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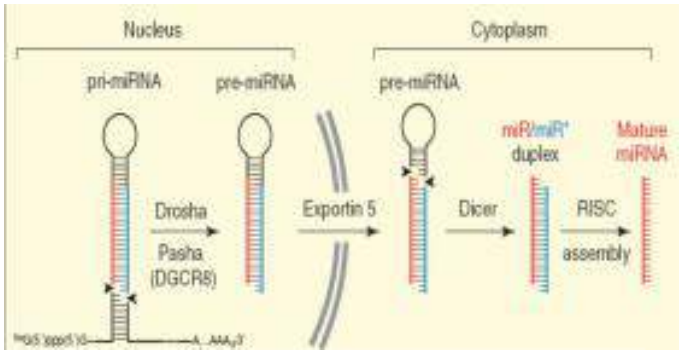
Top News

New test: Hope for liver disease in dogs

BBC News, Aug2018



The test, which is based on insights from human patients, could help vets begin treating sick animals earlier. It works by tracking levels of the blood molecule miR-122, a marker of liver disease in humans. Current diagnosis in dogs is based on biopsies, which are expensive and may lead to complications. Vets based at the University of Edinburgh's Royal (Dick) School of Veterinary Studies teamed up with medical doctors to look at miR-122 in 250 dogs.

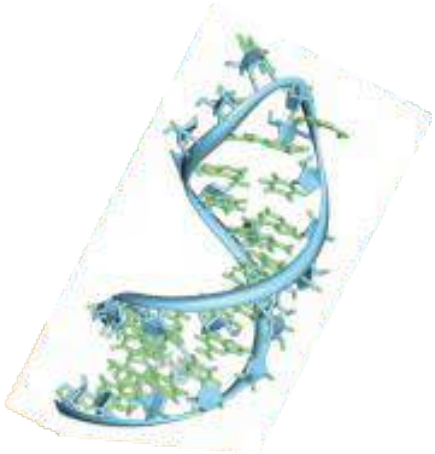
A revolution in regenerative medicine

Medical Express June2019

Drawn from biomedical and industrial engineering, textiles and veterinary medicine, the group is exploring how to apply 3-D printing and nonwoven fiber manufacturing to create new tissues that can grow in the human body. There are three components in tissue engineering, scaffolds, cells and active molecules that encourage further cell growth. The three work together to generate new tissues, with the scaffold giving form and structure to the tissue growth catalyzed by the active molecules. It would also develop the capability to produce, on a mass scale, scaffolds that could grow a range of different tissue types.

Positive Regulation of Hepatitis E Virus Replication by MicroRNA-122

Bangari Haldipur, Prudhvi Lal Bhukya, Vidya Arankalle, Kavita Lole



MicroRNAs (miRNAs) are known to modulate viral pathogenesis either by directly altering viral gene expression or by enhancing cellular antiviral responses. miR-122 facilitates HEV-1 replication. HEV-1 genomes showed a highly (97%) conserved miR-122 target site in the RNA-dependent RNA polymerase (RdRp) region. HEV infection did not change the basal levels of miR-122 in hepatoma cells. However, transfection of these cells with miR-122 mimics enhanced HEV-1/3 replication and depletion of miR-122 with inhibitors led to suppression of HEV-1/3 replication. The positive role of miR-122 in viral replication presents opportunities for antiviral therapy & Hepatitis E management.

Cd8+ T-Cell Response-Associated Evolution of Hepatitis B Virus Core Protein and Disease Progress

Journal of Virology

Under the immune pressure of cytotoxic T cells (CTLs), hepatitis B virus (HBV) evolves to accumulate mutations. However, little is known about the specific patterns of the immune pressure-associated HBV mutation of T-cell epitopes and their link to disease progression.

