Acute Sheep Poisoning from a Copper Sulfate Footbath

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ABSTRACT. An outbreak of footrot occurred in a flock of Corriedale sheep; 27 animals were treated with antibiotic and footbathed in a 5% copper sulfate solution. Being deprived of water for > 17 h, many sheep drank the footbath solution. After 6 h 16 sheep became ill with acute copper poisoning, 10 animals died within 10 h; 6 were severely ill and were sent to Veterinary Hospital, and 4 had mild signs and recovered without treatment. The sick sheep had anorexia, dullness, grinding teeth, moaning, rumen atony, dehydration, dark blue-green diarrheic feces and congested membranes. They were treated with 3.4 mg tetrathiomolybdate/kg body weight and lactated Ringer's solution iv, oral molybdate, sulfate, kaolin and pectin, and drenched with antacids. Two of the 6 sheep died during hospitalization. The ingestion of copper solution caused an intense gastrointestinal injury that resulted in ulcers, petechial and echymotic hemorrhages in the mucosa, mild hemolysis detected by microscopic hemoglobinuria and a lowered packed cell volume, severe hepatic injury that raised the AST and γGT blood values, and moderate kidney lesions with increasing serum blood urea and nitrogen creatinine levels.

The Brazilian sheep industry is increasing productivity rapidly in some southern states. Nevertheless, the principal bottleneck for the advance of this industry is the lack of control of many diseases that cause significant economical losses, such as gastrointestinal helminthiasis, some infectious diseases and poisonings (1).

Footrot is one of the most prevalent infectious diseases in sheep raised in this southern area (2). The lesions of footrot are caused by infection with *Dichelobacter nodosus*. Ovine footrot can spread rapidly through a susceptible flock when environmental conditions are favorable for transmission. Abundant moisture, warm weather, and the presence of infected sheep are usually required for transmission (3).

Footbathing is a practical approach to topical treatment of footrot when dealing with large numbers of sheep. Preparations suitable for footbaths include 5% copper sulfate, 5% formalin or 10% zinc sulfate with or without a surfactant to aid in the wetting of tissues. Among these preparations the farmers prefer to use copper sulfate because of its low price and high availability. The use of formalin is many times rejected for its unpleasant smell and for promotion of allergic reactions and dermatitis in man (4); zinc sulfate has not been adopted principally for its high price.

The copper present in copper sulfate is readily available for ruminants, being used as a source of this element in free-mineral mixes for herd supplementation. On other hand, excessive ingestion of copper sulfate causes copper poisoning, principally in sheep. The most common form of poisoning is the cumulative (chronic) form and follows the repeated ingestion of copper for weeks, months or even years. Conversely, acute copper poisoning in sheep is relatively infrequent and may arise from oral ingestion or the parenteral administration of copper complexes (5). A single or small number of large oral doses of copper for a short period may be followed by signs of acute copper poisoning. The use of large doses of injectable copper preparations for prevention and treatment of copper deficiency states have been cited more frequently as causes of acute poisoning (5).

This paper reports an outbreak of acute copper poisoning in a sheep flock footbathed with copper sulfate solution.

CASE REPORT

History

Two purchased Suffolk rams were introduced without quarantine into a flock of 223 Corriedale sheep apparently free of foot rot. One of the rams was mated with 47 ewes in a damp paddock, 2 km from the sheep house. About 7 d later this ram became lame, showed pain when walking and had reduced food intake. The veterinarian's clinical examination revealed swelling and moistness of the skin of the interdigital cleft, areas of necrosis at the margin of underlying separated horn, by heat and some tenderness of the sole, and a distinctive fetid smell. A preliminary diagnosis of footrot was made. The ram was treated with a single im injection of 70,000 IU procaine penicillin G and 70 mg streptomycin/kg body weight (3). For 10 d during the clinical recovery the animal was segregated in a dry environment and then returned to the same flock.

