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Industrial Copper Intoxication of Iranian Fat-Tailed Sheep in Kerman Province, Iran

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Abstract: An outbreak of industrial chronic copper poisoning in Iranian fat-tailed sheep in a region of southeast Iran is described. At least 10,000 out of 75,000 sheep (13.3%) originating from 50 flocks died over a period of 3 years in the Khatoon Abad region in Kerman province, Iran. The diagnosis was based on epidemiological, clinical, hematological, serum biochemical, urinalysis, necropsy and histopathological evaluations. Then findings were confirmed by toxicological analysis of liver and kidney samples.

The details of epidemiological, clinical, laboratory and pathologic findings are described in the manuscript. These findings were indicative of chronic copper poisoning in affected sheep. Toxicological analysis showed an exceeding of the permitted limit (150 mg/kg w.w.) of copper in liver samples by 7.97 times (1196.9 ± 20.6 mg/kg) (wet weight-w.w.). In kidney samples the excess was by 9.14 times (137.2 ± 8.96 mg/kg) (w.w.). The copper concentrations in water and pasture plant samples around the factory were very high, suggesting that water and pasture plants were the sources of intoxication.

In conclusion, the environmental pollution by a copper smelter factory in the region was the source of intoxication. Chronic copper intoxication through inhalation and ingestion may result in severe economic losses and a public health hazard. Thus it is necessary to determine copper concentrations in the organs (liver, kidney, muscle, and lung) of sheep in the influence area of chemical units.

Key Words: Copper, industrial, intoxication, Iranian fat-tailed sheep

Introduction

Copper is both an essential nutrient and a toxic element for all domestic animals (1,2) and especially for sheep. Copper toxicosis can be acute or chronic (3). Chronic copper poisoning is the most common form of copper toxicity in sheep. Its occurrence is associated with a long-term intake of copper compounds of different origin. The characteristic feature of industrial copper intoxication is that the animals are reared close to industrial plants, and ingest copper from industrial deposits through feed or from the air mostly together with other toxic elements throughout their entire lives (4). As a result animals reared under such conditions adapt to a certain degree to chronic intake of increased doses of copper and clinical and pathological manifestations of intoxication are not always characteristic. Due to the factors mentioned, the prevention of industrial copper intoxication raises some problems and its effectiveness depends, in addition to the amount and the period of copper intake, also on the nutritional status of animals, season, level of productivity, and reproductive phase (5). To the best of the authors'

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knowledge, industrial copper intoxication has not been reported in Iran and specifically in Iranian fat-tailed sheep. This paper describes the epidemiological, clinical, hematological, serum biochemical, urinalysis, toxicological, and pathological findings of an outbreak of chronic industrial copper intoxication in sheep reared in an area close to a copper smelter factory in Kerman province, Iran.

Materials and Methods

Epidemiology

Epidemiological data about the poisoning were obtained from the owners of the affected flocks and from the veterinary practitioner. The affected animals were examined clinically and clinical findings were recorded.

Hematology

Whole blood samples (n = 25) were prepared by jugular venipuncture for assessment of hematological parameters and gathered in tubes containing an anticoagulant (sodium citrate). Packed cell volume, hemoglobin, total RBC, total and differential WBC count, MCV, MCHC, and platelets were measured by an automatic cell counter: model Sysmex KX-21, Japan.

Serum biochemical analysis

Serum samples (n = 25) were sent for biochemical analysis including AST activity, and concentrations of total protein, bilirubins, ceroluplasmin, BUN, and creatinine. Serum biochemical analysis was done by an autoanalyzer: model RA-1000, US.

Urinalysis

Urine samples (n = 25) were examined for the presence of proteins, glucose, ketones, blood, bilirubin, urobilinogen, nitrate, WBC, RBC, epithelial cells, bacteria, crystals, and cast. Color, appearance, specific gravity, and pH were also assessed. Urinalysis was done using urine indicator paper (Arak Chemical Co, Iran, IROST).

Histopathology

Dead animals (n = 25) were necropsied immediately after death and assessed for macroscopic lesions. Samples of internal organs were processed using a standard procedure, i.e. they were fixed in 10% neutral formalin and embedded in paraffin. Sections 5-6 µm thick were stained with hematoxylin-eosin.

Toxicology

The concentrations of copper in the liver, kidney, pasture plants, and water samples (n = 25) were determined by means of atomic absorption spectrophotometer: GB 30, model 906, Australia.

Statistical analysis

Results are expressed as mean ± SD. Data from hematological indices, and serum biochemical and toxicological analysis were compared with normal values by sample t-test. Significance was accepted at $P < 0.05$ level.

