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Review

Contrast-enhanced ultrasonography (CEUS) in canine liver examination

M. Wdowiak, A. Rychlik, R. Nieradka, M. Nowicki

Department of Clinical Diagnostics,
University of Warmia and Mazury M. Oczapowskiego 14, 10-719 Olsztyn, Poland

Abstract

Ultrasonography is a noninvasive diagnostic tool used to image size, shape, parenchyma and vascularization of various body organs. Unfortunately, the ultrasonographic image is characterized by a low contrast due to similar acoustic properties of the soft tissue. The Doppler mode provides information about blood flow, but is incapable of imaging small vessels and capillaries because of their low blood flow velocity (1 mm/s). However, a possibility to increase the effectiveness of ultrasonographic diagnostics exists, thanks to intravenous ultrasound contrast agents (UCAs) consisted of gas microbubbles.

The purpose of this review paper is to characterize specific imaging techniques necessary to conduct a contrast-enhanced liver examination and indications for CEUS as an alternative diagnostic method.

Key words: ultrasonography, liver, canine, neoplasm, differential diagnostics

Specific contrast-enhanced imaging techniques

The basic B-mode (Haers and Saunders 2009) is not effective enough for ultrasound contrast agent detection in tissue, so it can not be used in contrast-enhanced ultrasonography (CEUS). All Doppler modalities are too sensitive to the microbubbles and this hypersensitivity causes artifacts such as "color blooming" (color Doppler) and "flash" (power Doppler), which decreases the usefulness of these modes. "Color blooming" artifact (Nilsson 2001) presents itself as extravascular color induced by reverberation and/or a high gain setting. The "flash" artifact appears when tissue or transducer movement disturbs the desired flow signal.

Specific contrast-enhanced imaging techniques (Haers and Saunders 2009) are deprived of these defects and bring satisfying clinical results and artifact reduction. Many techniques have been created e.g. second harmonic imaging, pulse/phase inversion harmonic imaging, cadence-contrast pulse sequencing and power (amplitude) modulation.

These specific imaging techniques take advantage of the nonlinear properties of UCAs which manifests in asymmetrical oscillation of the gas microbubbles under ultrasonic wave influence. The nonlinear properties of UCAs (Quaia 2007) increase contrast detection in tissues, thus increasing the contrast to tissue ratio and decreasing artifacts and noise.

The principles of the second harmonic imaging are to create an image based on a frequency twice the value of the wave emitted by the transducer. The fundamental wave is filtered. If the fundamental wave is 2 MHz, then the second harmonic is 4 MHz, third harmonic 6 MHz and so on. Conventional USG sys-

Correspondence to: M. Wdowiak, e-mail: ktmtombstone@interia.pl

Table 1. Ultrasound contrast agents characteristics.

Name	Optison	Definity	Levovist	Sonovue	Sonazoid
Manufacturer	GE Healthcare	Bristol-Myers Squibb, North Billerica, Mass.	Schering	Bracco Pharmaceutical Geneva	GE Healthcare
Shell	Human serum albumin	Lipid Microsphere	Galactose (999 mg) + palmitic acid (1 mg)	Phospholipid	Phospholipid
Gas	Perflutren C3F8	Perflutren C3F8	Air	Sulfur hexafluoride SF6	Perfluorobutane C4F10

tems emit and receive sound waves with identical frequencies. On the contrary to conventional methods (Szatmári et al. 2003), second harmonic imaging receives echoes of twice the frequency than the fundamental pulse. The limitations of this technique are low spatial axial resolution and a possibility of contrast resolution reduction if the transmit pulse bandwidth and the receive bandwidth overlap (harmonic signal contamination). Harmonic imaging (O'Brien and Holmes 2007, Quaia 2007) improves the "good artifacts" (shadow and through transmission – diagnosis of mineralization and fluid) and reduces the "bad" artifacts (ring-down).

Pulse (phase) inversion (Szatmári et al. 2003) imaging also is based on harmonic frequencies, but every other emitted pulse is inverted. The two subsequent echoes are added and each scanning line consists of two pulses. The added tissue echoes are nulled, but the echoes from the "nonlinear" microbubbles' are not. This results in a strong harmonic signal. The advantages of this technique (Stewart and Sidhu 2006, Quaia 2007), are good spatial resolution and no transmission spectrum restrictions. The disadvantage is a reduction in frame rate due to the need to interrogate each scan line twice.

