

EDITORIAL



From a 21st century perspective it may seem odd that for many pre-Christian societies the liver was the subject of not only debate and conjecture but of myth and mysticism. In some cultures the organ was regarded as a powerful force, an authority that controlled the body and directed potent passions such as anger, jealousy or spite, whilst at least one ancient civilization used the liver as a means of divination and prophecy. Although we may dismiss such fanciful notions today we should remember that there is still a lingering idea that this, the largest of the visceral organs, commands a compelling power within an individual; in many countries someone who is regarded as disagreeable or irritable may be described as “liverish”, whilst on a more positive note more than one of the world’s languages employs the word “liver” as a figure of speech to signify courage and strength.

Despite the fact that mysticism may now have given way to scientific study, the liver continues to fascinate and frustrate. The only mammalian organ capable of regeneration to a large extent; the ancients were right: it is the powerhouse of the body, the central factory responsible for undertaking literally hundreds of chemical processes every day to ensure body homeostasis and continuing health, and a failing or diseased liver can be a disaster for an individual. Nowadays our knowledge of hepatic physiology and pathology is extensive but when it comes to successful treatment of liver dysfunction clinicians may feel that we lag behind other veterinary disciplines, partly as there are few drugs which one may regard as being specific for this one organ and partly because the organ’s omnipotence makes reversal of disease so very challenging.

In this edition of FOCUS we attempt to unravel some of the unknowns of this organ, but recognize that because the liver is so powerful, so essential, we cannot cover the subject in its entirety. That said, the experts who have contributed toward this issue have together produced a reference work that should benefit it all small animal veterinarians, and we believe that the reader will profit so that the liver is less of a mystery than before.

*Ewan McNeill
Editor-in-Chief*

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The licensing arrangements for therapeutic agents intended for use in small animal species vary greatly worldwide. In the absence of a specific license, consideration should be given to issuing an appropriate cautionary warning prior to administration of any such drug.

Feline inflammatory liver disease - an overview



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◆ Introduction

In the broadest terms, conditions affecting the feline liver may be divided into inflammatory and non-inflammatory diseases. Unlike dogs, where inflammatory conditions tend to affect the hepatic parenchyma, in cats the biliary system is more commonly affected, with extension into the hepatic parenchyma only in more severe cases. As a result, cats with hepatic disease will frequently present

with jaundice (*Figure 1*) but rarely develop a small cirrhotic liver hence; with the exception of portosystemic shunts, the finding of a small liver is unusual in the feline patient. It should also be remembered that the liver may be affected by systemic diseases such as feline infectious peritonitis (FIP). This article reviews the complex group of disorders that is feline inflammatory liver disease. Outwith the scope of this article, but mentioned where appropriate when considering differential diagnosis, are the non-inflammatory conditions, the most important of which is hepatic lipidosis (HL). Other non-inflammatory causes include liver neoplasia, portosystemic shunts, amyloidosis, and polycystic liver disease.

➔ KEY POINTS

- ➔ Conditions affecting the feline liver may be divided into inflammatory and non-inflammatory diseases. Causes of liver disease in cats are frequently different from those identified in dogs.
- ➔ Patients with liver disease may vary in their presentation from mild, vague clinical signs to severely ill patients with multiple metabolic complications.
- ➔ Differential diagnosis of the most common clinical signs (jaundice, ascites and hepatomegaly) are considered. Specific diagnosis of the underlying disease usually requires biopsy.
- ➔ Naso-esophageal, esophageal or gastrostomy tube feeding are necessary in anorexic cats.

◆ Inflammatory liver disease

Given that feline inflammatory liver disease more typically affects the biliary system it should more correctly be called cholangitis, and in the UK cholangitis is the most commonly identified feline liver disorder. Different forms of cholangitis may be identified by histopathology of liver biopsies. Unfortunately, numerous different terminologies have been used over the years in the veterinary literature, hence the WSAVA have produced guidelines in an attempt to standardize the diagnosis (1).

The cholangitis complex comprises lymphocytic cholangitis (LC), neutrophilic cholangitis (NC), and chronic cholangitis associated with liver fluke (1). Mild lymphocytic portal hepatitis should not be over-interpreted as it is believed to be a non-specific reactive change possibly reflecting extra-hepatic disease or resolving hepatitis: >80% of cats over 10 years of age were reported to have these mild changes in one study (2).

Lymphocytic cholangitis

Previously termed non-suppurative or lymphocytic-plasmacytic cholangitis/cholangiohepatitis, the etiology of this condition is not known but an immune-mediated mechanism may play a role.

Presentation

Cats of any age may be affected, but disease is seen most typically in young to middle aged cats; Persian cats may have an increased risk (3). Clinical signs are usually chronic and insidious in nature, but may be acute. Affected cats are typically jaundiced, but appear to be clinically well, and are often polyphagic. Weight loss may be seen, despite a good appetite. Anorexia can also be seen, as can vomiting and/or diarrhea. Cats may have a palpably enlarged liver, and enlarged mesenteric lymph nodes; mild generalized lymphadenopathy may also be present. The disease may progress to causing chronic biliary cirrhosis with ascites, hepatic encephalopathy, and bleeding tendencies. Ascites may be present – in acute cases due to a hepatic exudate (which can make this condition difficult to differentiate from wet FIP), and in chronic cases from portal hypertension resulting from periportal fibrosis and cirrhosis.

Diagnosis

Serum biochemistry often reveals mild/moderately (occasionally severely) increased liver enzymes, increased bile acids, hyperbilirubinemia, hyperglobulinemia, and hypoalbuminemia. Hematology may reveal mild anemia, lymphopenia or lymphocytosis, monocytosis, and/or thrombocytopenia. Blood clotting times are frequently prolonged. Ascitic fluid, if present, is typically high in protein. Ultrasound examination may show heterogeneous hepatic parenchyma, which is often hyperechoic. Histopathology typically shows periportal lymphocytic infiltration. Portal fibrosis and proliferation of biliary ductules may also be seen.



Figure 1.

Cats with liver disease exhibiting jaundice.

©Danielle Gumm-Moore

Differential diagnoses

Differential diagnoses in a cat presenting with some or all of the above signs are numerous and are summarized in **Tables 1-3**. The primary possibilities to consider would be FIP (especially if ascites is present), NC, hepatic lipidosis (HL), lymphoma, and if weight loss with a good appetite is present, hyperthyroidism or exocrine pancreatic insufficiency.

Treatment

Treatment is largely empirical, as it is important to remember that there are NO specific treatments for liver disease. Supportive therapies for hepatic disease may be beneficial (*see Table 4*).

- Prednisolone (1-2 mg/kg q 12h *per os*). Due to the suspected immune-mediated etiology, immunosuppression is considered appropriate. Once remission of clinical signs is achieved, the dose should be tapered over 6-12 weeks to 1 mg/kg q 48h) and maintained on every other day if needed.
- Other immunosuppressive agents may be considered *e.g.* methotrexate (0.13 mg/cat *per os* q 8-12h for 3 doses; given every 7-10 days), chlorambucil (2-5 mg/m² *per os* up to once every 48h or 2-4 mg *per os* every 1-3 weeks), or cyclosporin A (measure serum levels, start at 2 mg/kg *per os* q 12h). Note that these drugs are all potentially hepatotoxic. Do not give azathioprine; it is toxic to cats.

Table 1.**Differential diagnoses of jaundice.**

Pre-hepatic	Hepatic	Post-hepatic	Sepsis
<ul style="list-style-type: none"> • Infection <ul style="list-style-type: none"> - <i>M. hemofelis</i> - <i>M. hemominutum</i> - <i>M. turicensis</i> - <i>B. felis</i> - FeLV • Immune-mediated <ul style="list-style-type: none"> - Primary IMHA - Drugs - methimazole - Toxin - Paracetamol/acetaminophen - Propofol - Onions - Methylene blue • Metabolic <ul style="list-style-type: none"> - Hypophosphatemia - Diabetic ketoacidosis • Anomalous <ul style="list-style-type: none"> - Pyruvate kinase deficiency (Abyssinian, Somali cats) - Porphyria - Erythrocyte fragility (Abyssinian, Somali cats) 	<ul style="list-style-type: none"> • Cholangitis <ul style="list-style-type: none"> - Lymphocytic - Acute neutrophilic - Chronic neutrophilic - Hepatic lipidosis • Toxin <ul style="list-style-type: none"> - Drugs - Heavy metals • Neoplasia <ul style="list-style-type: none"> - Hepatocellular carcinoma - Hepatoma - Biliary carcinoma • Infection <ul style="list-style-type: none"> - FIP - Toxoplasmosis • Amyloidosis (Siamese cats) • Cystic disease 	<ul style="list-style-type: none"> - Cholelithiasis - Rupture of bile duct or gallbladder - Neoplasia - Pancreatitis - Pancreatic mass lesions - Obstruction of common bile duct by duodenal disease 	<ul style="list-style-type: none"> - <i>Salmonella</i> - <i>E. coli</i> - <i>Streptococcus</i> spp. - <i>Staphylococcus</i> spp.

Prognosis

Limited studies are available on the outcome of cats with LC, but the authors' clinical experience is that cats with this condition generally respond well to treatment. Cats with ascites carry a poorer prognosis than those presenting with jaundice alone, as ascites may represent more marked fibrosis due to more advanced disease. Although clinical signs may resolve, owners should be warned of the potential for relapse, and some cats may require chronic therapy with prednisolone to prevent relapses when medication is stopped.

Neutrophilic cholangitis

Previously termed suppurative or exudative cholangitis/cholangiohepatitis, both acute (ANC) and chronic (CNC) forms are described. Neutrophilic cholangitis is attributed to ascending bacterial infection from the gastrointestinal tract. Infection may also ascend up the pancreatic bile duct, hence the frequent association with NC and pancreatitis. Inflammatory bowel disease (IBD) is also frequently associated with these conditions, resulting in the term "triaditis". Recently *Helicobacter* infection has been suggested to play a role in development of NC (4).

Presentation

Cats of any age may be affected, but middle-aged to older cats are reported to be affected more typically. With ANC clinical signs are typically severe, and include fever, anorexia, vomiting and lethargy. Vomiting is frequent in all types of biliary disease, possibly because inflammation of the bile ducts stimulates their rich autonomic nerve supply and triggers the emetic center of the brain. Cats with ANC may be jaundiced and/or demonstrate abdominal pain. Acute disease may progress to chronic disease. CNC typically has a waxing and waning time course of months to years, with periods of anorexia, vomiting and weight loss. Cats with CNC may be jaundiced and show hepatomegaly; ascites is rare. Systemic signs may be associated with secondary infections, typically of the liver and/or pancreas and "triaditis" is common: in one study 83% of cases had concurrent IBD whilst 50% had pancreatitis (5).

Diagnosis

Initially with ANC, when the inflammation is limited to the larger bile ducts and gallbladder, there may be little or no changes in the total

Table 2.

Differential diagnoses of ascites.

Transudate	Modified Transudate	Exudate	Other
<ul style="list-style-type: none"> • Decreased oncotic pressure <ul style="list-style-type: none"> - Protein-losing nephropathy - Protein-losing enteropathy - Decreased synthesis (liver disease) - Malnutrition - Severe burns • Increased hydrostatic pressure <ul style="list-style-type: none"> - Cardiac failure - Portal hypertension - Cirrhosis - Portal vein thrombosis - Portal vein hypoplasia 	<ul style="list-style-type: none"> • Neoplasia <ul style="list-style-type: none"> - Abdominal carcinomatosis - Lymphangiosarcoma • Inflammation <ul style="list-style-type: none"> - Pancreatitis - Cholangitis 	<ul style="list-style-type: none"> • Septic peritonitis • FIP • Bile peritonitis • Toxoplasmosis 	<ul style="list-style-type: none"> • Hemoabdomen • Uroabdomen

bilirubin and even the liver enzymes. More typically, there are mild to moderate increases in ALT, ALP, GGT, bile acids and bilirubin. Most CNC cases have raised liver enzymes (with GGT typically being proportionately higher than ALP). Hematology may reveal a mild to moderate leukocytosis, typically due to neutrophilia. Chronic or severe disease may result in mild anemia, lymphopenia or lymphocytosis, monocytosis, and/or thrombocytopenia. Prolonged clotting times may also be identified. Radiographs are often unhelpful, with ultrasound examination being the imaging modality of choice. The liver typically appears heterogeneous with increased echogenicity. The bile ducts are typically distended (> 5 mm) and often tortuous in appearance. The gallbladder may appear distended, with a thickened wall (> 1 mm) which suggests cholecystitis. Cholelithiasis (gallstones) may occasionally develop. Associated findings may include enlarged mesenteric lymph nodes, pancreatic irregularity, and/or thickening of the duodenal walls. Ultrasound guidance may also be used to obtain an aspirate of bile from the gallbladder (taken through the right medial liver lobe where the gallbladder is attached to the liver to reduce the risk of intra-peritoneal leakage of bile). This procedure carries the risk of bile peritonitis, particularly if cholecystitis is present or a large bore needle is required for centesis due to the inspissated nature of the bile. Aspirates from both the liver and bile should ideally be sent for cytological assessment and bacterial culture (preferably aerobic and anaerobic culture). Liver biopsies are more reliable for assessment of hepatic architecture.

Table 3.

Differential diagnosis of hepatomegaly.

Neoplasia	Lymphoma Hepatocellular carcinoma Hepatoma Biliary carcinoma Mastocytosis
Inflammatory	Cholangitis (lymphocytic, neutrophilic)
Metabolic/ endocrine	Hepatic lipidosis Diabetes mellitus Hyperadrenocorticism Acromegaly
Anomalous	Lysosomal storage diseases Polycystic liver/kidney disease Amyloidosis Telangectasis/peliosis
Congestion	Cardiac disease
Infectious	FIP Toxoplasmosis (especially kittens)

Differential diagnosis

Primary differential diagnoses to consider for acute disease include pancreatitis, sepsis, systemic infections (e.g. *Salmonella*, FIP, toxoplasmosis), HL, hepatotoxicity or biliary tract obstruction.

Treatment

Due to the association with infection, anti-bacterials are administered, along with supportive therapies (Table 4). Antibacterials may need to

Table 4.**Supportive therapies for feline hepatic disease.**

Medication	Dose	Comments
s-adenosyl methionine (SAMe)	20-40 mg/kg PO q 24h	Nucleotide essential for major hepatic biochemical pathways with antioxidant and hepatoprotective properties. A higher dose is required if enteric coated tablets are crushed for administration via feeding tube.
Ursodeoxycholic acid (UDCA)	10-15 mg/kg PO q 24h	Synthetic hydrophilic bile acids that aid bile flow. It has anti-inflammatory, immuno-modulatory, and anti-fibrotic activities, and is cytoprotective to hepatocytes.
Milk thistle (common name for <i>Silybum marianum</i>)	Optimal dosage unknown, range from 20-50 mg/kg PO q 24h	Contains silybinin (silybin/silymarin); may be useful for treatment of chronic and acute liver disease, including exposure to hepatotoxins (e.g. <i>Amanita phalloide</i> : "Death Cap mushrooms") and cirrhosis.
Cobalamin (vitamin B12)	0.125-0.25 mg/cat SC q 7-14 days	Liver cobalamin stores may be depleted in the face of normal serum cobalamin; may help stimulate appetite.
Vitamin K1	0.5-1.0 mg/kg PO or SC q 12h for 3 doses	Use 25G needle if given by injection to reduce risk of hematoma formation. Vitamin K required for various clotting factors.
Vitamin E	10 IU/kg PO q 24h	Antioxidant often depleted with anorexia or mal-absorption.
Maropitant	0.5-1.0 mg/kg SC q 24h	Anti-emetic.
Metoclopramide	1 mg/kg q 24h IV as constant rate infusion	Central anti-emetic effect in cats questionable due to lack of dopaminergic receptors, but peripheral prokinetic effects.
Ranitidine	2 mg/kg PO or SC or IV q 8-12h	Preferred to cimetidine due to intestinal prokinetic effect.

be given for 1-3 months. *E. coli* is the most frequently isolated organism, but mixed infections are not uncommon. Ideally, antibacterials should be administered as directed by culture and sensitivity of bile and/or liver samples, but empiric choices are as follows:

- Amoxicillin/clavulanate (11-22 mg/kg *per os* q 8-12h) *or*
- Cephalexin (10-35 mg/kg *per os* q 8-12h) plus a fluoroquinolone (well concentrated in bile). The authors prefer marbofloxacin (2 mg/kg *per os* q 24h) over enrofloxacin because of the risk of irreversible blindness in cats *plus*
- Metronidazole for its effect against anaerobes and its immune-modulating effects (7.5-10 mg/kg *per os* q 12h). Do not use higher doses, as these

can be hepatotoxic, neurotoxic and potentially teratogenic.

- Clindamycin (5.5 mg/kg *per os* q 12h) has efficacy against anaerobes and gram positive organisms, but not gram negative organisms. It is concentrated in the bile, but care should be taken with hepatic impairment, as this is the main route of metabolism.
- In very severe cases give according to '4 quadrant cover' IV (*i.e.* to cover gram positive and negative, anaerobes and aerobes – e.g. amoxicillin/clavulanate + fluoroquinolone + clindamycin or metronidazole).

Supportive therapies may be required for management of pancreatitis (e.g. intravenous fluid therapy, analgesia, antibiotics) and if IBD is

present, concurrent therapy with prednisolone may also be required.

Prognosis

Prognosis may depend in part on the severity of the disease at presentation and any concurrent problems. The majority of cats are reported to survive in excess of 1 year, with 1 study reporting a median survival time of 29 months (6).

