https://doi.org/10.5281/zenodo.2550273
UDC: 636.7.09:616.411-073.432.19

ULTRASONOGRAPHIC EVALUATION OF THE CANINE SPLEEN: A REVIEW

Emilian Shabani[1], Dardan Shehdula, Kastriot Belegu[2]
[1] Department of Preclinic Subjects, Faculty of Veterinary Medicine
Agricultural University, Tirana, Albania
[2] Department of morphophunctional Subjects
*Corresponding author; emilian3shabani@yahoo.it

ABSTRACT

The spleen is one of the most susceptible organs to primary tumours, metastasis, and a wide range of diseases of the hematopoietic system. Therefore, detailed assessment of the spleen is of great importance in veterinary medicine, especially in dogs, due to their economic and sentimental value, and as an experimental model in human medicine. Considering the recent advances in diagnostic imaging in companion animals, this review aims to describe the applicability of acoustic radiation force impulse (ARFI) elastography (qualitative and quantitative), Doppler, and contrast-enhanced ultrasonography for evaluating the spleen in dogs. The ARFI elastography is a recent ultrasound method that can provide reference values and aid in the diagnosis and evaluation of splenic abnormalities routinely encountered in veterinary practice. Conventional ultrasonography of the spleen combined with haemodynamic analysis by Doppler and contrast enhanced ultrasonography is an important tool in diagnosis and triage.

Keywords: B-mode, contrast enhanced ultrasonography, dog spleen, Doppler, elastography.

Introduction

Morphologic abnormalities of the spleen, including focal masses and diffuse alterations with or without overall splenic enlargement, are common in the dog (Couto, 2010). The spleen is the largest reticuloendothelial organ in mammals and one of the main sites for primary and metastatic tumours and diseases of the haematopoietic system (Morris and Dobson, 2007).

The diagnosis of splenic alterations is of particular interest due to its involvement in lymphatic, immune, circulatory, and haematopoietic functions (Fry and McGavin, 2007).

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

Nodular splenic disease is also common and can be caused by non-neoplastic (focal nodular hyperplasia, haematoma, infarct, or abscess) or neoplastic processes (haemangiosarcoma, lymphoma, malignant histiocytosis, or other mesenchymal tumours; Buergelt *et al*., 2001).

Ultrasonography is a useful imaging tool to evaluation of splenic abnormalities such as splenomegaly and changes in parenchyma echogenicity and echotexture (Feliciano et al., 2015a). Focal splenic lesions are frequently identified during routine ultrasonographic examination, especially in older animals. These are often incidental findings and represent a diagnostic challenge (Mattoon and Nyland, 2015). However, conclusive diagnosis of various splenic pathologies can only be obtained by histopathological analysis of samples collected via an ultrasound-guided biopsy or after splenectomy (Rodaski and Piekarz, 2009).

Currently, there is an increasing interest in non-invasive diagnostic procedures using novel ultrasonography-based imaging modalities (Maronezi et al., 2015b). B-mode, Doppler, elastography [strain, shear wave, and acoustic radiation force impulse (ARFI)], and contrast-enhanced ultrasonography can be used in splenic evaluation. B-mode and Doppler ultrasound provide real time information on the vascular architecture and hemodynamic aspects of the vessels (Carvalho *et al*., 2008). The ARFI elastography is safe, non -invasive, and provides quantitative and qualitative measures of tissue rigidity with reduced inter-observer variability (Dudea *et al.,* 2011; Feliciano *et al*., 2014a). Microbubble contrast-enhanced ultrasound increases the Doppler signal and detects blood flows that are hardly detectable by traditional methods (Nogueira *et al.,* 2002). Considering the importance of these imaging techniques, this review aims to describe the main applicability of each ultrasonographic method in the evaluation of the canine spleen.

