Diagnosis and Monitoring of Avian Hepatic Disease

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Clinical Signs

The clinical presentation of birds with liver disease is 'typically' non-specific and variable. Presenting signs may include any of the following:

- anorexia
- lethargy
- weight loss
- weakness
- diarrhoea
- poor feathering
- self trauma
- PU/PD
- ascites
- coagulopathy
- melaena
- overgrown beak
- "Sick Bird Look"
- personality or behavioural change
- anaemia
- obesity
- regurgitation
- respiratory compromise
- seizures
- paresis
- "sudden death"
- biliverdinuria
- abdominal enlargement
- pruritus

Serum/Plasma Biochemistry

Biochemical evaluation of a blood sample can increase the suspicion of liver disease. The distribution of enzymes in different organs varies between avian species, causing differences in test specificity, but overall, plasma enzymes are considered non-specific indicators of liver disease. Elevated plasma levels indicate recent damage to liver cells, but give no indication of liver function. Fluctuation within the course of disease are common, including all values being normal in the presence of chronic hepatic disease. Repeated sampling may improve the diagnostic value of biochemical testing. Some of the common biochemical measurements and their diagnostic value are listed on the next page.

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AST	Wide distribution (liver, heart, skeletal muscle, kidney, brain etc). Not liver specific, but the most sensitive enzyme for detecting liver disease. Elevations also recorded with septicaemia, infections stress, trauma, toxins, neoplasia and IM injections
ALT	Mainly liver and kidney. Long half life. Elevation may only occur with severe liver damage. Also elevated with intoxications and muscle trauma.
LDH	Wide distribution and non-specific. Short half life. May rise and fall in the early stages of liver disease, when AST is still elevating. Has often returned to the normal range by the time biochemical testing is performed.
GLDH	Liver specific in many species, but only elevated with hepatocyte necrosis as it is a mitochondrial enzyme. Useful in detecting chronic active hepatitis.
GGT	Liver specific enzyme, but less sensitive than AST. May be elevated in liver disease due to synthesis during cholestatic disease.
AP	Wide distribution, and is rarely elevated with liver disease.
CK	Specific and sensitive indicator of muscle damage. Short half life (may decrease before AST after muscle trauma resulting in a misleading indication of liver disease). Assists in differentiating hepatic/muscle source of plasma enzymes, but cannot rule out concurrent hepatic and muscle damage.
Bile Pigment	Biliverdinaemia can occur with severe hepatic disease (concurrent renal disease should be suspected with reduced renal clearance). Bilirubin may also be detectable with liver disease in some species, but will be rapidly cleared by the kidneys.
Protein	Both albumin and globulins may be decreased with decreased liver function

Protein Electrophoresis

Alterations in the protein electrophoresis profile are not diagnostic, but can be useful in narrowing a differential list. Paired samples can aid in prognosis and serial samples can be useful for monitoring response to treatment. Typical changes for a range of conditions have been defined, and include:

Acute hepatitis	Decreased alpha fraction, increased beta and gamma fractions with beta-gamma bridging
Acute chlamydiosis	Decreased albumin, alpha increased, beta increased, gamma increased, A/G decreased (nb this pattern also consistent with acute nephritis)
Chronic active hepatitis	Albumin decreased, alpha increased, beta increased, gamma increased, A/G ratio decreased (nb this pattern also consistent with acute nephritis)

Bile Acids

Measurement of serum bile acids (SBA) is a specific and sensitive test for liver function. It provides information on the combined effectiveness of extraction, conjugation and excretion of bile acids. In mammals SBA have been found to correlate well with, and provide additional information to plasma enzyme testing. In birds, the correlation with enzyme studies has not been well defined, although bile acids are considered to provide useful additional data, for prognosis and monitoring, or to differentiate between muscle damage and liver disease in cases with AST elevation. Results can be affected by haemolysis or lipaemia, depending on the test system used. A single post-prandial sample is usually recommended ideally collected 2 hours after a meal. Species differences in bile acid composition can be determined by HPLC, but the significance of changes in bile composition have not been defined in birds.

Radiography

Radiography is indicated when clinical signs and blood analysis are suggestive of hepatic disease, and are recommended before proceeding to more invasive diagnostic methods (eg biopsy). General anaesthesia is usually required. Positioning is critical, or false alterations of the hepatic shadow can be produced. Species variation in hepatic shadow should be considered. Change in hepatic shadow can be evaluated by change in the normal "slope" of the proventriculus on a lateral view (steeper=atrophy, shallower=hepatomegaly), or displacement of other organs. A contrast series may be required to evaluate space occupying lesions (may require multiple anaesthetic episodes with increased risk of regurgitation). It is important to differentiate between hepatomegaly and hepato-cardiomegaly as the second may imply primary cardiac disease and secondary hepatic congestion. Ascites or peritonitis may obscure hepatic enlargement. Peritoneal drainage may improve the diagnostic quality of the radiographs.

