Liver trouble

By Catherine Harvey

The diagnosis and treatment of canine chronic hepatitis.

An 11-year-old female spayed Huntaway dog had a history of several years of worsening hind-quarter lameness with weakness and falling, and a pain response on extension of her left hip, especially after a lot of exercise. On diagnosis of arthritis (chronic degenerative joint disease) the dog had been occasionally treated with Carprieve (carprofen, Norbrook Laboratories). On a worsening of signs and increased conscious proprioception deficits/weakness in the left compared to the right hip, pain relief and daily treatment with Carprieve was initiated.

Blood work carried out in the veterinary clinic demonstrated mildly increased serum alanine aminotransferase (ALT) enzyme activity and normal serum alkaline phosphatase (ALP) activity.

ALT	344 IU/L	(reference interval: 10–125 IU/L)
ALP	163 IU/L	(reference interval: 23–212 IU/L)

On a recheck one month later, the dog was anorexic, lethargic and depressed. In-clinic blood testing indicated significantly elevated liver activities and hyperbilirubinemia.

ALT	unable to read	(reference interval: 10–125 IU/L)
ALP	518 IU/L	(reference interval: 23–212 IU/L)
GGT	17 IU/L	(reference interval: 0-11 IU/L)
Bilirubin	53.0 umol/L	(reference interval: 0-15 umol/L)

On abdominal ultrasound the liver appeared sonographically normal, with mild sludge in the gall bladder. A fine needle aspirate of the liver was taken for cytology. Cytological examination showed hepatocellular vacuolar degeneration, possible mild-mixed inflammation and mild hepatocellular atypia. Histopathology was recommended to rule in or rule out inflammation and better determine the severity and microanatomic location.

Biochemistry and haematology testing was performed at Gribbles Veterinary prior to surgery, and confirmed marked increased serum ALT activity – ALT 831 IU/L (reference interval 0-88 IU/L). Thrombin clotting time was mildly increased – 6.9 sec (4.0–6.0 normal).

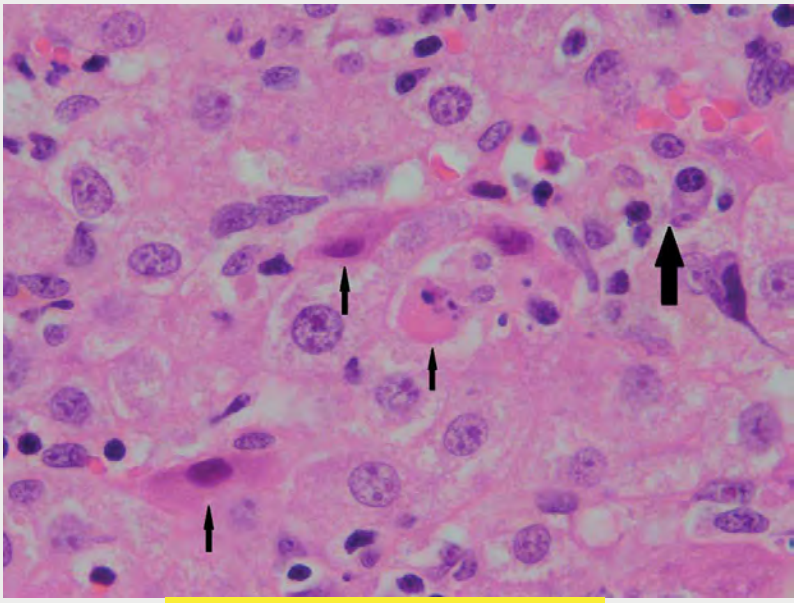

FIGURE 1: Liver H&E stain, 1,000x. Small arrows: apoptotic/necrotic hepatocytes; large arrow: inflammatory cells.

Thrombin clotting is generally used to assess fibrinogen, and prolongation may be seen with liver failure, disseminated intravascular coagulation, marked hyperglobulinaemia or heparin therapy. Prothrombin time (PT) and activated partial thrombin time (aPTT) were both normal.

At surgery, the right medial liver was swollen and all of the liver appeared hyperaemic and inflamed. The gall bladder appeared distended. Punch biopsies (5–7mm) were taken from the right and left liver lobes for culture and histopathology, and an aspirate of bile was taken for bacterial culture.

There was no growth on the aerobic culture of the bile and liver samples.

On histopathology of the liver biopsies, there was a moderate, chronic-active, necrotising hepatitis, which had multifocal individual hepatocellular necrosis, as well as areas of bridging centrilobular hepatocellular necrosis, dropout, fibrosis and small-to-moderate infiltrates of lymphocytes, plasma cells, neutrophils and Kupffer cells. Hepatocytes had mild-to-moderate anisocytosis and anisokaryosis, and areas of nodular regeneration. Histological activity/grading for hepatitis and necrosis was mild to moderate (grade 2–3). Activity/grade ranged from absent (grade 0) to very marked (grade 5).

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In a special stain for copper (rhodamine copper stain) there was a small amount of copper pigment in the cytoplasm of small groups of hepatocytes in centrilobular areas and in areas of nodular regeneration = score 2+.

