NOTE

Internal Medicine

Copper-associated hepatitis in a young Dalmatian dog in Japan

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ABSTRACT. A male 25-month-old Dalmatian dog attended our veterinary hospital because of anorexia and high circulating liver enzyme activities. Abdominal computed tomography showed a slightly small liver with rounded edges, and laparoscopic examination showed that the liver was yellowish. Histopathological examination revealed multifocal necrosis of hepatocytes and severe chronic hepatitis. Rhodanine staining showed severe copper accumulation in hepatocytes and a quantitative analysis of the copper content of the liver showed substantial accumulation (10.3 mg/g dry mass), suggesting a diagnosis of copper-associated hepatitis. Previously reported canine mutation in the *COMMD1,* the gene responsible for the copper-associated hepatitis in the Bedlington terrier, was not identified. To our knowledge, this is the first report of copper-associated hepatitis in a Dalmatian in Japan.

KEY WORDS: *COMMD1*, copper, Dalmatian, hepatitis, laparoscopy

Copper is an essential trace element, but it causes severe damage to various organs when it accumulates excessively [4, 7]. Copper-associated hepatitis is a disease in which copper accumulates in the liver because of impaired excretion of copper, resulting in inflammation in the affected organs. In the veterinary field, copper-associated hepatitis is recognized in the Bedlington terrier, and molecular biological research has identified the cause of this disease to be an autosomal recessive defect in exon 2 of the *COMMD1* gene, encoding copper metabolism Murr1 domain-containing protein 1, which is involved in copper excretion [5, 9, 10]. In addition, many other canine pure breeds, such as the West Highland white terrier, Skye terrier, Labrador retriever, and Doberman, have been reported to develop a similar copper-associated hepatitis to that of Bedlington terriers [6, 8, 11, 15–18]. However, the precise mechanism underpinning this disease in breeds other than Bedlington terriers remains to be determined. Furthermore, there have been some reports of copper-associated hepatitis in Dalmatians, although these have been few in number, and have mainly come from North America [1, 2, 12, 13, 19]. To date, no cases of copper-associated hepatitis in the Dalmatian have been reported in Japan. Here, we report a case of copper-associated hepatitis in a young Dalmatian, which was diagnosed on the basis of histopathological examination and quantitative analysis of the copper content of a laparoscopic liver biopsy specimen.

A 25-month-old male Dalmatian, weighing 25.5 kg, was referred to our Animal Medical Center with a loss of appetite and high circulating liver enzyme activities. The dog was bred in Japan according to the pedigree. At the first visit, the patient was in good general condition and showed no abnormalities in appearance. However, blood biochemical testing revealed high circulating aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, and c-reactive protein (CRP) concentration (Table 1). Radiographic examination showed that the dog's liver was slightly small, but revealed no other abnormalities in the abdominal or thoracic cavities. Abdominal computed tomography (CT) was then performed under general anesthesia, with a particular focus on the liver. The liver was considered to be slightly small in size and to have rounded edges (Fig. 1). Contrast-enhanced CT examination confirmed the absence of a portosystemic shunt, on the basis of the courses of the portal veins. In addition, no lesions were identified in other organs on CT examination. Subsequently, an ultrasound-guided liver biopsy was performed, using an 18-G biopsy needle, which provided limited information regarding any liver pathology, because of the small size of the samples obtained. However, the presence of chronic hepatitis with moderate infiltration of lymphocytes and plasma cells

Table 1. Blood testing data at the first admission of the dog and 13 days later

	At first visit	At 13th day	Unit	Normal range
Complete clood count				
RBC	894	852	×10⁴/µl	630−880
Hb	20.8	19.8	g/dl	13.0−19.0
PCV	58	56	%	40−56
TP	7.2	7.2	g/dl	6.0−8.0
PLT	17.1	16.2	×10⁴/µl	20−50
WBC	8,750	17,880	/µl	6,000−17,000
Blood biochemical test				
BUN	24.1	15.9	mg/dl	9.2−29.2
Cre	1.23	1.29	mg/dl	0.4−1.4
AST	369	1,887	U/l	17−44
ALT	524	2,146	U/l	17−78
ALP	337	2,309	U/l	47−254
Alb	4.0	3.6	g/dl	2.6−4.0
T-Bil	0.8	2.2	mg/dl	0.1−0.5
Glu	104	105	mg/dl	75−128
Na	151	153	mEq/l	141−152
K	4.6	3.6	mEq/l	3.8−5.0
Cl	117	108	mEq/l	102−117
CRP	2.25	6.9	mg/dl	0−1.0
Coagulation system test				
PT		8.2	sec	6.1−9.6
APTT		37.5	sec	8.7−20.6
Fibrinogen		94.5	mg/dl	178−480
ATIII		71.2	%	116−161

