATGACTAAATTACACAGCTTCTACTTACT	360		
Sbjct 492	CCAGTATCAAACTCCCTGTTTATGATGACTAAATTACACAGCTTCTACTTACT	551		
Query 361	ATCCCTGCTGTACTGGCTGCTGGAATTAACATCTTTTAACAGACCGGAATCTTAATAC	420		
Sbjct 552	ATCCCTGCTGTACTGGCTGCTGGAATTAACATCTTTTAACAGACCGGAATCTTAATAC	611		
Query 421	AACATTTTGTATCCCGCTGAGGAGGAGACCTATCTATAT	463		
Sbjct 612	AACATTTTGTATCCCGCTGAGGAGGAGACCTATCTATAT	654		

of protective histones, compromised DNA repair mechanism and exposure to free radical injury during oxidative phosphorylation (Miyazono et al., 2002). Though somatic mtDNA mutations have been reported in canine neoplastic transformations (Slaska et al., 2015), very few studies have focussed on the role of COX1 in canine mammary tumours (CMT). Hence this study was done as a preliminary one employing two cases of CMT to gain insights into the role of COX1 subunit-I sequence variations in CMT, using next generation sequencing.

The samples included in the study were two CMT resected masses which

were collected with the owner's consent. Tissue samples were collected in 10 per cent Neutral Buffered Formalin (NBF) for histopathological analysis (Bancroft and Gamble, 2008). Samples were also preserved in ethanol for sequencing studies. Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following the manufacturer's instructions. The PCR amplification for COX1 (Folmer et al., 1994) was performed using PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the forward primer JGLCO F (5'-TITCIACIAAYCAYAARGAYATTGG-3') and reverse primer JGLCO R (5'-

TAIACYTCIGGRTGICCRARAAYCA -3'). The removal of unwanted primers and dNTPs from a PCR product mixture was done using ExoSAP-IT (GE Healthcare). After the clean-up, sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) following the manufacturer's protocol. Mitochondrial genome sequence of 466nt from one sample and 463 nt in the other sample were analysed against standard sequences available in the NCBI database using the BLAST tool.

The tumours were subjected to histopathology and were found to be carcinomas as depicted in Fig.1. In this study, two canine cancer tissues were analysed to study whether mtDNA mutations in CMTs had any significant role to play in tumorigenesis. The sequences obtained from both the cases of CMT blasted using NCBI BLASTn revealed 100 per cent identity with the prevailing GenBank submissions of partial mitochondrial genomes of *Canis lupus familiaris*. The BLAST result in both samples did not show any variation in the COX1 subunit I as represented in Fig.2.

There have been reports on COX1 mutation in many human cancers including prostate cancers and breast cancers (Petros et al., 2005 and Omasanggar et al., 2020). However, the partial genome analysis of COX1 subunit I in the two samples did not show any variation. Further studies involving the whole genome of mtDNA and corresponding normal tissues in CMT patients may shed more light on the role of mtDNA mutations in carcinogenesis. As only two samples were analysed, a detailed analysis with more number of samples with complete mtDNA analysis will provide more authentic data to confirm its statistical significance. This study also sheds light on the possibility of analysing CMTs as a model to study mtDNA biology in human breast cancers.

Acknowledgement

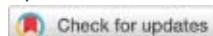
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References

- Bancroft, J.D. and Gamble, M. 2008. Theory and Practice of Histological Techniques. 6th edn. Churchill Livingstone, United States of America, p. 725.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299
- Grzybowska-Szatkowska, L. and Slaska, B. 2012. Mitochondrial DNA and carcinogenesis (Review). *Mol Med Rep.* 6: 923–930.
- Haakensen, V.D., Bjoro, T., Lüders, T., Riis, M., Bukholm, I.K., Kristensen, V.N., Troester, M.A., Homen, M.M., Ursin, G., Borresen-Dale, A.L., et al. 2011. Serum estradiol levels associated with specific gene expression patterns in normal breast tissue and in breast carcinomas. *BMC Cancer.*, 11: 332.

