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Copper associated hepatitis

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Budapest
2023

I hereby confirm that I am familiar with the content of the thesis entitled

Copper associated chronic hepatitis

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ÁLLATORVOSTUDOMÁNYI EGYETEM Belgyógyászati Tanszék és Klinika 1787 Tanszékvezető BUDAPEST

INTERNAL MEDICINE

Department

Thesis progress report for veterinary students

Name of student: Lina Hedman-Borg

Neptun code of the student: CUZCH9

Name and title of the supervisor: Dr. Agnes Sterczer PhD

Department: Internal Medicine

Thesis title: Copper caused chronic hepatitis

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	11	11	Discussion of the topic	
2.	2022	12	03	Literature suggestion	
3.					
4.					
5.					

Grade achieved at the end of the first semester: .....

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	03	21.	Discussion of the structure of the thesis	
2.	2023	05.	16.	outline of the thesis	
3.	2023	09.	12.	Consultation of the thesis	
4.	2023	10	09	Correction of thesis	

5.					

Grade achieved at the end of the second semester: 4

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

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signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: Katalin-Sólyom Renáta

Date of handing the thesis in: 10. 11. 2023

Content

Abstract

Chronic hepatitis (CH) is a group of inflammatory-necrotizing liver diseases with clinical and biochemical hepatocellular dysfunction. It can be characterized by periportal mononuclear or mixed cell typed inflammatory infiltration and periportal necrosis and fibrosis. CH in dogs is a quite common condition that can be caused by many different reasons, all from ingestion of toxic substances, different drugs, viruses, bacteria, immune-mediated and the accumulation of copper. Copper is a trace element that is mainly ingested with the food the dogs eat. The liver is the main place where copper is stored and it is then excreted by the bile. As the copper concentration gets higher than what the hepatocytes can transport and the bind, free copper will cause oxidative stress damage which will lead to hepatocellular degeneration and necrosis, this will the lead to acute or chronic inflammation of the liver, or sometimes both. The diagnostic criteria of copper-caused chronic hepatitis (CuCH) are histological evidence of CH and centrilobular Cu accumulation; positive histochemical Cu staining (rhodanine) and Cu concentration > 1000 ug/g dw liver (ppm).

Primary copper accumulation inside the liver is a pathological condition and is caused by a failure in the copper excretion into bile, this will lead to an increased copper concentration inside the liver especially centrilobular. In primary CuCH the progressive Cu accumulation resulting from failure of normal hepatic biliary excretion in transport from lysosomes to biliary tract and this leads to chronic hepatitis.

There is also a secondary form of the copper associated hepatitis. This form is often due to cholestasis which can be caused by an intrahepatic or an extrahepatic disease and the Cu accumulates mostly periportally and not throughout the life.

The copper associated hepatitis can be seen in many different dog breeds but there are some breeds that are predisposed for the disease, especially Bedlington terrier, Labrador retrievers, Dalmatians, Doberman pinschers, and West Highland White Terrier.

In my thesis I have focused on to map out why these breeds are more prone for the disease, what are the pathophysiological reasons behind it. I have focused on trying to find the reason behind it and if there are any differences between the breeds, that could explain the disease a bit more in detail.

When it comes to Bedlington Terriers and Labrador retriever, these are the dog breeds where most studies and research have been made. A primary form of the disease has been seen in both these dog breeds and evidence have been found that there is an inheritable defect on several genes. These genes are a part of different proteins that participate or influence the copper excretion from the liver and blood. In other dog breeds like Dalmatians and West Highland White Terrier the research done can also prove that the primary form of the disease can be seen in them as well, but due to the low number of studies made the result can not exactly tell us the background. In Dobermann pinscher the published studies mainly show that there is a secondary form of the disease, caused by another pathological condition inside the liver.

Abnormal copper concentration in the liver can come from altered copper excretion into the bile, and or excessive dietary Cu intake. Beginning in the late 1990s, there were a growing number of CH cases. This increase is related to the change in the premixes used to add copper in commercial dog food, which increased the amount of accessible copper in diets.

1. Introduction

Definition of chronic hepatitis is an inflammation of the liver that can be caused by many possible underlaying causes. The clinical signs of chronic hepatitis are often unspecific but can be seen general signs like loss of body weight, vomiting, skin problems and anorexia. Other symptoms we can see are polydipsia and polyuria, even in some cases a problem with the coagulation of the blood can be seen. If the disease gets more serious even icterus and ascites can be detected (Watson, 2014). More specific findings can be found when looking closer of the liver upon histopathological examination we can find signs of inflammation, necrosis, and fibrosis.

Hepatitis in dog is a quite common disease and, in a study, from 200 dogs, that were euthanized due to various reasons like general illness, signs of severe liver failure, age and economic issues, chronic hepatitis was diagnosed in 12% of the dogs (Watson et al 2010).

Already in the late 20th century, studies were made conserving the copper associated chronic hepatitis and it could be proven that Bedlington terrier were predisposed for this disease (Hardy et al 1975). Since these old studies were made, the disease has been seen in more breeds.

In my thesis I will focus on the most predisposed breeds such as Bedlington terrier and Labrador retriever. I will look at the possible causes to why these breeds are predisposed. I will look at the pathophysiological mechanism that might be behind the copper associated chronic hepatitis in dogs.

Copper is one of the essential trace elements that the dog will take in with its food. In a fully functional body, the copper is mainly stored in the liver and the excess copper are then excreted via the bile. If the accumulation or the excretion is malfunctioning it is a pathological condition due to a defect in the copper metabolism. The science behind how copper associated chronic hepatitis can cause inflammation and/or fibrosis is not fully understanded yet, but some studies say it due to the free copper ions can cause oxidative stress which then can cause inflammation and damage the surrounding tissue.

Increased copper concentration can also occur secondarily to other liver diseases.

When it comes to research that have already been done, the above-mentioned breeds have had the best mapping and are the most understood. It has been shown that in both these breeds the primary copper accumulation can occur, often in association with an inherited defect on several different genes. The genes that are mutated are part of the genes that participates or influence the excretion of copper from the liver to the bile and the blood. It must be remembered that the copper associated chronic hepatitis can be both due to primary causes, like the ones mentioned, but it can also be due to secondary causes. Secondary causes can be for example the intra or extrahepatic cholestasis or the high copper intake via the diet.

3. Literature overview

3.1 Liver as an organ and its mechanism.

In the body of a dog, liver is a major organ facilitating a lot of different functions. We can call it the treatment plant of the body. The position of the liver is in the cranial part of the abdominal cavity, just caudal to diaphragm. The structure of the liver consists of lobuli, in which we can find the v. centralis and portal triads, where the triads consist of a. hepatica and v. portae and bile duct. At this triad the oxygen poor blood from the v. aortae will be mixed with the oxygen rich blood from a. hepatica and will then continue into the sinusoids, which is the basis of the capillary system of the liver. Around the sinusoids can be found fenestrated endothelial cells. Another very important structure in the liver is "disses space", which created a form of cavity between the fenestrated endothel and hepatocytes, at this space the nutrients can be absorbed from the blood while the circulating blood cells remain. Sinusoids will then empty into the c. centralis which in their turn will empty into the v. cava caudalis, direction towards the heart (Sjaastad et al 2016).

As it was mentioned above, liver is a very important organ in the body. Some of the most important tasks of the liver is to metabolize and inactivate toxins, drugs, and hormones. Liver will also take part in the metabolism and absorption of lipids from the intestines, by the help of bile, in other word bile is important. Bile is produced by the hepatocytes and in it can be found bile acids and bile salts. It is by the help of bile the liver can excrete metabolites and inactivated toxins. These waste products will then be eliminated together with bile, via the intestines and out with the feces. Also, the storage of glycogen is an important task of the liver (Sjaastad et al 2016).

3.1.1 Chronic liver disease

When talking about liver diseases we can categorize it into either acute or chronic liver disease. In my thesis I will mainly focus on the chronic path.

Definition of chronic hepatitis according to the World Small Animal Veterinary Association is the following "Chronic hepatitis is defined as a combination of inflammation, liver cell death, fibrosis, and regeneration". The cause of chronic liver disease can be many, for example can it be infectious, metabolic, toxic, and immune mediated, or idiopathic.

3.1.1.1 Infectious causes to chronic hepatitis.

Today there are no strong evidence on how the viral and bacterial causes can cause chronic hepatitis. There have been seen in some cases that Leptospirosis, Leishmaniosis, Bacillus piriformis or Helicobacter canis have caused chronic hepatitis in dogs (Table 1).

Table 1: Infectious agents that can cause liver failure [1]

Infectious agent	Evidence that it causes CH
Leptospirosis	Moderate
Leishmaniasis	Moderate to strong
Mycobacteria	Moderate
Histoplasmosis	Moderate
Protozoal causes - Neosporidia - Sarcocystis - Toxoplasma	Moderate

Leptospirosis

Leptospirosis is a disease that can be found all around the world, it can affect both animals but also humans [2]. In dogs the disease is mainly caused by the Leptospira interrogans and Leptospira kirschneri species, the serogroup icterohemorrhagiae, canicola, pomonoa and gryppotyphosa. Dogs can be infected from the environment by contact with urine (direct transmission) or by contact with contaminated water (indirect transmission) [2].

The disease will manifest several different symptoms and the severity of the symptoms will be very varying. Clinical symptoms include such as fever, myalgia, and vascular injury. As the pathogen mainly target the kidney, we will also see signs that indicate kidney failure such as polyuria, polydipsia, vomiting, and diarrhea [4, 5]. Dogs may also have signs of liver failure, in this case icterus could be one of the leading symptoms [6, 7], and therefore leptospirosis should be on the differential diagnosis list when a dog present with above mentioned symptoms. Also, pulmonary hemorrhages and hemorrhagic diathesis can be seen [8]. The diagnosis of leptospirosis is important, as this disease can spread between animals but also it is classifying as a zoonosis and can therefore infect humans as well. Further tests that can be used is also PCR test, serology, or microscopic agglutination tests (MAT).

Leishmania

Leishmania associated chronic hepatitis is more common in tropical regions such as Spain, Greece, Portugal, and France[9]. The main causative agent is L. infantum. It is transmitted by vectors, especially sand flies, it is also by these vectors that dogs get inoculated with the causative agent. A dog diseased with leishmania can show several clinical signs such as emaciation, anemia, ulcerations of the mucocutaneous junctions. Leishmania will primarily affect the kidney and the main reason for death is kidney failure, but also a fully developed hepatic failure have been seen.

In a study made [9] histopathological examination showed that the hepatic tissue had multifocal hepatocellular necrosis, they could see vacuolar degeneration and parasitized macrophages that had infiltrated the hepatic tissue. On a biochemical note, there was increase in serum ALT, AST, and ALP.

Mycobacterium

Mycobacterium is a very large bacterial family and there are several species that technically could infect dogs. Dogs that are diseased with a mycobacterial disease (tuberculosis) will

show various signs. The clinical symptoms can be both pulmonary, gastrointestinal, or cutaneous. The most common form of tuberculosis (TB) in dogs is the pulmonary form.

Mycobacterium tuberculosis: A case where a female dog showed signs of systemic illness such as fever, dyspnea, cough, emaciation, emesis has been reported. The anamnesis of the dog was that she had been in close contact to her owner, who passed in pulmonary tuberculosis Upon postmortem examination there were found multifocal grey-white circumscribed lesions on organs such as pleura, spleen, and liver [10]. Dogs may also be infected by the ***M. bovis, M. avium*** complex.

