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Short Communication

Investigation and management of nitrate poisoning in cattle: a case study

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A R T I C L E I N F O

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A B S T R A C T

Nitrate poisoning is generally caused by ingestion of high levels of nitrate containing fodder and it usually occurs in late autumn in Bangladesh, mainly during a flush growth of green grasses after a dry period. This case report was based on a suspected nitrate poisoning in two Holstein Friesian (2 years age, pregnant; 3.5 years age milking) cows in a commercial dairy farm at Hathazari, Chattogram. The cows had a history of feeding German grass from grassland that was flooded by a nearby fertilizer company's wastewater and after 6 hours of feeding of that grass the animals were showing clinical signs of inappetite, diarrhoea, mild dyspnea and distended abdomen. The suspected fodder samples were brought to the Department of Physiology, Biochemistry and Pharmacology laboratory, Chattogram Veterinary and Animal Sciences University, Chattogram for toxicological testing. In diphenylamine test, we detected a high concentration of nitrate in the supplied fodder. However, we could not measure the plasma nitrate concentration of affected animals. The affected cows were treated with slow intravenous administration of 1% methylene blue (20mg/kg body weight) and ascorbic acid (15mg/kg body weight) for every 8 hours until completely remission of all clinical signs. In addition, cattle were medicated liver tonic (15 ml per animals) for a period of one week. Feeding of nitrate containing grass was restricted. After three days of treatment, the animals were recovered.

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1. INTRODUCTION

Nitrate toxicosis is frequently associated with grazing or feeding the stem or stalk portions of green, high-yielding grass may causes the death of a ruminant. Drought stress and excessive nitrogen fertilization are commonly observed as etiological conditions for nitrate accumulation in forages (Nagarajan et al., 2015). Regardless of the type of nitrogen fertilizer (including

manure), plants generally take up nitrogen from the soil in the form of nitrate. When plant grows normally, very little nitrate accumulates as the nitrate is readily converted to the amino acids and protein by the stem and leaves. However, under certain circumstances, this equilibrium can be broken, causing the roots to absorb nitrate quicker than the plant can convert it to the protein (Nicholls, 1980; Shaikat et al., 2012). Three factors influence the nitrate-to-protein conversion in plants: availability of water, sunlight energy and temperature that favors chemical reactions. If any of these elements is lacking, the root of the plant continues to absorb nitrate at the same frequency and store it in the stalk and lower sections of the leaves. In addition, using of excess nitrogen fertilizer and high amount of manure, may cause nitrate accumulation in plants (Burrows et al., 1987). In ruminant, particularly in cattle, nitrate poisoning usually develop after the ingestion of large amount of nitrate-containing grasses or water or accidental ingestion of nitrate containing fertilizers (Shaikat et al., 2012). Consumption of nitrate at apparently 0.05% of total body weight an animal can achieve minimum lethal dose (Yeruham et al., 1997).

Under normal circumstances, ruminants like cattle, sheep, and goats can turn nitrate into ammonia, which is then converted into protein in the rumen by bacterial fermentation (Blood, 1991). The rate of nitrate to nitrite conversion is quicker than the rate of nitrite to ammonia conversion. As a result, when nitrate is consumed in excess, nitrite may accumulate in the rumen. After then, nitrite enters the bloodstream. Nitrites in the bloodstream convert ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}), which results in methemoglobin (Wood, 1980). Oxygen cannot be carried by methemoglobin for cellular respiration. Moreover, nitrite is a vasodilator that may cause accumulation of blood into the peripheral blood vessels which can also contributes to develop hypoxia in the central circulation (Ozmen et al., 2005). In acute nitrate poisoning, animals are experience an increased heart and respiratory rates. The visible mucous membranes and skin around the nose become bluish in color. The blood of the acute nitrate intoxicated animal becomes chocolate brown tinged (Norberg and Llewellyn, 2014). Therefore, an animal's death from acute nitrate toxicity is caused by cerebral hypoxia (Bruning-Fann and Kaneene, 1993).

