

## **Several Flaws in Study Describing Hepatic Copper Concentrations Make Conclusions Unfounded**

As Internal Medicine specialists with expertise in treating dogs with liver disease, we have significant concerns with the recent publication by Amundson.<sup>1</sup> The authors describe hepatic copper concentrations in 336 dogs, indicating 38% had concentrations below the published reference range. Figure 1 shows ~25 dogs (~7%) with copper concentrations <50 ppm, some approaching zero; low enough to cause clinical copper deficiency.<sup>2</sup> In a review of 4559 liver biopsies, other authors found 13% had low hepatic copper, with most having significant liver disease.<sup>3</sup> Subnormal hepatic copper results from copper deficient diets, genetic factors, liver disease, or over-chelation therapy. The most likely explanation for overwhelming low copper concentrations in this series we believe is due to flawed copper quantitation. The short and likely inadequate sample drying time of 4 hours (most laboratories require 12 hours minimum) could explain erroneous low copper values.<sup>4</sup> The authors did not provide specific quantitation details, including biopsy sample size and validation methods using appropriate certified standards. Also lacking are liver histopathological descriptions, including histochemical copper staining for each case.

Another concern is the lack of information on diet histories, despite the dogs being in a colony maintained by a major pet food company. Without this information it is impossible to make any statements as to whether dietary intake had any influence on hepatic copper concentrations. The study also merged 55 dogs from a previous publication<sup>5</sup> combined with 281 dogs from the colony. In that publication, 2/55 dogs had a hepatic copper > 1000 ppm, but in the current publication there were no dogs reported with values this high. Were these cases censored from these data, and if so, why?

Finally, the authors also make several unsubstantiated conclusions. Twenty-seven dogs (8%) had high hepatic copper, yet the authors state that hepatic copper increase is not clinically significant. This is incorrect, and if their data is representative, it implies 8% of the canine population should have abnormally high hepatic copper concentrations that we as clinicians would treat. They also suggest that 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.

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