Histoplasmosis

Histoplasma capsulatum is a fungal agent that can mainly be found in United Sates. It's a quite common fungal infection found in our companion animals. If the infection becomes disseminated it will mainly affect the liver, spleen, and the gastrointestinal system. Clinical signs of a histoplasmosis infection include chronic wasting, anorexia, respiratory problems, and lameness. In dogs the gastrointestinal symptoms are more common [11].

Treatment in the case of an infectious cause to chronic hepatitis is based on the etiology behind it. In case of infective agents caused chronic hepatitis, by the histopathological examination a characteristic pyogranulomatous abnormalities can be detected in the liver. We need to screen for the possible pathogen and then base our treatment on that. In most cases an antibiotic treatment is needed.

3.1.1.2 Drugs and toxins

As mentioned in above, liver is the treatment plant of the body, so many drugs and toxins will end up in the liver for its further destiny. If the dog ingests something that can be harmful for the liver it may result in chronic hepatitis. The outcome of the toxicosis highly depends on the dose, the duration of the drug, also the general condition of the animals and other drugs applies together. The effect can be dose dependent or idiosyncratic. There are several drugs that could cause a liver failure (Table 2). There is strong evidence that drugs like phenobarbital, carprofen, primidone and lomustine can cause chronic hepatitis.

Table 2: Pharmacological agents that can cause liver damage

Drug name	Usage	Adverse effects
Phenobarbital	Anticonvulsant	Idiosyncratic hepatic injury, especially with long-term usage.
Primidone	Anticonvulsant	Long-term usage can cause liver cirrhosis.
Carprofen	NSAID	Labrador retrievers are overrepresented.
Paracetamol	NSAID	Especially toxic in cats.
Potentiated sulphonamides	Antibioticum	Hepatocellular, cholestatic or mixed pattern.

Itraconazole	Antifungal	Can cause a mild and self-limiting increase in AST. In severe cases it can cause severe hepatitis and liver failure.
Fluconazole	Antifungal	May cause hepatocellular liver damage.

When a dog suffers from an intoxication of toxins/drugs that can be hepatotoxic the administration of the drug or the removal of the toxin is indicated. If the dog has ingested a substance, it is very important to get the details about the substance, the concentration and if possible, when did the dog ingest it? If it is inside the time frame it is possible to give the dog drugs to induce emesis, activated charcoal to prevent the absorption of the toxic substances.

Table 3: Other toxins that are hepatotoxic

Compound	**Where it can be found**	**Adverse effects**
Xilit/xylitol	Artificial sweetener (food, chewing gums)	Xylitol-induced hypoglycemia [12]. Xylitol-induced liver failure [13].
Blue green algae	Fresh, brackish, and marine water bodies.	Affects cellular proteins and will affect the normal cytoskeletal structure which will lead to hepatic necrosis and liver failure [14].
Amatoxins	Fungal toxin	Hepatorenal failure. It will penetrate hepatocytes. It will prevent transcription of DNA into mRNA → protein synthesis is inhibiting which leads to cell death and necrosis [15].

3.1.1.3 Immune mediated chronic hepatitis

Immune mediated chronic hepatitis is more investigated when it comes to humans and is thought to occur in certain individuals when they are exposed for a certain trigger such as some pathogens, drugs, toxins, or a change in the intestinal microflora. This factors will then provoke a T-cell mediated immune response that will target specific epitopes belonging to the liver.

In human medicine the research and evidence have come a long way, this is a big difference between humans and dogs. The research when it comes to dogs still needs further research and evidence. However, there are some criteria that could suggest that the chronic hepatitis is of the idiopathic form, these could be according to the ACVIM consensus statement on the diagnosis and treatment of chronic hepatitis in dogs [1]. Lymphocytic infiltrates [16–18], abnormal expression of major histocompatibility complex II proteins [19–21], positive serum antibodies [22–25], familial history of hepatic diseases [26–28], and other immune mediated disorders.

To be able to diagnose the idiopathic cause to a chronic hepatitis in dogs we need to first eliminate every other possible etiology and a positive response to immunosuppressive treatment. When it comes to the treatment protocol in the case of immune-mediated chronic hepatitis a few studies have been made when using prednisolone, azathioprine or cyclosporin's[17, 29, 30]. In a study made by Ullai et al. 48 dogs with idiopathic chronic hepatitis were treated with cyclosporin. The results showed that using cyclosporin could decrease the biochemical parameters in most of the 48 dogs [31]. In these studies a part of the diseased individuals showed an improvement, but even though this it is hard to make a final determination whether it works or not, and therefore there is no recommendations for an optimal protocol using these types of drugs.

Table 3: Treatment of immune mediated chronic hepatitis. [1]

Drug name	Recommended dose	Adverse effects to take into consideration
Prednisolone	2mg/kg SID then gradually decreased to 0.5 mg/kg EOD	- Induce liver enzymes: ALP, GGT - PU/PD - Hypercoagulability - Nausea and vomiting
Azathioprine	2 mg/kg SID for 14 days then EOD	- Bone marrow suppression - Hepatotoxic
Cyclosporine	5mg/kg BID and then SID	- Nausea and vomiting - Gingival hyperplasia - Hepatotoxic

3.1.1.4 Reactive hepatitis

Reactive hepatitis can be detected when different extern factors will induce a response from the liver. These factors were associated to other organ systems such as urinary, reproductive, and gastrointestinal, mainly. This response will cause lesions in the liver with infiltration of neutrophils if the disease is acute, and lymphocytic and plasmocytic in chronic cases. This is a big challenge to distinguish the primary hepatitis from the secondary reactive hepatitis. On the histopathological evaluation, periportal infiltration of plasma cells and lymphocytes are common but cannot be seen liver cell necrosis [32] and only a mild grade of fibrosis can be detected.

A study was made by Neumann and Danner in 2012 using 114 dogs with diagnosed liver disease, out of these 114 dogs, 55 dogs showed to have reactive hepatitis. The main organ system that could be caused the reactive hepatitis were the urinary system and the reproductive system. The study could also show that some of the dogs had problems with the respiratory, gastrointestinal, and the cardiovascular system [32].

3.1.2 Clinical signs of chronic hepatitis.

These are usually nonspecific and include decreased appetite and lethargy and PU/PD More specific signs but that are seen in a fewer cases are such as jaundice, ascites, bleeding tendencies. However, even if there are some clinical signs that point towards the chronic disease, it is more reliable to look at the liver enzymes, and function tests [1].

Table 4: clinical signs of chronic hepatitis [1]

Clinical signs	Physical examination findings
- Abdominal distension - Emesis/diarrhea - Hepatoencephalopathy - Hyporexia - Jaundice - Lethargy - Polydipsia/Polyuria - Weight loss	- Ascites - Abnormal mucous membrane color caused by jaundice - Abnormal capillary refill time (normally 1-2 seconds) caused by hypovolemia - Icterus

3.1.3 Diagnosis of chronic hepatitis

Hematology

Hematology itself is not used that much as a diagnostic tool in liver disease, but it can give us a suspicion.

Microcytic hypochromic anemia: In one third of affected dogs, we can see a mild anemic picture. The reason behind this is that dogs with a chronic liver disease are predisposed to duodenal ulcerations, but also a hepcidin dysregulation can be in the background [33].

Thrombocytopenia: a mild subclinical form can be seen in around 23% of affected dogs. These dogs are often in a later stage of the disease and can be to the decreased production of thrombopoietin done by the hepatocytes [34, 35].

Biochemical analysis

Chronic hepatitis usually has a longer subclinical phase in which can be seen an increase in liver enzymes. 27 different studies were made, and they documented an increase of ALT activity in the early stages of chronic hepatitis, and therefor ALT activity is the best screening test for chronic hepatitis [27, 30, 36–43]. However, even if the ALT is the best parameter to detect chronic hepatitis in the early stages, histopathological evidence of chronic hepatitis can be found without the presence of elevated liver enzymes [27, 44–47]. Another valuable parameter is alkaline phosphatase (ALP), but this parameter elevates in later stages. If both ALT and ALP is elevated, the elevation of ALT is often higher than the one of ALP.

Table 5: Other biochemical parameters that can be abnormal in dogs with chronic hepatitis [1].

Parameter	Percent increased	Number of dogs examined
Increased Alanine Aminotransferase	85 +/- 16	10 out of 250
Increased Alkaline Phosphatase	84 +/- 19	10 out of 250
Increased Aspartate Aminotransferase	78 +/- 10	3 out of 56
Increased Gamma-glutamyl Transferase	61 +/- 12	5 out of 121
Increased Total serum bile acid	75 +/- 14	9 out of 109
Decreased Blood urea nitrogen	40 +/- 29	5 out of 65
Decreased albumin	49 +/- 19	15 out of 323
Decreased cholesterol	40 +/- 12	4 out of 118

[27, 37, 38, 48–50]

Function test

Bilirubin

Serum bilirubin is based on the rate which heme pigmentation is formed, albumin binding, hepatobiliary circulation, hepatic storage, conjugation, and elimination. Therefore, hyperbilirubinemia can be caused by increased production (prehepatic), decreased uptake (hepatic), or decreased elimination (post hepatic). A study made in dogs that were suspected to have a liver disease, the total bilirubin had a high specificity but unfordable, a low sensitivity [51].

Normally the serum bilirubin levels should be less and 6 umol/L [52]. In around 50% of the dogs that suffers from chronic hepatitis can be seen an increase in the serum bilirubin causing hyperbilirubinemia [53]. Liver is also responsible for the albumin synthesis and in the case of chronic hepatitis we can see a decrease in albumin, especially in late stages of hepatic failure.

Total serum bile acid (TSBA)

TSBA is the most sensitive function test for diagnosing chronic hepatitis, mainly to diagnose the later stages of it[51, 54, 55]. Since its sensitivity in early stages is non-valuable this marker should not be used to decide whether to perform a biopsy. TBSA is however good to use as a diagnostic tool for detecting portosystemic shunting and therefore it's a good indicator for the detecting liver cirrhosis [56].

Even if the sensitivity in early stages is not that specific and sensitive this is the test we should perform first when we suspect liver disease in an animal without jaundice. Increases in the serum bile acid may be caused by an abnormal enterohepatic circulation (portosystemic shunt), reduced uptake of the already used bile acids from the portal blood due to a diffuse hepatic disease or, due to an increased backflow of bile into the circulation due to biliary stasis [57].

Hyperammonemia

This function test has an equal sensitivity as the one mention above. One big difference however is that the hyperammonemia test will be slightly more specific as it is not affected by cholestasis, which the TSBA can be [58]. The one negative part of testing for increased ammonia levels are the measuring technique. The test is very sensitive to external factors and it cannot be stored or even sent by post [59]. It has also been shown in studies that arterial blood is to be preferred when testing the ammonium concentration as it will be significantly higher compared to venous ammonia concentration [59].

Urea

The measure of urea can indicate a hepatic disease in dogs. When we find a low urea (BUN) concentration it can tell us that there is a low ability to form urea from ammonia. The Urea test is however not sensitive and specific enough to be used as a diagnostic tool [60].

Urinalysis

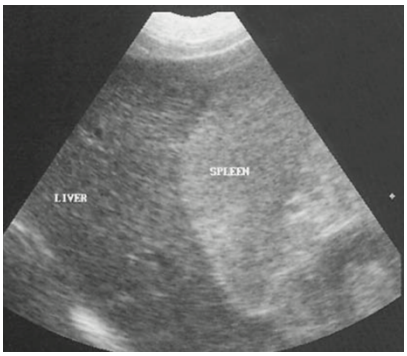
We can also test urine. Isosthenuria can be seen in dogs that have polyuria and polydipsia. Also, a transient acquired Fanconi like syndrome with concurrent euglycemic glucosuria can be seen in dogs with copper associated chronic hepatitis.