- Miyazono, F., Schneider, P.M., Metzger, R., Warnecke-Eberz, U., Baldus, S.E., Dienes, H.P., Aikou, T. and Hoelscher AH. 2002. Mutations in the mitochondrial DNA D-Loop region occur frequently in adenocarcinoma in Barrett's esophagus. *Oncogene*. **21**:3780-3783.
- Omasanggar, R., Yu, C.Y., Ang, G.Y., Emran, N.A., Kitan, N., Baghawi, A., Ahmad, A.F., Abdullah, M.A., The, L.K. and Maniam, S. 2020. Mitochondrial DNA mutations in Malaysian female breast cancer patients. *PLoS ONE* 15(5): e0233461.
- Petros, J.A., Baumann, A.K., Ruiz-Pesini, E., et al., "MtDNA mutations increase tumorigenicity in prostate cancer. In: Proceedings of the National Academy of Sciences of the United States of America, 2005, vol. 102, no. 3, pp. 719–724.
- Slaska, B., Grzybowska-Szatowska, L., Nisztuk, S., Surdyka, M. and Rozanska D. 2015 Mitochondrial DNA polymorphism in genes encoding ND1, COI and CYTB in canine malignant cancers. *Mitochondr DNA*. **26**:452–8.
- Warburg, O. 1956. On the origin of cancer cells. *Science*. 124: 269-270





Minor salivary glands in oral mucosa of two dogs: A pilot study



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Abstract

Presence of minor salivary glands in the oral mucosa and its potential use as an autograft for treating dry eye disease has been widely reported in humans. But there is a paucity of literature regarding the histological evidence for the presence of minor salivary glands in the oral mucosa of dogs. The present pilot study histologically identified and topographically described the minor salivary glands in the oral mucosa of two dogs, as a potential autograft for keratoconjunctivitis sicca. The samples were collected from different sites of the upper and lower lips and the commissures. Samples were processed for routine paraffin embedding; 5 µm sections were taken and were stained using Hematoxyline and Eosin staining. Histological examination revealed exocrine, compound, tubulo-acinar glands and the secretion was mixed type.

Keywords: Minor salivary glands, canine, Keratoconjunctivitis sicca, H and E staining

Salivary glands are mainly classified into minor and major salivary glands. The parotid, mandibular, monostomatic and polystomatic sublingual, and zygomatic glands are categorised as major salivary glands, while the labial, buccal, molar, lingual, and palatine are grouped under the minor salivary glands in the canine species (Evans and de Lahunta, 2013; Gioso and Carvalho, 2005). The minor salivary glands may be mucous, serous or mixed, and are the miniaturized forms of the major salivary glands (Frappier, 2006). These glands can be located in close proximity to the epithelial lining and in the interior of the lamina propria-submucosa in dogs (Dyce *et al.*, 2004; Frappier, 2006). Murube (1997) reported the existence of a large number of minor salivary glands between the quadratus labii and the lip or the buccal mucosa and had described labial minor salivary glands as a potential alternative lubricant source for the ocular surface in humans for dry eye disorder.

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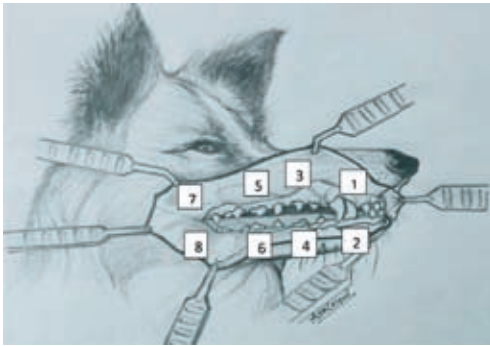


Fig. 1 Sample collection sites within the oral cavity. Samples were collected from the upper rostral labial mucosa (1), lower rostral labial mucosa (2), upper labial mucosa from canine to third premolar (3) and (5), lower labial mucosa from canine to third premolar (4) and (6) and buccal mucosa near the commissure (7) and (8).

There are contradictions associated with the presence of minor salivary glands in dogs (Cherry *et al.*, 2018). There is a paucity of literature regarding histological evidence for presence of minor salivary glands in oral mucosa of dogs. The present paper describes a pilot study on histological identification and topographical description of minor salivary glands in oral mucosa of two dogs.

Oral mucosal samples were collected from two adult dogs (one female Labrador aged 7 years and another Non-descript male aged 2 years) that had both died of reasons unrelated to the present study, for topographical identification of minor salivary glands in oral mucosa, as a potential autograft for dry eye disease in dogs. The samples were randomly collected from multiple sites (Fig. 1) of labial muco-cutaneous junction, labial mucosa of the upper and lower lips, and buccal mucosa. At every location, samples were collected from mucosa to the depth of muscularis. The collected samples were fixed in 10 per cent neutral buffered formaldehyde for 48 hours, in ambient temperature and processed using conventional histological techniques to embed in paraffin. Sections of 5 μ m thickness were taken and stained with Haematoxylin-Eosin for histological evaluation.