Cadence-contrast pulse sequencing (CPS) is a technique developed by Siemens, which principles are to emit a sequence of sound waves effects in microbubble oscillation, and the echoes originating from the microbubbles are filtered from tissue echoes by computer software. This technique (Quaia 2007) uses amplitude and phase modulation, and enables the visualization of all nonlinear contrast response, even the strong, nonlinear fundamental frequency. This technique (O'Brien and Holmes 2007) allows imaging with higher frequencies resulting in increased spatial resolution with the disadvantage of decreased temporal resolution. CPS, pairing the linear fundamental signal beside the nonlinear fundamental image, also allows anatomical orientation.

Power (amplitude) modulation (Quaia 2007) – in every scanning line two pulses are emitted, one with half and the other with a full frequency. Then, the received echoes are subtracted in the scanner to separate microbubble and tissue signals.

Ultrasound contrast agents (UCAs)

The first efforts (Calliada 1998) to enhance ultrasonic signals were based on i.v. admission of agitated 0.9% NaCl and receive sound wave reflections from the air microbubbles diluted in the patients blood. Feinstein continued research in this field and discovered that blood albumins improve microbubble stability. His investigations resulted in the development of the first pharmaceutical contrast agent, Albunex™. The first echo-enhancing substances were applied in cardiology and were incapable of penetrating the pulmonary bed. Ultrasound contrast media (Nyman et al. 2005) are classified into two generations according to the gas present inside the microbubbles: I gen agents (Albumex, Levovist) contain air, while II gen agents contain perfluorocarbon or sulfur hexafluoride (SonoVue, Optison, Definity).

New generation UCAs consist of small, gas-filled, mainly by prefluorane derivatives of saturated hydrocarbons (e.g. perfluoropropane, perfluorohexane, octafluoropropane) microbubbles stabilized by an external shell (denaturated human albumin, surfactant or phospholipids). The gas enhances the sound wave reflection and the gas type determines the quality of enhancement and microbubble durability. Gases with high molecular mass are poorly dissolved in serum thus are more durable and extend the enhancement effect. The shell determines elasticity which allows the ability of oscillation in a ultrasonic field and a more effective and longer enhancement effect.

The diameter (Szatmári et al. 2003) of the microbubbles (1-7 μm) is smaller than the red blood cells diameter; this excludes the possibility of capillary embolization. In comparison to contrast agents used in computer tomography or magnetic resonance imaging, UCAs remain in the intravascular space and do not diffuse outside blood vessels. Not all UCAs (Nyman et al. 2005) allow the imaging of the third, delayed phase (parenchymal, Kuppfer). This probably depends (Stewart and Sidhu 2006, Kanemoto et al. 2008) on UCAs elimination by phagocytes, e.g. Sonazoid (99% is phagocytosed) and Levovist (47% is phagocytosed) image the parenchymal phase, but Sonovue does not.

Table 2. Liver parameters in 3 phases by various researchers.

Author	Hepatic arterial phase (HA)		Portal vein phase (PV)		Hepatic Parenchymal phase (HP)		UCAs
	Phase start (time post injection)	Duration	Phase start (time post injection)	Duration	Phase start (time post injection)	Duration	
1	7-10 s	10-15 s	30-45 s	2 min		4-20 min	
2	10-15 s		30-60 s	150-200 s			Sonovue®
3	15-25 s		80-90 s (45-60s early PV phase)	180-240 s			Sonovue®
4	10 s		30s (peak)		45 s (peak)	15 min	Sonazoid®
5		do 20 s	20 s	40 s	8 min	7 min	Sonazoid®
6	5.47±1.52 s 13.5±3.37 s (peak)		16.03±3.39 s 27.5±4.09 s (peak)				Levovist®

1. Haers H., Saunders J.H. (2009)
2. Nyman H.T., Kristensen A.T., Kjelgaard-Hansen M., McEvoy F.J. (2005)
3. Nyman H.T., Kristensen A.T., Flagstad A., McEvoy F.J. (2004)
4. Kanemoto H., Ohno K., Nakashima K., Takahashi M., Fujino Y., Tsujimoto H. (2008)
5. Kanemoto K. Ohno K., Nakashima K., Takahashi M., Fujino Y., Nishimura R., Tsujimoto H. (2009)
6. Kutara K., Asano K., Kiot A., Teshima K., Kato Y., Sasaki Y., Edamura K., Shibuya H., Sato T., Hasegawa A., Tanaka S. (2006)

CEUS clinical applications

Contrast-enhanced ultrasonography (Haers and Saunders 2009) is mainly used to evaluate the liver and spleen. Other organs in case of which CEUS is useful are the kidneys, pancreas, lymph nodes and portal systemic shunts (PSS) diagnostics. CEUS liver evaluation consists of lesion detection (mainly focal, but also diffuse), lesion characteristics and monitoring after tumor resection.