Mixed cholangitis

Some cases of disease are difficult to categorize, due to the mixed nature of the inflammatory disease. This is especially true in more chronic cases. Unless infection can be ruled out by negative culture of bile and hepatic tissue, it is wise to initiate antibacterial therapy in these cases, accepting that prednisolone may also be required if the response to antibacterials alone is not adequate.

Chronic cholangitis associated with liver flukes

This condition is reported in association with a number of species of the *Opisthorchiidae* family which require both snails and fish as intermediate hosts. Endemic areas for fluke species include the Americas, India, China, Japan and Northern Europe. Cats acquire the infection by eating raw fish, with the immature flukes migrating from the intestine to the liver via the bile ducts. Low grade infections may be asymptomatic, but heavy burdens may cause anorexia, vomiting, weight loss, abdominal pain, lethargy and icterus. A peripheral eosinophilia may be present, but elevations in liver enzymes may be transient. Diagnosis requires demonstration of the adult flukes or operculated eggs on liver biopsy samples or aspirated bile. Treatment comprises praziquantel (30 mg/kg *per os* q 24h for 5-10 days), but surgical cannulation and drainage of bile may be required for more severe cases.

Other causes of inflammatory liver disease

FIP may be associated with elevated liver enzymes and bilirubin due to pyogranuloma formation and hepatic necrosis. Usually other clinical signs will be present to suggest a systemic disease process, but differentiation of LC with ascites may require biopsy. Toxoplasmosis may be associated with



Figure 2.

A naso-esophageal feeding tube providing nutrition to a cat.



Figure 3.

Placement of an esophagostomy feeding tube in a cat.

elevated liver enzymes, and less commonly, icterus. Diagnosis is usually based on elevated IgM antibody titers.

Drug reactions may induce hepatic damage either through toxicity or idiosyncratic reactions. The most commonly recognized drugs causing toxicity include diazepam, methimazole and carbimazole, potentiated sulphonamides, tetracyclines, phenobarbitone, griseofulvin (especially FIV+ve cats) and paracetamol (acetaminophen).

Nutrition in cholangitis cases

Whilst some cats with cholangitis may continue to eat well, or if inappetent may respond to tempting, hand feeding or syringe feeding, many cats may require placement of feeding tubes. In the first instance, naso-esophageal tubes are preferred, as they are easy to place and do not require

anesthesia (**Figure 2**). In addition, the risk of hemorrhage is minimal. However, the narrow diameter of the feeding tube limits the type of diet that can be administered via the tube, and irritation of the nasal passages may develop with prolonged use. Assuming the cat can tolerate a general anesthetic, an esophagostomy tube (**Figure 3**) is easy to place and allows for more prolonged feeding, potentially in the home environment. If the underlying disease merits it, a gastrostomy tube may be placed, although this is a more complicated technique requiring a more prolonged anesthetic. The cat's individual caloric requirement should be calculated so an appropriate feeding plan can be generated (*see Table 5*).

Conclusion

Inflammatory feline liver disease is a relatively common problem in general practice. Accurate diagnosis requires examination of biopsy material obtained from the liver, ideally by histopathology. With appropriate supportive treatment, the prognosis for these conditions is generally favorable, although recurrence is possible and an owner should always be informed of this.

Table 5.

Calculation of nutritional requirements.

Step 1 - Calculate calorie requirements based on resting energy requirements (RER).

$$\text{RER (kcal)} = [70 \times \text{bodyweight (kg)}] 0.75$$

Step 2 - Identify the calorie content of the diet (kcal/mL).

$$\text{Daily volume of food (mL)} = \frac{\text{RER (kcal)}}{\text{Diet kcal/mL}}$$

Step 3 - Calculate maximal volume to feed at one meal (10 mL/kg). Allow for fluid used to flush the feeding tube. Calculate the number of meals required per day.

Patients that have been anorexic for a number of days may develop metabolic complications (re-feeding syndrome) as a result of changing from a catabolic state. To try to prevent this, feeding should be gradually re-introduced, for example on day one give 1/3 of calculated RER, day two 2/3 of RER and day three total RER.

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Canine portosystemic shunts: an overview of diagnosis and treatment options



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◆ Introduction

Portosystemic shunts (PSS) are vascular communications taking blood directly from the portal circulation to the systemic circulation, bypassing the liver in the process. Anomalous vessels have been identified connecting between the portal circulation and a wide variety of vessels including the caudal vena cava, the azygous vein, and the renal veins (**Figure 1**). Animals with PSS may have single, double or multiple shunts and may have concurrent abnormalities such as complete portal vein atresia or portal vein hypoplasia (1). PSS may be acquired or congenital, intrahepatic or extra-hepatic. Congenital PSS are diagnosed more commonly in purebred dogs than crossbreeds, with a reportedly high incidence in cairn terriers, dachshunds, miniature schnauzers, golden and Labrador retrievers, Irish wolfhounds (IWH), Maltese terriers and Australian cattle dogs (2). There is evidence to suggest that left division intrahepatic PSS in the IWH and extra-hepatic PSS in cairn and Yorkshire terriers are inherited and there is a suspicion that PSS may be inherited in Australian cattle dogs and other over-represented breeds. However there are no firm or consistent recommendations regarding breeding policies for affected individuals. Clinical signs caused by PSS can be both variable and vague but neurological signs are the most common presentation. However, some cases have no neurological signs at all and instead may present with a history of recurrent urinary tract infections, crystalluria and urate urolithiasis (3). In others the clinical signs are sufficiently vague that the diagnosis is not immediately obvious; for example, recurrent unexplained infections, ongoing nausea and vomiting, or failure to thrive. Diagnosis is based on combining the history and clinical findings with the results of appropriate laboratory tests and diagnostic imaging. A period of stabilization with

➔ KEY POINTS

- ➔ Congenital portosystemic shunts are the most common congenital anomaly of the hepatobiliary system in dogs.
- ➔ Hepatic dysfunction results from the presence of a portosystemic shunt, leading to a wide variety of clinical presentations of which hepatic encephalopathy is the most common.
- ➔ Signalment, history and clinical signs may be strongly suggestive of portosystemic shunt as a diagnosis, but they are by no means conclusive.
- ➔ Definitive diagnosis, and identification of concurrent or complicating problems, requires a combination of laboratory data and diagnostic imaging.
- ➔ Medical and dietary management is generally instituted to stabilize patients prior to surgery.
- ➔ In the absence of a known contraindication to surgery, surgical attenuation or ligation is the treatment of choice.

medical management is typically recommended prior to surgery to attenuate or close the shunt vessel but surgery should be considered the treatment of choice unless there is a specific contra-indication present, such as absence of a portal vein or multiple secondary acquired shunts (2,4,5).

◊ **Signalment and clinical signs**

- Extra-hepatic shunts are primarily reported in small breed dogs, with Maltese terriers and Australian silkies being over-represented in reports from Australia, while certain terrier breeds, miniature schnauzers and dachshunds have been reported as more common within Europe, although terrier breeds, Lhasa apsos and shi tzus seem to be frequently presented in the UK and Ireland.
- Intrahepatic shunts are typically associated with large and medium breed dogs with Australian cattle dogs being over-represented in some reports from Australia, while IWH, deerhounds and retrievers appear to be more commonly affected in Europe. It is worth noting that small breeds may have intrahepatic PSS and large breeds may have extra-hepatic PSS, so assumptions should not be made based on the size of the patient.

Affected animals are typically undersized, may have a history of poor appetite and weight gain and are often perceived as runts (**Figure 2**). Neurological abnormalities are the most common presenting sign and the prime reason for seeking veterinary assistance, and may include shaking, tremors, head-pressing, circling, lack of awareness of surroundings, amaurotic blindness, lethargy, ataxia, seizures and paddling (3,6). Clinical experience shows that affected animals can also present with pyrexia and anorexia due to persistent or recurrent infections where no other obvious signs exist e.g. recurrent abscesses in a young puppy, recurrent gastroenteritis or polyarthritis. Polyuria, stranguria and/or pollakiuria may be noted due to the presence of urate crystals, urolithiasis, increased ammonia excretion and low urea levels (6). Vomiting and diarrhea typically occur intermittently, sometimes associated with nausea.

The pathophysiology of hepatic encephalopathy (HE) is complex and still poorly understood both in veterinary and human patients but is thought to

reflect insufficient hepatic capability to deal with hormones, toxins and other components of metabolism. The pathogenesis is multifactorial, involving increased blood ammonia, other synergistic toxins, alterations in monoamine neurotransmitters, alterations in amino acid neurotransmitters, GABA, glutamate, glutamine and increased cerebral levels of endogenous benzodiazepine-like compounds (3,7). Elevated peripheral and central concentrations of manganese have been recently identified in affected animals (8,9). It seems that the only consistent finding is that none of these factors will reliably induce HE on their own, underlining that this is not a straightforward problem. It may also be that some dogs with neurological signs and a diagnosis of PSS actually have concurrent central neurological disease. HE can be triggered by many factors including feeding, constipation, gastrointestinal bleeding (effectively a high-protein 'feed'), sedatives, anesthetics, azotemia, hypoglycemia, hypovolemia and infection.

The majority of animals with congenital shunts are identified at less than 1 year of age, but those with moderate, mild or atypical signs may not be diagnosed until middle-age. Traditionally, patients above 2 years of age were held to have poorer outcomes; however the available clinical evidence does not support this and older animals should not be automatically viewed as non-surgical (10-12).

◊ **Diagnosis**

The signalment, clinical signs and history will often be suggestive of a PSS; however there are other conditions which may present in a very similar manner so a full diagnostic work-up is essential in every case and assumptions should not lead to shortcuts being taken. This also ensures that secondary complicating factors are not missed, such as clotting abnormalities requiring management prior to surgery.

Hematology and biochemistry

- Microcytosis +/- concurrent anemia is seen in up to 72% of dogs. This anemia typically resolves following surgical closure of the shunt, and is probably due to altered serum iron concentrations and functional defects in iron transport.
- Leukocytosis is variable, and may be related to inadequate clearance of bacteria and toxins through the portal system.

- Hypoalbuminemia and decreased total protein
- Low blood urea nitrogen concentrations
- Low cholesterol and glucose levels
- Elevated liver enzymes
- Increased fasting ammonia levels

Urinalysis and bacteriology

- Low urine specific gravity
- Ammonium biurate crystalluria or urolithiasis
- +/- secondary cystitis

Bile acids

Primary bile acids are synthesized in the liver, conjugated with taurine, secreted into the bile canaliculi and stored in the gallbladder. After a meal, the gallbladder contracts, releasing bile acids into the intestines, where they enhance fat digestion and absorption. In the distal ileum a bile acid-sodium coupled transporter catches the bile acids and returns them to the portal circulation. Efficient uptake and re-secretion by hepatocytes ensures "enterohepatic circulation" of > 95% of all bile acids, with the remaining 5% or so being lost in the feces. Shunting of resorbed bile acids to the systemic circulation means that most animals with PSS will have elevated bile acids, although there are other conditions that may also cause elevation of bile acids. In addition to measuring fasting bile acids, a bile acid stimulation test should be performed, to obtain paired pre- and post-prandial samples. High post-prandial bile acid level or fasting serum ammonia only denotes abnormal liver function, and is not specific for PSS.

Resting serum ammonia levels and ammonia tolerance tests

Reduced ammonia metabolism means that baseline ammonia levels are likely to be increased, although they can be normal in up to 20% of cases if there has been a period of prior medical management or a long period of fasting. Some breeds are known to have higher than normal ammonia levels (e.g. IWH). It is important to use the appropriate cut-off levels to maximize the sensitivity and specificity of these tests. The ammonia tolerance test is quite cumbersome, and carries inherent risks in animals with impaired liver function.

Scintigraphy

Nuclear scintigraphy is a non-invasive means of evaluating the presence of shunting. In normal

animals technetium pertechnetate infused into the mid-proximal colon is transported through the portal system to the liver. In animals with a shunt, a greater proportion of the technetium is carried rapidly past the liver and highlights the heart. The shunt fraction compares activity in the liver with activity in the heart on the first passage of the radiopharmaceutical. In a normal dog, the shunt fraction should be < 15%. Animals with congenital shunts usually have shunt fractions in excess of 60% although there is a lot of variability (13). Note that this method does not allow imaging of the portal circulation and cannot differentiate reliably between single and multiple shunts, nor intra-versus extra-hepatic shunts and therefore it is of limited use for pre-surgical planning. Trans-splenic scintigraphy provides superior scans with more reliable information regarding shunt location and number, but carries a greater risk associated with ultrasound-guided injection into the splenic parenchyma in patients prone to clotting abnormalities (14).

Ultrasonography

Ultrasonography can identify the presence of shunt vessels, and also allows evaluation of liver size, parenchymal texture and arborization of blood vessels. In addition, the bladder and kidneys can be evaluated for renal architecture, crystalluria and uroliths. Color flow Doppler, if available, allows identification of turbulence patterns within the vena cava and portal vein typically associated with (a) shunting vessel(s). Accuracy of ultrasound for identification of PSS varies widely, and it is generally recognized that the diagnostic usefulness of ultrasonography is heavily dependent on the skill and experience of the operator (15).

Portography

This is still widely considered to be the 'gold standard' for diagnosis. Operative portography with injection into a mesenteric vein provides excellent imaging of the portal system and vascular anomalies, but does of course require general anesthesia and a minor surgical procedure. Where advanced imaging is available then either CT or MRI can be used with peripheral contrast injection via an intravenous catheter to obtain excellent images. Depending on the preference of the individual surgeon, the facilities available and the stability of the patient under anesthesia, it is often

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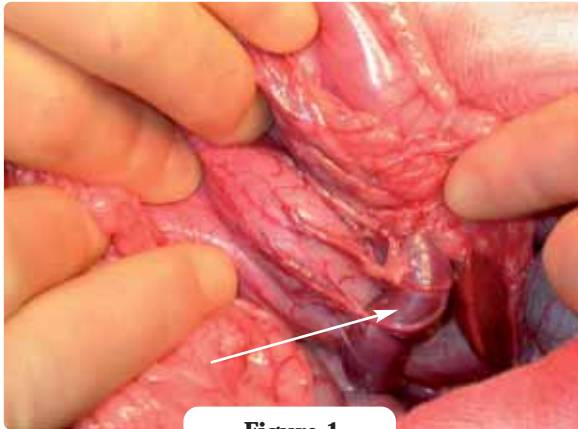


Figure 1.

Intraoperative view of an extra-hepatic shunt involving the gastroepiploic vessels over the greater curvature of the stomach. Note the shunt vessel (→) is of a wider diameter than adjacent vessels and thin-walled; turbulence is clearly visible within the lumen when viewed at surgery.

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Figure 2.

The dog on the left has a left division intrahepatic shunt; the dog on the right is his litter mate. Both dogs have been with the same owner since 8 weeks of age but there is a marked discrepancy in size and physical development.

possible to follow CT or MRI angiography with definitive surgery. If operative portography is preferred then availability of radiographic facilities in theater allows both imaging and surgical correction under the same anesthetic.

❖ Medical management

Where surgery is contraindicated (e.g. portal vein atresia) medical management is the only appropriate treatment option. Medical management can significantly improve survival times, but periodic assessment is essential, a normal life-span is very unlikely and quality of life can be variable.

It is generally considered beneficial for animals with PSS due to undergo surgery to have 2-3 weeks of medical therapy initially, unless a complicating factor creates a surgical emergency e.g. urinary tract obstruction with urate calculi. There is currently no information available to provide an evidence-based figure for the period of time that is most beneficial, or even to confirm whether it is essential to have this period of stabilization. Intuitively most clinicians would agree that anesthetizing and operating on a patient with overt signs of HE is not to be recommended. If the only presenting signs are related to the urinary tract it is less clear whether a period of medical stabilization is necessary.

Medical therapy involves correction of fluid, glucose and electrolyte imbalances, and prevention of HE. Either a prescription hepatic support diet, or home-made diet based on high biological value protein can be fed (*see article on page 16*). Although excessive protein should be avoided, over-enthusiastic protein restriction is also unhelpful as animals with abnormal liver function have a fundamental requirement for protein. Gastric inflammation or gastritis should be treated with sucralfate and a suitable antacid such as omeprazole. Oral antibiotics are administered to reduce colonic bacterial populations based on individual clinician preference; common choices include ampicillin, amoxicillin, and metronidazole. Lactulose can be administered to reduce colonic pH and thereby trap ammonium ions, inhibit protein and amino acid metabolism, increase fecal nitrogen excretion and decrease overall colonic transit time.

Some clinicians argue that medical management offers similar results to surgery in terms of survival times and quality of life, and that it should be considered an equally valid method of treatment. So far, however, the evidence to support this is lacking and it remains an opinion-based viewpoint rather than evidence-based medicine.

❖ Surgical options and issues

Initially surgical intervention for PSS produced poorer success rates than are now routinely achieved in specialist referral centers. Intrahepatic shunts in particular had morbidity and mortality that was significantly higher than what can be

achieved nowadays (2,11,16). Improvement in anesthetic drugs and protocols, better intensive care provision, and changes in the surgical approach to management of PSS have brought about marked improvements in surgical outcomes in the last decade and now the general expectation is that extra-hepatic PSS should have a success rate in the region of 90-95%, and intrahepatic PSS 75-80% (2,10,15). There are various options available. Surgical ligation was the initial method of choice but techniques allowing gradual progressive attenuation of PSS are now widely considered to be the preferred approach. Advantages of such techniques include a reduction in the incidence of postoperative portal hypertension, and increased time for the cardiovascular and central nervous systems to adapt to changing hepatic metabolism. The two main techniques in current use are ameroid constrictors and cellophane banding, although there are isolated reports of the use of extra-vascular hydraulic occluders as well as more recent reports of minimally invasive techniques (e.g. percutaneous coil embolization).

