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Spontaneously Occurring Chronic Copper Toxicosis in Pattanam Breed of Sheep

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ABSTRACT

Background: Copper (Cu), an essential trace element, is toxic if consumed in excessive amounts. Ruminants, particularly sheep, are highly susceptible to chronic copper poisoning and cause acute death. Spontaneous copper poisoning in sheep was reported from many parts of the world however limited reports are available from India. Hence the present investigation was undertaken to report the occurrence of chronic copper poisoning in an intensively maintained Pattanam breed of sheep flock and its therapeutic management.

Methods: An investigation was carried out to ascertain the possible cause of increased mortality in an intensively maintained 130 male lambs aged between 8 to 10 month old belongs to Pattanam breed during the month of September 2020 following death of 21 animals within a period of 15 days. Affected flock was inspected and samples were collected for biochemical analysis, toxicological, bacteriological and pathological examination.

Result: Affected animals showed depression, anorexia, jaundice, hemoglobinurea and accelerated breathing. At necropsy, the dead animals showed generalized icterus, lung edema, yellow to orange coloured liver and gun metal kidney. Histopathological lesions include lung edema, centrilobular hepatic necrosis, bile stasis, renal tubular necrosis and formation of tubular cast. Toxicological analysis of liver revealed the copper level of 781 mg/kg dry matter basis. Source of copper was identified as a commercial mineral mixture supplement intended for cattle was supplemented along with concentrate feed. The flock was treated with chelating agent (D-penicllamine) and supportive therapies.

Key words: Copper, Pathology, Sheep, Toxicosis, Therapy.

INTRODUCTION

Copper (Cu) is an essential trace element for various biological processes of animals such as normal iron metabolism, synthesis of elastin and collagen, production of melanin and keratin (wool), integrity of the central nervous system and effective immune response. Copper poisoning occurs when the amount of Cu ingestion exceeds the requirement of animal and it may be either acute or chronic. Copper is a well-documented cause of liver toxicity in many domestic species, including sheep, dogs, cats, horses, cattle, goats, pigs and camelids, however wide variation was noticed in their susceptibility. Among the domestic animal sheep is the most sensitive to Cu toxicity because of their less efficient Cu excretory mechanism (Carmalt *et al*., 2001; Morgan *et al*., 2014).

Poisoning in sheep is usually chronic type in which Cu is accumulate in the liver for a long time, without any clinical manifestations (Lewis *et al*., 1997; Humann- Ziehank *et al*., 2001; Ortolani *et al*., 2003). When maximum hepatic storage capacity is reached, Cu is released into the circulation causing acute hemolysis, renal and liver failure and ultimately death in 2–3 days (Garcia-Fernandez *et al*., 1999). Spontaneous copper poisoning in sheep was reported from many parts of the world including Australia, New Zealand, USA, Great Britain and South Africa (Kimberling, 1988), however limited reports are available from India (Banerjee, 2009). Hence the present study report the occurrence of chronic copper poisoning in an intensively maintained Pattanam breed of sheep flock and its therapeutic management.

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MATERIALS AND METHODS

Flock history and management

An investigation was carried out to ascertain the possible cause of increased mortality in an intensively maintained 130 male lambs aged between 8 to 10 month old belongs to Pattanam breed during the month of September 2020

following death of 21 animals within a period of 15 days. Lambs were purchased at the age of 2 to 3 months of age from adjoining districts of Namakkal, Tamil Nadu, India and reared in slatted floor system for mutton purpose. All the lambs were dewormed and vaccinated against PPR, Blue tongue and Enterotoxaemia. Animals were fed sorghum fodder and commercially prepared concentrate. Lambs exhibited red coloured urination, icteric mucous membrane and breathing difficulties prior to death. Initially the cases were suspected for ovine babesiosis and treated by local Veterinarian. In spite of the rigorous treatment, affected animals were died within 24 to 48 hours after the onset of clinical signs.

Sample collection

Affected flock was inspected, random blood samples and peripheral blood smears were collected from six live lambs, including two lambs with the clinical signs of depression and anorexia. Sorghum fodder, concentrate feed and water samples were collected for toxin analysis. Detailed necropsy examination was carried out on three dead lambs and the lesions were recorded. Heart blood and lung swabs were collected for microbiological examination. Urine sample was collected for screening of *Leptospira sp*., by dark field microscopy. Frozen ruminal content and liver samples were sent to Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), Madhavaram Milk Colony, Chennai for toxicological evaluation. Representative tissue samples from heart, liver, lung, spleen and kidney were fixed in 10% neutral buffered formalin for histopathological examination. The samples were processed by routine paraffin embedding technique, sectioned at five micron thickness and stained with hematoxylin and eosin method (Luna, 1968).