Liver examination – the examination of focal lesions is the main CEUS indication in the canine and feline patient. The evaluation of focal lesions (Haers and Saunders 2009) by conventional ultrasonography is based on lesion morphology (echogenicity) and blood flow parameters (Doppler mode – hyper/hypovascularization). This enables the diagnose of liver cysts (anechogenic) or calcifications (acoustic shadow), but not always liver soft tissue lesions.

Hepatic nodules are common in dogs, focal nodular hyperplasia (FNH) occurs in 70% of the canine population over the age of six, and in all dogs over 14 years of age. Other causes of hepatic nodules (O'Brien et al. 2007, Haers and Saunders 2009) are haematomas, abscesses, focal hepatic necrosis, primary neoplasm (hepatocellular carcinoma, cholangiocellular carcinoma, sarcoma, carcinoid) and metastases (hemangiosarcoma, pancreatic cancer, neuroendocrine tumors). The assessment of liver lesions in CEUS is based on two necessary elements: lesion detection and lesion characteristics. After a lesion is detected (O'Brien et al. 2007, Haers and Saunders 2009) a description (lesion characteristics) is useful in differentiating between neoplasm/non neoplasm lesions, benign/malignant neoplasm, and even between certain types of malignant tumors.

The CEUS pattern of healthy dogs has been described. As a result of the double vascularization of the hepatic tissue by the hepatic artery (20% to 30% of blood) and the portal vein (70% to 80% of blood), the following phases have been described (Nyman et al. 2004, Kutara et al. 2006, Kanemoto et al. 2008, Kanemoto et al. 2009, Haers and Saunders 2009):

1. Hepatic arterial phase (HA) – enhancement of the hepatic artery and its tributaries – begins at 7 to 10 seconds post UCAs injection and has a duration time of 10 to 15 seconds.
2. Portal vein phase (PV) – begins at 30 to 45 seconds post UCAs injection, duration time up to 2 minutes post injection. In cirrhotic patients (Szatmári et al. 2003) a delay is possible.
3. Hepatic parenchymal phase (HP) – also known as the Kuppfer or delayed phase – enhancement of the hepatic parenchymal sinusoids – lasts until the UCAs are eliminated from the parenchyma, about 4 to 20 minutes, depending on contrast agent type.

Lesion detection – CEUS (Haers and Saunders 2009) is useful in detecting small and unclear malignant or isoechoegenic lesions, which are often invisible on a conventional USG image.

