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AN OVERVIEW ON CANINE BABESIOSIS

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INTRODUCTION:

The *Babesia* species belongs to the super kingdom eukaryotes, kingdom Protista, phylum Protozoa, subphylum Apicomplexa, class *sporozoea*, order piroplasmida, family *Babesiidae* and Genus *Babesia*. The family consists of several species and in that four species have been described as important in dogs and cats were *Babesia canis*, *Babesia gibsoni*, *Babesia vogeli* and *Babesia felis* (Soulsby, 1982). The relatively larger forms referred to as *B. canis* and a smaller parasite, *B. gibsoni*.The larger forms of *Babesia* spp. include *B. canis*, *B. canis rossi* and *B. canis vogeli*.With regard to the smaller piroplasms, three genetically and clinically distinct species are currently recognized causing the disease in dogs. They are *B. gibsoni*, *B. conradae* and a *B. microti* like piroplasm (named *Theileria annae*). *Babesia gibsoni* has a worldwide distribution and is transmitted by *Haemaphysalis* spp. with a variable degree of virulence (Augustine, 2013).*Babesia gibsoni*was first reported in hounds and jackals in India and since then it was recognized in Asia, America, northern and eastern Africa and rarely in Europe (Zahler *et al*., 2000). *Babesia gibsoni*, a smaller form of parasite causing canine babesiosis was reported principally in the Middle East, Southern Asia, Japan, North Africa and South America, and considered as an emerging infection in the United States of America as well as having been detected later in Australia, Hungary and Italy (Muhlnickel *et al*., 2002).

Kerala

Canine babesiosis due to *B. canis* and *B. gibsoni* were first reported from Thrissur in Kerala (Sabu *et al*., 2002; Sabu, 2005) followed by Karunakaran *et al*. (2011) in a German shepherd dog from Palakkad district in Kerala. Later, Augustine (2013) reported that 30 (25.86 per cent)and four (3.45 per cent) out of 116 dogs were found to be infected with *B. gibsoni* and *Babesia canis* respectively in a study on morphological and molecular detection of *Babesia* spp. in dogs in Thrissur. Tresamol *et al*. (2013) documented a case report of cerebral babesiosis due to *B. gibsoni* infection in a Boxer dog from Thrissur district in Kerala.

EPIDEMIOLOGY

The young dogs were highly susceptible to babesiosis and most frequently had severe infections and suffered from acute and hyper acute forms than the older dogs (Muhlnickel *et al*., 2002; Boozer and Macintire, 2005). *B. canis* was observed in 36 hours old puppies and *B. gibsoni* in three day old puppies and concluded transmission of piroplasms by transplacental route because the age was shorter than the prepatent period.The incidence of canine

babesiosis was 10.6 times higher in less than two years of age when compared to older dogs while older dogs were predisposed to more complications when compared to younger ones (Bashir *et al*., 2009).Dogs screened for haemoprotozoal infections were positive for *B. gibsoni*, among them 58 per cent of neonate population were less than three month old and 18 per cent were less than one year old (Selvaraj *et al*. 2010). *B. gibsoni* was found to be higher among dogs less than two years of age (Augustine, 2013). Canine babesiosis was most common in male dogs when compared to female dogs due to behavioural activities like roaming, searching for mates and establishment of territories that resulted in them getting infested with ticks (Amuta *et al*., 2009; Bashir *et al.* 2009). Majority of dogs diagnosed with *B. gibsoni* in the United States were American Staffordshire terriers and American Pit Bull Terriers (APBT) (Birkenheuer *et al*. 2003). Babesiosis in dogs from northern Portugal occurred during auntum and winter months *viz.,* October (18 per cent), November (27per cent), December (20 per cent), February (13per cent) and March (9 per cent) (Cardoso *et al*. (2008). canine babesiosis was more prevalent during the months of January, July, August, September and November compared to February, March, April, May, October and December in a detailed epidemiological and vector identification study in Pakistan (Bashir *et al.* 2009).

TRANSMISSION

Babesia gibsoni infection in dogs was transmitted by ticks *Rhipicephalus sanguineus* and *Haemaphysalis bispinosa* (Taboada and Lobetti, 2006; Aysul *et al*., 2013; Augustine 2013). Other mode of transmission included transplacental transmission, the direct transmission of blood during dog bites, direct transmission of blood by iatrogenic means and shipping of dogs from endemic areas (Macintire *et al*., 2002).

PATHOGENESIS

Pathophysiology of canine babesiosis varied from mild anaemia to widespread multiple organ failure and death

The anaemia caused by *B. gibsoni* was due to destruction of erythrocytes, resulted from a combination of the direct mechanical disruption caused by the parasite as it leaves the red blood cell, along with intravascular haemolysis, which may be immune mediated or non-immune mediated destruction of erythrocytes. The indirect pathways of RBC destruction included immune mediated destruction secondary to the development of antierythrocytic membrane antibodies, inhibition of erythrocyte 5' nucleosidase, development of methemoglobinemia secondary to oxidative stress, induction of serum haemolytic proteins and increased macrophage erythrophgocytic activity. Oxidative damage of erythrocytes induced by *B. gibsoni* infection, even in the presence of low parasitaemia led to severe anaemia (Otsuka *et al*., 2002). Significant increase in the level of anti-erythrocyte membrane antibodies (IgG andIgM) in sera of dogs naturally infected with *B. gibsoni*. However the antibodies were suggested as possible enhancers of erythrocyte destruction and this was confirmed by ELISA and immunoblotting technique. Immunosuppression was observed in dogs suffered from relapses of clinical *B. gibsoni* infection and it could be due to prominent depression of lymphocyte blastogenesis and anti-parasitic antibody production (Adachi *et al*., 1993). Dogs experimentally infected with *B. gibsoni* had anatomic lesions *viz.,*diffuse nonsuppurative periportal hepatitis, centrilobular hepatitis, multifocal necrotizing arteritis, membranoproliferative glomerulonephritis, reactive lymphadenopathy, diffuse erythrophagocytosis, and extramedullary haematopoiesis (Wozniak *et al*., 1997).

Infection with *B. gibsoni* resulted in more severe clinical manifestations with multiple organ failure than infection with *B. canis* in Japan hence infection caused by *B. gibsoni* was considered clinically more important than *B. canis* (Miyama *et al*. 2005). Anaemia in *B. gibsoni* infection might be due to direct parasite- induced red-cell damage, increased osmotic fragility of infected red blood cells, oxidative and secondary immune-mediated injury of the erythrocyte membrane resulting in a combination of intravascular and extravascular haemolysis (Irwin, 2009).

SEVERE OR COMPLICATED BABESIOSIS:

Acute renal failure, hepatopathy, coagulopathy, secondary immune mediated haemolytic anaemia, haemoconcentration, hypotension, cardiac related alterations, acute pancreatitis and acid base disturbances were reported in complicated or severe form of babesiosis. Cerebral babesiosis characterized by combination of incoordination, pelvic limb paresis, muscle tremors, nystagmus, anisocoria, intermittent loss of consciousness, seizures, stupor, coma, aggression, paddling, or vocalization (Birkenheuer, 2012). Multi-system disease and multiple organ dysfunction syndromes (MODS) developed from Systemic Inflammatory Response Syndrome (SIRS), were responsible for complicated cases of canine babesiosis by *Babesia canis*. Systemic Inflammatory Response Syndrome (SIRS) was characterized by uncontrolled inflammatory response with one or more organ dysfunction including cerebral, acute renal, hepatic dysfunction, rhabdomyolysis, adult respiratory distress syndrome, pancreatitis, dermal necrosis, haemorrhagic diathesis and secondary immune mediated haemolytic anaemia. Shock in babesiosis resulted from severe anaemia or release of inflammatory mediators associated with MODS (Jacobson and Clark, 1994; Matijatko *et al*., 2010). Acute Respiratory Distress Syndrome (ARDS) was common and an important complication of the more pathogenic strains of *Babesia* spp. characterized by tachypnoea, dyspnoea, a moist cough, serosanguinous frothy respiratory secretions and hypoxemia. Radiographs revealed either diffuse or caudo dorsal patchy alveolar infiltrate with normal cardiac silhouette and vessel size (Ayoob *et al.*2010).

CLINICAL SIGNS

Acute form of canine babesiosis was characterized by pyrexia, weakness, pallor of mucous membranes, depression, lymphadenopathy, splenomegaly and general malaise (Muhlnickel *et al.*, 2002; Jefferies *et al*., 2007). The fatal form of disease exhibited some signs like melena and local erythema of skin. Bleeding from vein puncture site was attributed to thrombocytopenia and coagulopathy which was the major presenting clinical sign and complicating factor in more number of cases (Johan Schoeman and Leisewitz 2006; Selvaraj *et al*. 2010). Intermittent vomiting also reported in a *B. gibsoni* infected dog from Italy (Trotta *et al*. 2009). Wide range of clinical presentations from subclinical disease to serious illness characterised by fever, pallor, jaundice, splenomegaly, weakness, systemic inflammation, hyperglobulinemia, neutrophilia with left shift,thrombocytopenia and pigmenturia in canine babesiosis (Jacobson *et al*. 2006).

Chronic form of *B. gibsoni* infection was manifested as a subclinical infection or associated with weight loss and weaknes (Solano-Gallego and Baneth, 2011). Clinical signs of cerebral babesiosis due to *B. gibsoni* in a dog consisted of anorexia, weakness, ataxia, occasional seizures and depression (Tresamol *et al*. 2013). Dogs experimentally infected with *B. canis* were characterized by fever, increase in pulse, tachycardia, anorexia, lethargy, pallor of the mucous membrane of the mouth and

eye, emaciation, muscle tremor, respiratory distress, nervousness, drooling salivation, haemoglobinuria, mucoid ocular discharge and followed by death if not treated (Konto *et al*. 2014). Few cases of canine babesiosis caused by *B. gibsoni* were complicated with multi-organ failure, hepatopathy, acute renal failure, immune-mediated haemolytic anaemia and cerebral babesiosis (Vijayalakshmi *et al*. 2014). Rarely cutaneous lesions manifested as oral or cutaneous petechial and ecchymotic hemorrhages associated with thrombocytopenia or disseminated intravascular coagulation, subadjacentl eukocytoclastic vasculitis with or without vascular necrosis. Clinical signs include edema, ecchymosis, ulceration, and necrosis, which can be seen on the pinnae, axillae, groin, lower limbs, ear tips or scrotum. (Miller *et al*. 2013).

DIAGNOSIS

Three fundamental techniques for diagnosis incorporates microscopic examination, serological testing and nucleic acid based detection by molecular methods

Single to multiple, variable sized (1 - 3µm in diameter), and round to oval to band like piroplasms within many red blood cells consistent with small form of *Babesia spp* in Wright's staining technique (Trotta *et al*. 2009). *Babesia gibsoni* piroplasms appeared highly pleomorphic and exhibiting different forms such as linear, amoeboid, reticulate, paired pyriform, and signet ring form (Armando *et al*., 2001). These piroplasms were pleomorphic and exhibited linear, reticulate (network forming), pyriform, amoeboid, and signet ring forms and the latter was reported as the most common form (Fukumoto *et al*., 2000;Augustine 2013). *Babesia* parasites were usually visualized in blood smears only during the acute phase and the reason for lower percentage of *Babesia* positive cases by direct examination of blood smears was attributed to less number of infected cells in peripheral blood (M'ghirbi and Bouattour 2008). Indirect fluorescent antibody test is the most regularly utilized test for identification of anti-babesial antibodies. Ano *et al*. (2001) conducted the molecular survey of *Babesia*s pp. infection among dogs in Japan and developed a new method of nested PCR. Seminested PCR for the diagnosis of canine babesiosis which differentiated *B. gibsoni* (Asian genotype), *B.canisvogeli*, *B.caniscanis* and *B.canisrossi* with defined limit of detection. Outer primer pairs 455-479F, 793-772R and species specific primers BgibAsia-F, was used in first and second round of PCR respectively to successfully amplify a 185bp products of *B. gibsoni* (Asian genotype) (Birkenheuer *et al*. 2003). Multiplex PCR can be utilized to at the same time distinguish potential co-infection with numerous tick-borne pathogens including *Babesia* spp.

TREATMENT

Diminzeneaceturate for the treatment of both small and large *Babesia* spp. infections should be used with caution account of a relatively small dose safety margin with a large inter-individual pharmokinetic variation (Miller *et al*., 2005).

Wulansari *et al*. (2003) reported that clindamycin, a dose dependant antibiotic with the property of immune enhancing ability inactivated or damaged *B. gibsoni* organisms in infected dogs. Combination therapy of clindamycin, doxycycline and metronidazole showed a rapid recovery from anaemia and thrombocytopenia or a long disease-free period compared to the untreated control dogs (Suzuki *et al*. 2007).

Atovaquone (13.3 mg/kg body weight, PO, q8h and azithromycin (10 mg/kg body weight, PO, q24h hours) for 10 days could eliminate chronic *B. gibsoni* (Asian genotype) infections or suppress the parasitemia below

the limit of detection in majority of the treated dogs (Birkenheur *et al*. 2004). Atovaquone, a hydroxynaphthoquinone acts primarily to inhibit the parasite's mitochondrial electron transport chain, possibly by mimicking the natural substrate ubiquinone. Resistance to atovaquone was rapid and resulted from a single point mutation in the gene for cytochrome b (Rang *et al*., 2012).

PREVENTION:

Doxycycline @ 10 mg /kg body weight BID for eleven consecutive days was effective in preventing babesiosis due to *B. gibsoni* but infection could not be cleared by the same (Vercammen *et al*., 1996).

Soluble parasitic antigen (SPA) from plasma of *Babesia canis* infected animals or supernatant of in vitro cultures of these parasites could be used as vaccine. The antigen treated with formalin and freeze dried when used as a vaccine against *B. canis* in dogs could decrease the incidence of babesiosis from 16 per cent to almost zero in vaccinated dogs (Schetters, 2005). A preliminary study on the safety of a new vaccine against canine babesiosis containing *B. canis* soluble parasitic antigen (SPA) and reported that the vaccine stimulated the development of antibodies prevented the development of the disease. It was found to be considered to be safe without any adverse effects (Adaszek, *et al*. 2012). Two vaccines were used against canine babesiosis *viz.*NobivacPiro® (NP) and Pirodog® (P) in France (Freyburger *et al*. 2011).

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MANAGEMENT OF LIVESTOCK WASTE

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INTRODUCTION

Livestock sector plays a critical role in the welfare of India's rural population. Livestock sector includes animal husbandry, dairy and fisheries sector which are considered major sectors. It plays an important role in the national economy and in the socio-economic development of the country. Its role is very crucial in the rural economy as supplements family incomes and generates gainful employment in the rural sector (Kumbhar, 2011).

The total livestock population consisting of cattle, buffalo, sheep, goat, pig, horses & ponies, mules, donkeys, camels, mithun and yak in the country is 512.05 million numbers in 2012. In 19th Livestock Census, 37.28 percent were cattle, 21.23 percent buffaloes, 12.71percent sheep, 26.40 percent goats and 2.01 percent pigs.The livestock sector alone contributes nearly 25.6 percent of value of output atcurrent prices of total value of output in agriculture, fishing & forestry sector. Theoverall contribution of livestock sector in total GDP is nearly 4.11 percent at current pricesduring 2012-13(19^{th} Livestock Census-2012).

The presence of livestock invariably generates wastes. In India, livestock wastes are managed generally in three ways. The waste excreted by livestock are removed by dumping into heaps nearby the cattle sheds. The heaps get converted into manure, which are spread subsequently in the fields as an organic matter. Much of the livestock waste is utilized for energy purpose in village level where the waste are made into small cakes and dried and later used as fuel for cooking purposes. Another method of livestock waste management is the establishment of bio-gas plant where waste is used for the production of methane under anaerobic (lack of oxygen) conditions. The methane gas is used for cooking purpose,and the slurry after methane extraction is used as farm manure(Gautam, 2006).

Among the livestock wastes, animal dung and wastewater constitute the maximum of the total.Animal dung waste produced ranges from 3 MT/ day to 6000MT/ day. Other types of wastes like fodder and hay, fat and grease may lie in the range of 25 kg/day to 1500 kg/day. More than 60 percent of the waste generated is treated aerobically and less than 5 percent by anaerobic treatment. It is estimated that only 9 percent of the livestock sector is involved into methane recovery and utilization projects (FICCI,2009).In decades past, livestock waste management was not considered as a big problem. However, as milk and meat production needs increased, herd size and waste production also increased.

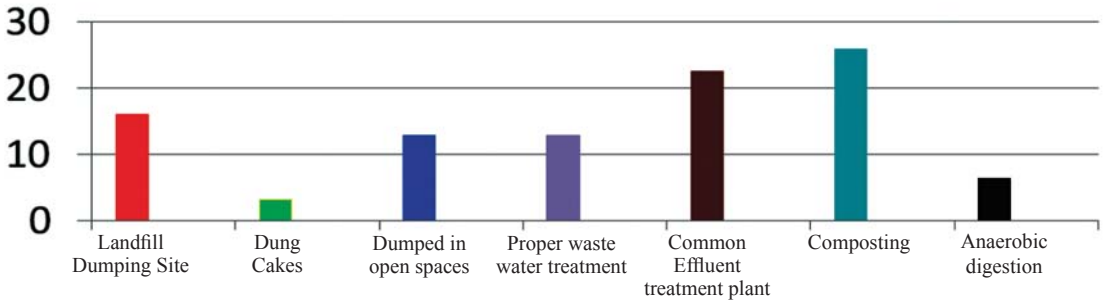
This has heightened the awareness for waste management.

Livestock production systems are intensifying worldwide, particularly in urban and peri-urban areas. As a result, livestock waste is emerging as a serious environmental and public health concern. Livestock waste can lead to huge nutrient surpluses concentrated in areas close to humans leading to soil and water pollution and has even been implicated in climate change. (Martinez *et al*., 2009). Untreated and ill-disposed hog waste can cause air pollution by the release of noxious gases like hydrogen sulfide, methane, and ammonia leading to health effects such as respiratory ailments, skin irritation, "blue baby syndrome," and cognitive impairments due to the growth of *Pfiesteria* in the air and water at high nitrate concentrations. Accumulation of livestock waste attracts flies and parasites causing bad odour and other nuisance. The health issues generated from waste accumulation affects public health, livestock health, farm staff health and food quality(Martinez and Burton,2003).