RBC: red blood cell, Hb: hemoglobin, PCV: packed cell volume, TP: total protein, PLT: platelet, WBC: white blood cell, BUN: blood urea nitrogen, Cre: creatinine, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, Alb: albumin, T-Bil: total bilirubin, Glu: glucose, CRP: C-reactive protein, PT: prothrombin time, APTT: activated partial thromboplastin time, ATIII: antithrombinIII.

was suggested, and no pathogens or neoplastic changes were observed.

To perform a more detailed assessment of the dog's liver, laparoscopic liver biopsy was scheduled 13 days after the first visit. The general condition of the dog on the day of the laparoscopy was good, but ALT and AST activities had increased, as had the serum concentrations of total bilirubin and CRP. In addition, blood coagulation testing showed a coagulopathy, indicated by a high activated partial thromboplastin time (APTT) and a low antithrombin III (ATIII) concentration, which was likely because of progression of the dog's hepatic disease (Table 1).

Laparoscopic liver biopsy was performed under general anesthesia, after preparation for routine abdominal surgery and the induction of pneumoperitoneum. After a camera port was prepared in the abdominal wall, a laparoscopic examination, focusing on the liver, was performed using a camera inserted *via* the port. This showed that all the lobes of the dog's liver were yellowish in color (Fig. 2A), and had rounded edges and irregular surfaces (Fig. 2B). To facilitate a detailed histopathological assessment, several tissue samples were obtained from the liver using a 5-mm cup-shaped tissue biopsy device that was inserted through another abdominal port. At that time, the liver appeared to be firmer than normal. Hemostasis from the biopsy site was unremarkable. Some of the collected samples were formalin-fixed and used for examination of HE staining in a routine manner, and rhodanine copper staining was also performed. Other biopsy samples were immediately frozen and prepared for the quantitative analysis of copper content, because some young Dalmatians have previously been reported to have copper-associated hepatitis. The serum concentration of copper was also measured. The postoperative recovery of the dog was unremarkable; therefore, it was discharged the day after the laparoscopy. However, the dog suddenly deteriorated on the day following discharge and the dog died at home. Necropsy could not be performed.

