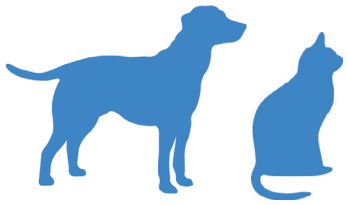
Characterization of liver focal lesions in dogs and cats by contrast-enhanced ultrasonography



Introduction and aim of the study - The use of ultrasonography for the detection of parenchymal disorders of the liver is rarely specific. Contrast-enhanced ultrasound (CEUS) improves the accuracy of focal liver lesion characterization. Nevertheless, in veterinary medicine, data on liver lesion characterization by CEUS are scant and based on a small number of cases in dogs, and totally missing in cats. Aim of this study is to describe the contrast enhancement pattern of focal liver lesions in dogs and cats.
Materials and methods - 34 dogs and 6 cats with hepatic lesions were considered. Each animal underwent a qualitative contrast-enhanced ultrasound using a contrast agent consisting of sulphur hexafluoride; cytohistology was used as a gold standard for the diagnosis. Sensitivity, specificity, positive and negative predictive values and positive and negative likelihood ratios were determined in order to classify the liver focal lesions examined with Sonovue® as benign or malignant.
Results - CEUS showed that 86.4% of benign lesions had homogeneous enhancement in the portal and late phase compared with the adjacent liver parenchyma; all (100%) malignant lesions showed hypoenhancement in the late phase, while the portal phase ranged from iso- to hypoechoic in primary and metastatic carcinomas.
Discussion - The results of our study confirm the usefulness of CEUS in differentiating benign from malignant lesions. Nevertheless, three cases of benign lesions with atypical behaviour were detected. The main differences in terms of vascular behaviour were registered in the late phase, with hypoenhancement in malignant lesions in both the dog and the cat. In four cases (10%), hypoenhancement was detected in the advanced late phase, suggesting that observation must be prolonged for at least 2 minutes in order to avoid possible false negatives. Pathognomonic patterns capable of characterizing specific lesions were not recognized. In our experience, Sonovue® showed a high sensitivity and specificity in the differentiation of benign or malignant liver focal lesions.
CEUS, a non-invasive and relatively cheap technique, could have an important role in the diagnostic approach to characterize hepatic lesions in dogs and cats, particularly when more complete exams (i.e. CT) and/or biopsy are not available.

Paola Paradies*, Vet, PhD, EVPC, Associate Professor in Clinical Medicine, Veterinary Medicine Campus, University of Bari, Italy

Italia Mele, Vet, freelance sonographer, Apulia, Italy

Francesca Palma, Vet, Bari, Italy

Gabriele Barella, Vet, PhD, Milan, Italy

Stefano Faverzani, Vet, PhD, Associate Professor in Clinical Medicine, Department of Veterinary Medicine, University of Milan, Italy

Key words - Ultrasound, dogs and cats, contrast agents.

*Veterinary Clinics, Department of Emergencies and Organ Transplantation, Veterinary Medicine Campus, University of Bari Strada Provinciale per Casamassima km 3, 70010 Valenzano, Bari, Italy

*Corresponding Author (paola.paradies@uniba.it)

Received: 13/03/2018 - Accepted: 31/01/2019

INTRODUCTION

In human medicine[1], contrast-enhanced ultrasonography (CEUS) is part of the diagnostic workup to liver focal lesions as it allows physicians to discern between benign/malignant nodules and to identify characteristic patterns associated with specific pathologies[2,3]. In veterinary medicine, the potential of CEUS has been documented for more than 10 years[4,5,6,7] but in dogs, scientific work on the use of contrast agents (CA) for the assessment of liver focal lesions is limited, outdated and performed on a small number of lesions[8,9,10]; results are also not easily comparable in view of the different contrast agents used[11,12]. Moreover, in the literature, no feline cases have been reported. Aim of this study is to describe the ultrasound contrast enhancement patterns of liver focal lesions in dogs and cats using a CA containing sulphur hexafluoride (Sonovue®, Bracco, Italy).

Although CEUS is a technique that has entered into clinical practice, the data published in veterinary literature on its use for focal liver lesions is scant and dated in the dog and absent in the cat.

MATERIALS AND METHODS

The ultrasound records of dogs and cats with single or multiple focal lesions or with a diffuse heterogeneous hepatic pattern and for which a qualitative contrast enhanced study with Sonovue® was available were reviewed, as were the results of cyto-histopathology, the gold standard to establish the definitive diagnosis. The cytological examination was used for the definitive diagnosis only in those cases in which the cytologist considered it to be definitely diagnostic (i.e. steatosis) or if it was undoubtedly contextualized within a systemic context (i.e. lymphoma) or if it eventually indicated a diagnosis of neoplasm that could at least be characterized by cellular type (epithelial, mesenchymal, etc.). In all other cases the diagnosis was based on histology, performed by means of an ultrasound-guided Tru-cut biopsy, surgical excision or necropsy.

Using cyto-histology as the gold standard, the study analysed CEUS scans of focal liver lesions in dogs and cats using a contrast agent marketed in Italy containing sulphur hexafluoride (Sonovue®, Bracco, Italy).

Patients were examined at two different sites: at the Veterinary Clinic of the Department of Emergencies and Organ Transplantation of the University of Bari (site A) and at the Extracardiac Ultrasound clinic of the Department of Veterinary Medicine, University of Milan (site B). Ultrasound scanners with dedicated software and probe (MyLab 30 and MyLab 70, Esaote®, Italy, in site A and site B, respectively) were used for both conventional ultrasonography and for CEUS.

Having confirmed the uniformity of the approach used the results were evaluated collectively as a single sample.

Procedure

The recumbency of the animals and the scans used for the conventional ultrasound examination and for CEUS were chosen based on the location of the lesions (left/right lateral and dorsal recumbency; transverse and longitudinal scans). Patients were manually restrained. In all subjects a 22-gauge intravenous catheter was placed in the right or left cephalic vein for the infusion of the contrast agent. With the scanner set in Contrast Tuned Imagining (CnTI) mode the acoustic power was set at 35 kPa. The gains were adjusted so that only few background noises were detected and a single focal point was set just below the lesion under investigation. For the contrast enhanced scan a dedicated linear probe with a variable frequency of 3-10 MHz was used, being the size of the subjects included in the study and the depth of the lesions compatible with such a probe. The contemporary use of grey-scale and CnTI mode was only possible at site B. The CA was used at a dosage of 0.05 ml/kg[13]. In order to allow the microbubbles to arrive as a bolus in the area to be examined, the intravenous administration of the CA was followed by inoculation of approximately 2 ml of saline in the cat and of 5 ml in the dog.

In order to evaluate the behaviour of multiple focal lesions or to improve the setting, in some patients at times additinal boluses were necessary; in such cases, the mechanical index was increased in the interval between the individual boluses, this to eliminate any residues of the contrast agent administered with the previous inoculation.