The copper scoring system ranges from 0 to 5+. Scores of 0 to 2+ are observed in normal dogs. Scores of 3, 4 and 5+ have been associated with pathological copper accumulation and copper-associated hepatitis.

In a special stain for collagen/fibrosis (Mason's trichrome stain) there was a mild amount of collagen (fibrosis) in the areas of hepatocellular dropout and in periportal areas. The degree of fibrosis was classified as mild (stage 2).

Degree of fibrosis ranges from absent (stage 0) to very marked (stage 4) (van den Ingh et al., 2021; Vince et al., 2014).

The dog (figure 4) was taken off carprofen and is improving (doing well, eating and has good energy). Follow-up liver biochemistry testing carried out at Gribbles Veterinary showed improvement.

ALT	546 IU/L	(reference interval 0-88)
ALP	838 IU/L	(reference interval 0-87)
Bilirubin	5.5 umol/L	(reference interval 1.0-3.0)

Discussion:
Carprofen is a non-steroidal anti-inflammatory drug indicated for the relief of pain and inflammation associated with osteoarthritis and for the control of postoperative pain associated with soft tissue and orthopaedic surgeries in dogs. Carprofen is eliminated in the dog primarily by biotransformation in the liver followed by excretion in the faeces and urine. Some enterohepatic circulation is observed. Contraindications are in dogs exhibiting previous hypersensitivity to carprofen. Adverse hepatic reactions include inappetence, vomiting, jaundice, acute hepatic toxicity, hepatic enzyme elevation, and abnormal liver function tests, especially in Labrador Retrievers (Norbrook, 2023).

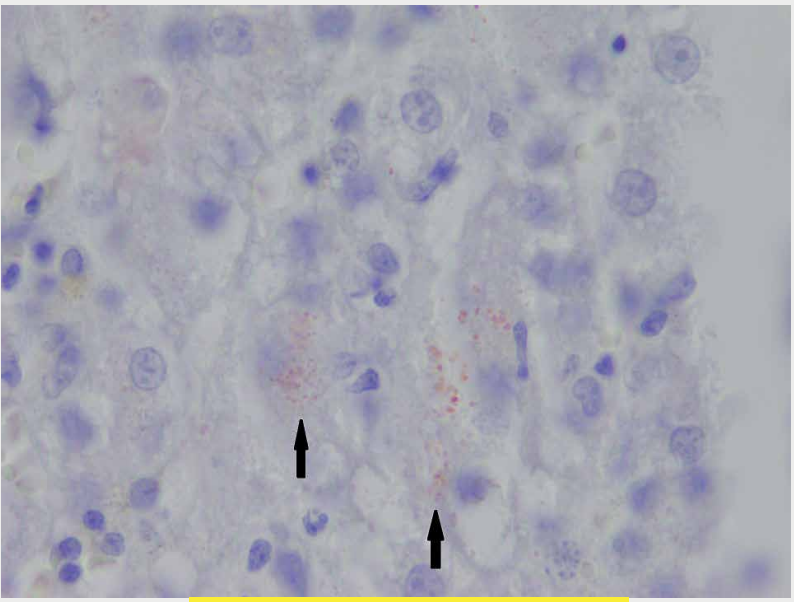

FIGURE 2: Liver rhodamine copper stain 1,000x. Arrows: copper pigment.

The diagnosis and treatment of chronic hepatitis (CH) in the dog is a complex process that requires an integration of clinical presentation and clinical pathology, diagnostic imaging and hepatic biopsy. Primary hepatopathies are typically accompanied by evidence of hepatocyte necrosis/apoptosis, as well as varying degrees of ductular proliferation and fibrosis. The key histologic features include the presence of lymphocytic, plasmacytic or granulomatous inflammation (portal, multifocal, zonal or panlobular) and some combination of these along with hepatocyte cell death and variable severities of fibrosis and regeneration. Although there is evidence for infectious, metabolic, toxic and immune causes of CH, most cases in the dog are classified as idiopathic.

Several drugs and toxins have been implicated in causing liver injury. Strong evidence indicates that treatment with phenobarbital, primidone, phenytoin and lomustine can result in CH. Several other drugs or toxins, including carprofen, oxibendazole, amiodarone, aflatoxin and cycasin, may lead to CH. In humans, herbal and dietary supplements can cause drug-induced liver injury.

The most common toxic injury causing CH in dogs is a consequence of hepatic copper excess, which may develop in any breed, including mixed breeds, but the Bedlington Terrier, Dalmatian, Labrador Retriever, Doberman Pinscher and West Highland White Terrier are predisposed. Infectious aetiologies are an uncommon cause of CH; however, a search for an infectious agent should be undertaken in cases with pertinent clinical findings or when there is pyogranulomatous hepatitis.

A definitive diagnosis of copper-associated CH in all breeds requires a liver biopsy and copper quantitation. Excessive dietary copper intake strongly influences hepatic copper accumulation in both predisposed and non-predisposed breeds. Every liver biopsy should be evaluated for abnormal hepatic copper, as this is a common cause of liver injury and is treatable.