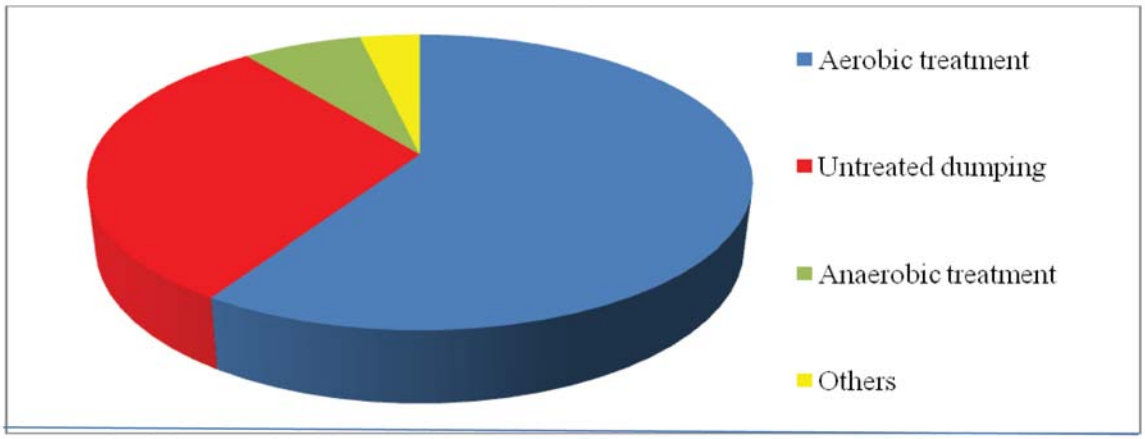
FARM ANIMAL WASTE MANAGEMENT

Composting

Composting is a naturally occurring process in which bacteria, fungi, and other microorganisms convert organic material into a stabilized product known as compost. Within the carcass, anaerobic microorganisms work to degrade it, releasing fluids and odorous gases such as hydrogen sulfide and ammonia. These



WASTE MANAGEMENT AND DISPOSAL METHODS IN INDIAN LIVESTOCK SECTOR (%) (FICCI Report 2009)



LIVESTOCK WASTE MANAGEMENT SCENARIO IN INDIA(FICCI Report, 2009)

diffuse into the surrounding bulking agent. In this bulking agent, aerobic microorganisms degrade these materials to odour-free carbon dioxide (CO_2) and water (H_2O). The aerobic process produces considerable heat, causing the temperature of the compost pile to rise. The active bacteria in both the aerobic and anaerobic zones are heat tolerant. However, the heat kills common viruses and bacteria that may be present in the carcass. Odour is controlled by having an adequate quantity of bulking agent around the carcass. It is a simple way to add nutrient-rich humus which fuels plant growth and restores vitality to depleted soil(Morse *et al*., 2001).

Vermicomposting

Vermicomposting is a simple bio technological process of composting in which certain species of earthworms (*Eisenia fetida, Eisenia andrei, Eudrilus eugeniae Dendrobaenaveneta, Perionyx excavates*) are used to enhance waste conve rsion and to enhance a better endproduct. Vermicomposting involves bio-oxidation and stabilization of organic materials by the joint action of earthworms and microorganisms.and (Dominguez Edwards, 2010)

Combining the two systems in an experiment in Spain resulted in a superior product with more stability and homogeneity. In this system, Composting combined with subsequent vermicomposting was carried out by composting the manure for 15 days and then vermicomposting in a $1m^3$vermireactor containing a stable and very active population of the earthworm *Eisenia andrei* for 40days. Samples were collected from the vermireactor 40 days after the addition of the third layer of composted manure. (Lazcano *et al*. 2008)

Type of by-product	% of live weight	Uses
BY-PRODUCTS FROM PRODUCTION PHASE		
Poultry litter and manure	-	Recycled feed, surface dressing of agricultural land
HATCHERY BY-PRODUCTS		
Egg shells, infertile eggs, unhatched eggs and dead as well as culled chicks	-	Hatchery by-product meal upto 3–5% into feed. Egg shell meal as high calcium diet
BY-PRODUCTS OF POULTRY DRESSING PLANT		
Feathers	7–8	Bedding material, decorative purpose, sporting equipment, manure or fertilizers, feather meal.
Heads	2.5–3.0	Poultry meal
Blood	3.2–3.7	Blood meal
Gizzard and proventriculus	3.5–4.2	Edible,source of chitinolyticenzyme.
Feet	3.5–4.0	Soup, technical fat/poultry grease
Intestines and glands	8.5–9.0	Sportgats, meat meal, poultry grease and active principles (hormones and enzymes)

BIOMETHANATION TECHNOLOGY

In this method, production of methane occurs from livestock waste under anaerobic condition through biodegradation of organic materials (used in biogas technology).Biogas plants help in total recycling of organic wastes in an environment-friendly manner. This is the best alternate source of energy from organic waste. It is used as fuel for cooking and lighting purposes.It can also be used in diesel engines to substitute diesel-oil up to 80 per cent. In recent years, with advanced processes of biomethanation, the technology is further being expanded as a solution to waste handling and mitigating environmental problems. The left over decomposed slurry is a good source of manure for agricultural lands. Biomethanation can be applied as a profitable waste management plan in institutions that generate large quantities of organic waste, like schools, markets, restaurants, and hotels (Ngumah, 2013). The methane potential in manure is assessed on the basis of the content of volatile solids in the manure and empirical standards for the production of methane per kg of Volatile Solids. The methane potential has been estimated to be 0.29 m^3 CH_4/kg of Volatile Solidsin pig manure, 0.21m^3 CH_4/kg of Volatile Solids in cattle manure.(Nasir *et al*., 2012).

GENERATION OF ELECTRICITY

From 1 ton of manure with 20 percent solid content, 20–25 cubic meter biogas can be produced with a total energy value of 100–125 kWh and the same can be utilized to generate 35–40 kWh of electricity and 55–75 kWh of heat energy (Burton and Turner, 2003).

UTILISATION OF COW URINE

Roughly about 11.4- 22.8 crore litres of cow urine is produced each day and it can be utilized in many ways. This has to be considered as a precious natural resource, and not as a waste generated from livestock.Cow urine is one of the ingredients of 'Panchagawya' capable of treating many curable as well as incurable diseases and has been used extensively in ayurvedic preparations (Pathak and Kumar, 2003).'Panchgawya' is also used as fertilizer and pesticide in agricultural operations. Cow urine is basically an excellent germicide and a potent antibiotic. Distillate cow's urine is an activity enhancer and availability facilitator for bio active molecules (Mohanty*et al*., 2014).

METHODS OF POULTRY WASTES UTILIZATION (Sams, 2001)

It is assumed that every year, approxi mately 1.2 croretonne manure is produced from the broiler and layer industry and more

Animal	Kg/Head	% of animal weight
Bovine	83	27.5
Goat/Sheep	2.5	17
Pig	2.3	4

Quantity of solid waste generated from the bovine, goat, sheep and pig slaughter houses (USDA,2001)

Waste Source	BOD Value(mg/l)
Cattle slurry	10,000-20,000
Pig slurry	20,000-30,000
Silage effluents	30,000-80,000
Slaughter house wastes	10,000-30,000

Ranges of BOD concentrations from various wastes(MAFF,1998)

than 14.1 crore kilogram of slaughter waste from broiler birds produced in India.

SLAUGHTER HOUSE WASTES AND ITS MANAGEMENT

The changing dietary trends of future population is expected to increase the consumption of livestock products. Percentage increase in consumption of beef, pork and poultry by 2030 is estimated to be 51 percent, 160 percent, 844 percent respectively of that in 2000 (FAO 2011). Most of the slaughter houses in the country perform the killing and dressing of animals without an onsite renderingoperations and are more than 50 years old. These slaughter houses are without adequate basic amenities like proper flooring, ventilation, water supply, lairageetc. In addition to these deficiencies, slaughter houses suffer from very low hygiene standard posing a major public health and environmental hazard. This is attributed to the in-discrete disposal of waste and highly polluted effluent discharge. Unauthorizedand illicit slaughtering has also increased many fold leading to the related problems.Most of the meat industry does not meet the standards for discharge of effluents as laid down and notified under the Environment (Protection) Act, 1986. (Govt of India. 2000)

The wastes from slaughter houses and packaging houses are similar chemically to domestic sewage, but are considerably more concentrated. They are almost wholly organic, chiefly having dissolved and suspended material. The principal deleterious effect of these wastes on streams and water courses is their deoxygenation(Chakraborty and Mukhopadhyay, 2014**)**.

SLAUGHTER HOUSE WASTE MANAGEMENT

Different methods for the disposal of such wastes exist, including composting, anaerobic digestion (AD), alkaline hydrolysis (AH), burial, aerobic fermentation, rendering, incineration and burning. (Whittle and Insam, 2013).

Utilization of blood for blood sausages, blood pudding, biscuits bread, blood curd and for non-food items such as fertilizer, feedstuffs and binders and in pharmaceutical industry (Ghost, 2001)

Utilization of hides and skins for manufac turing leather shoes and bags, athletic equipments, reformed sausage casing and sausage skins, cosmetic products, edible gelatine and glue. (Benjakul *et al*. 2009)

Utilization of edible tallow and lard for preparation of french fries and other fast foods, Margarine,sausages or emulsified products (Ghotra *et al*. 2002)

Treatment of meat industry waste waters by ultrafiltration – reverse osmosis, chemical precipitation – reverse osmosis, chemical precipitation –ultrafiltration–reverse osmosis (Bohdziewicz and Sroka, 2005)

UTILIZATION OF WASTE AS BIOFUEL

Due to sanitary, environmental problems and operational costs related to the discharge, land disposal and re-use of wastes and the utilization of biofuel for steam generation has shown to be a viable alternative. **(**Jayathilakan *et al*., 2012**)**.

Poultry litter with 9percent or less water content can be burnt without extra fuel, hence can be used as fuel for generation of electrical power (Davalo s *et al.,* 2002).

Commercial ferric sulfate treatment as coagulant allows the retention of 0.83–0.87 kg of biomass fuel for each cubic metre of treated wastewater **(**Jayathilakan *et al*., 2012**)**

Thermal recycling of residues in power plant (Arvanitoyannis and Ladas, 2008)

Compressed Natural Gas: New system of biogas purification and bottling was developed at IIT, New Delhi (Vijay,

2011). Biogas can be purified up to 98 % methane content and can be stored into CNG cylinder compressed to 150 bar pressure and can be easily used any time anywhere as LPG cylinders. Further, the stored biogas was used to run petrol-based auto rickshaws (Kapdi *et al*. 2006) and diesel engines (Ilyas, 2006).

Generation of biodiesel from animal fat–"Biodiesel is a mono alkyl ester of long chain fatty acids derived from renewable sources (vegetable oil or animal fat)for use in diesel engines" (National Biodiesel board, 1996). Manure can also be combined with plant and animal fat to make biodiesel. (HSUS Report, 2009). The use of biodiesel can reduce the engine emission of smoke level by 47.14% when compared to petrol and diesel used in an engine test rig. Importing of crude oil can be reduced to an extend by blending of 20% biodiesel (John Abraham *et al*., 2014).

SOLID WASTE POLICY IN INDIA

(Municipal Solid Waste Rules, 2000).

1. Prohibit littering on the streets by ensuring storage of waste at source in two bins; one for biodegradable waste and another for recyclable material.
2. Primary collection of biodegradable and non-biodegradable waste from the doorstep, (including slums and squatter areas) at pre-informed timings on a day-to-day basis using containerized tricycle/ handcarts/pick up vans.
3. Street sweeping covering all the residential and commercial areas on all the days of the year.
4. Abolition of open waste storage depots and provision of covered containers or closed body waste storage depots.
5. Transportation of waste in covered vehicles on a day to day basis.
6. Treatment of biodegradable waste using composting or waste to energy technologies meeting the standards laid down.
7. Minimize the waste going to the land fill and dispose of only rejects from the treatment plants and inert material at the landfills as per the standards laid down in the rules.

FUTURE TRENDS IN LIVESTOCK WASTE MANAGEMENT

(Martinez *et al*., 2009 and Martinez and Burton, 2003)

Early separation of liquids from solids in livestock houses can reduce the gaseous emissions in the buildings and it generates liquid and solids that can be processed separately.

Development of techniques allowing nutrient recycling from wastes, especially phosphorus.

Amendment of environmental protection policies by notifying about new "emerging" pollutant like antibiotics, endocrine disrupters, antibiotics-resistant pathogens etc

Finding new global methods to assess the viability of production chain and food supply.

Manure-soil interactions studies are required to study the effect that various treatments have on the subsequent interactions of the manure with the soil in order to verify that subsequent pollution is reduced. Development of newer technologies for the re-use of diluted effluents for washing and irrigation purposes

Development of methods to work on both the inputs and the outputs of livestock production and the integration in "regional" or geographical aspects.

Better use of the nutrients in organic material.

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ANAPLASMA AND ANAPLASMOSIS IN LIVESTOCK OF KARNATAKA – A RETROSPECTIVE

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Anaplasma is an obligate intraerythrocytic bacteria of family Anaplasmataceae of the order Rickettsiales which causes the dreadful disease, anaplasmosis among livestock and this organism is always dealt along with other blood protozoans, *Theileria* and *Babesia*. Though anaplasmosis caused by *A. marginale* was known to be prevalent in native cattle of Karnataka, its occurrence in cross-bred dairy cattle of Bengaluru was reported by Jagadish Kumar *et al*. (1977). This disease along with other haemoprotozoans gained more attention in late sixties in Karnataka state after the impact of import of foreign breeds of cattle for upgrading indigenous stock to improve their milk yield. The cross-bred progenies from these cattle are more susceptible to these disease compare to indigenous stock which acted as carriers of infection to the susceptible animals through ticks and other haematophagous insects. The carrier status of infection existed in indigenous animals as well as the newly recovered ones from this disease. By considering the importance of this infection in dairy cattle in terms of loss in milk reduction, cost of treatment, mortality and morbidity, an ICAR scheme of five years duration "On the biology and control of *Anaplasma*" was implemented at Veterinary College, Bengaluru during 1974 which contributed important information on different aspects of the disease. Another major source of information on this disease was from the accumulated data for about seven years period provided by the four Veterinary Diagnostic Laboratories (VDL) located at strategic places like Bengaluru, Mysuru, Tumukuru and Hassan, established by the Karnataka Dairy Development Project with the financial assistance of World Bank and the technical support from the University of Agricultural Sciences, Bengaluru. These laboratories helped for the prompt recognition disease problems especially of the bovine population of dairy co-operative societies (DCS) of the eight southern districts of Karnataka such as Bengaluru, Kolar, Mysuru, Mandya, Kodagu, Tumukuru, Hassan and Chikkamagaluru. Incidences of anaplasmosis in livestock in various parts of Karnataka, its pathogenesis, diagnostic methods adopted, advanced serological investigations, surveillance, recognition of vectors and treatment followed were enlightened in this review. Further, attempts were made to know its occurrence in certain captive animals. Success achieved in growing *A. marginale* organisms in tissue-culture and in attenuating them widened the scope for developing suitable vaccine.

SPECIES OF ORGANISMS

Two species of *Anaplasma* occur in

Karnataka, *A.marginale* in cattle and buffaloes and *A. ovis* in sheep.

MORPHOLOGY

The Giemsa-stained smears prepared out of the blood of positive cattle showed typical *A. marginale* bodies in about 60.0% of affected erythrocytes, one or two bodies per cell (Jagadish Kumar *et al*., 1977; Ravindranath *et al*., 1982). When the fresh positive blood was mixed with equal quantity of 0.5% new methylene blue stain and examined as wet smear preparation, the *Anaplasma* bodies appeared as small bright dots or tiny blue particles of 0.5µ size inside the erythrocytes exhibiting a floating type of movement with a tendency to be at the periphery (Setty, 1983; 2002).

INCIDENCE

The available data on the incidence of *A. marginale* infection in cattle and buffaloes based on stained blood smear examinations have been presented in Table I. It could be inferred from the Table that the overall incidence of infection in affected animals was within the range of 0.37%-46.45% with one exception of 60.87% infection of an epizootic proportion in cross-bred heifers. The data on incidence have been analyzed seasonal, breed as well as age-wise and noted below.

Seasonal incidence

Analysis of data of positive cases of *A. marginale* infection for the six years (1978-1984) period of VDL, Bengaluru and Mysuru indicated higher seasonal infection was observed in south-west (SW) monsoon followed by north-east (NE) monsoon seasons. Data from VDL, Hassan revealed that summer season was more favorable for the spread of infection while VDL, Tumukuru recorded higher prevalence during cold weather followed by SW monsoon and no infection was noticed in NE monsoon (Seshadri *et al*., 1985). The seasonal studies indicated that *A. marginale* infections occurred throughout the year, initiated from the pre-monsoon months (Muraleedharan *et al*., 2005). But Ravindranth *et al*. (1982) reported this disease in September-October as an outbreak-form.

Breed incidence

Among exotic pure breeds Holstein-Friesian had shown 42.8% and Jersey had 26.5% infection whereas cross-bred cattle had higher rate of 46.4% infection compared with 16.7% in non-descript indigenous cattle (Jagannath and Krishna Murthy, 1980). Ravindranath *et al*. (1982) reported very high percentage (60.86%) of infection in cross-bred heifers at a farm in suburban Bengaluru. In the jurisdiction of VDL, Mysuru, Muraleedharan *et al*. (2005) noticed that the cross-bred cattle showed more infection (1.51%) than indigenous cattle (0.86%) while pure-bred exotic cattle did not show infection. Among the cross (x) between exotic breeds and local cattle, Red Dane x (2.67%) followed by Jersey x (2.08%) showed more susceptibility to infection than by Holstein-Friesian x (1.24%).

