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Confirmation of acute nitrate poisoning differentiating from anthrax in three Indian indigenous cattle

Kumaresan Nagarajan[1,*], Jayaraman Selvaraj[2], Govindan Balakrishnan[3], Kaliyaperumal Manimaran[4], Krishnan Padmanath[4], Namakkal Rajamanickam Senthil[4] and Roy Parimal[4]

[1]*Department of Veterinary Pathology, Madras Veterinary College, Vepery, Chennai-600007, India;*
[2]*Department of Veterinary Pathology, Veterinary College and Research Institute, Orathanadu, Thanjavur-614625, India;*
[3]*Department of Veterinary Microbiology, Veterinary College and Research Institute, Orathanadu, Thanjavur-614625, India;*
[4]*Central University Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Madhavaram, Chennai- 600051, India.*
[*]*Corresponding author's e-mail: nagavet@gmail.com*

ABSTRACT

This article reports cases of nitrate poisoning in Indian indigenous cattle breeds comprising two Gir cows aging 4 years each, and one Barugur cow at 1.5 years of age. The cattle with case history of sudden death and oozing of partially clotted blood from the anal opening were brought to the Central University Laboratory (CUL), Center for Animal Health Studies (CAHS), Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) for diagnostic investigation with a suspicion of anthrax. According to anamnesis, all the animals were clinically normal and did not reveal any abnormality on the previous day. The animals were fed with recently harvested sorghum leaves and stalks. Smears examined for anthrax were found negative. Biological test (mice inoculation) for anthrax was also negative. Gross lesions on necropsy examination of the carcases were suggestive of nitrate intoxication. Finally, nitrate intoxication of these cattle was confirmed by chemical and toxicological analysis of fodder, rumen content, aqueous humor, liver, kidney and urine.

Keywords

Cow, Nitrate, Nitrite poisoning, Pathology, Ruminants, Sudden death

ARTICLE HISTORY

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INTRODUCTION

Nitrate toxicosis resulting in cattle loss is commonly associated with grazing or feeding stem or stalk portions of sorghum, sorghum–Sudan grass hybrids, corn, oats, Johnson grass, pigweed, thistle, lamb's-quarter, and nightshade etc. Environmental factors such as drought stress and excessive nitrogen fertilization are usually considered as etiological conditions for nitrate accumulation in forages. Nitrate-nitrite poisonings are observed mostly in bovines. Orally taken nitrate is converted to very toxic nitrite by bovine ruminal microflora which is further converted to ammonia (Blood et al., 1991). Poisonings usually develop after consuming nitrate-containing forage of fodder crops or water or taking nitrate-containing substances accidentally. Acute poisonings are frequently observed in ruminants because they consume ammonium nitrate-containing fertilizers with appetite (Issi et al., 2008). Acute poisonings result in the increase of methaemoglobin ratios in blood. Consequently, the tissue is not supplied with sufficient oxygen and anaemic hypoxia develops (Schneider, 1998). This article describes acute nitrate-poisoning in Indian indigenous cattle differentiating from anthrax.

MATERIALS AND METHODS

Animals: Indian indigenous cattle of various breeds are maintained in the University Research Farm (URF), Madhavaram milk colony, a constituent research unit of Tamilnadu Veterinary and Animal Sciences

University (TANUVAS) Chennai. Carcasses of two Gir cows at 4 years of age and one Barugur cow at 1.5 years of age from the unit were brought to the Central University Laboratory (CUL), Centre for Animal Health Studies (CAHS), (TANUVAS) for diagnostic postmortem investigation.

Clinical history: Three cattle were found dead with oozing of partially clotted blood around the rectal opening in the early morning. All the carcasses were presented and requested to screen for anthrax. On the previous day of the incidence, all the animals were found to be active and normal in all aspects.

Blood smear examination: Peripheral blood smear at the ear tip was collected and subjected to staining with polychrome methylene blue to observe the McFadyean's reaction (McFadyen and Stevens, 1936) to rule out the anthrax.

Mice inoculation test: Whole blood was collected in a vacutainer from jugular vein with a 2 mL syringe using 24 gauge needles and properly labelled. Respective blood sample of each cow were inoculated to three different adult mice through intraperitoneal route and one adult mouse injected with normal saline kept as control and observed for 48 h (OIE, 2012).