Immune-mediated CH is presumed to occur in dogs; however, a careful search for a primary underlying aetiology should be undertaken before instituting immunosuppressive therapy for suspected immune-mediated CH.

Persistent (>2 months) unexplained increases in serum ALT activity, with or without other laboratory changes, is the

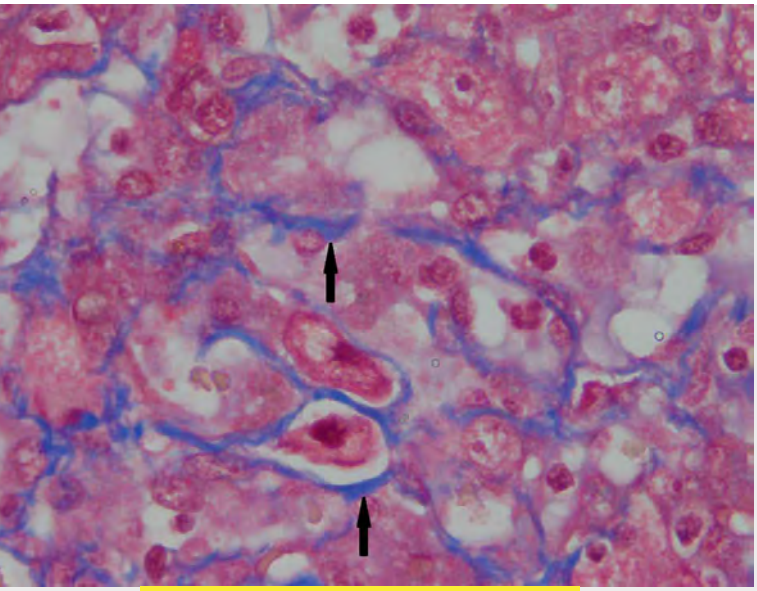

FIGURE 3: Liver Mason's trichrome stain 1,000x. Arrows: collagen.

best screening test currently available for early detection of CH. However, histopathologic evidence of CH can exist in the absence of increased serum liver enzyme activity. Increased serum ALP activity occurs later in CH. If both ALT and ALP activities are increased, the magnitude of ALT increase often exceeds that of ALP. As CH progresses and hepatic parenchyma decreases, ALP and gamma-glutamyl transpeptidase (GGT) activities increase compared to ALT. In late-stage cirrhosis, transaminases may decrease with parenchymal loss. Hyperbilirubinemia is reported in approximately 50% of dogs with CH and is a negative prognostic indicator. Total serum bile acid concentrations are the most sensitive hepatic function test for CH. However, their sensitivity, particularly for early-stage disease, is inadequate, which makes them poor screening tests for CH and thus they should not be used as the basis for deciding to pursue hepatic biopsy.

Abdominal ultrasound is the most useful and informative imaging modality for dogs with suspected CH, but it is highly operator dependent, its sensitivity is low and no changes are pathognomonic or diagnostic for CH. Ultrasound helps to identify complications associated with CH, such as acquired portosystemic shunts, ascites, splanchnic thrombi and gastrointestinal ulceration. Although coagulation parameters are unreliable indicators of the risk of haemorrhage after liver biopsy, they are recommended to be obtained before hepatic sampling to assess for high-risk patients. In dogs considered high risk for bleeding complications (PT/PTT > 1.5× the upper limit of normal, platelets <50,000/uL, fibrinogen concentration <100 µg/dL and/or packed cell volume < 30%), clinicians should exercise caution.

Laparoscopy is the method of choice for liver biopsy in dogs with suspected CH, as this minimally invasive method enables gross evaluation of the liver, extra-hepatic biliary system and adjacent structures, and the safe acquisition of large, targeted biopsies from multiple liver lobes. Laparotomy has similar advantages and disadvantages, but is considerably more invasive with greater postoperative pain and recovery time.

A minimum of five laparoscopic or surgical biopsies from at least two liver lobes should be obtained for histopathology, aerobic/anaerobic culture and quantitative copper analysis. Ultrasound-guided hepatic biopsy is least invasive, but small sample sizes can compromise diagnostic accuracy. Accuracy is increased with a larger gauge needle (14 or 16) and the obtaining of biopsies from multiple sites, but the latter carries a greater risk of post-biopsy haemorrhage.

FIGURE 4: The dog in this case study enjoying life.

The diagnosis of CH requires histopathologic evaluation of hepatic biopsy. Hepatic fine needle aspirate and cytology cannot make a definitive diagnosis of CH.

Hepatic biopsy interpretation should include a scored (mild, moderate or severe) evaluation of type and degree of inflammation/degeneration (grade) and fibrosis/nodularity (stage), as well as an evaluation of the copper staining pattern and a semiquantitative score of copper staining intensity.

The presence of (pyo)granulomatous inflammation should prompt a search for an infectious aetiology. An exchange of information between clinician and pathologist optimises biopsy interpretation.

Factors with the strongest association with poor prognosis are hyperbilirubinemia, prolongations in PT and aPTT, hypoalbuminemia, the presence of ascites, and the degree of fibrosis on biopsy (Webster et al, 2019; Willard, 2015). VS

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