WSV18-0265

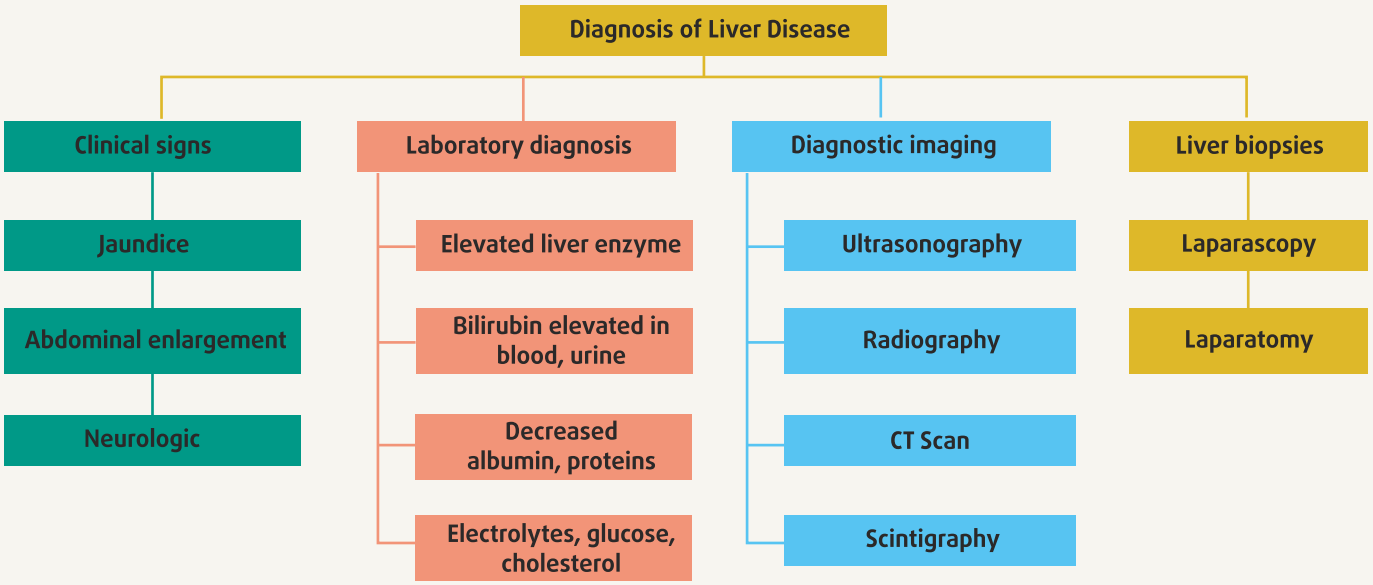
GASTROENTEROLOGY / LIVER

CANINE CHRONIC HEPATITIS : CAUSES AND TREATMENT

P. Waston'
''Department of Veterinary Medicine, University of Cambridge, UK

In chronic hepatitis, the factors to consider and treat are:

1. Ascites: Treated with spironolactone as the primary diuretic and addition of loop diuretics as necessary
2. Vomiting, diarrhoea, GI ulceration: common in animals with portal hypertension and treated by careful, little and often feeding to provide nutrition for gut wall healing.
3. Jaundice: When pre hepatic causes and post hepatic obstruction have been ruled out, to be treated with ursodeoxycholic acid and anti-oxidants.
4. Hepatic encephalopathy: Found mostly in young dogs with congenital portosystemic shunt. Highly digestible, high quality diet to be given often & little. Antibiotic & lactulose therapy to be considered.
5. Protein-calorie malnutrition: Nutrition to be given priority and type of highly digestible and high quality diet to be given often and little. Diet should not be protein-restricted.



DIAGNOSIS & MANAGEMENT OF LIVER DISEASES IN DOGS

Article by
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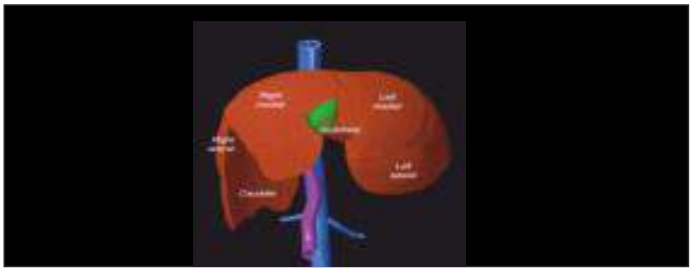
Madras Veterinary College

Functions of Liver

- Immunoregulation
- Storage of vitamins and trace minerals, glycogen, triglycerides
- Detoxification
- Metabolism of carbohydrates, lipids, proteins, vitamins and hormones
- Extreme regeneration capacity



Liver toxins

Environmental
Cycad palms, Aflatoxin, Blue- green algae
Food additives
Xylitol
Chemicals
Heavy metals, arsenic
Drugs
Paracetamol, Azathioprine, Corticosteroids, Doxycyline, Halothane, Lomustine, Mitotane, Nitrofurantoin, Phenobarbital, Sulfonamides Tetracyclines

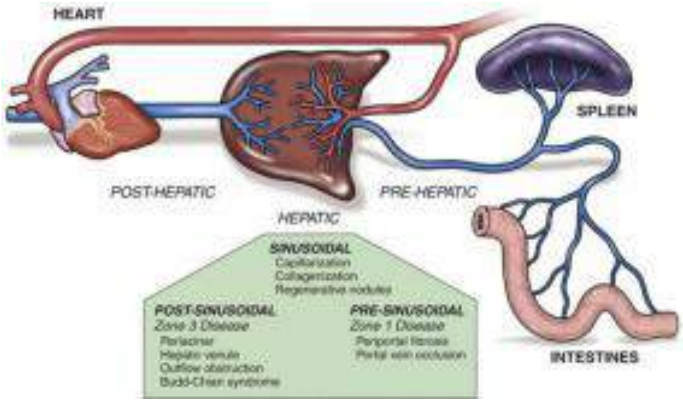
Infectious agents

Canine adenovirus - 1
Leptospirosis
E.coli
Enterococcus
Bacteroides
Streptococcus
Clostridium
E.canis
B.gibsonii

