Copper toxicosis with hemoglobinuric nephrosis in three adult sheep

Adrian STANCU
Banat's University of Agricultural Science and Veterinary Medicine Timisoara
"King Michael of Romania",
Faculty of Veterinary Medicine, 300645, Calea Aradului, no 119, Timișoara, Romania;
astancu2002@yahoo.com

Abstract

Acute and, particularly, chronic copper exposures, along with defects in hepatic copper metabolism, altered excretion of copper, and/or nutritional imbalances between copper and other trace elements, can lead to hepatic accumulation of copper and primary copper toxicosis. There is interspecies variation in susceptibility to copper toxicosis, with sheep being the species most likely to develop this condition. The current report is rather unusual in that it describes instances of naturally occurring copper toxicosis with hemolysis and hemoglobinuric nephrosis in 3 adult sheep. In 2 of these sheep, a possible source of excessive dietary copper was investigated but not definitively identified. In the third goat, the etiologic factors associated with the copper toxicosis were not determined. It appears that mature sheep are susceptible to the hemolytic stage of chronic copper toxicosis, which was not observed in a recent, large-scale copper intoxication involving lactating dairy sheep (3, 5, 6, 12). Copper analyses on both kidney samples were necessary to confirm the diagnosis of copper toxicosis in all 3 sheep. All feedstuffs associated with instances of copper toxicosis should be analyzed for iron, molybdenum, sulphur, and zinc as well as copper to determine what nutritional factors are contributing to the pathogenesis of this disease. Consideration also should be given to the ingestion of hepatotoxic plants and other toxic exposures, which could predispose an animal to secondary chronic copper toxicosis (4, 7, 8, 11). It is thought that sheep are predisposed to chronic copper toxicosis because of their reduced biliary and urinary excretion of copper, the distribution of zinc- and copper-binding proteins in the liver, and the relatively small difference between the copper concentrations reported to be adequate for sheep rations (5–10 mg/kg, 7–11 mg/kg, or 10–20 mg/kg on a dry matter basis, depending on the reference) and those dietary copper concentrations considered to be potentially toxic (>15, 20, or 30 mg/kg on a dry matter basis). In contrast, cattle, horses, swine, and poultry tend to be more resistant to copper accumulation and chronic copper toxicosis, with maximum tolerable dry matter concentrations of dietary copper being approximately 50 mg/kg for cattle and horses, 250 mg/kg for swine, and 300–500 mg/kg for poultry. In a previous study, ponies were even reported to tolerate dietary copper concentrations approaching 800 mg/kg for 6 months. However, histopathologic examinations of the kidney were not apparently performed, and it is extremely important to recognize that copper bioavailability and dietary concentrations of molybdenum also play important roles in the pathogenesis of chronic copper toxicosis (9, 10, 13).

Key word: *copper, sheep, kidney*

Materials and methods

It was performed an 3 adult sheep post-mortem examination, following a sudden death. There were taken spleen samples for histopathological examination.

The samples preparation was carried out as follows: 24 h alcohol fixation at room temperature (prevent the tissue alteration due to the enzymes activity; preserve the tissue texture; improves the optical differentiation), alcohol dehydration (five steps: 70, 80, 90, 100% and 100% alcohol, each step for two hours), clearing with benzene, paraffin wax at 56°C, embedding tissues into paraffin blocks, trimming of paraffin blocks (6 μm), sections mounting on the glass slides (using Meyer albumin), hematoxylin - eosin- methylene blue staining.Staining was performed as follows: deparaffination of tissue sections in benzene, rehydration using decreasing concentrations of alcohol, rinsing in distilled water, hematoxylin staining, alcohol, eosin staining and methylene blue staining, water removal using increasing concentrations of alcohol, cover slide mounting. Hematoxylin will stain the nuclei in blue and the mucins in light blue. Eosin will stain the

cytoplasm in pink, collagenin pale pink, red blood cells in bright red, and colloid in red. Methylene blue improves the blue colour of the nuclei, making them more observable.The microscopical examination is useful as differentiating diagnosis method only if chemical preparation of samples is applied (1, 2).

Result and discution

Based on the history and clinical signs, as well as the gross necropsy and clinical pathology results, chronic copper toxicosis was suspected in the 3 goats. Diagnosis was corroborated by the observed histopathologic findings, all of which were consistent with the pathogenesis of chronic copper toxicosis as well as the absence of severe gastroenteritis, which would have suggested a larger and more acute copper exposure. Macoscopic on the renal surface observed black discoloration of the kidneys due to concentration of (met)hemoglobin. Massive acute hemolysis caused by chronic copper poisoning.

Microscopicaly observed proteinaceous material in tubular lumina resulting from hemoglobin filtration. Green-blue homogeneous globules (hyalindroplets) in tubularepithelial cells, are due to reabsorption anlysosomal accumulation of the filtered hemoglobin. Moreover, hydropidegeneration and necrosis of tubularcells.

