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Hepatitis and increased copper levels in a Dalmatian

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Inherited disorders of copper metabolism characterized by increased hepatic copper concentrations have been linked with hepatitis and cirrhosis in many breeds of dogs. References to this syndrome and research has focused on Bedlington terriers, West Highland white terriers, and doberman pinschers.^{2,5,6} Hepatic lesions with corresponding increases in liver copper concentration have been recorded in cocker spaniels, German shepherd dogs, keeshonds, kerry blue terriers, Labrador retrievers, old English sheep dogs, samoyeds, and Skye terriers.^{3,4} Abnormal increases of liver copper concentration (> 400 ppm dry weight [dw]) have not been clearly defined as a cause of liver disease.⁴ Increased liver copper concentration has not been shown to augment preexisting liver disease in any breed other than Bedlington terriers.⁶ Hepatitis with an associated abnormal increase in liver copper has not been described in Dalmatian dogs.

A 2-year-old ovariohysterectomized Dalmatian was presented with acute onset of lethargy, clear vomiting, and pale

mucous membranes. Physical exam revealed 5% dehydration, icterus, and a slight elevation in rectal temperature. Complete blood count, serum chemistry analysis, and urinalysis were conducted.

Total white cell counts were moderately elevated at 22.4×10^9 cells/liter. Serum samples were icteric on subjective visual evaluation. Alkaline phosphatase (ALP) was markedly elevated at 919 IU (upper normal limits [UNL] = 300 IU from in-house clinical laboratory). Alanine aminotransferase (ALT) was also markedly elevated at 1,639 IU (UNL = 67 IU). Total bilirubin (TBIL) was also elevated at 12.18 mg/dl (UNL = 0.5 mg/dl). Elevated ALT was considered suggestive of acute hepatic injury and/or hepatocellular necrosis. Increased levels of ALP and TBIL, in conjunction with clinical icterus, were considered to be related to cholestasis.

Despite initiation of supportive therapy, including intravenous fluids, antibiotics, and vitamin K, the dog continued to deteriorate. Euthanasia was elected, and a gross necropsy was performed. Icterus was diffuse and marked. Prominent hepatomegaly with associated rounding and blunting of liver margins was seen. Hepatic parenchyma was deep yellow-green, and there was an accentuated lobular pattern.

Formalin-fixed liver samples were submitted to the Vet-

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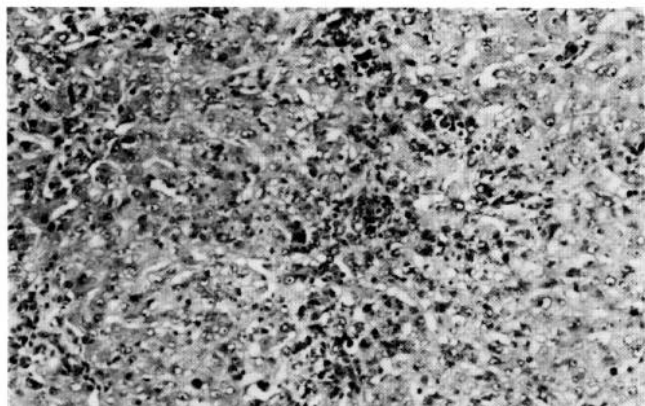


Figure 1. Hepatic lesions in a 2-year-old Dalmatian dog. Small multiple foci of necrosis with infiltrates of macrophages, neutrophils, and rare lymphocytes HE.