Physical examination:

- Icterus (sclera)-bilirubin >3.0mg/dl
- Icteric plasma 0.5 to 1.0 mg/dl
- Hepatocutaneous syndrome-superficial necrolytic dermatitis
- Ascites

Portal hypertension:



Prehepatic:

Increased resistance in the extra hepatic portal vein due to mural, intraluminal or extraluminal obstruction (congenital atresia, fibrosis, thrombosis, neoplasia, AV fistulas)

Intrahepatic:

Increased resistance in the microscopic portal vein tributaries, sinusoids or small hepatic veins.
Presinusoidal, sinusoidal and post sinusoidal. e.g. Chronic hepatitis with fibrosis or cirrhosis

Posthepatic:

Secondary to obstruction of larger hepatic veins such as posthepatic caudal vena cava or the right atrium.

Ascetic fluid:

Low protein (< 2.5g/ dl) – prehepatic, presinusoidal and sinusoidal intrahepatic PH High protein (>2.5g/dl) – posthepatic, postsinusoidal and sinusoidal intrahepatic PH
PH leads to multiple acquired portosystemic shunt and hepatic encephalopathy

Hepatic encephalopathy

- Brain dysfunction secondary to liver disease
- Toxins from GI tract bypass hepatic metabolism
- Ammonia
- Others – aromatic amino acids, bile acids, glutamine, phenol, short chain fatty acid, tryptophan

Acute encephalopathy in fulminant hepatic failure and Chronic in PSS acquired or congenital
Signs – decreased mental alertness, head pressing, ataxia, circling, salivation, stupor, coma

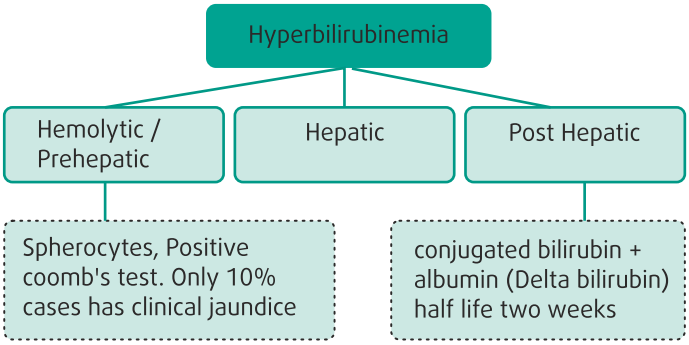
Liver enzymes:

Causes of liver enzymes elevation in the absence of primary Hepatobiliary diseases

- Medication – Glucocorticoid, Phenobarbital
- Inflammation/infection – sepsis, GI disorders, systemic infection, muscle injury
- Endocrine – Hyperadrenocorticism, Diabetes, Hyperthyroidism, Hypothyroidism
- Hypoxia – CHF, Status epilepticus, severe hypotension, shock
- Others – osteosarcoma, acute severe haemolysis, mammary tumor, young growing, lab error

Bilirubin:

- Measurement of direct/indirect bilirubin is of no value in distinguishing causes of hyperbilirubinemia
- Liver enzymes are of little value in distinguishing the causes of hyperbilirubinemia
- Imaging is the best to distinguish hepatic or post hepatic



Bile acids:

- Synthesized by liver exclusively from cholesterol and Cholecystokinin secreted in the duodenum is the major stimulus for gall bladder contraction.
- Bile acids solubilize dietary lipid and reabsorbed in the ileum and back to liver via portal vein
- More than 95% of bile acids are removed by this enterohepatic circulation
- Elevated serum bile acids in liver dysfunction (parenchymal disease or cholestasis) or portosystemic shunt.

Estimation of serum bile acid:

- Bile acid estimation 12 hours preprandial and then 2 hours postprandial
  Preprandial – 15umol/L Postprandial –25umol/L
- 99% specific and 95-100% sensitive for PSS in dogs, 100% cirrhosis

Ammonia:

- Produced by action of colonic bacteria on break down products of ingested protein
- Intestinal ammonia is absorbed and enters portal vein and converted to urea in urea cycle
- 70% reduction in urea cycle function for hyperammonemia
- 98% sensitive and 89% specific for PSS in dogs
- Measured in fasted patient from non-haemolysed sample in heparin tubes, spun in refrigerated centrifuge within 30 minutes after collection
- Normal levels are < 45mcmol/L