Age incidence

As per the data of VDL, Mysuru, the cattle below six month of age were not susceptible to *A. marginale* infection. The minimum age at which infection was observed was one year. Other age group-wise incidences was as follows: 6 month to 1 year-1.33%; 1 to 4 year-1.38%; 4 to 8 year-1.48% and <8years-1.06% indicating a declined trend. The animals of one year of age alone showed higher rate of infection indicating more susceptibility in comparison with other age groups with four years class-intervals (Muraleedharan *et al*., 2005).

CONCURRENT INFECTION WITH HAEMOPROTOZOANS

A combined infection of *A. marginale* and *T. annulata* was seen in four cattle (Anon,

1979). Of the 60 positive cattle (n=4521) identified at VDL, Mysuru (Muraleedharan *et al*., 2005), 10 had concurrent infection of *A. marginale* and *T. annulata* with predominance of *A. marginale* in three cases, theilerial infection in two cases and the remaining five cases shared almost equal number of these parasites.

PATHOGENESIS

Cattle positive for *A. marginale* infection had pyrexia of 40-41^{o}C marked anaemia, dyspnoea, increased pulse rate, lachrymation, salivation, shivering, pale bulbar conjunctiva, visible mucus membrane icteric including vulvar, swelling of sub-maxillary region enlarged lymph nodes, increased pulse rate and respirations were shallow and labored. The udder, teats and muzzle of one of the affected cows were distinctly yellow giving the appearance of "yellow cow" (Jagadish Kumar *et al*., 1977; Ravindranath *et al*., 1982; Muraleedharan *et al*., 2008).

Haematology

Jagadish Kumar *et al*. (1977) and Ravindranath *et al*. (1982) reported that most of the clinical anaplasmosis about 60% of the erythrocytes (60.0%) had infected with one or two organisms per cell. The infected erythrocytes had a punched out appearance with marked anisocytosis, polychromasia, basophilic stippling with presence of many megaloblasts and normoblasts. The haematologial values of acute anaplasmsis were: Hb: 3-8g/dl, TEC: 2 m/cmm, TLC: 10700/cmm, ESR: nil/hr and PCV: 9% (Jagadish Kumar *et al*., 1977 and Ravindranath *et al*., 1982). Scanty to high parasitaemia (2.0-60.0%) was recorded by Muraleedharan *et al*. (2005) in cattle of Mysuru-Mandya area.

Experimental infection of calves

Experimental infections of *A. marginale* were induced to 25 splenectomized and 5 intact calves maintained in tick proof sheds, by injecting varying quantities of blood with 80% parasitaemia and the blood collected from them were stored for 52 days at +4^{0}C. The calves on recovery from *Anaplasma* infection remained carriers. Infected erythrocytes were stored in liquid nitrogen at –196^{0}C as per procedures after adding 10% dimethyl sulphoxide (DMSO) to minimize haemolysis. The recovered calves from clinical form remained as carriers. For rapid multiplication of *A. marginale*, cortisone (decadron) was administered to *Anaplasma* carrier experimental calves for four consecutive days, but it did not flare up the infection (Anon, 1979; Krishna Murthy and Jagannath, 1980a).

Experimental infection of laboratory animals

Inoculation of 0.2 ml of 60% *Anaplasma* infected blood intravenously (i/v) to rabbits, guinea-pigs, mice, hamsters and to four a day-old embryonated eggs by intra-yolk-sac route failed to set up infection. Capillary agglutination test conducted in these laboratory animals also proved negative showing their non-susceptibility to infection. No visible lesions were observed in chicks-embryos except for haemorrhages and no sign of disease in experimental animals. The administration of decadron (2mg) to these animals did not promote the establishment of this organism (Krishna Murthy and Jagannath, 1980b).

SEROLOGICAL DIAGNOSIS

Various serological tests using indig enous antigens had been tried to detect *Anaplasma* infection, especially in the carrier animals whose did not always exhibit the organisms by GBS examinations (Anon, 1979).

1. Capillary-tube agglutination test

Krishna Murthy *et al*. (1978; 1994) conducted capillary-tube agglutination (CA) test using antigen prepared using local strain of *A. marginale* as per the method of Ristic

with minor modifications such as the blood samples were frozen at –20°C instead of –65°C and antigen was stored both at –20°C as well as at +4°C. Sera collected from clinical cases and experimentally infected calves were found 100% positive to CA test whereas as high as 80.95% of the 21 suspected cattle and 3.08% of 130 apparently healthy cows were shown positive instead of 4.76% and 2.31% corresponding to GBS examination. The CA test further indicated a higher prevalence of infection in exotic and cross-bred cattle (20.72%) compared with that of local cattle (6.07%). The clinically cured exotic cows following oxytetracycline therapy remained positive for CA test up to 100 days, but they were found negative when tested after 300 days. Inoculation of 20ml blood of these animals to two splenectomized calves failed to establish infection in them for an observation period of 30 days which indicated the complete clearing of organisms as well as antibodies. The efficacy of locally prepared CA test antigen was found equivalent to that of imported antigen supplied by Miodrag Ristic of the University of Illinois, USA. It was also noted that the antigen did show any cross or non-specific reaction with the sera of normal sheep, goats, dogs, rabbits as well as man and those of cattle recently suffered from theileriosis, babesiosis, brucellosis and foot and mouth diseases.

2. Card agglutination test

The coloured card agglutination antigen was prepared with local strain of *A. marginale* following the method of Todorovic and Kuttler for *Babesia bigemina* antigen. Out of 251 sera samples tested, 31.07% showed positive reaction to card test as against 27.89% to CA test and 25.90% by GBS examination. The test was found to be sensitive and dilutions 1:4 and 1:6 of antigen gave better results than the higher dilutions. The blood smears and sera collected from indigenous cattle were all negative for *Anaplasma* while those of cross-bred cattle from Bengaluru and its suburbs were, however, found to be carriers of infection by card agglutination tests and CA tests. In detection of carrier condition the card test appeared to be more efficient than CA test (Anon, 1979).

3. Gel diffusion test

Gel diffusion test was done as per Ouchterlony method using CA antigen. Positive reactions were noted with precipitation band formation in the agar in between the wells containing corresponding antigen and sera. Out of 15 sera samples including four known positives, only one (6.67%) was found positive indicating that the method was not satisfactory (Anon, 1979).

4. Complement fixation test

The antigen for complement fixation test (CFT) was prepared as per Gates *et al*. Out of 30 sera samples tested, only six (20.0%) were found positive to CFT. Sera positive for *Theleria*, *Babesia*, Foot and Mouth disease and *Brucella* when tested against known antigen by CFT showed negative results. It was observed that the CFT appeared to be more sensitive in detecting the *Anaplasma* positive cases than CA test and Card test (Anon, 1979).

5. Fluorescent antibody technique

Two methods of fluorescent antibody technique (FAT) were tried. Direct and indirect FAT were applied on unknown and known blood smears using fluorescein isothiocynate tagged antibodies. In the indirect method, tagging was effected to antibovine globulins.

a) Direct method

Serum containing antibodies against *A. marginale* was used for conjugation with fluorescein isothiocynate. The hyperimmune serum against *Anaplasma* in the rabbit was used for conjugation and test was conducted following standard procedure. When 20 known positive slides were examined, all gave

sufficient fluorescence to detect the organisms whereas known negative slides did not give any fluorescence. Another 30 blood smears were also examined, of which 10 (33.33%) found to be positive and the remaining showed negative results. In the direct method, non-specific reactions were observed. In the direct method, non-specific reactions were observed (Anon, 1979).

b) Indirect method

Thirteen known positive slides were also positive and five known negative cases did not show fluorescence**.** The sera samples of 30 more suspected cases for anaplasmosis from Bengaluru were tested by indirect FAT and proved to be negative. Indirect FAT was appeared to be more sensitive than direct FAT (Anon, 1979).

6. Immuno-peroxidase test

The immuno-peroxidase test (IPT) using *Anaplasma*-induced globulin was developed for detection of *A. marginale* in cattle. The test was performed for the first time in protozoan disease diagnosis. Antigen-antibody reaction was observed by the blue colour reaction under ordinary microscope. It was possible to detect more number of positive animals (53.0%) compared to that of conventional GBS examinations (40.9%) when 66 blood smears of field cases had been subjected to these two tests. The blood smears positive for *Theileria. annulata, Trypanosoma evansi* and *Babesia bigemina* failed to show a positive reaction with IPT and thereby confirmed the specificity of this test (Krishna Murthy and Jagannath, 1980a).

7. Leucocyte migration inhibition test

The cells involved in producing cellular immunity were mainly sensitized T. lymphocytes and macrophages which acted directly on *Anaplasma* organisms or attempted to phagocytose and digest them. The procedure of Ristic and Nyindo was adopted for conducting leucocyte migration inhibition test. The leucocyte migration was inhibited in two splenectomized infected calves as against one healthy control calf indicating that the test appeared to be sensitive (Anon, 1979).

8. Delayed cutaneous hypersensitivity test

Out of 6 recovered calves received injection of 0.1 ml of *Anaplasma* CA test antigen, 5 (88.33%) showed cutaneous swelling. By application of above two tests (Sl. No. 7 and 8) the status of cellular immunity of *A. marginale* among cattle could be assessed (Anon, 1979).

Tissue culture

a) Bovine lymph node culture

Pure lymph node cells (LNC) from a foetal bovine calf, negative for *A. marginale* were obtained and grown into a monolayer as well as inoculum of washed erythrocytes containing *A. marginale* organisms were prepared following standard procedures. Monolayer cells were serially passaged at least 8 times to obtain a well established cell line. Monolayer cell of each passage up to 8th passage was inoculated with infected blood from different sources. The multiplication of the organisms in the LNC was not rapid for the first 24-48 hr, but later it was hastened 3-4 times by repeated passages. The organisms were seen on the cytoplasm of the LNC and in certain places inclusion bodies were also observed inside cytoplasm of the cells. The treatment of LNC with diethylaminoethyl dextran (DEAE-D) solution and phytohaemagglutinin (PHA) did not improve the multiplication of the organisms. One ml of the inoculum of *A. marginale* organisms grown on 8th passage were inoculated into splenectomized calf. The calf showed 1.0 percent parasitaemia on the 48th day which reached 20.0 percent on 56th day. The parasitaemia came down to 2.0 percent and

later calf became negative for infection. But it reappeared on 90th day and subsequently calf died following extreme anaemia (Anon, 1979).

b) Leucocyte cell culture

The leucocyte cell culture was attempted following the standard procedure. Monocytic leucocyte cells (MLC) were collected for tissue culture from the blood of a calf below six months. Multiplication of leucocytes was fast during the 2nd and 3rd day. The leucocytic culture was obtained on 9th day and the MLC were sub-cultured till a good cell line was obtained. *Anaplasma* organisms were inoculated to cell line of leucocytes. The MLC was also treated with DEAE dextran and PHA at the rate of 30mg/ml respectively to encourage the multiplication of *Anaplasma* organisms. Repeated passage of *A. marginale* organisms in MLC tissue culture showed limited multiplication of organisms in spite of treatment with DEAE dextran and PHA. The multiplication of organisms in MLC was much less compared to the LNC monolayer tissue culture (Anon, 1979).

Premunition trials

Preimmunization with co-infectious immunization is an effective means of producing immunity to anaplasmosis. Non-splenectomized calf which did not contain any antibodies for *A. marginale*, was injected 20ml blood i/v from a carrier cattle having about 1 percent parasitaemia and revealed *A. marginale* on the 19th day of post-inoculation and clinical disease with lasted for a week. After 45 days of initial infection when CA test detected antibody titre 1:2, the calf was challenged with blood 15ml blood having 37 percent parasitaemia, resulted in the rise of serum titre to 1:16. On challenging with infected blood, the calf did not exhibit clinical disease, but with occasional *A. marginale*. Five more calves were preimmunized in the same way. Similarly a splenectomized calf with a low parasitaemia of 3 percent was inoculated with blood having 60 percent parasitaemia remained normal without any increase in parasitaemia (Anon, 1979).

Irradiation of *Anaplasma* organisms

Ten ml of *Anaplasma* infected blood with 60 percent parasitaemia after exposure to UV rays for 20 minutes was inoculated to a healthy calf while control calf was inoculated with the same quantity of positive blood without irradiation. Higher parasitaemia of 32 percent was observed early on the 19th day of infection in experimental calf whereas it was only 18 percent on the 22nd day in the case of control. The result was suggestive of enhancement of infection rather than attenuation of organisms by the effect of UV radiation (Anon, 1979).

Vectors of *Anaplasma*

Common ixodid ticks of 1830 cattle of Bengaluru were identified and their seasonal incidence was studied along with the biology of ixodid tick, *Rhipicephalus* (*Boophilus*) *annulatus* (Jagannath *et al*. (1979; 1982). The engorged ticks collected soon after their dropping off from *Anaplasma* positive experimental calf showed many round bodies in the gut contents and the smears of gut contents and salivary gland was subjected to direct FAT and fluorescence was observed only in salivary gland smears after 72h confirming the presence of *Anaplasma*.

Transmission trials in vectors

The transmission of *A. marginale* by *R.* (*B.*) *annulatus* was investigated in splenectomized calves. The hatched out larvae from 2g eggs from the engorged females collected from splenectomized calf infected with *A. marginale* and they were utilized to infect another healthy uninfected splenectomized calf on which the ticks completed their life cycle. During the infection period, the blood smears of calf were examined

daily, but smears did not show the presence of *Anaplasma* organisms, and Hb as well as PCV values of the calf remained normal indicating that trans-ovarian transfer of infection did not occur. Further the experiments on stage to stage transmission also proved negative (Anon, 1979; Jagannath, 1988).

Tick tissue culture

Culturing of tick tissue of *R.* (*B.*) *annulatus* in the laboratory for growing *Anaplasma* organisms as per the methods of Yunker and Cory was attempted. The clumps of tissue and tissue debris were seen floating in the culture medium after 1-2 days of setting up of culture. Slowly the clumps began to attach to the surface and cell attachment was enhanced after a week. Large granular fibroblastic type of cells appeared in between the tissue clumps. Half of the medium was replaced every week with fresh medium. This process gradually eliminated the tissue debris and by about 4-5 weeks, the surface was covered with closely packed epithelial cells of *R.* (*B.*) *annulatus*. In total, success had been achieved in preparing 15 primary cultures and 9 sub-cultures of tick tissue (Anon, 1979).

Infection in other animals

Sheep

Concurrent infection of *A. ovis* with *Theileria hirsi* was noted among two (4.4%) out of 45 sheep examined (Muraleedharan *et al.*, 1994).

Deer and elephants

Four out five Sambar deer (80.0%) of Shimoga zoo acted as carriers of *Anaplasma* whereas 37 Indian elephants of Chikkamagaluru district did not show infection (Anon, 1979).

Treatment with oxytetrcycline

Jagadish Kumar *et al*. (1977) and Ravindranath *et al*. (1982) successfully treated cattle with oxytetracycline hydrochloride (OTC) 5mg/kg b. w. injections by i/m for 3-5 days and the same treatment was also followed in 72 clinical cases in cattle (Anon, 1979). Supplementary treatment with haematinics hastened the recovery. The same treatment was undertaken in 11 carrier cattle and 10 experimental calves. Treated cases showed marked clinical improvement in 2-3 days with normothermia and disappearance of causative organisms. The animals recovered to normal state within a fortnight. The blood picture of these animals became normal, i. e. normocytic and normochromic appearance and Hb concentration ranged between 7.5 and 9g/dl (Ravindranath *et al*., 1982). In clinical cases where Hb content reduced to 4g/dl and below, did not respond to OTC treatment and died due to anoxia and asphyxia (Anon, 1979). Ananda *et al*. (2014) also treated positive cases with OTC in 500ml normal saline by i/v.

Treatment with hyperimmune sera

Hyperimmune sera having high antibody titre were used in acute clinical cases for producing passive immunity. The immune sera were collected from the experimental calves, the recovered animals from acute cases and also from the convalescent animals. The serum was stored at -20^0C in deep freeze after adding 1: 1000 dilution of merthiolate solution. The inoculation of hyperimmune sera 50mg i/v for 2 days had relieved clinical symptoms and timely recovery was observed in severely affected cases (Anon, 1979).

CONCLUSION

Contributions on anaplasmosis from scientists of Karnataka are a very significant as they expressed the technical capabilities to carry out advanced researches on the diagnosis and to contain the highly spreading nature of this vector-borne disease. Now is the appropriate time for pursuing molecular aspects of diagnostic assays and proteomic studies to detect early and minute levels of *A. marginale*

Table. I.