Within the next 20 d, 27 ewes presented with the same clinical picture. The veterinarian suggested a topical footrot to the farmer, besides antibiotic therapy. The flock was displaced from the paddock to the sheep house at 3 pm for treatment. The sick ewes were segregated from the rest of the flock and were initially treated with the same doses of the antibiotics cited before. Afterwards the hooves of all sheep were trimmed and any undermined horns were removed to expose anaerobically infected tissues to air and to the action of the footbath. The trimming work was finished late at 6 pm, and for this reason the farmer decided to postpone the footbathing until the next morning. Thus, the ewes were kept overnight in the sheep house with no access to water and food.

A footbath solution of 5% copper sulfate solution ($CuSO_4.5H_2O$) was prepared at twilight time and placed within the 8 m long, 10 cm depth and 70 cm wide permanent concrete stand-in trough located near the sheep house. The footbathing started at 8 am, and each ewe was kept standing in the trough for at least 8 min. It was noticed that a few thirsty

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sheep drank the trough content. For at least 3 h the treated sheep were held in a dry concrete floored yard before being turned out to a paddock adjacent to the sheep house.

About 6 h after the footbathing, 16 ewes became anorexic and somnolent followed by depression and weakness. Diarrhea was seen 3 h later in most sheep. Veterinary assistance was urgently called. Ten early-affected ewes succumbed within 10 h after the footbathing; 6 ewes were seriously ill and were sent to the Veterinary Hospital at the University of Sao Paulo, Brazil. Another 4 ewes had mild clinical signs, but recovered after 1-2 d without treatment. The remaining ewes did not show any clinical effects.

Clinical Findings and Therapy

On arrival at the Veterinary Hospital 1 ewe was in extremis and died suddenly. The remaining 5 sheep were dull, and somnolent, but still responded to external stimulus; 3 of them were able to walk. Two ewes were in sternal recumbency and had their heads turned to the flank, with grinding of the teeth and moaning. The heart rates (120 ± 10 beats/min) and the respiratory rates (72 ± 14 breaths/min) were increased and accompanied by a fall in rectal temperature (36.2 ± 1.3 C). No rumen movement was recorded, but no signs of gas bloat were seen. Two ewes excreted light-red urine. The 3 less depressed ewes were very thirsty and drank > 0.5 L of fresh water, but they still were anorexic. All the ewes were diarrheic, passing dark blue-green metal brilliant reflective stool. The feces had large amounts of mucus. Mucus membranes were slightly congested. Dehydration was a common feature in the animals. The colder the rectal temperature, the higher the degree of dehydration.

According to the history and clinical findings, a preliminary diagnosis of acute copper poisoning was made and medical treatment was carried out immediately. The following treatment was given once daily for the next 4 d: 3.4 mg/kg bw of ammonium tetrathiomolybdate and 20 mL/kg bw of lactated Ringer's solution iv; a drench containing 150 mg ammonium molybdate and 1.5 g anhydrous sodium sulfate diluted in 100 mL of water, and a solution containing 80 g kaolin + 2 g pectin orally. During the first day of treatment the sheep were abomasally injected with 3 doses of 10 mL of an antacid solution (400 mg aluminum hydroxide + 400 mg magnesium hydroxide) interspaced 3 h between doses. One of the ewes that was unable to walk was treated iv daily with 3 L of lactated Ringer solution and drenched with a 150 mL glycerol solution for 4 d.

One of the most depressed ewes died about 15 h later. The sheep that could walk (n = 3) on admittance to the Veterinary Hospital improved rapidly until the end of treatment. The diarrheic feces lasted about 3 d; appetites were fully restored by the 5th d.

The remaining ewe made gradual recovery within the next 9 d. She was able to stand and walk only on the 3rd d of treatment. Anorexia was noted for > 2 d, returning initially with the intake of grass, then hay and finally commercial concentrate 9 d later; diarrheic feces were excreted for > 6 d, decreasing progressively in the amount of mucus in the feces. An occult blood test carried out on the feces had positive reactions throughout the first 5 d of treatment, rumen movements were recorded only by the 3rd d. The mucus membranes were congested until the 2nd d, being slightly yellow for 3-4 d, and then pale for the next 15 d.