Sections from labial and buccal mucosa revealed mucosal, submucosal and the muscularis layers. Mucosa was lined by non-keratinised stratified squamous epithelium,

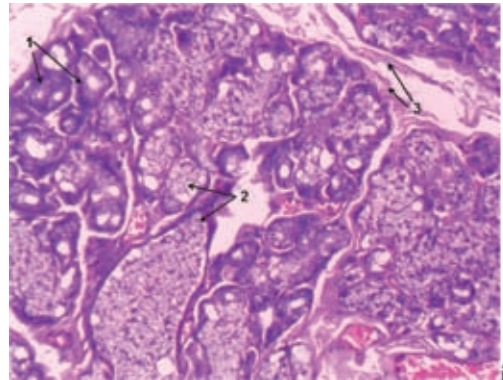


Fig. 2 Photomicrograph of oral mucosal sample from canine to third premolar in dogs showing minor salivary glands. H and E x 150. (1) Serous glands, (2) Mucus glands, (3). Connective tissue septa

while submucosa was composed of well vascularised dense irregular connective tissue. Small minor salivary glands were observed in sections of oral mucosa collected from the level of canine to third premolar. The glands were well encapsulated by connective tissue. The septa from the capsule divided the gland into lobules which enclosed secretory acini. Both serous and mucous secretory acini were present (Fig. 2). This indicated that the oral mucosa from the level of canine to third premolar contained plenty of mixed minor salivary glands. The glands were observed in the submucosal layer very close to the epithelium. The section of the oral mucosa from the other sites did not reveal any type of glands.

The present pilot study revealed that minor salivary glands were present in the oral mucosa at the level of canine to third premolar area and is in accordance with that reported by Nickel *et al.* (1979) who reported that minor salivary glands in dogs are localised in the area demarcated from canine to third cheek tooth. The minor salivary glands in the present study were observed close to the mucosal lining and a few were present close to the submucosa and were surrounded by connective tissue. These glands were mixed in nature.

Minor oral salivary glands account for half the baseline secretion of saliva and they exist in large numbers in the labial, buccal, and palatal mucosa in humans. Murube (1997) described transplantation of labial salivary

glands for the treatment of severe dry eyes in humans. Labial salivary glands along with the overlying mucosa as an auto-graft have been transplanted to the eyelids to improve the ocular lubrication and to reduce discomfort in patients with kerato-conjunctivitis sicca (Marinho *et al.*, 2010). Occurrences of minor salivary glands are reported in dogs. There are contradictions on the topography of glands and their existence in dogs. Cherry *et al.* (2018) reported that no minor salivary glands were observed in the canine oral mucosa after examining the oral biopsies of six randomly selected dogs.

The present pilot study in two dogs revealed that minor salivary glands were present in the area demarcated from canine to third premolar and were observed close to the mucosal lining. The presence of these minor salivary glands in oral mucosa potentiates its use as an auto-graft for treating dry eye disease in dogs. Further histological studies in a larger population and varied breeds of dogs are warranted.

References

- Cherry, R. L., Smith, J. D. and Ben-Shlomo, G. 2018. Canine Oral Mucosa Evaluation as a Potential Autograft Tissue for the Treatment of Unresponsive Keratoconjunctivitis Sicca. *Veterinary Ophthalmology* **21**(1):48–51
- Dyce, K. M., Sack, W. O. and Wensing, C. J. G. 2004. Digestive Appartus. *Treaty of Veterinary Anatomy*. 3rd edn., pp. 99-145. Rio de Janeiro: Elsevier.
- Evans, E. and de Lahunta, A. 2013. *Miller's Anatomy of the Dog*. 4th edn., 299p, Philadelphia, PA: Elsevier.
- Frappier, B. L. 2006. Digestive system. *Dellmann's Textbook of Veterinary Histology*. pp. 170-211, 6th edn., Iowa, Blackwell.
- Gioso, M. A. and Carvalho, V. G. G. (2005). Oral Anatomy of the Dog and Cat in *Veterinary Dentistry Practice*. *Veterinary Clinics of North America: Small Animal Practice*. **35**(4): 763-780.
- Marinho, D.R., Burmann, T.G and Kwitko, S. 2010. Labial Salivary Gland Transplantation for Severe Dry Eye due to Chemical Burns and Stevens - Johnson syndrome. *Ophthalmic Plastic and Reconstructive Surgery*. **26**(3):182-4.
- Murube, J. 1997. Substitute Surgery for Dry Eye and Glandular Transplants. *Dry Eye*. 1st edn., pp. 207–221, Tecnimedia Editorial, Madrid.
- Nickel, R., Schummer, A., Seiferle, E. and Sack, W.O. 1979. *The Viscera of the Domestic Animals*. 2nd edn., 401p, Berlin, Hamburg, Parey.



