

Elemental Sulphur Toxicosis in Cattle and Sheep in Botswana

MG Binta^{1*}, Baipoledi EK¹, Nyange JC¹ and Mushi EZ²

¹National Veterinary Laboratory, Gaborone, Botswana

²Botswana College of Agriculture, Gaborone, Botswana

Abstract

Acute deaths involving 141 Tswana breed of cattle and 15 Black Head breed of sheep were reported within 2 to 48 hours of drinking water at Mahibitswana crush, Shoshong village in Mahalapye district, Botswana. The water was from an open well at the crush, the latter being a designated communal livestock collection area used for watering, inspection, treatment and vaccination of livestock by Veterinary services personnel. At the time of the ambulatory visit, 7 sheep and 41 cattle belonging to this crush were reported dead. Donkeys and goats watered from the same source were not affected. The signs were acute and included hind limb weakness with subsequent recumbency, severe dyspnoea, tachycardia, a thready pulse and mud colored mucus membranes. The latter were injected in some animals. Slight frothing at the mouth was evident. The menace reflex to check for visual acuity was blunted. Parenteral administration of Vitamin B complex (Virbac) using the intramuscular route of inoculation in addition to dexta-tomanol and long acting terramycin, resulted in amelioration of the clinical signs. Recovery was uneventful and relapses were not reported.

This is the first published report of suspected elemental sulphur poisoning among ruminants in Botswana.

Keywords: Elemental sulphur; Poisoning; Cattle; Sheep; Parenteral vitamin B complex; Amelioration

Introduction

Sulphur is an important element in the diets of ruminants. It is required for the synthesis of protein by ruminal microbes and in sheep, for wool production. The chemical substance is generally not regarded as a hazardous or toxic substance and is often added to diets of ruminants as a substitute for sulphur-containing amino acids namely, methionine, leucine and cysteine, especially animals consuming non-protein sources of nitrogen [1]. Most of the dietary sulphur, whether as amino acid or inorganic sulphur, ingested by the ruminant is reduced to sulphide by ruminal microorganisms and then either incorporated into microbial protein or absorbed and oxidized to sulphate in the liver [2]. These authors observed that generation of large quantities of hydrogen sulphide in the rumen may depress ruminal motility and cause severe depression of the nervous and respiratory systems.

This chemical substance is also used as an anti-ectoparasitic agent as well as chemoprophylactic agent to reduce the incidence of enterotoxaemia in sheep [3-5]. Poisoning of animals with sulphur from environmental pollution has been reported [6]. However, the impact of industrial sulphur emissions on the environment has not been assessed in this country.

Sulphate containing supplements are often fed to ruminants to limit feed intake. A critical review of the toxicology of sulfur in ruminants has previously been given [1]. Although specific toxic doses are not known, concomitant feeding of sulphur with a high protein diet has resulted in toxicity [7-9].

To date, there are no published reports of sulphur poisoning among ruminants in Botswana. It is possible that some undiagnosed cases of massive acute deaths among cattle in this country may have been associated with iatrogenic poisoning with this chemical substance.

The report describes the clinical signs observed in an investigation of an outbreak of suspected sulphur toxicosis among cattle and sheep at a crush in Mahalapye district, therapeutic intervention and subsequent management of the syndrome.

Case History

In March, 2002, Veterinarians at the Botswana National Veterinary Laboratory (BNVL), Gaborone, Botswana were requested to attend to a case of acute death among cattle and sheep after drinking water from an open well at Mahibitswana crush, Shoshong location in Mahalapye district.

As many as 156 animals comprising 141 head of cattle and 15 sheep belonging to three farmers had died suddenly after drinking water from an open well. The herd boys had observed no premonitory signs. Donkeys and goats watered from the same well did not show any signs of sickness.

Clinical signs

On initial examination, of affected animals 4 days after the first deaths, approximately, 30% of the cattle comprising mainly of adult cows were found to be in sternal recumbency. They were showing signs of somnolence, lethargy, abdominal discomfort and in some cases, prostration.

Clinical examination of recumbent animals showed subnormal temperature, a weak thready pulse, tachycardia, depressed respiratory rate, reduced ruminal sounds and cold extremities. Some animals appeared to have occasional facial muscle twitching. Apart from a blunted menace reflex, the rest of the animals did not appear to have impaired vision. Cattle that were able to stand were seen wandering aimlessly and elicited a blunted menace reflex without evidence of

*Corresponding author: MG Binta, National Veterinary Laboratory, Private Bag 0035, Gaborone, Botswana, E-mail: mary@naftec.org

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complete blindness. Other noteworthy signs manifested by these animals included severe depression, frequent extrusion and retraction of the tongue, frothing at the mouth, curling of lips and grinding of teeth. None of the animals appeared to be grazing or ruminating. Most of the cattle in the herd appeared to be heavily infested with ticks. There was no previous treatment against ectoparasites.