Suture ligation

This involves ligating the shunt either partially or completely with silk suture, depending on changes in the portal and systemic pressures in response to ligation. A particular issue with suture ligation is that in many cases full ligation induces unacceptable portal hypertension, but up to 50% of cases with only partial shunt ligation can have recurrence of clinical signs (17). The degree of occlusion achieved at surgery does not subsequently change significantly; therefore cases with recurrence of clinical signs due to persistent shunting require repeat surgery to achieve resolution. This of course both increases the risks and the expense. Where suture ligation is used, it is recommended that portal pressures be monitored during occlusion, to ensure that the degree of ligation will not induce life-threatening portal hypertension, whether full or partial. The surgeon can use subjective measures such as the color of the pancreas and intestines, stagnation of blood flow through mesenteric vessels, and development of random disordered motility of intestinal segments together with objective measurements such as changes in central venous pressure (decrease of >1 cm H₂O is a cause for concern) and arterial pressure (changes in excess of 5 mm Hg are again a cause for concern).



Figure 3.

A 5 mm diameter ameroid constrictor placed at the junction of an extra-hepatic portocaval shunt with the pre-hepatic vena cava, which is dilated where the flow from the shunt enters the caval lumen.



Figure 4.

A cellophane band prior to placement.



Figure 5.

Intraoperative picture of a cellophane band in place around an intrahepatic portosystemic shunt, which has been isolated within the parenchyma of a liver lobe. The band is secured with ligaclips, although placement of the clips is perhaps suboptimal in this case. Ideally clips should be larger relative to the band and placed in an alternating manner.

Provided the surgeon has the appropriate level of experience and surgical judgment these measurements are very reliable. Manometric measurement of portal pressures via a mesenteric vein, although theoretically providing direct values for portal pressures and therefore preferable, is in the author's experience time-consuming, inaccurate, frustrating and unreliable.

Ameroid constrictors

These have been used clinically since 1996 (18) although they were employed as long ago as the 1950's to induce experimental models of vascular stenosis. Ameroid constrictors consist of hygroscopic casein clay contained in a stainless steel ring. When implanted into the peritoneal cavity (*Figure 3*) the clay absorbs fluid and expands. The presence of the rigid stainless steel casing forces expansion inwards, occluding the vessel. In addition to physical occlusion the ameroid stimulates a fibrous tissue reaction that completes vessel closure. This means there is an initial rapid phase of vessel attenuation (3-14 days after implantation) followed by a slower phase (probably 2-3 weeks). Concerns have been raised regarding the initial fast phase of closure that the rate may in fact be too rapid, leading to development of multiple secondary acquired 'pressure relief' shunt vessels, but there is little clinical evidence to support this concern. Ameroid constrictors come in various diameters, and although there are no definitive guidelines regarding selection, the author's preference is to choose a constrictor that produces minimal reduction in vessel diameter after placement.

Cellophane bands

The use of cellophane (*Figure 4*) was first reported in a clinical case in 1990 (19). As with ameroid constrictors, cellophane had previously been used experimentally as a model of portal hypertension. When placed around blood vessels cellophane incites an initial acute inflammatory reaction followed by a chronic low grade foreign body type reaction. This response is still ongoing as long as 6 weeks after surgery. The cellophane is cut in a rectangle, and then folded into 3-4 layers to form a band approximately 1 cm wide. Tapering the end that is to be advanced around the shunt vessel facilitates placement, and once the band is in place it can then be secured with ligaclips or sutures,

although sutures are extremely difficult to place without damaging the cellophane whilst ensuring that the band is held securely in place (*Figure 5*). Again there are no definitive guidelines available regarding the degree of occlusion one should aim for. Most surgeons will place a band around the vessel to produce little immediate reduction in diameter, as there is some evidence that placing cellophane to actively induce 40-50% attenuation in diameter is associated with a poorer long-term outcome. The advantages of cellophane over ameroid constrictors include ease of availability, low cost, low weight and bulk, and greater flexibility. The disadvantage is that cellophane cannot be steam sterilized so an alternative sterilization method such as ethylene oxide is required.

Percutaneous embolization

Percutaneous embolization with intravascular coils has been used to occlude both extra-hepatic and intrahepatic PSS. This technique has the advantage of being minimally invasive, but this does not mean that the procedure is risk-free. Problems can include loss of coils to the systemic and pulmonary circulation, with resulting complications varying from mild to fatal. Recent adaptations have significantly reduced the risk of coil embolization, particularly the use of intra-caval expandable stents which act as 'traps' to prevent coils moving out of the shunt. Success rates similar to those achieved with surgery are reported (20) although availability of this technique is limited by a requirement for specialized facilities and expertise, the costs involved and the fact that more than one procedure may be required; limited follow-up is available as yet.

⊕ Postoperative care

- Acute portal hypertension has been recognized as a risk within the first 12 hours after surgery, although with ameroid constrictors and cellophane bands this is really only likely to be a problem if there has been a surgical error in terms of the size of implant chosen or inappropriate implant placement. Typical signs of portal hypertension include abdominal pain and distension, endotoxic or hypovolemic shock, hemorrhagic diarrhea, hypothermia or severe systemic hypotension. It is a complication that requires immediate surgical intervention to relieve the obstruction to portal flow.

- Ascites may develop secondary to chronic mild portal hypertension present pre-operatively. It is generally not life-threatening, and will spontaneously resolve with time.
- Post-ligation seizure activity is of unknown etiology; it can occur up to 4-5 days after surgery and can sometimes be difficult to control. Treatment generally consists of supportive care and administration of anti-seizure medications such as diazepam or (preferably) levetiracetam. Severely affected cases may require propofol by bolus or continuous rate infusion to control their seizures.
- Severe hypoglycemia can occasionally be seen, especially in toy breeds. Careful monitoring of blood glucose levels through surgery and recovery is essential.
- Portal vein thrombosis is a rare complication (better described in humans) but the author has seen this occasionally after partial suture ligation resulting in mild-moderate portal hypertension with development of multiple acquired shunts. Acute portal vein obstruction could potentially be fatal.
- Uncontrollable intra- or post-operative hemorrhage. This complication is heavily influenced by surgeon experience and ability, and is more likely with intrahepatic shunts, reflecting the typical location of such shunts and the degree of surgical complexity involved.

◊ Conclusion

Appropriate case selection and stabilization, the pre-operative presence of hepatic encephalopathy and/or other complicating factors and the location/morphology of the shunt all influence outcome; however the experience of the anesthetic and surgical team are also paramount. Where surgery is performed by a team of clinicians and nurses with the appropriate experience and the facilities for adequate postoperative care, the outcome for dogs with single extra-hepatic shunts is excellent. Intrahepatic shunt surgery is significantly more challenging, and this is reflected in the somewhat lower success rate. ◊

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Dietary management of liver disease

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The liver is essential for the digestion, absorption, metabolism and storage of most nutrients. It is also involved in the detoxification, catabolism and excretion of numerous toxins, hormones and xenobiotics. Decompensated liver function will thus be associated with malnutrition, intoxication, fluid imbalance and major metabolic abnormalities. Fortunately, the liver has tremendous functional reserves and the hepatic parenchyma has an amazing capacity to regenerate.

Nutritional support has been shown in both humans and companion animals to be key in the management of patients with hepatic diseases. This is the only effective treatment in cats with hepatic lipidosis.

In dogs and cats, the four main objectives of the dietary management of liver disease are:

- To correct malnutrition by fulfilling the basic energy and nutrient requirements (amino acids, potassium and zinc, as well as some vitamins, especially vitamins B, C and K).
- To support hepatocellular regeneration by providing the limiting nutrients, especially proteins.
- To limit liver damage by preventing copper accumulation and scavenging free radicals. Low dietary copper has been shown to significantly reduce hepatic copper in dogs with storage disease.
- To prevent or minimize complications such as hepatic encephalopathy, portal hypertension and ascites. Highly digestible plant and milk proteins are better tolerated than animal proteins in patients with hepatic encephalopathy.

Anorexia is a common complication of liver disease. Clinicians must consider tube feeding to ensure adequate energy and nutrient intakes. If the animal is anorexic or eats very little, it is important to reintroduce the diet progressively (gradually reaching 100% of requirements over 5-7 days). To avoid supersaturating the liver, daily intake should be divided into 3 to 6 meals.

Nutritional support must be adapted to each individual case based on the type of liver disease, extent of hepatic dysfunction, tolerance to dietary protein and nutritional status. Following surgical (hepatic shunts) and/or medical (hepatic lipidosis) treatment, the liver may recover normal function, the patient might then be returned progressively to a normal maintenance diet. In other cases, dietary treatment will be lifelong. ☺

Jaundice in the dog



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Introduction

Jaundice (or icterus) is a syndrome characterized by a yellow discoloration of the mucosae and teguments, caused by an increase in the serum concentration of bilirubin (hyperbilirubinemia). Although often indicative of hepatic disease, extra-hepatic conditions can also result in icterus. Detection of jaundice in the dog is relatively easy, but determining the exact etiology can prove more challenging and a rigorous diagnostic work-up is essential. Given the complexity of the mechanisms

KEY POINTS

- ➔ Icterus can be classified into 3 pathological and etiological types; it can have a variety of causes and the mechanisms behind it are complex.
- ➔ Careful, in-depth history taking is essential, as is a thorough clinical examination.
- ➔ A variety of laboratory and other diagnostic tests are usually necessary to reach a definitive diagnosis; shortcuts and assumptions should be avoided.



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behind icterus, it is important to follow a clear and consistent diagnostic approach; this article describes the authors' preferred method of approaching a dog with icterus.

Pathophysiology of icterus

Icterus can be classified into three pathophysiological and etiological types (1,2). Pre-hepatic or "hemolytic" icterus (as it is a consequence of hemolysis) occurs when the increase in bilirubin production exceeds the capacities of the hepatocytes to conjugate and excrete it. Hepatic icterus results from intrahepatic cholestasis, associated with diffuse disease of the bile ducts or hepatocytes, principally in the periportal zone (the so-called zone 1). Post-hepatic icterus results from extra-hepatic cholestasis due to impaired or obstructed flow of bile downstream from the liver.

The normal total bilirubin concentration in the blood is less than 0.4 mg/dL. The tissues start to discolor when the concentration exceeds 2 mg/dL, and icterus becomes frank at 4 mg/dL and above. Biochemical icterus therefore always precedes clinical icterus. The presence of massive bilirubinuria, which may occur long before icterus, should prompt the clinician to test the bilirubin concentration in the blood.

However, it is important to note that over the course of the disease several pathological components can be identified in cases of icterus and these often

Table 1.

Differential diagnosis for icterus.

Pre-hepatic icterus	Hepatic icterus	Post-hepatic icterus
<p>Primary immune-mediated hemolytic anemia:</p> <ul style="list-style-type: none"> - Idiopathic - Systemic lupus erythematosus - Blood transfusion <p>Paraneoplastic hemolytic anemia:</p> <ul style="list-style-type: none"> - Lymphoma - Hemangiosarcoma <p>Hemolytic anemia of infectious origin:</p> <ul style="list-style-type: none"> - Piroplasmosis, dirofilariasis, bacterial endocarditis, leptospirosis, ehrlichiosis <p>Hemolytic anemia of toxic origin:</p> <ul style="list-style-type: none"> - Onion, zinc, methylene blue, sulphonamides, copper, penicillins, or cephalosporins 	<p>Chronic hepatitis:</p> <ul style="list-style-type: none"> - Hereditary hepatitis (Bedlington terrier, Doberman, Dalmatian, Labrador, springer spaniel, cocker spaniel, West Highland white terrier, etc.) - Drug-induced chronic hepatitis (phenobarbital) <p>Acute hepatitis:</p> <ul style="list-style-type: none"> - Toxic (NSAIDs) - Infectious (leptospirosis, canine infectious hepatitis, <i>Yersinia</i>, <i>Salmonella</i>) <p>Neoplasia:</p> <ul style="list-style-type: none"> - Lymphoma, hepatic metastases, etc. <p>Acute cholangitis</p>	<p>Extra-hepatic bile duct obstruction (EHBDO):</p> <ul style="list-style-type: none"> • <i>Intra-luminal EHBDO:</i> <ul style="list-style-type: none"> - Lithiasis, biliary sludge - Stenosis - Cholangiocarcinoma • <i>Extra-luminal EHBDO:</i> <ul style="list-style-type: none"> - Pancreatitis • Duodenal or pancreatic mass, neoplasia or abscess <p>Cholecystitis and cholangitis</p> <p>Rupture of the extra-hepatic biliary tract:</p> <ul style="list-style-type: none"> - Phlegmonous cholecystitis, mucocele, trauma

become mixed: with significant hemolysis, oxygen deprivation of the hepatocytes may lead to edema then hepatocytic necrosis due to the accumulation of certain degradation products from the red blood cells and this results in a hepatic icterus. Similarly, extra-hepatic cholestasis progressively leads to the development of intrahepatic cholestasis and the onset of a mixed hepatic and post-hepatic icterus.

When presented with a case of icterus in a dog, it is important for the clinician to assess the intensity of the icterus and to try and correlate it with other symptoms that could help narrow down the differential diagnosis (*Table 1, Figure 1*). Pale-yellow mucosae (sub-icterus) and/or orange-brown or even dark brown urine (“coffee granules”) are suggestive of a pre-hepatic icterus with the proviso that anemia is confirmed on hematology. In the absence of anemia and signs of hemolysis, the clinician should focus on diseases of the liver or biliary tract.

History

A precise and detailed history is essential when working-up an icteric patient as it is a significant source of information. It is particularly important to

ascertain the time and mode of onset of the icterus from the owner. In general, a pre-hepatic icterus (hemolytic) has an acute onset and is accompanied by depression, anorexia, and discoloration of the urine in the event of intravascular hemolysis. Post-hepatic icterus associated with obstruction of the main bile duct can be chronic in onset, progressing over a period of several weeks (3,4). It may be well tolerated with the only clinical signs being a drop in appetite, a few non-specific gastrointestinal signs, and mild weight loss. However, extra-luminal biliary obstruction caused by pancreatitis or rupture of the extra-hepatic bile ducts may cause more severe clinical signs and be acute to subacute in nature (4).

Nausea and vomiting are common in inflammatory disease of the biliary tract, and more specifically of the gallbladder and common bile duct (5). This is probably related to their highly developed autonomic innervation. The high concentration of emetic receptors at this level leads to enhanced sensitivity to distension, inflammation, or neoplastic infiltration.

Hepatic icterus may be acute in onset (6). Leptospirosis is the most common cause of acute

hepatitis in the dog, the ictero-hemorrhagic form of which causes marked icterus and is accompanied by severe clinical signs (depression, gastrointestinal disorders, dehydration, and hemorrhagic diathesis). However, some forms of leptospirosis can be sub-clinical and chronic. Chronic hepatitis, irrespective of the etiology (infectious, toxic, immune-mediated, or breed related), has a more insidious nature and icterus is often the sign of very advanced disease (7-11).

Acute or chronic in onset, hepatic icterus is the result of a very severe deterioration in hepatic function, usually associated with systemic signs reflecting the severity of the disease and its repercussions.

The possibility of toxic processes should be explored if the animal has been exposed to certain toxins (lead, copper), has been on medication (NSAIDs, phenobarbital), or has recently undergone gaseous anesthesia (12). It is very important to take the breed of dog into consideration since numerous causes of chronic hepatitis are breed-related and are associated with the onset of icterus (8-11). Many breeds of dog are predisposed to the development of chronic idiopathic hepatitis (springer spaniel, Labrador, Doberman, etc.) (10,13) or are associated with metabolic anomalies such as copper overload (Bedlington terrier, Labrador, West Highland white terrier, etc.) (8,9,11), or an accumulation of alpha 1-antitrypsin (cocker spaniel) (14). Similarly the Shetland sheepdog and Scottish terrier are predisposed to the development of biliary mucocele, which can generate a severe extra-hepatic cholestasis (15).

The dog's environment and vaccination status are obviously important factors. Hunting dogs, or those that live outside, are more exposed to piroplasmiasis or leptospirosis. With the latter, several infective strains may not be covered by vaccination (*L. icterohemorrhagica* and *L. canicola*) and can be responsible for the onset of clinical leptospirosis.

◈ Clinical examination

The clinical examination should first and foremost concentrate on the detection of signs of hemolysis and any symptoms associated with the icterus such as ascites, abdominal pain, and neurological disorders (1,2). The yellow coloration may be

subtle, notably in cases with hemolytic syndrome, especially given that the mucosae are often pale at the time of examination; this is often referred to as sub-icterus (*see clinical case report*). However, this coloration may be very intense if it is accompanied by mucosal congestion.

Particular attention should be paid to the liver during abdominal palpation. The size, shape, firmness, and presence of any surface irregularities should be assessed. Palpation may reveal localized cranial abdominal pain in cases of gallbladder or pancreatic disease. Splenomegaly may indicate hemolytic anemia.

In the event of icterus, the urine becomes discolored (orange-brown or even dark brown - "coffee grounds") if intravascular hemolysis is present (hemoglobinuria). Pigmenturia is identified on examination of a urine dip stick, revealing bilirubinuria and urobilinogenuria in cases of pre-hepatic icterus. The presence of hemoglobin is indicative of hemolytic icterus, but the latter cannot be ruled out in its absence. Polyuria-polydipsia, common in cases of chronic hepatic disease, leads to a reduction in urine density and dilution of the bilirubin: bilirubinuria, even moderate, should be considered as significant when the urine is of low density.

Feces are usually dark colored with hepatic icterus, and pale in cases of extra-hepatic bile duct obstruction; complete bleaching occurs around one week after complete obstruction. Other clinical signs may also be present, such as purpura, respiratory signs (due to intoxication with agents that cause methemoglobinemia), and abdominal effusion.

◈ Further diagnostic tests

Further diagnostic tests should make it possible to quantify the icterus, and to determine its type and etiology. These include laboratory tests and imaging.