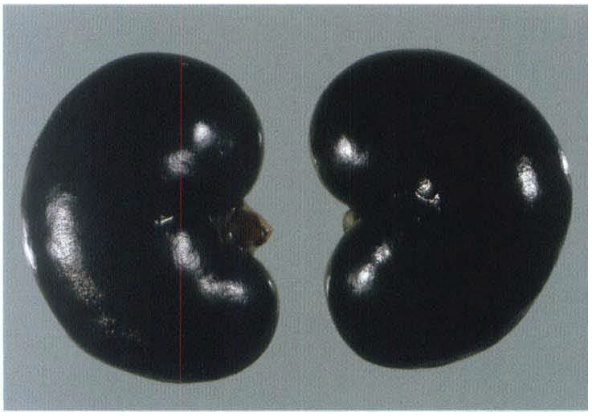

Fig 1. Hemoglobinuric nephrosis,. Massive acute hemolysis caused by chronic copper poisoning. Sheep.

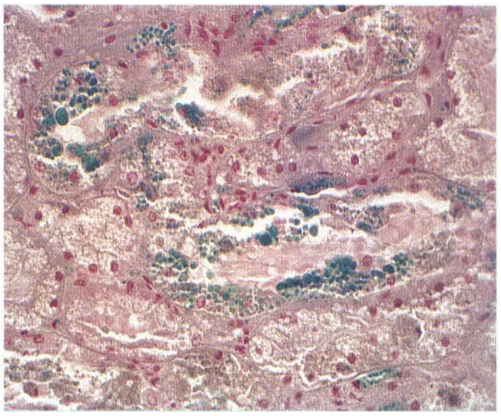

Fig 2. Renal cortex. Hemoglobinuria caused by chronic copper poisoning. Sheep. Fast blue stain for hemoglobin.

Conclusions

1. Chronic copper intoxication was diagnosed in 3 adult sheep based on the macroscopic examination, complete with histopathological examination.
2. Specific lesions were present in the kidneys.
3. Long-term exposure to Copper intoxication induces characteristic kidney damage.

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References

1. Adrian Stancu, Special veterinary pathological anatomy, Editura Agroprint, 978-606-8037-49-3, 2014
2. Adrian Stancu, Diagnostic necropsic veterinar, Editura Mirton 2013, 978-973-52-1395-4, 2013.
3. Bostwick JL: 1982, Copper toxicosis in sheep J Am Vet Med Assoc 180: 386–387.
4. Charles JA: Pancreas. *In*: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, Maxie MG, ed., 5th ed., Vol. 2, p. 393-394. Saunders Elsevier, Philadelphia, PA, 2007.
5. Gandini G, Bettini G, Pietra M, Mandrioli L, Carpene E: Clinical and pathological findings in acute Cupper intoxication in a puppy. J Small Anim Pract 43:539-42, 2002
6. George LW: Copper toxicosis. *In*: Large Animal Internal Medicine, Smith BP, ed., p. 1166-1169. Mosby Elsevier, St. Louis, MO, 2009.
7. Haywood S, Muller T, Muller W, Heinz-Erian P, Tanner MS Ross G: Copper-associated liver disease in North Ronaldsay sheep: a possible animal model for non-Wilsonian hepatic copper toxicosis of infancy and childhood. J Pathol 195:264-269, 2001.
8. Maxie MG, Newman SJ: The urinary system. *In*: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, Maxie MG, ed., 5th ed., Vol. 2, p. 475-476. Saunders Elsevier, Philadelphia, PA, 2007
9. Octavian Sorin Voia, Marioara Nicoleta Filimon, Dinu Găvojdian, Ludovic-Toma Cziszter, Estimating growth curves in unweaned lambs through stimulation of rumen function 15th International Multidisciplinary Scientific Geoconference SGEM Conference Proceedings, DOI: 10.5593/SGEM2015/B61/S25.057, ISBN 978-619-7105-42-1 / ISSN 1314-2704, June 18-24, 2015, Book6 Vol. 1, Bulgaria, 419-426.
10. Octavian Sorin Voia, Ioan Padeanu, Dinu Gajojdian, Maria Sauer, Walter Ioan Sauer, Carmen Dragomir, Mihaela Albulescu. Study on Quantity and Quality of Sheep Milk Sampled from Three Areas of Timis County, Animal Science and Biotechnologies, 2016, 49 (1), Timisoara, Romania.
11. Stalker MJ, Hayes MA: The Liver and biliary system. *In*: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, Maxie MG, ed., 5th ed., Vol. 2, p. 339-340. Saunders Elsevier, Philadelphia, PA, 2007
12. Valli VEO: The hematopoietic system. *In*: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, Maxie MG, ed., 5th ed., Vol. 3, p. 254-255. Saunders Elsevier, Philadelphia, PA, 2007.
13. Voia Octavian Sorin, Pădeanu Ioan. Creșterea ovinelor si caprinelor, 2013, Eurobit, Timisoara, 978-973-620-635-1.