Bengaluru dt.	*1375	5.24	CB, local	Seshadri *et al.*(1985)
Kolar dt.	*1234	1.70	CB, local	Seshadri *et al.*(1985)
Hassan dt.	*2726	0.37	CB, local	Seshadri *et al.*(1985)

Table. I. Incidence of *Anaplasma marginale* infection in cattle and buffaloes based on stained blood smear examinations

District/locality	No. exam.	Percent	Breeds	Reference
A. Cattle				
Bengaluru		8 cases	CB	Jagadish Kumar *et al.*(1977)
Karnataka	413	24.70		Anon (1979)
Bengaluru	134	2.31	CB	Krishna Murthy *et al*. (1978; 1994)
Bengaluru	42	42.85	HF	Jagannath & Krishna Murthy (1980)
Bengaluru	32	26.47	JR	Jagannath & Krishna Murthy (1980)
Bengaluru, Haasan, Mysuru, Tumukuru Chikkamagaluru	155	46.45	CB	Jagannath & Krishna Murthy (1980)
Bengaluru	18	16.67	Local	Jagannath & Krishna Murthy (1980)
Bengaluru	66	40.90	Cattle	Krishna Murthy & Jagannath (1980)
Bengaluru	24	60.87	CB	Ravindranath *et al.*(1982)
Bengaluru dt.	*1375	5.24	CB, local	Seshadri *et al.*(1985)
Hassan dt.	*2726	0.37	CB, local	Seshadri *et al.*(1985)
Chikkamagaluru dt.	*145	0.69	CB, local	Seshadri *et al.*(1985)
Tumukuru Dt.	*3154	0.73	CB	Seshadri *et al.*(1985)
Kodagu dt.	125	0.00	CB, local	Seshadri *et al.*(1985)
Bengaluru, Mysuru & Tumukuru	4081	2.30	Dairy herd	Setty *et al.*(1985)
Mysuru dt.	3318	1.51	Exotic, CB, local	Muraleedharan *et al.*(1994; 2005)
Mandya dt.	1174	0.85	Exotic, CB, local	Muraleedharan *et al.*(1994; 2005)
Karnataka, different areas	11,755	6.60		Harish *et al*.(2006)
Shivamogga, in and around	*566	2.83	JRx, HFx, Hallikar, Amrith- mahal	Ananda *et al.*(2014)
Shivamogga dt.	215	2.70		Krishna Murthy *et al.*(2014)
B. Buffaloes				
Shivamogga	93	0.00		Jagannath & Krishna Murthy (1980)
Shivamogga dt.	85	2.35		Krishna Murthy *et al.*(2014)
Tumukuru dt.	*3154	0.00		Seshadri *et al.*(1985)
Mysuru dt.	344	0.58		Muraleedharan *et al.*(1991)
Mandya dt.	117	0.00		Muraleedharan *et al.*(1991)

*Bovine (mainly cattle including a few buffaloes; HF= Holstein-Friesian; RD= Red Dane; JR= Jersey; CB or x = cross with exotic and indigenous breeds; ND= Non-descript.

carrier conditions in cattle, buffaloes and the wild-counter parts since success have already achieved in some of these aspects with respect to other haemoparasitic infections in Karnataka such as *Theileria*, *Babesia* and *Trypanosoma*. A highly sensitive and specific PCR-ELISA test developed abroad (Gale *et al*. 1996; Braz Junior *et al*., 2000) and the outcome of the studies on PCR conducted in Punjab (Singh *et al*., 2012; Sharma *et al*., 2015) could be adopted in Karnataka for detecting antibodies against *A. marginale* in sera of bovines in carrier status more efficaciously. Moreover the progress achieved in ICAR Scheme of the in the state in culturing the organisms could be utilized for developing an effective vaccine against anaplasmosis.

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IMPORTANCE OF RUMEN PH MONITORING IN DAIRY CATTLE

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INTRODUCTION

Dairy cattle have very well developed rumen microbial ecosystem, which is characterized by the presence of a large variety of microbial consortium. This ecosystem contains bacteria (10^{10}-10^{11} cell/ml, representing more than 50 genera), ciliate protozoa (10^{4}-10^{6} cell/ml from 25 genera), anaerobic fungi (10^{3}-10^{5} zoospores/ml, representing 5 genera) and bacteriophages (10^{8}-10^{9}/ml) (Mackie *et al*., 1996).The weight of rumen (10-15% of body weight of animal) itself explains its importance in digestion and metabolism of cattle. The enzymes produced by the microbial consortia are helpful to digest lignocellulosic feed in the diet of dairy cattle. None of the enzymes of animal origin have ever been found able to degrade lignocellulosic feeds. These microbial enzymes are boon to the dairy cattle if adequate environment (pH) is present in the rumen ecosystem.

Over the last few decades, due to intensive cross breeding programme, the productivity of dairy cows in Kerala have increased greatly. As a result, high energy density diets, which are high in grain starch/highly soluble starch but low in forage, are often fed to the dairy cows in order to meet the high energy demand during the lactation. The most nutritionally challenging time for dairy cows is the early lactation period, during which their feed intake still not fully developed but milk production increases quickly. Dairy farmers, then, tend to push the grain content in the diets to an even higher level in order to meet the dairy cow's productivity potential. Moreover the availability of fodder in Kerala is very low and this situation further worsens the ecosystem of rumen by reducing the pH.

OCCURRENCE OF SUBACUTE RUMINAL ACIDOSIS (SARA)

Naturally ruminants are designed to eat fibrous grasses, plants, and shrubs, which are digested slowly in rumen. In contrast, grains are high in rapidly fermentable carbohydrates that are rapidly broken down by ruminal microorganisms, and leads to the accumulation of acids in the rumen and a lower rumen pH. When rumen pH drops too much, the growth of many ruminal bacteria is inhibited, and concentration of toxic compounds in rumen digesta increases. This can lead to impaired animal health, including decreased feed intake and milk fat production, lowered body condition, inflammation of rumen, liver abscesses, and laminitis related hoof lesions. These signs indicate that dairy cows suffer from Sub acute Ruminal Acidosis (SARA). SARA is common in most of the high producing animals of Kerala which also leads to infertility.

MONITORING AND MANAGEMENT OF RUMEN pH

Effective rumen pH control is critical in maintaining a healthy rumen. Most of the diets in ruminants produce acidic products in the rumen and these are buffered with bicarbonate produced in saliva. A 400 kg cow can deliver as much as 100 liters per day of saliva rich in bicarbonate, phosphate and urea. The rate of saliva production is variable throughout the day depending on whether the cow is resting, eating or ruminating. A greater quantity of saliva is produced during rumination and the period, an animal spends ruminating depends on the active fiber of the diet. The management of microbial populations in the rumen is achieved by diet and through pH control.

Rumen pH fluctuates diurnally between nearly neutral before morning feeding and acidic after feeding. When cows are fed by high forage diets, rumen pH can be maintained between 6 and 7, which is considered to be the optimum for cellulolytic bacteria (Mould *et al*., 1983). Ruminal pH may decline periodically below 6 when dietary grain content increases. Generally, SARA occurs when ruminal pH stays in the range of 5.2 and 6 for a prolonged period. It is challenging to set up a specific threshold of rumen pH for defining SARA, since rumen pH varies among different sites inside the rumen. The use of different techniques to collect rumen fluid for pH determination introduces further variation. The highest rumen pH usually observed in the cranial dorsal sac, followed by the cranial ventral, caudal ventral, and the caudal dorsal sac. Rumen pH in the ventral sac and the center of rumen solid mat is the lowest (Shen *et al*., 2012). When rumen fluid is collected using an oral-stomach tube, the specific collection site is unknown but the sample will often be collected from the cranial dorsal sac and it could be contaminated by saliva. In contrast, rumen fluid collected via rumenocentesis is from the ventral sac. Duffield *et al.* (2004) observed that the pH of rumen fluid samples collected by a stomach tube was on average 0.35 pH units higher than the pH of rumen fluid samples collected by rumenocentesis. Oro-ruminal probes (Geishauser, 1993) and rumen cannulae were used but proved unsatisfactory for accurately characterising the dynamic pattern in rumen pH. The first attempts to continuously measure rumen pH in cattle (Lampila 1955) used in-dwelling glass electrodes in cannulated animals connected by a wire to a receiver located outside the rumen. In 1993, Dado and Allen developed a system enabling constant measurement of rumen pH in animals maintained in stanchions but reported difficulties in maintaining calibration of the electrode due to static build up, faulty solid-core electrode leads, and rumen fluid leakage. Recently, a new device has been reported, using a wireless indwelling probe, called a bolus (Mottram *et al,* 2008). The bolus measures pH continuously, stores the data and transmits it telemetrically via an in-built radio transmitter to a receiver station located around the cow. It gave reliable data for up to 40 days (Phillips *et al.*, 2010). In recent times, bolus technology has been improved and continuously recording rumen pH telemetry can give accurate data for over 150 days and can continue to be downloaded for 7 months after insertion.

CONCLUSION

Infertility and hoof problems are very common in most of the cross bred (HF-Cross) dairy cattle of Kerala. This situation is mostly made due to the fluctuation in the normal pH of rumen and often most of the cows are in sub acute ruminal acidosis. So it is very much essential to manage the pH of rumen for proper absorption of nutrients through ruminal epithelium and for preventing toxic end products of unwanted metabolism. It is observed that adequate amount of fodder (fibre) is essential to maintain the neutral pH

by providing salivary buffers from chewing activity. Introducing rumen pH bolus (wireless telemetry) in dairy farms of Kerala may help to monitor the pH of rumen. Further it helps to prevent acidosis and alkalosis and also helps to modify the diet to get neutral pH in rumen. Cost effective rumen pH bolus should be made available to dairy farmers to monitor the pH of rumen for saving the dairy cattle of Kerala.

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HERITABILITY OF BIRTH WEIGHT AMONG CROSSBRED CATTLE IN KERALA UNDER FARM CONDITIONS

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ABSTRACT

Study was focused to estimate the heritability of birth weight of calves and non-genetic factors affecting on it in farm conditions of Kerala. Data of 412 calf births between 2004 and 2009 were recorded from cattle breeding farm Thumburmuzhi, representing 41 sires. The overall mean birth weight recorded was 28.4±0.19 . To find out the effect of non genetic factors like year and season on birth weight the data were subjected to least squares analysis. Season of the year showed highly significant effect on birth weight whereas year of birth had no effect. After adjusting the data for non genetic factors, the heritability was estimated by paternal half sib regression method. In the present study the heritability estimate of birth weight was found to be 0.48.

Keywords: Heritability, Birth weight, Crossbred cattle

INTRODUCTION

Progress made on selection depends primarily on the heritability of the character, genetic correlation and intensity of selection. Estimates of heritability for characters in farm animals help one to understand the extent to which the echarecters of the animals in one generation may influence those of animals in the next generation genetically, or what proportion of change in certain characters from selected parents is to be found in the offspring. Calf birth weight is influenced by genes received from the sire and dam (direct effects), by maternal environment provided by the dam (maternal effects) and by interactions among direct and maternal effects (Bennett and Gregory, 2001). Significant genetic variation for birth weight exists within herd composed of commercially adapted *Bos taurus* germplasm (Grosz and Macneil, 2001). Although many studies have focused on these parameters, the information available on the heritability of birth weight under farm conditions of Kerala is still scarce.

The objectives of the present study are 1) to estimate the heritability of birth weight 2) to find out the average birth weight of crossbred calves 3) to assess the effect of season on birth weight 4) to determine the effect of year of birth on birth weight and 5) to evaluate the effect of sires on birth weight.

MATERIALS AND METHODS

Records of progenies of 41 bulls mated to crossbred cattle collected from the progeny testing scheme at College of Veterinary and Animal Sciences, Mannuthy were analysed. Sires with a minimum number of 3 progenies were selected for study. Birth records of 412

calves bred from the year 2004 to 2009 were recorded from the University Cattle Breeding Farm Thumburmuzhi, under Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala. The effect of non genetic factors like year and season on birth weight was determined using least squares analysis. In order to study the effect of season on birth weight, the whole year was divided in to four seasons based on climate *viz.* 1. December-February (Winter) 2. March-May (Summer) 3. June–August (Monsoon) and 4. September-November (Post monsoon). After adjusting the data for non genetic factors, the heritability was estimated by paternal half-sib regression method (Martin and Cecil, 1952).

RESULTS AND DISCUSSION

The heritability estimate in the present study was found to be 0.48±0.03.The current estimate of heritability is in agreement with those reported in the literature. The average value of heritability of birth weight of dairy cattle vary from 0 .44 to 0.51. Most of the estimate of heritability of birth weight of calves has come from beef cattle. In beef cattle heritability estimate was found to be 0.22 (Martin and Cecil, 1952), 0.49 (Arango *et al*., 2002) and 0.46 (Mujibi and Crews, 2009). According to Bennett and Gregory (2001) heritability estimate was 0.43 for direct (calf) genetic effects and 0.23 for maternal (heifer) genetic effects. The correlation between direct and maternal effect was -0.26. Direct effects were strongly positively correlated with birth weight and smaller negative correlations of maternal calving difficulty with direct effect of birth weight were also noticed (Eriksson *et al*., 2004). Intermediate to high heritability indicate that genetic changes in birth weight can be accomplished easily by selection.

In the current study, the overall mean birth weight recorded was 28.4±0.19 kg. The average birth weight for various breeds varies from 19.05 kg for Sindhi cattle to 47.90 kg for Charolaise cattle (Mujibi and Crews, 2009). In Holstein cattle, birth weight above the average of 40.3 kg had an exponentially increased risk of mortality (Aksakal and Bayram, 2009). Similarly, probabilities of perinatal mortality for birth weights of 29, 35, 40, 46, and 52 kg were 2.1, 2.5, 3.4, 5.1 and 9.6 per cent respectively, when other factors were set at their average value (Johanson and Berger, 2003). In middle aged and older human population there were inverse graded and independent associations between birth weight and type 2 diabetes (Peter *et al*., 2009). Marker-assisted selection can be used to reduce birth weight with minimal effect on postnatal growth (Grosz and Macneil, 2001).

Birth weight is an indicator of calving ease and perinatal mortality. Perinatal mortality (**PM**, defined as death before 48 hours of age) and dystocia are unfavorable traits for dairy producers. Calves that are lighter and heavier than average tend to have more PM. A difficult birth can cause trauma both for the cow and the calf. From a clinical point of view, most difficult birth occurs due to fetal-pelvic incompatibility because size of the calf (basically explained by birth weight) exceeds the pelvic opening. Calves born with difficulty were over 6-8 kg heavier than those born in easy calvings (Gutierrez, *et al*., 2007). The cow may experience reduced milk production or uterine infection, resulting in additional veterinary costs and decreased fertility, which may lead to premature culling. On rare occasions, the cow may need to be slaughtered or euthanized. A difficult parturition can substantially increase the calf's risk of death. It is quite costly to replace the dead calf, especially a dead heifer calf.

Dystocia may also contribute to additional management costs for continuous surveillance of parturient cows. Either 1) producers are ignoring the evaluations for calving ease and PM and are more interested in selecting for milk yield, 2) the evaluations

are inadequate to produce favorable genetic changes, or 3) a reduction in difficult births is not resulting in a reduction in PM (Johanson and Berger, 2003). Whatever the reason, PM is becoming a problem and should not be neglected any longer. Progress in reducing calving difficulty will likely require optimum birth weight. Genetic evaluation of sires and maternal grandsires for birth weight may be included in the breeding programme for control of dystocia and PM.

Because crossbreeding and selection can increase birth weight to a larger extend, a negative genetic correlation between birth weight and other traits can be expected. Hence, in the future, dairy farmers should measure birth weight of calves. Farmers often handle the calf with in the first 48 hr of birth, so measuring the weight by scale or even by heart girth tape or hip height would mean a small amount of additional handling.

Study showed significant effect ($p \leq 0.05$) of season on birth weight of calves. According to Johanson and Berger (2003) calves born in winter had 36 per cent higher risk of PM and 15 per cent higher risk of dystocia than calves born in summer. A positive association was reported between season and diseases, with fewest deaths occurring in summer. In Surti buffalo calves mortality rate was highest in winter (38.29 per cent) than during other seasons (Khan *et al*., 2007). Time series studies indicated that death losses increased during mid-summer and mid-winter, with mortality rates in winter months being 20 per cent greater than those in summer. Thus seasonal effect on birth weight can be included in mating plan (Gutierrez *et al.*, 2007).

The current study revealed that year of birth from 2004 to 2009 had no significant effect ($p \leq 0.05$) on the birth weight of calves. Relatively uniform management conditions might have been a factor in causing low variation in calf weights from year to year. Also, differences between sires were not significant ($p \leq 0.05$) and their effects on birth weight were also insignificant ($p \leq 0.05$). Martin and Cecil (1952) found that difference between Angus and short horn birth weight was not significant. They also found that the effect of sires on birth weight was insignificant as were the year effects.

CONCLUSION

The crossbreds in Kerala are managed under marginal conditions in semi intensive or extensive systems mostly depend on highly variable feeding, breeding and management inputs. Genetic relationship between production traits in purebred cattle cannot be directly extrapolated to crossbred cattle because the energy requirement for milk production are much lower for average crossbred yielders than for high producing purebred temperate breeds. Body tissue mobilization during lactation is also not as important for crossbred cows as it is in purebred exotic dairy cows. As a consequence, combined selection for production and reproduction traits would not have detrimental effect on crossbreds at least when feeding, breeding and management support is at its optimum. Moreover, due to high humidity and adverse environmental conditions farmers prefer dual purpose animals to milch animals. Thus, birth weight with high heritability than milk yield can be used for selection of bulls for breeding programmes. Selection would be effective for birth weight provided important correlated responses are also taken in to consideration. Selection for optimal birth weight for different seasons can also be considered.

In Kerala, much work has been done to improve production efficiency through crossbreeding and improved management techniques, although less has been done through direct selection. However, recording

data from direct measurements of production traits, along with improved methodologies to analyse such data suggests the opportunity for improving production through selection. Thus, although improvement in crossbred cattle in Kerala has traditionally focused on production traits, future breeding program should consider all traits of economic importance to optimize total genetic gain. The reported analysis can be useful to implement multi trait breeding value evaluation in different environmental conditions to aid in sire selection.

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ENDOHELMINTH PARASITES OF DOMESTIC FOWL *(Gallus Domesticus)* IN DODA DISTRICT OF JAMMU & KASHMIR STATE, INDIA

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ABSTRACT

Domestic fowl constitute an important source of protein mostly in rural areas. Different types of helminth parasites have been found infecting domestic fowl all over theworld. The decreased productivity and increased mortality in fowl is mainly due to mismanagement, lack of nutritional feeding and diseases. The aim of this study was to find out the prevalence of various endohelminth parasites infecting domestic fowl in district Doda of Jammu & Kashmir State, India. Visceral samples from domestic fowl were collected from different areas of Doda district and examined for any probable endohelminths. Study revealed that 67.85 percent of the birds were infected with endoparasites. Different types of helminth parasites recovered included *Raillietina tetragona*, followed by *Heterakis gallinarum* and *Ascaridia galli*. Occurrence of various edohelminth parasites calls for intervention measures like mass chemotherapy of fowl of district Doda.

INTRODUCTION

Poultry is of great importance in rural production system in small communities throughout the developing world. According to WATT, 1996 poultry production has been constantly increasing over the past decades and a survey made by FAO shows that whole poultry population in the world has reached about 14 billion of which 75 percent among these are in developing countries (FAO, 2000). The domestic fowl and eggs provide an important source of protein for human consumption. The increased mortality and decreased productivity in chickens is mainly due to mismanagement, lack of nutritional feeding, diseases and predation. Helminthiasis is more common in outdoor than indoor flocks. The nematodes are widely distributed causing nonspecific clinical signs of infection, such as loss in appetite and growth and on occasions even death. These parasites have either a species specific, direct bird to bird life cycle or they have indirect cycle requiring intermediate host.