The lesions were scanned and filmed continuously for two minutes since the starting of the timer, which occurred simultaneously with the injection of the CA; the contrast enhanced ultrasound was divided into three different phases: the arterial, portal and late phases. The timings of the vascular, arterial and portal phases were defined based on the results of a study carried out using Sonovue® as contrast agent[13]. Based on these results, the arterial phase occurs 10-15 seconds post-injection;

this is followed by the portal phase, which starts at least 30 seconds after the bolus[13]. The time interval between the inoculation of the CA and the enhancement peak (maximum contrastographic impregnation) was estimated at 46.3 seconds in non-sedated patients[13]. The late phase was defined as the one starting 60 seconds after the administration of the CA and in any case subsequent to the enhancement peak. By reviewing the images of conventional ultrasound scans the hepatic lesions were classified based on their distribution (single, unifocal and multifocal) and their echogenicity with respect to the adjacent parenchyma (hyperechoic, isoechoic or hypoechoic).

The contrast enhanced ultrasound was divided into three different phases: the arterial, portal and late phases.

As for CEUS, qualitative parameters were used in the evaluation, i.e. videos were analysed with a focus on the enhancement (contrastographic impregnation) of the lesions, which was then compared with the enhancement of the adjacent hepatic parenchyma in the three different phases. In the arterial phase the lesions were classified as hypervascular, isovascular or hypovascular, based on their vascularization compared to that of the adjacent liver. In the portal and late phases the perfusion of the lesions was classified as increased (hyper = hyperenhanced), equal (iso = isoenhanced) or reduced (hypo = hypoenhanced) compared to the adjacent liver.

When possible, the contrastographic enhancement pattern was defined according to the classification reported in human medicine[3] (Figure 1).

The usefulness of using Sonovue® for the differentiation of malignant lesions from benign ones was calculated by evaluating the diagnostic performance indices (Sensitivity, Specificity, Positive Likelyhood Ratio, Negative Likelyhood Ratio, Positive Predictive Value and Negative Predictive Value)[14] and was then compared to the results of the definitive diagnosis.

RESULTS

A total of 40 subjects were recruited (Table 1), specifically 34 dogs and 6 cats, with advanced mean age (11.7 years in dogs; 13.3 years in cats), weight between 3.5 and 5.5 kg in cats and between 9 and 22 kg in dogs. At conventional ultrasound examination all the animals presented focal or mass, single or multiple hepatic lesions, or areas of heterogeneous parenchyma, as required by the inclusion criteria (Table 2). In particular, 13 animals presented single focal lesions in number ≤ 3; 14 animals multiple focal lesions (11 with lesions with constant and superimposable characteristics, whereas 3 animals - CN11, CN19 and GT1 - exhibited multiple lesions with differing characteristics), 8 animals mass lesions with diameters ranging from 3.2 to 9.8 cm and 5 animals presented areas of heterogeneous parenchyma. Benign lesions were identified in 22 animals and malignant lesions in 18, based on the histological (n = 29) or cytological (n = 11) examination.

86.4% of the cases with benign lesions presented homogeneous enhancement in the portal and late phase; 100% of the malignant lesions presented hypoenhancement in the late phase.

Of the 6 cats included in the study, 3 presented a mass lesion and 2 showed focal lesions, diagnosed as primary or secondary malignancies; one cat presented heterogeneous areas subsequently diagnosed as multiple abscesses.

The retrospective CEUS examination showed that among the benign lesions (Table 3), 86.4% of the cases (19/22) presented homogeneous enhancement in the portal and late phase, such that the lesions were not distinguishable being isoechoic compared to the adjacent parenchyma (Figure 2). The exceptions were: 1. a cystic lesion (CN8) presenting the characteristic enhancement defect in all phases; 2. an inflammatory granuloma (CN26) which in the arterial phase presented a peripheral hypervascular pattern, followed by hypoenhancement in the portal and late phases and a nonenhanced

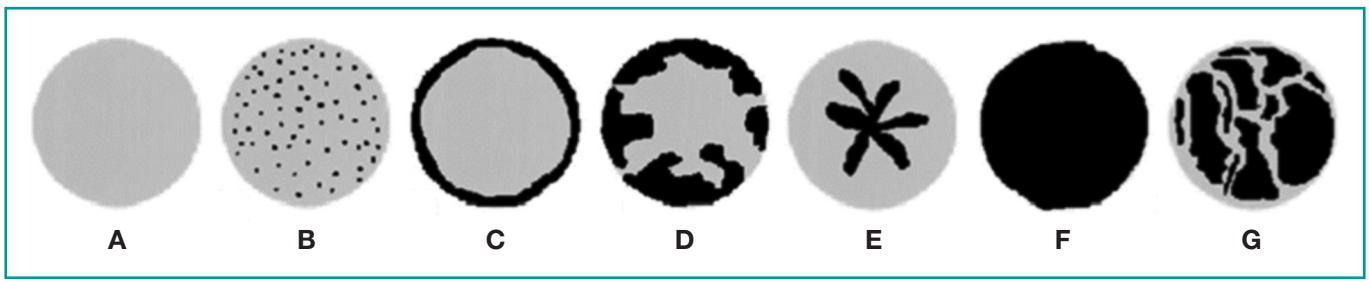


Figure 1 - Classification of enhancement patterns in focal liver lesions in human medicine3: absent **(A)**, dotted **(B)**, peripheral rim-like **(C)**, peripheral nodular **(D)**, central with spoke-wheel shape **(E)**, diffuse homogeneous **(F)**, diffuse heterogeneous **(G)**.

Table 1 - Summary table of the 40 cases reported in the study: signalment; appearance of liver lesions at conventional ultrasonography; CEUS patterns in the three phases: arterial, portal and late; cyto/histological diagnosis