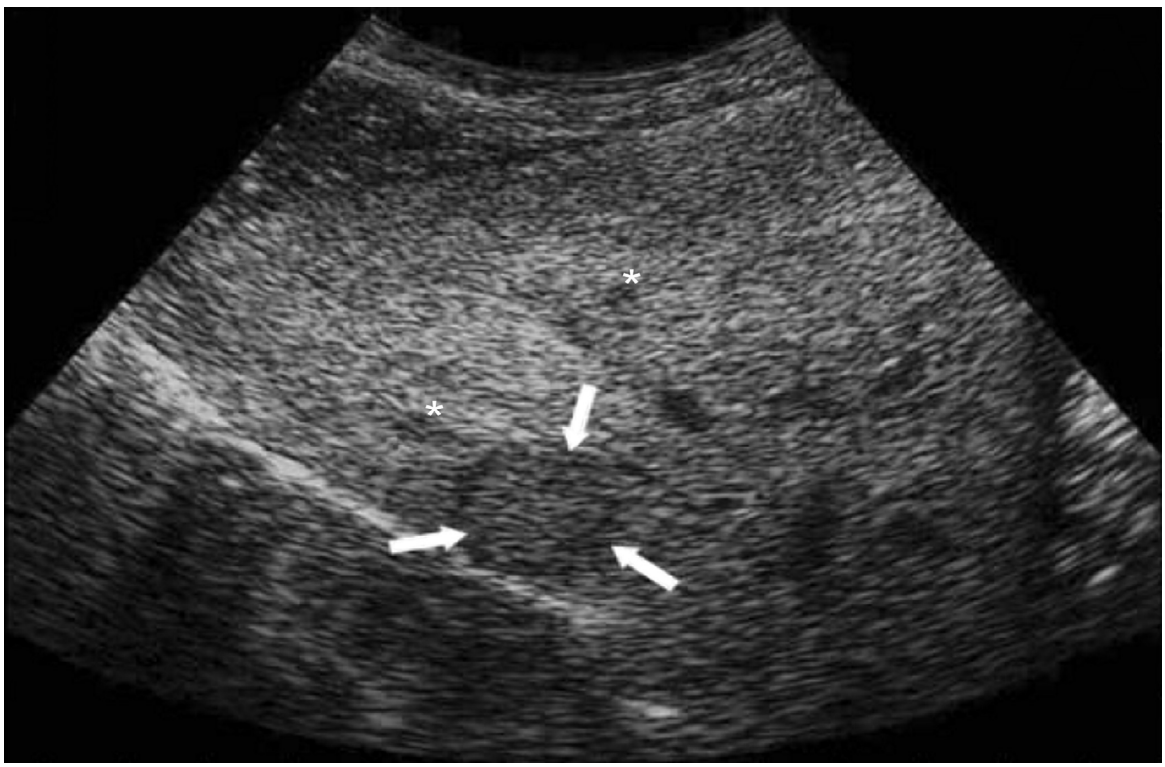

Fig. 1. A conventional US liver image with 3 hypoechoic lesions. Two small (asterisk) lesions and one large (white arrows) targeted lesion.

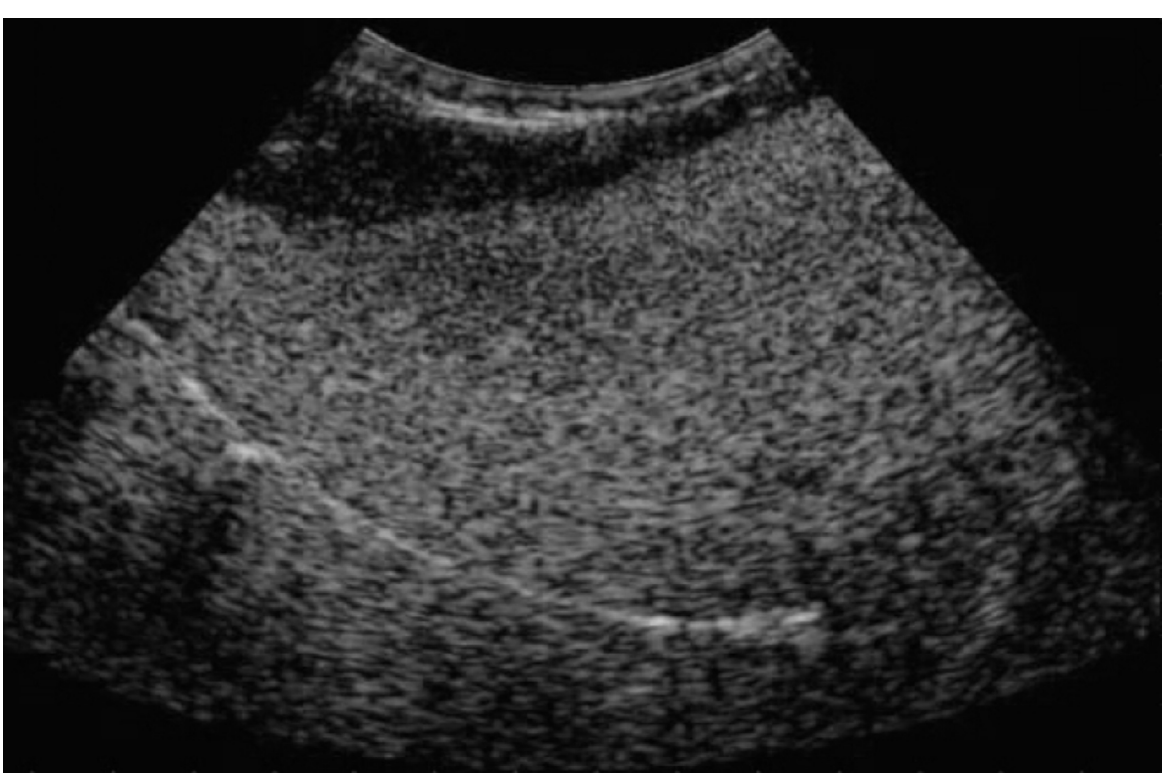

Fig. 2. Late phase CEUS image. The isoechogenic liver parenchyma indicates the benign nature of the hypoechoic lesions.