The present study was ventured to determine the prevalence of gastrointestinal helminth infections in domestic fowl.

MATERIAL AND METHODS

In the present study, regular visits were made to selected areas (Banihal, Thathri, Bhaderwah, Doda, Kishtwar) of district Doda to collect samples. Age and sex of the animals were also recorded. The viscera were thoroughly examined and the cestode and trematode parasites recovered were fixed in Carnoys fixative and then kept in 70 percent alcohol. Nematode parasites were fixed in hot 70

percent alcohol and preserved in 70% alcohol and glycerine. The parasites were then processed and mounts were prepared for their identification in parasitological laboratory of the department of Zoology, the University of Kashmir.

RESULTS

140 domestic fowl were examined during the present study. 67.85 percent (95/140) of the birds studied were infected with endohelminths. The different types of helminth parasites recovered during the study included *Raillietina tetragona*, with highest prevalence of 51.42 percent followed by *Heterakis gallinarum* (31.42 percent) and *Ascaridia galli* (30.71 percent). *Raillietina tetragona* was the most abundant parasite with mean abundance of 2.18 followed by *Heterakis gallinarum* (1.15) and *Ascaridia galli* (0.64) (Table 1).

Seasonal prevalence

Highest prevalence was noticed in summer (72.8 percent) followed by spring (68.75 percent), autumn (64.51 percent), and winter (55.5 percent). The results thus prove the role of warm and humid environmental conditions during summer season in higher prevalence of infections among domestic fowl (Table 2).

Table 2. Seasonal prevalence of helminth parasites in Domstic fowl

Season	No. examined	Infected (%)
Spring	32	22 (68.75)
Summer	59	43 (72.8)
Autumn	31	20 (64.51)
Winter	18	10 (55.5)

Sex wise prevalence

There seems to be some sex related link in the infection pattern in domestic fowl. Males were generally more infected by all the helminth parasites than females (Table 3). The prevalence of *Ascaridia galli* was 31.1 percent in males and 28.2% in females. *Heterakis gallinarum* prevalence was 35 percent and 23 percent in male and female fowl respectively. Likewise prevalence of *Raillietina tetragona* also peaked in males with prevalence of 55.3 percent than in females (43.49 percent).

DISCUSSION

Present study revealed a high prevalence (67.85 percent) of helminth infection in domestic fowl (*Gallus domesticus*). These figures when compared to the studies round the globe show that Doda is also an endemic

Table 1. Intensity and abundance of helminth species found in 140 domestic fowl (Gallus domesticus) in district Doda

Parasite	Total infected	%age	No. of parasites	Abundance	MI±SD
Ascaridia galli	43	30.71	90	90/140= 0.64	2.09±0.7
Heterakis gallinarum	44	31.42	161	161/140=1.15	3.65±0.5
Raillietina tetragona	72	51.42	306	306/140=2.18	4.25±1.7
Total	95	67.85	557	557/140=3.97	5.86±1.9

Table 3. Sex wise prevalence of parasite in domestic fowl (Gallus domesticus)

Sex	No. examined	Ascaridia galli	Heterakis gallinarum.	Raillietina tetragona.	P- value
		No. (%)	No. (%)	No. (%)	0.05
Male	94	30(31.19)	33(35)	52(55.3)	
Female	46	13 (28.2)	11(23)	20(43.49)	
Total	140	43(30.71)	44(31.42)	72(51.42)	

region of helminth infection of fowl.

The high prevalence of infection observed in domestic fowl can be due to the type of production system, their constant contact with soil and intermediate host, free ranging management system and climatic conditions which alter the population dynamics of the parasite. Similar reasons were shown to be related with high prevalence of helminthiosis in domestic fowl by Yadav and Tandon (1989) and Magwisha *et al*. (2002).

In the present study it was observed that male chicken had a higher prevalence of infection than females, which is in agreement with the reports by Soulsby, 1982, Negesse, 1991, Sanders and Schwartz, 1994, Magwisha *et al*, 2002, Phiri *et al,* 2007. In Nsukka, Eastern Nigeria,2003 , Fakae and Paul-Abiade reported that male fowls carried significantly ($P<0.05$) more parasite burden than female, Magwisha *et al*. 2002 observed that *Heterakis gallinarum* prevalence is higher in male than female chickens, which is in agreement with our observation. This may be due to hormonal influence.

In current study prevalence of helminth infection was generally high during warm months of spring and summer than in other seasons of the year. The difference in the helminthic prevalence might be due to various factors such as geographical and environmental conditions of the area. In cold temperature, lower level of infestation occurs as low temperature inhibit the development and survival of infective larval stages hence decrease access to intermediate hosts or final hosts. These findings are comparable with the previous reports from other parts of world. Mpoame and Agbede, 1995 reported that the parasitic prevalence and the worm burdens were generally higher during April to October. Magwishwa *et al*. 2002, observed that helminthic infection varied with the month in the rainy season, and showed that burdens of cestode such as *Hymenolepis* and *Raillietina tetragona* were higher ($P<0.05$) in March.

CONCLUSION

From the above results, it is clear that helminth infection is widely prevalent in fowls of district Doda. This calls for the institution of control measures like mass chemotherapy of all the fowl periodically at least once a year, so that the load of heminths could be lowered and productivity increased.

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PURIFICATION OF MAJOR FERTILITY-ASSOCIATED PROTEINS FROM SEMINAL FLUID OF VECHUR BULLS BY AFFINITY CHROMATOGRAPHY

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ABSTRACT

Bovine seminal plasma proteins (BSPs), is a family of threeacidic proteins, namely BSP-A1/-A2, BSP-A3 and BSP-30kDa, which form major fertility-associated proteins in bull seminal plasma. Present study was undertaken to purify homologous proteins from seminal plasma of Vechur bulls by affinity chromatography. For this, proteins in semen was precipitated by adding cold ethanol, dissolved in ammonium bicarbonate solution and lyophilized. The proteins were adsorbed to gelatin-agarose by affinity chromatography and then eluted by 8M urea in PBS. Absorbance of fractions was checked at 280 nm and protein containing fractions were pooled and proteins were precipitated. SDS-PAGEof gelatin-bound proteins revealed presence of three bands corresponding to molecular weight of BSP proteins. The results indicated the existence of gelatin binding proteins in Vechur bull seminal plasma and gelatin-agarose affinity chromatography was found to be reliable and effectivetool for purification of BSP proteins.

INTRODUCTION

Seminal plasma is an important reproductive secretion produced by male animals. It is rich in proteins which not only play role in pre-fertilization events occurring in sperms, like motility, capacitation, acrosome reaction, binding to oviduct epithelium, oocyte binding, etc., but also influence post-fertilization events like blocking polyspermy and early embryonic development.

A family of three acidic proteins, namely BSP-A1/-A2, BSP-A3 and BSP-30kDa, called as Bovine seminal plasma proteins (BSP proteins), occur in seminal plasma and account for 40 to 57 percent of proteins(Nauc and Manjunath, 2000).

BSP-A1 and BSP-A2 being glycoforms of same protein, they are regarded as single entity BSP-A1/A2 (Esch *et al*., 1983). The physiological roles of BSP proteins are well studied. At ejaculation, BSPs bind with choline phospholipids in sperm membrane,and remove (8 to 10 percent) cholesterol called 1st cholesterol efflux (Therien *et al*., 1998) and stabilize the membrane by limiting free movement of phospholipids. This inhibits premature acrosome reaction chieflyby blockingthe action of phospholipase A2(Manjunath *et al*., 1994).High density lipoprotein (HDL), along with heparin like glycosaminoglycans (GAG) present in the oviduct and/ follicular fluid remove sperm bound BSPs,while HDL cause 2nd cholesterol efflux (Therien *et al*., 1998). This elicits increase in membrane calcium permeability and intracellular pH leading to

capacitation, which is followed by acrosome reaction. BSP proteins also enable sperms to bind to oviductal epithelium (Gwathmey *et al*., 2006) for formation of sperm reservoir and maintaining motility.

However, BSPs are detrimental to sperms at higher concentration and/ at longer period of exposure that especially occursduring storage, wherein it causes continual cholesterol and phospholipid efflux decreasing the viability of sperms (Therien *et al*., 1998 and Therien *et al*., 1999). Low-density lipoproteins present in egg yolk based diluents are found to sequester BSP proteins thereby ameliorate their effects (Manjunath *et al*., 2002).

Homologs of BSP proteins are demonstrated installion, boar and buck but not so far in Vechur bulls. Hence this study is undertaken to standardize the technique gelatin–agarose affinity chromatography for purification of BSP homologs from Vechur bull seminal plasma.

MATERIALS AND METHODS

Materials

Semen from five Vechur bulls was obtained from Vechur farm, Vechur Cattle Conservation project, Centre for Advanced Studies in Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy. The following chemicals, glassware, commercial kits and equipment were used; Affigel-15(Bio-Rad, USA), Gelatin, Morpholino ethanesulphonic acid (MES), Sodium azide, Ethanolamine-hydrochloride, Tris-Hcl, Glycine, SDS and Urea (Himedia), Chromatography column: 300×10 mm (Borosil), Fraction collector (Model 2110), TGX: stain free fast cast kit, Low-range molecular weight protein standards and Mini-protean tetra cell (Bio-Rad, USA) and Lowry's protein estimation kit (Merck, Germany).

Methods

Coupling of gelatin to agarose and preparation of columns for affinity chromatography

As prescribed by Manjunath *et al*. (1987), contents of Affigel 15 were taken in muslin cloth todrain away the preservative liquid.25 mL gel slurry was washed with three bed volumes of cold (4^0 C) distilled water and was transferred to 50 mL falcon centrifuge tube. 25 mL gelatin solution (20 mg/mL in 0.1 M MES solution, pH 6.5) was added and kept for linear shaking at 25^0 C for 2 h. Two mL of 1 M ethanolamine-hydrochloride was added to neutralize any gelatin-unbound active esters, followed by an hour of linear shaking. After coupling reaction, the gel was transferred to chromatography glass column. Successive washing with 10 bed volumes of water, 8 M urea and PBS (pH 7.5) were carried out and the column was stored at room temperature by layering with 0.2 percent sodium azide.

Isolation of seminal plasma proteins from semen

Semen was collected in split ejaculates from single bull by artificial vagina method. As described by Manjunath (1984), the semen was centrifuged at 1000 ×*g* for 10 min.at 4^0C. The supernatant was aspirated and further centrifuged at 10,000 ×*g* for 10 min. at 4^0C to obtain seminal plasma. Nine volumes of cold (-20^0C) ethanol was added to seminal plasma and kept at 4^0 C for 90 min. with constant stirring to precipitate proteins,followed by centrifugation at 10,000×*g* for 10 minutes at 4^0 C to pellet the precipitate. The pellet was dissolved in 50 mM Ammonium bicarbonate and lyophilized (-84^0 C), to bestored in deep freezer (-20^0 C).

Gelatin-agarose affinity chromatography

As prescribed by Manjunath *et al*. (1987) and Boisvert*et al*. (2004), 'Phosphate buffered saline (PBS, pH 7.5) pre-equilibrated gelatin-agarose column' was loaded with 100 mg crude seminal plasma protein reconstituted with 3 mL PBS. Sample flow rate was maintained @

1 drop per 2 seconds. After sample has entered the column, stop cock was locked for 30 min. to allow proteins to interact with the column matrix. PBS and8 M urea in PBS were added in succession to wash out unadsorbed proteins and to elute bound proteins respectively. Fractions of 4 mL were collected during washing and elution, using fraction collector. The absorbance of fractions was checked at 280 nm and standard curve was plotted (Fig 1). The fractions under corresponding peaks were pooled: fraction A and fraction B (FA and FB), the protein content was in corresponding pool was estimated as per Lowry's protocol (Lowry *et al.,* 1951) and the proteins were precipitated, pelleted, dissolved in 50 mM Ammonium bicarbonate and lyophilized as per earlier procedure. Lyophilized FA and FB proteins were stored in deep freezer.

SDS-PAGE

Lyophilized crude seminal plasma proteins, unbound fraction (FA) and bound fraction (FB) were separated in 12% TGX stain free polyacrylamide gel using mini-protean tetra cell and molecular weight was estimated by referring to low-range molecular weight protein ladder.

RESULTS

Gelatin-agarose columns stored with preservative 0.2 percent sodium azide at room temperature could yield better results for8-10 times chromatography run in a period of 4-6 months without significant reduction in binding efficiency.

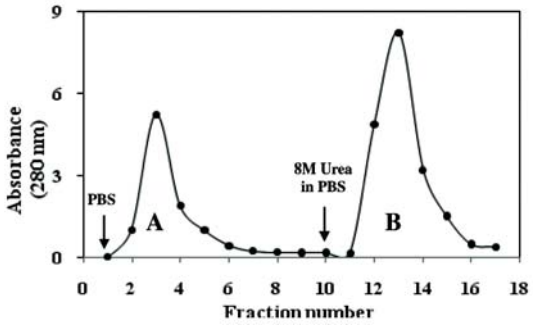

Fig.1 Gelatin-agarose affinity chromatography profile of Vechur Seminal plasma proteins; Graph plotted with absorbance (280 nm) against fraction number. Fractions under A and B peaks were pooled.

Figure 1 depicts the gelatin-agarose affinity chromatography profile of Vechur seminal plasma proteins. A and B represents the peaks under which the corresponding fractions contains unbound and bound proteins respectively. These fractions were separately pooled and protein content was estimated by Lowry's protocol (Lowry *et al*., 1951). Average protein content of FA and FB from all the bulls,as calculated by taking mean of individual values, was found to be 21.07 mg and 53 mg respectively. This indicates approximately 70 percent of the loaded proteins was recovered, out of which about 70 percent constituted bound proteins.

SDS-PAGE analysis of bound proteins (Fig 2) revealed presence of three protein bands, two of which showed molecular weight in the range of 15 to 17 kDa and one at 30 kDa.

DISCUSSION

Gelatin-agarose affinity chromatography is one of the standard techniques for isolation

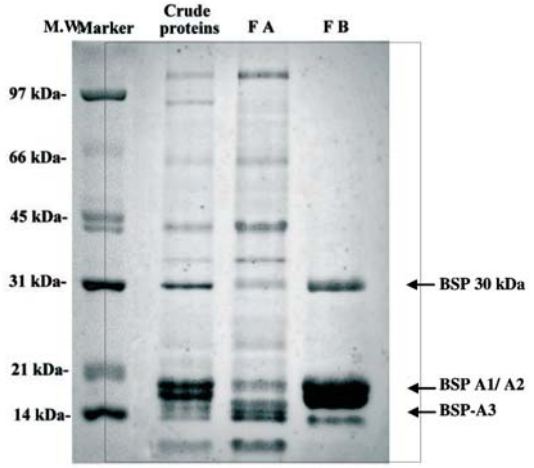

Fig. 2 SDS-PAGE of GAAC end products. 15 µg of crude seminal plasma proteins, 20 µg of FA (unbound proteins) and15 µg of FB (bound proteins) were reduced, denatured and electrophoresed in 12 % stain free gels. Low-range protein marker, diluted according to the supplier instructions, used to estimate molecular weight.

and purification of BSP proteins and it yielded good results with respect to Vechur bull seminal plasma in this study. The technique was proved to be simple and effective for isolation of BSP proteins.

Structure of BSP proteins show presence of two type-II domains which is identical to gelatin-binding domains of fibronectin (Esch *et al*., 1983; Seidah *et al*., 1987; Calvete*et al.*, 1996).This property was utilized here to purify BSP proteins as reported earlier byManjunath *et al.*(1987). Gelatin binding property of BSP homologs was also demonstrated in stallion, boar (Calvete *et al*., 1995; Calvete *et al*., 1997), goat (Michele *et al*., 2003) and bison (Boisvert *et al*., 2004). Thus it can be deduced that the gelatin-bound proteins obtained in our study may be homologs of BSP proteins with type-II domains.

Results of the study showed that, when 100 mg lyophilized crude seminal plasma proteins were loaded onto the column, after a series of elution with PBS and urea, about 70 percent of the proteins were recovered, out of which about 70% constituted gelatin-bound proteins. This indicates that gelatin binding proteins form major fraction in bull seminal plasma as reported earlier by Nauc and Manjunath (2000). SDS-PAGE of bound proteins showed 3 bands corresponding to molecular weight of BSP proteins;BSP-A1, BSP-A2, BSP-A3 and BSP-30kDa which have molecular weight of 16.5 kDa, 16 kDa, 15 kDa and 28 kDa respectively (Desnoyers *et al*., 1994). Since molecular weight of BSP-A1 and BSP-A2 differ only by 0.5 kDa, these two proteins may have migrated as single band during electrophoresis and also was found to be thickest of all the other bands..This observation coincides with the observations of Manjunath and Sairam (1987) that due to minute variation in molecular weight, BSP-A1 and A-2 migrated as single distinct band during electrophoresis, and these proteins alone constituted morethan 30% of seminal plasma proteins, resulting the band formed by them was intense. Hence the band may be composed of two proteins; BSP-A1 and BSP-A2.

To summarize, isolation of major fertility associated proteins from seminal fluid of Vechur bulls by gelatin-agarose affinity chromatography has been efficiently carried out. These proteins exhibited gelatin binding property, GAAC elution characteristics, abundance in seminal plasma and SDS-PAGE pattern similar to BSP proteins reported. Howeverfurther characterization shall be done by 2-D gel electrophoresis and amino acid sequencing study.