Histopathological examination of the liver biopsy specimens revealed multifocal areas of necrosis mainly observed in the centrilobular region and individual necrotic hepatocyte scattered throughout the sections (Fig. 3A, 3B). The necrotic foci contained various numbers of inflammatory cells that were consistent with macrophages, lymphocytes, plasma cells, and neutrophils, and bile duct proliferation was often observed. Occasionally, there was mild to moderate interlobular or central-portal bridging fibrosis. Furthermore, in some areas, there was an almost complete loss of hepatocytes: the lobular structures had collapsed and were replaced with cellular debris, inflammatory cells, and proliferated bile ducts (Fig. 3C). Rhodanine staining revealed diffuse, moderate-to-marked copper accumulation within hepatocytes and macrophages. The copper accumulation was observed diffusely and was often more pronounced in centrilobular regions. (Fig. 3D). This copper accumulation was more conspicuous in individual
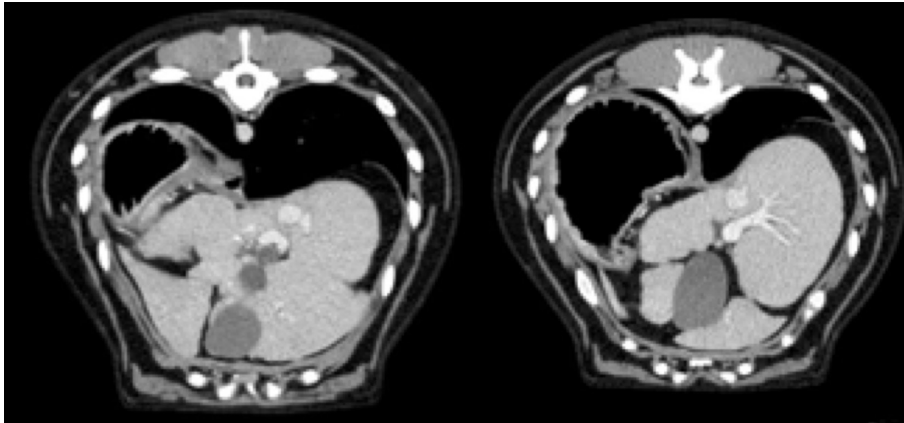
Fig. 1. Abdominal computed tomography findings in the dog at its first admission. The liver was slightly small in size and had rounded edges, but no other abnormalities were identified.
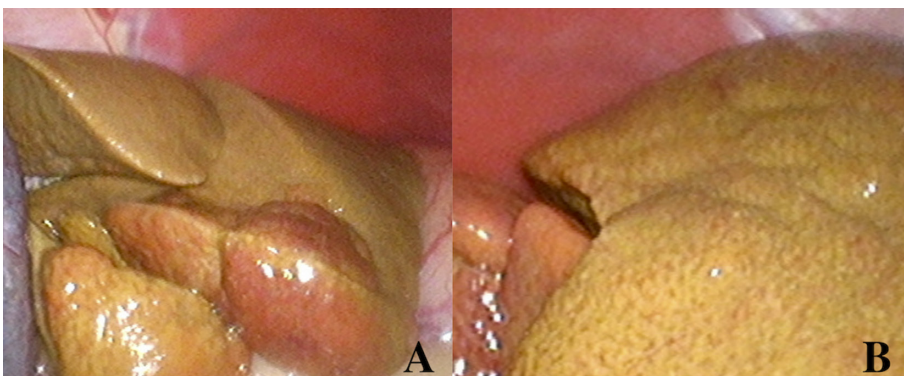

Fig. 2. Laparoscopic findings in the liver of the patient. The liver was yellowish in color and had rounded edges (A), and irregular surfaces (B) throughout the all lobes.
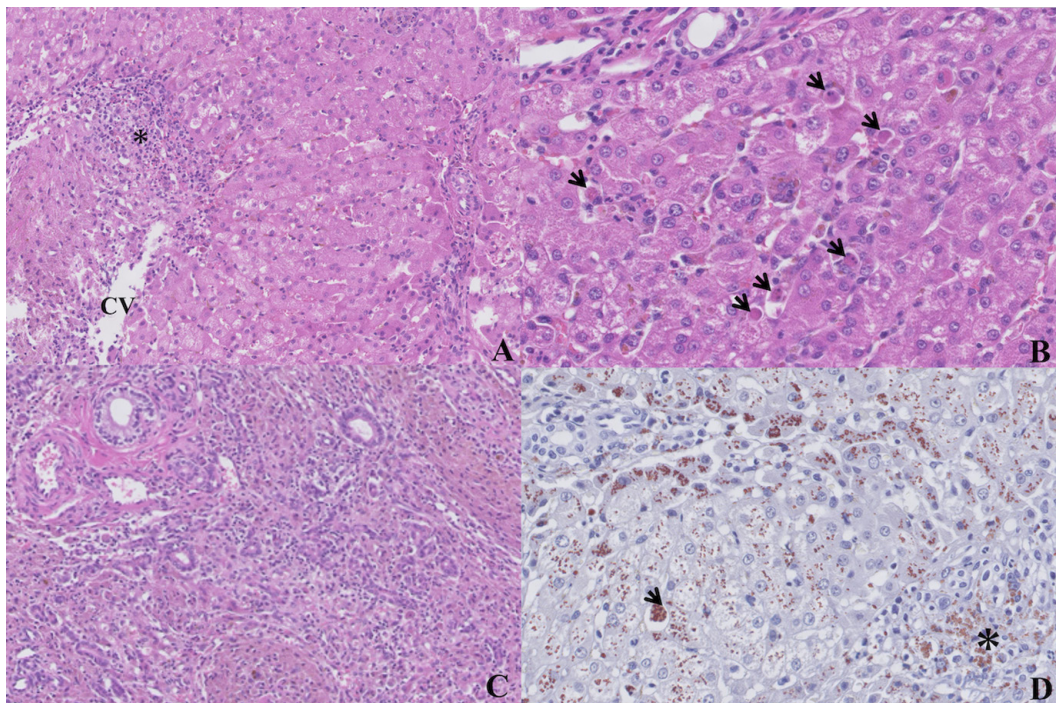