Results

Epidemiological findings

There is a copper smelter factory in the Khatoon Abad region of Kerman province, Iran. There are several pastures different distances from this factory. A total of 50 flocks graze in these pastures, with each flock consisting of 1500 sheep. Iranian fat-tailed sheep constitute the animal population in the flocks. In November 2005 a local veterinary practitioner reported that a large number of sheep from these flocks had died or were showing signs of hemolytic crisis over several months. Disease occurred in all age groups of these flocks. There was a particularly high incidence in grazing flocks, compared to intensively reared ones. Case mortality ranged from 95% to 100%. In this region cattle husbandry is uncommon and most of the population of animals comprises sheep. Moreover, goats are less susceptible to copper intoxication and had no signs of toxicity.

Clinical findings

A large number (n = 35) of randomly selected sick animals were examined clinically and the following clinical symptoms were observed. There was an extremely rapid, irregular pulse, accompanied by shallow, hurried respirations in practically all cases but the temperature seldom, if ever, rose above normal. The acceptance of feed was decreased and overall inappetence was observed finally. The intake of water was increased. Conjunctivae, the mucous membranes of the mouth and vagina, and cutis were pale and icteric. Chocolate-brown coloring of blood obtained by venipuncture and hemoglobinuria were observed. There was little disturbance of alimentary tract

function. The animal remained mostly in a lying position and exhibited signs of complete weakness. Nervous symptoms characterized by swaying movements, teeth grinding, and idle rumination were also observed. In the final period the animals were in lateral recumbency and failed to respond to external stimuli. Depression was profound and the disease ordinarily terminated fatally within 24 to 48 h of the first appearance of the characteristic symptoms but occasionally affected animals lingered for considerably longer. The clinical findings and their percentages of occurrence are presented in Table 1.

Hematological indices, serum biochemical parameters, and toxicological, necropsy, and histopathological findings are presented in Tables 2-5, respectively. Some macroscopic and microscopic findings are shown in Figures 1-4. Methemoglobinemia was also present in some cases. Urinalysis (n = 25) did not show any significant change, except for proteinuria and hemoglobinuria (100%).

Discussion

The diagnosis of copper poisoning was based on epidemiological, clinical, hematological, serum biochemical, necropsy, and histopathological findings and was confirmed by toxicological identification of high liver and kidney copper concentrations. Gummow et al. (6) reported that chemical industry development can lead to environmental pollution of industrial origin that negatively affects human and animal health. This is the case at Khatoon Abad in southeast Iran in Kerman province due to the existent chemical units (copper smelter factory). The chemical units pollute the area with copper. The pollution is observable in the area as fumes and sewage. High copper concentrations in pasture plants of different areas around the factory and the water confirmed the pollution of the area by this element. According to Galey et al. (3) chronic copper poisoning may be the result of grazing in pastures contaminated by smelter fumes or sewage. Gopinath et al. (7) showed that

Table 1. The clinical findings and their percentages of occurrence (number of examined cases = 35).

Clinical findings	Icterus	Nervous symptoms	Hemoglobinuria	Rapid, irregular pulse	Shallow, hurried respiration	Rising temperature	Inappetence
Number of cases	30	25	35	35	35	2	35
Percentage (%)	85.7	71.4	100	100	100	5.7	100

Table 2. Hematological indices (mean ± SD) in blood samples from sheep near the copper factory (numbers of samples = 25).

	Hemoglobin (g/dl)	Hematocrit (l/l)	RBC ($\times 10^{12}$/l)	WBC ($\times 10^{9}$/l)	Thrombocytes ($\times 10^{9}$/l)	MCV (fl)	MCHC (g/l)
Acquired values	75 ± 10*	0.23 ± 0.01*	5.73 ± 0.55*	8 ± 2.00	300 ± 50.00	46.1 ± 2.00*	280 ± 15*
Normal values	90-150	0.27- 0.45	8 - 18	4-12	250-750	28-40	310-340

In each column only those with * are significantly different from normal values.

Table 3. Serum biochemical parameters (mean ± SD) in samples from sheep near the copper factory (numbers of samples = 25).

	AST (units/l)	Total protein (g/l)	Total bilirubin (µmol/l)	Direct bilirubin (µmol/l)	Urea nitrogen (mmol/l)	Creatinine (µmol/l)	Ceruloplasmin (mg/dl)
Acquired values	1752 ± 150.00*	15.5 ± 1.00*	30 ± 0.45*	2 ± 0.04	90 ± 6.20*	150 ± 2.8*	28 ± 2.55*
Normal values	60-280	24-30	1.7-8.5	0-4.61	3-10	70-105	4.5-10

In each column only those means with * are significantly different from normal values ($P < 0.05$).

