HISTORICAL BACKGROUND

The first papers concerning the use of ultrasounds to examine the abdominal organs of the dog and cat were published in 1981 (Cartee, 1981, Nyland *et al.*, 1981). Since then ultrasonography has become an essential imaging tool for identifying abnormalities of the liver parenchyma, biliary tract and vascular system.

Initially papers described the positioning of the transducer, the topographical anatomy of the canine liver, the normal ultrasonographic appearance of the liver and cases of hepatic neoplasia, cirrhosis and cholelitiasis. The first Italian work was presented at S.I.S.Vet in 1982 (Bellucci *et al.*, 1982).

In 1985 ultrasound-guided liver biopsy was described (Hager *et al.*, 1985), but most of the publications concerned methodology and the ultrasonographic appearance of liver diseases such as chronic hepatitis and cirrhosis.

In 1987 the ultrasonographic diagnosis of portosystemic shunts was reported by Wrigley *et al.* (1987). In 1989 Kantrowitz *et al.* described the measurement of portal blood flow velocity by means of pulsed Doppler (Kantrowitz *et al.*, 1989).

Since 1990 the evolution of ultrasound machines and the wide availability of duplex Doppler allowed better description of the vascular anatomy of the liver (Carlisle *et al.*, 1991). In those years the ultrasonographic diagnosis of portosystemic shunts replaced portography (Lamb, 1996).

At the same time, the diffusion of ultrasound-guided liver biopsy increased the knowledge of hepatic pathology (Biller *et al.,* 1992).

The first report concerning the use of an ultrasound contrast medium was published in 2000 (Bahr *et al.*, 2000), but most of the papers on contrast media have appeared since 2003, when specific software was developed to improve their use.

Today the liver ultrasonography of the dog and cat is the synthesis of these 25 years of history. The technological improvement of ultrasound machines has certainly conditioned either the diagnostic accuracy of this diagnostic technique, or our skill in small animal clinic: now we can consider diseases such as cholecystitis or cholangitis in the differential

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diagnosis of jaundice, or we can make an early (sometimes serendipitous) diagnosis of a hepatic mass by examining the liver of a dog with biochemical abnormalities and no specific symptomatology.

LIMITS OF LIVER ULTRASONOGRAPHY

Even if everybody agrees with the benefit of performing hepatic echography every time the liver is suspected to be involved in a disease, ultrasonography alone is often more useful to exclude a diagnosis, rather than to specify the exact pathology.

The ultrasonographic abnormalities of the liver are well known and classified: diffuse liver diseases (decreased, increased and mixed echogenicity), focal parenchymal abnormalities, diseases of the gallbladder and biliary tract, hepatic and portal vein abnormalities. In spite of this simple classification, a single ultrasonographic pattern is shared by different pathologies or the same pathology may appear with different ultrasonographic patterns.

The clinical, laboratory and ultrasonographic data are usually sufficient to make the diagnosis when considering diseases such as steroid hepatopathy, cirrhosis, obstructive jaundice or portosystemic shunts. However, investigation of a mild to severe increase in liver enzymes, or the causes of a peritoneal transudation or to stage a malignancy, often reveal the inadequacy of using ultrasonography alone.

To improve the diagnostic accuracy of liver ultrasonography, a standardized protocol of investigation should always be followed. This includes choosing the highest frequency probe consistent with the animal's size, to carefully watch all the liver parenchyma, to compare it with spleen and renal cortex and to then perform a complete abdominal examination in order to assess the characteristics of the adrenal glands, the lymph-nodes, the pancreatic areas and all the other abdominal organs, especially when liver focal lesions are detected.

Abdominal ultrasonography is currently a strongly operator experience-conditioned diagnostic technique and yet there are no shared guidelines to perform similar examinations, to get images of comparable definition and to draw up a report by two different practitioners.

MODERN CONCEPTS IN LIVER ULTRASONOGRAPHY

This chapter introduces the goals of the research in small animal ultrasonography in order to define diagnostic algorithms and guidelines useful to improve the diagnostic accuracy of liver ultrasonography.

Considering diffuse liver abnormalities, hypoechoic parenchyma has been reported in dogs and cats with diffuse infiltrative processes such as lymphoma, leukaemia, amyloido- sis, acute hepatitis or passive congestion of the liver. Uniformly increased liver echogenicity usually indicates steroid hepatopathy, chronic hepatitis, cirrhosis and, less commoly, lymphoma.

Sometimes diffuse inflammatory, toxic or neoplastic conditions may produce a coarse echo texture with uneven echogenicity throughout a portion of the parenchyma or the entire liver and it may be difficult to determine whether the more echogenic or less echogenic areas, or both, are abnormal.

A liver biopsy is often required to differentiate diffuse abnormalities of the liver because its ultrasonographic appearance may be identical in different conditions. The decision to perform an aspiration or a tissue-core biopsy is determined by the lesion to be sampled and the suspected abnormality. Ultrasound-guided fine needle aspiration of the liver is a safe and harmless procedure which may usually be performed on conscious animals. It may be useful to confirm the diagnosis when round cell tumors (lymphoma, mast-cell tumor, plasmocytoma, etc.), fatty infiltration of the liver or steroid hepatopathy is suspected. Sometimes liver cytology shows neutrohilic hepatitis, but this condition may be misdiagnosed when also a neutrophilic leukocytosis is also present and the sample is blood diluted.

In order to provide samples with less blood dilution, we perform liver aspiration cytology using a 22–23 G needle and a 2 ml syringe.