ACKNOWLEDGEMENT

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ANATOMICAL STUDIES ON THE SKELETON OF PELVIC LIMB IN MOUSE DEER (*Moschiola indica*)

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ABSTRACT

The anatomical peculiarities of the skeleton of hind limb in mouse deer (*Moschiola indica*) was studied using three specimens brought for post mortem in the Veterinary College, Pookode. The os coxae in mouse deer resembled those of carnivores and rabbit. In the ilium, gluteal line was faint and the sacral surface was extensive. The ilium and ischium was positioned parallel to the back bone and and was fused with the sacrum. The lesser ischiatic notch was absent and the acetabulum was large and deep. Obturator foramen was typically oval. Femur was long; the head was prominent and placed at a higher level than the greater trochanter. Head of the femur was smaller than the acetabulum and the articular surface extended on to the neck. Medial and lateral supracondyloid fossae and the lateral supracondyloid crest were absent. Trochlear ridges were sagittal and equal. Patella was arched, narrow and elongated. Tibia was the longest bone of the pelvic limb and the distal end was notable for the great distal extent of its medial malleolus. Fibula was much reduced. There were five tarsal bones arranged in three rows. There were three metatarsals, of which the middle one formed the well-developed large metatarsal bone and the small medial and lateral metatarsals were very thin and long. Distally the large metatarsal presented two trochleae separated by a deep intertrochlear incisor. The two well-developed digits presented three phalanges and three sesamoids.

Key words: Mouse deer, pelvic limb skeleton, anatomy

INTRODUCTION

Mouse deer belongs to the family *Tragulidae* (Patton, 2004) and is the smallest ruminant in the world. This animal is found only in the tropical forests in Southern Asia, including the islands of Java, Sumatra and Kalimantan. The population of mouse deer is declining due to habitat destruction, hunting activity and the threat of predators (Najamudin *et al.,* 2012). They are solitary or live in pairs and feed more or less exclusively on plant resources. Each and every animal of the forest plays its particular role in keeping the ecological stability and hence the threat to this species has to be abruptly stopped so as to prevent further deterioration in population. The present study on the gross anatomical features of the pelvic limb of the mouse deer will form a basis for

further physiological and pathological studies and also aid in the diagnosis and treatment in this species.

MATERIALS AND METHODS

Three specimens brought for post mortem to the Department of Veterinary Pathology, Veterinary College, Pookode constituted the material for this study. The bones were prepared as described by Young (1980).

RESULTS AND DISCUSSION

The os coxae in mouse deer was formed of ilium, ischium and pubis and resembled those of carnivores and rabbit. The long axis of the ilium was almost in line with that of the ischium (Fig. 1) as seen in pig (Nickel *et al*., 1981). The gluteal line was faint. Greater ischiatic spine was low and everted. Sacral surface was extensive as in pig (Hilary and Flood, 1996) and smooth. The parallel position of ilium and ischium to the back bone and their fusion with the sacrum helps to transmit the thrust of hind limbs to body axis without any loss of force. Iliac surface was narrow and became thicker towards the sacral tuber. Iliac crest was convex, thick and rough in the middle which formed the highest point of the bone. Sacral and coxal tubers were lower than the crest. The lateral border of the ilium terminated abruptly. The sacro- pelvic surface presented a ridge at the distal extremity towards the body of the ilium.

Acetabulum was large and deep as reported in small ruminants and dog (Evans and Christensen, 1979, Siddiqui *et al.,* 2008 and Konig and Liebich, 2009). Acetabular notch was seen on the caudal part of the rim just in front of the obturator foramen. Ischiatic symphysis was not ossified. Obturator foramen was oval with an average length of 1.5 cm and width of 1 cm. The pelvic inlet was typically "U" shaped. Ischium was quadrilateral in shape and the dorso-pelvic surface was slightly concave. The lesser ischiatic notch was absent and the ischiatic spine was less prominent. Caudo-lateral angle or tuber ischii was thick, smooth and rudimentary. Strength and rigidity of pelvic girdle enable the animal to successfully withstand the thrust of the hind limbs during jumping.

Femur was long with a length of 8 cm. The prominent head of the femur was smaller than the acetabulum with the articular surface extending on to the neck. Head was at a higher level than the greater trochanter as in the cat (Arnbjerg and Heje, 1993 and Dyce *et al*., 2002). The neck was relatively long and the femoral head bowed medially as in *Apterodontinae* (Grohe *et al.,* 2012). The fovea

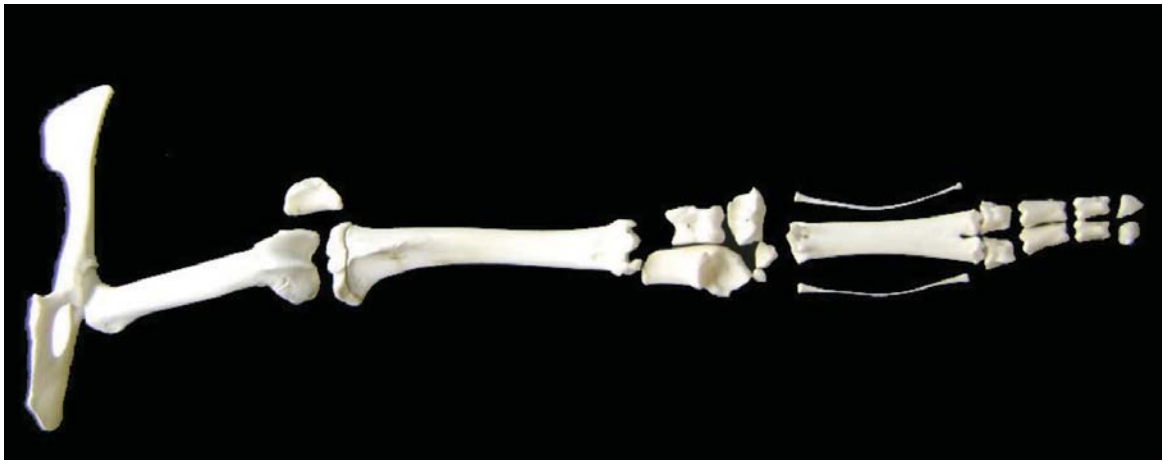

Fig.1. The skeleton of pelvic limb of the mouse deer

capitis was faint. Greater trochanter was small and continued distally on the caudal surface of the shaft as a thin faint ridge. The trochanteric fossa was wide and broad and guarded by the oblique intertrochanteric crest that extended from the inconspicuous lesser trochanter to the greater trochanter. Proximal aspect of the cranial surface presented the nutrient foramen. Medial and lateral supracondyloid fossae and the lateral supracondyloid crest were absent. Trochlear ridges were sagittal and equal and the intercondyloid fossa was wide.

Patella was arched, narrow and elongated (Fig. 1) as in rabbit (Popesko *et al*., 1992). It was compressed transversely with a wide base and presented two surfaces. Cranial surface was convex and smooth. Articular surface was divided by a vertical ridge into two strongly concave areas of which the lateral one was larger. Lateral border was convex and presented numerous foramina. Medial border was almost straight.

Tibia was the longest bone of the hind limb (9 cm) as reported in llama (Hilary and Flood, 1996). The triangular proximal end was much more massive than the anterio-posteriorly compressed distal end as in the new goat-like camelid (David and Webb, 2005). The popliteal surface was rough proximally and smooth distally and the lateral border presented a groove leading to the nutrient foramen. The popliteal surface presented a single popliteal line. The intercondyloid eminence was divided into two but the lateral and medial divisions were of the same height. The intercondylar area was well separated and showed numerous tiny foramina. Tibial tuberosity was well developed with a short and very prominent tibial crest. Fibula was much reduced. The distal end of the tibia was notable for the great distal extent of its medial malleolus as seen in new goat-like camelid (David and Webb, 2005). Fibula was much reduced. The lateral malleolus was fused with the tibia and reached up to the same level as that of the medial malleolus and on its lateral side there was a rough, highly pitted notch which extended upwards along the lateral border of the tibia.

Proximal row of tarsal bones consisted of the tibial tarsal and fibular tarsal; the middle row was constituted by the quadrilateral, well developed, fused central and fourth tarsal and the distal row presented the first and fused second and third bones which were very small. The centroquartal bone was pierced by a canal which was continuous with the large metatarsal for the lodgement of blood vessels. It also presented a hook-like plantaro-medial projection which articulated with the large metatarsal.

There were three metatarsals of which the middle one formed the well-developed large metatarsal bone (5.5 cm long) and the small metatarsals were very thin and long. Distally the large metatarsal presented two trochleae separated by the intertrochlear incisor which was very deep. The dorsal metatarsal groove was very wide and deep. Medial half of the dorsal surface presented a ridge which extended from the proximal to distal third of the large metatarsal. The two well-developed digits presented three phalanges and three sesamoids each as in other ruminants. The proximal phalanx was the biggest of all.

Bones of the pelvic limb in mouse deer are strong to keep the body clear off the ground when required and to take leaps by their straightening. Long segments of the hind limbs bent like a spring and increase the thrust produced by straightening of the hind limbs. The skeletal peculiarities represent adaptation of the species in sensing danger and making a rapid escape from its predators by running fast.

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CORRELATION BETWEEN DIFFERENT POST-THAW QUALITY PARAMETERS OF MALABARI BUCK SEMEN

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ABSTRACT

As single evaluation technique has limited value in predicting the actual fertilizing potential, adequate evaluation techniques must be used for evaluating a particular semen processing protocol. In this study, freezing of buck semen was carried out using egg yolk based and soybean lecithin based extenders at two extension rates. The parameters included for post- thaw evaluation were progressive motility, viability, acrosomal integrity, and functional membrane integrity (Hypo Osmotic Sperm swelling Test). Pearson's correlation coefficient between the parameters reveals the reliability of them in the post thaw evaluation of Malabari buck semen with the freezing protocol, adopted.

Key Words: Malabari buck semen, Motility, viability, acrosomal integrity, HOST, Pearson's correlation coefficient

INTRODUCTION:

Assessment of the degree of cryodamage happened to the spermatozoa during their cryopreservation is crucial in predicting their fertilizing potential. Therefore, selection of reliable methods of semen evaluation is one of the most important factors in a breeding programme. If two parameters are significantly correlated, one of them can be used to predict trend of the other (Taylor & Francis, 2006). At the same time, evaluation of one of the parameters may be enough instead of doing all the qualitative tests so that the evaluation procedure will be simplified.

Hence, we studied the correlation between post-thaw motility, viability, acrosomal integrity and functional membrane integrity of Malabari buck spermatozoa in the freezing protocol using egg yolk based and soybean lecithin based extenders at two extension rates.

MATERIALS AND METHODS:

Forty eight semen samples collected following double ejaculate regime from two Malabari bucks, maintained at the Artificial Insemination centre, Dept. of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur were utilised for the study. Sperm concentration was determined using haemocytometer after diluting the semen at 1:200 using hypertonic eosin- saline.

Semen samples with more than 80 per cent initial motility were divided into four groups. Samples of group I and II were extended with Tris egg yolk based extender and soyabean lecithin based extender (Andromed) respectively at the rate of 400

million spermatozoa per ml. Group III and IV were extended with Tris egg yolk based extender and soyabean lecithin based extender (Andromed) respectively at the rate of 800 million spermatozoa per ml. Group I and III were initially extended with non glycerolated fraction of the extender. Group II and IV were fully extended with Andromed. One hour after attaining 5ºC in the cold handling chamber, glycerolated fraction of the extender was added to the non glycerolated fraction in three steps at 10 minutes interval so that the fully extended samples of group I and III contained six per cent glycerol. French medium straws were used to fill the semen samples manually. After an equilibration period of 2 hours, the straws were undergone conventional freezing for 10 minutes and were plunged into liquid nitrogen.

Post thaw evaluation of semen from each group was done 24 h after freezing. Post-thaw quality parameters such as motility, viability, acrosomal integrity and functional membrane integrity were assessed after thawing the straws at 37°C for 60 seconds.

Motility was assessed at 400X objective of the light microscope. Sperm viability was assessed using eosin-nigrosin staining technique (Campbell *et al.,* 1953). Acrosomal integrity was assessed by Giemsa staining technique (Watson, 1975). Hypo osmotic sperm swelling test (HOST) was carried out to assess the functional membrane integrity of spermatozoa as per the method used for human spermatozoa described by Jeyendran *et al.* (1984). The data were statistically analysed using SPSS (Statistical Package for Social Studies) software. Correlation between the variables was assessed using Pearson correlation.

RESULTS AND DISCUSSION

Correlation between different parame ters is expressed in Fig1 to Fig.6. Here, progressive motility was found to be highly

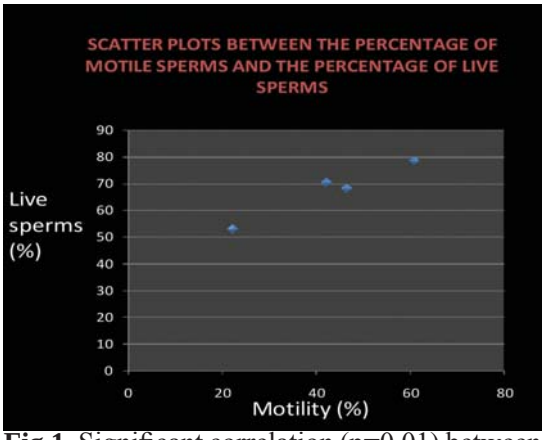

Fig.1. Significant correlation (p=0.01) between progressive motility and viability (R=0.848

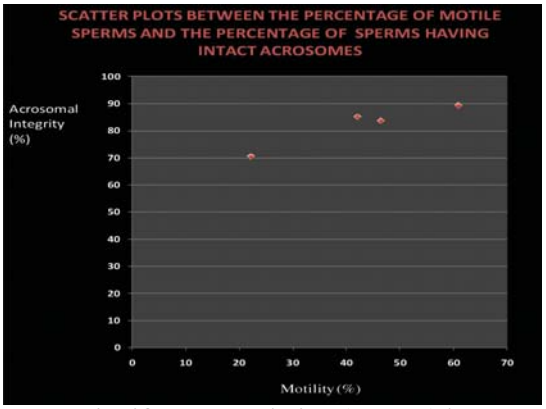

Fig.2. Significant correlation (p=0.01) between progressive motility and acrosomal integrity R=(0.760)

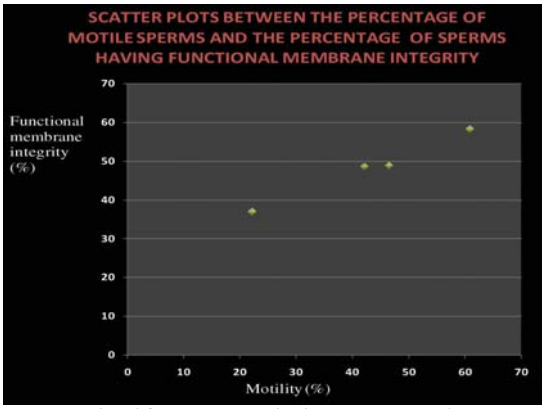

Fig.3. Significant correlation p=0.01) between progressive motility and functional membrane integrity (R=0.760)

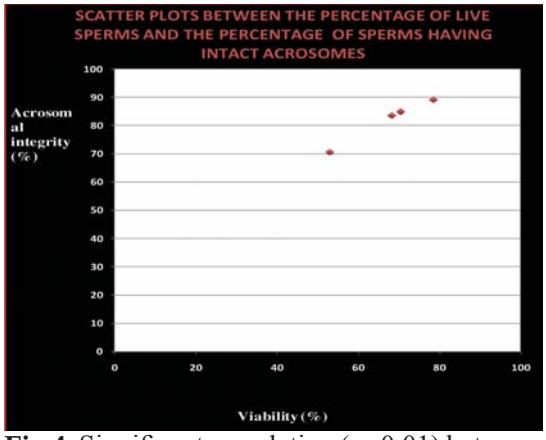

Fig.4. Significant correlation (p=0.01) between viability and acrosomal Integrity (R=0.790)

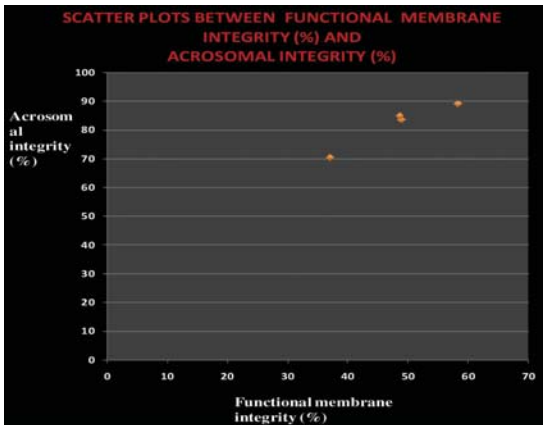

Fig.5. Significant correlation p=0.01) between viability and functional membrane Integrity (R=0.732)

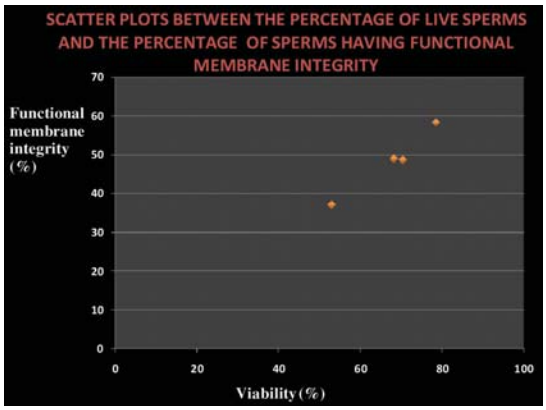

Fig.6. Significant correlation (p=0.01) between functional membrane integrity and acrosomal integrity (R=0.634).

correlated (p=0.01) with viability (R=0.848), acrosomal integrity (R=0.760) and functional membrane integrity (R=0.760). Percentage of viable spermatozoa was also highly correlated with (p=0.01) percentage of spermatozoa with intact acrosomes (R=0.790) and functional membrane integrity (R=0.732). Significant correlation (p=0.01) existed between functional membrane integrity and acrosomal integrity (R=0.634). Similar results were obtained previously by Jeyendran *et al*. (1984) in human semen, Mantovani *et al*. (2002) in equine semen Bohlooli S. *et al*. (2012) in ram semen.

SUMMARY

Results of the present study revealed the reliability of quality parameters such as motility, viability, acrosomal integrity and functional membrane integrity in the post-thaw evaluation of Malabari buck semen with the freezing protocol adopted using egg yolk based and soybean lecithin based extenders at two extension rates. However, research should be extended to find out the correlation of each of them to both *in vivo* and *in vitro* fertility rate of Malabari buck semen.