Hematology

Hematology is an essential part of the diagnostic work up in icterus as it can determine the presence of anemia. The hematocrit will reveal an anemia which should then be objectivized with a complete differential count.

Cases of hemolytic anemia that result in icterus are severe and regenerative, even if the regeneration only becomes maximal after two to four days, which may delay the onset of reticulocytosis. Anemia associated with hepatic disease is associated with chronic inflammatory reactions (defective iron utilization) and is usually moderate, non-regenerative, normocytic, and normochromic (2). If hemolysis is suspected, other examinations (blood smear, Coombs' test, serology for infectious diseases) should be considered.

The examination of a blood smear will provide a definitive diagnosis of babesiosis. Other morphological anomalies of red blood cells may help to orientate the diagnosis: spherocytes are suggestive of an autoimmune hemolytic anemia, and Heinz bodies are observed following the ingestion of certain toxins (zinc, onions, benzocaine).

A Coombs' test provides confirmation of the immunological character of anemia. It is then advisable to look for any possible primary infectious or parasitic etiology (babesiosis, ehrlichiosis, bartonella, leishmaniasis, dirofilariasis), neoplastic, iatrogenic, or toxic.

Biochemistry

These simple diagnostic tests are primarily oriented towards hepatic function (16) and usually make it possible to quantify and classify the icterus as pre-hepatic, hepatic, or post-hepatic (*Figure 1*).

Detecting cytolysis

Transaminases (ALT and AST) are markers of hepatic cytolysis. The magnitude of the increase in transaminase activity indicates the number of hepatocytes that have been injured, but not the reversibility of the phenomenon and therefore the prognosis. AST in dogs is also found in other organs and is not specific to the liver. An ALT assay is sufficient and simultaneous assay of both transaminases, common in human medicine, is not justified.

Investigating cholestasis

The ALPs (alkaline phosphatases) are enzymes that are excreted in the bile. Their activity increases in the event of cholestasis, but also under the effect of certain drugs, such as corticosteroids or

anticonvulsants. ALP is also present in numerous tissues, in particular bone. An increase in ALP is observed in growing dogs or in the event of bony disease (osteomyelitis, neoplasia, etc.). GGTs are also present in numerous tissues but the majority of their activity is hepatic. GGT assay is more specific but less sensitive; a combined GGT/ALP assay has a specificity of 94% in the diagnosis of hepatobiliary disease, whilst when ALP is tested alone the specificity drops to 50%.

Detecting hepatocellular insufficiency

Tests for hepatocellular insufficiency rely on the demonstration of a reduction in the synthetic capacities of the hepatocytes due to a reduction in serum proteins (in particular albumin) and clotting factors. Total proteins are usually normal as the frequent increase in inflammatory proteins masks the reduction in albumin levels. A reduction in the blood urea nitrogen may be indicative of an alteration in the liver's synthetic capacities.

Confirm the presence of inflammation

Serum protein electrophoresis will reveal inflammation and enables the evaluation of the synthetic capacities of the liver. An acute inflammatory condition provokes the synthesis of proteins that migrate in the α_2 zone of the electrophoresis, whilst chronic disease causes an elevation in the γ , and β zones, and may even result in a $\beta\gamma$ block in the event of cirrhosis.

Bilirubin assay

When icterus is clinically perceptible, the only advantage of performing a bilirubin assay is to monitor the progression of the disease and the efficacy of any treatment. Indeed icterus persists clinically even after a reduction in serum bilirubin levels. However, the distinction between conjugated and non-conjugated bilirubin is of no interest.

Other laboratory tests

Other tests may prove necessary to pinpoint the etiology of the icterus, in particular when considering infectious disease. In cases of hemolytic anemia, ehrlichiosis should be ruled out using PCR or serology. A detailed examination of a blood smear should enable the identification of babesiosis; this can be simplified by concentrating the blood to improve sensitivity. In cases of acute hepatic disease, leptospirosis should be ruled out using

Figure 1.

Diagnostic approach to icterus in the dog.

Icterus

- History
- Clinical examination
- Standard laboratory tests: CBC, biochemistry, urinalysis

- Moderate to severe regenerative anemia
- Hemolyzed plasma
- Normal to slightly raised hepatic enzymes
 - Blood smear
 - Slide agglutination test
- Hepatomegaly and/or splenomegaly

• Suspicion of hemolytic icterus

- Absence of anemia or moderate hyporegenerative anemia
- Plasma not hemolyzed
- Raised hepatic enzymes
- Liver normal, small, or increased in size

• Suspicion of hepatic or post-hepatic icterus

• Characterize the increase in hepatic enzymes

• Mostly hepatic or mixed icterus

• Post-hepatic icterus

• Abdominal ultrasonography

• Pancreatitis

• Effusion

• No dilation of the extra-hepatic biliary tract

• Dilation of the extra-hepatic biliary tract

- Mucocele
- Signs of severe cholecystitis

• Exudate

• Pure or modified transudate

• Clotting profile

• Hepatic fine-needle aspirate/biopsy

• Bile sample

• Laparoscopy

• Laparotomy

• Bilirubin in exudate > serum bilirubin

• Bile peritonitis

• Laparoscopy

• Laparotomy

• Primary hepatic disease:

- acute hepatitis
- chronic hepatitis
- neoplasia
- cholangitis

• EHBDO

• Cholecystitis

• Cholangitis

• Mucocele

serology or PCR. The antibody response is only detectable after one week and the dog's vaccination status should be taken into account when interpreting serology results.

Medical imaging **Radiography**

Radiography can provide valuable information in animals with suspected biliary tract disease. Up to 50% of choleliths (gallstones) are radiopaque because of their mineral content. The presence of gas around biliary structures is very indicative and compatible with emphysematous cholecystitis, abscessation, or severe cholangitis.

Ultrasonography

Ultrasonography is the examination of choice for differentiating intrahepatic cholestasis from extra-hepatic cholestasis. The sensitivity of this examination is significant in the confirmation of extra-hepatic cholestasis and in the diagnosis of the etiology of this obstruction. It is not unusual to diagnose an extra-hepatic obstruction of the bile ducts on ultrasonography before the onset of icterus. In the event of obstruction of the extra-hepatic biliary tract, dilation of the gallbladder is common but not systematic and in some cases it may even appear normal or reduced in size secondary to chronic inflammation. Dilation of the common bile duct is characteristic of extra-hepatic cholestasis. Certain diseases of

the gallbladder or biliary tract have a characteristic appearance on ultrasonography (cholangiocarcinoma, biliary mucocele). Cholecystocentesis can be performed under ultrasonographic guidance using a fine needle (20 - 22 G); although this is a relatively low-risk procedure it should not be performed if the extra-hepatic biliary tract is obstructed. Cytology and bacteriology can then be performed on the sample.

Laparoscopy

This examination enables assessment of the hepatic lobes to confirm any changes in color or appearance, visualization of the extra-hepatic biliary tract, gallbladder, cystic duct and common bile duct, and biopsies from specific sites.

Hepatic biopsy

Liver biopsy and histology is the final examination in the diagnostic tree and provides a diagnosis of the lesions, a prognosis, and the selection of appropriate treatment (17,18).

Conclusion

Icterus in the dog is usually easily identifiable on clinical examination, but determining the cause requires a reasoned, step-by-step diagnostic work-up. Further diagnostic tests often provide an indication of the etiology of the hyperbilirubinemia and will allow the veterinarian to pursue objective treatment strategies. ☺

Clinical case report

A 6-year-old female Doberman presented for anorexia, weight loss, polyuria-polydipsia, and vomiting with hematemesis. Clinical examination revealed a moderately pronounced yellow discoloration of the mucosal membranes (sub-icterus) (**Figure 1**). No anomalies were detected on abdominal palpation. Rectal examination revealed formed feces that were abnormally dark in color (melena); urine analysis confirmed hyperbilirubinuria.

Blood tests revealed moderate microcytic, hypochromic anemia, with slight regeneration (RBC $4.5 \times 10^{12}/L$ (N 6-9), Hg 100 g/L (N 13-19), Hct 35% (N 37-54), MCV 55 (N 60-77), MCHC 30% (N 31-34), reticulocytes $20 \times 10^9/L$ platelets $145,000 \times 10^9/L$), increases hepatic enzymes and hyper-bilirubinemia (ALT 502 U/L (N 10-100), ALP 945 U/L (N 18-94), GGT 17 IU/L (N 0-8), Bil 24 mg/L (N 0-9), hypoalbuminemia (Alb 20 g/L), and blood urea nitrogen (0.1 g/L).

Ultrasonography revealed a small liver, with a heterogeneous echostructure, and mild effusion (**Figure 2**).

Abdominal tap revealed a clear olive-brown colored liquid with a low protein content (10 g/L), a specific density of 1.012, and a low cell count (1,000 cells/mm³: a few macrophages, neutrophils, and mesothelial cells), indicative of a pure transudate (**Figure 3**).

Endoscopic examination confirmed the presence of gastric ulceration (**Figure 4**).

Laparoscopy revealed a notably altered liver and confirmed the presence of macronodular lesions (**Figure 5**). Biopsies were taken which demonstrated severe portal hepatitis (**Figure 6**) and macronodular cirrhosis. Specific staining (rhodamine) confirmed an abnormal accumulation of copper within the hepatocytes. Hepatic copper assay revealed a concentration of 2,300 µg/gram of dry liver (N < 400 µg).



Figure 1.

Moderate yellow discoloration of the mucosal membranes (sub-icterus).

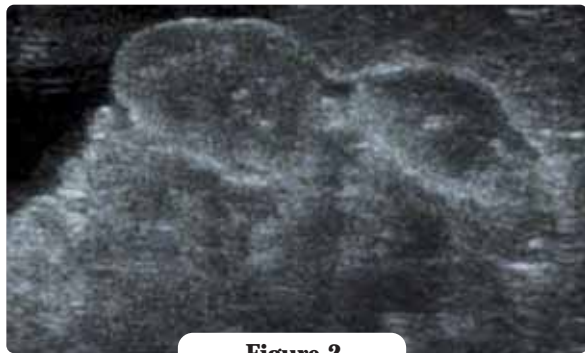


Figure 2.

Ultrasonography revealed a small liver, with a heterogeneous echostructure and mild effusion.

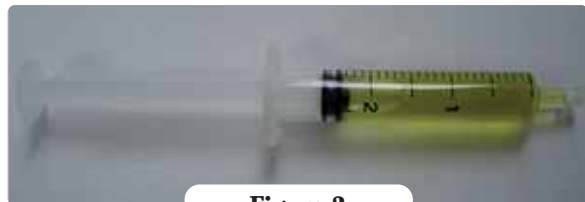


Figure 3.

Abdominal tap contents.

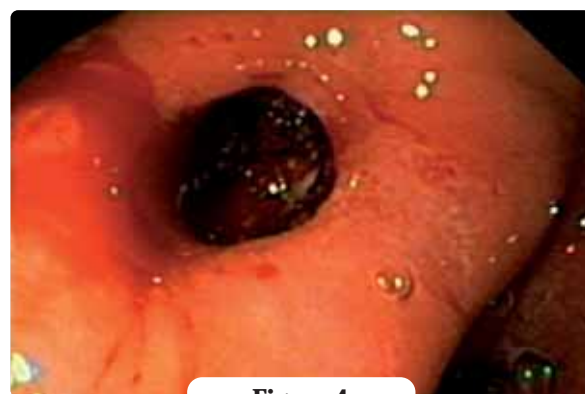


Figure 4.

Endoscopic examination showing gastric ulceration.

Discussion

This animal presented with icterus that was attenuated by anemia; the latter was probably due to chronic blood loss from the ulcerated gastrointestinal tract and hepatic insufficiency. The presence of a pure transudate cannot be explained by the hypoalbuminemia and orientates the diagnosis towards a probable portal hypertension. Laparoscopy and histology confirmed chronic hepatitis through copper overload with severe, advanced cirrhosis.



Figure 5.

Laparoscopy revealed a notably altered liver and the presence of macronodular lesions.

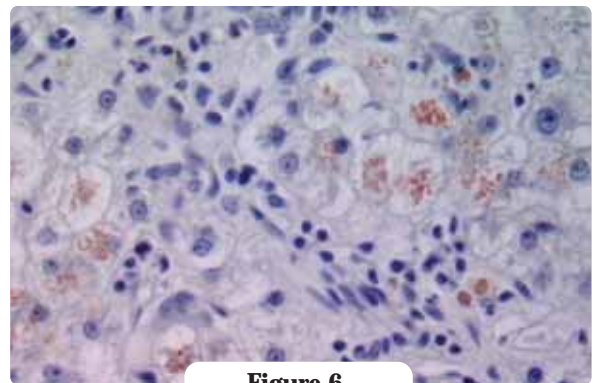


Figure 6.

Histology of the biopsies confirmed severe portal hepatitis.

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Extra-hepatic biliary system surgery



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Dr. Martinez graduated as a veterinarian from the Faculty of Veterinary Science at the University of Buenos Aires in 1991. Further studies allowed her to graduate from UBA in 2002 as a Specialist in Small Animal Surgery and she is currently a small animal surgery Specialty Instructor and tutor at the Faculty of Veterinary Sciences in UBA. She is a member of the Latin American Association of Veterinary Neurology (Neurolatinvet) and a founding member of Neurovet Argentina, the Association of Veterinary Neurology in Argentina.



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The extra-hepatic biliary system (EHBS) is a well-designed drainage structure that carries bile secreted from the liver to the duodenum, the bile being stored in the gallbladder before being excreted under the control of the gastrointestinal autonomic reflexes. The system consists of hepatic ducts, the gallbladder, cystic duct and bile duct (also known as the common bile duct), the latter running dorsal to the

gastrohepatic ligament and emptying into the duodenum (**Figures 1a and b**). The pathologies most commonly diagnosed with the EHBS that require surgery are related to obstruction or trauma of the system, and gallbladder mucoceles.

Obstructions are related to processes that may partially or completely involve the EHBS; they may be extra-luminal or intra-luminal in nature. Obstructions are rare but are usually associated with a significant degree of systemic involvement, which can pose a diagnostic and therapeutic challenge. Extra-luminal obstructions may be due to numerous pathologies: pancreatitis, neoplasia (which may be of pancreatic, hepatic, duodenal or pyloric origin), suppurative or non-suppurative cholangiohepatitis, or hepatic lipidosis. The most common intra-luminal obstructions result from cholelithiasis, choledocholithiasis (gallstones), biliary mucocele, or necrotizing cholecystitis (**Figures 2,3,4 and 5**).

Ruptures of the biliary system can be caused by external trauma or by pathologies that affect the

KEY POINTS

- The pathologies most commonly diagnosed with the extra-hepatic biliary system (EHBS) that require surgery are related to obstruction or trauma of the system and gallbladder mucoceles.
- The clinical signs of biliary disease can be both non-specific (anorexia, malaise, vomiting, diarrhea, weight loss, epigastric pain, and leukocytosis) and more specific (acholic feces, jaundice, hyperbilirubinemia and elevated transaminases).
- Hepatic biopsy and evaluation of the integrity of the EHBS are essential in all cases as a large percentage of EHBS problems are associated with liver disease.
- Surgical options include cholecystotomy, cholecystectomy, and biliary diversions (cholecystenterostomy); the clinician should proceed to laparotomy only after a full evaluation of the patient, using all necessary diagnostic tests.



Figures 1a and 1b.

- a.** EHBS anatomy - 1. gallbladder 2. cystic duct 3. hepatic duct 4. common bile duct (canine).
- b.** EHBS anatomy - gallbladder, cystic duct, hepatic duct and common bile duct (feline).

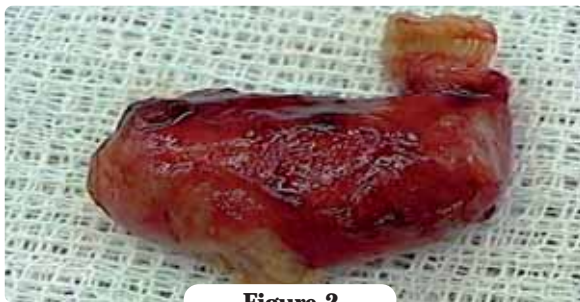


Figure 2.

Post-cystectomy - gallbladder showing severe cholecystitis.



Figure 3.

Post-cystectomy - interior of gallbladder with severe cholecystitis.

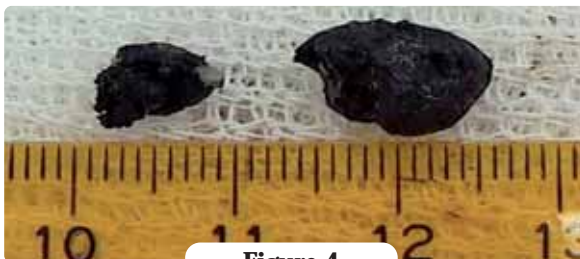


Figure 4.

Gallbladder calculi removed via cholecystotomy.

system, either primarily or secondarily, making the structures prone to tearing and subsequent loss of bile. The chemical characteristics of bile mean that it can cause extensive damage to adjacent tissues or structures, resulting in biliary peritonitis which may be aseptic or septic; the latter condition worsens the prognosis. It is also worth noting that in cats, differential diagnosis should include the inflammatory pathology complex known as “triaditis” (1-4), which includes cholangitis, pancreatitis and inflammatory bowel disease.

The clinical signs of biliary disease can be both non-specific (anorexia, malaise, vomiting, diarrhea, weight loss, epigastric pain, and leukocytosis) and more specific (acholia, jaundice, hyperbilirubinemia and elevated transaminases) (*Figures 6 and 7*).