B-mode ultrasonography

Ultrasonographic examination of the splenic parenchyma in dogs requires clipping the abdominal hair to facilitate transcutaneous ultrasonography (Feliciano *et al.,* 2007). The procedure should be performed with the animal in fasting state, following antiphysetics administration (Feliciano *et al.,* 2015a). Dorsal or lateral recumbence may be used, and curvilinear, convex or linear transducer (5 to 10 MHz frequency) is routinely used, depending on the size of the animal. The long axis of the spleen has a tongue-like shape and the cross-section a triangular or lenticular form. The cranial aspect is in dorsal location and visible between the gastric fundus and the left kidney (Hecht and Mai, 2015). In dogs, the splenic parenchyma has a homogeneous echotexture and is covered by a thin and very hyperechoic capsule. It is generally

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

hyperechoic to the liver and renal cortices (Matton and Nyland 2015). Branches of the splenic vein appear as tubular anechoic structures within the splenic parenchyma and can be seen leaving the spleen at the hilus. Splenic arteries, on the other hand, are usually not visible unless Colour-Doppler imaging is used (Hecht and Mai, 2015).

Ultrasonographic examination of the spleen is clinically useful to determine the location, size, and presence of parenchymal abnormalities when pathological conditions are suspected. The main indications for ultrasonographic evaluation of the spleen are generalized splenomegaly, abdominal or splenic mass, trauma, and hemoperitoneum (Matton and Nyland, 2015).

The ultrasonographic assessment of splenic size is subjective. In dogs with splenomegaly, the borders of the spleen appear rounded and the organ extends caudally and to the right side of the abdomen (Hecht and Mai, 2015). Splenomegaly with homogeneous echotexture is a frequent finding in dogs sedated with acepromazine, thiopental (O'Brien *et al*., 2004), ketamine, and diazepam (Wilson *et al.,* 2004). The maximum thickness of a normal spleen before anaesthesia (acepromazine, thiopental) is 1.7 cm. Splenic enlargement is observed approximately 15 min after acepromazine administration, resulting in a maximum splenic thickness of 2.6 cm (Wilson *et al.,* 2004)

Ultrasonography has proven to be highly sensitive to detect focal parenchymal abnormalities in dogs. However, a large overlap exists between the ultrasonographic appearance and the aetiology of lesions and, thus, the ultrasonographic appearance of an individual lesion is rarely, if ever, pathognomonic of the underlying disease. Therefore, it is very difficult to confidently differentiate benign from malignant nodules based on ultrasonographic appearance alone, and thus tissue sampling is usually required for the diagnosis (Warren-Smith, 2012). Fine needle aspiration and Tru-cut needle biopsy are commonly used to differentiate between benign and malignant lesions. However, these minimally invasive techniques generally require sedation or general anaesthesia (Matton and Nyland, 2015).

Doppler ultrasonography

Among the recent advances in ultrasound technology, Doppler ultrasonography has achieved prominence for being able to assess the function of various organs (Carrillo *et al.,* 2012). Triplex Doppler ultrasound involves the simultaneous use of two-dimensional ultrasonography, colour Doppler, and pulsed wave Doppler. It is used to obtain anatomical data of vessels and functional data of blood flow, such as presence or absence, direction and speed of flow, making it the method of choice for assessing the vascularity of several organs, including the spleen (Pozor and McDonnell, 2004; Wood *et al.,* 2010; Souza *et al.,* 2014). The splenic vasculature consists of the splenic

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

artery and the splenic vein. The arterial branches are difficult to see during routine scans, but Doppler ultrasonography can help determine their location and patency. In healthy dogs, the branches of the splenic vein can be easily visualized near the hilum of the spleen, but their course into the splenic parenchyma can be followed only for a short distance (Matton and Nyland, 2015).

The use of Doppler ultrasonography in animals is recent and has shown promising results. It is effective for the hemodynamic evaluation of tissues and is particularly useful in the analysis of tissue neovascularization in splenic disorders, neoplasia, and torsion; the latter being characterized by the absence of blood flow (Gil *et al.,* 2015). Furthermore, Doppler ultrasonography is useful in diagnosing splenic vein thrombosis in abdominal inflammatory disease or other pathologic conditions that could lead to hypercoagulability. It is also the procedure of choice for distinguishing splenic congestion and inflammation from severe vascular compromise caused by splenic torsion or vascular thrombosis (Matton and Nyland, 2015).