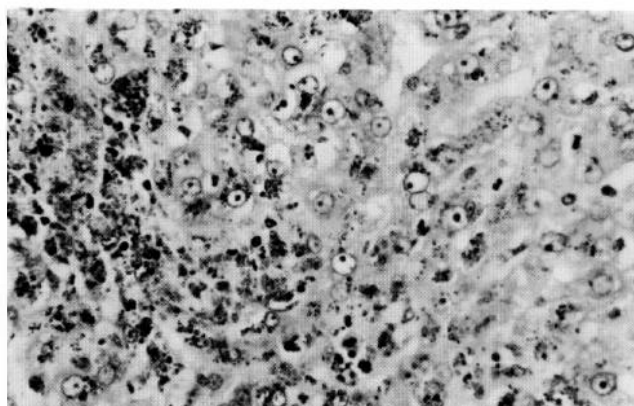


Figure 2. Positive staining of copper granules in the liver of a Dalmatian dog. RHOD.

erinary Diagnostic Center, University of Nebraska-Lincoln, for histopathologic evaluation. Tissues were processed by routine methods and stained with hematoxylin and eosin (HE), Brown & Brenn Gram stain (BB), Van Gieson stain for collagen (VANG), Prussian blue for iron (PBLU), and rhodamine (RHOD) and rubeanic (RUBN) stains for copper.

Multiple random foci of hepatocellular necrosis associated with mixed inflammatory infiltrates were distributed throughout hepatic parenchyma. Inflammatory infiltrates consisted predominantly of macrophages with occasional neutrophils and rare lymphocytes. Multifocal granulomas 5-10 cells in diameter were also observed. Hepatocytes adjacent to necrotic foci often contained coarse cytoplasmic granules or displayed vacuolar change (Fig. 1). Canaliculi were distended with bile. Periportal areas exhibited mild to moderate fibrosis and mild inflammatory infiltrates between connective tissue fibers. Diffuse positive staining for copper by RHOD (Fig. 2) and RUBN was observed. Staining was most prominent in broad bands of centrilobular to midzonal hepatocytes. Periportal hepatocytes also displayed prominent staining in focal areas. No differences in staining intensity between RHOD- and RUBN-treated tissues was noted. Staining with BB, PBLU, and VANG was unremarkable.

Histopathologic findings were suggestive of increased liver copper concentration. Toxicologic analysis of formalin-fixed liver was initiated. Formalin-fixed samples were cut into small pieces and dried for 4 hours at 100 C. Dried liver was divided into 2 portions. Each sample was digested in 1.0 ml of trace mineral-grade nitric acid in a teflon digestion vial at 60 C overnight. Digests were cooled to room temperature and diluted to 10.0 ml with distilled and deionized water. Analysis was performed by inductively coupled argon plasma atomic emission spectroscopy. Results were reported as mean concentration of replicate analyses. Copper levels in samples submitted were 7,940 ppm dw; compatible with an assessment of excessive copper accumulation and confirming the histopathologic interpretation. These findings were compared with those from control liver tissue from an 3-year-old dachshund. Histopathologic changes in this animal consisted of multifocal random hepatitis, severe cholestasis, and

multifocal hepatocellular coagulative necrosis. Comparable staining techniques were negative for copper. Copper concentration in formalin-fixed liver were 47.7 ppm dw.

Hepatic copper concentration > 2,000 ppm dw has been associated with liver pathology in dogs. Other reports indicate that levels as high as 6,800 ppm dw can be observed in normal animals without pathologic change.⁴ The area from which biopsy samples are obtained may explain the differences in copper concentrations found in normal animals.

Cholestasis has been suggested as a potential cause of increased liver copper concentration. Thus, any factors initiating cholestasis could be associated with increases in hepatic copper concentration; however, no published reports have substantiated this theory.⁴ In this study, tissues from an animal of similar age that showed analogous microscopic changes did not have a correlated increase in hepatic copper. Other theories of copper-induced hepatic injury include metallo-binding of hepatic enzymes. Copper binding to cytosolic proteins such as tubulin or to sulfhydryl groups in proteins are also favored theories in human medicine. Formation of free radicals, causing cellular injury,^{1,3} may also play a role.

Whether increased copper levels were a cause or an effect could not be determined. Environmental exposure to excess copper was ruled out based on patient and clinical history. An infectious cause of hepatic injury could not be determined by microscopic examination and histochemical techniques. Results of microscopic findings and toxicology results were well correlated in this case and indicated excess copper as a potential cause of hepatic disease in this Dalmatian.