Ultrasonography

Unlike radiography, ultrasonography allows examination of the liver parenchyma. Ultrasonography may be able to detect parenchymal masses earlier than they can be detected by radiography or endoscopy. A small acoustic window due to the positioning of the air sacs may limit the field in small to medium sized birds. Therefore it may not be possible to determine the overall hepatic shape or size. Ultrasonography can be used to perform minimally invasive guided biopsy or fine needle aspirates. An ultrasound examination may be able to rule out other GIT diseases.

Cytology/Biopsy

Indication for hepatic cytology or biopsy requires a high index of suspicion of a hepatic disease, as it is a procedure not without risk. Death rate in birds greater than 100g has been estimated at 1 in 300 biopsies. "Major complications" are reported to occur in 1.7% of cats. In mammals, the most common complication of liver biopsy is haemorrhage. This has not been widely reported in birds, but birds with cardiac disturbances may be at higher risk. The lack of reliable methods for determining coagulation ability in birds limits the preoperative work up available. The use of prophylactic pre-operative vitamin K1 injection has been recommended if there is a prolonged clotting time after blood collection. Hepatic samples can be collected from blind or ultrasound guided fine needle aspirate or needle biopsy, endoscopic or surgical biopsy. Samples are most commonly collected during endoscopy via a sub-sternal approach. It is preferable to use an endoscope with a biopsy channel rather than "walk" the biopsy equipment down the side of the endoscope, which is potentially traumatic. "Key hole surgery" can be used, but provides limited access to the liver, limiting the choice of biopsy site. An endoscopic approach allows good visualisation of the ventral surface of the liver, allowing more selective biopsy site selection, particularly important for focal lesions. The dorsal surface of the liver may not be fully visualised. Intra-abdominal fat deposits or ascites may limit visualisation. Biopsy findings may lead to a specific diagnosis, aiding the development of treatment plans and providing prognostic information.

Liver Function Testing

Liver function testing by clearance tests have been considered an important part of liver disease monitoring in humans for many years. Liver function tests have varied sensitivity and specificity depending on the marker and methodology used. They can be used to confirm liver dysfunction in birds with abnormal plasma enzymes or radiological signs, but will not provide a definitive diagnosis. They can also be used to monitor response to treatment in birds with confirmed hepatic disease.

A clearance test involves the intravenous injection of a marker, then single or multiple blood sample collection to calculate the rate of clearance of the marker from the blood stream. A wide range of markers have been described for liver function testing. The ideal liver function marker has the following characteristics:

- the marker is localised to the vascular system
- it is not metabolised or synthesised within the body
- will not cause variation in blood volume during the study
- is stable
- can be administered in a small volume

- concentration can be easily measured
- equilibrium is rapid
- clearance occurs only in the liver

A wide range of markers have been used in humans, of which several (including lignocaine and caffeine) are considered unsuitable for use in birds due to their potential toxicity. Several markers have been reported for use in determining blood volume in birds, however only one (indocyanine green - ICG) has been evaluated as a liver function test in birds.

Clearance Tests (Organic Dyes)

The most commonly used, and most accurate and repeatable marker in this group is ICG. It has been used to measure graft function post transplantation. In humans, ICG clearance has been shown to provide good prognostic indication, usually additional or superior to the Childs-Pugh score (the gold standard clinical measure), and is considered to be the best discrimination as to the degree of liver disease. ICG fulfills most of the characteristics listed above. It is administered intravenously at a dose rate of 0.5mg/kg. Either a single sample can be collected at 9 minutes (9 minute retention percentage) or three samples can be collected at 3,6 and 9 minutes to allow a clearance study. Clearance studies are considered more accurate, but require more samples and a calibration curve to be constructed. In either case, results need to compared to normal values determined for that species.

Cytosolic Function Tests

Galactose has been shown to provide good diagnostic information, either as a single measure or when measured serially in a variety of hepatic diseases. The main advantage of this test is its safety. There are few environmental influences which will alter the results, with the exception of hypoxaemia. Both retention and clearance tests have been developed in humans, allowing either single or multiple sampling to be used depending on the information required. Galactose undergoes extrahepatic metabolism, in both RBCs and the kidneys. In mammalian species, RBC metabolism is minor in comparison to hepatic metabolism, while renal metabolism is variable between species. The metabolism of galactose by avian RBCs and kidneys are to be determined.

Microsomal Function Tests

A number of markers has been evaluated, mostly to evaluate the P450 iso-enzyme family. These include antipyrine, aminopyrine, caffeine, erythromycin and lidocaine. Some of these markers are considered potentially toxic in birds (caffeine, lidocaine), others require the use of radio-labelled markers (some lidocaine and some caffeine methods). Antipyrine clearance is affected by a number of endogenous and exogenous chemicals, and aminopyrine clearance is not liver specific. Thus this group of agents has not been considered for study in birds at this time.

Flow Chart for Diagnosis and Monitoring of Hepatic Disease