Postmortem Findings and Laboratory Diagnosis

Necropsy of the ewe that succumbed revealed large amounts of a cloudy liquid in the peritoneum cavity. This fluid had high specific density (1.027) and 63 g/L protein. Within the abomasum was dark-brown content of pH 6.5. An occult blood test gave a strong positive reaction. Several gross lesions in the gastrointestinal tract were seen mainly in the abomasum and duodenum. Twelve ulcers were in the most ventral part of the fundic region of the abomasum and a few in the pyloric area. These ulcers measured 1 to 3 cm in diameter and were round or oval and filled with blood clots and necrotic material. The same ulcers were also in the duodenum. Petechial and echymotic hemorrhages were frequently seen from that segment of the small intestine and through the digestive tract. The liver and the kidneys were swollen, congested and hemorrhagic. Intense congestion and edema were seen in the lungs. Histological examination revealed necrosis of the hepatic parenchymal cells and slightly degeneration and necrosis in the lining cells in the proximal convulated tubules.

Samples of the liver from the 2 ewes that died at the Hospital were submitted for wet digestion with nitric acid-perchloric acid mixture and analysis for copper in an atomic absorption spectrophotometer. The liver copper levels were 1350 and 1830 ppm/kg dry matter.

Jugular venous blood was collected from the 4 ewes that survived before and after periodically the treatment for several laboratory analyses. The serum activities of aspartate aminotransferase (AST) and gamma glutamyltransferase (γGT) measured at 30 C, and the concentrations of copper, total bilirubin, and total protein were determined in commercial kits; the levels of serum urea and creatinine were assayed by the urease indophenol and Folin-Wu methods, respectively. The packed cell volume, total number of leucocytes and their differential cells were also determined. The results are presented in Table 1.

The AST and γGT increased markedly in serum activities throughout the treatment, but only the former had elevated values at day 0. The AST re-established normal values at the 5th d, but the γGT did that only after the 10th d. The serum copper concentration was elevated before the treatment, but decreased very rapidly with the beginning of treatment. Serum total bilirubin concentration was kept high for the first 2 d of treatment.

Table 1. Blood parameters before (day 0) and after the treatment of sheep that survived the outbreak of acute copper poisoning.

	0	2	5	10	20	Pooled SD	Reference (3, 9)
AST (U/L)	900	970	195	140	82	120	32-97
γGT (U/L)	22	65	60	55	47	6	26-40
Copper (μM/L)	34	14	10	15	15	4	10-18
Bilirubin (μM/L)	40	35	16	14	8	6	1.7-8.6
Packed cell volume (%)	55	25	22	18	24	3	27-45
Protein (g/L)	75	54	50	60	63	4	6-7.9
Urea (mM/L)	13	8	6	4	4	2	3.6-7.1
Creatinine (μM/L)	190	174	123	100	120	6	106-168
Leucocytes ($x10^3/\mu L^3$)	4.7	7.5	32	14	10	3	4-12

Both PCV and total serum protein were elevated before treatment, but decreased slightly thereafter. Urea and creatinine were elevated throughout the treatment; urea re-established its values on the 5th d, but creatinine showed a slight decrease only on day 10. An intense leucocytosis was seen on the 5th and 10th d as a relative neutrophilia (> 65%) with more than 5% band neutrophils and lymphopenia (< 25%).

Urine samples were collected by prepuce stimulation in the same 4 ewes. Urine analysis included color, specific gravity by refractometry; hemoglobin, occult blood, bilirubin and protein by analytical multistix stripes (Ames Co. Elkhart, IN); the urine sediment was examined by direct microscopy. Two ewes had light red-colored urine for 2-3 d, but all had microhemoglobinuria for > 1 d post-treatment. Hypostenuria (specific gravity < 1.012) was detected only on the 5th d in the most deteriorated ewe. Protein and occult blood were detected, and hemoglobin, granular and epithelial casts were scarcely seen in the ewes with better health status until the 5th d, but were recorded more intensively in the most clinically affected ewe.

Control

The outbreak was self-limiting. From that episode on footbathing was carried out during the day after the abundant offering of water to the flock. To avoid any other cases of copper poisoning the veterinarian decided to use zinc sulfate rather than copper sulfate in the footbath solution for topical footrot therapy.