Surgical intervention of the extra-hepatic biliary system is performed primarily to treat two problems: (i) primary obstructions and (ii) ruptures due to trauma or secondary to severe alterations of the gallbladder wall. Hepatic biopsy and evaluation of the integrity of the EHBS is essential in all cases as a large percentage of alterations to the EHBS are associated with hepatic disease. Controversy exists about the importance, during surgery, of ensuring the integrity of the bile duct by catheterization, either from the gallbladder (normograde/antegrade) or from the duodenal papilla (retrograde) via a duodenal enterotomy in all cases (5). In the authors’ experience, duodenotomy with retrograde flushing is not always necessary, only when manual compression or antegrade flushing have not provided positive results by emptying the gallbladder. It should be emphasized that the manual expression of the gallbladder must be attempted only when the organ has a normal macroscopic appearance, since there is a high risk of rupture in more advanced pathological stages.

⊗ Diagnosis

In addition to the clinical signs and the data produced by auxiliary diagnostic methods such as imaging (radiography and ultrasonography), laboratory results will assist in the diagnosis of hepatic disease, endocrine disorders or EHBS problems, and should differentiate patients with

obstructive disease from patients presenting with biliary peritonitis. Sampling by abdominocentesis or diagnostic peritoneal lavage (DPL) is very important in cases where abdominal fluid is present (**Figure 8**), and samples should be submitted for biochemical, cytological and culture/sensitivity analysis.

Ultrasound imaging can assist in the diagnosis of gallbladder mucocele, where a characteristic image of the gallbladder lumen has been described, the so-called “kiwi fruit sign” (6). Radiography assists in identifying animals with radiopaque gallbladder stones (**Figures 9**). While these auxiliary diagnostic methods can be helpful, in cases where doubts persist, the exploratory celiotomy is the alternative method of choice. Surgical exploration of the abdomen must be undertaken in cases where patient stabilization is not achieved or when there is no response to medical treatment. Cases identified as gallbladder mucocele, necrotizing cholecystitis, ruptured gallbladder/traumatic EHBS damage, and biliary stasis that has not responded to medical treatment are clear examples where surgical exploration is indicated.

The surgical procedures most frequently performed to resolve the various EHBS pathologies are as follows:

- Cholecystotomy:** this is the surgical procedure required for sampling gallbladder contents, for calculus removal, and for antegrade passage of a probe to evaluate the integrity of the bile duct. It is appropriate to use stay sutures to facilitate handling the gallbladder, thus reducing the possibility of instrumental trauma (**Figure 10**). Given the current progress in diagnosing EHBS pathologies, and the potential complications that can develop when this surgical technique is used on a damaged gallbladder (7), cholecystectomy is recommended only after verifying the integrity of the EHBS as previously described. If the general condition of the gallbladder allows, the incision is closed with 4/0-6/0 absorbable monofilament suture material using an apposition suture pattern. A permeability test should be performed, similar to that made after an intestinal anastomosis, to correct any defect in the gallbladder prior to abdominal closure.



Figure 5.

Biliary mucocele.

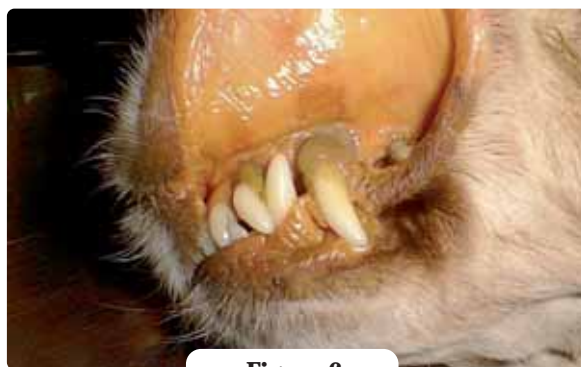


Figure 6.

Icteric oral mucosa.

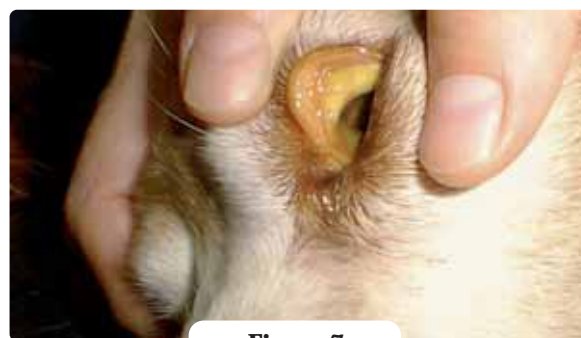


Figure 7.

Icteric conjunctival mucosa.



Figure 8.

Abdominocentesis performed to diagnose biliary peritonitis.



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Figure 9.

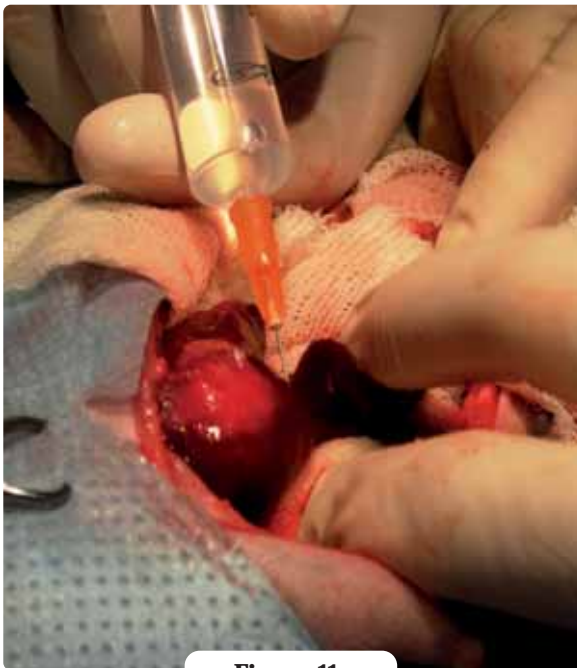
Abdominal radiograph demonstrating calculi within the gallbladder.



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Figure 10.

Cholecystotomy and sampling for culture and sensitivity.



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Figure 11.

Hydrodissection during cholecystectomy.

- **Cholecystectomy:** the advantage of this procedure over the previously described procedure is twofold; it is simple and it avoids the typically high morbidity/mortality indexes (22%-40%, respectively) (8-10) shown to occur with complications of surgical intervention or with pathologies related to EBHS rupture (*i.e.* peritonitis). A bold surgical approach is essential, and the surgeon should consider performing a right paracostal celiotomy in deep-chested patients. Once the gallbladder is opened, the contents should be sampled for culture and sensitivity.

In this procedure, the separation of the gallbladder, lying juxtaposed to the hepatic parenchyma, can be done either using an anterograde method (from the gallbladder fundus to the bile duct) or retrograde (from the common bile duct to the gallbladder fundus) via “hydrodissection”, which allows the gallbladder wall to be separated from the hepatic parenchyma by injecting saline into the gallbladder-liver interface to create a cleavage plane. Alongside this technique, blunt dissection instruments, the blunt tip of the suction piece, or cotton-wool buds can be used to delicately separate the gallbladder from the hepatic fossa where it normally lies (**Figure 11**). This method (described by Breznock - personal communication) allows better and more efficient, less traumatic removal of the bladder wall from the hepatic parenchyma to which it is closely adhered (**Figures 12 and 13**). This reduces not just operating time and blood loss, it also minimizes the chance of rupture by excessive manipulation of a gallbladder already weakened pathologically. Use of hemoclips is highly recommended for rapid ligation of vessels, especially for the cystic artery and duct. It is essential to verify the integrity of the bile duct by antegrade flushing before attempting this surgical technique (**Figures 14 and 15**). It is also advisable to avoid confusing early mucocoeles with vesicles containing thick bile (biliary sludge) via careful history-taking, the patient’s clinical signs and appropriate use of ultrasound.

Similarly, after the cholecystectomy has been completed, the bile duct must be re-evaluated by performing a small duodenotomy and passing a probe in a retrograde manner from the duodenal papilla (**Figure 16**); care should be taken to avoid the possibility of contamination of the area with extravasated bile.

Advances in minimally invasive surgery using laparoscopy allows the removal of biliary mucoceles and the treatment of uncomplicated cholelithiasis where obstruction of the EHBS has occurred. This type of surgical technique requires advanced training and specific instruments. Recently, there have been successes in the field of veterinary surgery with the new Natural Opening Transluminal Endoscopic Surgery technique (NOTES) (11). This technique looks promising in reducing morbidity and may allow surgeons to obtain better results in the not-too-distant future.

Biliary diversions or cholecystenterostomy

Based on the authors' experience and also taking into account literature reports, most pathological gallbladders in dogs are not sufficiently viable to withstand an enteral bypass, since in many situations gallbladder weakness progresses towards parietal necrosis, endangering the enteroanastomosis. Perhaps this is because the diagnosis of biliary pathology is frequently delayed; there is no doubt that advances in the knowledge of these pathologies, as well as improved diagnostic methods, will alter this situation in the future. The most frequently performed enteral diversions are cholecystoduodenostomy and cholecystojejunostomy. While the latter is a simpler technique to implement, it may facilitate the development of ulcers in the duodenum due to lack of bile flow; normally bile within the duodenum will protect against the acidity of gastric secretions. Care must be taken that the final stoma must be at least 2.5-3.5 cm in length to prevent possible stenosis of the new opening; due to the natural healing/retraction/ remodeling process, the original stoma can be reduced by up to 50% following surgery.

The placement of biliary stents for conservative management of EHBS obstruction is a recent development which has met with success, and presents an interesting alternative to known biliary bypass techniques and their potential problems, such as dehiscence, stenosis of the stoma and ascending cholangitis.

The use of tubes for pre-operative biliary drainage or cholecystostomy in high-risk patients remains controversial in veterinary surgery. They require more complex medical and hospital support for

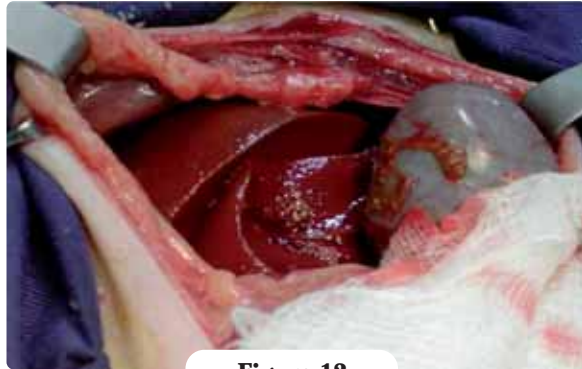


Figure 12.

Cholecystectomy - gallbladder separated from the hepatic parenchyma.

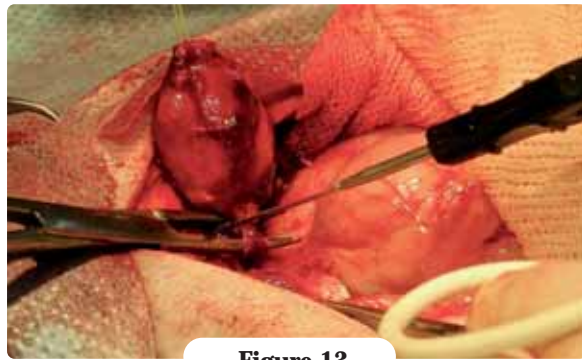


Figure 13.

Cholecystectomy - resection to complete removal of diseased gallbladder from the hepatic fossa.



Figure 14.

Testing the bile duct by normograde flushing (feline).



Figure 15.

Testing the bile duct by retrograde flushing (feline).

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Figure 16.

Testing the bile duct by retrograde flushing through the duodenal papilla (canine).

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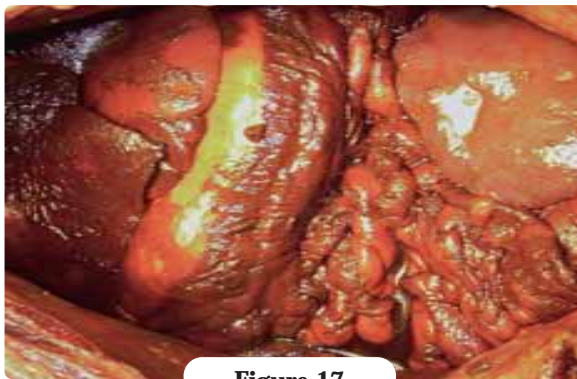


Figure 17.

Biliary peritonitis.

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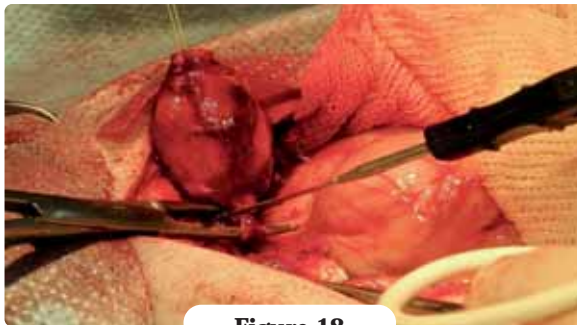


Figure 18.

Assessing a bile duct ruptured by trauma via the use of retrograde catheterization.

the proper monitoring of the drainage and the patient. The most frequent complications in this technique are premature obstruction and early drainage removal with subsequent intra-abdominal biliary loss.

❖ **EHBS/biliary peritonitis trauma**

In canines, the most common cause of bile loss is due to trauma or gallbladder pathology (12), while in felines trauma is the main reason. The

most reliable diagnostic method involves assessing the concentration of bilirubin from a peritoneal effusion sample when compared to that of serum. Where the effusion has a bilirubin value of at least double that of serum emergency exploratory celiotomy is indicated. It is well known that the irritable bile salts increase the local and systemic inflammatory response of the patient. When bacteria are present the prognosis is even more cautious. Signs of bile peritonitis (**Figure 17**) tend to have a late onset resulting in a deterioration of the patient in most cases before diagnosis; this means that almost all patients present as a clinical emergency. In the recent past, due to insufficient use of magnification equipment by surgeons, important details were ignored or missed (ischemia, necrosis) in the assessment of the damaged wall of the bile duct or other major branches of the biliary tree (**Figure 18**). This led in many cases to suture dehiscence. Currently, the use of surgical loupes, as well as improved microsurgery techniques/instruments and 5/0-6/0 absorbable monofilament suture material has led to better success in choledochotomy; a recent publication (5) has shown that dehiscence in primary repairs of the bile duct and choledochotomies was not as high as traditionally expected.

In all these procedures, the isolation of the viscera by the use of surgical drapes and laparotomy sponges is taken for granted, as well as the use of a Balfour abdominal retractor or similar to attain a better exposure of the surgical field. Once the source of bile leak is controlled (8) the abdominal cavity must be lavaged copiously, providing adequate and efficient suction is available (**Figure 19**). Care must be taken with the volume of fluid used for lavage, since an excess can yield a result opposite to that intended (*i.e.* dilution of opsonins). As a guide, the use of 100-200 mL/kg is suggested, depending on the size of the patient. Recently there has been controversy concerning open peritoneal drainage versus primary closure of the abdominal cavity after exploration. The authors use both methods, depending on the severity presented by the patient.

When considering postoperative care enteral nutrition deserves a prominent place. The

placement of a jejunostomy tube is necessary to maintain good nutrition to a critically ill patient until the patient's metabolism has improved. Early feeding of these patients can reduce morbidity and mortality, for example by increasing levels of plasma proteins within 48 hours of commencing administration of feeding.

Broad-spectrum antibiotic therapy must be started even before culture and sensitivity results are obtained. The possibility of contamination of the area by bile irritation and bacteria translocation is always present.

Conclusion

In summary, EHBS surgery can be very successful; however the clinician should proceed to laparotomy only after a full evaluation of the patient, using all necessary diagnostic tests – no shortcuts should be taken. Surgical competence is a prerequisite, as is adequate theater assistance and equipment, and the veterinarian should be capable of selecting the best option for each patient, if necessary adapting the chosen technique during the procedure, having assessed both the viability of the EHBS and the patient's general condition at surgery.



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Figure 19.

Aspiration after abdominal lavage.

Owners should be comprehensively briefed about possible peri-operative complications and postoperative care should be optimized to maximize recovery.

Although it is impossible to categorically predict patient outcome, informed and effective decision-making, taking all factors into account both before and during surgery, will help reduce the incidence of post-surgical problems. ☺

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Laboratory tests for liver disease



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◊ The role of the liver in intermediary metabolism

The liver is involved in many aspects of intermediary metabolism (1). The laboratory testing of liver function usually involves some aspect of the liver's role in intermediary metabolism.

Carbohydrate metabolism

The liver is at the center of carbohydrate metabolism through its role in maintaining

normoglycemia. Carbohydrate stored in the liver as glycogen is hydrolyzed to glucose via glycogenolysis when a need for glucose develops. When the glycogen available is insufficient, glucose is produced from amino acids by gluconeogenesis. Glucose is also produced from glycerol and intermediates of glycolysis, such as lactic acid and pyruvic acid. With inadequate carbohydrate in the diet, blood glucose is maintained at the expense of body proteins. Body lipid stores are also depleted during starvation, although lipids do not participate in the maintenance of blood glucose other than serving as an alternate source of energy, since glucose cannot be synthesized from fatty acids.

- Glycogen → glycogenolysis → glucose → normoglycemia
- Amino acids → gluconeogenesis → glucose → normoglycemia

Clinical relevance - Acute and chronic liver diseases may be accompanied by hypoglycemia.