Five recumbent ewes were seen thrashing their legs and showed convulsive seizures. Cattle and sheep belonging to other farmers were not available for examination for their owners had driven them away from the well suspected to have caused poisoning of their stock. It was learned that animals were raised only on grass with no supplementation apart from occasional salt licks administered to animals as blocks.

Treatment of the moribund calf

Two moribund calves were taken to the laboratory for further observation and treatment, of which one died upon arrival at the laboratory. The surviving calf was injected with a 1% (w/v) sterile solution of methylene blue at a rate of 1 mg/kg body weight using the intravenous (IV) route of inoculation. Using the subcutaneous route of inoculation, 5ml of Vitamin B Complex and Hepatitis Extraction (Virbac Animal Health) was also given to the calf. Each 50 ml of Vitamin B complex contains Vitamin B1, 10 mg; Vitamin B2, 4 mg; Nicotinamide, 25 mg; Calcium pantothenate, 5 mg; Vitamin B6, 4 mg; Liver Extract, 50 mg; methylparaben). Thereafter, a preparation of tetracycline was administered per os through drinking water. One ml of Dexa-tomanol [Centaur Laboratories: Each 50 ml of dexa-tomanol an anti-inflammatory agent, contains Ramifenazone-7.0 g, Sodium phenylbutazone-3.5 g, Dexamethazone-0.025 g, Cinchocaine hydrochloride-0.11 g and Benzalkonium chloride-0.0012% (w/v)] was also given only once using the intramuscular route. Fresh water and hay were given to the animal ad libitum throughout the study.

The treatment regime was given only once and the calf made an uneventful recovery without further therapeutic intervention. Relapses of paresis and signs of ataxia and general weakness manifested previously were not observed after treatment.

Administration of water implicated in the poisoning episode

Suspect water collected from the implicated well was administered per os ad libitum to three apparently healthy sheep obtained from an unaffected farm, for three consecutive days.

Laboratory Analysis

Collection of samples

Blood samples in Ethylene Diamine Tetra-Acetic Acid (EDTA) anticoagulant were collected from comatose cattle and sheep to determine the haematological profiles. Blood for the determination of lead was collected in vacutainer tubes containing heparin as an anticoagulant. During venipuncture, the blood had a characteristic salmon pink colour which immediately changed to brown on exposure to air. Sheep given water from the suspect well were also bled to establish the effect of this water on the haematological indices. Conventional techniques in haematology were used to establish the haematological parameters.

Clotted blood without anticoagulant was collected from both comatose and water-fed sheep for preparation of serum to establish the clinical chemistry profile. The levels of selected biochemical analytes, namely: calcium, inorganic phosphate, glucose, blood urea nitrogen, creatinine, uric acid, total protein, albumin, direct and indirect

bilirubin, as well as activity of the serum enzymes: Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST) Creatinine Kinase (CK) Gamma Glutamyl Transpeptidase (γ GT) were determined using a chemical analyzer (Vitalab Selectra, Merck Instruments) fitted with an isoselective (ISE) electrode for the determination of electrolytes-sodium, potassium and chloride. This chemical analyzer has a built-in automatic calibration system using commercial calibrators (SMT, calibrator, Merck) supplied with reagent kits

Samples of implicated well water were subcontracted to another laboratory to be tested qualitatively for the presence of toxic levels of the following analytes: lead nitrate/nitrite and sulphates using conventional methods [7].

Necropsy findings

At necropsy, the only significant observation on the integument of the carcasses was the high degree of dehydration and the presence of *Amblyomma hebraeum* ticks around the ears and escutcheon.

Ruminal contents collected from animals that suffered peracute death showed a ruminal pH between 6.0 and 6.5. Upon exposure of the ruminal contents, only a subtle smell of rotten egg, which mingled with the musty smell of ruminal contents, could be detected. However, no characteristic hydrogen sulphide (rotten egg) smell was detected from eructation gases emanating from affected animals. The most remarkable feature was the immediate blackening of the gastrointestinal contents even before opening. Also the blackening of lead acetate paper suspended over the ruminal contents in a capped test tube suggested the presence of hydrogen sulphide. However, unlike cattle that died after surviving the first poisoning episode, no overt gastrointestinal lesions were observed at post-mortem in animals that died peracutely.