DISCUSSION

This outbreak was caused by the inadvertent ingestion of copper sulfate solution by the affected ewes. Usually, this solution is used with success and without any detrimental consequences in the topical treatment of footrot in sheep and cattle (3). The deprivation of water for the flock was the decisive management failure that contributed to the intake of the copper solution. The ewes with footrot were kept without water for at least 17 h that resulted in intense thirst in most ewes (20 out of 27).

Usually, copper sulfate solution is very unpalatable to healthy sheep; this solution was offered to 15 ewes, with plenty access to fresh water, some of them smelled and tasted the solution, but none ingested it (Ortolani EL, University of Sao Paulo; unpublished data). Besides the deprivation of water, thirst was also intensified by the compulsory walking (2 km away) from the paddock to the sheep house, and the warm weather that persisted during that summer night (about 20 C). However, as far as the clinical picture was concerned, not all sheep (7/27 = 26%) apparently drank the footbath solution. The amount of copper ingested was enough to cause severe and significant acute enteric and internal lesions that culminated with the death of 12 ewes.

The availability of copper in the $Cu\ SO_4 5\ H_2O$ to ruminants may be very high (about 75%), indicating that a large part of the copper could be absorbed, causing marked internal disarrangements (eg hemolysis, hepatic and kidney lesions). Nevertheless, the copper, on its own, caused significant lesions in the gastrointestinal mucosa, such as ulcers, petechial and echymotic hemorrhages, by its corrosive proprieties. The clinical signs associated with these lesions were diarrheic feces, excretion of large amounts of mucus, melena, and slight anemia and hypoproteinemia.

It is classically known that copper sulfate solution strongly stimulates the esophageal groove bypassing the rumen and going directly to the abomasum (6). The presence of diarrheic feces with dark blue-green metal brilliance in the stool on the 2nd d after the copper sulfate ingestion indicated the gastrointestinal transit acceleration caused by the esophageal groove and increased intestinal peristalsis. The low abomasum pH ionizes the copper, turning it divalent and highly reactive and corrosive. It is likely that the use of copper antagonizing substances (ammonium molybdate and anhydrous sodium sulfate), antacids (aluminum hydroxide and magnesium hydroxide), and adsorbents (kaolin and pectin) decreased the caustic effects caused by soluble copper and should be considered in a therapy protocol for acute copper poisoning.

The hypercupremia and the high level of copper in the liver confirmed the high absorption of the copper sulfate by the sheep. Similar to cumulative copper poisoning, the high free copper in the bloodstream caused hemolysis, hepatic parenchymal necrosis and renal insufficiency. Nevertheless, the degree of these dysfunctions was much lower in the present outbreak than in that usually recorded in the cumulative form (7). While in this latter form the macroscopic hemoglobinuria lasts for at least 4-5 d producing urine with a heavy port wine color, in the present case it lasted for 2-3 d with light red-colored urine. This slight hemoglobinuria was a reflection of a lower degree of hemolysis, as seen by apparently high PCV values (18%) as compared to sheep with cumulative copper poisoning that have PCV levels as low as 8% (7). Nevertheless, the PCV value on the 1st d of hospitalization was very high (55%), probably as a result of transient dehydration that was promptly corrected by the adequate fluid therapy and high oral intake of water.

Comparative results of mean cupremia on the 2nd d of poisoning in untreated sheep indicated that the serum copper level was much lower in the acute form (30 μM/L) than in the cumulative form (59 μM/L) (7). Thus, if the serum copper concentration is low, the passage of free copper into the erythrocyte will be lower too, generating a less severe injury to the cytoplasmatic membrane of the erythrocytes and a lower rate of hemolysis (5).

As the serum copper concentration was not so high and the presence of free hemoglobin, as a result of the hemolysis, was not so intense, the renal insufficiency was also not so marked. Free copper and hemoglobin are the principal cause of marked injuries in the convoluted tubular cells and renal blood capillary vessels (8). Earlier studies demonstrated that renal insufficiency, rather than hemolysis and hepatic injury, was the *causa mortis* of cumulative copper poisoning in sheep (7); but in the same study it was shown that early medication with high doses of tetrathiomolybdate (3.4 mg/ kg bw), identical to the one used here, could markedly decrease deterioration of renal function. Even though, the most affected ewe presented hypostenuria and excreted different forms of casts indicating an evident renal injury, despite clinical improvement with tetrathiomolybdate treatment.

