

Acute encephalopathy and clinical pathology findings in a sheep with chronic copper poisoning

Nektarios D. GIADINIS^{1,*}, Nikolaos PAPAIOANNOU², Maria KRITSEPI-KONSTANTINOY³,
Nikolaos ROUBIES³, Nikolaos RAIKOS⁴, Harilaos KARATZIAS¹

¹Clinic of Farm Animals, School of Veterinary Medicine, Aristotle University of Thessaloniki, GREECE

²Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, GREECE

³Diagnostic Laboratory, School of Veterinary Medicine, Aristotle University of Thessaloniki, GREECE

⁴Laboratory of Forensic Medicine and Toxicology, Medical School, Aristotle University of Thessaloniki, GREECE

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Abstract: A 3-year-old East Friesian sheep from a sheep flock that suffered from chronic copper poisoning was forwarded to the Clinic of Farm Animals in the Aristotle University. The animal had signs of a hemolytic disease indicative of chronic copper poisoning and also nervous signs. Laboratory examinations were consistent with chronic copper poisoning. This was confirmed after necropsy and liver toxicological analysis. By blood smear examination, metarubricytosis, basophilic stippling, anisocytosis, and polychromasia were found. Also, brain histopathological examination revealed focal areas of gliosis in the cerebral cortex and white matter areas. Satellitosis, neuronal degeneration (chromatolysis) and neuronophagia were also observed. These findings are useful for the clinicians, as nervous signs in flocks suffering from chronic copper poisoning can be attributed to this toxicosis. Also, this study adds in the hematological findings of chronic copper poisoning, as the existing data in the literature are limited.

Key words: Sheep, chronic copper poisoning, acute encephalopathy, hematological findings

Introduction

Chronic copper poisoning is a hemolytic disease that affects mainly sheep. The disease is rather common in sheep industry, attributed to errors in flock management as has been pointed out in various studies (1-3).

The most common signs of chronic copper poisoning are anorexia, thirst, depression, jaundice, hemolytic anemia, hemoglobinuria, and increased respiratory and pulse rate (1,4). Sometimes nervous

signs have been mentioned in animals with chronic copper poisoning (1,3,5,6). It is accepted that copper causes histological changes of CNS in sheep (7,8). However, to date few studies have correlated the nervous signs of chronic copper poisoning in sheep with histopathological lesions of CNS in a small number of animals (5,6).

In the hemolytic phase, the hematological findings are typical of a hemolytic disease with reduced PCV and hemoglobin (9), and Heinz body anemia (10).

* E-mail: ngiadini@vet.auth.gr

Basophilic stippling and the presence of many metarubricytes (nucleated erythrocytes-nRBCs) in blood smears were also mentioned once (4).

This paper describes a case of chronic copper poisoning in a sheep with nervous signs and cerebral histological changes. Hematological, biochemical, and toxicological findings of the same animal are also described.

Case history

In an intensively reared sheep flock of Northern Greece that had losses due to simple chronic copper poisoning, the responsible copper containing concentrates were withdrawn, as well as sodium sulfate and ammonium molybdate were daily administered in the feed of the sheep and the mortality rate was dramatically reduced. One week after the start of the treatment a 3-year-old female East Friesian sheep of this flock was forwarded to the Clinic of Farm Animals. The animal was thirsty and inappetent, had jaundice, hemoglobinuria, and melena. Also the animal had nervous signs, as it was depressed, blind, and had tetraparesis. Two blood samples were taken with jugular veinpuncture, 1 with EDTA as anticoagulant for hematology, and 1 without anticoagulant for serum biochemical analysis. Hematological examination was conducted with an analyzer ADVIA 120 (Bayer), while blood smears were stained with Giemsa. After blood sampling the animal was euthanized.

Results and discussion

The blood hematological and biochemical values of the sheep are shown in Tables 1 and 2. In the blood smear were found 62 metarubricytes/100 white blood cells (WBC), basophilic stippling, anisocytosis, and polychromasia (Figures 1a and 1b).

Whole blood lead concentration was 30 µg/dL, while serum and whole blood were found negative for Maedi-Visna virus infection, as was pointed out with AGID, ELISA, and semi-nested PCR in whole blood.

In necropsy the carcass was icteric and the liver was discolored. Gallbladder and spleen were distended, while kidneys had a gunmetal color and the urine had hemoglobinuria. No gross lesions were detected in the brain and cerebellum. The brain was routinely fixed by immersion in 4% buffered formaldehyde. Coronal sections of different areas from the cerebral hemispheres (frontal, parietal, temporal, and occipital lobes) were embedded in paraffin by a routine procedure. Histopathological examination of the brain sections revealed focal areas of gliosis in the cerebral cortex and white matter areas. Satellitosis, neuronal degeneration (chromatolysis), and neuronophagia were also observed (Figure 2).

Liver copper and lead were determined and found to be 3800 mg/kg (dry matter basis) and 0.25 mg/kg (wet weight basis), respectively.