Coagulation proteins

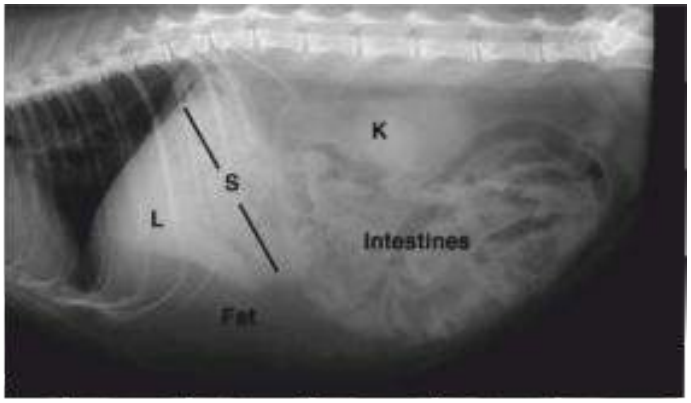
- All clotting factors except von Willebrand factor
- Cholestasis can cause malabsorption of fat –soluble vitamin K
- Vitamin K dependent clotting factors(II, VII, IX, X, Protein C, Protein S) are not activated

- Prolonged PT
- DIC- prolonged aPTT, PT, FDPs, D-dimers

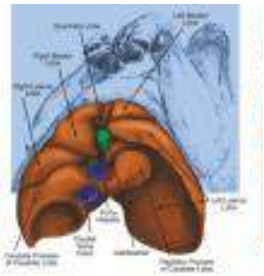
Hematologic findings:
- Microcytosis – impaired iron transport in vascular anomalies, target cells, poikilocytosis
- Anemia – GI ulceration bleeding, bleeding disorder

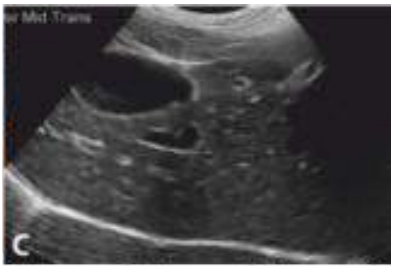
Urinalysis:
- PSS – ammonium biurate crystals
- Fanconi- like syndrome in copper storage hepatopathies secondary to accumulation of copper in the renal tubules

Radiography
- Liver size-right lateral-length-gastric axis based
- Rounded, caudoventral liver margins beyond costal arch and displacement of gastric axis
- Length – in cm from ventral border of caudal vena cava to apex of hepatic caudal border and comparing to T11
- Normal 5.5 ± 0.8 times the length of T11
- Microhepatica
- Loss of serosal details – ascites

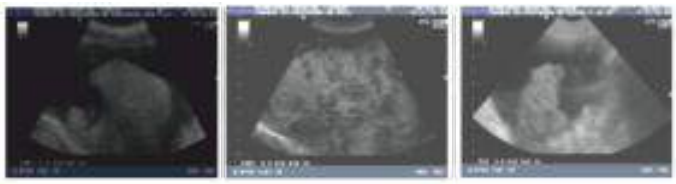


Ultrasonography
- Features evaluated –parenchymal echogenicity and uniformity, vascular structures, biliary structures
- Focal lesions, liver architecture and structure, diameter of extrahepatic and intrahepatic bile ducts and gall bladder
- Portal vein changes
- Free abdominal fluid
- Portal blood flow velocity and direction

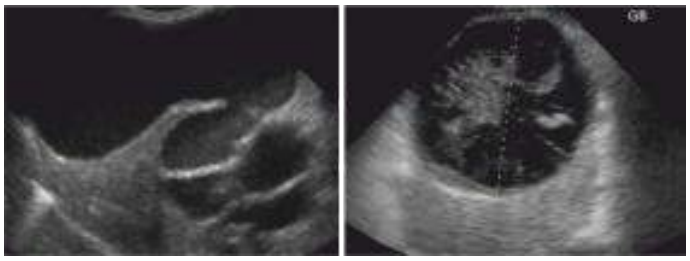




- Hyperechoic parenchyma –chronic hepatitis, lipidosis, steroid hepatopathy, vascular hepatopathies, toxic insult, lymphoma, histiocytic sarcoma, phenobarbital administration
- Hypoechoic parenchyma –acute hepatitis, amyloidosis, lymphoma, cholangitis/ cholangiohepatitis



Ultrasonography
- EHBDO – Neoplasia, pancreatitis, choleliths, sludge balls, gall bladder mucoceles
- Normal bile duct 3mm in diameter
- Normal sized gall bladder will not rule out EHBDO
- Color doppler to differentiate bile duct and vessels
- Gall bladder abnormalities- cholecystitis, choleliths, Emphysematous cholecystits- *E.coli*, *Clostridium perfringens*, diabetes mellitus
- Gall bladder mucocele - stellate or finely stranded pattern – kiwi fruit -like, also seen in gall bladder rupture
- PSS – Congenital PSS vs MAPSS
- PV: Ao ratio ≥ 0.8 and
- PV: CVC ratio ≥ 0.75 – consistently ruled out extrahepatic PSS
- MAPSS typically seen as a plexus of small tortuous splenic to renal vessels