Oestrus ovis larvae in nasal cavity of sheep: A case report



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Abstract

Oestrosis, caused by the larvae of Oestrus ovis, is an obligatory myiasis of sheep and goats. It is commonly known as sheep nasal bot. The present study reports the infestation of the nasal cavity of sheep by Oestrus ovis larvae in Nagpur. Sheep showing symptoms ranging from dyspnea, mild nasal discharge, and torticollis was brought to the Department of Veterinary Pathology, Nagpur Veterinary College, for postmortem examination. Grossly sinusitis, catarrhal discharge, purulent exudate, and congestion was observed. Microscopically, the cerebral cortex of infected animals revealed degeneration of neuron and softening of brain substance. Characteristic lesions included engorgement of blood vessels and vacuolation of brain substance with mild perivascular cuffing by mononuclear cells was seen. Nasal mucosa showed discontinuation of epithelial cells with mononuclear cell infiltration and edema and fibrosis. Based on parasitological and histopathological analysis, the case was confirmed to be of nasal myiasis.

Keywords: *Oestrus ovis, Histopathology, Nasal bot.*

The larvae of *Oestrus ovis* are commonly found parasites in the nasal cavity, frontal sinuses and the maxillary sinuses of domestic sheep, goats, and wild ruminants causing a clinical condition called oestrosis (Godara *et al.*, 2009). The females swarm around the heads of animals and deposit larvae into the nostrils in batches of one to several dozen. The larvae then migrate and molt twice in the nasal passage. The duration of the parasitic portion of the lifecycle varies considerably, depending on the season and climatic conditions (Cobbett *et al.*, 1941).

The larvae are deposited around nostrils and then crawl up the nasal passages and sinuses, causing inflammatory reactions, nasal discharge and frequent sneezing. Sometimes larvae enter into the cranial cavity and injure the brain, leading to the development of symptoms like in coordination of movement and high stepping gait (false gid). The affected animals become restless, go off-feed, and showed decreased weight gain and become anorectic.

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Clinical signs include seromucous or purulent nasal discharge, dyspnea etc. (Dorchies *et al.*, 1947). Although *O. ovis* causes nasal myiasis primarily in sheep, it sporadically affects goats, dogs and sometimes human beings (shepherds). In humans, ophthalmomyiasis, respiratory and non-respiratory infestation is also caused where small ruminants and humans live together (Susilathangam *et al.*, 2013). Pathological studies help in identifying the common lesions associated with disease pathology. The use of proper therapeutic control and prevention strategies helps in the prevention of future crises. Hence, the present study aims to study the occurrence and histopathological changes of *O. ovis* larvae in the nasal cavity of sheep.

A 17-day old sheep kid was received for a necropsy with a history of dyspnea, mild nasal discharge, and head torticollis. The clinical material was collected at the time of postmortem at the Department of Pathology, Nagpur Veterinary College, Nagpur, India. A detailed post mortem was conducted. Tissue samples of the nasopharynx and cerebral cortex were collected in 10 per cent buffered formalin for histopathological study. The formalin-fixed tissues were subjected to histopathological processing as per standard procedures (Luna, 1968). The larvae were collected from major sites, including the nasal cavity, middle meatus, and septum. The nasal cavity was opened, and larvae were collected in a petri plate, washed in physiological saline, and fixed in 70 per cent ethanol for parasitological analysis.

Based on the morphological features, i.e., dark bands on the dorsal surface and spines on the ventral surface, the larvae were identified as *O. ovis*. Also, the presence of D shaped, closed, dark black colored stigmal plates with radially arranged spiracles confirmed the identification of bots (Fig. 1).

Grossly, sinusitis, catarrhal discharge, purulent exudate, and congestion was observed. Increased goblet cell activity in the nasal mucosa and excessive mucosal secretion in submucosal glands was observed, which was in accordance with earlier reports (Fathy *et al.*, 2006).