Gross examination: Necropsy was conducted for all three carcasses systematically and gross lesions were recorded and photographed.

Microscopic examination: Suspected tissue specimens included of a piece of heart, lung, liver and kidney of all three cattle were collected and fixed in 10% neutral buffered formal saline for histopathology. Histopathological processing and examination was carried out as per Luna (1968).

Toxicological examination: Aqueous humor, ruminal content, urine, liver and kidney on ice were collected for toxicological examination. For a quick on-farm confirmation, the diphenylamine test a qualitative wet chemical test to detect the presence of the nitrate ion was carried out to detect the presence of nitrates in the fodder fed to the animal and in the suspected tissue samples. Briefly, a few drops of reagent (solution of diphenylamine and ammonium chloride in sulfuric acid) was applied over the tissues of the stem inside portion and observed for the development of an intense blue colour. In the presence of nitrates, diphenylamine is oxidized and giving a blue coloration (Roberts, 1949). Presence of organochlorine pesticides (Alpha, Beta, Gamma, Delta, BHC, DDT, Endosulphan) were estimated in the samples by thin layer chromatography technique.

Ethical approval: Ethical approval was issued by Institutional Animal Ethical Committee to Central University Laboratory, TANUVAS to conduct mice inoculation test. (Approval Lr. No. 2172/DFBS/DB/2013; dated 17/10/2013).

RESULTS AND DISCUSSION

During external examination, unclotted or partially clotted blood found in the anal opening of the carcasses was suggestive of anthrax (**Figure 1**). All the three cows had moderate tympany (**Figure 2**). Conjunctival mucosa was congested in one animal and cyanotic in others. Blood smears collected from those carcasses were stained with polychrome methylene blue stain to observe the McFadyean's reaction to rule out anthrax. None of the samples were positive for anthrax bacilli. Further to confirm, the blood sample of each cow was inoculated in a mice through intraperitoneal route and a mouse injected with normal saline was kept as control and observed for 48 hours. But all the mice were healthy and did not show any signs or mortality (OIE, 2012). Galey (2002) and Stober (2006) reported poor clotting of blood and tympany of the affected animals.

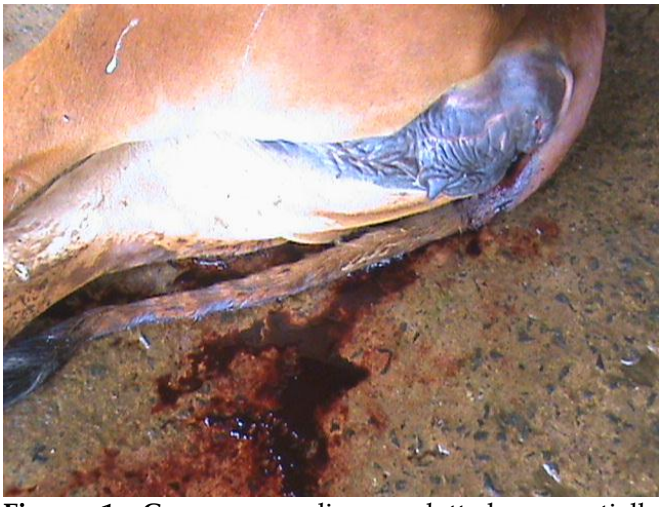

Figure 1: Carcass revealing unclotted or partially clotted blood near the anal opening simulating anthrax affected dead animal.

The cows in this case died suddenly without any clinical signs of disease. Spearman (1989) also reported that the cattle were found dead on the pasture without any overt clinical signs. Aslani and Vojdani (2007) reported clinical signs like posterior incoordination, weakness, trembling of the muscles of the hind limbs,

depression, diarrhea and extensive ventral subcutaneous edema in nitrate poisoning cases. In many cases, death followed in 1-14 days after onset of clinical signs. In acute cases clinical signs appear 2-6 h after intake of feed containing high level of nitrate. Signs include polypnoea, dyspnea, tachycardia, abdominal pain, tympany, diarrhea and frequent urination. Muscle tremor, weakness, intolerance to exercise, convulsions, and death is possible in just few hours (Stober, 2006). The cows in our case died without convulsions, which is not typical for poisoning with nitrate, a similar finding was also reported by Spearman (1989).