CASE	SIGNALMENT	PATHOLOGICAL PATTERN WITH STANDARD ULTRASONOGRAPHY	CEUS PATTERN			HISTOLOGY/CYTOLOGY DIAGNOSIS
			ARTERIAL	PORTAL	LATE	
CN1	Greyhound, FS, 11 year-old	Hypoechoic single focal lesion	Isovascular	Isoenhanced	Isoenhanced	Benign nodular hyperplasia
CN2	Poodle, FS, 13 year-old	Multiple hypoechoic focal lesions	Hypervascular with peripheral rapid rimlike pattern	Early wash-out (23"), hypoenhanced	Hypoenhanced	Malignant epithelial neoplasia/carcinoma
CN3	Mongrel, FS, 14 year-old	Hyperechogenic mass lesion, heterogeneous and with defined margins	Hypervascular	Isoenhanced	Isoenhanced	Hepatoma
CN4	Mongrel, MC, 12 year-old	Multiple hyperechoic focal lesions	Isovascular	Isoenhanced	Isoenhanced	Focal steatosis-C
CN5	Mongrel, M, 11 year-old	Multiple hypoechoic focal lesions	Isovascular	Early wash-out (23"), hypoenhanced	Hypoenhanced. New visible hypocaptating areas	Sarcoma
CN6	Cocker Spaniel, FS, 14 year-old	Multiple hypoechoic focal lesions	Areas with no uptake	Areas with no uptake	Areas with no uptake	Hemangiosarcoma metastases
CN7	Mongrel, FS, 11 year-old	Multiple hypoechoic focal lesions	Isovascular	Isoenhanced	Isoenhanced	Chronic purulent hepatitis and moderate fibrosis
CN8	Mongrel, FS, 11 year-old	Anechoic single focal lesion with posterior wall enhancement	No enhancement	No enhancement	No enhancement	Hepatic cysts
CN9	Labrador, M, 8 year-old	2 hypoechoic focal lesions	Isovascular	Isoenhanced	Isoenhanced	Benign nodular hyperplasia
CN10	Jack Russel, FS, 10 year-old	2 hyperechoic focal lesions	Isovascular	Isoenhanced	Isoenhanced	Focal steatosis-C
CN11	Mongrel, FS, 8 year-old	Multiple focal lesions, some hypoechoic, others "target"	Areas with no uptake	Areas with no uptake	Areas with no uptake, new lesions visible	Lymphoma
CN12	Mongrel, M, 14 year-old	Hypoechoic multiple focal lesions	Isovascular	Isoenhanced	Isoenhanced	Vacuolar degeneration
CN13	Cocker Spaniel, M, 13 year-old	Multiple mixed focal lesions: iso-hypo-anechoic	Areas with no uptake	Areas with no uptake	Areas with no uptake	Hemangiosarcoma metastases
CN14	Golden Retriever, FS, 13 year-old	Isoechoic mass lesion	Hypervascular	Isoenhanced	Isoenhanced	Hepatoma
CN15	Pincher, FS, 10 year-old	Hyperechoic mass lesion with undefined margins, presence of internal hypoechoic areas	Hypervascular	Heterogeneous isoenhancement (early wash-out of some intralesional areas)	Hypoenhanced	Hepatocellular carcinoma
CN16	Mongrel, FS, 17 year-old	Hypoechoic multiple focal lesions	Isovascular	Isoenhanced	Isoenhanced	Vacuolar degeneration
CN17	Beagle, FS, 12 year-old	3 hyperechoic focal lesions	Hypervascular	Isoenhanced	Mild hypoenhancement	Gastric carcinoma metastases
CN18	Mongrel, F, 13 year-old	Hypoechoic single focal lesion	Isovascular	Isoenhanced	Isoenhanced	Benign nodular hyperplasia
CN19	Labrador, M, 14 year-old	Hypo- or hyperechoic multiple focal lesions	Areas with no uptake	Areas with no uptake	Areas with no uptake	Hemangiosarcoma metastases
CN20	Mongrel, M, 10 year-old	Hypoechoic single focal lesion	Hypervascular	Isoenhanced	Isoenhanced	Moderate non-lipidosis vacuolar degeneration and cholestasis
CN21	Yorkshire, M, 12 year-old	Hyperechoic single focal lesion	Hypervascular	Isoenhanced	Isoenhanced	Vacuolar degeneration, of probable lipid origin
CN22	Mongrel, F, 12 year-old	Hyperechoic mass focal lesion	Early wash-in (13"), hypervascularr	Quick wash-out (24"), hypoenhancement with mild peripheral rimlike pattern	Hypoenhanced	Hepatocellular carcinoma/adenocarcinoma
CN23	Golden Retriever, M, 11 year-old	Iso/hypoechoic liver focal lesion	Isovascular	Quick and complete wash-out (20"), marked hypoenhancement with dotted pattern	Strong hypoenhancement. New lesions visible	Histiocytic sarcoma
CN24	Cocker Spaniel, M, 7 year-old	Hyperechoic single focal lesion	Hypovascular	Quick wash-out (23"), hypoenhanced	Hypoenhanced	Hemangiosarcoma metastases
CN25	Maltese, M, 11 year-old	Hypoechoic multiple focal lesions	Isovascular	Quick and complete wash-out, marked hypoenhancement with dotted pattern	Marked hypoenhancement. New lesions visible	Metastatic lymphoma (of intestinal origin)

continued

continued

CASE	SIGNALMENT	PATHOLOGICAL PATTERN WITH STANDARD ULTRASONOGRAPHY	CEUS PATTERN			HISTOLOGY/CYTOLOGY DIAGNOSIS
			ARTERIAL	PORTAL	LATE	
CN26	Mongrel, F, 9 year-old	Single target focal lesion with anechoic centre	Peripheral enhancement and centre with no uptake	Hypoenhancement in the periphery and centre with no uptake	Hypoenhancement in the periphery and centre with no uptake	Inflammatory granuloma
CN27	Pomeranian, F, 14 year-old	Hyperechoic mass lesion	Hypervascular	Isoenhanced	Hypoenhanced (wash-out at 1'12")	Metastatic carcinoma from right adrenal neoplasia
CN28	Cocker Spaniel, M, 9 year-old	Heterogeneous liver with 2 hyperechoic areas	Isovascular	Isoenhanced	Isoenhanced	Initial cirrhosis
CN29	Dachshund, M, 9 year-old	Hyperechoic liver. Hypoechoic multiple focal lesions	Isovascular	Isoenhanced	Isoenhanced	Turbid-vacuolar hepatocyte degeneration and macrovesicular steatosis
CN30	Irish Setter, M, 12 year-old	Hyperechoic multiple focal lesions	Isovascular	Isoenhanced	Isoenhanced	Turbid-vacuolar degeneration and glycogenosis. Multifocal findings of fibrosis
CN31	Yorkshire Terrier, M, 11 year-old	Hyperechoic, heterogeneous liver	Hypervascular areas	Homogeneous enhancement	Homogeneous enhancement	Vacuolar degeneration and steatosis
CN32	Flat-Coated Retriever, M, 8 year-old	Hyperechoic and heterogeneous liver	Homogeneous enhancement	Homogeneous enhancement	Homogeneous enhancement	Chronic hepatitis associated with copper accumulation
CN33	German Shepherd, F, 10 year-old	Diffuse macronodular heterogeneous pattern	Homogeneous enhancement	Homogeneous enhancement	Homogeneous enhancement	Vacuolar degeneration
CN34	Mongrel, M, 14 year-old	Single rounded lobe and diffuse heterogeneous pattern	Hypervascular	Homogeneous enhancement	Homogeneous enhancement	Hepatoma
GT1	European dog, FS, 15 year-old	Hypoechoic single focal lesion on heterogeneous liver due to the presence of hyperechoic multiple focal lesions	Hypovascular (single hypoechoic lesion)	Hypoenhanced (single hypoechoic lesion)	Hypoenhanced (single hypoechoic lesion)	Liver metastasis from pancreatic carcinoma
			Isovascular (multiple hyperechoic lesions)	Isoenhanced (multiple hyperechoic lesions)	Isoenhanced (multiple hyperechoic lesions)	Vacuolar degeneration
GT2	European dog, MC, 10 year-old	Mass lesion with a cystic portion with anechoic content in the centre	Hypervascular. No enhancement of the cystic portion	Hypoenhanced. No enhancement of the cystic portion	Hypoenhanced	Malignant epithelial neoplasia/carcinoma
GT3	European dog, M, 15 year-old	Hyperechoic single focal lesion	Extremely quick wash-in, hypervascular	Heterogeneous isoenhancement	Hypoenhanced	Cholangiocarcinoma
GT4	European dog, M, 11 year-old	Heterogeneous hypoechoic mass lesion	Hypervascular in the periphery, hypovascular in the centre	Hypoenhancement in the centre (persisting hyperenhancement in the periphery)	Hypoenhanced	Liver metastasis from pancreatic carcinoma
GT5	European dog, M, 14 year-old	Heterogeneous liver due to the presence of areas with different echogenicity	Hypovascular areas	Hypoenhancement areas	Hypoenhancement areas	Multiple abscesses-C
GT6	Persian, FS, 15 year-old	Heterogeneous hypoechoic mass lesion	Hypervascular	Hypoenhanced	Hypoenhanced, new visible lesions	Cholangiocarcinoma