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ASSESSMENT OF IMMUNOMODULATORY ACTIVITY OF METHANOLIC EXTRACT OF *Boerrhaviadiffusa L.*

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ABSTRACT

Boerrhavia diffusa L. (F. Nystaginaceae) has been used in Ayurvedic medicines for the treatment of liver, gall bladder, urinary and many other diseases since ages. The present study was aimed to assess the immunomodulatory effect of the methanolic extract by testing its effect in the haematological parameters of rats and circulating Abtitre and antibody forming cells in the spleen of Balb/c mice. The extract was given at a dose rates *i.e.*, 200 mg/kg and 400 mg/kg to 6 rats each for each parameter. The hematological parameters like total RBC count, total and differential WBC count and haemoglobin concentration did not show significant difference for both doses tested. The effect on plaque forming cells in the spleen was tested by the Jernes plaque assay using a modified slide technique. The extract at both dose rates produced increase in antibody forming cells in the spleen (as shown by the number of plaque forming cells) when compared to that of the control. Similarly the extract at both dose rates also produced increase in circulating Abtitre when compared to that of the control. The results showed for the methanolic extract at the dose rates tested had stimulant activity on the humoral immunity.

Keywords:- *Boerrhaviadiffusa* L., immuno modulatory, plaque forming cells.

INTRODUCTION

Boerrhavia diffusa L. has a long history of use in Ayurvedic medicine in India and has been used for the treatment of many ailments including liver, gall bladder, renal and urinary disorders (Abraham, 1975). Chopra *et al.* (1956) described the use of the plant as asthma, oedema, anaemia, jaundice, anasarca and as an antidote to snake poisoning.

Kirtikar and Basu (1975) recommended the use of the plant in anaemia, inflammations, vatha and kapha. The plant was included one of the extensively investigated medicinal plants in India (Vohora, 1989).

Mugantiwar *et al.* (1997) studied the effect of the alkaloidal fraction of *B. diffusa* on stress-induced changes in plasma and adrenal cortisol levels and immune responsiveness in rats. The drug was found to possess restorative activity against stress induced changes in plasma and adrenal cortisol levels and augmented antibody production.

The present study was undertaken to find out the immunomodulatory effect of the plant.

MATERIALS AND METHODS

The experiment was conducted in Balb/c mice of either sex. which were maintained on identical feeding and managemental practices in the laboratory for one week before the

commencement of study. Work was carried out after getting approval from Institutional Animal Ethics Committee. The whole plants were collected locally, dried under shade,pulverized and extracted with methanol using a Soxhlet extractor and evapourated to dryness using a vaccum flash evapourator. The study was performed using 3 different parameters.

1. Effect on haematological parameters

18 albino rats of either sex, divided into 3 groups of 6 each were used for the study. The first group was given treatment distilled water, the 2nd group was given the extract at the rate of 200 mg/kg and the 3rd group was given the extract at the rate of 400 mg/kg respectively orally for 5 consecutive days.

On the 6th day, blood was collected and the hematological parameters like total RBC count, total WBC count, Differential Count(DC) and Hemoglobin(Hb) count were assessed by standard procedures described by Schalm *et al.* (1975).

2. Effect on plaque forming cells in the spleen

This was performed by the Jernes' plaque assay (Jerne and Nordins, 1963)[7] using a modified slide technique described by Mehrotra (1983)[8]. 30 Balb/c mice of either sex divided into 3 groups of 10 each were used for the study. All the mice immunized with 0.1 ml each of 20% SRBC in PBS given i/p using aseptic precautions. The first group was kept as the control and was given no drug. The 2nd group was administered the extract @ 200 mg/kg orally daily till the mice were sacrificed. On the 3rd, 4th, 5th, 6th and 7th day after immunization, 2 mice from each group were sacrificed and spleen collected. Spleen cells were processed and plaque formation assay performed by the modified slide method, as given below.

The spleens were processed into single cell suspension ($8x10^6$ cells/ml) in HBSS. To 0.5 ml of spleen cell suspension were added, mixed well and poured over a glass slide. The slides were allowed to solidify and incubated with fresh rabbit serum as source of complement for 1 hour at 37°C. The plaque formed were counted over a light source and represented as PFCs/million spleen cells (Lefkovitz and Cosenza, 1979)[9].

3. Effect on circulating Antibody (Ab) titre/ Hemagglutinationtitre (HAtitre)

This was done by the method described by Nelson and Davy (1992)[10]. 15 Balb/c mice were divided into 3 groups of 5 each. The 3rd group was kept as the control. All the mice were immunized with 0.1 ml each of 20% SRBC given i/p. The 2nd group was administered the extract @ 200 ml/kg orally daily till day 25 of immunization. The 3rd group was administered the extract @ 400 mg/kg orally. Similarly blood was collected on day 5, 10, 15, 20 and 25 from all the mice. Sera were separated and heat inactivated as 56°C for

Table 1. Effect of methanolic extract of *B. diffusa* on hematological parameters in rats

Groups	Hb g%	RBC millions/ cu.mm	WBC 10^3/ cu.mm	DC		
				Lymphocyte %	Neutrophils %	Eosinophils %
Control	15.1 ± 1.0	8.28 ± 0.59	5.076± 1.328	61.9 ± 6.4	31.3 ± 6.4	1.3 ± 0.9
Extract @ 200 mg/kg	11.22 ± 0.41	8.68 ± 1.11	6.480 ± 2.423	67.8 ± 1.52	26.4 ± 1.13	4.8 ± 0.82
Extract@ 400 mg/kg	12.52 ± 0.17	8.09 ± 1.64	5.45 ± 0.987	63.0 ± 3.32	33.0 ± 2.52	4.0 ± 1.16

Table 2. Effect of methanolic extract of *B. diffusa* on plaque forming cells in spleen

Groups	Number of PFCs/10^6 spleen cells (days after immunization)				
	3	4	5	6	7
Control	40	112	200	280	96
Extract @ 200 mg/kg	56	152	192	416	104
Extract @ 400 mg/kg	32	136	120	304	194

30 min. 2 fold dilutions of sera samples were made using PBS (pH 7.2) in microtitre plates and mixed 1:1 with trypsinised suspension of SRBC in PBS. The plates were incubated at 37°C for 3h. The degree of agglutination was evaluated macroscopically. The HA titre was calculated as the reciprocal of highest dilution of serum which showed visible agglutination.

RESULTS

1. Effect on hematological parameters: There were no significant difference between the 3 groups in Hb concentration count, total and differential WBC counts and RBC count (Table 1).

2. Effect on plaque forming cells (PFCs) in the spleen: Administration of the extract at both dose rates produced increase in antibody forming cells in the spleen (as shown by the number of PFCs), when compared with that of the control (Table 2). The number of PFCs started increasing from day 3 and reached a peak level on day 6. The number gradually decreased in the higher dose group (group III), while it suddenly returned to near normal values at day 7 for the group II 3. Effect on circulating Ab titre: The experiment at both dose rates produced increase in circulating Ab titre when compared to that of the control group (Table 3). The peak titre was observed at day 15 for the 3 groups but it was maintained upto day 20 in the group III.

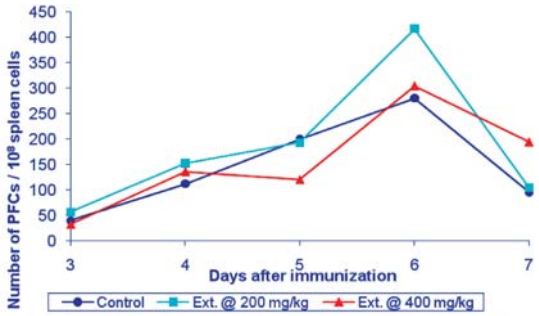

Fig. 1 Effect of methanolic extract of *B. diffusa* on plaque forming cells

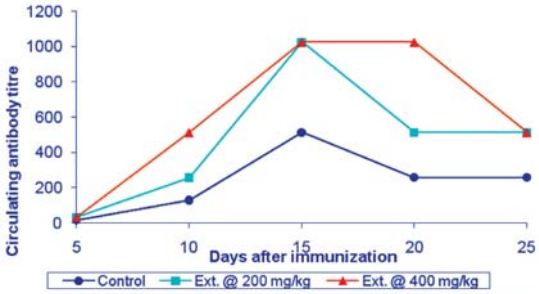

Fig. 2 Effect of methanolic extract of *B. diffusa* on circulating antibody titre

DISCUSSION

The results showed that the experiment at both dose rates stimulated the humoral immune response, as shown by an increase inAb forming cells in spleen and Abtitre against SRBC in Balb/c mice. The normal hematological values for rats are (1) RBC

Table 3. Effect of methanolic extract of *B. diffusa* on circulating antibody titre

Groups	Antibody titre (days after immunization)				
	5	10	15	20	25
Control	16	128	512	256	256
Extract @ 200 mg/kg	32	256	1024	512	512
Extract @ 400 mg/kg	32	512	1024	1024	512

count – 7-10 million/cu.mm (2) WBC count – 6-17 thousands/cu.mm. (3) Hb – 11-18 g/dl, (4) Neutrophil 9-34%, (5) Lymphocyte – 65-85% and (6) Eosinophil -0-6% (Hrapkewicz et al, 1998)[11]. Since the mean values for all the group fall within these ranges, there is no significant change in the hematological parameters.

Praveenkumar *et al.* (1999b) have found that 'rasayana', a multidrug herbal preparation could enhance humoral immune responses as seen from increased number of Ab forming cells & circulating Ab titres and hence had immune stimulant properties.

Geetha and Sangeetha (2000) conducted a controlled experimental study to assess the effect of *B. diffusa* extract @ 2.4 g/kg bodyweight orally to ward off post surgical infection and mortality in albino rats. The results showed that the drug caused (1) better prevention of post surgical infection, (2) better expectancy of life after surgery, (3) normal maintenance of level of water intake and urine output after surgery, (4) maintenance of total and differential WBC count after surgery and infection and (5) prevention of accumulation of peritoneal fluid and onset of gangrene.

Mugantiwar *et al.* (1997) have earlier reported that alkaloidal fraction of *B. diffusa* significantly reversed the depleted adrenal cortisol level and the elevated plasma cortisol level in stressed rats, thus appearing to have a corticosteroid sparing effect in experimental stress. The results of the present study were also complementary to these findings supporting the immunostimulant activity of alcoholic extract of *B. diffusa*.

CONCLUSION

The methanolic extract at the dose rates of 200 mg/kg and 400 mg/kg had immunostimulant properties as determined by an increase in the number of plaque forming cells in the spleen and circulating Ab titre.

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SKULL ABNORMALITIES ASSOCIATED WITH ANOPHTHALMOS CONDITION IN CHICKS

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ABSTRACT

Orbital asymmetry and the skeletal abnormalities associated with anophthalmos condition was studied using six each of hatched and unhatched chicks, using Toluidine Blue - Alizarin Red S staining of cartilage and bone protocol. Of these birds, one each of unilateral anophthalmos was also associated with beak deformities. Orbital part of the frontal bone, interorbital septum, horizontal plate of ethmoid bone and lacrimal bone were found defective in the affected side. Jugal bone was absent on the affected side and all these deformities collectively contributed to the deformed orbit. Etiology of these skeletal deformities and anophthalmos may be attributed to spontaneous and genetic aberrations, indicated by the mismatching between nucleotides at various locations in the ovomucoid gene.

Key Words: Anophthalmos, chicks, skull abnormalities.

INTRODUCTION

Anophthalmos or anophthalmia is the congenital lack of one or more eyes in chicks. Anophthalmus is an animal which lacks one or more eyes. True or primary anophthalmos, with complete absence of the ocular tissue within the orbit is very rare. Extreme microphthalmos is more common, where a very small globe is present within the orbital soft tissue, which is not visible on initial examination. Anophthalmos in chicks presents not only the absence of an eye but also the secondary disfigurement of the orbit, the lids, and the eye socket. The present study was undertaken to describe the skeletal anomalies associated with anophthalmos in chicks.

MATERIALS AND METHODS

The orbital asymmetry was studied using six hatched and six unhatched chicks with anophthalmos condition collected from Hatchery units of AICRP poultry, University Poultry Farm and Revolving fund Hatchery, Mannuthy. In all cases the birds showed orbital asymmetry. After recording the gross abnormalities the birds were sacrificed and the skeletal abnormalities were studied using Toluidine Blue - Alizarin Red S staining of cartilage and bone protocol (Alphonse, 1965). Cartilage and bone were differentiated in whole-mount preparations with toluidine blue-alizarin red S staining after formalin, acetic acid and alcohol (FAA) fixation. Specimens were fixed in FAA solution having the ratio of three components as 1:1:8 for approximately 40 minutes. Then they were stained in 0.06 per cent toluidine blue made in 70 per cent ethyl alcohol for 48 hours at room temperature. 20 volumes of stain solution to the estimated

volume of the specimen were used. Soft tissues were destained in 35 per cent ethyl alcohol for 20 hours; 5per cent for 28 hours and 70 per cent for 8 hours, respectively. The specimens were counterstained in a freshly prepared 1 per cent aqueous solution of KOH to which was added 2-3 drops of 0.1 per cent alizarin red S per 100 ml of solution. The specimens were transferred into the fresh 1 per cent KOH-alizarin mixture daily for 3 days, or until the bones had reached the desired intensity of red and soft tissues. The specimens were rinsed in water, placed in a 1:1 mixture of glycerol and ethyl alcohol for 1-2 hours and then transferred into fresh glycerol-alcohol for final clearing and storage.

RESULTS AND DISCUSSION

Of the twelve birds studied, eight were with bilateral anophthalmos, two with unilateral anophthalmos of left side (Fig. 1) and two with unilateral anophthalmos of right side. Of these birds, one each of unilateral anophthalmos was also associated with beak deformities. The beak as such was not deformed, but the upper beak crossed the lower beak so that the tip of the upper beak was deviated to the affected side with anophthalmos and the lower beak was normal (Fig. 1).

Among the bones of the neurocranium, frontal bone, especially its orbital part of the affected side showed deformities (Figs. 2&3). The interorbital septum formed by the orbital wings of sphenoid together with the more rostrally situated vertical plate of ethmoid bone, was also deformed. The horizontal plate of ethmoid bone lay below the processes of the frontal and nasal bones and corresponded to the lamina cribrosa of mammals and separated the orbit from the nasal cavity in normal birds (Fig. 4). It was also found defective in the affected side in the present study. Among the bones of the splanchnocranium, lacrimal bone was defective and reduced on the affected side. Zygomatic or jugal bone was absent on the affected side, collectively contributing the deformed orbit.Anophthalmia and microphthalmia (Fig. 5) may occur secondary to the arrest of development of the eye at various stages of growth of the optic vesicle during the embryonic period. It is important to recognize microphthalmia because the development of the orbital region, as well as the lids and the fornices, is dependent on the presence of a normal-sized eye *in utero.*

Pourlis (2011) opined that the etiology of these skeletal deformities and anophthalmos may be contributed to spontaneous and genetic aberrations. In the present study, as a means to detect the reduced hatchability of chicks, the genomic DNA samples of the affected chicks with structural anomalies were PCR-amplified for the ovomucoid gene, the PCR products sequenced, the results aligned and blast in the NCBI DNA database and compared

Fig.1. Day – old chick with anophthalmos condition on left side

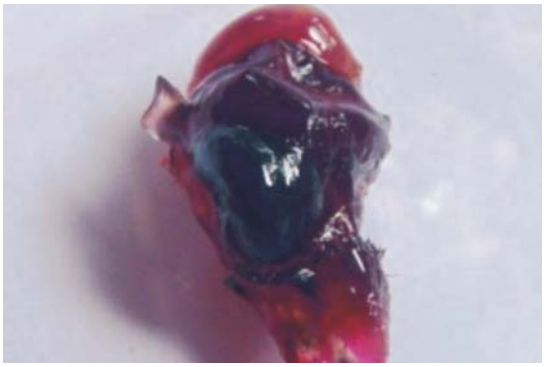

Fig. 2. Head of day–old chick with anoph thalmus condition on left side stained withToluidine Blue - Alizarin Red S Staining. Dorso- frontal view.

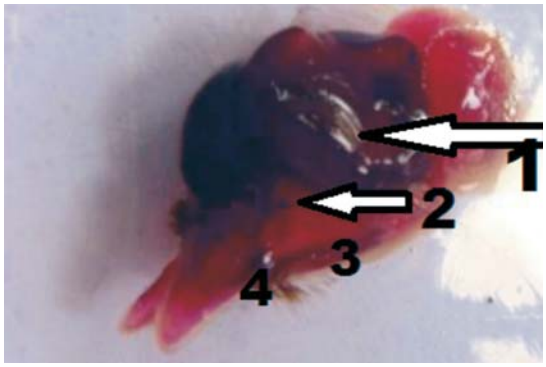

Fig.3. Head of day –old chick with anophthalmus condition on left side stained withToluidine Blue - Alizarin Red S Staining. Left view.
1. Frontal 2. Lacrimal 3. Mandible 4. Maxilla

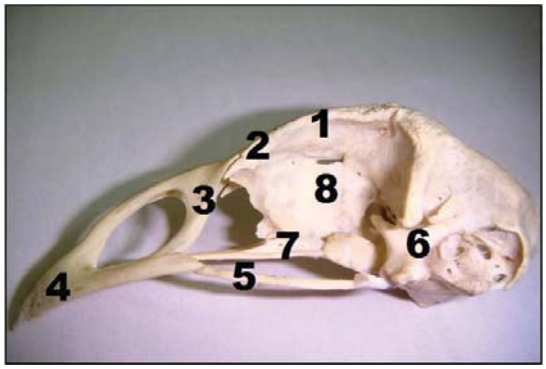

Fig 4. Upper skull of normal adult fowl. Left view.
1. Frontal 2. Lacrimal 3. Maxilla 4. Premaxilla 5. Jugal 6. Quadrate 7. Palatine 8. Interorbital septum

with those in the literature. Even though TAT deletion as noted in the ovomucoid gene (GenBank accession: HM776315) in ducks of low-hatchability group was not detected in the present study, the nucleotide sequences showed mismatching between nucleotides at various locations varying in number from 1-12, which might be contributed one reason for the deformed nature of the chicks.