Estimation of copper in tissue samples

Analysis of copper in the liver sample was performed using iCAP Q ICP-MS with Cetac ASX 520 auto sampler (Thermo Scientific). The iCAP Q ICP-MS performance was ascertained with tune solution (Thermo Scientific) before performing actual analysis. The plasma, auxiliary and nebulizer gas flows were set at 14, 0.8 and 1.04 L/min respectively. The Copper isotope $^{63}Cu^{+}$ was monitored under Kinetic Energy Discrimination (KED) mode in replicates of three for blanks, standards and the sample. Multi element standard solution (LGC Custom Multi Std 1320) having 100 mg/L was used for the preparation of working standards at the concentrations of 2.5 25, 50 and 100 µg/L (ppb) in 2 % trace metal grade nitric acid . About 0.5 g of the liver sample was digested with 10 mL concentrated trace metal grade nitric acid using aluminum block heater. The sample was then cooled and makeup to 50 mL with type-1 grade water. The sample was then diluted at 1:10 ratio with type-1 water in the auto sampler vials for analysis.

Therapeutic management

Animal with clinical signs were treated with polyionic fluids, D- Penicillamine (50 mg/kg/day, PO), Vitamin E (2,000 IU, PO), liver extract and B complex were administered parentally. Enrofloxacin (5 mg/kg by IM) was administered prophylactically to control secondary bacterial infection. Animals without any clinical signs were treated with D-Penicillamine, Vitamin E and liver extract.

RESULTS AND DISCUSSION

Sheep are the most susceptible species to copper poisoning because of their liver cells had a high affinity for copper and slow excretion of copper into the bile leading to a buildup of liver copper concentration over time (Orr, 1985). Chronic copper poisoning is a common problem in intensively maintained sheep flocks then pastured sheep and its early diagnosis in live animals is difficult because of the very slow progress and latent character of the disease for some weeks or even months (Ishmael *et al*., 1972). However there is eventually a sudden haemolytic crisis, jaundice and death usually follows in two to three days. This clinical syndrome has to be differentiated from leptospirosis and tick-borne diseases (*i.e.* babesiosis, theileriosis) that cause similar manifestations.

In the present study, among 130 lambs 21 were died and 12 sick lambs were sold within a period of 15 days. All the affected lambs showed depression, anorexia, dark brown to black coloured urine (hemoglobinurea), yellowish discolouration of sclera of the eye and visible mucus membranes, accelerated breathing, arched back and recumbency prior to death. Death was noticed within 24 to 48 hrs after the onset of clinical signs. Mendel *et al*., (2007) also reported similar signs in chronic copper poisoning of sheep and the appearance of hemoglobinuria indicates the transition of subclinical (copper accumulation phase) into clinical disease.

Serum aspartate aminotransferase (AST) levels ranged from 188 to 1130 U/L (normal upper limit, 125 U/L) and Alanine aminotransferase (ALT) ranged from 16 to 40 U/L (normal upper limit, 40 U/L). Lopez-Alonso *et al*., (2006) also reported that the serum AST activity was significantly correlated with liver copper concentrations and it will rise 2 weeks prior to the haemolytic crisis in experimentally induced chronic copper poisoning in goats. Results of the present study might indicate that the majority of sheep in the flock were affected with toxic levels of Cu, but most were pre-haemolytic and haemolysis had started only in a small percentage. Hence serum AST level might be used to detect the copper induced liver injury in natural cases so that therapy could be instituted beforehand.

At necropsy, all the three dead male lambs were good in body condition. All tissues and mucus membranes were grossly jaundiced (Fig 1). Blood was dark red in colour and watery in appearance. Subcutaneous and mesenteric fat showed intense yellowish discolouration with petechial haemorrhages. Trachea and bronchi contained yellowish frothy exudate and the mucosa was icteric. Lungs were edematous, dark brown in colour and on section oozing of yellowish froth was noticed in the major air ways (Fig 2).