Table 2 - Number of animals distributed based on the type of liver alterations found with conventional ultrasound examination

	Hypoechoic	Hyperechoic	Mixed	Other	Total No. (n=40)
Single focal lesion	3	3	0	3 (1 isoechoic, 1 anechoic, 1 target)	9
Multiple focal lesions	8	3	2	1 target	14
Mass lesions	2	3	1	2 (1 anechoic centre, 1 isoechoic)	8
Areas of heterogeneous parenchyma			5		5
Other (2-3 single lesions)	1	3			4

Table 3 - CEUS findings for benign lesions distributed based on cyto-histological diagnosis									
BENIGN LESIONS	**Arterial phase**			**Portal phase**			**Late phase**		
	Hyper	**Iso**	**Hypo**	**Hyper**	**Iso**	**Hypo**	**Hyper**	**Iso**	**Hypo**
Benign nodular hyperplasia n=3		3			3			3	
Hepatoma n=3	3				3			3	
Focal steatosis n=2		2			2			2	
Chronic purulent hepatitis/multiple abscesses n=2		1	1		1	1		1	1
Hepatic cyst n=1 °									
Vacuolar degeneration +/- steatosis or fibrosis n=8	3	5			8			8	
Inflammatory granuloma n=1	1*					1*			1*
Cirrhosis n=1		1			1			1	
Chronic hepatitis associated with copper accumulation n=1		1			1			1	
* with non-enhancing centre; ° non-enhancing at any phase.									

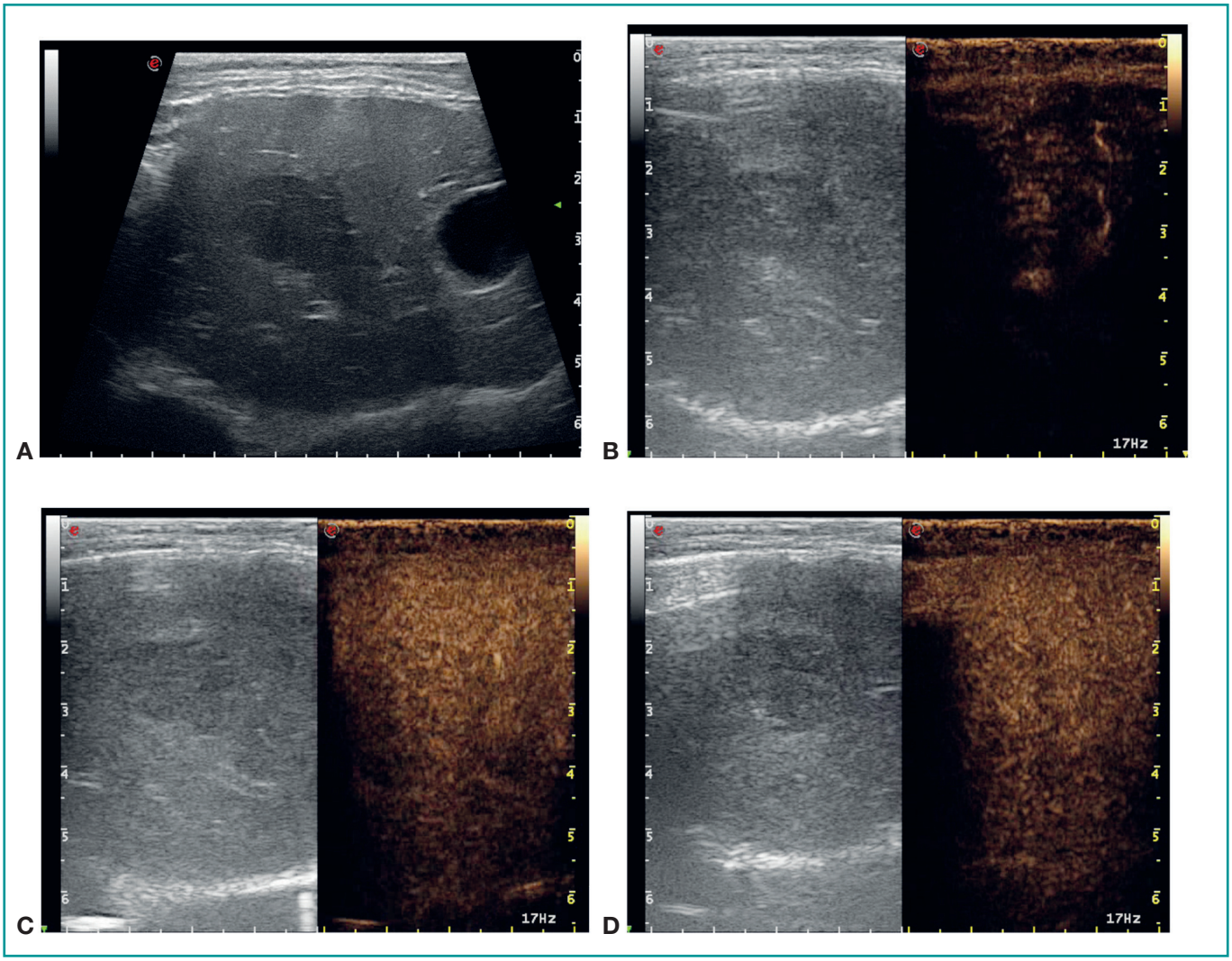


Figure 2 - 11 year-old female spayed Greyhound with diagnosis of benign nodular hyperplasia. At conventional ultrasonography, a single hypoechoic focal lesion of approximately 2 cm in diameter with irregular margins was detected **(A)**. At CEUS, at site B, both B-mode (left) and CnTi mode (right) images could be simultaneously assessed **(B, C, D)**. After injection of the CA the focal lesion presented an enhancement superimposable with that of the adjacent hepatic parenchyma during all three phases, i.e. isovascular in the arterial phase **(B)** and isoenhancement in the portal and late phases **(C, D)**.

centre in all three of the phases; 3. a case of multiple abscesses (GT5) with a hypovascular arterial phase and hypo-enhancement in the portal and late phases.