FNAC
- USG guided 22 to 25G needle
- No need for sedation or analgesia
- Needle without syringe –sewing machine technique compared to aspiration with syringe less blood contamination
- Sprayed on to glass slides and thin smear made
- Low cellularity

Biopsy
- Core needle biopsy
- Surgical biopsy
- Laparoscopic biopsy
- General anaesthesia required
- 1 Core biopsy = 1/50,000 of liver
- 14G large dogs and 16G small dogs
- Tru –cut type needles: manual, automatic, semiautomatic
- USG guided
- Check before for bleeding disorders

ACVIM consensus statement on the diagnosis and treatment of chronic hepatitis in dogs

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This consensus statement on chronic hepatitis (CH) in dogs is based on the expert opinion of 7 specialists with extensive experience in diagnosing, treating, and conducting clinical research in hepatology in dogs.

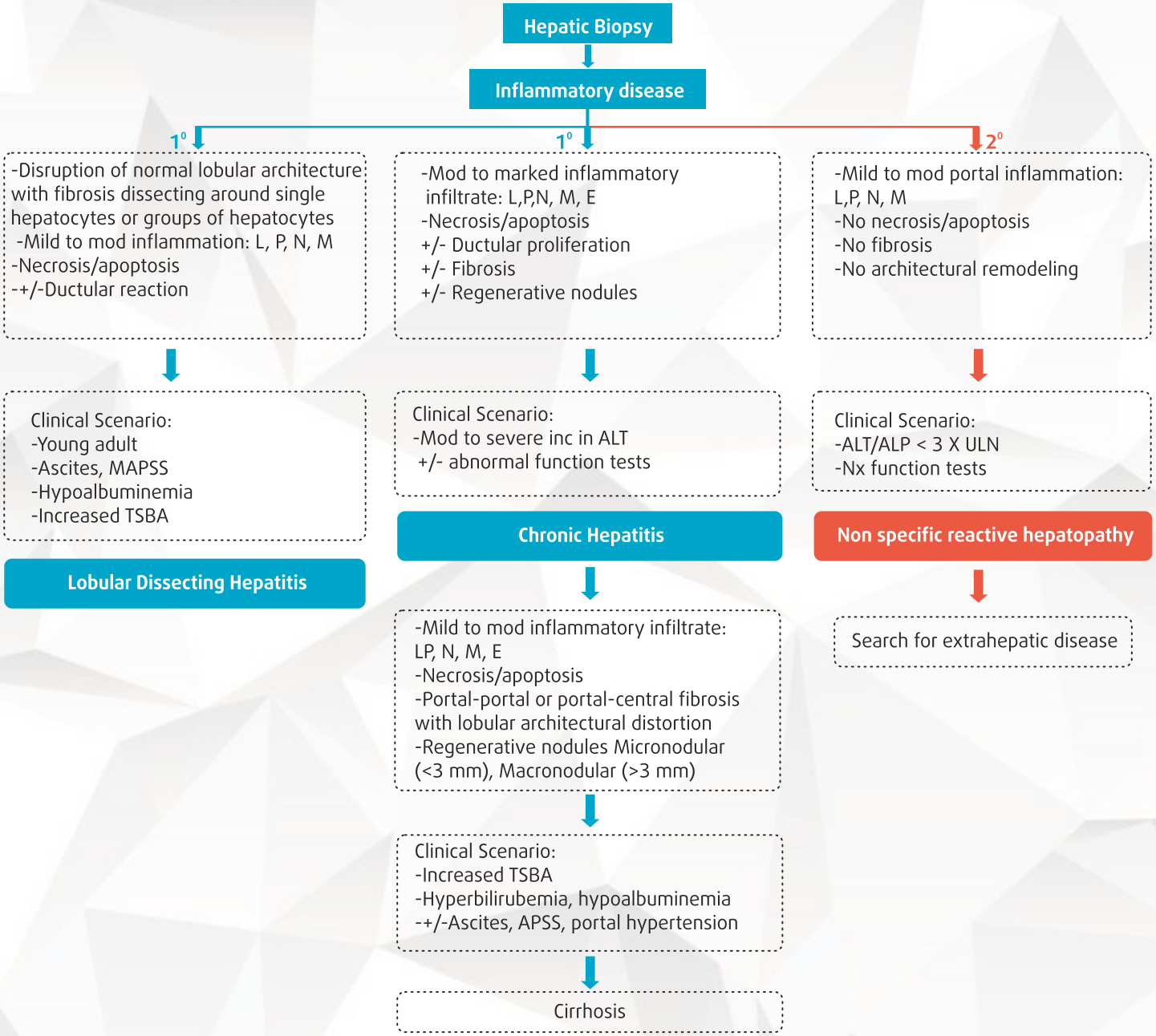


FIGURE 1 Primary and secondary chronic inflammatory hepatopathies in dogs. Inflammatory changes on a hepatic biopsy can be due to a primary or secondary hepatopathies. The primary hepatopathies represent a disease process centered on the liver and include chronic hepatitis which can progress to cirrhosis and lobular dissecting hepatitis. Primary hepatopathies are typically accompanied by evidence of hepatocyte necrosis/apoptosis as well as varying degrees of ductular proliferation and fibrosis. Secondary hepatopathies however occur due to a primary disease process elsewhere in the body, often involving the splanchnic circulation, that damage the liver. In this case inflammatory changes are limited to the portal areas and are not accompanied by fibrosis or hepatocyte necrosis/apoptosis. In this case the liver lesions do not represent the primary problem and one should search for the presence of an extrahepatic disorder. ALP, alkaline phosphatase; ALT, alanine aminotransferase; APSS, acquired portosystemic shunts; E, eosinophilic; L, lymphocytic; M, granulomatous; N, neutrophilic; P, plasmacytic; TSBS, total serum bile acids

Definition:
The presence of lymphocytic, plasmacytic, or granulomatous inflammation (portal, multifocal, zonal, or panlobular) or some combination of these along with hepatocyte cell death and variable severity of fibrosis and regeneration. Inflammation most commonly originates (or usually is more severe) in portal regions, often spilling over into the hepatic lobule (interface hepatitis). Cirrhosis reflects end-stage CH when substantial architectural distortion, fibrosis, and sinusoidal portal hypertension (PH) are present.

Etiology:

1. Infectious:
Leptospirosis causes acute hepatitis and can induce a chronic pyogranulomatous response.
Bacillus piliformis, Helicobacter canis, and Bartonella spp have been identified in dogs with CH. *Ehrlichia canis* has been associated with CH.
Nonsuppurative hepatitis has been reported with babesiosis.
Leishmaniasis is associated with granulomatous inflammation.

2. Drugs & Toxins:
1. Treatment with phenobarbital, primidone, phenytoin, and lomustine can result in CH