Fig. 3. Histopathological findings in liver biopsy samples. A: An area of necrosis around central vein (CV), which contains cellular debris and inflammatory cells (*) ×200, hematoxylin and eosin (HE). B: Many individual necrotic hepatocytes (arrows) composed of eosinophilic cytoplasm and often nuclear debris, which are scattered throughout a section. ×400, HE. C: Another section demonstrates an almost complete loss of hepatocytes. Collapsed lobules are replaced with cellular debris and inflammatory cells, including macrophages, lymphocytes, plasma cells, and neutrophils. Proliferated bile ducts are also observed. ×200, HE. D: Moderate-to-marked brown globular staining within hepatocytes and macrophages, indicating copper accumulation. Copper accumulation is more conspicuous in individual necrotic hepatocytes (arrow) and macrophages infiltrating necrotic foci (*). ×400, rhodanine stain.

necrotic hepatocytes and macrophages infiltrating the necrotic foci, and the cellular debris and macrophages filling the collapsed lobules showed marked copper accumulation. Semiquantitative evaluation of hepatic copper concentration with rhodanine staining revealed Grade 4–5 copper accumulation (grading scale of 0 to 5) [7].

Several frozen liver tissue samples were used for the quantitative analysis of copper content. The copper concentration in the liver tissue was determined in dry-ashed liver samples by atomic absorption analysis, which yielded an extremely high level of copper accumulation (10.3 mg/g dry mass of the liver; normal range: <400–500 µg/g dry mass). The serum copper concentration was 246 µg/dl, which was above the normal range (66–130 µg/dl).

A multiplex polymerase chain reaction (PCR) assay was performed to detect a deletion of exon 2 in the *COMMD1* gene associated with copper-associated hepatitis in Bedlington terrier, which was described previously, with some modifications [5]. Briefly, genomic DNA was extracted from the frozen liver samples using the QIAamp DNA Mini Kit (QIAGEN, Tokyo, Japan). The PCR amplification was performed using KOD FX DNA polymerase (TOYOBO, Tokyo, Japan) under the following reaction condition: 95°C for 10 min, followed by 35 cycles of 95°C for 30 sec, 30 sec at the annealing temperature and 72°C for 60 sec, and final elongation stage of 72°C for 10 min with PCR primers previously described. The PCR products were visualized on a 2% agarose gel. As a result, PCR products derived from a part of the intron present in the deleted region of the copper-related hepatitis in the Bedlington terrier were present, indicating that the *COMMD1* mutation similar to that of affected Bedlington terriers did not occur. In addition, the multiplex PCR showed that the *COMMD1* gene in the present case was neither mutant homozygous nor heterozygous. On the basis of these results, the Dalmatian was diagnosed as copper-associated hepatitis; however, the precise etiology of the case remained unclear, because the previously reported canine mutation in the *COMMD1* gene was not identified.

In small animal practice, copper-associated hepatitis in the Bedlington terrier is a recognized disease. Recently, genetic analysis of the disease in the Bedlington terrier has been carried out and the causative gene has been identified [5]. However, although copper-associated hepatitis in other breeds has also been reported, these reports have been sporadic, and in the Dalmatian, the disease has mainly been reported in North America [2, 12, 13, 19]. A large study of canine hepatitis in the United Kingdom also included several cases in Dalmatians, which were characterized by their onset at an early age [1]. In Japan, there is fragmentary information regarding this disease and no case reports have been published. To our knowledge, the present report represents the first clinical case report of copper-associated hepatitis in Dalmatians in Japan.