Fig. 1 : Presence of D shaped, closed, dark black colored stigmal plates with radially arranged spiracles

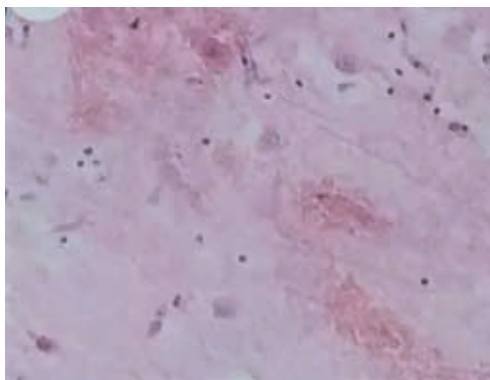


Fig. 2 : Section of cerebral cortex showing the degeneration of neuron and softening of brain substance

Microscopically, the cerebral cortex of infected animals revealed the degeneration of neuron and softening of brain substance (Fig. 2). Also, engorgement of blood vessels and vacuolation of brain substance with mild perivascular cuffing by mononuclear cells was seen. Nasal mucosa showed discontinuation of epithelial cells with mononuclear cell infiltration and edema, fibrosis, and engorgement of sub-mucosal blood Vessels, which was in agreement with previous reports (Sharma *et al.*, 2014). (Maxie, 2015) reported the presence of mucoid or catarrhal rhinitis, mucopurulent exudates, and degeneration of neurons with increased peri-neuronal space and softening of brain substance in the cerebral cortex of sheep and goats infected with *O. ovis*.

The zoonotic impact of the nasal bot is prominent, causing catarrhal conjunctivitis,

corneal opacity, and even stomatitis (Dorchies *et al.*, 1998). The infection of man with larvae of *O. ovis* often leads to the development of external ophtho-mo-myiasis, at times resulting in complete loss of vision (Pampiglione *et al.*, 1997). Nasal myiasis in small ruminant flocks leads to extensive seasonal morbidity, even mortality, and overall huge economic losses to their nomadic owners (Sharma *et al.*, 2014). Thus, it is the need of the hour to establish a proper management and treatment regime for the establishment of an appropriate control strategy.

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References

- Cobbett NG, Mitchell WC. 1941. Further Observations on the Lifecycle and Incidence of the Sheep Bot, oestrus ovis, in New Mexico and Texas. *Am J Vet Res.* 2:358–366.
- Dorchies P. and Alzieu J.P.L. 1947. Oestrose ovine: Revue. *Revue de Medicine Veterinaire.* 148: 565-574.
- Dorchies P., Duranton C. and Jacquet P. 1998. Pathophysiology of oestrus ovis Infection in Sheep and Goats: A Review. *Veterinary Record.* 142: 487-489.
- Fathy F.M., El-Barghathi A., El-Ahwal, A. and El-Bagar S. 2006. Study on Human Ophtho-mo-Myiasis Externa Caused by Oestrus ovis Larva in Sirte-Libya: Parasite features, Clinical Presentation and Management. *Journal of Egyptian society of parasitology.* 36: 265-282.
- Godara R., Sharma R.L. and Sharma C.S. 2009. Aberrant Infestation of Goat Mandibles with oestrus ovis Larvae. *Tropical Animal Health and Production.* 42: 137-139.
- Luna, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology (3rd ed.). McGraw-Hill, New York. pp. 4–8.
- Maxie G. 2015. In Jubb, Kennedy & Palmer's pathology of domestic animals-Ebook, vol. 2., 5 Ed., Elsevier Health Sciences, Philadelphia.
- Pampiglione S., Giannetto S. and Virga A. 1997. Persistence of Human Myiasis by oestrus ovis L (Diptera: Oestridae) among Shepherds of the Etnean Area (Sicily) for over 150 years. *Parasitologia.* 39: 415-418.
- Sharma N., Nayakwadi S., Pawaiya R.S., Kumar S., Tailie W.A., Paul S., Mishra A.K., Gururaj K., Kumar, A.Gupta, V.K. and Chaturvedi V. 2014. Parasitic Encephalomyelitis in Goats due to Aberrant Infestation with Botfly oestrus ovis larvae. *Advances in Animal and Veterinary Sciences.* 2: 8-11.
- Sucilathangam G, Meenakshisundram A, Hariramasubramanian S, Anandhi D, Palaniappan N, Anna T. 2013. External Ophthalmomyiasis which was caused by Sheep Bot Fly (oestrus ovis) larva: A Report of 10 Cases. *J Clin Diagn Res.* 7(3):539–542. ■