Liver was enlarged and diffusely yellow to orange in colour (Fig 3). Gall bladder was distended and contained dark yellowish bile but the bile duct was patent. Kidneys showed bilateral enlargement, dark brown to brown black in colour with metallic sheen (gun metal) (Fig 4). On section of kidney, dark brown colour was noticed in the cortex and it extended the medullary region also. The renal pelvis was icteric. Urinary bladder was distended with port wine coloured urine (Fig 5). Spleen was enlarged with soft pulp and cut surface appeared dark brown in colour (Fig 6) due to hemolytic crisis.

Histopathologically, lung alveoli contained pink stained homogenous material which might be due to damage of endothelial cells that constitute blood air barrier by hemolytic anemia induced oxygen deprivation (Zachery, 2017) along with multifocal deposits of golden brown pigment (Fig 7). Liver showed widespread hepatocellular cytoplasmic vacuolation, degeneration and necrosis frequently in the centrilobular areas (Fig 8). Kupffer cells with brown, granular cytoplasm were noticed close to central vein and mid zonal areas. The areas of degeneration and necrosis are devoid of inflammation, although foci of polymorphonuclear leukocytic infiltrations were occasionally observed. Accumulation of bile pigment was noticed in canaliculi between hepatic cords, hepatocytes and bile ductules. Mild

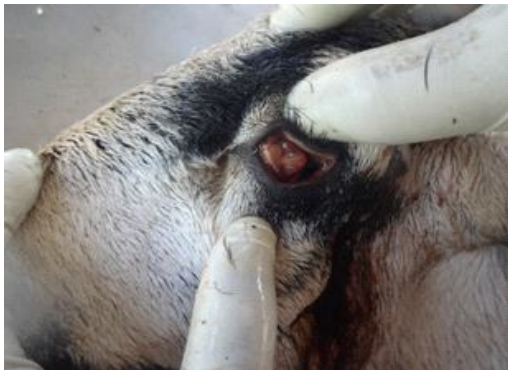

Fig 1: Conjunctival mucus membrane showing yellowish discolouration.

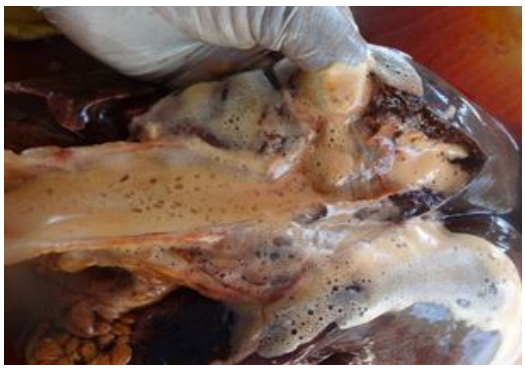

Fig 2: Lung showing dark brownish discolouration with oozing of yellowish frorh from the major air ways.

Fig 3: Liver showing diffuse yellowish orange discolouration.

Fig 4: Kidney showing gun metal appearance.

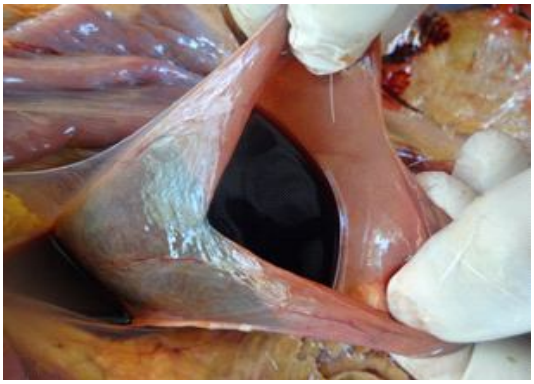

Fig 5: Urinary bladder showing retention of port wine coloured urine.