Imaging

Abdominal radiographs can be helpful to estimate the size of the liver, the opacity, and the presence of ascites. We prefer to use the ultrasound for dogs with suspected chronic hepatitis. By using the ultrasound (US), can be detected changes in echogenicity that can correlate to disease, identified the process of the disease, other abnormalities like vascular and biliary abnormalities, and it can also help us during biopsy sampling [61]. Even is the US providing us good information about size, shape, echogenicity and echotexture of the liver, the imaging of the liver in dogs with CH can be challenging.

It is very important to keep in mind that there are several hepatic diseases in dogs that will cause different forms of alterations. When using the ultrasound, it is important to check several parameters like size, contour, parenchymal density, and the degree of abnormalities. Some diseases have characteristics alterations but ultrasound itself is often non-pathognomic [62].

The procedure of doing an ultrasound examination of the liver is performed with the dog laying on its back most commonly but can be done in lateral recumbency as well. The liver can be found just caudally to the xiphopid process. We examine the liver in both transverse and longitudinal sections. The normal appearance of the liver is very variable and can be ranging from slightly coarsened to a fine-grained texture [63].



Picture: Mannion, P. Ultrasound in Small Animal practice. Picture shows an ultrasound picture of the liver.

3.1.4 Biopsy in case of chronic hepatitis

To be able to diagnose the chronic hepatitis it is required to perform histopathological evaluation of liver biopsy specimens. This procedure can be done, by using US-guided percutaneous core biopsy, laparoscopy, or by using the wedge biopsy. The latter supplies us bigger specimens and are also therefore more reliable in the diagnosis of chronic hepatitis.

We should collect the specimen from the area that is least affected to be able to avoid the regenerative nodules and the lobes that are highly fibrotic. We should collect a minimum of 12-15 portal triads to be able to perform a proper evaluation of the collected samples [63]. In the ACVIM consensus statement publication of Webster et al. is recommended to collect the following specimen:

- < 5 laparoscopic/surgical specimens from <2 liver lobes should be collected for histopathological, aerobic, and anaerobic culture and for a quantitative Cu analysis.

There are research going on to try to find biomarkers for the disease to be able to easier diagnose and put a final diagnosis [1].

Table 6: Different procedures to obtain liver sample for diagnosis [64]

Technique	**Percutaneous ultrasound guided needle biopsy**	**Laparoscopic biopsy using biopsy forceps**	**Surgical biopsy during laparotomy**
Invasiveness	Least invasive and low pain level	Less invasive than laparotomy but requires analgesics.	Most invasive method, require analgesics
Sedation/anesthesia	Requires heavy sedation but for a shorter period	General anesthesia is requiring	General anesthesia is required
Sufficiency of sample	Poor to adequate. The sufficiency will depend on the size of the needle used. 14G is recommended in most dogs	Usually enough for diagnosis. Around 8-13 triads per biopsy.	Usually enough for diagnosis
Post procedure complications	No direct control is possible and post-procedure complications are hard to control. Pre-procedure check is highly recommended.	Direct control of post-procedures is possible and possible to correct.	Direct control of post-procedures is possible and possible to correct.
Post procedure	Sometimes analgesics may be needed. Coagulation factors and blood parameters should be checked. Patients can go home same day or the day after	Analgesics are needed. Patient can go home same day or the day after. The vital parameters should be checked.	Analgesics are needed. Patient should be hospitalized for 24hr. Vital parameters should be monitored.

Before biopsy

Liver biopsies do always come with a risk, and in already ill patients the illness may even further complicate the procedure and the post-procedure recovery. Risks that could possible show are hypotension, hemorrhages, damage to surrounding structures and peritonitis [65, 66].

As the liver is responsible for the coagulation factor production, we must always remember that there can be abnormalities in the coagulation process and biopsy always comes with a risk of post-biopsy hemorrhages. Studies have been made that have stated that there is a 1.2-3.3% risk of bleeding complications [1, 67]. Even though there is evidence that a biopsy can be risky in patients with coagulation problems, the biopsies are still performed. One solution to the risk is to pre-treat the patients with fresh frozen plasma transfusions.

Before the biopsy it is therefore recommended to perform coagulation tests such as: evaluation of the prothrombin time (PT), activated partial thromboplastin time (aPTT), plasma fibrinogen concentration, platelet count, and buccal mucosal bleeding time (BMBT).

Table 7: Tests to perform to assess the bleeding tendencies [28]

Tests that can be used to determine the risk of bleeding after a biopsy	
Test	High risk
PCV	<30%
Platelet count	< 50 000/uL
PT and aPTT	> 1.5 x ULN
Fibrinogen	< 100 mg/dL
Von Willebrand factor activity	< 50%
BMBT	> 5 minutes

After biopsy

When the procedure is done, we should monitor the patient carefully and look out for possible complications, especially hemorrhage. We can prescribe pain medication, and we should closely monitor the viral signs.

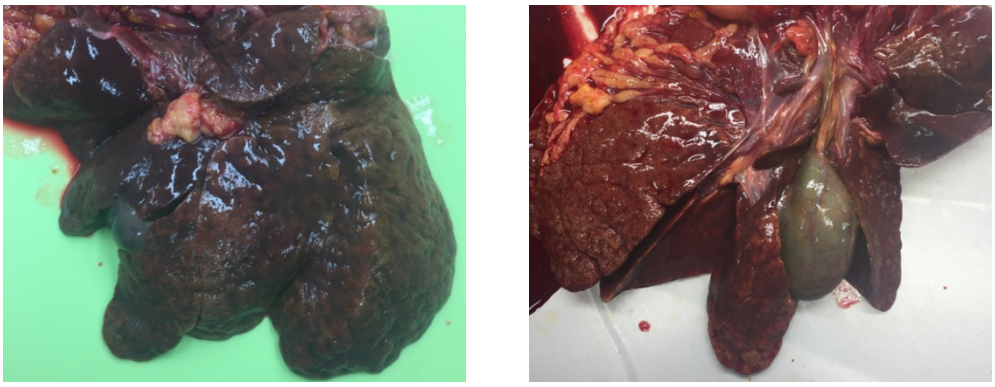
Interpretation of biopsy specimens.

What we look for when interpreting the specimens are: inflammation, cellular injury or death, fibrosis, ductular reaction and if there is any pigment deposition. To be able to evaluate the specimens special staining methods can be needed, this is to be able to better evaluate the remodeling, Cu, Iron, and connective tissue deposition.

When evaluating the inflammation, must be seen what type of inflammation, for example: neutrophilic, suppurative, granulomatous or lymphocytic, plasmocytic or eosinophilic. We also look at the location (periportal, centrilobular or diffuse) of the inflammation and the extent of the inflammation. Necrosis in the case of chronic hepatitis can affect different cells, hepatocytes, biliary epithelium, sinusoidal endothelium. The cell types that are affected by necrosis should be identified and we should also look at the extent and the distribution of

the necrosis. The cell death can be classified into massive, multifocal, centrilobular etc. The main feature in this case is that we can see the presence of lymphocytic, plasmocytic, or granulomatous inflammation. These features can be seen in both portal, multifocal, zonal, or panlobular areas. Liver cell death and different extent of fibrosis and regeneration might also be seen. The inflammation seen most often originates in the portal regions and spread into the hepatic lobule. Cirrhosis will be seen and this will reflect the so-called end stage chronic hepatitis. In this stage we will see architectural changes, fibrosis, and hypertension of the sinusoidal portals.

Fibrosis and the necrotic areas are a key feature in the diagnosis of chronic hepatitis, especially the location and extent. To be able to perform a good interpretation of the fibrosis can be used the Sirius red or Massons trichrome stains.



Picture. (Jubb, Kennedy & Palmer's Pathology of Domestic Animals, 6th Edition, Volume 2.pdf) The same liver specimen seen from 2 different views shows a liver with chronic hepatitis. Small and rigid liver with irregular borders can be seen [68].

As mentioned earlier in my thesis there are also another form of hepatitis, the reactive form. This form can be differentiated from the chronic hepatitis as we cannot see any hepatocyte necrosis. The main feature from the histopathological view when it comes to the reactive hepatitis is that in acute cases can be seen neutrophilic infiltration and in the chronic form, plasma cells and lymphocytic infiltrations.

3.2 Copper associated chronic hepatitis

Copper is a small molecule, an essential micronutrient that is needed for all the living organisms at earth. This small metal will participate in several different system in the body such as: different enzymes, help with electron transport, and antioxidizes copper is a transition element which means that it can either be in the form of oxidized Cu2+ or reduced Cu+ and thanks to this copper can act as both a donor and acceptor and participate in electron transports [69].Copper is also an important factor in the cytochrome-c enzyme, blood coagulation and more important functions. In a normal liver the Copper-concentration should be less than 400 ppm.

One branch of the chronic hepatitis is the copper associated chronic hepatitis. This form of hepatitis can be seen in a primary form or a secondary form. This type of hepatitis occurs as breed related disease in Bedlington terriers [70], Labrador retrievers [71], Doberman Pinchers [72], WHWT [40] and Dalmatians [41]. What happens in this form of hepatitis is that copper will accumulate in liver cells, which starts at the centrilobular regions and then progress into liver cell necrosis, inflammation, and finally chronic hepatitis and cirrhosis.

Primary copper-associated chronic hepatitis (CuCH) is mainly due to a genetic defect in the copper metabolism and this form have been reported in several breeds (see chapter 3.2.2). The secondary form is when copper accumulates inside the liver due to a malfunction of the biliary copper excretion or too high copper intake. Also, these two forms will differ in where the copper accumulates. In the primary form we will mainly see copper-accumulation in the centrilobular area, but in the secondary form it is mainly seen in the periportal areas [37, 50, 73].

3.2.1 Copper metabolism

Even if copper is a very important trace element, important for the normal physiological processes in the body of a dog. Copper can be found in both a reduced form and an oxidized form, this means it can act both as an electron giver but also a receiver. Dogs get their major intake of copper from their food. Its absorption takes place in small intestines, via enterocytes. From this, ATP7A-carriers will further transport the copper ion to the blood. In the blood copper will then be bound to ceruloplasmin and albumin. In this form the copper complexes will be transported through v. portae to the liver where the primary accumulation takes place. By the help of transmembrane protein copper transporter 1, copper can get into the hepatocytes. Copper is as mentioned a very important element, but as it is good, it can also be harmful. The free reduced copper ions can cause oxidative injury and therefore can be found these copper ions bound to metallothionein and glutathione inside the hepatocytes and can be protected and transported to specific targets inside the cell. In the cell these copper ions are very important in the mitochondrial respiratory chain and in the defense against oxidative stress.

Two very important transport proteins can be found in the liver. These are the ATP7A and ATP7B. These proteins are ATP-dependent membrane-bound transport proteins which will help with the excretion of copper from the liver. ATP7B task is to attach copper onto ceruloplasmins for further transport to blood.

To summarize the main steps in copper metabolism we can say that the homeostasis of copper, which is very important, is maintained by intake from diet, and excretion via the bile. When copper exceeds the hepatocyte transport and copper-binding capacity, free copper

will cause oxidative stress which will lead to hepatocellular degeneration and to cell death with chronic hepatic inflammation, or both [68, 74].