Colour and power Doppler evaluation of blood flow in splenic masses do not allow differentiating between benign and malignant cases in dogs. However, the presence of a wide or tortuous vessel within the mass is suggestive of malignancy, as is the peritoneal effusion (Sharpley, 2012).

The main splenic characteristics evaluated by spectral Doppler ultrasonography are the presence or absence of neovascularization and the calculation of vascular indices: peak systolic velocity (PSV), end -diastolic velocity (EDV), and resistance index (RI = [PSV - EDV] / PSV) of the splenic artery (Feliciano *et al.,* 2013). These indices have an advantage over flow velocity colour Doppler measurements, which are independent of the angle of insonation (Nelson and Pretorius, 1988; Middleton *et al.,* 1989; Gumbsch *et al.*, 2002; Carvalho *et al.,* 2008; Figure 1). Evaluation of splenic blood flow by Doppler often reveals low flow resistivity with broad and continuous peak systolic flow, increased diastolic peak flow or full diastole, and peak systolic flow without a spectral window, similar to the spectral pattern of hepatic and renal arteries (Gil *et al.*, 2015).

Information of the Doppler technique is limited to the characterization of the splenic vascularization using pulsed Doppler tracing (Albernaz *et al.,* 2007; Santarém *et al.,* 2008). The reported vascular indices of the splenic artery in dogs with subclinical ehrlichiosis are PSV of 22.59 ± 8.07 cm/s, EDV of 5.25 ± 4.66 cm/s, and RI of 0.71 ± 0.14. These vascular indices are important in animals infected with haemoparasites and can be useful in the therapeutic monitoring of patients that lack clinical signs. It is important to mention that splenic artery Doppler scanning may be difficult due to respiratory and intentional movements of the patient, which hinder the tracing and prolong the examination time (Maronezi *et al.,* 2015a).

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

Elastography

Currently, there is an increasing interest in the non-invasive assessment of viscoelastic properties of internal organs and tissues with novel ultrasonography-based imaging modalities (Maronezi *et al*., 2015b). In human medicine, new imaging techniques, such as elastography, have been developed. This ultrasound method is the technical evolution of one of the oldest medical tools, palpation; and thus evaluates the shape and stiffness of the organ of interest (Ophir, 2002).

To evaluate tissue stiffness, several methods have been proposed and grouped as elastographic techniques, among them stands out: Strain rate by manual compression, acoustic radiation force impulse (ARFI), and real-time shear velocity (RSV; Dudea *et al*., 2011) . Additionally, tissue hardness can be categorized qualitatively (e.g. strain rate, strain intensity) or quantitatively (e.g. shear wave velocity; Feliciano *et al*., 2015a).

In qualitative assessment, the tissue is subjected to a force (automatic or manual) and the image software measures tissue response to this force, creating an image known as elastogram (black and white or colourful scales). On applied form, the harshest tissue deforms less by compression and is classified in the scale as hard, while the less stiff has greater deformation and classifies as soft (Feliciano *et al*., 2015a). These techniques are able to detect movements, compression and extension, of the tissue along the axis. It can be performed in real time or off-line, but in clinical practice are commonly applied in real time. Ultrasound requires specific software adapted for evaluating the physical properties of a tissue, determining its relative degree of hardness compared with adjacent tissues through local compression. (Carvalho *et al*., 2013).

The Compression elastography technique applies a manual or automatic mechanical pressure with the transducer, and the software measures the resulting deformation. This technique is based on tissue tension estimated by the time "delay" between ultrasound signals during pre and post-compression. The stress caused by compression typically changes according to depth (i.e. stress and elasticity decrease when depth increases). Thus, tissue depth becomes a limiting factor for this technique, particularly for assessing deep organs or obese patients (Carvalho *et al*., 2015; Feliciano *et al*., 2015a). Furthermore, in this technique the acquisition and interpretation of elastography image data is largely dependent on the experience of the examiner (Burnside *et al*., 2007; Chang *et al*., 2011). As an advantage, this technique is commercially available, and it has shown promising results and clinical utility in human medicine (Carvalho *et al*., 2015; Feliciano *et al*., 2015a).

