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Case Report

Acute Phorate Poisoning in a Flock of Sheep- A Case Report

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Abstract

Organophosphate compound pesticides represent one of the major classes of insecticides used in agriculture and animal husbandry in India. Among these, Phorate is one of the commonly used OPC compounds. A flock of Hassan breed sheep from a village near Hassan district, Karnataka, India was presented with history of severe mortality (61%) in a span of 2 days. There was circumstantial evidence of spraying of ragi straw with Phorate in fields for the control of termites. Clinical examination revealed watery salivation, tachypnoea, tachycardia, frequent urination, lacrimation, discharges from nostrils, dynpnoea, tremors, ataxia and pungent odour. The carcasses appeared bloated with congested conjunctival and oral mucus membranes, sticky lacrimal secretion and rectal prolapse. Microscopically, severe congestion and hemorrhoages was in urinary bladder in most of the animals. Other pathological changes were observed in liver, heart and spleen. Thin Layer Chromatography confirmed the presence of Phorate. The survived animals (n= 33) were treated with 2-PAM, Atropine Sulphate, parentral fluids and all ailing animals recovered from the OPC poisoning. This manuscript describes clinical, pathological and toxicological features of Phorate toxicity in a flock of sheep.

Keywords: Histopathology, Necropsy, Pesticide, Phorate, Toxicity

Introduction

Organophosphate pesticides also referred to as OPCs are a class of pesticides developed almost 100 years ago. They are used in agriculture for the control of insects in various crops such as maize, cotton, potatoes, and millets and to control external parasites in livestock. The mechanism of action of these compounds is inhibition of acetylcholinesterase in the nervous system of man and mammals. The use of OPC in the post era of green revolution has increased so much that today they represent the major class of insecticides under use in India (Mahajan *et al.* 2006).

Increased use of pesticides, plastics and drugs regrettably is associated with their propagation in food chain and persistence in the environment leading to harmful effects in man, plants, animals and birds including wildlife (Kazemi*et al.* 2017, Marcoccia*et al.*2017). In addition, animal feed may sometimes be contaminated with pesticide residuesand these residues may be passed into the organ systems and in turn animal products and later may get excreted by the biological system (Peter *et al*. 2017). These chemicals are also linked to causation of cancer (Schinasi and Leon, 2014), Central nervous system anomaliesand Endocrine disturbances (Starks *et al*. 2012).

Phorate {O, O-diethyl S-[(ethylthio) methyl] phosphorothioate (trade name: thimet) is one of the commonly used OPC compounds. Through accidental to malicious ingestion of Phorate and similar OP compounds, there are increasing incidences of clinical toxicities in man and animals being reported in the recent past (Puschner *et al.* 2013). Following exposure to OPC, clinical signs such as salivation, lacrimation, urination, defecation, gastric cramps, emesis (acronymed as SLUDGE) symptoms in man and mammals including domestic animals are anticipated (Laetitia *et al., 2018)*. Necropsy examination of dead dairy cows with Phorate toxicity has been reported to produce nonspecific post mortem lesions with garlic-like odour in the rumen contents (Peter *et al.,* 2017).

In the current investigation symptomology, gross lesions, microscopic pathology and clinical intervention in flock of sheep with a size of 85 that were accidentally exposed to Phorate were studied.

Case History, Investigation and Treatment

The flock belonged to a farmer from a village called Nagenahally Gollarahatti of Arasikere taluk of Hassan district of Karnataka. Among the 85 Hassan breed sheep 9 were rams and 76 ewes. The Animals belonged to varying age groups from 6 months to 2 years. Total of 52 sheep that died in a span of 2 days (49 on day 1 and 3 on day 2) formed the basis of pathological investigation. The investigation included collection of history, observation, physical examination and recording of clinical parameters of affected animals, necropsy examination of the deceased animals, detailed histopathological evaluation of the collected morbid organs and tissues, collection of feed and gut materials for the confirmation of toxin and therapeutic intervention with pharmacologically relevant antidotes.