Protein metabolism

The liver is an important site of protein metabolism. Amino acids and proteins absorbed from the intestine or produced in the body are delivered to the liver. The liver deaminates amino acids and can convert them to carbohydrates and lipids depending upon nutritional needs. Deamination produces alpha-keto acids, which can be metabolized for energy or used for synthesis of monosaccharides and fatty acids. The liver synthesizes amino acids from intermediates of carbohydrate and lipid metabolism by amination and transamination. Examples of amino acid transaminations include:

- Alanine + alpha-ketoglutarate ↔ pyruvate + glutamate
- Aspartate + alpha-ketoglutarate ↔ oxaloacetate + glutamate

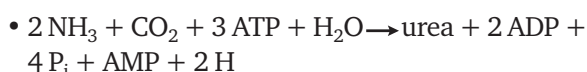
The liver synthesizes many proteins including albumin and fibrinogen, most of the alpha-

KEY POINTS

- ➔ The laboratory testing of liver function usually involves some aspect of the liver's role in intermediary metabolism. A good understanding of the liver's basic functions will assist interpretation of results.
- ➔ In animals with suspected liver disease, the minimum database should include complete blood count, serum chemistry, urinalysis, and fecal flotation.
- ➔ Survey abdominal radiography should be performed as part of the minimum database in any animal suspected as having liver disease. Additional laboratory tests and imaging studies (e.g. ultrasound) may be considered following development of the minimum database.
- ➔ Acute and chronic liver diseases may be accompanied by a diverse range of other abnormalities including hyperammonemia, elevated bile acids, heavy metal accumulations and coagulopathies.

globulins, some of the beta-globulins, ceruloplasmin, ferritin, and other serum enzyme activities.

The urea cycle is involved in the oxidative degradation of amino acids. Ammonia is a primary metabolite of amino acid metabolism. The gastrointestinal tract, and especially the colon, is the most important source, through the action of bacterial urease on endogenous urea that diffuses in the intestine and on degraded dietary amines. Ammonia produced by the colonic bacteria enters the portal vein and is transported to the liver to be transformed by the urea cycle.



Clinical relevance - Acute and chronic liver diseases may be accompanied by (i) increases in serum aminotransferase activities, (ii) hypoalbuminemia, (iii) hyperammonemia, and (iv) decreases in blood urea nitrogen concentration.

Lipid metabolism

The liver is involved in the intermediary metabolism of lipids from (i) triglyceride synthesis and storage to (ii) fatty acid oxidation, and (iii) cholesterol synthesis, storage, secretion and transport (2).

- **(i) Synthesis and storage:** Acetyl-CoA + malonyl-CoA + NADPH \rightarrow triacylglycerol + CO₂ + NADP + H₂O
- **(ii) Fatty acid oxidation:** Triacylglycerol + CoA + NAD + FAD \rightarrow acetyl-CoA + NADH + FADH
- **(iii) Cholesterol:** Intestine \rightarrow cholesterol in chylomicrons \rightarrow apoprotein B48 \rightarrow liver muscle, connective tissue \rightarrow cholesterol in HDLs \rightarrow liver
- Liver \rightarrow cholesterol in VLDLs \rightarrow serum and bile
- Blood \rightarrow cholesterol in LDLs \rightarrow apoprotein B100 \rightarrow liver

Clinical relevance - Acute and chronic liver diseases may be accompanied by hypocholesterolemia. Biliary obstructive disorders may be accompanied by steatorrhea.

Coagulation factors

The liver synthesizes plasma clotting factors I (fibrinogen), II (prothrombin), V, VII, VIII, IX, and X. Factors II, VII, IX, and X are vitamin K-dependent clotting factors. The most important factors in

liver disease are those with the shortest half-lives, factors VII and VIII.

Clinical relevance - Acute and chronic liver diseases may be accompanied by (i) prolongations in prothrombin and partial thromboplastin times and (ii) associated coagulopathies.

Bile secretion

Bile is a slightly alkaline iso-osmotic solution of bile salts, bile pigments, phospholipids, cholesterol, electrolytes, and water. Bile acids and bile salts are the primary component of bile. Bile acids are synthesized from cholesterol and conjugated to an amino acid (usually taurine or glycine) to become a bile salt. They are secreted into the biliary tract where they undergo storage in the gallbladder and are subsequently emptied into the small intestine during feeding. Bile salts carry out the emulsification of ingested lipids to facilitate pancreatic lipase digestion, and the micellarization of free fatty acids to facilitate enterocyte absorption. Ileal re-absorption of bile salts facilitates the return of bile acids to the liver for re-uptake, re-conjugation, and re-secretion.

- Emulsification \rightarrow pancreatic lipase digestion \rightarrow β -monoglycerides free fatty acids + glycerol \rightarrow micellarization by bile acids \rightarrow enterocyte absorption \rightarrow re-esterification \rightarrow triglyceridemia

Clinical relevance - Biliary obstructive disorders may be accompanied by icterus and steatorrhea.

Porphyrin metabolism

Porphyryns are intermediates of the heme biosynthetic pathway. In health, the porphyryns are converted into the oxygen-carrying heme compounds of hemoglobin, myoglobin, cytochromes, catalase, and peroxidase. The liver serves as a synthetic as well as excretory pathway for the porphyryns.

- δ -aminolevulinic acid \rightarrow porphobilinogen \rightarrow protoporphyrin IX \rightarrow globin + Fe²⁺ + bilirubin

Clinical relevance - Acute and chronic liver diseases may be accompanied by (i) porphyrin accumulation and the syndrome of porphyria, but more often (ii) bilirubin accumulation and icterus.

Metal metabolism

The liver stores iron which can be toxic in excessive amounts (hemochromatosis). The amount of iron in the body is largely determined by regulation of its absorption in the upper small intestine. Iron is stored intracellularly as ferritin in a number of tissues, with the liver having a large storage capacity. When the capacity of the liver is exceeded, iron accumulates as hemosiderin. The liver incorporates copper into specific copper proteins such as cytochrome c oxidase, mitochondrial monoamine oxidase, and ceruloplasmin. Mobilization of copper from hepatocytes takes place by two mechanisms - ceruloplasmin binding, and biliary secretion.

- Dietary Fe^{2+} → absorption → ferritin binding → transferrin transport → liver storage → biliary secretion
- Dietary Cu^{2+} → absorption → albumin binding → albumin transport → liver storage → biliary secretion

Clinical relevance - Cholestatic liver disease may be accompanied by secondary iron and copper retention which may then induce hepatocyte injury through apoptosis and oxygen free radical generation.

Vitamin metabolism

The liver has several major roles in vitamin metabolism. As well as producing bile for absorption of fat-soluble vitamins (A, D, E, K), it is an important site for vitamin storage. Water-soluble vitamins, except for vitamin B₁₂ (cobalamin), are readily absorbed from the small intestine. These vitamins are used primarily as co-enzyme precursors for use in metabolic processes. Large amounts of all water-soluble vitamins, except vitamin C, are stored in the liver.

Clinical relevance - Cholestatic liver disease may be accompanied by steatorrhea and fat-soluble vitamin malabsorption.

Glutathione metabolism

Glutathione (GSH) is synthesized in most, if not all, mammalian cells; the liver has relatively high levels of GSH. GSH performs a variety of physiologic and metabolic functions including thiol transfer reactions that protect cell membranes and protein and also promotes thiol-disulfide reactions involved in protein synthesis/degradation and catalysis. GSH

provides reducing capacity for other reactions, and detoxifies hydrogen peroxide, organic peroxides, free radicals, and foreign compounds.

- Glutamate + cysteine + glycine → glutathione → methylation, sulfuration, aminopropylation reactions

Clinical relevance - Acute and chronic liver diseases may be accompanied by glutathione deficiency and increased apoptosis, oxygen free radical generation, and lipid peroxidation.

Xenobiotic metabolism

Numerous foreign compounds, including drugs, are so hydrophobic that they would remain in the body indefinitely were it not for hepatic biotransformation. The liver is an important site in drug toxicity and oxidative stress because of its proximity and relationship to the gastrointestinal tract. 75-80% of hepatic blood flow comes directly from the gastrointestinal tract and spleen via the main portal vein. Portal blood flow transports nutrients, bacteria and bacterial antigens, drugs, and xenobiotic agents absorbed from the gut to the liver in more concentrated forms. Drug-metabolizing enzymes detoxify many xenobiotics but may activate the toxicity of others. The major mechanisms of hepatotoxicity include bile acid-induced hepatocyte apoptosis, cytochrome P₄₅₀E₁-dependent toxicity, peroxynitrite-induced hepatocyte toxicity, adhesion molecules and oxidant stress in inflammatory liver injury, microvesicular and non-alcoholic steatosis.

- Oxidation, reduction, hydrolysis, methylation, sulfuration, acetylation, glucuronidation → inactivation

Clinical relevance - Acute and chronic liver diseases may be accompanied by accumulation of xenobiotics as well as endogenous hormones (e.g. glucocorticoids).

Hormone metabolism

Natural and synthetic hormones including mineralocorticoids (aldosterone), glucocorticoids (cortisol, corticosterone), and sex steroids (androgens, estrogens, progesterone) are metabolized in the liver. Liver disease reduces the capacity for metabolic transformation.

Clinical relevance - Acute and chronic liver diseases may be accompanied by accumulation of endogenous hormones.

Immune surveillance

The reticuloendothelial system of the liver removes microbes, endotoxins, enterotoxins, and exotoxins from the portal circulation. The liver regulates T-cell homeostasis, induces T-cell tolerance, and supports intrahepatic T-cell responses against hepatotropic pathogens.

Clinical relevance - Acute and chronic liver diseases may be accompanied by portal bacteremia and predisposition to systemic infection.

Laboratory tests of liver disease

In animals with suspected liver disease, the minimum database should include: i) complete blood count - red blood cell, white blood cell, and platelet counts; ii) serum chemistries - electrolytes, urea nitrogen, creatinine, glucose, cholesterol, albumin, globulins, bilirubin, ALT, AST, and ALP or gamma-glutamyltranspeptidase (GGT) activities; iii) urinalysis; and iv) fecal flotation. Survey abdominal radiographs should be performed as part of the minimum database in any animal suspected as having liver disease. Additional laboratory tests and imaging studies may be considered following development of the minimum database.

(i) Complete blood count

Complete blood count will be useful in the assessment of severity and chronicity of anemia, as well as in the characterization of inflammatory response and thrombocytopenia. Hematology usually reveals only non-specific changes in liver disease such as microcytic anemia, or normocytic, normochromic non-regenerative anemia. Erythrocyte dysmorphias, e.g. schistocytes and leptocytes, may be evident in the blood smears of more severely affected animals with liver failure and dyslipidemia (**Figure 1**). Severe leukocytosis and neutrophilia may be observed in animals with bacterial, viral, or granulomatous hepatitis, hepatic necrosis, hepatic abscessation, and hepatic neoplasia.

(ii) Serum chemistry

Routine serum biochemical analysis helps identify



Figure 1.

Erythrocyte dysmorphias (leptocytes and schistocytes) in a young dog with portosystemic shunting.

©Dr. Jed Overmann

metabolic causes of disease, including liver disease (increases in serum ALT, AST, ALP, GGT and bilirubin; decreases in serum glucose, albumin and cholesterol), renal disease (increases in BUN, serum creatinine and phosphorus), as well as endocrine disorders such as diabetes mellitus, hyperadrenocorticism and hypoadrenocorticism. An exaggerated BUN/creatinine ratio (often > 50:1) secondary to gastrointestinal hemorrhage may accompany liver disease. Paraneoplastic changes (e.g. hypercalcemia, hyperglobulinemia) associated with hepatic or systemic neoplasia (e.g. lymphoma, extramedullary plasmacytoma) may also be identified by routine serum biochemical analysis.

(iii) Urinalysis

Urinalysis (**Figure 2**) will prove useful in the determination of hyposthenuria (e.g. urea depletion), hematuria (e.g. coagulopathy), and crystalluria (e.g. ammonium biurate – **Figure 3**). Urine protein-creatinine ratio determinations may be necessary to exclude protein-losing nephropathy as a cause of hypoalbuminemia. It may be difficult to obtain a cystocentesis sample in an animal with concurrent ascites, in which case cystocentesis should be via guided ultrasound.

(iv) Fecal examination

Direct fecal smears and fecal flotations should always be part of the initial screening tests even in suspected cases of liver disease. While many parasites and microbes preferentially infect one part of the G.I. tract, others may induce pathology throughout the whole tract (e.g. *Salmonella*, *Campylobacter*, *Pythium*, *Histoplasma*), including the pancreas, liver, and biliary tract.

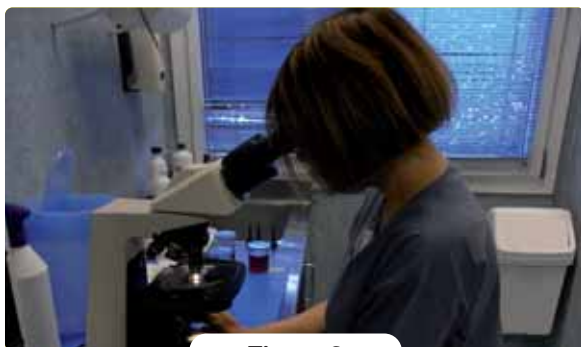


Figure 2.

Urinalysis and the detection of crystalluria may assist the clinician in diagnosis.

◆ Additional diagnostic tests

Further tests may be necessary depending upon the outcome of the initial medical investigation. Liver function tests (*e.g.* PT and PTT, plasma NH_4^+ , and serum bile acids) should be performed in any animal in which there is suspicion, but not yet definitive proof, of liver disease. This is particularly true in cases of suspected hepatic cirrhosis in which there may be only mild elevation in serum liver enzyme (ALT, AST, ALP, GGT) activities.

- **Coagulation** - In addition to coagulation factor synthesis, the liver is involved in the clearance of activated clotting factors and products of fibrinolysis. Therefore assessment of prothrombin (PT) and activated partial thromboplastin (APTT) times are assays of liver function, and should always be assessed prior to invasive procedures such as liver biopsy. The APTT and PT are both likely to be prolonged in severe, acute hepatic necrosis or parenchymal collapse, while only the APTT tends to be prolonged in dogs with congenital portosystemic shunts (3).
- **Plasma ammonia** - Ammonia is primarily a byproduct of intestinal bacterial metabolism that is normally transported to the liver via the portal vein, and further metabolized to urea by hepatocytes in the Krebs-Henseleit cycle. With portosystemic shunting or severe liver disease, ammonia accumulates in the systemic circulation and subsequently the brain where it rapidly saturates transformation capacity giving rise to the syndrome of hepatoencephalopathy. Fasting plasma ammonia should be measured before oral tolerance testing; a markedly

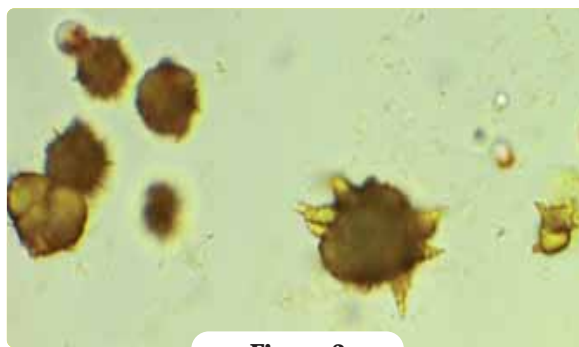


Figure 3.

Ammonium biurate crystalluria in a young dog with portosystemic shunting.

elevated level should obviate any further need for tolerance testing. If findings are equivocal, plasma concentrations can be measured before and after the administration of ammonium chloride (100 mg/kg) orally or per rectum. Blood is collected into ammonia-free heparinized tubes, placed on ice, and assays should be performed within 20 minutes of collection (4).

- **Serum bile acids** - Total serum bile acid concentrations will be increased when pathology alters the enterohepatic recirculation. The uptake and resecretion of bile acids into bile is diminished in many forms of primary liver disease, from inflammation to infection, malignancy, and portosystemic shunting. The finding of a fasting and/or postprandial serum bile acid concentration greater than $15 \mu\text{mol/L}$ in cats and $25 \mu\text{mol/L}$ in dogs is supportive of a diagnosis of liver pathology or portosystemic shunt (5).
- **Heavy metal analysis** - Normal hepatic copper concentrations in the dog are maintained at approximately 200-400 $\mu\text{g/g}$ dry weight liver (6). Cholestatic liver diseases are often accompanied by heavy metal (Cu^{2+} , Fe^{2+}) retention. Copper retention in particular produces hepatocyte injury primarily through generation of oxygen free radicals, lipid peroxidation, and apoptosis. Tissues assays of copper content are readily available through many toxicology laboratories.

◆ Imaging studies

The diagnosis of liver disease should never rely solely on laboratory testing (7). Survey abdominal radiographs should be obtained as part



Figure 4.

Ultrasonography is an invaluable diagnostic aid when investigating liver disease.

of the minimum database in all cases of suspected liver disease. Negative or equivocal radiographic findings should be further investigated with other imaging studies such as abdominal ultrasound. Ultrasonography (**Figure 4**) permits the assessment of size, shape, and density of parenchymatous organs, and is therefore a more useful tool in the further delineation of hepatic, renal, splenic, pancreatic, and mesenteric disease. Confirmation and definitive diagnosis of liver disease may require tissue biopsy by percutaneous or direct surgical sampling. Percutaneous ultrasound-guided liver biopsy may be difficult or challenging in some cases and laparoscopy or open surgical biopsy may offer a safer approach in these patients.

Conclusion

The complexity of liver function (**Table 1**) and the diversity of liver disease in dogs and cats are such that a veterinarian cannot place reliability on any one test or indeed one group of tests in reaching a definitive diagnosis. A conscientious clinician will take a holistic approach in assessing potential

Table 1.

Relationship between clinical and laboratory findings in liver disease.