With the exception of these last 3 peculiar cases, in benign lesions the arterial phase was mostly isovascular, except for in hepatoma nodules (CN3, CN14, CN34) and in 3 cases of degenerative disorders (CN20, CN21, CN31), in which the arterial phase appeared, instead, hypervascular.

Among the malignant lesions (Table 4), primary hepatic neoplasms - hepatocarcinomas in dogs and cholangiocarcinomas in cats (CN15, CN22, GT3, GT6) -, presented a similar behavioural pattern, i.e. a hypervascular arterial phase with a quick and vivid wash-in, a portal phase characterized by iso- or hypoenhancement and a persistent late phase of hypoenhancement (Figure 3).

Metastases from carcinoma or malignant epithelial neoplasia (CN2, CN17, CN27, GT1, GT2, GT4) exhibited a variable CEUS behaviour in the arterial and portal phase, but a constant behaviour in the late phase, with the characteristic hypoenhancement. In 4 cases out of 6 the arterial phase was hypervascular; the portal phase was isoechoic in 2 subjects and hypoechoic in 4.

Pancreatic carcinoma metastases (GT1, GT4) were constantly hypoechoic in all phases, with a hypervascular peripheral rim in one of the 2 cases.

The only case of undifferentiated sarcoma (CN5) presented an isovascular arterial phase, followed by a quick wash-out in the portal phase and persistent hypoenhancement in the late phase.

Haemangiosarcoma metastasis (CN6, CN13, CN19, CN24) presented no enhancement in all three of the

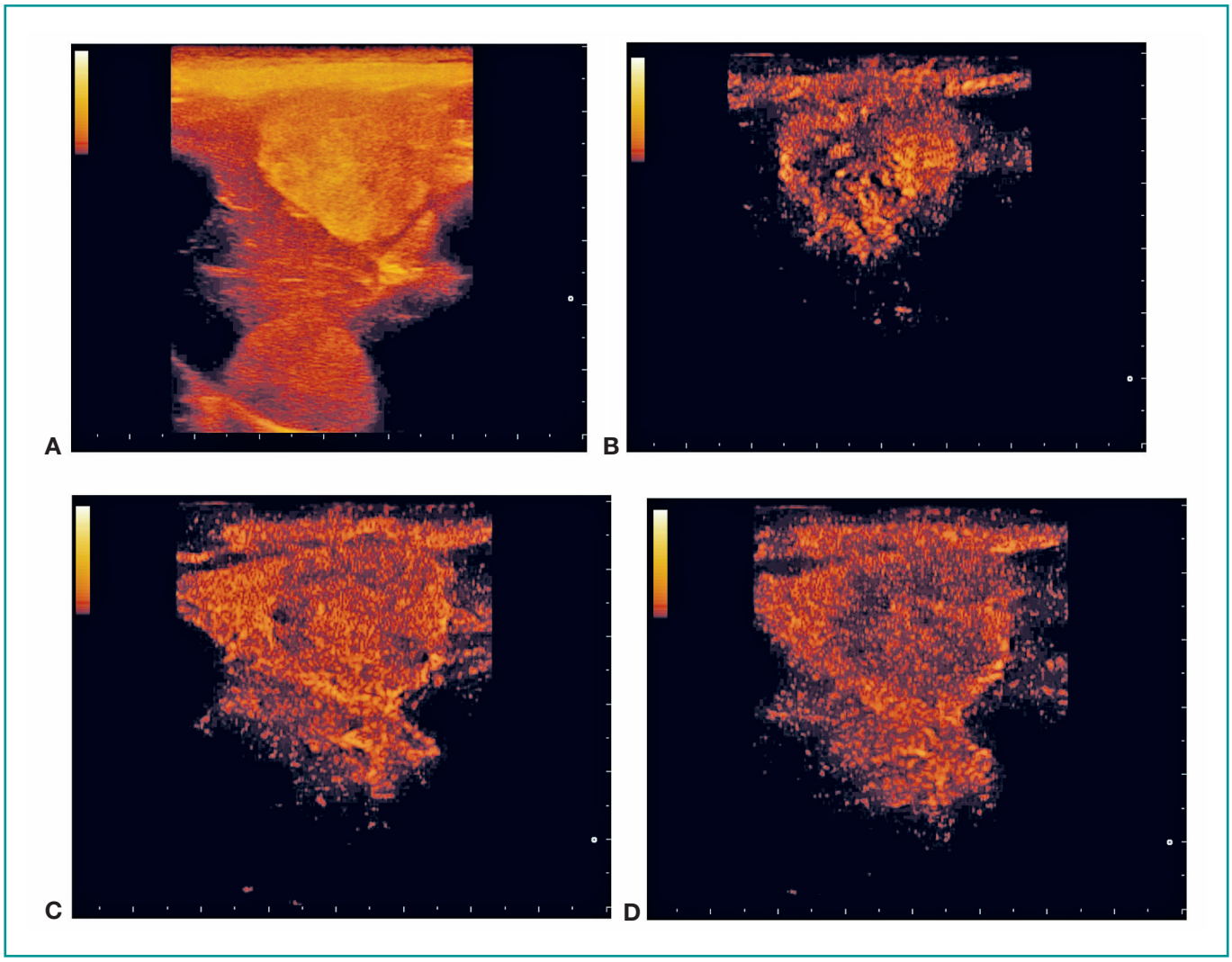


Figure 3 - 12 year-old female mongrel with diagnosis of hepatocellular carcinoma/adenocarcinoma (CN22). At conventional ultrasonography the presence of a hyperechoic mass lesion was detected at hepatic level deforming the organ profile **(A)**. After injection of the CA the lesion exhibited a quick and intense wash-in (at 13") in the arterial phase, hypervascular compared to the adjacent hepatic parenchyma **(B)**. The portal phase was characterized by a quick wash-out (24") and the mass presented hypoenhancement with a mild peripheral rim-like pattern **(C)**. In the late phase, hypoenhancement of the lesion persisted **(D)**.

Table 4 - CEUS findings for malignant lesions distributed based on cyto-histological diagnosis												
MALIGNANT LESIONS	**Arterial phase**				**Portal phase**				**Late phase**			
	Hyper	**Iso**	**Hypo**	**No E.**	**Hyper**	**Iso**	**Hypo**	**No E.**	**Hyper**	**Iso**	**Hypo**	**No E.**
Hepatocellular carcinoma n=2	2					1	1				2	
Cholangiocarcinoma n=2	2					1	1				2	
Carcinoma/epithelial neoplasia metastasis n=6	4		2			2	4				6	
Sarcoma n=1		1					1				1	
Hemangiosarcoma metastases n=4			1	3			1	3			1	3
Lymphoma n=2		1		1			1	1			1	1
Malignant histiocytosis n=1		1						1				1