2. Hepatic copper (Cu) excess: When Cu exceeds the hepatocyte transport and Cu-binding capacity, free Cu causes oxidative stress leading to hepatocellular degeneration and cell death with acute or chronic hepatic inflammation or both.

3. Metabolic conditions:
1. Alpha-1 antitrypsin (AAT) deficiency: retention of abnormally folded proteins causing CH.

4. Immune-mediated CH:
1. The presence of lymphocytic infiltrates in the liver
2. Abnormal expression of major histocompatibility complex class II proteins
3. Positive serum autoantibodies, familial history of liver disease
4. Female predisposition
5. Favourable response to immunosuppression

Signalment and clinical signs:

1. Signalment:
Increased prevalence of CH in BT, Doberman Pinschers, Dalmatians, American and English Cocker Spaniels, English Springer Spaniels, and West Highland White Terriers in several countries.
The overall mean age when clinical signs are reported is 7.2 years. The age of presentation for CuCH and idiopathic CH do not appear to be different. Dogs with LDH present younger than do dogs with CH, with an average age of 2 years

2. Clinical signs:
The clinical signs in descending order of prevalence are:
- Decreased appetite
- Lethargy/depression
- Icterus
- Ascites
- PU/PD
- Vomiting
- Diarrhea
- Hepatic encephalopathy
- Melena
- Abdominal pain
- Gingival bleeding
- Hematochezia
- Hemoperitoneum

Clinical pathology

1. Serum enzymology:
Serum ALT activity is the best screening test for chronic hepatitis. Increased serum alkaline phosphatase (ALP) activity occurs later in CH. As CH progresses and hepatic parenchyma decreases, ALP and gamma-glutamyl transpeptidase (GGT) activities increase compared to ALT. In late stage cirrhosis, transaminases may decrease with parenchymal loss.

2. Function tests:
Hyperbilirubinemia in 50% (approx.) of dogs with CH.
Hypoalbuminemia, a late marker of hepatic synthetic failure
Decreased concentrations of blood urea nitrogen and cholesterol.
Hypoglycemia more often is associated with acute liver failure.
TSBA (total serum bile acid) concentrations are uniformly increased when portosystemic shunting is present.
Hyperammonemia has similar sensitivity to detecting CH or cirrhosis

3. Hematology and coagulation time

4. Urinalysis
Isosthenuria is seen in dogs with polyuria and polydipsia. A transient acquired euglycemic glucosuria may develop in dogs with CuCH and in other toxin-induced liver injuries when concurrent renal tubular injury occurs

Imaging

Hepatomegaly when the liver extends beyond the costal arch with rounded edges.
Hepatic ultrasonography is the preferred imaging modality for initial evaluation of dogs. It provides information regarding size, shape, echogenicity, echotexture of the parenchyma, the biliary tract & main vessels.
The normally hypoechoic liver, as compared to spleen, tends to hyperechoic in CH because of the presence of fibrosis or glycogen-type vacuolation.
Ultrasound helps to identify complications associated with CH such as acquired portosytemic shunts (APSS), ascites, splanchnic thrombi, and gastrointestinal ulceration.
Advanced imaging modalities such as CT angiography may be necessary to diagnose vascular anomalies like portal vein thrombi or APSS.