Collection of history involved details pertaining to circumstantial evidence. Physical examination included recording of clinical signs and symptoms, temperature, behaviour, abdominal auscultation and general health. Total eight sheep (four each male and female) were subjected to detailed post mortem investigation. Representative tissue samples collected were fixed immediately in 10% Neutral Buffered Formalin and were processed by routine paraffin embedding technique. Sections of five-micron thickness were cut using Leica microtome with disposable blades. The tissue sections were then stained with routine Hematoxylin and Eosin method (Luna, 1968). Thin Layer Chromatography was employed for confirmation of the toxin at Institute of
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Animal Health and Veterinary Biologicals, Bengaluru. Thin Layer Chromatography confirmed the presence of the toxin at Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore, KVAFSU, Karnataka as per the procedure (Vijay and Dhananjay, 2009). The TLC plates were spotted with Phorate standard solution and samples obtained from the Sheep. which was then developed the distance of approximately 10 cm with chloroform

- acetone 7:3 as mobile phase in a presaturated TLC chamber. The plate was removed from the chamber, left to dry at room temperature and sprayed with 5% p-Dimethylaminobenzaldehyde solution. The plate was then placed in UV chamber and observed the pink spot with R_F 0.55.

The survived ailing animals (n=33) were administered with 0.5 mg/Kg of Atropine (Trade name: ATN-MB, Martin and Brown Biosciences) at four hour interval intravenously and 10 mg/Kg of 2-PAM (Trade name:Pralidoxiealth, BioPharma Pvt Ltd) along with intravenous fluids (Ringers lactate and Dextrose (5%) parentral fluids) and corticosteroid injection at 1 mg/Kg dose (Prednisilone Acetate, Intervet India Pvt. Ltd) . All the thirty-three sheep were recovered from the OPC poisoning.

Results and Discussion

History revealed circumstantial evidence of spraying of ragi crop with Phorate in fields near to the pond where the sheep were taken for drinking water.Clinical examination of surviving sheep or sheep prior to death revealed watery salivation, tachypnoea, tachycardia, frequent urination, lacrimation, watery discharges from nostrils, dynpnoea, tremors, convulsions, ataxia and pungent odour emanating from affected animals. Body temperature was found to be subnormal ranging from 96 to 98°F.Thin Layer Chromatography confirmed the presence of the toxin at Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore, KVAFSU, Karnataka as per the procedure (Vijay and Dhananjay, 2009). The TLC plates were spotted with Phorate standard solution and samples obtained from the Sheep. which was then developed the distance of approximately 10 cm with chloroform - acetone 7:3 as mobile phase in a presaturated TLC chamber. The plate was removed from the chamber, left to dry at room temperature and sprayed with 5% p-Dimethylaminobenzaldehyde solution. The plate was then placed in UV chamber and observed the pink spot with R_F 0.55. The OP insecticides are known to produce toxicity by irreversibly inhibiting the acetylcholinesterase (AChE). Cholinergic signs are due to the muscarinic, nicotinic, and central effect (Starks *et al*., 2012; Peter *et al.*, 2017). This would result in the synaptic build-up of ACh, the eventual toxicant. Death follows due to paralysis of the respiratory muscles mainly diaphragm and intercostal muscles (Gupta and Crissman, 2013).

On necropsy examination the carcasses appeared bloated with congested conjunctival and oral mucus membranes, sticky lacrimal secretion and rectal prolapse. Foul to pungent smelling ruminal contents with undigested feed material, mucosal hemorrhages in the intestines devoid of contents were observed. Internally, they presented increased secretions from bronchi, congested tracheal mucus membranes, congestion and hemorrhages on the mucosa of urinary bladder (Fig. 1), congested and pale areas in liver (Fig. 2) pulmonary edema hemorrhages (Fig. 3) and petechial hemorrhages on epicardium of heart (Fig. 4). Also, injected cerebral vessels in the brain (Fig. 5) and hemorrhages and softening of kidneys (Fig. 6) were seen. Oliveira-Filho *et al*. (2010) and Puschner *et al*. (2013) recorded that post mortem findings would be non-specific in cows with Phorate toxicity. Kalaivanan *et al.* (2011) were described the lesions such as pulmonary edema, dilated and fluid-filled intestinal tract with degenerative, haemorrhagic and necrotic changes in kidney and liver while investigating secondary Phorate toxicity in large carnivores.