3.2.2. Copper accumulation

When we study the reasons behind copper accumulation we can differentiate between primary and secondary causes. The primary cause is due to failure in the hepatic copper metabolism while the secondary is caused by cholestasis due to an intrahepatic or extrahepatic reason [37].

Primary copper accumulation in the liver is associated with gene defects and this form have been reported in Bedlington terriers mainly but have also been seen in other breeds. In the case of a primary copper accumulation, we will see an increased copper concentration and inflammation in zone 3 [37]. The inherited form of copper associated hepatitis the copper concentration is always located in the centrilobular areas.

The secondary form of the copper associated hepatitis is when there is a failure of the copper excretion into the bile, often due to an extrahepatic disease that cause cholestasis. In the case of the secondary form, we most often see the copper accumulation in the periportal areas, in the hepatocytes [71].

Table 8: Differences between primary and secondary copper-associated chronic hepatitis

	Primary form	**Secondary form**
Location of copper accumulation	Centrilobular areas	Periportal areas
Copper concentration (ug/g dw)	600-2200 ppm [71]	Average 997 ppm [75]

As mentioned, the main difference between the primary and the secondary form is the etiology behind it and this was also proven in a study was also made by Azumi in 1982, where the examined dogs were divided into two groups. Both groups had their ductus choledochus ligated. One part of the dogs was given copper intra venously, in the dose of 0.5 mg/kg/bw. In the study there were also a control group of dogs that had neither the surgery, nor the administration of copper. According to their results, there was an increase of copper concentration in the liver of the dogs that have been through the ligation of ductus choledochus in addition to copper administration. There were not seen any remarkable difference between the control group and the group that did not receive any copper intra venously [76].

The copper concentration in the liver can be measured quantitative either by spectrometric methods or by radiation of tissue pieces from the liver which then are measured on copper radioactivity[74]. In a healthy dog we can see a copper concentration in the liver up to 400 microgram/gram dry matter of liver (parts pro million ppm), this is the reference value. In case of primary copper accumulation, the concentration of copper can be as high as 2000 microgram/gram dry mass of liver. If there is a secondary cause of copper accumulation, we rarely see a higher value than in primary copper accumulation [68].

3.2.3 Diagnosis of copper-associated chronic hepatitis

When it comes to the diagnosis of the copper associated chronic hepatitis there are certain criteria that must be taken into consideration before a final diagnosis can be made:

- Histological evidence of chronic hepatitis, and centrilobular copper accumulation
- Histochemical copper staining done by using stains like rhodanine or rubeanic acid, and
- A copper concentration >1000 ug/g dw (ppm)

The samples are collected by the help of biopsy (which is discussed in detail: biopsy). It is important that the samples are not only collected from the periphery liver lobes if these lobes have a fibrotic appearance [77]. To avoid an underrepresent hepatic copper concentration, the sample should be taken from the least affected area, to avoid the regenerative nodules and liver lobes that are highly affected by fibrosis [78, 79]. For the diagnosis of copper associated chronic hepatitis we need around 20-40 milligrams of wet liver weight. The atomic absorption spectrometry can be used.

According to the Webster et al 2019, the diagnosis an abnormal copper accumulation requires special stains such as rhodanine or rubeanic acid [1]. By the help of these staining, we can detect copper-binding proteins presence, acinar distribution and with this information can be estimated the severity of copper-accumulation. The areas that are most affected in dogs are the centrilobular areas, this accumulation might extend into the midzonal and periportal hepatocytes as well. However, the periportal copper accumulation are often not very specific and the copper concentration n might be false negative. In the article made by G. Hoffman in 2009 it is suggested to then use a subjective grading scale to score the degree of copper accumulation [78, 80]. When we decide how much of a clinical relevance the copper accumulation have, we must also take the necrotic hepatocytes, and the development of so-called copper-granulomas. The definition of copper granulomas is a specific form of macrophages that aggregate together with lymphocytes, pigmented macrophages, neutrophils and sometimes we can see plasma cells.

The main diagnostic test for the copper-associated hepatitis is the so-called atomic absorption spectroscopy. This test requires only a small amount of tissue, around 20-40 milligrams [1, 75].

Conclusion of the diagnosis is that it can be hard to get a correct diagnosis. The copper concentration may vary between the different liver lobes and therefore the pathologist should have the experience and knowledge to be able to identify the different liver lobes during a histopathological evaluation.

The reference for copper concentration in the liver should be lower than 400 ppm. If it is above >1000 ppm we consider this as definitely abnormal. If the value exceeds 2000 ppm, we will see ruptured lysosomes will be seen which means that the different liver zones and structures are destroyed and clinical signs will appear. However, there are no studies that can prove what copper is needed to trigger the copper-associated chronic hepatitis, for example is Cu-concentration exceeds 1000 ppm, changes in ALT and morphology have been seen, but there are also cases where Cu exceeds 1500 ppm and no changes is seen. Also, there are several phenotypic differences. In some individuals a severe damage of the liver can be seen already if copper is lower than 1000 ppm while in some cases no evidence of

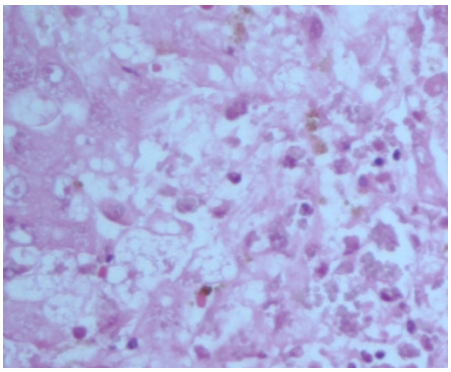
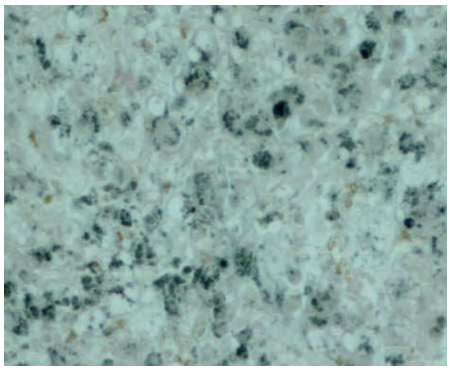
liver damage can be seen even if copper exceeds 1000 ppm. The individual threshold is influenced by environmental factors, genetic factors, and physiologic factors. This is also one of my big questions in my thesis, what is the difference between different breeds?

Histopathological changes.

When we stain the samples with a HE is staining, copper will look like grayish yellow to grey-brown granules inside the hepatocytes and the Kupffer-cells cytoplasm. When using the special stains like rhodanine or rubeanic acid, it is easier to confirm the accumulation.

Primary copper-associated chronic hepatitis is often characterized by centrilobular copper-accumulation and inflammation. The inflammatory changes are characterized by the infiltration of plasma cells, lymphocytes, macrophages, and even sometimes neutrophil granulocytes. Inside the inflammatory zones there may also be possible to identify apoptosis and necrosis of the hepatocytes. If the chronic hepatitis is far exceeded, even cirrhosis of the liver can be observed, which can be identified by pseudo lobules of the liver.

In secondary copper-associated chronic hepatitis, caused by cholestasis, the accumulation of copper will mainly be seen in the periportal zones. In addition, bile duct proliferation and periportal fibrosis with infiltration of mixed inflammatory cells can be detected [40, 68, 74].

	
HE-staining: The inflammatory cells and necrosis seen inside the hepatocytes is seen because of 3copper-accumulation. Photo: Söderström F and Andersson E 2019	Rodanin-staining: This is a special staining where copper will be seen as blackish granules. Photo: Söderström F and Andersson E 2019

The usage of a histological semiquantitative grading of the copper-concentration inside the liver have become praxis in copper-associated chronic hepatitis. This grading consists of a scale between 0-5, between 3-5 will indicate a primary copper-associated chronic hepatitis. This grading system were founded by Johnson et al [81].

Table 9: Grading scale based on histochemical examination [37]

0	No copper could be found in hepatocytes.
1	Some hepatocytes contain a low number of copper molecules.
2	Small hepatocyte groups contain a moderate amount of copper molecules.
3	Larger areas of hepatocytes or certain areas of the liver contains a moderate amount of copper and/or copper containing macrophages.
4	Large areas of the liver contain large amount of copper, we often see copper containing macrophages.
5	Diffuse areas with large amount of copper. Copper macrophages can be seen.

Bedlington terriers

Bedlington terrier is the most examined dog breed when it comes to copper-associated chronic hepatitis. This dog breed is affected with an autosomal recessive defect in copper metabolism [82, 83]. It has been shown that homozygous recessive individual will accumulate a higher concentration of hepatic copper. These concentrations can be as high as 10.000 ppm [84, 85] . The copper accumulation is almost in all cellular compartments inside the living cells can be seen. The lowest hepatic concentrations can be found in the young dogs and the concentrations will then increase with age, with its peak when the dog is around 6-year-old [86]. In the youngest dogs we can see copper accumulation in the so-called centrilobular liver cells and its related lysozymes. During this period the expected copper concentration is expected to be around 400-1500 ug/g, these dogs are often asymptomatic and the diagnosis is very hard. As the disease proceeds the copper concentration is between 1500-2000 ug/g and copper can be found in the midzonal and in the periportal liver cells, as well. Even in this stage clinical signs are absent but focal hepatic inflammation, lymphoplasmacytic inflammation and copper-laden macrophages can be found. In the second stage also an increase in serum ALT can be seen. When the disease reaches the most severe stage the copper concentration exceeds 2000ug/g we can find morphological alterations that will reveal chronic hepatitis. This chronic hepatitis might proceed into cirrhosis and clinical signs and biochemical alterations can be seen.

Table 10: Stages of CuCH in Bedlington terriers [37]

Stages	Clinical signs	Copper	Liver histology
1	No clinical signs can be seen	Copper can be found in zone 3. 400-1500 ppm	No morphological changes
2	No clinical signs	Copper can be found in all liver areas. 1500-2000 ppm	Hepatic inflammation can be seen.
3	Clinical illness can be seen	Copper is found in all liver areas. >2000 ppm	Hepatic inflammation and liver cirrhosis

Diagnosis of CuCH in Bedlington terriers is best achieved by performing liver biopsies in early age. It is recommended to perform liver biopsies in individuals we want to use for breeding. Screening of individuals with no symptoms at the age of 6 months and 15 months can help us determine whether an affected dog is homozygous or heterozygous (carrier) [87]. Dogs being affected, both homozygous and heterozygous, will have an increased copper concentration by the age of 6 months. The difference is that by the age of 1 year the copper concentration will decrease in heterozygous individuals while in the homozygous individualist will keep on increasing [88]. The specimens are then examined with special staining methods to be able to determine the amount of copper. (See: Interpretation of biopsy specimen)

In the study that was made by Hardy et al in 1975, blood samples were taken from 21 Bedlington terriers (BT) dogs that showed signs of liver disease, out of these 21 dogs, 19 of them had abnormal biochemical values which indicated liver disease. The copper concentration was then measured and in which a massive increase could be proven. According to the author, the disease was inheritable [88].

Year 2002 it was detected that a mutation could be the reason behind the disease. This mutation was found as a deletion of exon 2 on copper metabolism MURR1 domain containing 1-gene on chromosome 10. When this was detected the function of this gene was still unknown [72]. It was detected that abnormal gene could be connected to the absence of COMMD1-protein in the liver. According to the author of the article, this protein could be involved in several functions and in the regulation of copper excretion [89]. In another study by S. Haywood, it was however proven individuals that lack this mutation, still can develop CuCH (non-COMMD1) [90]. The study that Haywood did sample 30 BT, 15 of these dogs had CuCH and lacked the COMMD1-gene defect.