In the young Dalmatian reported herein, chronic hepatitis and necrotic foci around the central veins were observed histologically. These findings were highly compatible with the previously reported histopathological features of copper-associated hepatitis [2, 7, 13, 15, 17]. The clinically observed smaller liver may be due to areas of lobular necrosis and collapse, and the firmer appearance of the liver could be caused by chronic fibrosis. In addition to these pathological findings, significant copper accumulation, exceeding 10 mg/g dry mass, was confirmed in the liver. The quantification of copper in the livers of nine previous cases of copper-associated hepatitis in Dalmatians showed a mean copper concentration of 3.20 mg/g dry mass and a maximum of 8.39 mg/g dry mass [19]. Thus, the present case demonstrated an extremely high concentration of copper in its liver, which might be sufficient to induce severe hepatic failure and be fatal. Furthermore, the clinical features of Dalmatians with copper-associated hepatitis are that it develops at an early age, that gastrointestinal symptoms, such as vomiting and diarrhea, are the main clinical signs, that these clinical signs worsen rapidly, and that a large amount of copper accumulates in the liver [1, 2, 12, 13, 19]. The clinical features of the present case and its disease course are highly compatible with the clinical characteristics of the disease that have been reported to date in Dalmatians.

Two possible mechanisms of copper accumulation in the liver tissue have been proposed [7]. One is a genetic abnormality that leads to an impairment in copper excretion, which typically occurs in Bedlington terriers. The other is a secondary accumulation of copper in the liver as a result of a liver disease that causes impaired copper excretion. The precise cause of the substantial copper accumulation in the liver in the present case remains unclear, and previous reports have rarely mentioned the causes of copper-associated hepatitis of Dalmatians. The onset of disease in the present case was at an early age, and a significant amount of copper accumulated in the liver. In addition, centrilobular distribution of copper accumulation was observed, which suggested a primary copper accumulation [7]. Therefore, it is unlikely that it was secondary to another liver disorder. Because a genetic abnormality was suspected to be involved in the present case, we searched for mutations in *COMMD1*, but the mutation previously reported in Bedlington terrier was not identified. Although our genetic investigation was limited to *COMMD1*, copper turnover is strictly regulated by many proteins, and a mutation in *ATP7B* is known to be responsible for copper accumulation in the liver of humans, causing Wilson's disease [4, 7]. Furthermore, recent genetic investigations in the veterinary field have shown that mutations in the *ATP7B* gene are involved in copper accumulation in the livers of Labrador retrievers [3, 14, 20]. The precise cause of copper-related hepatitis in Dalmatians remains to be determined; therefore, future studies should aim to identify the causative genes.

The affected dog died the day after laparoscopy. The cause of death remained unclear, but it may be due to the progression of severe liver failure, abdominal hemorrhage due to coagulopathy and other various unforeseen causes. In this case, such a sudden deterioration could not be expected at discharge, but more careful and long-term hospitalization may have been required for animals with such severe underlying illness.

Some reports of copper-associated hepatitis in Dalmatians have shown that this disease has been demonstrated to be familial [12, 19]. As the dog in this case had been bred in Japan, it is possible that further cases will be recorded. At present, no similar disease in Japan has been reported to our knowledge; however, if a disease similar to this case is found, it is necessary to conduct a familial survey based on the pedigree, identify the carrier of this disease, and strictly manage the breeding. If the carrier can be further identified, it may be necessary to investigate the causative gene in Dalmatian in detail.