Although the hemolysis and renal injury was not so intense in the acute copper poisoning, the liver damage was consider-

able, as judged by the AST activities. While the levels of this enzyme reached between 900 to 970 U/L the first days of the present outbreak (Table 1), the highest elevation in sheep with cumulative copper poisoning did not surpass 780 U/L (7). Nevertheless, the liver copper concentration was about 2.3 times lower than in traditional cumulative copper poisoning (5, 7). It is likely that the free ionized copper absorbed suddenly in large amounts from the intestines probably caused the significant liver injury seen in the acute poisoning. During cumulative toxicity the copper loaded in the liver during the pre-hemolytic phase is mostly retained within cell organelles, principally the lysosomes, not as a free ion but conjugated with proteins, until a threshold level is reached when about 25 % of this copper is suddenly released into the bloodstream as ionized copper causing systemic and liver parenchyma damage (5).

The plasma γGT levels were significantly increased only 2 d after the onset of the clinical signs, but they persisted above the upper reference limits longer than the AST activities (Table 1). While AST is present in all parenchymal hepatocytes, γGT is normally found only in the hepatic biliary system in the bile duct epithelium and pericanicular zone. Although both parenchymal hepatocytes and the pericanicular zone could suffer necrosis from the ionized copper at same time, the magnitude and quantity of the lesions were probably higher in the former, increasing the blood AST activity earlier, but γGT persisted longer with its higher plasma half-life (80 h) and the continuous production and liberation of this enzyme after proliferation of the biliary epithelial cells during the copper poisoning process (9).

The use of tetrathiomolybdate was very effective in decreasing the plasma copper concentration to normal levels within 2 d of therapy. The high capacity of this chelate to combine tightly to free copper excretes it principally in the bile (1, 3, 10). The tetrathiomolybdate also improved kidney function, since plasma urea and creatinine levels returned to normal in the first days of treatment. Ionized copper associated with free hemoglobin originating from hemolysis and circulating free radicals are the major cause of renal injury. A recent study showed that treatment with tetrathiomolybdate sharply decreased the effects of those agents on the kidney, improving tubular and glomerular function during the post-hemolytic phase in sheep with cumulative copper poisoning (11).

Although the free copper could cause intense gastrointestinal damage, the oral and intrabomasum treatments alleviated this effect since the occult blood in the feces became negative the 5th d of therapy, and the mucus in the stools disappeared some days later. The ionized copper was probably neutralized by the ammonium molybdate and sodium sulfate drench. Free copper within the rumen complexes with molybdenum and sulfide to form stable and non-reactive thiomolybdate that is excreted in the feces (12). Part of the remaining copper may be adsorbed by the kaolin and pectin. The ulcers on the gastrointestinal tract were improved with the use of antacids such as aluminum hydroxide and magnesium hydroxide.

There was a marked leucocytosis caused by intense neutrophilia with a shift to the left and lymphopenia during the first 10 d of poisoning. A similar picture was also described in sheep after injecting iv 100 mg copper sulfate iv (13). This acute response was probably caused by a stress reaction associated with the intense inflammatory response. The former increases the blood levels of cortisol and adrenocorticotropic hormone (ACTH), that in turn induce liberation of granulocytes to the bloodstream. The blood levels of corticosteroids after stress are proportional to the severity and duration of the stress, but return to normal 12-24 h after the end of the stressful episode (14), while the inflammation, principally in the gut, results in increased granulopoiesis and the release of neutrophils to the bloodstream (14).

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The true lover of knowledge naturally strives for truth, and is not content with common opinion, but soars with undimmed and unwearied passion till he grasps the essential nature of things.

- Plato

In searching for the fundamental principles of the science of teaching, I find few axioms as indisputable as are the first principles of mathematics. One of these is this, He is The Best Teacher Who Makes The Best Use of His Own Time And That Of His Pupils. For Time is all that is given by God in which to do the work of improvement.

-Emma Hart Willard