Figure 1: Urinary bladder showing congestion and haemorrhages on mucosa

Figure 2: Liver showing congestion, paleness and discoloration

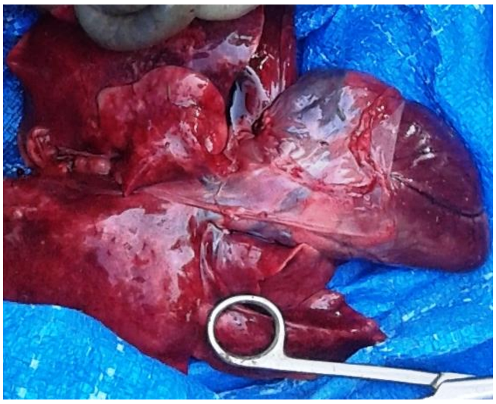

Figure 3: Liver showing congestion, paleness and discoloration

Figure 4: Liver showing congestion, paleness and discoloration

Figure 5: Brain showing injected cerebral vessels in the sulci of the cortex

Figure 6: Kidney showing degeneration of the cortex

Microscopic examination showed severe congestion and hemorrhoages in the submucosal blood vessels of urinary bladder consistently in most of the animals (Fig.7); Spleen displayed hypocellularity of the white pulp and increased hemosiderin pigmentation (Fig.8); liver revealed moderate to severe cloudy swelling, sinusoidal congestion, hemosiderosis, bile duct proliferation and vacuolar degeneration (Fig.9.10.11); Kidney revealed severe degenerative changes in both glomerulus and convoluted tubules; Moderate to severe hemorrhages with the presence of pigmentation surrounding cardiac arteries was noted in the heart (Fig.12); Severe alveolar hemorrhages and congestion of pulmonary vessels and pulmonary edema as visualized by eosinophilic homogenous fluid in the alveoli were noticed in the lungs (Fig. 13,14). Brain showed cerebral congestion and neuronal degenerative changes (Fig.15) and Brain with Arachnoid showing congestion in the meningeal vessels (Fig.16).

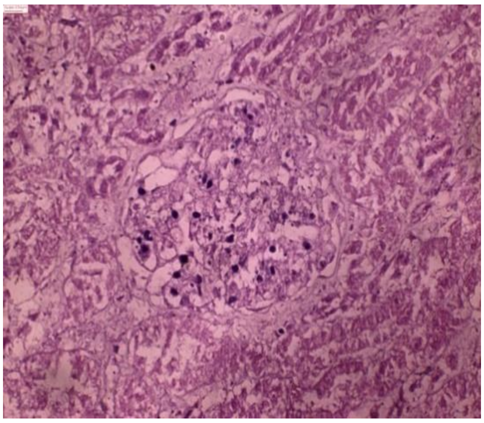

Figure 7: Urinary bladder showing congestion (10 X)

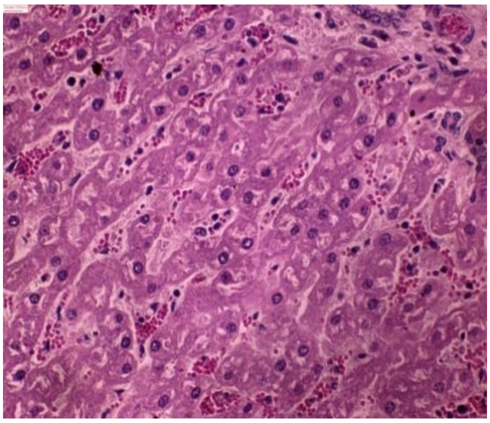

Figure 8: Spleen showing hemosiderin deposits (40X)