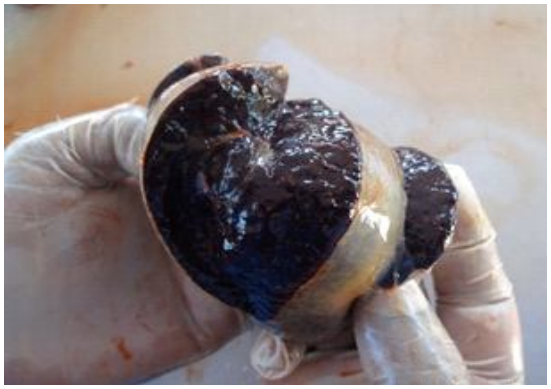

Fig 6: Spleen showing dark brown discolouration on cut surface.

to moderate bile duct hyperplasia was noticed in portal tracts. In kidney, epithelial cells of proximal convoluted tubules revealed degeneration, vacuolation, focal coagulative necrosis and desquamation. Distal convoluted tubules were dilated. Eosinophilic hyaline or coarsely granular hemoglobin casts were noticed both in the cortex and medulla (Fig 9). Multifocally, tubular epithelial cells contained intracytoplasmic dark brown pigment (hemosiderin) and tubular dilatation.

Copper storage begins in the centrilobular hepatocytic lysosomes, its membranes lose their integrity and release lysosomal hydrolases as Cu accumulates leads to irreversible injury to the cell (Rolfe and Twedt, 1995). The accelerated loss of hepatocytes by necrosis and apoptosis leads to acute massive Cu release, which in turn increase the free blood Cu concentrations, results in oxidative injury to hemoglobin and converting it into methemoglobin which cannot bind to the oxygen and also induces erythrocytic inclusions such as, Heinz-body formation. Moreover, the sulfhydryl groups of the erythrocyte membrane also undergo oxidative changes (lipid peroxidation), resulting in increased RBC fragility. Copper-induced fragility of RBCs and Heinz body might contribute to hemolysis in the present study and accumulation of hemoglobin casts in the renal tubules.

Spleen red pulp vascular spaces are distended by erythrocytes and contained numerous hemosiderin laden macrophages (Fig 10). Splenic congestion and hemosiderosis might be due to the processes of removal (phagocytosis) and storage of large numbers of altered erythrocytes (Heinz body) from the circulation (Zachery, 2017). Marked depletion of lymphocytes was noticed in the white pulp of the affected animals. Myocardial fibers revealed vacuolar degeneration and erythrocytes in between the fibers.

Fodder, feed, water samples and ruminal contents were negative for toxic agents. Peripheral blood smears revealed the absence of blood protozoan parasites. Heart blood and lung swabs did not show any etiological agents of pathological significance. Urine samples collected from dead animals were negative for *Leptospira sp*., on dark field microscopy. All the dead lambs showed similar clinical signs, gross and histological lesions which were consistent with previously described cases of chronic copper poisoning in sheep (Angus, 2000; Cullen and Stalker, 2016). The tentative

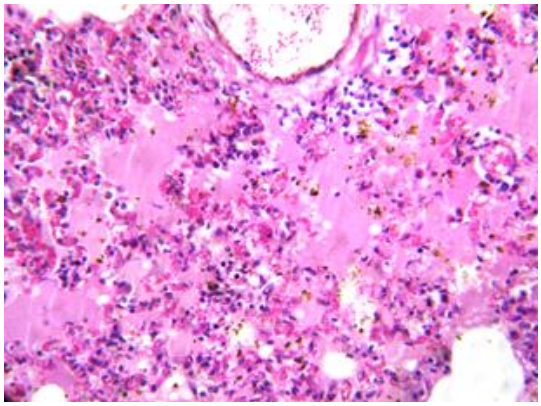

Fig 7: Lung alveoli containing pink stained homogenous fluid with multifocal deposits of golden brown pigment. H & E x 400.

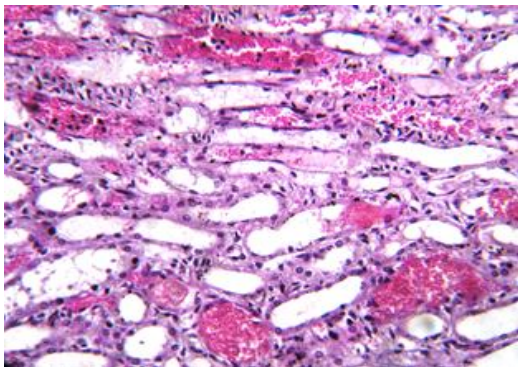

Fig 9: Kidney showing eosinophilic hyaline to coarse granular cast in the tubules. H & E x 400.