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***Alaria arisaemoides* in a black Labrador retriever pup**

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In December 1995, a 4-month-old male black Labrador retriever pup was presented for veterinary care with the primary complaint of vomiting and anorexia. The pup was admitted for 24 hours, symptomatically treated,^{a-c} and released. During the next several months, the pup continued to experience poor weight gain and intermittent episodes of vomiting. Clinical signs suggested chronic malnutrition, and a change in diet and supplemental vitamins and minerals were prescribed. On March 13, 1996, a recheck examination revealed flaky skin, easily epilated hair, bilateral carpal varus deformities, generalized weakness, and a normal temperature. A fecal flotation was negative. Abdominal radiographs revealed a radioopaque zone in the anterior duodenum. Di-

agnostic ultrasound demonstrated a hyperechoic duodenal lumen. During laparotomy, mild microhepatica and mild splenomegaly were found. An incision made in the duodenum revealed an aggregate of pyriform cream-colored parasites, each approximately 10 mm in length (Fig. 1). Repeated attempts to clear the parasites from the duodenal lumen by probing with forceps resulted in the extraction of 15-20 parasites. Several parasites and biopsies of the liver, spleen, and duodenum were placed in 10% neutral buffered formalin and submitted to the Veterinary Diagnostic Laboratory at North Dakota State University.

No significant microscopic lesions were seen in the liver, spleen, or duodenum. Initial examination of parasites resulted in a tentative diagnosis of fluke infection, and samples were submitted to the Department of Veterinary Pathology at Iowa State University for definitive identification. The parasites were subsequently identified as *Alaria arisaemoides*, a diplostomatid strigeoid trematode typically found in the intestines of red fox, gray fox, and coyotes. The species was determined from documented descriptions of the organism.^{2,6} Important features in histologic sections of the trem-

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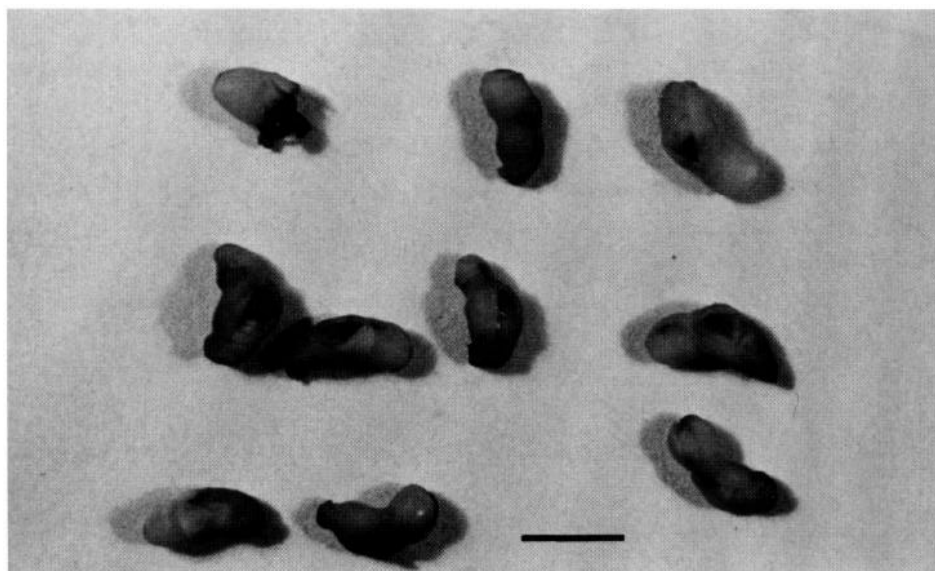


Figure 1. *Alaria arisaemoides*, adult (formalin fixed, contracted state), gross appearance. Bar = 5 mm.