Moderate pulmonary edema and pericardial effusion, the latter with a tinge of salmon pink colour, were observed. Petechiation was noted in the visceral organs and musculature of some but not in all cattle that died peracutely.

Portions of tissues were collected from various organs, namely: kidney, lung, liver, intestine and brain were collected into both 2.5% (w/v) Boric acid and 10% (v/v) formol-saline for bacteriological and histopathological examination, respectively. Portions of these tissues for toxicological analyses were placed into clean sampling polystyrene bottles, preserved on ice at 4-8°C and transported to the laboratory for analyses. Aqueous humour was collected from the eyes, and cerebral spinal fluid was collected the foramen magnum. Lead acetate impregnated paper strips were used to detect the presence of hydrogen sulphide using previously described methods [7].

Results of Haematology and Clinical Chemistry

Haematological and clinical chemistry profile of blood and serum collected from adult animals that survived the initial episode of poisoning were unremarkable. However, haematological analysis carried out on blood collected from the surviving calf showed an elevated leukocyte count (29.7×10^9 /L). The calf also showed a hypochromic, normocytic anaemia with the following erythron indices: red blood cell count 5.9×10^{12} /L; haemoglobin, 5.3 g/dL; haematocrit 23.7%. Judging from the erythron indices and microscopic assessment of the blood picture, there were no regenerative tendencies. The anemia went into remission after treatment to give the following indices: total white blood and red blood cell counts, haemoglobin, and haematocrit were 5.2×10^9 /L,

6.2x10¹²/L, 10.0 g/dL and 30.5%, respectively. The erythron indices were unremarkable apart from the elevated haemoglobin suggestive of mild haemoconcentration sequel to dehydration.

Increased activity in the serum enzymes γ GT and AST and also hypokalaemia (1.8 mmol/L) were evident from the clinical chemistry profile.

None of the sheep given suspect water showed any abnormalities on hematology and clinical chemistry.

Quantitative chemical analysis was conducted on well water sample by a water analysis laboratory in Gaborone, to determine the full complement of anions and cations using standard methods of analysis.

Well water, ruminal fluid, aqueous humour and cerebral spinal fluid (CSF) were tested for the presence of nitrate/nitrite using the diphenylamine test and Gries's test (α -naphthylamine plus sulphanilic acid) which are standard methods for these analytes [7]. Quantitative analysis of tissues and body fluids for nitrate/nitrite yielded negative results. This ruled out the presence of nitrite and nitrate in the water and body fluid samples. Water, blood, ruminal contents and body fluid samples also subcontracted for testing at Onderstepoort Veterinary Research Institute, Republic of South Africa, using conventional methods tested negative for the presence of pesticides, lead and nitrate/nitrite. Analysts however, stated that they could not preclude the involvement of sulphur.

Discussion

The presumptive diagnosis of sulphur poisoning in cattle and sheep was made based on suggestive clinical symptomatology, results of necropsy and admission by some farmers that they fed sulphur. The blackening of lead acetate impregnated filter paper held over ruminal contents was diagnostic of the presence of sulphur. Whereas the positive response to therapeutic intervention with vitamin B complex may have been suggestive of sulphur poisoning it was considered confirmatory. Vitamin B complex group are water-soluble vitamins comprising thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), biotin (vitamin H or B7) folic acid (vitamin B9), cobolamin (vitamin B12). Vitamins B1, B2, B3 and B7 work synergistically in the process of energy production. Failure to detect eructated hydrogen sulfide may probably have been due to the length of period after ingestion of the sulphur. Furthermore, absence of clinical signs in sheep given suspect crush water was ample evidence that water was not involved in this toxicosis.

The most likely differential diagnoses based on neurological manifestation would have been cerebrocortical necrosis due to administration of amprolium, a thiamine antagonist [10], lead poisoning [11], ingestion of Dichapetalum cymosum and therapeutic intervention with furazolidone [12]. There was no evidence that the animals at Mahibitswana crush were treated with amprolium and furazolidone or had access to lead. Nitrate poisoning suspected on the basis of the brownish blood on venipuncture was ruled out since ocular and cerebral spinal fluid nitrate was not detected in the laboratory. Dichapetalum cymosum, a poisonous plant known to interfere with the enzyme aconitase in the Krebs cycle, was not found growing anywhere in the vicinity of Mahibitswana location.