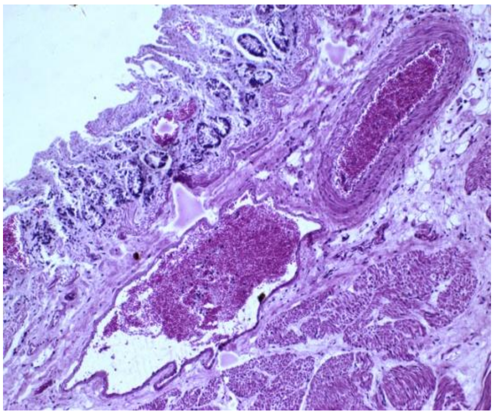

Figure 9: Kidney showing degeneration the cortex

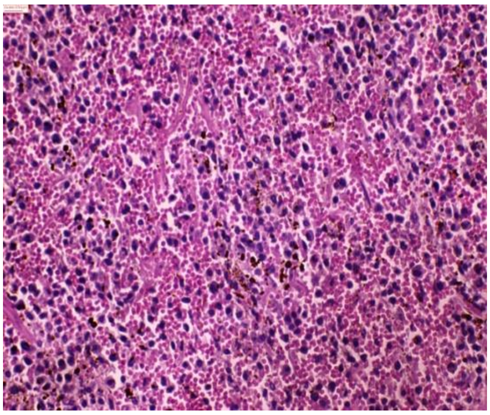

Figure 10: Liver showing sinusoidal congestion (40X)

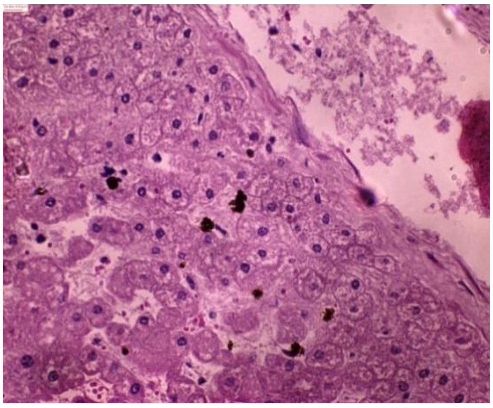

Figure 11: Liver showing cellular swelling and hemosiderin deposition (40X)

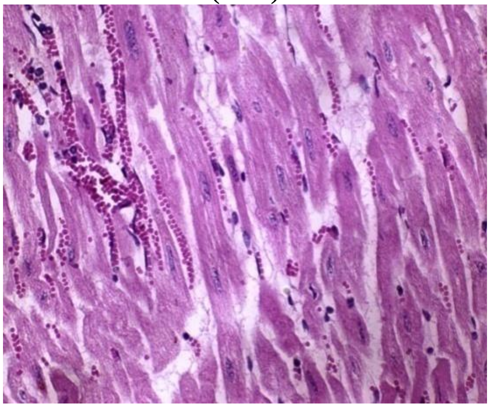

Figure 12: Heart showing hemorrhage between the myofiber bundles (40X)

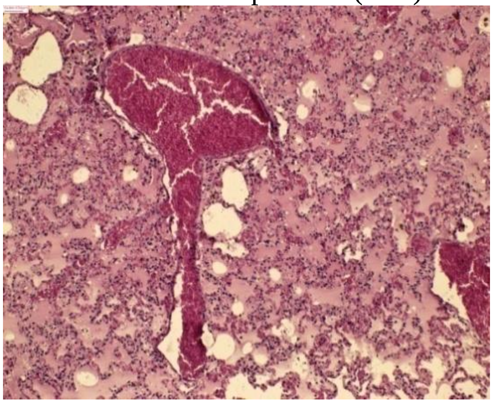

Figure 13: Lung showing hemorrhage and alveolar edema (40 X)

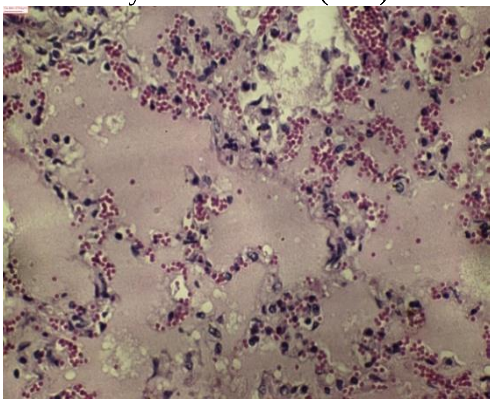

Figure 14: Lung showing hemorrhage and alveolar edema (40 X)