Table 4. The copper concentrations (mean ± SD) in the liver, kidney, pasture plants, and water samples from near the copper factory (numbers of samples = 25).

Sample type	Normal values (ppm)	Average copper concentration (ppm) (mean ± SD)
Liver (wet weight)	150	1196.9 ± 20.6 *
Kidney (wet weight)	15	137.2 ± 8.96 *
Pasture plant	12	6208 ± 58.69 *
Water	0.025-0.075	0.3 ± 0.02 *

In each row only those means with * are significantly different from normal values ($P < 0.05$).

Table 5. Macroscopic and microscopic findings in different organs.

Organ	Macroscopic findings	Microscopic findings
Intestine	Dark discoloration of the intestine (Figure 2)	Necrotic enteritis, denuded epithelium with villous atrophy
Liver	Swelling and yellow discoloration f liver (Figure 3)	Hepatocellular degeneration, bile retention
Kidney	Black cortex of the kidney in hemolysis crisis (Figure 4)	Capsule thickening, ATN, tubular dilatation, regenerated tubules with flattened epithelium, a few interstitial lymphocytic foci, glomerulonephritis, brown pigment of lipofuscin in tubular epithelium due to hemolysis, apoptosis, hyaline cast, mild fibrosis, deposition of calcium in tubules
Heart	Endocarditis and necrosis of the heart	Rhabdomyolysis with formation of granulation tissue
Lung	Hemorrhagic with inflammatory exudates, atelectasis, emphysema	Purulent pneumonia, atelectasis, emphysema, intra alveolar hemorrhage, hemorrhagic pleuritis
Spinal cord	Mild hyperemic	Axonal degeneration, progressive degeneration of the spinal cord evidenced by lipofuscin pigment stored in cell body of the neurons
Brain	Petechiation	Demyelination, endothelial hyperplasia, extravasation of RBCs into Virchow Rabin space, microgliosis
Urinary bladder	Hyperemic	Hyperemia
Lymph node	Enlarged	Lymphatic depletion
Spleen	Dark and enlarged	Severe hemosiderosis
Uterus	Dark and hyperemic	Lipofuscinosis

Figure 1. Chronic Cu intoxication in sheep. Hemoglobinuria.

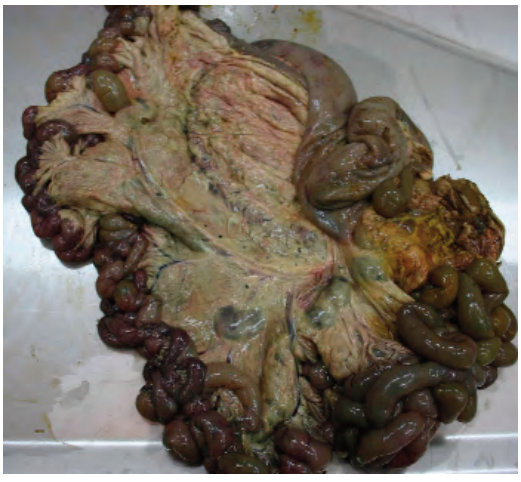

Figure 2. Chronic Cu intoxication in sheep. Dark discoloration of the intestine.

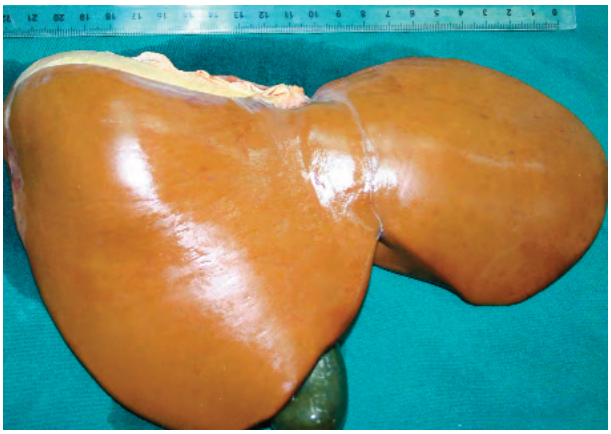

Figure 3. Chronic Cu intoxication in sheep. Swelling and yellow discoloration of the liver.