Whenever evaluation of the liver parenchyma architecture is needed (for chronic hepatitis, cirrhosis, amyloidosis, fibrosis, etc.), a tissue core biopsy should be performed. Today, safe anesthesiologic protocols are also available for animals with mild to moderate hepatic failure but liver biopsy should be avoided when a severe hemostatic disorder or obstructive jaundice are detected.

Doppler techniques assessing flow within the main portal vein are becoming more useful to obtain quantitative functional information in diffuse liver diseases for which conventional ultrasonography has been less valuable (Lee, 1999; Nyland and Mattoon, 2002).

Today the measurement of Portal Blood Flow Velocity (PBFV) is one of the most important parameters for the Doppler evaluation of the liver. The portal vein is visualized by either a ventral or right intercostal approach, whereby it can be seen entering the liver at the porta hepatis. Doppler measurements of the PBFV are obtained with the portal vein oriented in a longitudinal axis view and the probe directed cranially to obtain an incident angle of less than 60 degrees.

Mean PBFV in normal, unsedated dogs has been reported between 10 and 25 cm/s. The Portal Blood Flow (PBF) is calculated by the formula:

PBF = portal vein cross sectional area(cm2)
× PBFV(cm/s)/body weight (kg) = ml/min/kg

The normal range of PBF is considered between 20 and 40 ml/min/kg.

The portal vein Congestion Index (CI) is a measure of vascular resistance which may increase with pre-hepatic, hepatic or post-hepatic portal hypertension (normal range 0.04 ± 0.015 cm × s).

CI = portal vein cross sectional area(cm2)/PBFV (cm/s) = cm × s

Reduced PBFV and PBF are associated with increased resistance to PBF through the liver and the development of multiple extrahepatic portosystemic shunts.

The portal vein CI may increase with hepatic cirrhosis and other liver diseases.

The prognostic value of these parameters has still to be confirmed, but in future they may be used for evaluating the progression of disease and the effectiveness of treatment.

In ultrasonography, the interpretation of focal parenchymal lesions is always a challenging problem (Cuccovillo and Lamb, 2002; Stockhaus *et al.*, 2004). Even if single or multiple focal lesions may or not represent primary or metastatic liver neoplasia, this diagnosis should always be considered.

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The appearance of hepatic neoplasia is variable and different histologic types of tumors may appear similar ultrasonographically or the same tumor may have a variety of appear- ances, even within the same animal. So the diagnosis of focal or multifocal liver lesions must be always confirmed by fine needle or tissue-core biopsy of the liver.

Discovery of a single liver lesion in an otherwise healthy animal raises the possibility that the lesion is benign, especially if the liver size is normal. However, primary liver tumors or metastatic neoplasia sometimes present with solitary lesions.

Furthermore, the operator is often required to specify the extent of the lesion in cases where a medical or surgical treatment is being considered.

Ultrasound-guided liver biopsy is still considered the gold standard test for the diagnosis of hepatic masses, but sometimes it may be difficult to perform, especially when the lesion is deep in the liver or a hemangiosarcoma is suspected

Moreover, malignancies are often characterized by wide areas of necrosis and these can affect the histologic interpretation.

Fine needle aspiration of liver masses may be useful in cases of epithelial or round cell tumors, even if it may be difficult to distinguish between hepatoma and hepatocarcinoma. Cytologic diagnosis of sarcomas is usually hard.

To improve the diagnostic sensitivity of cytology it may be useful to take multiple samples of the lesion, especially when it appears with mixed echogenicity: mixed echogenicity may in fact be caused by the presence of different pathologic processes, such as inflammation, necrosis, neovascularity, etc..

When the clinical staging of a round cell tumor is required, it is always advisable to perform fine needle aspiration of the liver parenchyma, even if no ultrasonographic le- sions are detected, as neoplastic infltration of the liver (and the spleen) may be present anyway.

Doppler ultrasound evaluation of focal liver lesions has been used in an effort to differen- tiate benign from malignant diseases and to characterize various tumor types (Nyland and Mattoon, 2002).

High-frequency shifts compatible with neovascularity and arterio-venous shunts may be seen with hepatocellular carcinoma and other highly vascular tumors. Although these

signals are not seen with all malignant tumors, they are never detected with benign diseases. Conventional color Doppler and, above all, power Doppler imaging are sensitive in the visualization of disorganised and irregular vessels of malignant masses.

These Doppler ultrasound techniques need further studies to classify the vascular patterns of the most common liver neoplasia. Nevertheless, they may be useful in better identification of the target of ultrasound guided liver biopsy, avoiding less vascular areas where necrosis is most likely present.

Contrast enhanced ultrasonography is a new imaging modality in veterinary medicine (Nyland and Mattoon, 2002). Intravenous ultrasound contrast media are formulated as stable microbubble suspensions that serve as microreflectors upon intravenous injection. Contrast microbubbles distribute within the vascular space. When ultrasound waves emitted at a given fundamental frequency interact with the contrast media, the microbubbles resonate, generating returning ultrasound at both fundamental and harmonic frequencies.

After injection of the contrast agent, it is possible to follow the distribution of the agent within the liver. The mean greyscale intensity increases as the medium enters the hepatic

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arteries. To reach the portal veins the microbubbles have to traverse three microvascular beds: the capillaries of the lung, the intestine and the liver sinusoids, resulting in enhancement of the portal veins after 30–60 s. (Nyman *et al.,* 2005).

These contrast agents appear to be safe and well tolerated for use in dogs and provide data on tissue perfusion, as well as improving detection and characterization of smaller tumors, and those that are isoechoic with liver parenchyma.

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