Labrador Retriever

In a publication that was made in the early 1900 century there was evidence an increased incidence of chronic liver disease in this breed as well, and especially in females [80]

In one of the studies compared Labradors with copper-associated chronic hepatitis to Labradors without any clinical signs of liver disease were found that from 14 asymptomatic Labradors, 8 was related and 6 unrelated. In the related Labradors there could be seen an increase of copper in the liver but in the dogs that were unrelated, this increase could not be seen [80]

Smedley et al in 2009 measured the copper concentration in 12 Labradors with histopathological changes, like centrilobular inflammation and copper accumulation. In 10 of these 12 dogs the copper concentration in the liver could be measured to above 2000 ppm. The authors of the study could then confirm that primary copper-associated chronic hepatitis most likely occurs in Labradors [91].

When viewing pedigrees and biopsies from the liver from 146 Labradors, the inheritance occurrence of copper-associated chronic hepatitis has been evaluated to be around 39-52% [92]. In a Gene Wide association study of 235 Labradors a miss-mutation could be identified on two chromosomal loci. These miss-mutations were found on the genes that codes for the copper-transport-protein ATP7B and ATP7A. There was one mutation found on ATP7B-gene, on chromosome 22, this was associated with increased copper-accumulation in the liver, this mutation was most prominent in bitches. According to the authors to the study

these miss-mutations could explain up to 12.5% of the total inheritance of copper-accumulation [91].

Copper mediated liver injury in Labrador retrievers may be influenced by mutations in ATP7B gene, which predispose for the accumulation of copper, and mutations on gene ATP7A may influence the intestinal copper transporter, which protects against the copper accumulation. Although there have been tests to try to diagnose these mutations and test available, the predictive and diagnostic utility of these tests is currently unknown.

A study made by Fieten in 2016 found that the genes that encodes for the copper carrying proteins ATP7A and ATP7B had mutations [48]. The mutation on ATP7A have later been associated with partial protection against accumulation of copper inside the liver while the mutation on ATP7B could be connected to an increased copper accumulation inside the liver. In the study that Fieten made, only 12.5% of these mutations could explain the inheritance of copper accumulation in the liver. I think that the results show that there could be an inheritance, like the Bedlington terrier, of copper-associated chronic hepatitis.

Doberman Pinscher

In this breed the chronic hepatitis has mainly been observed in bitches. In Doberman pinschers we mainly see CuCH in dogs of the age 1-3 years old, with increase in ALT and sub-clinical hepatitis. The clinical sign of CH usually develops around 4-7 years of age.

In a study made in the late of 1900th, 8 Doberman with chronic hepatitis and cirrhosis were compared to 17 healthy dogs of the same breed. In all, except one, of the affected dogs, intrahepatic cholestasis could be seen. The copper-concentration were appreciated to be grade 3 by a semiquantitative grading, while in the dogs without chronic hepatitis and cirrhosis were graded between 0-2 [72].

Later, a new study was made by Mandigers et al in 2006 [92]. In this study, 106 randomly chosen Dobermans were picked and in 50 of these a copper-containing granula could be found by a fine needle aspiration examination of the liver. An increased median copper-concentration in the liver were measured in dogs with hepatitis compared to dogs without hepatitis. When further following up of 16 of these dogs after ca 2 years, the liver inflammation was persistent in 6 of the dogs, which also showed an increased copper-concentration. According to the authors to this study, the results being found indicated a connection between copper-accumulation and hepatitis [93].

Spee et al did a study in 2005, to try to see on what gene and miss-mutation could explain the findings [94]. In this study, 6 Dobermans with copper-associated chronic hepatitis were examined. In these dogs, a significant decrease of the mRNA encoding for ATP7A, ATP7B, ceruloplasmin, metallothionein and COMMD1-protein could be found, this compared to a healthy control group with normal copper-concentration in the liver. It has been thought to be a primary copper-associated hepatitis [94].

Another important form of chronic hepatitis that must be taken into consideration when it comes to this breed is the immune mediated form of the chronic hepatitis. This form of hepatitis is mainly seen in female dogs in the age of 4 to 7 years old. The chronic hepatitis in Doberman pinschers is mainly characterized by micronodular cirrhosis, fibrosis, infiltration by plasma cells and lymphocytes of the portal triads. There is some suspicion

that Doberman hepatitis could have some genetic background but the complete inheritance pattern has not yet been confirmed.

The etiology behind Doberman hepatitis (DH) is not completely clear yet. The suspicion of a idiopathic cause were raised when it could be found mononuclear cell infiltrates inside the liver cells and also an abnormal expression of MHC class II antigens found on liver cells that can correlate with the degree of inflammation found in the liver [93]. The author of this article studied different parameters that could indicate the prevalence of a genetic background, these parameters DLA class II antigens that could be associated with DH. Variations of the promoter areas of homozygous DLA-DRB1*00601/DQA1*00401/DQB1*01303 that could explain why some individuals are more prone for DH. Circulating serum anti-nuclear autoantibodies, autoantigens that can be found in the liver and might be associated with DH, autoantibodies that could be used as biomarker for DH. The author could conclude that there was evidence when investigating these factors that the DH could have an idiopathic background especially in homozygous individuals [43].

When it comes to Doberman Pinschers, conclusions have been made that the copper-associated hepatitis seen in this breed could be explained to occur secondarily due to a problem with cholestasis. We need more studies here to be able to draw a complete conclusion about the pathophysiological that is in the background of the disease in this breed. The fact that the evidence is so limited could be explained by the fact that the disease is not as widespread in this breed, or that the breed itself is rarer than Labrador for example.

West Highland White Terrier

In a study made in Sweden, by Andersson and Sevelius in 1991. 250 histopathological samples were examined from dogs with mixed breeds and chronic liver diseases, there was an increase in the incidence of chronic liver disease in WHWT. In 2 out of 18 WHWT, according to the authors, they could see a moderate amount of copper in the liver. This finding according to the author were seen to be secondary to the chronic hepatitis [94].

In a published study that examined 71 WHWT, there could be proven that in 44 of these dogs had higher copper-concentration in the liver. In dogs with increased copper-concentration, 20 of them had normal hepatic histomorphology. In the remaining 15 dogs, 10 of them had multifocal hepatitis with centrilobular placed inflammatory foci consisting of mixed inflammatory cells and necrosis. The median value of the copper-concentration of 10 dogs with the multifocal hepatitis were 2460 ppm [37].

Summarizing, in WHWT there is probably a secondary Cu accumulation associated with chronic hepatitis.

Dalmatians

In a study that was based on 10 dalmatians with suspected copper associated chronic hepatitis, these dogs showed signs of gastrointestinal disease such as anorexia and vomiting. In the study the age of the dogs varied from 2-10 years of age, in the group there were both females and males, spayed and non-spayed individuals [95].

When checking the liver enzymes there was an increase of the ALT in all 10 dogs, the increase was around 6.4x higher than normal, and in 9 out of 10 they could also see an

increase in ALP, as high as 7.0x higher than normal. Three individuals also shower low serum albumin [97].

A biopsy was made on the affected dogs where the copper concentration was measured. An average concentration of 3197 ug/gram dwl of copper in the liver could be proven. On histopathological examination there were found necrosis, fibrosis, and signs of inflammation. These histopathological findings together with the high Cu-concentration, the author of the study could draw the conclusion that even dalmatians could be susceptible for primary copper-associated chronic hepatitis [74].

I have studied 2 different case reports [1, 71, 96] in which both authors state that the most likely explanation for the copper-associated chronic hepatitis in dalmatian is due to a primary disease. In the mentioned studies, both individuals were below 2 years of age, healthy, and presented with signs that could be connected to gastrointestinal disease. Upon further investigation of the blood parameters there could be found an increase in both ALT and ALP. On histopathological examination there were found liver cell necrosis, mixed inflammatory cell infiltration and cirrhosis of the liver. The copper accumulation was mainly found in the centrilobular areas. The copper concentration was measured and determined to be between 7.664 ug/g/dwl-9.424 ug/g/dwl.

In Dalmatians there is probably a primary Cu associated hepatitis, where the Cu accumulation develops already in early age [95]

Table 11: Genes of different breeds that are believed to be behind the disease.

Breed	**Type of CuCH**
Bedlington terrier	**Primary form. Mutations of gene:** - **COMMD1 protein** - **ATP7B** - **ATP7A** - **ABCA12**
Labrador retriever	**Primary form** - **ATP7B**
Dobermann Pinscher	
West highland white terrier	
Dalmatians	

4. Treatment of chronic hepatitis

When treating the chronic hepatitis our goal is to treat the underlaying cause/causative agent, but as mentioned, chronic hepatitis in dogs is often idiopathic and therefore no underlaying treatable cause. In this case, when we have excluded all other possible causes, a treatment with immunosuppressive drugs and nonspecific hepatoprotective agents may be indicated.

4.1 Treatment of copper associated chronic hepatitis

The increased copper concentration in the liver is an abnormal condition and will also come with consequences, therefore it needs treatment. When a dog is diagnosed with copper-associated chronic hepatitis the treatment first is chelator drug until the Cu concentration in the liver is normalized together a lifelong low copper diet. When the chelator treatment was stopped than Zink supplementation is necessary.

Chelator

A chelating agent is used to lower the blood and tissue levels of a heavy metal that can cause injury. These agents have special metals that they target. The one commonly used chelating agent in the case of copper associated chronic hepatitis is the D-Penicillamine. The function of this drug is that it will bind the copper, makes complex with it and help the excretion via urine [100, 101]. Other positive characteristics of this drug is that it will increase the metallothionein and the enterocytes, in addition it also has a milder anti-inflammatory and anti-fibrotic properties [102–105].

Table 14: D-penicillamine

Mechanism of action	**Dose and duration of the treatment**	**Other relevant information about the drug**	**Side effects**
It will bind/chelate to the copper and excrete it with urine. It will also upregulate the hepatic metallothien to bind intracellular copper.	10-15 mg/kg twice a day. Should be given 30 minutes before the food or 2 hours after the meal. The duration of the treatment depends on repeated measurement of hepatic copper concentration or by monitoring the ALT.	This drug is also anti-inflammatory, anti-fibrotic. Should not be used together with Zinc.	Common side effects are related to the gastrointestinal system such as nausea, vomiting, hyporexia. These side effects are common. Rare side effects include immunologic reactions and bone marrow dyscrasia

When combining the D-penicillamine together with a copper-restricted diet the effects are often good and this combination can normalize copper-concentrations as high as 1500ug/g dw in 6 months. If the copper concentration is around 2000-3000 ug/kg dw we can see a decrease in copper concentration within 9 months.

Diet

In the beginning of the late 1990s, there was an increase in the number of cases of chronic hepatitis. This increase could be connected to the change in premixes which was used to add copper into the commercial dog food, which then increased the accessible copper in diets [37, 96, 97].

The national Research Council (NRC) and Association of American Feed Control Officials (AAFCO) dietary guidelines, along with a change to more bioavailable Cu chelate premixes in commercial dog food, are linked with an increased prevalence of hepatic Cu accumulation in dogs. The NRC has recommendations for the adult dogs that are based on puppies that were given diets that had 0.11-0.19 mg/kcal/me/day, this diet reduced the serum ceruloplasmin. However, serum ceruloplasmin do not reflect the copper bioavailability and therefore it is not for dietary recommendations [1]. AAFCO recommends that adult dogs need a minimum of 7.3 mg/kg/DM/day, this is regardless of the copper source. Copper is then often added as a form of premix, the problem here is when copper is added, the copper level in the base feed is often forgotten, resulting in a higher copper level.