Biopsy acquisitions

1. Pre-biopsy considerations
The primary concern for any hepatic sampling technique is post-procedural hemorrhage.
Assessment of bleeding risk in CH is challenging because of the liver's complex and antagonistic role in the synthesis and degradation of pro- and anti-thrombotic proteins and its role in fibrinolysis.
Coagulation and hematological abnormalities are common in dogs with CH.

2. Sampling methods:
- Conventional" coagulation parameters are unreliable indicators of the risk of haemorrhage after liver biopsy.
- Prothrombin time (PT), activated partial thromboplastin time (aPTT), platelet count, fibrinogen concentration, and PCV should be obtained before hepatic sampling to assess for high-risk patients.
- Laparoscopy, a minimally invasive method enables gross evaluation of the liver, extrahepatic biliary system and adjacent structures, and safe acquisition of large targeted biopsies from multiple liver lobes.
- Laparotomy is considerably more invasive with greater

postoperative pain & recovery time.

- A minimum of 5 laparoscopic or surgical biopsies from at least 2 liver lobes should be obtained for histopathology (3), aerobic/anaerobic culture (1) and quantitative copper analysis (1).
- Ultrasound-guided hepatic biopsy is least invasive with increased accuracy with a larger gauge needle (14 or 16) and by obtaining biopsies from multiple sites.

3. Biopsy specimen interpretation

- Hepatic biopsy interpretation should include a scored (mild, moderate, or severe) evaluation of type and degree of inflammation/degeneration, fibrosis/nodularity (stage), as well as an evaluation of the copper staining pattern & semi-quantitative score of Cu-staining intensity.
- The presence of (pyo)granulomatous inflammation should prompt an infectious etiology.
- An exchange of information between clinician & pathologist optimizes biopsy interpretation.
- Icterus (sclera)-bilirubin >3.0mg/dl
- Icteric plasma 0.5 to 1.0 mg/dl
- Hepatocutaneous syndrome-superficial necrolytic dermatitis
- Ascites

Treatment

1. Infectious

Antimicrobials

2. Drugs & Toxins

Suspected hepatotoxic drug or supplement exposure should be promptly discontinued and hepatic recovery monitored by serial biochemical evaluations. Preferably, antioxidant treatment is indicated

3. Hepatoprotective agents and anti-oxidants

SAMe (S-adenosyl methionine)	⬆ Intracellular cysteine ⬆ Hepatic glutathione synthesis ⬆ Hepatoprotective polyamines Promotes membrane stability, Controls production of inflammatory cytokines	20 mg/kg PO once a day on an empty stomach Phytate salt: 8-10 mg/kg once a Day
Sylimarin	Antioxidant Anti-inflammatory Anti-fibrotic Choleretic	Native extract: 4-8 mg/kg/d for 2-3 times a day PC complexed 0.7-6 mg/kg/d PO once a day
Ursodeoxycholate	Antioxidant Choleretic Immunomodulatory Anti-inflammatory	15 mg/kg once a day PO given with food to increase bioavailability
Vitamin E	Protects against lipid peroxidation Anti-fibrotic Anti-inflammatory	10 IU/kg once a day PO not to exceed 400 IU given with food to increase bioavailability

4. Copper associated toxicity

Dietary copper restriction- Limit Cu uptake in intestine, Water: limit Cu intake in water to <0.1µg/g

D-Penicillamine- Chelates Cu, with urinary excretion. Upregulates hepatic metallothien binding intracellular Cu

Trientine-Copper chelator

Zinc- Interferes with enteric zinc absorption by inducing intestinal metallothionein that binds Cu.

5. Immune mediated hepatitis

Corticosteroids are efficacious as first-line treatment, but acknowledged the limiting impact of drug-related adverse effects (eg, catabolic effects, polyuria, polydipsia, hepatocyte glycogen vacuolation for degeneration, serum liver enzyme induction).

Corticosteroids, azathioprine, cyclosporine, and mycophenolate are used in single or in combination.