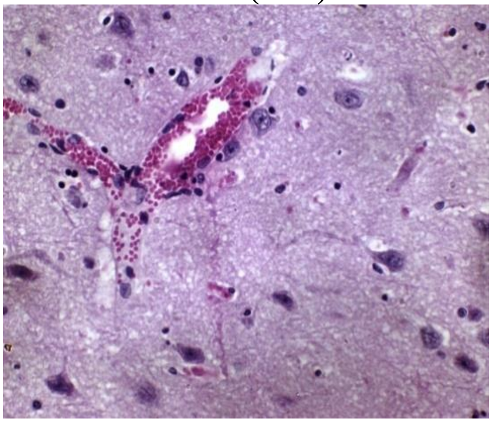

Figure 15: Brain showing cerebral congestion and neuronal degenerative changes (40X)

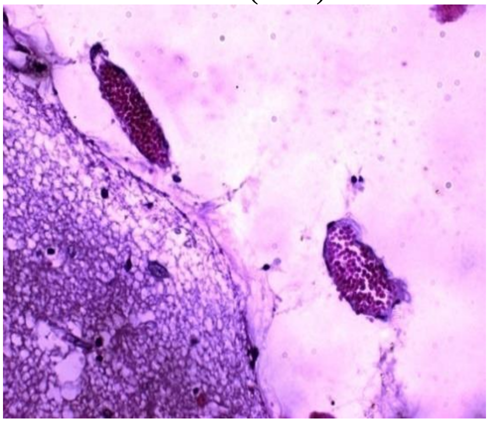

Figure 16: Brain with arachnoid showing congestion in the meningeal vessels.

These changes are anticipated observations associated with gross pathological lesions recorded. There is paucity of recent information on microscopic changes in Phorate toxicity in ruminants. Gupta and Crissman (2013) have reported that acute histopathological effects of AChE inhibitors in rats were neuronal death different parts of the brain such as hippocampus, thalamus, cerebral cortex and amygdala. Further, they also record skeletal muscle necrosis which was not noticed in the current study. Explaining the molecular mechanisms behind Phorate toxicity in wistar rats, Saquip *et al.* (2012) explained that Phorate induces transcriptional changes and enhances the activities of caspases 3 and 9 leading to apoptosis and oxidative stress and phoratecan also damage bone marrow cells.

Clinical presentation, pathological and toxicological features of Phorate toxicity in a flock of sheep was described. Though macroscopic and microscopic lesions have been regarded as non-specific in the past, there were some consistent lesions observed in the urinary bladder and liver in the current study. More insights are needed in the near future to understand the mechanism of toxicity apart from inhibition of AChE. The role of urinary bladder and other vital organs as probable target organs of Phorate toxicity needs further confirmation.

Conclusion

In the present case a flock of Hassan breed sheep (85) from a village near Hassan district, Karnataka, India was presented phorate poisoning after consuming of ragi straw sparyed with phorate to control termites. The 52 sheeps were died to acute phorate poisoning. The Clinical signs, gross pathological lesions and histopathological changes revealed the phorate poisoning. The survived animals (n= 33) were treated with 2-PAM, Atropine Sulphate, parentral fluids and all ailing animals recovered from the OPC poisoning. This clinical case helpful for the field veterinarians for the identification, diagnosis and treatment of the animals suffered from the phorate poisoning.

Acknowledgments

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Conflict of Interests

In the field condition only few OPC poisoning cases were reporting in case of sheep. This kind of histopathology finding in the brain and urinary bladder observed in sheep similar to the human cases. The sick animals treated with 2 PAM and Atropine were survived. This is the information helpful for all the field veterinary doctor and researchers for the diagnosis and treatment of phorate poisoning in animals.

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