Chronic nitrate poisoning is a form of nitrate toxicity where no specific signs are observed. It may reduce growth rate, lower milk production, inappetence, and make animals susceptible to infections. Nitrate consumption at a level of 0.5-1.0% of the feed (on a dry matter basis) normally develops chronic nitrate toxicity in cattle (Ishigami and Inoue, 1976).Excess dietary intake of nitrate via feed and forage has been associated with last trimester bovine abortion and other reproductive difficulties, but there is no record of abortion at the first trimester (Ozmen et al., 2005). In a study, it has been found that, nitrate or nitrite causes a rapid decline in transplacental oxygen to fetal blood. Intrauterine death and abortion occur due to a rapid decrease of oxygen transfer to fetal blood (Johnson et al., 1983; Sanli et al., 1983).

In this study, we investigated a suspected case of intoxication of two cattle of a single farm through forage feeding. From our investigation, we identified that the forages those were given to cattle were contaminated with excessive amount of nitrate content. We also observed a positive response of affected cattle to treatment by methylene blue which is specific antidot for nitrate poisoning.

Based on the case history, detected nitrate contamination of forage, clinical symptoms and positive response to treatment for nitrate poisoning, we concluded that the cattle might have been suffering from nitrate poisoning. Note that in this study, we did not measure the plasma nitrate concentration of the affected cattle, therefore lack the direct evidence for nitrate poisoning in these cattle.

2. MATERIALS AND METHODS

Case definitions

A 2 years old pregnant cow and a 3.5 years oldmilch cow of Holstein Friesian cross breed at Zarif Dairy Farm, Hathazari, Chattogram, Bangladesh was investigated based on owner's complain as suspected poisoning. The manager of the farm stated that the animals had been fed German grass from grassland and after 6 hours

of feeding, animals were showing clinical signs of inappetence, diarrhea, mild dyspnea and distended abdomen (Figure 1). After showing those signs the owner immediately administered anti-diarrheal feed supplement named Diarrhoea-Dysentery Nil® (DD Nil) powder (100 grams powder in 1 liter water) and cholera saline containing sodium chloride 0.5gm, potassium chloride 0.1gm, and sodium acetate 0.393gm per 100ml but the animals didn't respond to treatment.

After 24 hours the animals were examined, clinical history and clinical signs had been recorded. It had been found that the grassland from where the animals were fed the grass was flooded by a nearby fertilizer industry's wastewater.

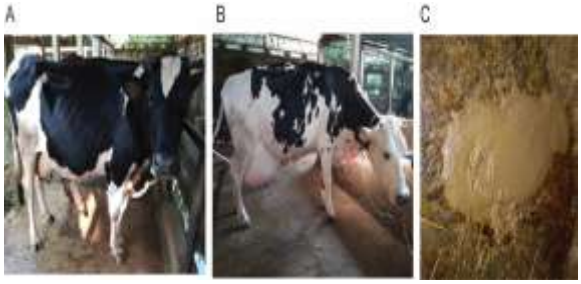

Figure 1. Clinical findings of affected cow. A) Distended abdomen of affected pregnant cow, B) Mild dyspnea of affected milch cow and C) representative fecal content of affected cow.

Toxicological investigations

Approximately 100 gm fresh German green grass had been collected from 4 different places of that fodder land and brought to the toxicology laboratory of the department of physiology, biochemistry, and pharmacology, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh. Nitrate poisoning test and cyanide poisoning test had been performed to detect the nitrate and cyanide, respectively (Table 1).

Table 1. The test methods and the results of cyanide and nitrate poisoning

Type of sample	Animals	Nitrate Poisoning Test	Cyanide Poisoning Test
German Grass	Cow 1	+	-
	Cow 2	+	-

Nitrate poisoning test

Collected samples were chopped into small pieces. The chopped grasses were then fine-grinded using mortar and pestle. A few drops of fresh-prepared reagent (Diphenylamine 500mg + Concentrated Sulphuric Acid 100ml + Distilled Water 20ml) was added to the grinded grass sample. The content was then incubated for 2-3 minutes at room temperature. A blue or violet discoloration of the sample indicates the presence of nitrate in the forage sample (Figure 2).

Figure 2. Diphenylamine test for the detection of nitrate in German grass sample. The content is after incubating processed grass samples with diphenylamine reagents.