Table 12: Recommended copper level in food. [1]

NRC minimum	AAFCO minimum	Average dog food	Hepatic diets	
Copper concentration (mg(kg/DM/d)	6	7.3	Approx. 15-25	

A copper restricted diet is needed in all dogs that have a copper concentration higher than >600 ug/g dw [1, 37] and it is recommended that the food should contain less than 0.12 mg/100 kcal. Another thing that must be kept into consideration is that these copper restricted diets are often low in protein and therefore additional protein is advised. Copper restricted diets usually contain between 3.9-4.1 mg/100 kcal of protein, and the minimum protein requirement is 4.5 mg/100 kcal.

It is also advised to make sure that the copper content in water do not exceed 0.1 ug/g, this can be done by flushing the pipes for 5 minutes.

Commercial diets are available today. There are a lot of commercial diets that could be suitable for a dog that suffers from copper-associated chronic hepatitis. As mentioned earlier the main feature of a diet that suits these patients are to find a food that have no more than 12 mg copper per 100 kcal. The variation of these diets are many and many of these commercial diets exceeds the NRC recommendation very much [98, 99].

Table 13: Commercial dog foods for dogs with liver diseases.

Brand name	Total amount of copper/kg
Hills liver care (I/D)	2.9 mg/kg
Purina Hepatic	5 mg/kg
Royal Canin Hepatic	3 mg/kg

A research study made by G. Hoffman et al 2009, studied 29 Labrador retrieves that had been diagnosed with copper associated chronic hepatitis. The aim of the study was to see how the copper associated chronic hepatitis could be managed by the help of both drug and diet. All the examined dogs had completed their treatment with the D-penicillamine and had a good response to it where a decrease in copper concentration in liver could be seen. All dog owners were then supplied with the same commercial dog food that contained 2 ppm copper, 43 ppm zinc, no further salts were added into the diet. The owners got the instructions to only feed the dogs with this food during the study and avoid any treats or supplements. In 11 of the examined dogs, a new copper concentration was measured, after the treatment with the D-penicillamine but before starting the diet. The result showed that in all but one dog, the concentration of copper had increased for 8 months. Further results drawn from the study did show that during the special diet the copper concentrations did decrease and stabilize. The authors of this study did in the end recommend that dogs with abnormal high copper concentration in the liver should be kept on a life-long copper restricted diet to avoid re-accumulation of copper [97].

We can determine whether the combination between chelating agents and a copper restricted diet is effective by repeating the quantification of hepatic copper accumulation. Also, the ALT level can be used as an indicator of success.

Zinc

Zinc is a substance that will interfere with the copper uptake from the gastrointestinal tract by inducing metallothionein in the enterocytes [1, 69]. The metallothionein binds to copper of the diet and eliminates with the feces. Co-treatment with D-penicillamine and Zinc is strictly contraindicated because each negates the benefits of the other.

A study was made in 1992 where 3 Bedlington terriers and 3 WHWT were treated with zinc to treat and prevent copper accumulation in the liver. 2 out of 3 dogs from both breeds were treated with zinc for 2 years while 1 from each breed were only treated for 1 year. The first observation of clinical signs was good. Three dogs had only mild to moderate liver disease and higher copper concentration than they had in the beginning of the treatment. A biopsy was made in which the copper associated chronic hepatitis and the copper concentration had been decreased. The authors of this study believed that zinc could be a good and nontoxic treatment for the copper associated chronic hepatitis [106].

Table 14: Zinc [109]

Mechanism of action	Dose	Other useful information	Side effects
It will induce the intestinal metallothionein that binds the copper.	8-10 mg/kg/day. Divided in 3-4 portions. Should be used for long term treatment and maintenance.	Serum levels for effective administration should be >200 ug/dL	Nausea and vomiting are common side effects while hemolytic anemia, and zinc-toxicity are rare

Antioxidants

When we are treating the copper-associated chronic hepatitis by the help of a chelator, it is advised to also supplement with antioxidants. This is because copper will cause an oxidative injury on the liver [5, 106–109]. The antioxidant treatment is also suggested in secondary Cu accumulation.

Table 15: Different antioxidants that can be used. [1, 5, 108, 109]

Drug name	Formulation	Dose	Mechanism of action	Side effects
S-adenosylmethionine (SAMe)	Very unstable compound, use stabilized salts. Also, available in the form of phytate salt	Stabilized salt:20 mg/kg PO SID on an empty stomach Phytate salt: 8-10 mg/kg SID	Increase intracellular cysteine which leads to an increase in the formation of liver glutathione	Nausea can be seen in rare cases
Vitamin E		10 IU/kg SID PO. It should be given with food as this will increase its absorption.	It protects against lipid peroxidation, work as anti-fibrotic and anti-inflammatory	If an overdose is seen it may impair the vitamin K activity and may increase the risk of oxidative injury.
Ursodeoxycholate	Stable secondary bile acid.	15 mg/kg SID PO with food	Have antioxidant, choleretic, anti-inflammatory effects	Side effects are rare but gastrointestinal signs may be seen.

Silymarin		Undefined, but between 4-8 mg/kg/day can be given BID-TID	Have similar effects as ursodeocycholate.	

5. Discussion, results, conclusion

There are 2 dog breeds that are overrepresented when it comes to the copper-associated chronic hepatitis, these are the Bedlington terrier and Labrador retriever, and in these two breeds we can find most studies and research. In the study, made by Johnson et al. 1980, we can see that the inheritance of the disease in Bedlington terrier is an autosomal pattern. At first sight this pattern could not be found in Labrador retrievers but in the book written by G. Hoffmann (2006), it has been proven that the copper-concentration in the liver is higher in related individuals compared to unrelated. According to this information a conclusion can be made that there can be some inheritance of the disease in Labrador retrievers as well. When seeing these results, this is very important information to take into consideration when breeding these breeds. In Bedlington terrier there is a compulsory genetical filtration of Cu and checking the pedigree before breeding. This control would be also important in Labrador retriever as well. I think this will be also the part of the breeding program in the future.

In the other dog breeds where we have currently less studies, the diagnosis of the copper associated chronic hepatitis have mainly been concluded to be due to a primary or secondary form of the accumulation. These conclusions have been made by studying the copper concentrations found inside the liver. Thanks to these studies we can see an indication about what pathophysiological processes that might be behind the disease. In my opinion, these studies still miss some important evidence about the background of the disease, compared to the well-studied breeds. Additional studies are required in those breeds about the pathomechanisms of the Cu accumulation.

The conclusion of my work here is that there are 2 different forms of the same disease with different etiology. In Bedlington terrier we can see a recessive inheritance with mutations on several different genes. Probably a similar pathomechanism goes for Labrador Retrievers. These breeds have been more studied and the researcher have come a long way in the science behind it. In Dalmatians there is probably also primer Cu accumulation, which starts very early age and cause chronic hepatitis. The exact pathomechanism must be clarified in this breed. In other dog breeds like West Highland White Terrier and Doberman Pinschers have another form of it, the secondary form, which originates from intra- or extrahepatic bile duct obstruction most commonly. The Doberman has also an immune-mediated chronic hepatitis.

One thing that I find very interesting when it comes to this topic is that there is evidence that the disease is more common in Labrador retrievers and Bedlington terrier females [37, 72, 74]. This is very interesting because the gene that encodes for ATP7A can be found on an X-chromosome. Later, in another study however, no difference between the two genders could be seen [37, 72, 74].

Studying this topic have been very interesting and giving. How come that the Bedlington terrier and the Labrador retriever are so overrepresented when it comes to this disease? A lot of evidence can be found that it can be genetic predisposition like mutations on different genes. But what I find interesting is how one disease can be so different with different background in different breeds. How come that we can find a lot of evidence in two of the breeds while in the others it is unexplored ground. I see a big opportunity to precede this research to be able to map out more information about it and more tools to be able to manage it and eventually prevent it. I see that this is possible as the science and the tools we can use is being developed all the time. I hope to see a progress in this topic in the future.

1. Webster CRL, Center SA, Cullen JM, Penninck DG, Richter KP, Twedt DC, Watson PJ (2019) ACVIM consensus statement on the diagnosis and treatment of chronic hepatitis in dogs. J Vet Intern Med 33:1173–1200. https://doi.org/10.1111/jvim.15467
2. Levett PN (2001) Leptospirosis. Clin Microbiol Rev 14:296–326. https://doi.org/10.1128/CMR.14.2.296-326.2001
3. McCallum KE, Constantino-Casas F, Cullen JM, Warland JH, Swales H, Linghley N, Kortum AJ, Sterritt AJ, Cogan T, Watson PJ (2019) Hepatic leptospiral infections in dogs without obvious renal involvement. J Vet Intern Med 33:141–150. https://doi.org/10.1111/jvim.15340
4. Adin CA, Cowgill LD (2000) Treatment and outcome of dogs with leptospirosis: 36 cases (1990–1998). J Am Vet Med Assoc 216:371–375. https://doi.org/10.2460/javma.2000.216.371
5. Center SA, Warner KL, Erb HN (2002) Liver glutathione concentrations in dogs and cats with naturally occurring liver disease. Am J Vet Res 63:1187–1197. https://doi.org/10.2460/ajvr.2002.63.1187
6. Birnbaum N, Barr SC, Center SA, Schermerhorn T, Randolph JF, Simpson KW (1998) Naturally acquired leptospirosis in 36 dogs: serological and clinicopathological features. J Small Anim Pract 39:231–236. https://doi.org/10.1111/j.1748-5827.1998.tb03640.x
7. Kohn B, Steinicke K, Arndt G, Gruber AD, Guerra B, Jansen A, Kaser-Hotz B, Klopfleisch R, Lotz F, Luge E, Nöckler K (2010) Pulmonary Abnormalities in Dogs with Leptospirosis: Leptospirosis in Dogs. J Vet Intern Med 24:1277–1282. https://doi.org/10.1111/j.1939-1676.2010.0585.x
8. Schuller S, Francey T, Hartmann K, Hugonnard M, Kohn B, Nally JE, Sykes J (2015) European consensus statement on leptospirosis in dogs and cats. J Small Anim Pract 56:159–179. https://doi.org/10.1111/jsap.12328
9. Rallis T, Day MJ, Saridomichelakis MN, Adamama-Moraitou KK, Papazoglou L, Fytianou A, Koutinas AF (2005) Chronic Hepatitis Associated with Canine Leishmaniosis (Leishmania infantum): a Clinicopathological Study of 26 Cases. J Comp Pathol 132:145–152. https://doi.org/10.1016/j.jcpa.2004.09.004
10. Martinho APV, Franco MMJ, Ribeiro MG, Perrotti IBM, Mangia SH, Megid J, Vulcano LC, Lara GHB, Santos ACB, Leite CQF, de Carvalho Sanches O, Paes AC (2013) Disseminated Mycobacterium tuberculosis infection in a dog. Am J Trop Med Hyg 88:596–600. https://doi.org/10.4269/ajtmh.12-0332
11. Brömel C, Sykes JE (2005) Histoplasmosis in Dogs and Cats. Clin Tech Small Anim Pract 20:227–232. https://doi.org/10.1053/j.ctsap.2005.07.003
12. Dunayer EK, Gwaltney-Brant SM (2006) Acute hepatic failure and coagulopathy associated with xylitol ingestion in eight dogs. J Am Vet Med Assoc 229:1113–1117. https://doi.org/10.2460/javma.229.7.1113
13. Todd JM, Powell LL (2007) Xylitol intoxication associated with fulminant hepatic failure in a dog. J Vet Emerg Crit Care 17:286–289. https://doi.org/10.1111/j.1476-4431.2007.00243.x
14. Falconer IR, Yeung DSK (1992) Cytoskeletal changes in hepatocytes induced by Microcystis toxins and their relation to hyperphosphorylation of cell proteins. Chem Biol Interact 81:181–196. https://doi.org/10.1016/0009-2797(92)90033-H