Abdominal pain - congestion, inflammation, infection, thrombosis
Acholic feces - biliary obstruction
Anemia - bone marrow suppression, hemolysis, gastrointestinal blood loss
Anorexia/weight loss - inflammation, infection, protein-calorie malnutrition, vomiting, diarrhea, disturbances in intermediary metabolism
Ascites - portal hypertension, renal sodium retention, hypoproteinemia
Depression/weakness - hypoglycemia, hepato-encephalopathy (NH ₃ , fatty acids, mercaptans, alkalosis)
Diarrhea - biliary obstruction, concurrent intestinal disease, portal hypertension
Fever - infection, inflammation, neoplasia
Hematuria - infection, coagulopathy, calculi
Icterus - primary liver disease, biliary tract disease
Melena - bleeding disorders, gastrointestinal inflammation, ulceration
Petechiae - thrombocytopenia, thrombocytopathy
Polyuria/polydipsia - renal medullary solute washout, potassium depletion, hyperadrenocorticism
Pruritis - photoactivation of bile salts
Vomiting - central mechanisms (hepatotoxins), inflammation, hypergastrinemia, ketonemia

hepatic patients and beware the many pitfalls that can trap the unwary, and in particular the possibility that normal results do not necessarily rule out a liver problem. ☹

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Clinical epidemiology – liver enzyme testing



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Editor's note:

We are delighted to introduce a new feature for Veterinary FOCUS, utilizing information obtained from the computer database of Banfield, The Pet Hospitals. The extensive records generated by Banfield veterinarians can be analyzed to examine a huge variety of factors within pet populations; here we take a brief look at "healthy" pets and whether their hepatic biochemistry results correlate with their clinical appearance.



Introduction

There is limited literature on sensitivity, specificity and predictive values for liver enzyme activity testing. Canine alanine aminotransferase (ALT) activity testing has been reported to have a sensitivity ranging from 45-100%, depending on the liver pathology (acute or chronic hepatopathy, etc.) (1). The predictive value cited for ALT activity testing in this paper was 18%, *i.e.*, an elevated ALT activity testing was predictive for liver disease in 1 of

5 patients. Specificity for canine alkaline phosphatase (ALP) ranged from 44-90% depending on the liver disease in question; its predictive value was 21%, about the same as ALT activity testing. In general, these tests are not ideal for establishing a definitive diagnosis, and follow-up with more specific tests is essential. In this paper we describe Banfield database results for liver enzyme testing, specifically for pets that are reported to be healthy when presented to the veterinarian.

Table 1.

Result ranges in tested "normal animals".

Cats < 6 months of age		Dogs < 6 months of age	
ALP	14.0 – 192.0 U/L	ALP	46.0 – 337.0 U/L
ALT	12.0 – 115.0 U/L	ALT	8.0 – 75.0 U/L
BIL	0.0 – 0.9 mg/dL	BIL	0.0 – 0.9 mg/dL
Cats > 6 months of age		Dogs > 6 months of age	
ALP	14.0 – 111.0 U/L	ALP	23.0 – 212.0 U/L
ALT	12.0 – 130.0 U/L	ALT	10.0 – 100.0 U/L
BIL	0.0 – 0.9 mg/dL	BIL	0.0 – 0.9 mg/dL

Methods of analysis

Banfield data captured in the PetWare® Banfield's electronic medical record system throughout 2007 was used to generate the healthy canine and feline test sample distribution (mean, median and range) by age group (< 6 months, 6 months - 7 years, 8 years and older) for ALP and ALT activities and total bilirubin (BIL) concentration. The proportion of "healthy" pets was also calculated by age group that had test elevations for ALP and ALT activities and BIL concentration. A "healthy" pet was defined as one for whom overall condition in PetWare was not defined as "Needs Improvement." Normal result ranges for ALT, ALP and BIL concentration were defined as in **Table 1**.

Table 2.

2007 Banfield canine population proportion with elevated liver test results – healthy* pets.

Test	Age group	Elevated healthy*	Total count	Healthy* count	% Elevated in healthy*
ALP	< 6 months	439	56,296	23,461	1.9
	> 6 months	12,998	329,586	143,796	9.0
	Total unique pets*	13,428	382,599	166,541	8.1
ALT	< 6 months	1,606	56,292	23,467	6.8
	> 6 months	14,586	329,606	143,832	10.1
	Total unique pets*	16,147	382,604	166,577	9.7
BIL	< 6 months	640	56,285	23,457	2.7
	> 6 months	10,008	329,465	143,735	7.0
	Total unique pets*	10,647	382,469	166,476	6.4

Results

PetWare records from 382,856 dogs were evaluated for ALP activity, ALT activity or BIL concentration; 166,700 or 43.5% of these dogs were healthy as defined above. 89,598 cats were evaluated for ALP activity, ALT activity or BIL concentration; 34,073 or 38% of these cats were by definition healthy.

Nearly 21% of healthy dogs had one or more elevation in ALP activity, ALT activity or BIL concentration; 15.5% of healthy cats had one or more parameters elevated. *Tables 2 and 3* provide

the proportion of the healthy pets evaluated that had elevations for ALP activity, ALT activity or BIL concentration individually. For dogs, these proportions range from just under 2% for ALP activity in dogs < 6 months old to just over 10% for ALT activity in dogs > 6 months. For the cat population, the proportion with elevated ALP ranged from 4.4% for ALT activity in cats < 6 months old to nearly 11% for ALP activity for cats > 6 months old. *Tables 4 and 5* display the distribution of these liver test results for healthy cats and dogs overall and by age group. Of the healthy

Table 3.

2007 Banfield feline population proportion with elevated liver test results – healthy** pets.

Test	Age group	Elevated healthy*	Total count	Healthy* count	% Elevated in healthy*
ALP	< 6 months	702	16,378	6,533	10.7
	> 6 months	1,406	73,608	27,617	5.1
	Total unique pets*	2,100	89,534	34,038	6.2
ALT	< 6 months	384	16,377	6,529	5.9
	> 6 months	1,467	73,635	27,636	5.3
	Total unique pets*	1,842	89,557	34,050	5.4
BIL	< 6 months	288	16,369	6,528	4.4
	> 6 months	1,622	73,603	27,617	5.9
	Total unique pets*	1,909	89,521	34,033	5.6

* Overall condition in PetWare not defined as “Needs improvement”

** Count of unique pets will be less than sum of counts because one pet could have multiple tests

Table 4.

2007 Canine population liver test results by age group – all healthy* pet samples.

Test	Age group	Total count	Mean	Median	Range
ALP U/L	< 6 months	24,058	183.2	173.0	10.0 - 1,880.0
	6 months - 7 years	137,463	95.9	72.0	3.0 - 3,330.0
	> 8 years	35,723	203.4	102.0	0.2 - 7,373.0
	All ages	197,244	126.0	84.0	0.2 - 7,373.0
ALT U/L	< 6 months	24,043	44.2	38.0	0.9 - 3,491.0
	6 months - 7 years	137,515	56.9	46.0	0.1 - 2,000.0
	> 8 years	35,706	72.9	53.0	0.1 - 2,677.0
	All ages	197,264	58.3	46.0	0.1 - 3,491.0
BIL mg/dL	< 6 months	24,067	0.2	0.1	0.0 - 11.0
	6 months - 7 years	137,761	0.3	0.2	0.0 - 20.0
	> 8 years	35,831	0.3	0.2	0.0 - 20.0
	All ages	197,659	0.3	0.2	0.0 - 20.0

* Overall condition in PetWare not defined as "Needs improvement"

Table 5.

2007 feline population liver test results by age group – all healthy* pet samples.

Test	Age group	Total count	Mean	Median	Range
ALP U/L	< 6 months	6,741	126.4	115.0	10.0 - 1,950.0
	6 months - 7 years	21,912	55.2	46.0	5.0 - 13.50
	> 8 years	8,851	55.1	46.0	2.0 - 1,547.0
	All ages	37,504	68.0	51.0	2.0 - 1,950
ALT U/L	< 6 months	6,757	71.4	64.0	1.0 - 785.0
	6 months - 7 years	22,012	69.9	62.0	5.0 - 2,815.0
	> 8 years	8,878	72.3	62.0	1.5 - 2,129.0
	All ages	37,647	58.1	63.0	1.0 - 2,815.0
BIL mg/dL	< 6 months	6,753	0.4	0.3	0.0 - 12.0
	6 months - 7 years	21,977	0.4	0.3	0.0 - 18.4
	> 8 years	8,864	0.4	0.3	0.0 - 18.8
	All ages	37,594	0.4	0.2	0.0 - 18.8

* Overall condition in PetWare not defined as "Needs improvement"

dogs that had elevations in ALP activity and ALT activity, about 8% (291/3785) had a bile acids concentration test performed (within 30 days of ALP/ALT activity elevations) to assess liver function. For this canine subset, 39% had an elevated bile acids concentration ($> 25 \mu\text{mol/L}$). Of the healthy cats that had elevations in ALP activity and ALT activity about 5% (15/316) had a bile acids concentration test to assess liver function within 30 days. For this feline subset, 40% had an elevated bile acids result ($> 20 \mu\text{mol/L}$).

Discussion

Two major points can be identified in summarizing the results from the Banfield database:

- Liver enzyme activity testing has limited predictive value for definitively diagnosing liver disease in dogs and cats.
- Bile acids concentration results from the healthy population reveal that there may be undetected disease in apparently healthy animals since 40% of these animals with elevated ALT and ALP activities that had bile acids concentration tested were abnormally elevated; less than 10% of healthy dogs and cats with elevated ALT and ALP activities had a bile acid concentration test performed within 30 days of the elevated enzyme activities results.

The limitations of the analysis include the potential for misclassification of pets as healthy based on our analysis definition. In addition the bile acids concentration test used for this analysis was a single sample test rather than the two-sample stimulation test, the single test having limited sensitivity and specificity. However, the results do underscore the importance of further case work-up with liver function tests, imaging, fine-needle aspirate or biopsy when indicated by history, physical examination and clinical signs. ☹

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Liver tumors: diagnosis and treatment



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Introduction

Primary liver neoplasms are infrequent in the dog and cat, with an estimated prevalence in necropsy studies of 0.6-2.6% in canines and 1.5-2.3% in felines. In the dog liver metastases are much more frequent than primary hepatic tumors, and affect

30.6-36.8% of all animals with non-hepatic neoplasms, the spleen, pancreas and gastrointestinal tract being the most common primary tumor locations implicated in such metastases. In cats it is estimated that 20% of all liver tumors are metastases of primary malignancies located in the pancreas, gastrointestinal tract or kidneys (1,2,3,4,5).

KEY POINTS

- Primary hepatobiliary neoplasms are rare in the dog and cat, and tend to affect older animals which present with non-specific or gastrointestinal clinical signs.
- The clinico-morphological presentation of the disease, its cellular origin and its nature (benign or malignant) usually determine the therapeutic options and prognosis.
- The clinical approach to such cases involves multiple laboratory tests including urine and coagulation analysis, survey radiographs and abdominal ultrasound, as well as liver cytology or biopsy.
- Surgery only offers a good prognosis in the case of lobar presentations or benign neoplasms. Chemotherapy is usually ineffective for primary liver tumors.
- Most animals with malignant lesions tend to have metastases or diffuse disease presentations at the time of diagnosis, with a correspondingly poor prognosis.

Primary liver neoplasms are usually classified according to their cellular origin and macroscopic appearance. With respect to their cellular origin, these tumors may be hepatobiliary, hematopoietic, sarcomas, or metastases of other tumors (**Table 1**). In relation to their morphological presentation, they can be classified as lobular, multiple nodular or diffuse (**Table 2**). The combination of histopathological and morphological classification has consequences for the prognosis and treatment strategy in these animals; a clinician must therefore always address these factors in order to take correct management decisions. In dogs, malignant tumors are more common than benign lesions. In cats, biliary neoplasms are the most common presentation, particularly intrahepatic forms (6,7).

Table 1.

Classification of liver neoplasms according to cellular origin.

Primary	<ul style="list-style-type: none"> • Hepatobiliary neoplasm: <ul style="list-style-type: none"> - Hepatocellular carcinoma - Biliary carcinoma (cholangiocarcinoma, biliary adenocarcinoma) - Hepatocellular adenoma (hepatoma) - Biliary duct adenoma (cystadenoma) - Carcinoid tumor (neuroectodermal neoplasm)
	<ul style="list-style-type: none"> • Hematopoietic neoplasm: <ul style="list-style-type: none"> - Lymphoma - Leukemia
	<ul style="list-style-type: none"> • Sarcomas: <ul style="list-style-type: none"> - Hemangiosarcoma - Sarcoma - Leiomyosarcoma - Rhabdomyosarcoma - Osteosarcoma - Chondrosarcoma
Metastatic	<ul style="list-style-type: none"> • Gastrointestinal tract • Spleen • Pancreas • Kidneys • Mammary tissue • Prostate

❖ Clinical presentation and clinical signs

Most animals with liver neoplasia present with non-specific clinical signs such as anorexia and weight loss; it is estimated that these may be noted in 75% of canine and 50% of feline cases. Less frequent signs may include vomiting or diarrhea, although in cats vomiting can be common. Approximately 50% of the animals may present as polydipsic/polyuric, whilst others may

show pale mucosal membranes or acute weakness due to anemia and hypovolemic shock secondary to tumor rupture. However, up to 25% of all animals show no clinical signs, and evidence of the presence of a liver tumor is only established upon identifying an increase in liver enzymes (7,8).

The most common findings on physical examination are a mass in the cephalad portion of the abdomen and abdominal bloating (30% of cases) or jaundice (18% of cases). In cases of metastasis, jaundice is very infrequent. Other less commonly described manifestations are neurological signs due to hepatic encephalopathy, or paraneoplastic syndromes such as hypoglycemia or skin alterations. There have been reports of myasthenia gravis associated with biliary carcinoma (7).

❖ Clinical approach and staging

The clinical approach to an animal with a suspected liver neoplasm should include basic information such as a complete blood count, blood biochemistry, coagulation tests, urinalysis, thoracic and abdominal radiographs, abdominal ultrasound (**Figure 1**), and fine-needle aspiration biopsy of the liver where possible.

Laboratory test findings

Table 3 shows the most frequent hematological and biochemical alterations in animals with liver neoplasms. Leukocytosis associated with liver neoplasia is a result of inflammation and necrosis of large tumors. Anemia tends to be moderate and non-regenerative, and is thought to be due to chronic illness, inflammation or iron deficiency. Thrombocytosis can be seen in 50% of animals with hepatocellular carcinoma, and is attributable

Table 2.

Definition and percentage incidence of the morphological presentation of different liver tumors in the dog.

Morphological presentation	Definition	Incidence in hepatocellular carcinoma	Incidence in biliary carcinoma	Incidence in sarcoma	Incidence in carcinoid tumor
Lobular or massive	Nodule or large mass in a single liver lobe	53-84%	37-46%	36%	0%
Multiple nodular	Several nodules throughout the liver parenchyma, or several affected liver lobes	16-25%	0-21%	64%	33%
Diffuse or infiltrating	Multiple coalescent nodules in all the lobes, or diffuse disappearance of the liver parenchyma	0-19%	17-54%	67%	0%

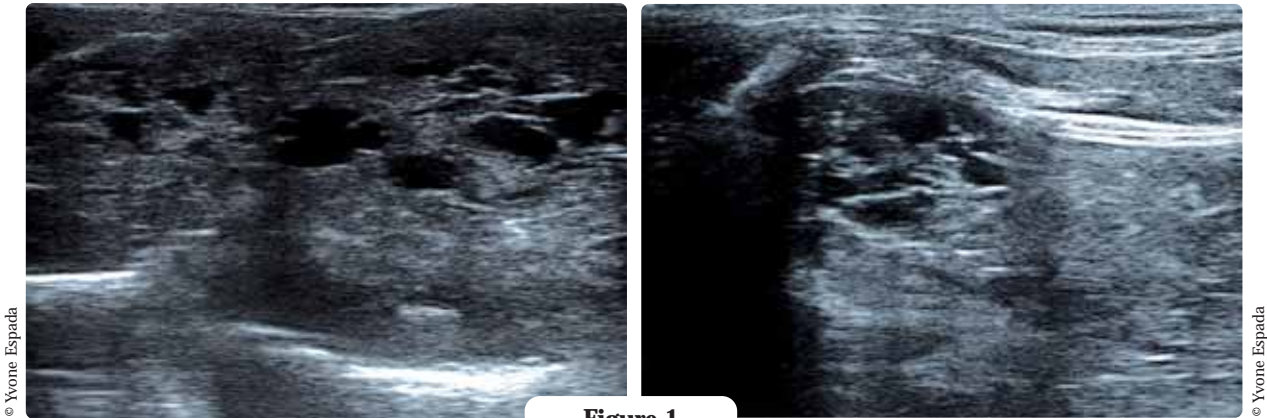


Figure 1.

Ultrasound appearance of biliary adenoma in two cats.

to a paraneoplastic syndrome characterized by thrombopoietin production, iron deficiency or anemia.

Liver enzyme elevation is a frequent, but not universal, finding in animals with liver neoplasms, and the degree of enzyme elevation does not correlate with the degree of liver involvement or the severity of the disease. According to one study (9), animals with primary liver tumors tend to show a more marked increase in ALT and ALP than animals with metastasis, while the latter tend to show a greater increase in bilirubin and AST. It also has been suggested that an AST/ALT ratio of < 1 is more compatible with carcinoma, while a ratio of > 1 is more indicative of sarcoma or carcinoid tumor. Other reported biochemical changes include hypoglycemia, hypo- or hyper-albuminemia and increased bile acids. Hypoglycemia as a paraneoplastic syndrome associated with hepatocellular carcinoma is attributed to the secretion of insulin-like growth factor II (IGF-II). Unlike dogs, cats usually present with a high incidence of nitrogenated compound elevation (9,10).

Coagulation factor changes are more commonly associated with hemangiosarcoma, although in end-stage liver neoplasia or in decompensated animals it is possible to observe coagulation factor deficiency or disseminated intravascular coagulation. Consequently a coagulation study is recommended before deciding on any invasive procedure in these animals (8).