Renal and hepatic compromise as indicated by elevation in activity of the serum enzyme γ GT may have occurred. Reduced renal perfusion culminating in pre-renal azotaemia may have resulted from haemoconcentration associated with dehydration.

Hemoconcentration may also have falsely elevated other analytes, namely, sodium and calcium: inorganic phosphate ratio imbalance. Results of the clinical chemistry profiles concurred with observations made by previous authors [13,14].

Amelioration of neurological manifestations in the calf successfully treated at the National Veterinary Laboratory concurred with the observations of previous authors [4,13,15,16] on the protective effects of Vitamin B Complex in cases of sulphur-induced encephalomalacia. Furthermore, the neurological clinical signs observed in cattle and sheep that survived acute death at Mahibitswana crush may have been attributed to the toxic effect of hydrogen sulphide on the central nervous system [4]. The latter authors reported signs of lethargy, abdominal discomfort and prostration among sheep as were noted in the present case.

Interference in the metabolism of thiamine associated with hydrogen sulphide may have accounted for neurological signs among affected cattle and sheep from this crush [17,18]. Neurological evaluation of affected animals involved, orientation, head posture, inco-ordination and cranial nerve integrity. The most predominant neurological signs were severe depression and coma. The latter finding may have been suggestive of disturbances in the cerebrum, diencephalon or mid-brain. Defective visual acuity with apparent blindness was found only in a few cattle seen wandering aimlessly in the kraal. Some animals in extremis presented with seizures, a documented manifestation of Vitamin B6 deficiency [16].

Polioencephalomalacia is reportedly associated with the feeding of diets and water containing high levels of sulphates [2,13,11,16,19]. However, failure to demonstrate the typical polioencephalomalacia lesion on histopathological examination of brain tissue in this study may partly have been attributed to acute death of the animals. Perhaps, some of the survivors of acute death may have sustained various degrees of this lesion but they were not necropsied.

Non-specific oedema and congestion observed in the brain tissue of cattle and sheep in this study have also been cited in cases of sulphur intoxication arising from ingestion of hydrogen sulphide or sulphates [5,13,17,20].

Sulphur toxicosis is essentially hydrogen sulphide poisoning. Upon ingestion, sulphur in the rumen is changed to hydrogen sulphide by rumen microorganisms and is absorbed across the rumen wall at a fast rate. Absorption has been shown to be a function of the sulphide concentration and the rumen pH [3,21]. This finding was corroborated by the authors after necropsy of the animals that died at Mahibitswana crush. Hydrogen sulphide may also have accounted for the blackening of the gastrointestinal contents upon exposure to air.

The sulphide that is absorbed affects oxidative metabolism by inhibiting the action of enzymes in the cytochrome system [2,4,17]. This generalized effect on energy producing metabolic processes may explain the general weakness shown by all affected animals. Some of the survivors remained moribund for some time. The dramatic response to therapeutic intervention with Vitamin B parenterally was most likely attributed to energy boosting by speeding the detoxification of hydrogen sulphide in the liver and formation of haemoglobin.

Sulphide also has a direct effect on the carotid body resulting in inhibition of respiration which may have accounted for some deaths sequel to respiratory embarrassment. The latter may have also been exacerbated by the sulphide combining with haemoglobin causing the observed cyanosis of the visible mucous membranes. This reaction

may also have partly explained the abnormal dirty brown color of the blood during venipuncture. Compromised respiration due to this phenomenon may also have accounted for the cyanotic membrane and cold extremes of the comatose animals during clinical examination. The observed respiratory depression in this study may also have been due to the fact that the hydrogen sulphide gas formed after ingestion of sulphur collects in the gas cap and is eructated. It is possible that the sulphide crossed the respiratory epithelial barrier and caused severe respiratory distress.

Although, necropsy results were unremarkable, the cause of signs of colic and discomfort as well as the soiled perineum in cattle and sheep on the affected farms was suggestive of diarrhoea that may have been due to vasculitis and necrosis of ruminal and abdominal wall. This inflammatory reaction was seen on histopathological examination of tissues collected from gastro intestinal area.

One of the risk factors cited that may exacerbate sulphur toxicosis was well water containing high levels of sulphate which is aggravated by concentration in deep wells during periods of drought [4,22]. Analysis of open well water at Mahibitswana crush yielded clinically insignificant levels of sulphate and nitrate. This ruled out the well water as one of the instigating factors in this case study. There was no evidence of industrial emission of sulphur into the environment.

On the other hand, excessive dietary deficiency may result in long term secondary metabolic problems such as copper deficiency [23,24]. Although clinical signs suggestive of copper deficiency were not observed at the time of the ambulatory visit, it was speculated that perhaps the moderate anaemia noted in the calf admitted to the laboratory may have in part been sequel to this problem.