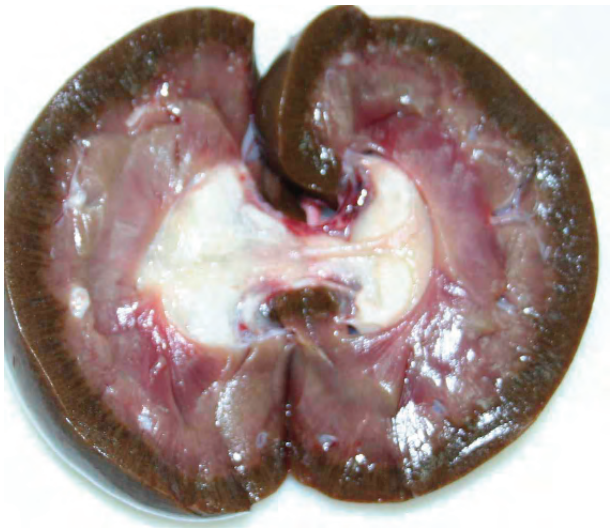

Figure 4. Chronic Cu intoxication in sheep. Black cortex of the kidney in hemolysis crisis.

inhalation of fumes in the area by sheep could also lead to chronic copper intoxication. Copper accumulates in the tissues and progressive histological and histochemical changes occur in the liver as defined by Ishmael et al. (8). Ishmael et al. (9) demonstrated some changes in serum enzyme levels that indicate that a functional disturbance of the liver can occur before the hemolytic crisis. At the time of the hemolytic crisis, copper is released into the blood stream and the animal becomes acutely ill; hemoglobinemia, methemoglobinemia, hemoglobinuria, and jaundice occur at this time (7). Animals usually die during or shortly after a hemolytic crisis and in these animals liver and kidney damage can be observed. The

clinical findings described in the present description are in agreement with those of previous reports (1,10-12).

Although nervous signs are not the major manifestation of chronic copper poisoning, in this study the nervous signs were prominent, interestingly.

Evaluation of hematological indices showed that hematocrit, number of RBC, and hemoglobin concentration decreased sharply, which suggested anemia. These findings are in agreement with those described by Maiorka et al. (13) and Christodoulopoulos and Roubies (14). Hypochromic anemia is characterized by MCHC falling 30%. Increasing MCV can be suggestive of macrocytic and regenerative anemia as explained by Jain (15). Anemia can occur in chronic copper poisoning following the hemolytic crisis (10). Numbers of WBC and platelets were within the normal ranges. Serum biochemical analysis indicated that AST activity increased significantly. Ortolani et al. (10) concluded that increasing GGT followed by AST are the best enzymes to assess copper load in sheep during the pre-hemolytic phase. Severe liver damage in chronic copper poisoning could lead to an increase in AST activity as suggested by Maiorka et al. (13). Total protein decreased significantly, which can be due to severe liver and kidney damage. Copper toxicosis hepatopathy was described by Maiorka et al. (13) and Mendel et al. (12). Copper poisoning can cause toxic tubular nephrosis as explained by Maiorka et al. (13), Jha and Chugh (16), and Mendel et al. (12), which can lead to proteinuria (17). Kidney damage can also lead to increasing BUN and serum creatinine concentrations. High blood urea nitrogen and nephropathy were reported in poisoned sheep by Maiorka et al. (13). In the present study significant increases in total and indirect bilirubin may be due to the hemolytic crisis, as suggested by Gopinath et al. (7) and Ayiannidis et al. (18). In our study the mean concentration of serum cerulopalsmin increased to 28 mg/dl. Increased levels of serum ceruloplasmin level may be attributed to an increase in copper concentration in the liver, which can be explained by the delivery of copper, accumulating in the non-metallothionein-bound form, to ceruloplasmin outside the Golgi apparatus of the liver (19), although Jain (15) indicated that decreased ceruloplasmin levels may be found in liver disease associated with copper toxicosis. In addition, rapid decreases in serum ceruloplasmin concentration occur at least 80 days before overt clinical signs of copper deficiency (17).

Toxicological analysis showed that the permitted limits of copper concentration in liver (150 mg/kg w.w.) and kidney (15 mg/kg w.w.) samples (20) from areas situated around the chemical unit were exceeded by 7.97 and 9.14 times. The copper concentrations in water and pasture plant samples around the factory were very high, which could suggest that water and pasture plants were the sources of intoxication. Necropsy and histopathologic findings described in this paper are in agreement with those described by Howell et al. (21), Edelsten (11), and Christodoulopoulos and Roubies (14). The environmental pollution by a copper smelter factory in the region was the source of intoxication. Chronic copper intoxication through inhalation and ingestion may result in severe economic losses and a public health hazard. Thus it is necessary to determine copper concentrations in the organs (liver, kidney, muscle, and lung) of sheep in the influence area of chemical units.

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