The liver is frequently the first organ in which neoplasm's metastasize from primary tumors in the abdominal cavity by blood vessels (Nyman et al. 2005). The presence of a metastatic tumor with a diameter of 1.5 cm results in portal hypertension (blood vessel compression theory). To maintain a constant blood flow (Nyman et al. 2005) (energy) the metastasis supplements its shortage from the hepatic artery or by arteiovenous shunts. The CEUS image (Nyman et al. 2005) of metastatic tumors is various and is characterized by: a vascular ring, growing peripheral, necrotic center and various degree of calcification and density.

The effectiveness of ultrasonic imaging of hepatic lesions (Nyman et al. 2005) with a diameter smaller than 2 cm (depending on the information source) is 53% to 84%. The sensitivity for nodular lesions below 1 cm in diameter is 20%. The presumed threshold for detection is 0.5 cm.

Lesion characteristics – the change in enhancement throughout the phases gives a basis to focal hepatic lesion diagnosis. The arterial phase (Haers and Saunders 2009) provides information about the degree and pattern of vascularization, while the portal vein and parenchymal phases inform about UCAs elimination. Most benign lesions are characterized by a constant enhancement. This determines that the focal lesion is enhanced in a degree equal or higher than the hepatic parenchyma during the portal vein phase. Thus, benign lesions can be differentiated from most malignant lesions which are strongly enhanced in the early, hepatic artery phase, and poorly enhanced and hypoechogenic to the hepatic parenchyma in the portal vein and parenchymal phase (early washout phenomenon). This is due to the malignant tumors blood supply, the hepatic artery.

Focal hepatic lesion characteristics in dogs have been described in three phases (Kanemoto et al. 2009):

1. Hepatic arterial phase – degree of vascularization in comparison to the hepatic parenchyma (hyper-/iso-/hypovascular).
2. Portal vein phase – lesion perfusion (hyper-/iso-/hypoperfusion).
3. Hepatic parenchymal phase – echogenicity enhancement was classified as no lesion presence, unclear lesion or clear lesion visualization in the hepatic parenchyma.

The study was conducted on 25 dogs, 16 had malignant lesions and 9 benign lesions; 6 dogs with hepatocellular carcinoma (HCC), 3 with cholangiocellular carcinoma (CCC), 1 with leiomyosarcoma, 1 with liposarcoma, 1 with sarcoma of unknown origin, 1 with lymphoma, 1 with histiocytosis, 1 with mast cell tumor, 6 with nodular hyperplasia HN and 3 with cirrhotic nodules.

The lesion characteristics in each phase are: 5/6 dogs with HCC were hypervascular in the arterial phase, 4/6 hyperperfusion was observered in the portal vein phase and 4/6 had a dysmorphic image in the parenchymal phase. In the parenchymal phase, an uncomplete, irregular or partially enhanced lesion was detected which is a HCC mark.

Dogs with CCC: the lesion was hipoechogenic during the artery and portal vein phase in comparison to the hepatic parenchyma. The vascular pattern in the arterial phase was peripheral, rim-like. A clear lesion was apparent in the parenchymal phase.

The hemopoietic tumors (lymphoma, malignant histiocytosis and mast cell tumor) were almost identical. Hypovascularity and early washout was observed in the arterial and venous phase. A stippled vascular pattern was observed in the dog with lymphoma, and a peripheral (rim pattern) vascular pattern characterized the malignant histiocytosis and the mast cell tumor. Clear parenchymal lesion was observed.

Dogs with leiomyosarcoma and sarcoma of unknown origin: hypovascularity in the artery phase, early washout in the portal vein phase and a clear lesion in the parenchymal phase.

Liposarcoma is hypoechogenic to the hepatic parenchyma and has an unclear lesion image in the parenchymal phase.

Dogs with benign lesions (6 nodular hyperplasia HN and 3 cirrhotic nodules): 8/9 no lesions visible in the parenchymal phase. Five out of six dogs with HN had a diffused and homogenic pattern, which was isoechogenic or hypoechogenic in the arterial phase. One dog had a decrease in vascularization and was aechogenic in the vessel phases and a clear lesion was imaged in the parenchymal phase.

Three dogs with cirrhotic nodules had identical changes in all three enhancement phases with minimally hypoechogenic characteristics.