Whereas excessive ruminal sulphide production without concurrent thiamine deficiency is often implicated as the cause of polioencephalomalacia [9,18,25]. This claim could not be proved in this study using the limited histopathological observations. Similarly, determination of levels of thiamine in blood and erythrocytes was not done for it was beyond the scope of this study. It was assumed that since ruminants synthesize their own Vitamin B complex in the rumen, there was no dietary deficiency of thiamine. Also, measurement of ruminal hydrogen sulphide gas cap as advocated in the literature [18,16] is not a routine diagnostic test at laboratories in this region. The tentative diagnosis of sulphur poisoning was based on the clinical history, clinical signs, necropsy and eliminative toxicology results

In the present study, some of the owners admitted giving feed containing elemental sulphur to their animals before driving them to the well which was a clear implication of sulphur toxicosis. This finding concurred with the negative observations of the subcontracting laboratory for toxic levels of lead, nitrate/nitrite and sulphates in the drinking water. Corollary to this was the observation made from the water feeding study in apparently, clinically normal sheep that the water did not have any deleterious effects on the recipient sheep in the trial. Well water at Mahibitswana crush was therefore excluded as the cause of the syndrome although in the literature well water has been implicated in such poisoning episodes [4].

In the light of the foregoing discussion, it would appear that there is urgent need to establish the safe upper limit of dietary sulphur for ruminant animals in Botswana. Equally important is the need to assess the potential risk of feeding sulphur to ruminants countrywide, as has previously been described elsewhere, in the literature [16,26].

Animal health personnel need to be appraised of the management

and effective practical control measures of these poisoning episodes. In the literature, reducing the dissociation of sulphate using bismuth carbonate which imparts a higher ruminal pH thus limiting absorption of hydrogen sulphide from the rumen has yielded success. This however, needs to be carried out by informed personnel to obtain the desired pH.

Oral administration of glucose an energy supplement to boost metabolism is imperative since energy generating processes are compromised due to hypokalaemia. Success with this treatment has been alluded to in the literature [2]. These authors also administered glycerine mixed with bismuth carbonate by mouth or the latter in form of cubes. The rationale behind this treatment is that sulphide from the rumen is limited by reducing the absorption rate constant by increasing ruminal pH hypokalaemia probably caused by homeostatic disturbances was noted in the calf that survived acute death. Anaemia observed in the treated calf notably went into remission. It is possible that administration of energy boosting Vitamin B complex may have stimulated erythropoiesis. The successful use of intravenous calcium gluconate previously by some authors explains why the calf treated with glucose in the present study recovered from the poisoning episode. These authors advocate the use of chemical antidotal treatment comprising 31.1 g of iron sulphate and 15.6 g of liquor ammonia fortis dissolved in 1.135 liters of warm water given as one dose.

The beneficial effects of administration of Vitamin B complex to supply thiamine (Vitamin B1) has been documented and advocated. Therapeutic intervention with Vitamin B complex was supported by the work done on sulphur induced encephalomalacia in lambs [25]. These authors have, however, discounted the thiamine deficiency involvement since blood thiamine levels were unaltered in experimental sulphur toxicosis in cattle

Determination of thiamine was out of the scope of this investigation. This warrants a separate study considering that currently, sulphur toxicosis in ruminants in Botswana is proving to be an emerging livestock and environment management problem.

Parenteral administration of dexamethasone has successfully been used to manage sulphur induced polio-encephalomalacia in lambs [25]. Since the use of dexamethasone in food animals is prohibited in this country, a non-steroidal anti-inflammatory drug would have to be substituted. The authors reported that oral administration of antibiotics whereby sulphide producing ruminal microflora would be killed off thus reducing the risk of hydrogen sulphide build up, had a beneficial effect. In the present study, per os administration of tetracycline in the drinking water for the calf admitted for observation corroborated the latter claim.

Diagnosis of sulphur poisoning requires a detailed history for other differential diagnosis possibilities [10-12]. The need for this is even more pressing considering that chemical determination of sulphur in samples requires specialized equipment [27] which may not be available to clinicians in the field.

Conclusion and Recommendation

It is of paramount importance that farmers are cautioned about the dangers of feeding sulphur without veterinary advice. While the latter practice may be a sustainable alternative to supplementation with expensive sulphur containing amino acids, the potential danger of inadvertent overdosing of animals culminating in stock losses outweigh the benefits.

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