

04/08/2024

Dear Dr. Fortier,

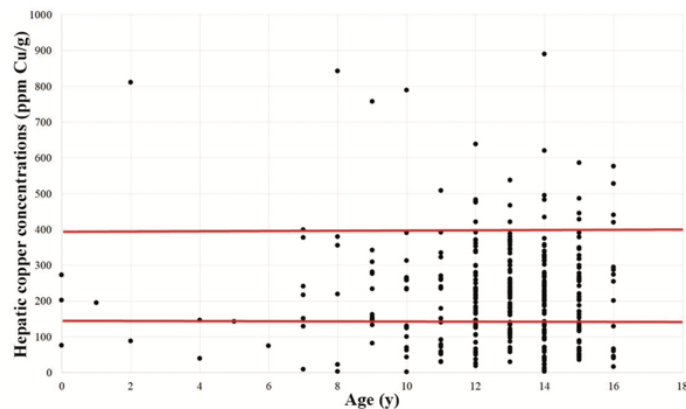
We are ACVIM Small Internal Medicine specialists that have spent much of our professional career diagnosing and treating canine liver disease. We each have dealt personally with hundreds of dogs suffering from copper associated hepatopathies and are considered experts in this area through our publications, research, consulting, lecturing, and managing patients. We were very troubled after reading the recent publication: *Amundson MD, Motsinger LA, Brejda J, Hancock L. Sixteen years of canine hepatic copper concentrations within normal reference ranges in dogs fed a broad range of commercial diets. J Am Vet Med Assoc. 2024 Mar 7:1-6.* As a result of our concerns, we submitted comments in a Letter to the Editor. However, being limited to only 400 words we are further motivated to write you personally to express in more detail specific concerns regarding this publication.

The authors state the objective of this study is to “*examine the effects of age, sex, breed, liver histopathology, and year of death on liver copper concentrations in dogs fed commercial dog foods*”. We believe the real underlying objective was to prove that commercial dog foods are not responsible for increasing hepatic copper concentrations and copper associated hepatopathies. This is even further evident by simply reading their conclusion in the last paragraph where they state “*that although there is an increase in copper concentrations, it is not clinically significant and changes in AAFCO copper recommendations are not resulting in hepatic copper toxicity*”. We would be the first to applaud them if they successfully proved their objective, but they did not. Their experimental of design combining a selected small number of clinical cases with a much larger number of research Beagles is concerning. The hepatic copper quantitation methodology appears flawed, resulting in highly implausible copper concentration values never described before to our knowledge. This, among other things we will point out below, raises serious questions as to the reliability of the data described in this paper. We also believe the statistical approach of the data is naïve and incorrect and we suggest seeking an opinion of a statistician knowledgeable in medical biostatistics. And finally, we find their summary and conclusions to be incorrect and misleading. It is obvious to us the authors have little if any knowledge of copper associated hepatopathies and hepatic copper metabolism. Below are specific bullet point issues leading us to this opinion.

- The study combined dogs from a previous publication<sup>1</sup> and colony dogs (predominately Beagles) from a nutrition research colony. The previous publication of 55 dogs includes a selected group of dogs with liver disease. In that study they evaluated their pathology, hepatic copper content and markers of oxidative damage. The authors included this small group with a research colony of mostly Beagle dogs without evidence of liver disease. We would question the wisdom of including those dogs in this study.
- Archived frozen liver samples were analyzed for hepatic copper content and values expressed on a dry weight (dw) basis. Under Sample Analysis in their paper, they state liver samples were dried for 3-4 hours at 104°C. This short drying time is inappropriate and if samples are not completely dried the remaining moisture contributes to total weight and the measured values will be falsely lower when reported on a dw basis. Most all Veterinary Diagnostic Laboratories we are familiar routinely dry samples for a minimum of 12 hours and some for 24 to 48 hours. We have personally contacted different diagnostic laboratories (KSU, CSU and UC-Davis) and

talked to their directors of toxicology and they all stated a 3-4 hour dry time is inappropriate and that inadequate drying would likely effect the results (we would be happy to provide that documentation). The authors also did not report validation methods using appropriate certified standards. There were also no details given for the analysis of 281 samples, only the 55 samples from the first study. Additionally, the laboratory in which the analysis was performed was not mentioned. We believe there are serious questions concerning the validity of their data.

- Of the 336 dogs, best shown in figure 1 (attached), 38% of the dogs (majority considered to have normal livers) were below the reported normal reference range. Approximately 7% had less than 50 ppm dw with some approaching zero. If the copper measurements were accurate, those with the very low hepatic copper content should be exhibiting signs of copper deficiency. Other publications evaluating dogs with liver disease find a much smaller percentage having copper concentrations below the lower reference interval. It is known liver conditions such as nodular regeneration, significant fibrosis, hepatic atrophy or extensive inflammation will affect copper quantification. A recent study by Ullal et al<sup>2</sup> examining over 4000 hepatic biopsies in dogs showed that only 13% had low copper values and an unpublished survey of hepatic copper determinations at Cummings School of Veterinary Medicine at Tufts University found only 2.3% (7/302) of samples to have values below the reference range. There is no explanation we are aware of why such a high percentage of normal dogs would have such low hepatic copper concentrations and the authors failed to discuss why so many of their cases had copper concentrations below the normal reference range. We strongly believe it is due to erroneous



**Figure 1**—Hepatic copper concentrations of dogs aged < 1 to 16 years old from the years 2006 to 2022. Red lines represent the lower (150 ppm) and upper (400 ppm) limits of the normal copper range.<sup>4,5</sup>

copper quantitation.