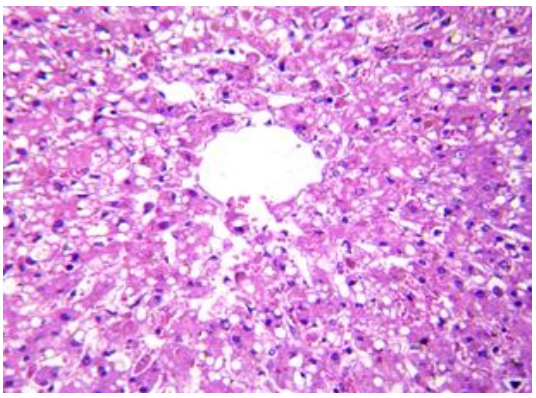

Fig 8: Liver showing hepatocytic vaculoation, degeneration, necrosis and bile stasis. H & E x 400.

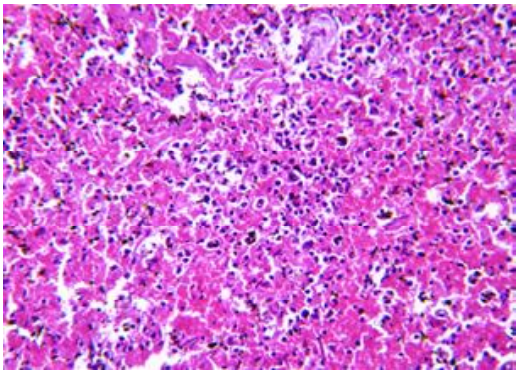

Fig 10: Spleen showing distension of vascular spaces with erythrocytes in the red pulp and diffuse infiltration of hemosiderin laden macrophages. H & E x 400.

diagnosis of copper toxicosis was confirmed by the elevated concentration of Cu (781mg/kg DM) in the liver by toxicological evaluation. Concentrations of Cu in the liver greater than 350 mg/kg DM are diagnostic of this intoxication (Angus, 2000; Radostits *et al*., 2007). Liver Cu concentration may be normal after hemolytic crisis due to release into the blood stream (Kimberling, 1988). In the present study, liver Cu concentration was twice the normal value in spite of the samples were collected from the hemolytic crisis affected animals, hence the detected Cu level clearly indicate toxic concentration.

The recommended dietary copper requirement for sheep was 5 mg/kg/day (Clegg *et al*., 1986; Puls, 1994). In the present study, all the lambs were maintained by intensive system of management and fed with commercially prepared concentrate feed, hence their Cu requirement may be less than the recommended level, moreover the daily requirement may be met out from the concentrate feed which usually contain adequate copper required for the lambs (Angus, 2000; Constable *et al*., 2017). The farmer was questioned about all medications given to the animal and found that he has given 10 gm of commercial mineral mixture supplement per lamb per day for a period of two months which contained 1200 mg of Cu per kg. This means that every lamb received 12 mg of Cu per day only from the supplementation for almost two months before the first case was observed. Mineral supplement used in the present incidence was formulated for cattle since the farmer lacks the knowledge on the consequences of furnishing a cattle based mineral supplement to sheep. Hence the source of copper toxicosis in the present study could be attributed to the chronic ingestion of a commercially prepared mineral supplement destined for cattle.

Treating the copper poisoning is very difficult and challenging in most cases, hence, severely affected animals often die despite treatment. D-penicillamine (degradation product of penicillin) is a chelating agent used to enhance copper mobilization and urinary excretion (10- to 20-fold) in sheep (Humann-Ziehank *et al*., 2001) and has been identified as an useful agent for maximize copper removal from the body in chronic copper toxicosis (Gooneratne and Christiansen, 1997). Hence in the present study affected and apparently normal animals were treated with penicillamine (@ 50mg/kg Bwt for seven days through oral route) and found that no further occurrence of fresh cases in the investigated flock. Moreover the animals were maintained for meat purpose, hence the farmer sold all the animals within 15 days due to attainment of required body weight and also to avoid further economic loss.

CONCLUSION

Increased mortality in the sheep flock was diagnosed as chronic Copper toxicosis based on toxicological analysis of liver samples as well as the supportive medical history, clinical signs, necropsy lesions and microscopic findings. The source of Cu was attributed to the use of commercial mineral mixture supplement intended for cattle. This communication highlights the need to consider chronic copper poisoning as one of the differential diagnosis in sheep presented with signs hemolytic crisis. Further study on the level of dietary copper which Indian sheep breeds can tolerate and ovine breed resistance to copper intoxication may be key feature to reduce economic losses in modern sheep industry.

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