6. Dietary management

If signs of HE are suspected, feeding a prescription diet with protein restricted to 2.1-2.5 g protein per kg body weight can be a starting point. However, it is prudent to individually titrate these diets

Complications

1. Portal hypertension
2. Ascites
3. Hepatic encephalopathy
4. Coagulation
5. Gastroduodenal ulceration
6. Infection

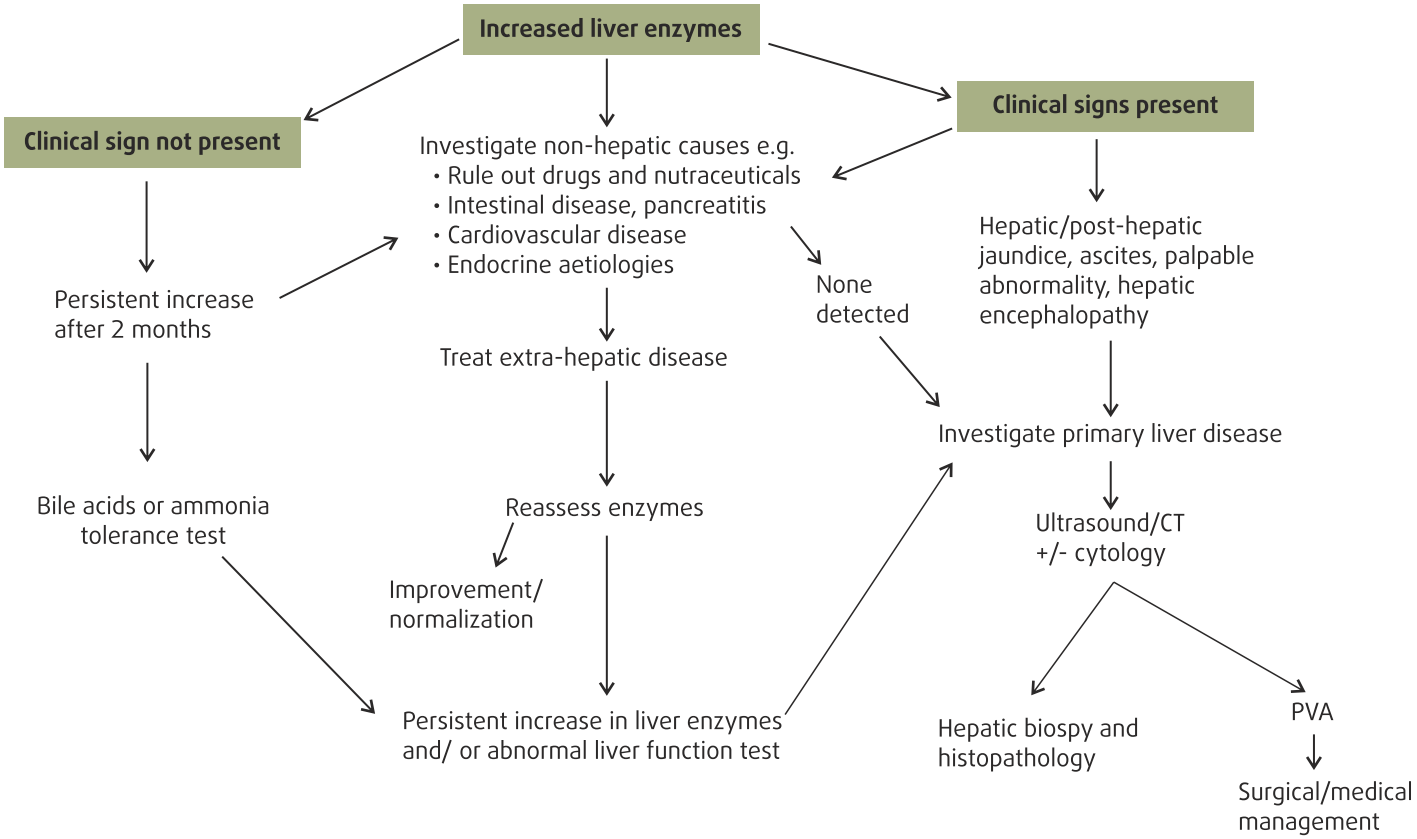
Future perspectives

- Validation of grading & staging systems for hepatic biopsy in dogs with chronic hepatitis
- Biomarkers for detection of hepatic inflammation, immunemediated hepatitis, and copper-associated chronic hepatitis (CuCH).
- Clinical trials to study the pharmacology, pharmacodynamics, and efficacy of immunosuppressive protocols in dogs with suspected immune hepatitis.
- Define the role of infectious agents as direct pathogens versus triggers for immune hepatitis.
- Address the accumulating body of evidence that high dietary copper levels are casually associated with copper induced liver damage.
- Genome-wide sequencing studies to clarify the impact of genotypes with phenotypic severity of CuCH in dogs with suspected breed predilection.
- Expand clinical assessment of coagulation status in dogs with CH to establish standard of care assessment tests that are most predictive of liver biopsy provoked hemorrhage.
- Determine a standard of care for interventional control of coagulopathies in dogs with CH.

WSV18-0224
TIPS FROM THE EXPERTS FOR THE MANAGEMENT OF HIGH LIVER ENZYMES

N.Prakash'

Approach



Approach to Increased Liver Enzymes