- The author reports 27 dogs (8%) had coppers above the normal reference range stating that this is clinically insignificant. This is not insignificant. Abnormal hepatic copper causes liver damage and could lead to liver failure. The authors do not appear to understand this. In the 55 cases from the previous publication that were also included in this study, 2 dogs had hepatic copper > 1000 ppm dw. Looking at Figure 1 shows no dogs with copper above 1000 ppm. Did the authors censor these out? That previous study also had an additional 23 dogs reported to have been analyzed but not used for that study (as stated in their paper under sample analysis). We also wonder why those dogs were not used, were they censored for some reason? We are also concerned that there is no information on liver histology and specific

copper staining (Rhodanine stain) of samples. Most pathologists include histochemical staining for copper as a routine quality check done in conjunction with hepatic copper quantitation. They also did not report on the hepatic tissue procurement methods and specimen sizes, which are known to affect both histologic interpretation and copper quantitation.

- The statistical analysis is unusual with authors looking at age of animals and the year that animals had copper quantitation determined (time of death). Authors determined means with SE in Table 1 and SEM in Table 2. The authors should have tested for normality first, then decided on the best measure of central tendency and dispersion using either mean and SD, or median and percentiles. Most all authors referring to hepatic copper concentrations in publications use mean and SD that shows true variation in each group. This would be more meaningful to the reader. We are not sure why these comparisons were made to copper concentration and a specific age or the year a dog died. In Figure 2 the authors show a tenuous cubic model of hepatic copper concentration to year. The figure makes one wonder if something changed around 2013 in the colony that dramatically changed copper concentrations. The data before that time seem to fit a flat line best. Having such small numbers in the early years (2009-2013) may have skewed the data and compounds the confusion of this figure. If one uses their formula and data and then extrapolate ahead approximately 5 years from 2022, the hepatic copper concentrations would be zero or negative.

- In the discussion the authors suggest that the year of death and copper concentrations they observed reflects dietary trends in the pet food industry suggesting copper chelates, avoidance of cereal grain, GMOs, and glutes being likely responsible. Those suggestions are nonsensical without knowing the diets being fed to the animals in this study. We find it ironic that the diet history was unknown, when the vast majority of dogs were in a controlled housing environment maintained at a major pet food company. They state that “*the majority of the dogs in the present study represented a population being fed various dog foods from the pet food industry*”. The implication is that these dogs are eating common commercial dog foods. Presumably these diets all met AAFCO minimums, but perhaps just barely so (the point is, we have no idea). We should also point out that one of the foods made by this company is a therapeutic diet for liver disease that is designed to have low copper concentrations (Hills l/d). It is very interesting that the author’s do not know what their dogs are eating but they try to extrapolate their findings suggesting things like increased sales in grain free diets are likely responsible. Similarly, they discuss the possible role of mineral-to-mineral interactions, zinc therapy and organic meat ingredients. Again, this is nonsensical as they do not provide any dietary information, and thus, no conclusions about these factors and hepatic copper can be drawn.

- The discussion has many assumptions and implications without any supporting data. The authors even misquoted at least one reference<sup>2</sup> saying Bedlington terriers lack ceruloplasmin, although the reference they use states they have normal or increased ceruloplasmin concentrations. The authors even suggest the reason Labradors have lower copper concentrations is their Beagles had increased energy requirements due to increased activity resulting in higher dietary intake of copper. Would not a better explanation simply be that the dogs with liver disease may be more likely to have hepatic pathology affecting hepatic copper concentrations? It is very evident the authors do not understand liver disease and copper associated hepatopathies. Also in the discussion they say “*Copper-associated hepatopathy is commonly observed in breeds such as Bedlington Terriers, Labrador Retrievers, Doberman*

*Pinschers, and other suspected predisposed breeds due to a defect in copper metabolism.*<sup>35</sup> The reference they used<sup>3</sup> has nothing to do showing a defect in copper metabolism, it is a retrospective clinical report. Even if they did cite the correct manuscripts the genetic defect (ATP7B) has been shown to account for only about 12-13% of the cases of copper associated hepatopathy in Labradors and the COMMD1 mutation is confined only to Bedlington terriers.<sup>4,5,6,7,8</sup>

•They say in the discussion “*changes in AAFCO dietary copper recommendations have not resulted in hepatic copper toxicity*”, but without a dietary history and failure to evaluate dogs prior to changes in AAFCO dietary copper guidelines, this conclusion is unfounded. At most, the only conclusion that can be drawn is that a select population of purpose-bred dogs, 86% of which were Beagles, eating completely unknown diets, had no evidence of copper associated hepatopathy. Additionally, their statement would be only true if one were to believe their hepatic copper quantitation.

•Finally in the discussion they dismiss the fact that 8% of the dogs had increased hepatic copper concentration as clinically insignificant. But if their data is representative, then one would assume 8% of the canine population has abnormally high hepatic copper concentrations. This would represent millions of dogs with high levels of a known hepatic toxin in their liver.

In summary, we hope you understand our concerns regarding this publication and the incorrect implications made in this paper. It is our opinion that because of the questionable experimental design, likely inaccurate copper quantitation, misinformation presented, or implied and many unsubstantiated conclusions this paper should be retracted. We would be happy to discuss this further if you have any specific questions.

Respectfully submitted,

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