O'Brien (2007) on the basis of his studies stated that UCAs decrease the visibility of benign focal lesions, causing isoechogenicity in the ultrasonographic image during the enhancements peak. Also the enhancement patterns of malignant lesions were totally different from benign lesions patterns. Namely, during the peak of parenchymal enhancement all malignant focal lesions were hypoechogenic. This occurrence (O'Brien et al. 2004) correlates with the malignancy with a very high sensitivity, specificity, positive predictive value, negative predictive and accuracy (100%, 94.1%, 93.8%, 100% and 96.9%, respectively).

Table 3. Ultrasound appearance of liver lesions by O'Brien et al. *(2004).

Histological type	Hypoechoic	Hyperechoic	Target**	Mixed	Complex	Isoechoic
BENIGN						
Regenerative hyperplasia (n=13)	11	6	1	1		
Hepatoma (n=4)		3		1		
MALIGNANT						
Metastatic neuroendocrine tumor (n=5)	3	1	2	1		
Hemangiosarcoma (n=3)	1				2	
Hepatocellular carcinoma (n=1)	1	1				
Histiocytic sarcoma (n=1)			1			
Lymphoma (n=1)			1			
Metastatic spindle cell carcinoma (n=1)						1
Metastatic carcinoma (n=1)				1		
Sclerosing adenocarcinoma (n=1)	1					
Fibrosarcoma (n=1)	1					1
TOTAL (n=32)	18	11	4	4	2	2

* The sum of dogs in all categories may exceed the total number of dogs if nodules had more than one appearance in a dog.
** Target lesions – hyperechoic center and hypoechoic outer rim (O'Brien et al. 2004, Nyman et al. 2005).

Quantitative CEUS examination via time-intensity curves

Quantification of UCAs (Nyman et al. 2005) is necessary for mathematical evaluation of the degree of tissue perfusion and the detection of diffused lesions in tissue. UCAs are used as markers for the dynamic evaluation of organs such as the liver, brain and kidneys. The results presented as time-intensity curves for a ROI (region of interest) are calculated by built-in software installed in ultrasound systems. The following parameters (Ziegler et al. 2003, Nyman et al. 2005) are computed: upslope, downslope, baseline, peak, change and time to peak and additionally: area under curve (AUC), intensity peak (IP), and mean Transit Time (mTT). Arteriovenous UCAs transit times are digitally imaged as time-intensity curves which allow UCAs uptake and clearance measurement in a ROI.

The shape of the time-intensity curve (Haers and Saunders 2009) is dependent upon the method of UCAs administration. A bolus injection causes a dual phase response, and a constant infusion is characterized by a progressive enhancement with a subsequent plateau that persists until the end of the infusion.

Changes in vascuarization and blood flow secondary to pathological processes (Nyman et al. 2005, Haers and Saunders 2009) alternate the curves shape.

Comparison of CEUS with other Diagnostic Techniques

CEUS, compared to contrast-enhanced CT or MRI (Stewart and Sidhu 2006, Haers and Saunders 2009), allows the analysis of tumor perfusion in real-time without anesthesia. Additionally CEUS is more cost-effective, faster and does not involve ionizing radiation.

Compared with cytological examinations (Haers and Saunders 2009), CEUS is noninvasive and relatively easy to perform with the capability of differentiating benign and malignant lesions.

In conclusion, contrast-enhanced ultrasonography is an alternative and competitive method to other imaging and cytological techniques.

Equipment

CEUS requires (Haers and Saunders 2009) a sophisticated ultrasound system, equipped with Doppler modalities, low frequency transducers of 1 to 3 MHz (such low frequencies are untypical for most US systems used in veterinary medicine), software capable of specific contrast-enhanced imaging and eventually software for quantitative evaluation.

Ultrasound contrast agents necessary for the examination need to be reconstituted before usage. Route of administration is intravenous. The half-time of stability after reconstitution depends on the UCAs used (e.g. Definity 5 min, SonoVue 1h to 2 h, Sonazoid 2 h). To reduce costs, patients should be scheduled within the same period.

Conclusion

Contrast-enhanced ultrasonography increases the intensity of echo signals in the canine patient. The main indication for CEUS is focal liver lesion evaluation, especially differentiating between benign and malignant lesions, and malignant lesion diagnosis.

Noninvasiveness, no anesthesia and the ability to evaluate in real-time are the main advantages of CEUS. The disadvantages are equipment requirements and cost of UCAs. Also the elimination of contrast agents via lungs may limit the use of CEUS in patients with pulmonary diseases. Adverse effects were observed in human medicine after UCAs administration (headaches, diarrhea, neutropenia, nausea, skin reactions, dyspnoe, rhinorrhagia). No adverse effects were observerd in animals.

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