15. Vetter J (1998) Toxins of Amanita phalloides. Toxicon 36:13–24. https://doi.org/10.1016/S0041-0101(97)00074-3
16. van den Ingh TSGAM, Van Winkle TJ, Cullen JM, et al Morphological classification of parenchymal disorders of the canine and feline liver: hepatocellular death, hepatitis, and cirrhosis-2 (updated version). 2006:
17. Boisclair J, Doré M, Beauchamp G, Chouinard L, Girard C. Characterization of the inflammatory infiltrate in canine chronic hepatitis. In: Vet Pathol. pp 628–635
18. Sakai M, Otani I, Ishigaki K, et al Phenotypic analysis of hepatic T lymphocytes in a dog with chronic hepatitis. J Vet Med Sci 2006:1219–1221
19. Bexfield NH, Watson PJ, Aguirre-Hernandez J, et al DLA class II alleles and haplotypes are associated with risk for and protection from chronic hepatitis in the English Springer Spaniel. J Vet Med Sci 2012:
20. Speeti M, Ståhls A, Meri S, Westermarck E. Upregulation of major histocompatibility complex class II antigens in hepatocytes in Doberman hepatitis. Vet Immunol Immunopathol 1–12
21. Dyggve H, Meri S, Spillmann T, Lohi H, Kennedy LJ, Speeti M (2011) Evaluation of DLA promoters in Doberman hepatitis. Tissue Antigens 78:446–450. https://doi.org/10.1111/j.1399-0039.2011.01784.x
22. Weiss DJ, Armstrong PJ, Mruthyunjaya A. Anti-liver membrane protein antibodies in dogs with chronic hepatitis. J Vet Intern Med 1995:267–271
23. Andersson M, Sevelius E. Circulating autoantibodies in dogs with chronic liver disease. J Small Ani Pr 1992:389–394
24. Dyggve H, Jarva H, Spillmann T, Speeti M, Meri S Identification of glyceraldehyde-3-phosphate and alcohol dehydrogenases as autoantigens in Doberman hepatitis. Scand J Immuno 2017:156–164
25. Dyggve H, Meri S, Spillmann T, Jarva H, Speeti M Antihistone autoantibodies in Dobermans with hepatitis. J Vet Intern Med 2017:1717–1723
26. Speeti M, Eriksson J, Saari S, Westermarck E Lesions of subclinical Doberman hepatitis. Vet Pathol 1998:361–369
27. Crawford MA, Schall WD, Jensen RK, Tasker JB Chronic active hepatitis in 26 Doberman Pinschers. J Am Vet Med Assoc 1985:1343–1350
28. Hoffmann G, Heuven HC, Leegwater PA, et al Heritabilities of copper-accumulating traits in Labrador Retrievers. p 454
29. Strombeck DR, Miller LM, Harrold D (1988) Effects of corticosteroid treatment on survival time in dogs with chronic hepatitis: 151 cases (1977-1985). J Am Vet Med Assoc 193:1109–1113
30. Favier RP, Poldervaart JH, van den Ingh TS, et al A retrospective study of oral prednisolone treatment in canine chronic hepatitis. Vet Q 2013:113–120
31. Ullal T, Ambrosini Y, Rao S, Webster CRL, Twedt D (2019) Retrospective evaluation of cyclosporine in the treatment of presumed idiopathic chronic hepatitis in dogs. J Vet Intern Med 33:2046–2056. https://doi.org/10.1111/jvim.15591
32. Neumann Stephan Reactive hepatitis in dogs. Glob Vet 2012:454–459
33. Daure E, Ross L, Webster CRL (2017) Gastroduodenal Ulceration in Small Animals: Part 1. Pathophysiology and Epidemiology. J Am Anim Hosp Assoc 53:1–10. https://doi.org/10.5326/JAAHA-MS-6635

34. Shih JL, Keating JH, Freeman LM, Webster CRL (2007) Chronic Hepatitis in Labrador Retrievers: Clinical Presentation and Prognostic Factors. J Vet Intern Med 21:33–39. https://doi.org/10.1111/j.1939-1676.2007.tb02925.x

35. Fry W, Lester C, Etedali NM, Shaw S, DeLaforcade A, Webster CRL Thromboelastography in dogs with chronic hepatopathies. J Vet Intern Med 2017:419–426

36. Fieten H, Biourge VC, Watson AL, Leegwater PAJ, van den Ingh TSGAM, Rothuizen J Dietary management of Labrador Retrievers with subclinicalhepatic copper accumulation. J Vet Intern Med 2015:822–827

37. Hoffmann G (2009) Copper-Associated Liver Diseases. Vet Clin North Am Small Anim Pract 39:489–511. https://doi.org/10.1016/j.cvsm.2009.02.001

38. Johnson GF, Zawie DA, Gilbertson SR, Sternlieb I Chronic active hepatitis in Doberman Pinschers. J Vet Med Assoc 1982:1438–1442

39. Hutchins RG, Breitschwerdt EB, Cullen JM, Bissett SA, Gookin JL Limited yield of diagnoses of intrahepatic infectious causes of canine granulomatous hepatitis from archival liver tissue. J Vet Diagn Invest 2012:888–894

40. Thornburg LP, Shaw D, Dolan M, et al Hereditary copper toxicosis in West Highland White Terriers. Vet Pathol 1986:148–145

41. Webb CB, Twedt DC, Meyer DJ. Copper-associated liver disease in Dalmatians: a review of 10 dogs (1998-2001). J Vet Intern Med 2002:665–668

42. Dirksen K, Verzijl T, Grinwis GC, et al Use of serum microRNAs as biomarker for hepatobiliary diseases in dogs. J Vet Intern Med 2016:1816–1823

43. Andersson M, Sevelius E Breed, sex and age distribution with chronic liver disease: a demographic study. J Small Anim Pr 1991:1–5

44. Dirksen K, Verzijl T, van den Ingh TS, et al Hepatocyte-derived microRNAs as sensitive serum biomarkers of hepatocellular injury in Labrador Retrievers. Vet J 2016:75–81

45. Koenig EM, Fisher C, Bernard H, et al The beagle dog microRNA tissue atlas: identifying translatable biomarkers of organ toxicity. BMC Genomics 2916:649

46. Oosthuyzen W, Ten Berg PWL, Francis B, et al Sensitivity and specificity of microRNA-122 for liver disease in dogs. J Vet Intern Med 2018:1637–1644

47. Daure E, Ross L, Webster CRL (2017) Gastroduodenal Ulceration in Small Animals: Part 2. Proton Pump Inhibitors and Histamine-2 Receptor Antagonists. J Am Anim Hosp Assoc 53:11–23. https://doi.org/10.5326/JAAHA-MS-6634

48. Fieten H, Dirksen K, van den Ingh TS, et al D-penicillamine treatment of copper-associated hepatitis in Labrador Retrievers. Vet J 2013:522–527

49. Poldervaart JH, Favier RP, Penning LC, van den Ingh TSGAM, Rothuizen J Primary hepatitis in dogs: a retrospective review (2002-2006). J Vet Intern Med 2009:72–80

50. Nakaichi M, Iseri T, Horikirizono H, Komine M, Itoh H, Sunahara H, Nemoto Y, Itamoto K, Tani K (2021) Copper-associated hepatitis in a young Dalmatian dog in Japan. J Vet Med Sci 83:911–915. https://doi.org/10.1292/jvms.21-0061

51. Center SA, ManWarren T, Slater MR, Wilentz E Evaluation of twelve-hour prepardial and two-hour postpradial serum bile acids concentration for diagnosis of hepatibiliary disease in dogs. J AM Vet Med Assoc 1991:217–226

52. Gómez Selgas A, Bexfield N, Scase TJ, Holmes MA, Watson P Total serum bilirubin as a negative prognostic factor in idiopathic canine chronic hepatitis. J Vet Diagn Invest 2014:246–251
53. Center SA Serum bile acids in companion animal medicine. J Vet Diagn Invest 2014:246–657
54. Center SA, Baldwin BH, Erb HN, Tennant BC Bile acid concentrations in the diagnosis of hepatobiliary disease in the dog. J AM Vet Med Assoc 1993:625–657
55. Toulza O, Center SA, Brooks MB, Erb HN, Warner KL, Deal W Evaluation of plasma protein C activity for detection of hepatobiliary disease and portosystemic shunting in dogs. J Am Vet Assoc 2006:1761–1771
56. Toulza O, Center SA, Brooks MB, Erb HN, Warner KL, Deal W (2006) Evaluation of plasma protein C activity for detection of hepatobiliary disease and portosystemic shunting in dogs. J Am Vet Med Assoc 229:1761–1771. https://doi.org/10.2460/javma.229.11.1761
57. Gerritzen-Bruning MJ, Ingh TSGAM, Rothuizen J (2006) Diagnostic Value of Fasting Plasma Ammonia and Bile Acid Concentrations in the Identification of Portosystemic Shunting in Dogs. J Vet Intern Med 20:13–19. https://doi.org/10.1111/j.1939-1676.2006.tb02818.x
58. Goggs R, Serrano S, Szladovits B, Keir I, Ong R, Hughes D (2008) Clinical investigation of a point-of-care blood ammonia analyzer. Vet Clin Pathol 37:198–206. https://doi.org/10.1111/j.1939-165X.2008.00024.x
59. Rothuizen J, van den Ingh TS (1982) Arterial and venous ammonia concentrations in the diagnosis of canine hepato-encephalopathy. Res Vet Sci 33:17–21
60. Partington BP, Biller DS (1995) Hepatic Imaging with Radiology and Ultrasound. Vet Clin North Am Small Anim Pract 25:305–335. https://doi.org/10.1016/S0195-5616(95)50029-4
61. Penninck, Dominique, and Marc-André d'Anjou, eds Atlas of small animal ultrasonography
62. Dominique Pennick, Marc-André d'Anjou Atlas of Small Animal Ultrasonography. John Wilay & Sons
63. Bravo AA, Sheth SG, Chopra S (2001) Liver Biopsy. N Engl J Med 344:495–500. https://doi.org/10.1056/NEJM200102153440706
64. Rothuizen J, Twedt DC (2009) Liver Biopsy Techniques. Vet Clin North Am Small Anim Pract 39:469–480. https://doi.org/10.1016/j.cvsm.2009.02.006
65. Kerwin SC (1995) Hepatic Aspiration and Biopsy Techniques. Vet Clin North Am Small Anim Pract 25:275–291. https://doi.org/10.1016/S0195-5616(95)50027-0
66. Westgren F, Hjorth T, Uhlhorn M, Etterlin PE, Ley CJ (2014) Hepatic emphysema associated with ultrasound-guided liver biopsy in a dog. Acta Vet Scand 56:25. https://doi.org/10.1186/1751-0147-56-25
67. McDevitt HL, Mayhew PD, Giuffrida MA, Brown DC, Culp WTN, Runge JJ (2016) Short-term clinical outcome of laparoscopic liver biopsy in dogs: 106 cases (2003–2013). J Am Vet Med Assoc 248:83–90. https://doi.org/10.2460/javma.248.1.83
68. Cullen JM, Stalker MJ (2016) Liver and Biliary System. In: Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 2. Elsevier, pp 258-352.e1