Cyanide poisoning test

Collected sample (5gm) had been grinded and transferred to a test tube. About 3 drops of chloroform had been added to test tube. After that, a piece of moistened sodium-picrate paper was inserted in it without contacting the sample. After few minutes incubation (37^{o} C), gradual orange or brick red discoloration of the sodium picrate paper indicate the positive result and no color change indicate the negative result.

Management of cattle affected by nitrate poisoning

Nitrate toxicity can be treated with reducing chemicals such as methylene blue and ascorbic acid. Methylene blue was given intravenously in doses ranging from 20 mg/kg B. weight, depending on the severity of the poisoning (Smith, 1990). 2 doses of 1% Methylene blue were administered intravenously at a dose of 20mg/kg in this current investigation. Besides, ascorbic acid (15mg/Kg body weight) and liver

tonic (Liq. Liva vit® 15 ml per animal) was also administered in oral route, respectively in both cases. Feeding the forage from that grassland was withdrawn and after three days, all clinical signs were subsided. The pregnant cow gave birth to a healthy offspring after 1.5 months of the incident. Further, no case of abortion and congenital abnormalities had been noticed on that farm.

3. RESULTS AND DISCUSSION

Diphenylamine reacts with nitrate/nitrite and converted to colorless diphenyl benzidine, which become oxidized to form violet or bluish color diphenyl benzidine. An intense blue color in the diphenylamine reaction test was recorded (Figure 2), suggesting the presence of high amount of nitrate in the German grasses. On the contrary, the result of the cyanide poisoning test was negative for that sample.

Dietary nitrate intake may cause inflammation of the rumen and intestine which results increase their permeability which further facilitates nitrate absorption to the blood. Nitrate is converted to the nitrite, an intermediary product, by bacteria of the ruminant digestive tract. On the contrary, nitrite is converted to ammonium in the same way. Blood nitrite ions rise and may produce methemoglobinemia by co-oxidizing iron in hemoglobin, which leads to anemic hypoxia if nitrite-ammonium transformation is insufficient (van't Klooster et al., 1990). Around 24 hours after ingestion, nitrates can produce diarrhea and other related abdominal symptoms due to their direct irritant action on the mucosa of the digestive tract (Brown et al., 1990; Alam et al., 2018; Uddin et al., 2020). Potentially hazardous substances nitrate and nitrite are becoming more prevalent in the environment. The rising dispersion of nitrogenous compounds in the environment, notably in surface and groundwater, is mostly due to the widespread use of nitrates as agricultural fertilizers and the growing volumes of nitrogenous waste produced by industries and feedlots.(Issi et al., 2008). Human and animal exposure to nitrate in environment is possible, but excessive nitrate concentration in feed and forage poses a greater risk of poisoning(Ruffin et al., 1987).

Ruminants can tolerate a variety of nitrates if they progressively increase their consumption of nitrate-containing foods and other substances. Additionally, healthy animals are less likely to become nitrate poisoned than animals that are malnourished or feeble. Furthermore, animal nitrate toxicity is decreased by feed that is high in carbohydrates (grain).

Concern should be shown when supplying fodder since nitrate poisoning may result in circumstances that are life-threatening. Cattle could become nitrate poisoned by eating grasses from nearby grasslands that are near fertilizer companies. It is advised to test the grasses for nitrate poisoning before to feeding the cow.

4. CONCLUSION

In conclusion, the clinical examination, laboratory investigation, and treatment response suggested that these animals were suffering from nitrate poisoning, which was cured with methylene blue treatment. Nitrate poisoning is a serious problem that may cause significant loss in livestock production. Caution must be taken while feeding of green grass from the fodder filed specially and also be aware of using nitrogen fertilizers to avoid environmental contamination and animal exposure to lethal quantities of nitrates.

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REFERENCES

Alam, M. B., Mahmud. T., Khan, S. A., Islam, A., Hai, M. A. and Hassan, M. M. 2018. Occurrence of diseases and disease conditions in cattle and goats at the Upazilla Veterinary Hospital, Debidwar, Comilla. Journal of Advanced Veterinary and Animal Research, 5 (2): 117-122.

Blood, D. H. J. A. and Rodstititis, O. M. 1991. Veterinary medicine. London: Bailliere, Tindall.