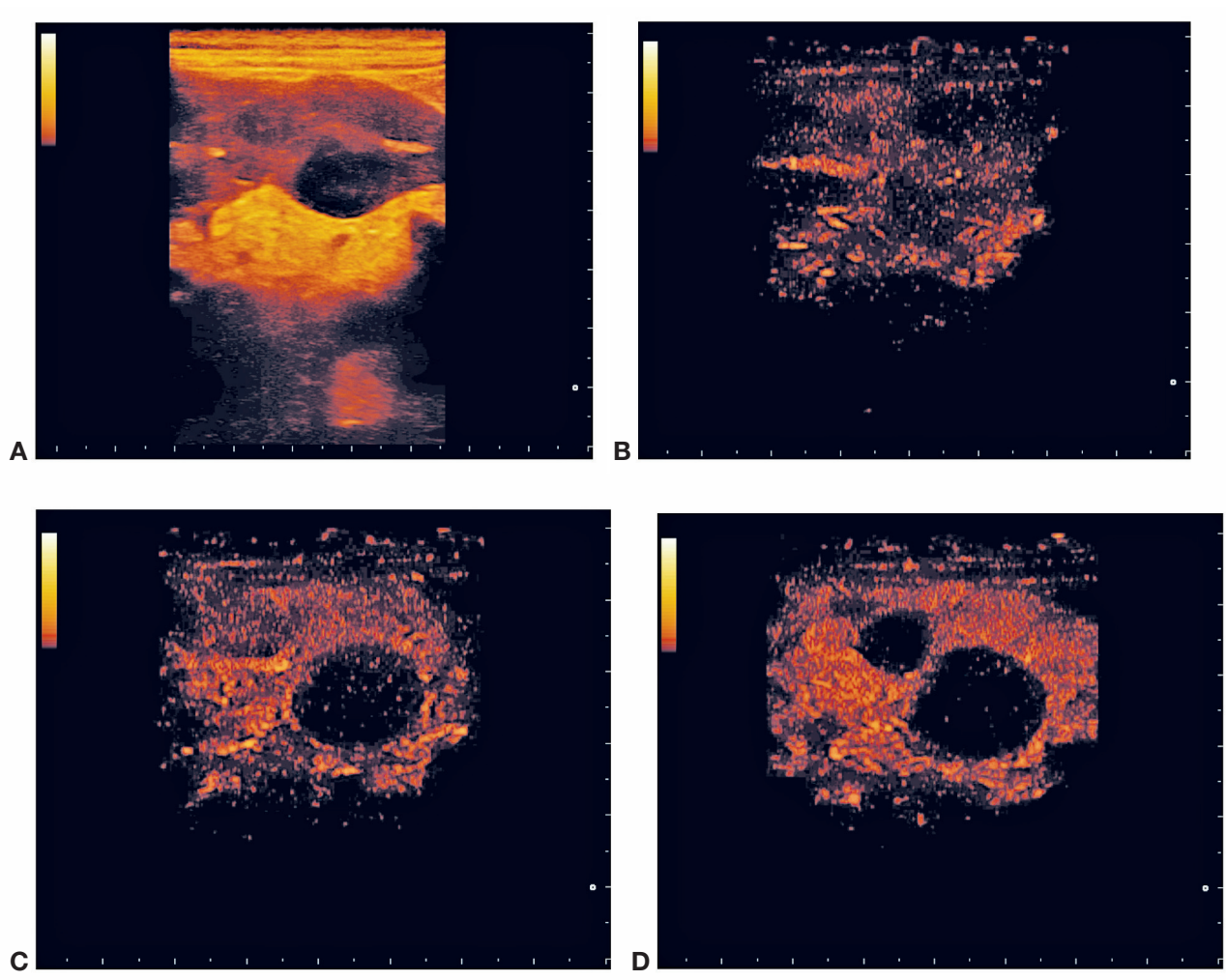


Figure 4 - 11 year-old male Golden Retriever with a diagnosis of histiocytic sarcoma (CN23). At conventional ultrasonography a single focal hepatic, mixed iso- and hypoechoic lesion of approximately 2 cm in diameter was detected **(A)**. At CEUS the lesion was isovascular in the arterial phase **(B)**, with filling superimposable with that of the adjacent parenchyma. A quick and complete wash-out (already at 20") followed, with marked hypoenhancement at the enhancement peak **(C)** and with a dotted pattern in the portal and late phase **(C, D)**. In the late phase, additional focal hepatic lesions not visible with conventional ultrasonography **(D)** were detected.

phases due to the total absence or the reduced presence of contrastographic impregnation.

Among the 3 round-cell tumours (lymphoma or histiocytic sarcoma), in 1 case (CN11) no contrast uptake was detected in any phase, while in the remaining two (CN23 and CN25) a dotted pattern was detected (according to the description by Quaia et al., 2004[3]) in the portal phase, which followed an isovascular arterial phase characterized by an ultra-quick wash-out (Figure 4). In all three cases, additional lesions not visible with conventional ultrasound were identified in the late phase.

Based on the above results, in our case series the descriptive statistics analysis showed a CEUS sensitivity of 100%, specificity of 91.67%, Positive Likelyhood Ratio of 12 (95% CI; 3.18-45.23), Negative Likelyhood Ratio of 0, Positive Predictive Value of 90% (95% CI; 70.48-97.14%) and a Negative Predictive Value of 95.24% (95% CI; 83.84-99.42%).

DISCUSSION

The results of our study first confirm, as already reported, that conventional ultrasonography cannot conclusively characterize liver focal lesions[15,16,17,18,19].

With regards to contrast enhanced ultrasonography with Sonovue®, the predominant portal and late phase isoenhancement in benign lesions has already been documented in both human[3,20] and registered in this study in veterinary medicine[8,10] and is presumably due to a similar vascular structure between the lesion itself and the normal hepatic parenchyma, both in terms of vessel conformation and blood flow velocity[3]. The ultrasound contrastographic behaviour of quick wash-in in the hypervascular arterial phase observed in all 3 cases of hepatoma reflects what has been described in human medicine[1]; however, this was also recorded in 3 cases of degenerative lesions and is therefore non-pathognomonic.

With regards to benign lesions, atypical behaviour was recorded in 3 cases. In one case, i.e. the cystic lesion characterized by the absence of uptake in all phases, the peculiar behaviour was easily and correctly interpreted as benign. In the inflammatory granuloma, the pattern of peripheral arterial enhancement followed by hypoenhancement in the portal and late phase and with absence of central enhancement was instead erroneously interpreted as a behaviour referable to a malignant lesion with central necrosis. However, in human medicine, such behaviour has been described in association with or in the presence of abscesses [21,22]. In the cat with multiple abscesses, the CEUS performed in the patient without sedation had also been suggestive of a malignant neoplastic behaviour, which was however not confirmed by histology. In all phases the lesions showed a weak wash-in, with hypo-enhancement comparable to that of the adjacent parenchyma. It should be emphasized that in scarcely collaborative animals, sedation may be necessary and useful in order to reduce as much as possible mistakes in the interpretation. In general, no characteristic pattern was identified allowing to differentiate, among the benign lesions, between inflammatory, degenerative and hyperplastic lesions. In the 3 cases of benign nodular hyperplasia the typical behaviour described in humans of rapid centrifugal filling and non-enhancing central scar was not found[20].

CEUS showed a sensitivity of 100% and a specificity of 91.67%. Among the benign lesions the cases of inflammatory granuloma and of multiple liver abscesses exhibited an atypical CEUS behaviour, misleading for diagnostic purposes.