According to the clinical, gross-pathological, hematological, biochemical, and toxicological findings, it was confirmed that the sheep suffered

Table 1. Hematological values of the examined sheep.

Parameter	Value	Reference Values	Parameter	Value	Reference Values
RBC (× 10 ⁶ /µL)	4.32	9-15	WBC (/µL)	24,300	4000-12,000
Hb (g/dL)	6.1	9-15	WBC (/µL) corrected	15,000	-
PCV (%)	19.3	27-45	Neutrophils (/µL)	3300	700-6000
MCV (fL)	45	28-40	Neutrophils (band) (/µL)	450	Rare
MCH (pg)	14.1	8-12	Lymphocytes (/µL)	1800	2000-9000
MCHC (%)	31.59	31-34	Monocytes (/µL)	150	0-750
PLT (×10 ⁵ /µL)	4.67	2.5-7.5	Metarubricytes (nRBCs)	62/100	Rare
			WBCs		

¹Schalm, O.W. (1986) In: Schalm's Veterinary Hematology, 4th ed., Lea and Febiger, Philadelphia, USA.

Table 2. Biochemical values of the examined sheep.

Parameter	Value	Reference Values
AST (U/L)	440	6-280 ¹
γ-GT (U/l)	87	20-52 ¹
LDH (U/l)	2,250	238-440 ¹
Total Bilirubin (mg/dL)	2.2	0.1-0.5 ¹
Creatinine (mg/dL)	1.1	1.2-1.9 ¹
Serum copper (μg/dL)	200	50-200 ¹
Liver copper	3,800 mg/Kg (d.m.)	100-350 ¹
Whole blood lead	30 μg/dL	1-31 μg/dl ²
Liver lead	0.25 mg/Kg (w.w.)	< 20 mg/Kg (w.w.) ²

¹Pugh D.G. (2002) Sheep and Goat Medicine, Saunders, USA

²Underwood and Suttle (2004) The mineral nutrition of livestock, CABI, USA

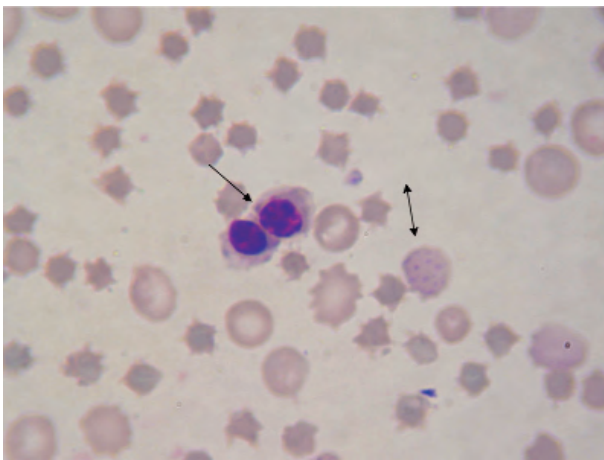


Figure 1a. Anisocytosis, polychromasia, poikilocytosis, basophilic stippling (double arrow), and 2 metarubricytes (nRBCs-arrow) (Giemsa, ×1000).

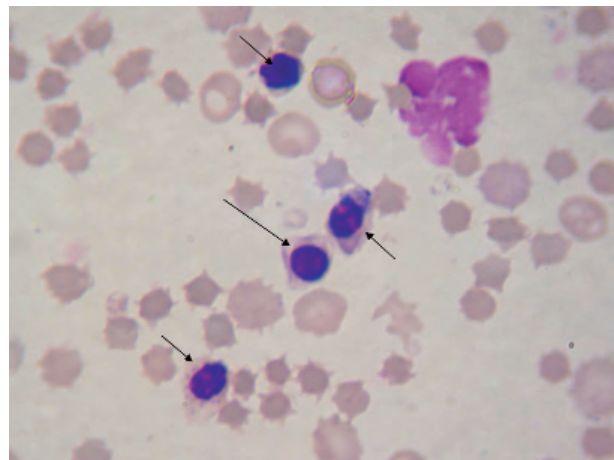


Figure 1b. Four metarubricytes (nRBCs-arrows) and polychromasia (Giemsa, ×1000).

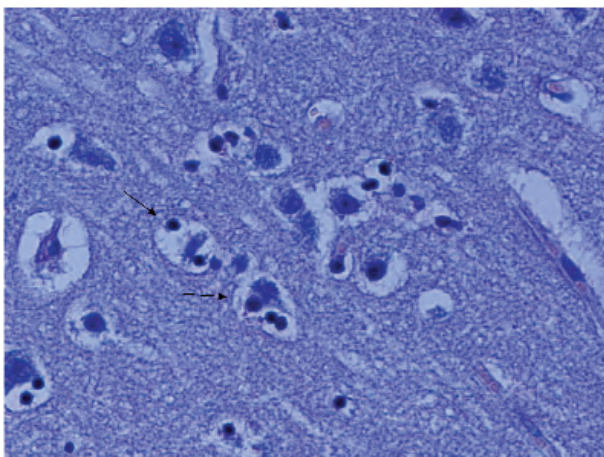


Figure 2. Parietal lobe: Presence of satellite cells secondary to neuronal degeneration and neuronophagia (arrows) (H&E, ×200).

from chronic copper poisoning. In chronic copper poisoning nervous signs are not very common (3,5). Copper seems to cause status spongiosus in sheep CNS as a result of altered glial transport mechanisms (7,8), though nervous signs have not been reported in these cases. Also, polioencephalomalacia with gliosis that was attributed to liver insufficiency and increased plasma sulfur amino acids has been found in a sheep with chronic copper poisoning (6). A similar gliosis has been found at the junction of the molecular and granular layers of cerebellum in sheep with chronic copper poisoning (5).