Brown, C., Burrows, G. and Edwards, W. 1990. Nitrate intoxication. Veterinary Human Toxicology, 32 (5): 481-482.

Bruning-Fann, C, and Kaneene, J. 1993. The effects of nitrate, nitrite, and N-nitroso compounds on

animal health. Veterinary Human Toxicology, 35 (3): 237-253.

Burrows, G., Horn, G., McNew, R., Croy, L., Keeton, R. and Kyle, J. 1987. The prophylactic effect of corn supplementation on experimental nitrate intoxication in cattle. Journal of Animal Science, 64 (6): 1682-1689.

Ishigami, K. and Inoue, K. 1976. Metabolism of nitrate and methemoglobinemia in ruminants. Research Bulletin of Obihiro University. Series I.

Issi, M., Gul, Y., Atessahin, A. and Karahan, I. 2008. Acute nitrate poisoning in two cattle. Toxicological Environment Chemistry, 90 (1): 135-140.

Johnson, J., Schneider, N., Kelling, C. and Doster, A. 1983. Nitrate exposure in perinatal beef calves [Potential toxicants]. In proceedings of annual meeting American Association of Veterinary Laboratory Diagnosticians.

Nagarajan, K., Selvaraj, J., Balakrishnan, G., Manimaran, K., Padmanath, K., Senthil, N. R. and Parimal, R. 2015. Confirmation of acute nitrate poisoning differentiating from anthrax in three Indian indigenous cattle. Journal of Advanced Veterinary and Animal Research, 2 (1): 30-33.

Nicholls, T. J. and Miles, E. J. 1980. Nitrate/nitrite poisoning of cattle on ryegrass pasture. Australian Veterinary Journal, 56 (2): 95-96.

Norberg, S. and Llewellyn, D. A. 2014. Nitrate poisoning in ruminants. Washington State University. Extension Fact Sheet. FS139E.(https://rex.libraries.wsu.edu/discovery/delivery/01ALLIANCE_WSU:ResearchRepository/99900502660701842#13332922070001842), USA, 2014. Accessed 10 January 2022.

Ozmen, O., Mor, F., Sahinduran, S. and Unsal, A. 2005. Pathological and toxicological investigations of chronic nitrate poisoning in cattle. Toxicological Environmental Chemistry, 87 (1): 99-106.

Ruffin, B., Ball, D. and Kjar, H. 1987. Nitrate poisoning of cattle in Alabama. Circular ANR-Cooperative Extension Service.

Sanli, Y., Imren, H., Kaya, S., Koc, B. and Kahraman, M. 1983. The investigation of the relationship between the chronic nitrate poisoning in the pregnant cows and amaurosis in calves reared on the area of Isparta. Journal of Veterinary Faculty Ankara, 30: 657-673.

Shaikat, A. H., Hasan, M. M., Hasan, S. A., Khan, S. A., Haque, M. A., Islam, M. N. and Hossain, M. B. 2012. Non-protein nitrogen compound poisoning in cattle. University Journal of Zoology, Rajshahi University, 31: 65-68.

Smith, B. 1990. Large animal internal medicine—diseases of horse, cattle, sheep and goat. Mosby, Missouri, 846-848 pp.

Uddin, M. B., Moniruzzaman, M., Islam, M., Nayem, M. R. K., Dutta, P. and Hassan, M. M. 2020. Occurrence of diseases of ruminants admitted at the Santhia upazila veterinary hospital of Pabna, Bangladesh. Bangladesh Journal of Veterinary and Animal Sciences, 8 (1): 60-65.

van't Klooster, A. T., Taverne, M., Malestein, A. and Akkersdijk, E. 1990. On the pathogenesis of abortion in acute nitrite toxicosis of pregnant dairy cows. Theriogenology, 33 (5): 1075-1089.

Wood, P. 1980. The molecular pathology of chronic nitrate intoxication in domestic animals: A hypothesis. Veterinary Human Toxicology, 22 (1): 26-27.

Yeruham, I., Shlosberg, A., Hanji, V., Bellaiche, M., Marcus, M. and Liberboim, M. 1997. Nitrate toxicosis in beef and dairy cattle herds due to contamination of drinking water and whey. Veterinary Human Toxicology, 39 (5): 296-298.