As for the malignant lesions, they all showed hypoenhancement in the late phase and were therefore clearly distinguishable from benign lesions. In primary and metastatic carcinomas the portal phase varied from iso- to hypoechoic.

In primary malignant tumours the vigorously hypervascular arterial phase and the early contrast loss with respect to the adjacent parenchyma corresponded to what has been reported in both human[23] and veterinary literature[8,10]. However, the different iso- or hypoechoic presentation in the portal phase is in disagreement with other works carried out using Sonovue®[8,10] which report a quick wash-out already in the portal phase; despite this, such data should be interpreted in the light of the limited number of cases, i.e. one case of hepatocellular carcinoma[8] and one case of cholangiocarcinoma[10]. In other studies using different contrast agents (i.e. parenchimal CA)[11,12], performed on a higher number of subjects, the arterial phase was mostly hypervascular and all the studies showed a parenchymal (or late) phase of hypo-enhancement. In the portal phase, instead, hypoenhancement was not a constant feature; in fact, most of the lesions appeared isoechoic or mixed[11,12]. Despite the substantial difference in the CA used, it should be emphasized that in our study 2 cases of primary neoplasia out of 4 (CN15 and GT3) were isoechoic at the enhancement peak and became hypoechoic only at a later stage, after a minute of observation. In human medicine, hepatocellular carcinomas characterized by prolonged enhancement and late wash-out after the enhancement peak have been reported; this atypical behaviour has been associated with hepatocellular carcinomas characterized by a greatly differentiated tumour histotype[23]. In the dog, one study[24] focused on cholangiocellular adenoma, a mass-shaped benign neo-

plasm for which CEUS may erroneously exhibit a malignant behaviour.

Again among primary tumours, in our study we examined 2 cats with cholangiocarcinoma, which is the most common primary hepatic tumour in the feline species; the arterial phase appeared markedly hypervascular and intense. The other rare cases of documented cholangiocarcinoma have been reported in the dog[10,11].

With regards to metastatic neoplasms, these showed a quick wash-out in the portal or late phase, as previously reported[8,10]. However, interestingly, also secondary neoplasms - similarly to what previously said for primary neoplasms - can be isoechoic at the enhancement peak and become hypoechoic only in the late phase, as recorded in the 2 cases of carcinoma metastases (CN17 and CN27). These findings differ from those of O'Brien et al.[8], who in 15 focal malignant lesions (mainly metastatic) reported a hypoechoic pattern at the enhancement peak, i.e. less than one minute from the injection. Our data are in agreement with those of a large study carried out in human medicine with Sonovue® (452 lesions studied), in which it was concluded that the main difference between benign and malignant lesions is detected only during the late phase[3], suggesting the need to extend observation times for a minimum of 2 minutes in order to avoid false negative results. In fact, although in the dog the vascular behaviour is often already defined at the enhancement peak (46.2 seconds from the injection of Sonovue® in non-sedated dogs[13]), the loss of contrast can also occur at a later stage.

Observation times must be extended for at least 2 minutes in order to avoid false negative results as in malignant lesions a late washout is possible.

It is believed that malignant lesions have an early loss of contrast given their purely arterial blood perfusion, unlike the hepatic parenchyma which is instead characterized by a double vascularization[25,26]. The role of portal vessels in nourishing malignant lesions is controversial. In experimental studies it has been hypothesized that the portal flow can penetrate hepatic tumours through shunts between portal branches and the arterial system afferent to the lesion[27]: this could explain the wash-out of the lesion only in the late phase, and not in the portal phase, as we detected.

Among the metastatic lesions, those from splenic hemangiosarcoma exhibited a characteristic behaviour already described by other authors[8,9,28], i.e. the absence of enhancement or reduced enhancement in all phases, confirming the importance of CEUS in the staging of this tumour. However, this aspect is not pathognomonic of hemangiosarcoma, as in another study this was also detected in a case of lymphoma (CN11) and in different metastatic forms[8].

In round-cell tumours, the dotted vascular pattern displayed in CN23 and CN25 was previously documented in another case of lymphoma[11]. In our study we found it difficult to evaluate the distribution pattern of the CA in the arterial phase, as was instead done in another study[11]. The enhancement patterns were more easily appreciated in the portal phase; we could not however recognize any pattern univocally associated with specific lesions. It should be considered that the lymphoma cases included in the study were only 2; they derived from multicentric lymphomas and presented nodular lesions, while it is known that lymphomas more commonly induce a diffuse infiltrate. In view of its extremely variable ultrasonographic presentation our data cannot be generalized to cases of lymphoma.

CEUS findings in the cats of the study, despite few in number, were of interest in view of the total absence in the literature of studies which have tried to characterize focal liver lesions in this species. Regardless of there being a mass or a focal lesion, the cases of primary or metastatic malignant neoplasia presented a hypovascular late phase, as expected from data in humans and dogs.

The results of our study, although still susceptible to further confirmation and conducted on a rather limited series of cases, at least as far as cats are concerned, confirm that CEUS can distinguish between benign and malignant lesions, with a specificity greater than 90% (91,67%) and with 2 false positives out of 22 cases (CN26, GT5). However, this said, the elective diagnostic techniques - such as liver biopsy – should not be considered of lesser importance. In fact, a "morphological" diagnosis is always necessary, as it allows to define the prognosis and consequently the necessary therapy: this also applies to liver alterations which, although definable as benign, could still be the expression of a severe liver disease, even if not neoplastic. CEUS should therefore be considered as a useful alternative for all those conditions in which, due to reduced owner's compliance or because of a greater anaesthesia risk, the use of 2nd level diagnostic techniques (i.e. CT or MRI) for the study of focal liver alterations may prove to be more difficult. In addition, the behaviour of focal liver lesions when exposed to an ultrasound contrast agent can provide useful indications in choosing (when necessary) which lesions should be sampled first for diagnostic purposes.

In fact, in human medicine, CEUS is not the first-choice approach to liver lesions. Guidelines[1] report MRI as the first choice, followed by CT; CEUS is considered

more of a niche examination, i.e. for controls during therapy, or for interventional procedures, or for patients with ascertained liver cirrhosis in order to assess possible carcinomatous lesions other than regenerative nodules. Contrast CT has become broadly available also in veterinary medicine and is now considered a more complete method for the characterization of focal liver lesions, this in spite of it being more expensive and necessarily requiring anaesthesia. CT can give the same perfusion information as CEUS and in addition it allows the complete staging of the patient, something non possible with CEUS. On the other hand, when a biopsy is necessary, the ultrasound-guided technique is usually easier to perform compared to CT-guided sampling techniques.

CEUS is a useful non-invasive and relatively inexpensive technique for the diagnostic approach to focal liver lesions in clinical veterinary practice.