Field diagnosis of paramyxoviral infection in psittacines

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Abstract

There are about 356 species of psittacines in the world and number of people keeping parrots as pets is innumerable. In spite of the rapid growth of Indian aviculture industry, lack of proper field diagnosis of diseases in these birds is a burning issue faced by both veterinarians and owners. Paramyxoviral infection is a threat to the aviculture industry since it spreads quickly and results in high mortality. Differential diagnosis in parrots showing neurological and respiratory signs is of paramount importance. In the present study 11 parrots namely cockatiels and alexandrine parakeets showing neurological and respiratory signs were screened for the presence of paramyxoviral infection using NDV antigen lateral flow kits. All the birds tested were positive for paramyxoviral infection. These lateral flow kits can act as a good field diagnosis for paramyxoviral infection in psittacines. The present study was the first to use lateral flow kits to identify infectious causes of neurological signs in birds of Kerala.

Keywords: Paramyxoviral infection, psittacines, field diagnosis

Bird-keeping is an emerging trend of the era. Indian aviculture industry has gone a long way since the past few decades. People keep birds either as a hobby or as a source of income. But the field of avian medicine is still at the budding stage in the country. Proper diagnosis or treatment of diseases in birds is not practised by veterinarians. Of all the diseases, neurological diseases pose a grave threat to the whole aviculture industry. Neurological signs can be due to infectious and non-infectious causes. The infectious etiologies of nervous signs includes Newcastle disease by avian paramyxovirus serotype 1, avian encephalomyelitis, septicemic form of avian listeriosis, otitis interna. Out of the many diseases, paramyxoviral infection is a threat as it spreads quickly

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in the aviary and causes high mortality. Lack of vaccinations for paramyxoviral infection in psittacines is another factor of concern. If proper diagnosis is done at an earlier stage, the whole aviary can be protected by isolating the affected birds. Most of the tests for diagnosis of paramyxoviral infection is laborious and expensive. It is high time that the veterinarian start to rely on field diagnostic kits like NDV antigen lateral flow kits for easy diagnosis of diseases in birds.

Birds from three different aviaries were presented with neurological and respiratory signs. Birds presented included seven cockatiels and four alexandrine parakeets. Faecal samples of these birds were collected and NDV antigen lateral flow kits (Bionote, Korea) were used to detect the presence of paramyxoviral infection.

Out of the 11 birds tested for presence of paramyxoviral infection using NDV antigen lateral flow kits, all were positive which was indicated by the formation of bands in test and control in the kit. That is, 100 per cent positivity was obtained in the birds that showed nervous signs. This is the first time that these kits are being used to diagnose the presence of paramyxoviral infection in psittacines in India.

The main reason for the occurrence of paramyxoviral infection in these birds can be attributed to the presence of feral pigeons that visited the aviaries to feed on the waste materials thrown in the aviary premises. According to De Sousa *et al.* (2010) feral pigeons are reservoirs of paramyxoviral infection and can act as source of infection to other birds. Diagnosis of paramyxoviral infection in psittacines was

earlier done using RT-PCR by Peroulis and O'Riley (2004). The antigen NDV kits used in this study are 94.7 per cent sensitive and 96.4 per cent specific compared to RT-PCR. The kits have been used in Japanese quails by Al Ajeeli and Amer (2016).

In India, there are frequent reports of psittacines being presented with neurological signs. As of now, a field diagnostic test that is simple and efficient is not been used in Indian avian diagnostic protocols. The rapid antigen NDV kits used in this study are non-invasive there by presenting an easy method for the detection of viruses and can act as a field diagnostic test for detection of NDV in pigeons.

References

- Al-Ajeeli, K.S. and Amer, A.A. 2016. Serological study for detection of newcastle disease virus in Japanese quails in some state of Diyala province, Iraq. *Diyala Agric. Sci. J.* **8**:13-21.
- De Sousa, E., Júnior, A.B., Pinto, A.A., Machado, R.Z., Carrasco, A.D.O.T., Marciano, J.A. and Werther, K. 2010. Prevalence of *Salmonella* spp. antibodies to *Toxoplasma gondii*, and Newcastle disease virus in feral pigeons (*Columba livia*) in the city of Jaboticabal, Brazil. *J. Zoo Wildl. Med.* **41**:603-607.
- Peroulis, I. and O'Riley, K. 2004. Detection of avian paramyxoviruses and influenza viruses amongst wild bird populations in Victoria. *Aus. Vet. J.* **82**:79-82.

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