Fig. 5. Day –old chick with microphthalmos condition on both sides.

ACKNOWLEDGEMENT

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GROSS MORPHOLOGICAL STUDIES ON PLACENTA IN PIG (*Sus domesticus*)

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ABSTRACT

The porcine placenta may be grouped as less advanced but tremendous changes were noticed during its development. The present gross morphologic study was done on 58 specimens of gestational age ranging from 20 to 110 days. Marked hyperaemia of uterine endometrium was observed as early as around 20 days. The demarcation between locular and interlocular zones amplified very much during the later stages. The vascularisation of allantochorionic sac was distinguishable at around 20 days that extended to its extremities in time. Five different zones: central placental zone and two each of paraplacental and necrotic tips positioned on either side were distinguished on the fully formed chorionic sac. Specialised structures called areolae were noticed from around 30 days of gestation. The umbilical cord was of primitive nature up to 42 days of gestation. The formation of necrotic tips may prevent freemartinism.

Key words: Porcine placenta, allanto chorionic sac, areolae, endometrium

Placenta may be defined as "an approximation or combination of an embryo's tissues with those of its natural or surrogate parent for physiological interchange". The placenta acts as a multi-functional organ of physiological exchange between mother and conceptus. The initially small area of foeto-maternal apposition is usually enormously increased by folding and refolding as it proliferates in parallel with the growth of the developing embryo or foetus (Fowden and Moore, 2012). The placenta of pig is of diffuse, chorio-allantoic, non-deciduate, epitheliochorial type. The foetal part, allanto chorionic membrane and the maternal part, endometrium are highly specialised during morphogenesis of placenta and the growth rated is rapid. Even if structurally porcine placenta may be considered as of less specialised type to consider it as a separate entity, the changes occurring during its development is tremendous. Though some studies had been made by Ashdown and Marble (1967) and Flood (1973), the progressive changes in mixed bred Large White York Shire pigs are less. So the present study was undertaken to depict the gross changes during pregnancy.

MATERIALS AND METHODS

The specimens were collected from mixed bred Large White York Shire pigs of around 20 days to 105 days of gestation from authorised slaughter places in Bengalooru. The gestational age of the specimens was determined according to the crown-rump length

of the embryos and/or foetuses (Marrable, 1971). The specimens from early (up to 40 days) (n=20), mid (41 days to 80 days) (n=26) and late (above 80 days) (n=12) gestational stages were used for the present study.

RESULTS AND DISCUSSION

The placenta of pig comprised of maternal and foetal parts. The maternal component of porcine placenta was contributed by the endometrium while the foetal element *via* allantochorion. Umbilical cord formed the connecting stalk between foetus and the placenta

Early gestation:

On gross examination, the non pregnant uterine endometrium was uniformly vascular throughout the entire length. Small circular primary folds and smooth secondary folds were also noticed on its surface. However at around 20 days of gestation, the locular endometrium (placental attachment regions) exhibited intense hyperaemia (Fig. 1). The interlocular regions did not intensify in vascularisation as of the locular zones so that these zones appeared as pale, oedematous and translucent. The number and dimensions of primary and secondary folds of the locular endometrium augmented by around 35 days so that they were more prominent than of interlocular regions. The demarcation between the two zones became intensified during later stages of gestation. These changes are in accordance with the reports of Flood (1973) in pig.

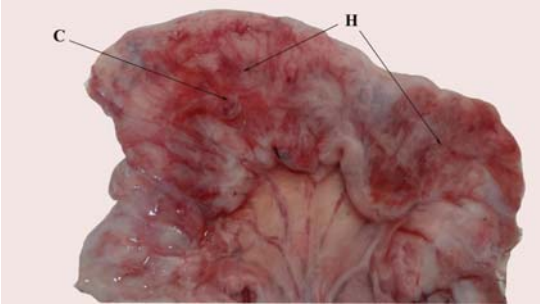

Fig. 1 Uterus of pig from around 20 days of gestation
C- Conceptus, H- Hyperaemia of endometrium

The vascularisation of the foetal part- allantochorionic sac was noticed in the equatorial zone by 20 days which extended to extremities by around 30 days (Fig. 2). This central region of chorionic sac created the placental zone. Though the hyperaemia was uniform at initial stages; by around 35-40 days the extremities revealed dull brown spots or discrete brown caps that indicated ischemic changes. These regions were designated as necrotic tips. Two allantoic arteries and veins which formed the axial vessels of the allantochorionic sac were obvious from 30 days of gestation along its mesometrial or concave border (Fig. 3). These vessels diverged in opposite direction from the distal element of umbilical cord which gave off branches on its course within the placental zone. In the paraplacental zone, only a few straight brown terminal branches were observed while the terminal necrotic regions were devoid of any vascular supply.

Specialised structures called areolae were appreciated macroscopically on the placental zone of the allantochorion from 30 days of gestation onwards in the present study. The emergence of areolae made possible to divide this zone into areolar and inter areolar regions. Two types of areolae were noticed:

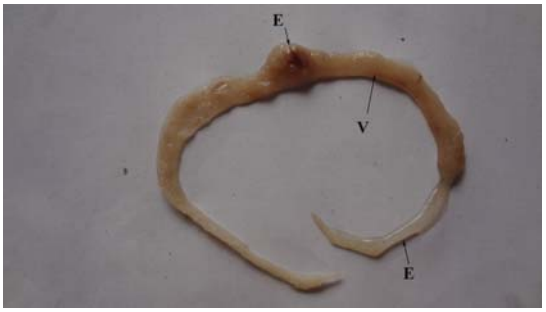

Fig.2. Embryo of around 30 days within allantochorionic sac
E- Embryo in the equatorial zone, V- Allantoic vessels, E- Extremities of chorionic sac

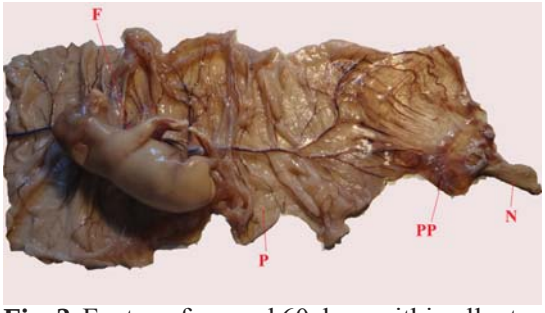

Fig. 3 Foetus of around 60 days within allantochorionic sac (cut open)F- Foetus, P- Placental zone, PP- Paraplacental zone, N- Necrotic tips

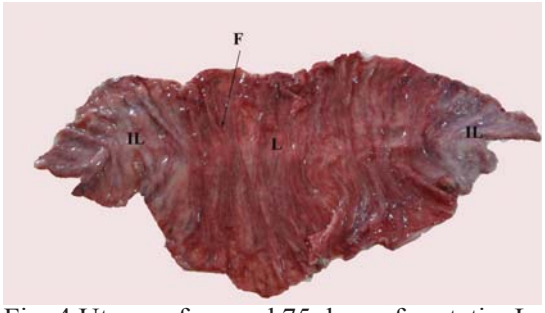

Fig. 4 Uterus of around 75 days of gestationL- Locular endometrium, Il- Interlocular endometrium, F- Endometrial folds

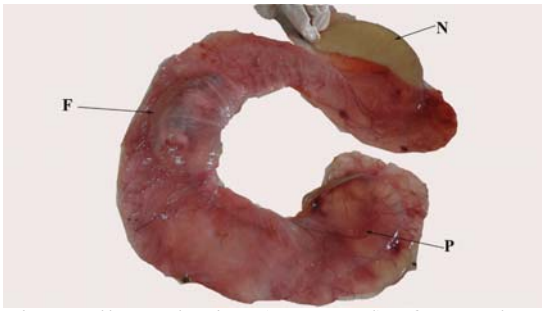

Fig. 5 Allantochorion (separated) of around 55 days of gestation
F- Foetus in the placental zone, P- Paraplacental zones, N- Necrotic tips

regular and irregular. The regular variety appeared as small circular opaque or pale spots of about 2 mm in size with a darker centre while the irregular as large, translucent polymorphic areas. The regular areolae were more in number and uniformly distributed than the irregular type. The irregular areolae were more frequent on the mesometrial side of the uterus and close to the large allantoic vessels. Irregular areolae have been reported in pig (Jamuna, 1993).

The umbilical cord of pig (Fig. 3) was short and had an average length of 1.5 cm, 3 cm, 5.5 cm, 8.5 cm, 9.5 cm and 12 cm respectively at 35 days, 42 days, 62 days, 70 days, 85 days and 105 days of gestation. The umbilical cord was of primitive nature up to 42 days. The average circumference of primitive cord at 42 days was 2.8 cm whereas that of the definitive cord of later periods was 1.5 cm. The cord revealed two spirally coiled allantoic arteries. Though two allantoic veins were distinct in the distal part only one noticed in the proximal part as these vessels fused within the distal part of cord itself as recorded by Mc Geady *et al*. (2006) in pig.

Mid gestation

The demarcation of locular and interlocular endometrium was much augmented from around 50 days (Fig. 4) and the interlocular regions was about 4-5 cm long. The endometrial folds of locular zones also amplified in time. The interlocular regions contained a few medium sized folds.

The necrotic tips of allantochorionic sac from mid gestation onwards appeared dry, wrinkled and layered with dark brown sticky material. An intermediate region separating placental zone from necrotic tips termed paraplacentae was observed from around 45 days (Figs. 3 and 5). These paraplacentae were clearly demarcated by constrictions with necrotic tips at around 50 days. These zones were smooth and glistening that enclosed almost straight longitudinal brown vessels. Similar findings were also recorded by Ashdown and Marble (1967) and Dantzer (1984). The formation of necrotic tips plus paraplacentae may avoid placental anastomoses of adjacent foetuses and thus may play a role in prevention of freemartinism as suggested by Flood (1973) in pig. The number as well as the size of areolae

of the placental zone improved rapidly during this period.

The allantochorion when separated from the properly infusion fixed gravid uterus had a crescent-shape at around 45-55 days of gestation that enclosed embryo in its centre (Fig. 5). Five different zones were clearly distinct on this sac: a large central placental zone, limited on either side by paraplacental regions and necrotic tips at the extremities. The large highly vascular central placental zone enclosed numerous irregular concentrically arranged macroscopic and microscopic folds which gave it a matt-like velvety appearance. These folds though apparent to some extent at 30 days became clear and distinct by 50 days of gestation. The folds were prominent on the concave border of the chorionic sac which faded away on its convex surface. The plentiful irregular concentric macroscopic and microscopic folds of the placental zone of allantochorionic sac interlocked with the complementary furrows of the endometrium during the morphogenesis of placenta. The increasing complexity of these folds in time may correspond to the intimate and intricate association of maternal and foetal parts as gestation advanced. In improperly perfusion fixed specimens, the allantochorionic sac was seen detached from the endometrium. Such separated sac had an apparent increase in length to that of the corresponding endometrium. The apparent enlargement of improperly fixed allantochorionic sac can be attributed to the sac's temporary microscopic folds which vanished following its separation from the endometrium whereas on the maternal side both macroscopic and microscopic folds remained permanent as suggested by Dantzer (1984).

Late gestation

The allantochorionic sac at around 85 days revealed an apparent transition of the necrotic tips from the terminal to sub terminal position along with terminal displacement of paraplacental zones. The number and demarcation of both types of areolae enhanced along with progress of gestation.

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HYPOKALEMIC POLYMYOPATHY IN A KITTEN - A CASE REPORT

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INTRODUCTION

In healthy animals, plasma potassium levels are maintained within narrow limits of 4-5 mmol/L by complex neuroendocrine /homeostatic mechanisms. Hypokalaemia can occur because of reduced potassium intake, translocation of potassium from the extracellular to the intracellular space, or an increased loss of potassium via the kidneys or the gastrointestinal tract. Acute or chronic vomiting and / or diarrhoea can lead to increased gastrointestinal loss of potassium. Small intestinal diarrhoea can result in significant faecal loss of potassium, particularly if malabsorption compromises resorption of the ion (Mardell and Sparkes, 2004).

The relative sodium and potassium concentrations are important in maintaining the correct electrical charge across the membranes of nerve and muscle cells. Potassium has a major role in acid-base balance, as it diffuses easily across cell membranes.

CASE HISTORY AND OBSERVATION

A three month old kitten was presented to the University Veterinary Hospital Kokkalai with the complaint of inability to walk and lowering of head. The animal was having diarrheoa for the past five days.

On examination, animal was having generalized muscular weakness and ventroflexion of neck (Fig. 1). Hypokalemic polymyopathy secondary to diarrheoa was suspected. Serum potassium level was estimated and was 3.4 mmol/L. The animal was given 40 ml ringer's lactate and 10 ml dextrose normal saline mixed with B complex vitamins as intravenous infusion. Prescribed Potassium chloride @500 mg per day orally and Amoxycillin clavulanate combination @12 mg/ kg body weight BID orally for 5 days with an advise to give tender coconut water orally. The animal became normal within 3 days.

DISCUSSION

Hypokalaemia is commonly encoun

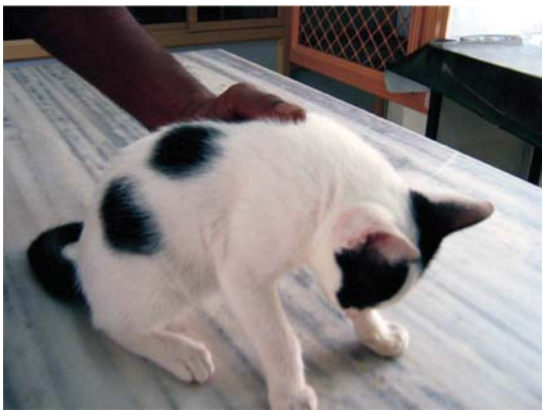

Fig. 1 Ventroflexion of the neck in the kitten with hypokalemic poly myopathy. Serum potassium concentration was 3.4 mmol/L.

tered in feline patients. Signs typically include generalized skeletal muscular weakness, which can be profound. Affected cats classically present with a plantigrade stance on the hind limbs, and ventroflexion of the neck. Ventroflexion of the neck occurs as the cat lacks a nuchal ligament. Other signs include reluctance to move, poor exercise tolerance and muscle pain (Fox and Jones, 2003). Early potassium defficiency causes hyperpolarization of muscle fibre membranes, increasing their resting potential and thus reducing their excitability (i.e. a larger influx of sodium is required to depolarize the cells) and thus inducing muscle weakness. More chronic and severe hypokalaemia causes muscle fibre hypopolarization, leading to extreme muscle weakness and eventual rhabdomyolysis. This can be accompanied by, sometimes very profound, elevations of circulating Creatinine Kinase concentrations.

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EAR MITE INFESTATION IN A BEAGLE- A CASE REPORT

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ABSTRACT

Ear mite infestation is one of the primary ear diseases of dogs and is common in young puppies. A six months old male Beagle dogwas presented to the Teaching Veterinary Clinical Complex, Mannuthy with the history of head shaking and reddish lesions in the ear. Clinical examination revealed erythematous lesions with coffee colouredcerumin in the ear canal. Sterile ear swab was used to collect cerumin and it was processed with 10% KOH. Microscopic examination of the 10% KOH processed cerumin under low power revealed the presence of ear mite *Otodectes cynotis.* Based on the history, clinical signs and laboratory examination, the condition was diagnosed as *Otodectes cynotis* ear mite infestation. The animal was successfully treated with ivermectin and was supported with syrup immunol @ 2ml PO BID.

INTRODUCTION

Otodectus cyanotis is one of the most common primary ear infestation of dogs and cats especially puppies and kittens caused by non-burrowing psoroptic mite that lives and feeds on skin and ear. The host range extends up to foxes, ferrets and sometimes humans. It is gaining importance because of its highly contagious and zoonotic in nature (Miller *et al*. 2013)

CASE HISTORY AND CLINICAL FINDINGS

A six months old male Beagle dog was presented to the Teaching Veterinary Clinical Complex, Mannuthy with the history of head shaking and reddish lesions in the ear for more than a week. Clinical examination of the dog revealed erythematous lesions with coffee colouredcerumin in the ear canal.Sterile ear swab was used to collect cerumin and it was processed with 10% KOH. Microscopic examination of the 10% KOH processed cerumin under low power revealed the presence of parasitic ova and adultear mite of *Otodectescynotis.*

TREATMENT

The dog was treated with two injections of Ivermectin @ 200mcg/kg body weight 14 days interval and was supported with syrup immunol @ 2ml PO BID. Dog showed clinical improvement after two days. The dog had uneventful recovery after two weeks.

DISCUSSION

Otodectus cyanotis nourishes on tissue liquid and epidermal debris from the superficial epidermis. The life cycle goes on for 21 days. Off host survival time least of twelve days. Coffee ground appearance of ceruminous discharge was common presenting

signs in *Otodectus cyanotis* infestation and became purulent with auxiliary bacterial contaminations like staphylococcus and pseudomonas and so forth (Miller *et al*. 2013). The feline assumes a key part in transmission of ear parasite in grown-up canines, rabbit and ferrets (Sasikala *et al*., 2011). Because of pruritis stamped self-mutilated trauma and hot spots over the external ear pinnae and may lead to aural haematoma. Ectopic infestations are conceivable in felines and basically manifested as papular crested eruptions in neck, rump and tail. History, clinical signs, Otoscopyand microscopic examination of ceruminous wax from ear and superficial scrappings were valuable in diagnosing this condition. Clinical signs and examination of ceruminous wax were useful in diagnosing this condition. Treatment should be carried out not only in affected animal but all animals in contact.Mild ceruminolytic agents should be used before other topical ear medications will ensure high penetration. Some of the drugs were used as extra label purpose for treating ear mite infestation which includes 10% fipronil solution 2 drops into the ear twice weekly for 3–4 weeks and 1% injectable ivermectin diluted 1:9 with propylene glycol 2–4 drops into the ear daily for 3-4 weeks.Ivermectin 0.3 mg/kg po, scevery 10 days for 3 applications andmoxidectin (dogs) 0.2 mg/kg po every 10 days for 3 applications (Sue Patterson, 2008).

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