In conclusion, our work confirms the ability of CEUS to distinguish between malignant and benign lesions; we therefore believe that this non-invasive and relatively inexpensive methodology may represent a useful step in the diagnostic approach to focal liver lesions in clinical veterinary practice, particularly when more complete examinations such as CT cannot be proposed or when a biopsy is not possible. The data of our study support the need to perform a correct and standardized contrast enhanced examination; in particular, observation times must be extended beyond the enhancement peak for a minimum of 2 minutes and considerable attention must be paid to the late phase in order to avoid false negative results. It was not possible to define CEUS patterns that univocally characterize the different types of neoplasms; for this purpose, additional studies comprehensive of more cases are necessary.

KEY POINTS

- Although CEUS has become a clinical practice in companion animals, scant data is available on the use of a contrast agent containing sulphur hexafluoride (Sonovue®) for focal liver lesions in the dog and totally missing in the cat.
- The results of this qualitative contrast enhanced ultrasound study of liver lesions using the Sonovue® contrast agent in 34 dogs and 6 cats confirm that CEUS may be useful in distinguishing between malignant and benign lesions; however, the possibility of having benign lesions with atypical behaviour should not be dismissed.
- The main difference in vascular behaviour between malignant and benign lesions is revealed in the late phase, with hypoenhancement of malignant lesions in both the dog and the cat.
- Observation times must be extended for at least 2 minutes beyond the enhancement peak in order to reveal a possible late washout.
- In 2 cats with cholangiocarcinoma the arterial phase appeared markedly hypervascular and intense. Hemangiosarcoma metastases showed a characteristic behaviour with absence of enhancement in all phases.
- CEUS is a useful non-invasive and relatively inexpensive technique for the diagnostic approach to focal liver lesions in clinical veterinary practice.

REFERENCES

1. Claudon M, Dietrich CF, Choi BI et al. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver- Update 2012. Ultrasound in Medicine & Biology 39:187-210, 2013.
2. Isozaki T, Numata K, Takayoshi K et al. Differential diagnosis of hepatic tumors by using contrast enhancement patterns at US. Radiology 229:798-805, 2003.
3. Quaia E, Calliada F, Bertolotto M et al. Characterization of focal liver lesions with contrast-specific US modes and a sulfurhexafluoride contrast agent: diagnostic performance and confidence. Radiology 232:420-430, 2004.
4. Szatmàry V, Harkaanyi Z, Vöaröas K. A review of non-conventional ultrasound techniques and contrast enhanced ultrasonography of non-cardiac canine disorders. Veterinary Radiology&Ultrasound 44:380-391, 2003.
5. Nyman HT, Kristensen AT, Flagstad A et al. A review of the sonographic assestment of tumor metasteses in liver and superficial lymphnodes. Veterinary Radiology&Ultrasound 45:438-448, 2004.
6. Ohlert S, O'Brien RT. Contrast ultrasound: General principles and veterinary clinical application. The Veterinary Journal 174:501-512, 2007.
7. Haers H, Saunders JH. Review of clinical characteristics and applications of contrast-enhanced ultrasonography in dogs. Journal of the American Veterinary Medical Association 234:460-470, 2009.
8. O'Brien RT, Matheson J, Delaney F et al. Contrast harmonic ultrasound of spontaneous liver nodules in 32 dogs. Veterinary Radiology&Ultrasound 45:547-553, 2004.
9. Ivancic M, Long F, Seiler GS. Contrast harmonic ultrasonography of splenic masses and associated liver nodules in dogs. Journal of the American Veterinary Medical Association 234:88-94, 2009.
10. Volta A, Gnudi G, Manfredi S et al. The use of contrast-enhanced ultrasound with a second-generation contrast medium: preliminary report in the dog. Veterinary Research Communications 33:221-223, 2009.
11. Kanemoto H, Ohno K, Nakashima K et al. Characterization of canine focal liver lesions with contrast-enhanced ultrasound using a novel contrast agent-Sonazoid. Veterinary Radiology&Ultrasound 50:188-194, 2009.
12. Nakamura K, Takagi S, Sasaki N et al. Contrast-enhanced ultrasonography for characterization of canine focal liver lesions. Veterinary Radiology&Ultrasound 51:79-85, 2010.
13. Nyman HT, Kristensen AT, Kjelgaard-Hansen M et al. Contrast-enhanced ultrasonography in normal canine liver. Evaluation of imaging and safety parameters. Veterinary Radiology&Ultrasound 3:243-250, 2005.
14. Sardanelli F, Di Leo G. Biostatistica in Radiologia, Ed. Springer-Verlag Italia, Milano, 2008.
15. Winklev W et al. Standards for clinical and histological diagnosis of canine and feline liver diseases, AAVV, Ed. Wsava, 2006.
16. Feeney DA, Johnston GR, Hardy RM. Two-dimensional, gray-scale ultrasonography for assessment of hepatic and splenic neoplasia in the dog and cat. Journal of the American Veterinary Medical Association 184:68-81, 1984.
17. Nyland TG. Ultrasonic patterns of canine hepatic lymphosarcoma. Veterinary Radiology&Ultrasound 25:167-172, 1984.
18. Lamb CR. Ultrasonography of the liver and biliary tract. Problems in Veterinary Medicine 3:555-573, 1991.
19. Whiteley MB, Feeney DA, Whiteley LO et al. Ultrasonographic appearance of primary and metastatic canine hepatic tumors: A review of 48 cases. Journal of Ultrasound in Medicine 8:621-630, 1989.
20. Dietrich CF, Schuessler G, Trojan J et al. Differentiation of focal nodular hyperplasia and hepatocellular adenoma by contrast-enhanced ultrasound. The British Journal of Radiology 78:704-707, 2005.
21. Catalano O, Sandomenico F, Raso MM et al. Low mechanical index contrast- enhanced sonographic findings of pyogenic hepatic abscesses. American Journal of Roentgenology 182:447-450, 2004.
22. Liu GJ, Lu MD, Xie XY et al. Real-time contrast-enhanced ultrasound imaging of infected focal liver lesions. Journal of Ultrasound Medicine 27:657-666, 2008.
23. Solbiati L, Tonolini M, Cova L et al. The role of contrast enhanced ultrasound in the detection of focal liver lesions. European Radiology 11:15-26, 2001.
24. Baron Toaldo M, Diana A, Bettini G, Di Donato P et al. Imaging Diagnosis-cholangiocellular adenoma: contrast-enhanced ultrasonographic findings of a benign tumor mimicking malignant neoplasia in a dog. Veterinary Radiology and Ultrasound 54(1):71-74, 2013.
25. Jang HJ, Kim TK, Burns PN et al. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. Radiology 244:898-906, 2007.
26. Martegani A et al. Tumori maligni-Metastasi. In: Rossi S, Callida F, Mertegani A. Ed. Mezzi di contrasto in ecografia, Testo Atlante. Poletto ed. Gaggiano Milano, 2004.
27. Ackerman NB. Experimental studies on the role of the portal circulation in hepatic tumor vascularity. Cancer 58:1653-1657, 1986.
28. O'Brien RT. Improved detection of metastatic hepatic hemangiosarcoma nodules with contrast ultrasound in three dogs. Veterinary Radiology&Ultrasound 48:146-148, 2007.