An interesting finding of this study was intense metarubricytosis accompanied by basophilic stippling, polychromasia, and anisocytosis. Basophilic stippling accompanied by marked

metarubricytosis and minimal polychromasia can be an indication of lead toxicosis (11). In the present study lead poisoning was excluded, as lead levels in blood and liver were within normal limits (12,13). Also, lead poisoning in sheep is manifested mainly with signs of osteoporosis (14). Lewis et al. (4) had similar hematological findings in sheep suffering from chronic copper poisoning without signs of the central nervous system. The difference of the present study is that metarubricytes were

62/100 WBC, while 24/100 WBC in the study of Lewis et al. (4).

Metarubricytosis found could be attributed to intense erythrocytic regeneration or to premature release from the bone marrow following anemia-induced hypoxia (15).

In conclusion, nervous signs with CNS gliosis as well as metarubricytosis, basophilic stippling, anisocytosis and polychromasia can be findings of sheep chronic copper poisoning.

References

1. García-Fernández, A.J., Motas-Guzmán, M., Navas, I., María-Mojica, P., Romero, D.: Sunflower meal as a cause of chronic copper poisoning in lambs in southeastern Spain. *Can. Vet. J.*, 1999; 40: 799-801.
2. Christodouloupolous, G., Roubies, N.: Diagnosis and treatment of copper poisoning caused by accidental feeding on poultry litter in a sheep flock. *Aust. Vet. J.*, 2007; 85: 451-453.
3. Roubies, N., Giadinis, N.D., Polizopoulou, Z., Argiroudis, S.: A retrospective study of chronic copper poisoning in 79 sheep flocks in Greece (1987-2007). *J. Vet. Pharmacol. Ther.*, 2008; 31: 181-183.
4. Lewis, N.J., Fallah-Rad, A.H., Connor, M.L.: Copper toxicity in confinement-housed ram lambs. *Can. Vet. J.*, 1997; 38: 496-498.
5. Bostwick, J.L.: Copper toxicosis in sheep. *J. Am. Vet. Med. Assoc.*, 1982; 180: 386-387.
6. Sargison, N.D., Scott, P.R., Penny, C.D., Pirie, R.S.: Polioencephalomalacia associated with chronic copper poisoning in a Suffolk ram lamb. *Vet. Rec.*, 1994; 135: 556-557.
7. Doherty, P.C., Barlow, R.M., Angus, K.W.: Spongy changes in the brains of sheep poisoned by excess dietary copper. *Res. Vet. Sci.*, 1969; 10: 303-304.
8. Howell, J. M., Blakemore, W.F., Gopinath, C., Hall, G.A., Parker, J.H.: Chronic copper poisoning and changes in the central nervous system of sheep. *Acta Neuropathol.*, 1974; 29: 9-24.
9. Edelsten, R.M.: Chronic copper poisoning of sheep in Nigeria. *Trop. Anim. Health Prod.*, 1980; 12: 69-76.
10. Kerr, L.A., McGavin, H.D.: Chronic copper poisoning in sheep grazing pastures fertilized with swine manure. *J. Am. Vet. Med. Assoc.*, 1991; 198: 99-101.
11. Brockus, C.W., Andreasen, C.B.: Erythrocytes. In: Duncan and Prasse's *Veterinary Laboratory Medicine-Clinical Pathology*, 4th edn., Blackwell Publishing, 2003; 3-45.
12. Frøslie, A., Norheim, G., Rambaek, J.P., Steinnes, E.: Heavy metals in lamb liver: contribution from atmospheric fallout. *Bull. Environ. Contam. Toxicol.*, 1985; 34: 175-182.
13. Underwood, E.J., Suttle, N.F.: Essentially toxic elements. In: *The Mineral Nutrition of Livestock*, 3rd edn., CABI Publishing, 2004; 543-586.
14. Quarterman, J.: Lead. In: Mertz, W., Ed., *Trace Elements in Human and Animal Nutrition*. 5th edn., Academic Press, USA, 1986; 281-317.
15. Latimer, K.S., Mahaffey, E.A., Prasse, K.W.: Case 2- Autoimmune hemolytic anemia and thrombocytopenia. In: *Duncan and Prasse's Veterinary Laboratory Medicine-Clinical Pathology*, 4th edn., Blackwell Publishing, 2003; 347-348.