CONFLICT OF INTEREST. None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

REFERENCES

1. Bexfield, N. H., Buxton, R. J., Vicek, T. J., Day, M. J., Bailey, S. M., Haugland, S. P., Morrison, L. R., Else, R. W., Constantino-Casas, F. and Watson, P. J. 2012. Breed, age and gender distribution of dogs with chronic hepatitis in the United Kingdom. *Vet. J.* **193**: 124–128. [Medline] [CrossRef]
2. Cooper, V. L., Carlson, M. P., Jacobson, J. and Schneider, N. R. 1997. Hepatitis and increased copper levels in a dalmatian. *J. Vet. Diagn. Invest.* **9**: 201–203. [Medline] [CrossRef]
3. Dirksen, K., Spee, B., Penning, L. C., van den Ingh, T. S. G. A. M., Burgener, I. A., Watson, A. L., Groot Koerkamp, M., Rothuizen, J., van Steenbeek, F. G. and Fieten, H. 2017. Gene expression patterns in the progression of canine copper-associated chronic hepatitis. *PLoS One* **12**: e0176826 [CrossRef]. [Medline]
4. Fieten, H., Leegwater, P. A. J., Watson, A. L. and Rothuizen, J. 2012. Canine models of copper toxicosis for understanding mammalian copper metabolism. *Mamm. Genome* **23**: 62–75. [Medline] [CrossRef]
5. Forman, O. P., Boursnell, M. E., Dunmore, B. J., Stendall, N., van den Sluis, B., Fretwell, N., Jones, C., Wijmenga, C., Rothuizen, J., van Oost, B. A., Holmes, N. G., Binns, M. M. and Jones, P. 2005. Characterization of the *COMMD1 (MURR1)* mutation causing copper toxicosis in Bedlington terriers. *Anim. Genet.* **36**: 497–501. [Medline]
6. Haywood, S., Rutgers, H. C. and Christian, M. K. 1988. Hepatitis and copper accumulation in Skye terriers. *Vet. Pathol.* **25**: 408–414. [Medline] [CrossRef]
7. Hoffmann, G. 2009. Copper-associated liver diseases. *Vet. Clin. North Am. Small Anim. Pract.* **39**: 489–511. [Medline] [CrossRef]
8. Hoffmann, G., van den Ingh, T. S., Bode, P. and Rothuizen, J. 2006. Copper-associated chronic hepatitis in Labrador Retrievers. *J. Vet. Intern. Med.* **20**: 856–861. [Medline] [CrossRef]
9. Kim, Y. G., Kim, S. Y., Kim, J. H., Lee, K. K. and Yun, Y. M. 2016. Prevalence and clinical relevance of exon 2 deletion of *COMMD1* in Bedlington terriers in Korea. *J. Vet. Intern. Med.* **30**: 1846–1850. [Medline] [CrossRef]
10. Lee, S. A., Fillipich, L. J. and Hyun, C. 2007. Prevalence of the exon 2 deletion of the COMMD1 gene in Australian Bedlington terriers. *J. Genet.* **86**: 289–291. [Medline] [CrossRef]
11. Mandigers, P. J., van den Ingh, T. S., Bode, P., Teske, E. and Rothuizen, J. 2004. Association between liver copper concentration and subclinical hepatitis in Doberman Pinschers. *J. Vet. Intern. Med.* **18**: 647–650. [Medline] [CrossRef]
12. Napier, P. 1996. Hepatic necrosis with toxic copper levels in a two-year-old Dalmation. *Can. Vet. J.* **37**: 45. [Medline]
13. Noaker, L. J., Washabau, R. J., Detrisac, C. J., Heldmann, E. and Hendrick, M. J. 1999. Copper associated acute hepatic failure in a dog. *J. Am. Vet. Med. Assoc.* **214**: 1502–1506, 1495. [Medline]
14. Pindar, S. and Ramirez, C. 2019. Predicting copper toxicosis: relationship between the ATP7A and ATP7B gene mutations and hepatic copper quantification in dogs. *Hum. Genet.* **138**: 541–546. [Medline] [CrossRef]
15. Rifkin, J. and Miller, M. D. 2014. Copper-associated hepatitis in a Pembroke Welsh corgi. *Can. Vet. J.* **55**: 573–576. [Medline]
16. Smedley, R., Mullaney, T. and Rumbeiha, W. 2009. Copper-associated hepatitis in Labrador retrievers. *Vet. Pathol.* **46**: 484–490. [Medline] [CrossRef]
17. Thornburg, L. P., Shaw, D., Dolan, M., Raisbeck, M., Crawford, S., Dennis, G. L. and Olwin, D. B. 1986. Hereditary copper toxicosis in West Highland white terriers. *Vet. Pathol.* **23**: 148–154. [Medline] [CrossRef]
18. van De Sluis, B., Rothuizen, J., Pearson, P. L., van Oost, B. A. and Wijmenga, C. 2002. Identification of a new copper metabolism gene by positional cloning in a purebred dog population. *Hum. Mol. Genet.* **11**: 165–173. [Medline] [CrossRef]
19. Webb, C. B., Twedt, D. C. and Meyer, D. J. 2002. Copper-associated liver disease in Dalmatians: a review of 10 dogs (1998–2001). *J. Vet. Intern. Med.* **16**: 665–668. [Medline]
20. Wu, X., den Boer, E. R., Vos-Loohuis, M., Steenbeek, F. G. V., Monroe, G. R., Nijman, I. J., Leegwater, P. A. J. and Fieten, H. 2020. Investigation of genetic modifiers of copper toxicosis in Labrador retrievers. *Life (Basel)* **10**: 266. [Medline]