Figure 2: Carcass revealing moderate tympany.

On necropsy examination, oral mucosa was cyanotic, rectal mucosa near anus revealed slight congestion in all the cows. These carcasses also revealed subcutaneous hemorrhages and oedema of the abdomen and rumen. In two cows, green fibrous contents were found in the rumen, ruminitis and obstipation of omasum were also ascertained. Balooning of the intestine was evident in two of the animals and it was not obvious in the other. Hyperaemia, oedema of lungs and dilatation of heart were also seen. Heart revealed petechial, partly confluent haemorrhages in subendothelial and subepicardial areas. The lungs were emphysematous and one animal revealed minor petechial haemorrhages in the submucosa of trachea. Tracheal lumen contained frothy exudates and tracheal mucosa was slightly congested in a cow. Right lung revealed scattered areas of pneumonic patches. Subpleural hemorrhage was evident in right lung of the other animal. In pericardium 2-3 dL of clear yellowish red fluid was found. The kidneys were slightly congested and swollen with accumulation of fluid around the kidney suggestive of perirenal oedema. Liver of all the cows were pale with scattered reddish areas. Gall bladder was distended with thin yellowish green bile in a cow. Gall bladder was distended with brownish yellow bile in another cow. Urinary bladder of these cattle was highly distended with around 2-3L of urine.

The results of post mortem examination confirmed the suspicion of poisoning. On personal visit to the farm after this incident happened and anamnesis was collected again to arrive at a precise diagnosis. It was found that the forage which was provided to the cows were contained high nitrate content. The established pathomorphological changes agreed with the published reports. These reports opined that in cows with acute nitrate-nitrite poisoning, increased quantity of fluid in body cavities, subcutaneous oedema and petechial haemorrhages on serous surfaces are common. Perirenal oedema, swollen kidneys with petechial haemorrhages were also being reported (Aslani and Vojdani, 2007) and similar changes were established also in pigs (Salyi et al., 1996).

Microscopical examination of cardiac muscle revealed minor intramuscular haemorrhages and focal areas of muscular degeneration characterized by loss of striation and granulation. In one of the animals, liver showed a mild acute stasis along with some mild centrolobular parenchymatous degeneration of hepatocytes. The kidneys were hyperaemic at the cortico-medullary junction with mild degenerative vacuolating changes in some proximal tubules. In one animal, kidney revealed degeneration of the epithelium of the proximal tubules and marked engorgement of glomerular capillaries. Horner (1982) reported similar findings in the animals died of acute toxicity with nitrate-nitrite poisoning experiments and concluded that the main toxic substance acting on these animals was ammonia. The reduction of nitrate to ammonia by the rumen flora may initially increase the amount of ammonia and thus cause the acute toxicity in ruminants (Deebs and Sloan, 1975).

Diphenylamine test of the fodder developed blue color within a few seconds. Urine of all three animal was having high nitrate content on toxicological test (2%). Similarly, high nitrate content was detected in aqueous humour of all the three cows. Nitrite was not detected in any of the sample. Organochlorine pesticides (Alpha, Beta, Gamma, Delta, BHC, DDT, Endosulphan) were not detected in any sample by thin layer chromate-graphy.

Nitrate and nitrite are closely related in effects of poisoning. Besides, excess intake of nitrates may cause inflammation of rumen and intestines. They are the routes to a supply of more toxic product, before and

after absorption. Nitrate is reduced to nitrite, an intermediary-product, by bacteria of the digestive tract. On the other hand, nitrite is converted to ammonia in the same way. If nitrite-ammonia transformation is not effective, blood nitrite ions increase and can cause methaemoglobinaemia by co-oxidation of iron in haemoglobin and anaemic hypoxia results (Smith, 1995; Blood, 1991).

CONCLUSION

The laboratory findings suggested that the sudden mortality of three indegenous cattle was due to acute nitrate poisoning. Differential diagnostic tests for the other possible etilogy was ruled out in detail by carrying out various laboratory and biological tests. Gross and histopathological lesions of the affected animals correlated well with the lesions observed by other authors.

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