Alpha-fetoprotein has been evaluated in the dog, and is seen to be increased in 75% of animals with

hepatocellular carcinoma and in 55% of those with biliary carcinomas. However, the use of this tumor marker is limited by the fact that it is also increased in cases of hepatic lymphoma and other liver neoplasms/diseases, and only very significant increases in alpha-fetoprotein may be taken to indicate hepatocellular carcinoma.

Radiography

Abdominal radiographies may reveal the presence of a mass in the cephalad abdominal space, although this depends on the size of the neoplasm or metastases. Other reported findings are dorsal displacement of the stomach, hepatomegaly, loss of abdominal contrast (due to the presence of free fluid) and, occasionally, biliary tract calcification. Thoracic radiography can be useful and should be

Table 3.

Hematological and biochemical changes observed in dogs and cats with liver neoplasms.

Parameter	Change	Incidence in dog	Incidence in cat
Hematocrit	decrease	27-50%	?
Leukocytes	increase	54-73%	?
Platelets	increase	50% (in hepatocellular carcinoma)	?
ALP	increase	61-100%	10-64%
ALT	increase	44-75%	10-78%
GGT	increase	39%	78%
Total bilirubin	increase	18-33%	33-78%
Bile acids	increase	50-75%	67%
Albumin	decrease	52-83%	?
Albumin	increase	occasionally	?
Glucose	decrease	occasionally	?



Figure 2a.

Ultrasound view of a 10-year-old mixed-breed dog with a large hyperechoic mass in the liver.

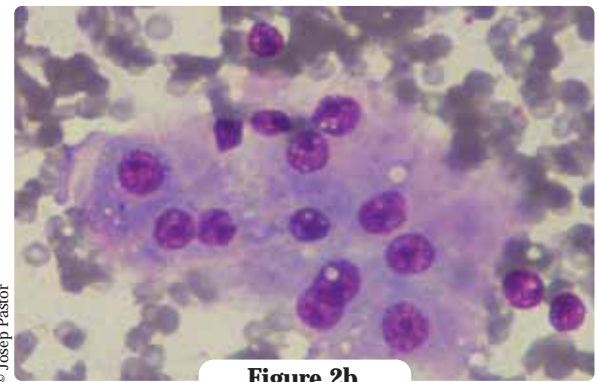


Figure 2b.

Ultrasound-guided cytology revealed abundant normal-appearing liver cells suggestive of nodular hyperplasia.

included as part of the staging procedure of animals with a view to evidencing metastatic disease (11).

Abdominal ultrasound

Ultrasound examination in veterinary practice commonly demonstrates changes within an animal's liver which may take a variety of forms. Most liver ultrasound changes are not pathognomic of a given disease process, and the final

diagnosis is established on the basis of the clinical and blood test findings, and the cytological or histopathological results (Figure 2). The basic ultrasound patterns are described in Table 4. Ultrasound is also very useful for evaluating the rest of the abdomen in animals with suspected liver neoplasia, particularly for tumors of the liver parenchyma and lymph nodes, and for the staging of such tumors (11-15).

Table 4.	
Basic ultrasound patterns in liver neoplasia.	
Diffuse or multifocal	<ul style="list-style-type: none"> Diffuse or multifocal liver neoplasms tend to present with hepatomegaly, but this depends on the degree of infiltration. Liver carcinomas can be diffuse or affect multiple lobes, with variable ultrasound characteristics depending on the presence of necrosis, inflammation, hemorrhage or cavitation. In these malignant tumors it is common to observe a mixed echogenicity pattern. Lymphoma can affect the liver without detectable ultrasound changes, or cause diffuse hypoechogenicity, hyperechogenicity or mixed echogenicity with or without hypoechoic nodules. Consequently, if lymphoma is suspected, even if the liver ultrasound findings appear normal, fine-needle aspiration cytology is recommended. Histiocytic neoplasms are more often associated with multiple nodules and hypoechoic masses, though diffuse liver hypoechogenicity has also been described. Mast cell infiltration of the liver tends to produce diffuse hyperechogenicity.
Nodular patterns	<ul style="list-style-type: none"> Benign nodular hyperplasia is common, particularly in dogs, and accounts for many of the focal liver lesions identified on ultrasound exploration. It has been estimated that 25-36% of all nodular masses detected in the liver are nodular hyperplasias. Benign liver adenomas or hepatomas can manifest as a focal mass of variable size and of normally hyperechoic characteristics. Primary liver neoplasms such as hepatocellular carcinoma can present as focal or multifocal masses, though less often so than in the case of metastases. Focal hypoechoic lesions with a hyperechoic center or core (known as target or bull's-eye lesions) are usually associated with metastases, though some benign processes such as nodular hyperplasia can generate similar patterns.
Biliary obstruction	<ul style="list-style-type: none"> Ultrasound has become an important tool for evaluating biliary obstruction in icteric dogs and cats. Primary tumors of the liver, biliary tract, duodenum and pancreas have been shown to be capable of causing biliary obstruction.



Figure 3.

Macroscopic appearance of diffuse hepatocellular carcinoma during exploratory laparotomy in a dog.



Figure 4.

Macroscopic appearance of intrahepatic biliary carcinoma during exploratory laparotomy in a dog.

Liver cytology and biopsy

Liver cytology is useful for the initial evaluation of hepatomegaly and normally allows differentiation between primary tumors, metastatic disease and focal infection. However, cytology is unable to distinguish between benign focal inflammatory disease and progressive chronic disease, and cannot establish the extent of a lesion. Likewise, a definitive diagnosis of regenerative nodular hyperplasia cannot be established, and the technique is unable to differentiate a benign inflammatory reaction from the cell changes associated with other pathologies that cause liver damage.

Contraindications to ultrasound-guided cytology include the following:

- Coagulation abnormalities: if one or more coagulation test parameters are altered, it is advisable to administer vitamin K1 via the subcutaneous route 12 hours before cytology.
- Cavitory masses: the ultrasound detection of a large cavitory lesion in an elderly dog usually contraindicates cytology, particularly in male German shepherds or golden retrievers, due to the high probability that such lesions correspond to hemangiosarcoma.

One of the inconveniences of liver cytology is that it cannot correctly distinguish between liver adenoma and a regenerative nodule, and histological differentiation is moreover often problematic. For example, some hepatocellular carcinomas may be composed of apparently normal hepatocytes, while others present obvious

features of malignancy. As a result, in many cases it proves necessary to resort to ultrasound-guided biopsy, laparoscopy or exploratory laparotomy. However, cytology can determine the presence of lymphoma, mastocytoma or histiocytic sarcoma, and contribute to the initial classification of the cellular type of the neoplasm (*Table 1*). While the concordance between the cytological and histopathological findings is generally good the reported rate varies from 14-86% (12-15).

⊕ Treatment and prognosis

The treatment to be provided and the prognosis of the animals with primary liver neoplasms depend on the cellular origin of the tumor, its benign or malignant nature, and the morphological presentation. The clinician should decide if surgery or palliative care is the treatment of choice for individual patients. Palliative treatment is the option for animals that are not surgical candidates and consists of pain management and general liver failure treatment recommendations. The success of newer options such as metronomic therapy or the use of antiangiogenics or tyrosin kinase inhibitors in the treatment of these patients is as yet unproven.

Hepatocellular carcinomas

No clear breed predisposition is observed with canine liver neoplasms, although poodles, fox terriers and Labrador retrievers may have a greater incidence of hepatocellular carcinoma (*Figure 3*). The macroscopic presentation is clinically very important, since 100% of the diffuse forms show metastasis at the time of diagnosis, *versus* 40% of the isolated presentations. Metastatic spread



Figure 5.

Multiple liver nodules in a dog with splenic hemangiosarcoma. Biopsy confirmed their metastatic nature.

usually affects the regional lymph nodes, lungs and peritoneum. The treatment of choice is surgical resection where possible; however surgical complications are reported in over 28% of cases, with a mortality rate of almost 12%. The right and caudad lobes of the liver pose the greatest surgical challenge, due to the proximity of the caudal vena cava. If the neoplasm is lobular and without metastases, the prognosis is good. However, this is rare, since most animals have metastases at the time of diagnosis; early diagnosis of this tumor would be ideal, but the nonspecific clinical presentation makes this difficult. Without surgery, the average life expectancy is 270 days and prognosis is generally poor. No effective chemotherapy options have been described, though mitoxantrone has been reported to be helpful in some cases. The most common situation is a lack of treatment response due to p-glycoprotein expression in the liver cells. In cats, hepatocellular carcinoma is less frequent, and less data is available (16-19).

Hepatocellular adenomas

These tumors are also known as hepatomas and are more common in cats than in dogs. In the latter it is sometimes very difficult to distinguish adenoma from reactive nodular hyperplasia, and biopsy is needed to clarify the diagnosis. The prognosis is usually good, but it is advisable to resect the lesions if they cause problems; they can grow very large and spontaneous rupture is frequent.

Bile duct carcinoma/adenocarcinoma/ cholangiocarcinoma

These are the most common liver malignancies in cats, and the second most common liver malignancy

in dogs (**Figure 4**). Tumor behavior is very aggressive in both species, and metastases are present at the time of diagnosis in 60-88% of cases. Biliary carcinomas usually metastasize to the regional lymph nodes, lungs and peritoneum, but also to the kidneys, heart, adrenal glands, eye or bone. These tumors are usually classified as either intrahepatic or extra-hepatic bile duct lesions – intrahepatic tumors being more common in dogs, and extra-hepatic neoplasms in cats. Three morphological forms or presentations have been described (lobular, multifocal and diffuse); in general, only the lobular form should be subjected to surgical removal, and only if there is no evidence of metastasis. In the other presentations the prognosis is very poor, and surgery is usually not feasible. Even if removal is possible, the prognosis remains poor, since most animals die within 6 months of surgery. No effective chemotherapeutic options have been described in application to these malignancies (20).

Bile duct adenomas

Also known as biliary cystadenomas, biliary adenomas or cholangiocellular adenomas, these tumors are infrequent in the dog, but are the most common lesions in cats, where males appear to be more frequently affected than females. In cats, 50% of these lesions are isolated or lobular, and 50% are multifocal. The prognosis is usually good, since these lesions constitute incidental findings, but they tend to grow until their associated mass effect or compressive action upon other organs gives rise to clinical symptoms – in which case surgical resection is usually required (21).

Other neoplasms

Neuroendocrine (carcinoid) tumors are infrequent in the dog and cat. They tend to present as diffuse lesions, but should not be mistaken for metastasis or tumors of other origins. They have also been described in the gallbladder, and can show partial response to cholecystectomy. However, the prognosis is generally poor, and metastatic disease is considered to be present in 90% of the cases at the time of diagnosis (7).

Primary liver sarcomas are again infrequent in the dog and cat. Leiomyoma is the most common canine presentation, although there have also

been reports of hemangiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma and histiocytic sarcomas. In cats, hemangiosarcoma is the most common primary sarcoma. These are usually very aggressive tumors, metastasizing in 86-100% of cases or spreading diffusely within the liver. The response to chemotherapy is similar to that seen in other sarcomas. As an example, histiocytic sarcomas respond partially to lomustine (CCNU), with a mean duration of remission of 85 days and a survival of 172 days (22).

There have also been descriptions of benign neoplasms such as fibrosarcoma and hemangioma, but these are much less frequent. On the other hand, metastases (**Figure 5**) must always be considered as a possibility when a hepatic tumor is diagnosed.

Conclusion

Primary hepatobiliary neoplasms are infrequent in the dog and cat, and tend to affect elderly animals with nonspecific or gastrointestinal clinical manifestations. The clinico-morphological presentation of the disease, its cellular origin and benign or malignant nature usually determine the therapeutic options and prognosis. Definitive diagnosis and accurate classification require complete laboratory testing, radiography and ultrasound, as well as liver cytology or biopsy. Surgical resection of a hepatobiliary tumor offers a good prognosis in only a few cases and should only be undertaken after due consideration of all the factors. ☺

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Liver biopsy sampling

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Liver diseases are an extensive and heterogeneous group of pathologies that can affect dogs and cats of any breed and age. Although laboratory tests often provide a clear indication of a hepatic disorder, and imaging techniques may be an essential clinical tool, a definitive diagnosis of the liver disease in progress can be made only by examination of the cell lines involved in the pathological process.

The primary indications for performing a liver biopsy include the following:

- Abnormal liver size
- Differential diagnosis of jaundice
- Differential diagnosis of non-homogeneous parenchymal patterns on ultrasound
- Identifying the type of hepatic masses or nodules
- Clarifying the cause of changes in the biochemical profile

Although sampling procedures are generally safe for the patient, there are some contraindications, particularly in the case of histologic biopsy samples:

- Absolute contraindications:
 1. Elevated risk of bleeding (severe coagulopathy, intraparenchymal vascular dilatation)
 2. Fluid-containing cystic lesions (abscess, cystic lesions with unknown content)
 3. Severe anemia
- Relative contraindications:
 1. Ascites
 2. Operator inexperience
 3. Extra-hepatic biliary obstruction
 4. Congestive heart failure

Even if all precautions are followed when taking the sample, some complications have been reported as a result of liver biopsy sampling. The primary complications include the following:

- Severe hemorrhage
- Gallbladder laceration
- Septic peritonitis
- Biliary peritonitis

There are various methods of sampling biopsy material from the liver: generally, the first sampling attempt is for cytologic examination using fine-needle aspiration. If this technique provides insufficient or inconclusive material the next step involves taking a tissue sample for histologic analysis. This material may be collected by ultrasound-guided technique using a core biopsy needle, a laparoscopic mini-invasive procedure, or conventional surgical approach.

🔍 Cytological sampling using a freehand technique

Fine-needle aspiration sampling may be performed 'blind' or via ultrasound guidance.

In the first case the sample is taken with the patient standing or in right lateral decubitus position (**Figure 1**). Depending on the size of the patient, a regular syringe needle or spinal needle may be used. Generally, the initial attempt involves using the fine-needle capillary method, without aspiration by a syringe attached to the needle. A different approach (**Figure 2**) is preferred in cats.

Material sampled in this way is deposited on a slide and smeared to obtain a single-cell layer that is air-dried and stained using the preselected staining technique.

🔍 Ultrasound-guided biopsy sampling

Interventional ultrasound is the most commonly used procedure in veterinary medicine to obtain hepatic, cytologic, or histologic biopsies (**Figure 3**).



The great advantage of this technique is the continuous control of the various sampling phases, which allows the sample to be optimized while minimizing the risks. Two main techniques for liver biopsy using ultrasound are described. The first (ultrasound-guided) uses a guide applied to the ultrasound transducer through which the needle is moved along a fixed track. In the second technique (ultrasound-assisted), the needle is inserted using the freehand technique under ultrasonographic vision, allowing the technician to have greater freedom of maneuverability but theoretically less precision in taking the sample.

In the case of a single lesion the cytology needle (23 - 27 G) is passed 2 or 3 times inside the lesion to maximize the collection of cells without creating excessive hematic contamination. If the ultrasonographic view shows diffuse pathologies, it is good practice to take additional samples from different lobes.

The needle contents are deposited carefully on a microscope slide and smeared, ensuring that the smear is stopped before the end of the slide so as not to lose larger cell elements such as clusters of hepatocytes, which are often the most representative and useful for a diagnosis.

The histologic sample is taken using the Menghini, Tru-Cut®, or BARD® needle. Generally, the patient is placed in dorsal decubitus, and the needle is inserted according to the indications resulting from the ultrasound investigation. Once the correct positioning of the needle inside the tissue to be sampled is checked (**Figure 4**), the needle cutting mechanism is released. When the tissue is extracted it is checked to ensure that the amount taken is adequate. The sample is then placed carefully inside a histologic sample cassette and immersed in formalin. An ultrasound test performed about an hour after the sample has been taken should confirm the absence of significant bleeding.

Generally, the procedures described above do not require anesthesia or sedation, except for particularly nervous or agitated patients. ☺

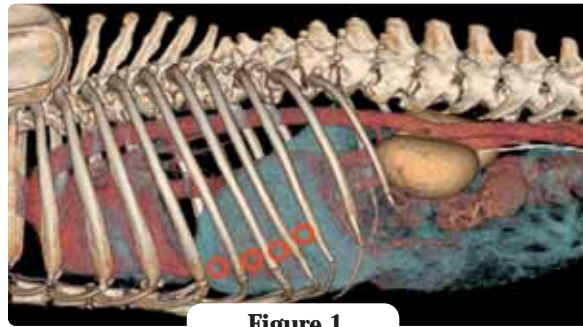


Figure 1.

An area between 7th - 10th left intercostal space is surgically prepared from sternum to the mid-thorax, disinfected and a 22 - 24 G needle, inserted craniodorsally, just dorsal to the costochondral junction.

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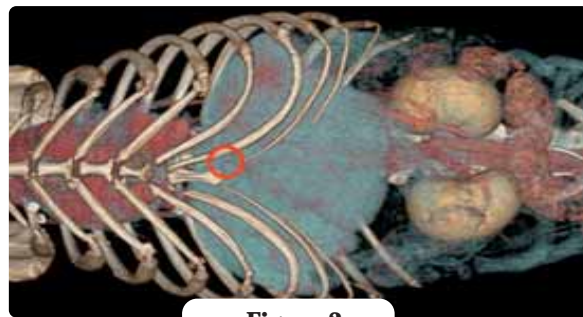


Figure 2.

With the cat in dorsal decubitus insert the needle cranio-caudally in the space between the left margin of the xiphoid process and left costal margin.

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Figure 3.

Ultrasound is the most commonly used procedure to obtain hepatic biopsies.

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Figure 4.

Ensure correct positioning of the needle inside the tissue to be sampled before the needle cutting mechanism is released.

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