69. Brewer GJ, Dick RD, Schall W, Yuzbasiyan-Gurkan V, Mullaney TP, Pace C, Lindgren J, Thomas M, Padgett G (1992) Use of zinc acetate to treat copper toxicosis in dogs. J Am Vet Med Assoc 201:564–568

70. Brewer G (1998) Wilson disease and canine copper toxicosis. Am J Clin Nutr 67:1087S-1090S. https://doi.org/10.1093/ajcn/67.5.1087S

71. Hoffmann G, van den Ingh TS, Bode P, et al Copper-associated chronic hepatitis in Labrador Retrievers. J Vet Intern Med 2006:856–861

72. Mandigers PJJ, van den Ingh TSG a. M, Spee B, Penning LC, Bode P, Rothuizen J (2004) Chronic hepatitis in Doberman pinschers. A review. Vet Q 26:98–106. https://doi.org/10.1080/01652176.2004.9695173

73. Rifkin J, Miller MD (2014) Copper-associated hepatitis in a Pembroke Welsh corgi. Can Vet J Rev Veterinaire Can 55:573–576

74. Dirksen K, Fieten H (2017) Canine Copper-Associated Hepatitis. Vet Clin North Am Small Anim Pract 47:631–644. https://doi.org/10.1016/j.cvsm.2016.11.011

75. Spee B, Arends B, Ingh TSGAM, Penning LC, Rothuizen J (2006) Copper Metabolism and Oxidative Stress in Chronic Inflammatory and Cholestatic Liver Diseases in Dogs. J Vet Intern Med 20:1085–1092. https://doi.org/10.1111/j.1939-1676.2006.tb00706.x

76. Azumi N (1982) Copper and liver injury--experimental studies on the dogs with biliary obstruction and copper loading. Hokkaido Igaku Zasshi 57:331–349

77. Vasanjee SC, Bubenik LJ, Hosgood G, Bauer R (2006) Evaluation of Hemorrhage, Sample Size, and Collateral Damage for Five Hepatic Biopsy Methods in Dogs. Vet Surg 35:86–93. https://doi.org/10.1111/j.1532-950X.2005.00117.x

78. Center SA, McDonough SP, Bogdanovic L (2013) Digital image analysis of rhodanine-stained liver biopsy specimens for calculation of hepatic copper concentrations in dogs. Am J Vet Res 74:1474–1480. https://doi.org/10.2460/ajvr.74.12.1474

79. Johnston AN, Center SA, McDonough SP, Warner KL (2009) Influence of biopsy specimen size, tissue fixation, and assay variation on copper, iron, and zinc concentrations in canine livers. Am J Vet Res 70:1502–1511. https://doi.org/10.2460/ajvr.70.12.1502

80. Johnston AN, Center SA, McDonough SP, Wakshlag JJ, Warner KL (2013) Hepatic copper concentrations in Labrador Retrievers with and without chronic hepatitis: 72 cases (1980–2010). J Am Vet Med Assoc 242:372–380. https://doi.org/10.2460/javma.242.3.372

81. Johnson GF, Gilbertson SR, Goldfischer S, Grushoff PS, Sternlieb I (1984) Cytochemical Detection of Inherited Copper Toxicosis of Bedlington Terriers. Vet Pathol 21:57–60. https://doi.org/10.1177/030098588402100110

82. Johnson GF, Sternlieb I, Twedt DC, Grushoff PS, Scheinberg I (1980) Inheritance of copper toxicosis in Bedlington terriers. Am J Vet Res 41:1865–1866

83. Su LC, Ravanshad S, Owen CA, McCall JT, Zollman PE, Hardy RM (1982) A comparison of copper-loading disease in Bedlington terriers and Wilson's disease in humans. Am J Physiol-Gastrointest Liver Physiol 243:G226–G230. https://doi.org/10.1152/ajpgi.1982.243.3.G226

84. Johnson GF, Morell AG, Stockert RJ, Sternlieb I (1981) Hepatic lysosomal copper protein in dogs with an inherited copper toxicosis. Hepatology 1:243–248. https://doi.org/10.1002/hep.1840010309

85. Lerch K, Johnson GF, Grushoff PS, Sternlieb I (1985) Canine hepatic lysosomal copper protein: Identification as metallothionein. Arch Biochem Biophys 243:108–114. https://doi.org/10.1016/0003-9861(85)90778-7

86. Van De Sluis B (2002) Identification of a new copper metabolism gene by positional cloning in a purebred dog population. Hum Mol Genet 11:165–173. https://doi.org/10.1093/hmg/11.2.165

87. Willard M, Ettinger S, Feldman E Textbook of veterinary internal medicine, 7th ed

88. Rolfe DS, Twedt DC (1995) Copper-Associated Hepatopathies in Dogs. Vet Clin North Am Small Anim Pract 25:399–417. https://doi.org/10.1016/S0195-5616(95)50034-8

89. Tao TY, Liu F, Klomp L, Wijmenga C, Gitlin JD (2003) The Copper Toxicosis Gene Product Murr1 Directly Interacts with the Wilson Disease Protein. J Biol Chem 278:41593–41596. https://doi.org/10.1074/jbc.C300391200

90. Haywood S, Boursnell M, Loughran MJ, Trafford J, Isherwood D, Liu X, Olohan L, Carter SD (2016) Copper toxicosis in non- COMMD1 Bedlington terriers is associated with metal transport gene ABCA12. J Trace Elem Med Biol 35:83–89. https://doi.org/10.1016/j.jtemb.2016.01.015

91. Fieten H, Gill Y, Martin AJ, Concilli M, Dirksen K, Van Steenbeek FG, Spee B, Van Den Ingh TSGAM, Martens ECCP, Festa P, Chesi G, Van De Sluis B, Houwen RHJH, Watson AL, Aulchenko YS, Hodgkinson VL, Zhu S, Petris MJ, Polishchuk RS, Leegwater PAJ, Rothuizen J (2016) The Menkes and Wilson disease genes counteract in copper toxicosis in Labrador retrievers: a new canine model for copper-metabolism disorders. Dis Model Mech 9:25–38. https://doi.org/10.1242/dmm.020263

92. Mandigers PJJ, Ingh TSGAM, Bode P, Teske E, Rothuizen J (2004) Association between Liver Copper Concentration and Subclinical Hepatitis in Doberman Pinschers. J Vet Intern Med 18:647–650. https://doi.org/10.1111/j.1939-1676.2004.tb02600.x

93. Noaker LJ, Washabau RJ, Detrisac CJ, Heldmann E, Hendrick MJ (1999) Copper associated acute hepatic failure in a dog. J Am Vet Med Assoc 214:1502–1506, 1495

94. Sevelius E (1995) Diagnosis and prognosis of chronic hepatitis and cirrhosis in dogs. J Small Anim Pract 36:521–528. https://doi.org/10.1111/j.1748-5827.1995.tb02801.x

95. Webb CB, Twedt DC, Meyer DJ Copper-associated liver disease in Dalmatians: a review of 10 dogs (1998-2001. J Vet Intern Med 2002:665–668

96. Fieten H, Biourge VC, Watson AL, Leegwater PAJ, Van Den Ingh TSGAM, Rothuizen J (2014) Nutritional management of inherited copper-associated hepatitis in the Labrador retriever. Vet J 199:429–433. https://doi.org/10.1016/j.tvjl.2013.12.017

97. Hoffmann G, Jones PG, Biourge V, Van Den Ingh TSGAM, Mesu SJ, Bode P, Rothuizen J (2009) Dietary Management of Hepatic Copper Accumulation in Labrador Retrievers. J Vet Intern Med 23:957–963. https://doi.org/10.1111/j.1939-1676.2009.0352.x

98. Gagné JW, Wakshlag JJ, Center SA, Rutzke MA, Glahn RP (2013) Evaluation of calcium, phosphorus, and selected trace mineral status in commercially available dry

foods formulated for dogs. J Am Vet Med Assoc 243:658–666. https://doi.org/10.2460/javma.243.5.658

99. Fieten H, Hooijer-Nouwens BD, Biourge VC, Leegwater PAJ, Watson AL, Van Den Ingh TSGAM, Rothuizen J (2012) Association of Dietary Copper and Zinc Levels with Hepatic Copper and Zinc Concentration in Labrador Retrievers. J Vet Intern Med 26:1274–1280. https://doi.org/10.1111/j.1939-1676.2012.01001.x
100. Bergstrom RF, Kay DR, Wagner JG (1981) The pharmacokinetics of penicillamine in a female mongrel dog. J Pharmacokinet Biopharm 9:603–621. https://doi.org/10.1007/BF01061028
101. Schumacher K (1975) [Basis of immunosuppressive effect of D-penecillamine]. Internist 16:460–465
102. Kazemi K, Geramizadeh B, Nikeghbalian S, Salahi H, Bahador A, Reza Nejatollahi SM, Dehghani SM, Dehghani M, Kakaei F, Malek-Hosseini SA (2008) Effect of D-penicillamine on liver fibrosis and inflammation in Wilson disease. Exp Clin Transplant Off J Middle East Soc Organ Transplant 6:261–263
103. Epstein O, De Villiers D, Jain S, Potter BJ, Thomas HC, Sherlock S (1979) Reduction of Immune Complexes and Immunoglobulins Induced by D-Penicillamine in Primary Biliary Cirrhosis. N Engl J Med 300:274–278. https://doi.org/10.1056/NEJM197902083000602
104. DeVore DP, Gruebel RJ, Hutson TB, Turner RH (1979) Effects of D-Penicillamine on lymphocyte modulation of synovial collagenase production. Agents Actions 9:172–176. https://doi.org/10.1007/BF02024730
105. Członkowska A, Litwin T (2017) Wilson disease – currently used anticopper therapy. In: Handbook of Clinical Neurology. Elsevier, pp 181–191
106. Nagasaka H, Inoue I, Inui A, Komatsu H, Sogo T, Murayama K, Murakami T, Yorifuji T, Asayama K, Katayama S, Uemoto S, Kobayashi K, Takayanagi M, Fujisawa T, Tsukahara H (2006) Relationship Between Oxidative Stress and Antioxidant Systems in the Liver of Patients With Wilson Disease: Hepatic Manifestation in Wilson Disease as a Consequence of Augmented Oxidative Stress. Pediatr Res 60:472–477. https://doi.org/10.1203/01.pdr.0000238341.12229.d3
107. Gaetke LM, Chow-Johnson HS, Chow CK (2014) Copper: toxicological relevance and mechanisms. Arch Toxicol 88:1929–1938. https://doi.org/10.1007/s00204-014-1355-y
108. Sokol RJ, Devereaux M, Khandwala RA (1991) Effect of dietary lipid and vitamin E on mitochondrial lipid peroxidation and hepatic injury in the bile duct-ligated rat. J Lipid Res 32:1349–1357
109. von Herbay A, de Groot H, Hegi U, Stremmel W, Strohmeyer G, Sies H (1994) Low vitamin E content in plasma of patients with alcoholic liver disease, hemochromatosis and Wilson's disease. J Hepatol 20:41–46