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

The ARFI elastography is an imaging technique that provides tissue stiffness by means of qualitative and quantitative measurements with a reduced inter-observer variability (Feliciano *et al*., 2015b). In qualitative ARFI evaluation, short acoustic pulses at high intensity are used to deform tissue elements and create a static greyscale map (elastogram) of the relative tissue stiffness. Generally, lighter areas represent more pliable tissues (Goddi *et al.,* 2012; Figure 2). Using this technique, measurements are more objective, because it avoids the variation of compression force applied by the operator. Thus, the method becomes more accurate with less inter-observer variation and greater reproducibility (Carvalho *et al*., 2013). The elastography quantitative analysis can be performed by two different methods that require specific equipment and software: ARFI elastography imaging and supersonic shear wave imaging. In quantitative ARFI, the primary acoustic impulse directed towards the region of interest (ROI) promotes forming pressure waves capable of deforming the tissue, thus increasing the velocity of wave propagation (shear velocity). Wave velocity and the attenuation of acoustic pressure waves are related to tissue stiffness and viscoelasticity (Comstock, 2011; Figure 3). The shear wave velocity obtained by this method will be faster in hard tissues than in soft tissues. Also, the software may provide a color coded image displaying the shear wave velocity (m/sec) or elasticity (kPa) for each defined ROI and a variety of stiffness parameters may be measured, including the mean, maximum, and standard deviation of stiffness (Sebag *et al*., 2010; Bhatia *et al*., 2012). The ARFI elastography has several advantages over other techniques; this evaluation can be combined with routine real time ultrasound and moreover, due to its greater penetration compared with mechanical compression, this technique is of great help in ascites and obese patients. However, the high cost of the equipment and training for ARFI limits its clinical application (Carvalho *et al*., 2013).

In veterinary medicine, the ARFI technique is a recent development and has been used to evaluate focal liver lesions in rats (Carvalho *et al.,* 2012), mammary tumours in bitches (Feliciano *et al.,* 2014a), and queens (Feliciano *et al*., 2015c), prostate and testes in dogs (Feliciano *et al.,* 2015b), kidneys (Garcia *et al,* 2015), testes (Brito *et al*., 2015), and spleen in cats (Feliciano *et al.,* 2014b), spleen, liver, and kidneys in adults dogs (Holdsworth *et al.*, 2014), and spleen in healthy dogs of different age (Maronezi *et al.*, 2015b).

The manual compression elastography and ARFI techniques were related for evaluating canine splenic stiffness (Holdsworth *et al*., 2014; Jeon *et al.*, 2015; Maronezi *et al*., 2015b). Qualitative evaluation reported by Jeon *et al*. (2015) was performed on the colour elastogram and the calculated splenic strain intensity was compared with abdominal wall strain intensity, resulting in the splenic strain rate that may be useful for normal and abnormal spleen evaluation. A preliminary study published by

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

Holdsworth *et al*. (2014) determined the quantitative elastographic characteristics of the normal liver, spleen, and kidneys in 15 healthy dogs at predetermined depths within the parenchyma. The values obtained for the spleen were 1.59 to 2.4 m/s at 0 to 2 cm deep and 1.45 to 1.94 m/s at 2 to 4 cm. They observed that depth had a significant negative correlation with shear wave velocity. Furthermore, Maronezi *et al*. (2015) examined healthy splenic segments by qualitative elastography in dogs and related normal mean shear velocity values for the head (2.32 m/s), body (2.16 m/s), and tail (2.25 m/s) of the spleen, and did not find differences between age groups (Maronezi *et al.,* 2015b).

Quantitative and qualitative ARFI elastography of the spleen in dogs is easily performed and may provide additional criteria for the evaluation and diagnosis of splenic abnormalities typically obtained by B-mode ultrasonography. However, some patients may not cooperate, move and disrupt the acquisition of measurements. In these cases sedation may be necessary to obtain an appropriate assessment. Researchers suggest keeping subjects in a quiet environment with minimum restraint (Feliciano *et al.,* 2014b; Garcia *et al.,* 2015).

Contrast-enhanced ultrasonography

Contrast-enhanced ultrasonography (CEUS) is an innovative method that improves detection of perfusion and vascularity of organs (Nyman *et al.*, 2005). Ultrasound contrast agents are tiny bubbles of gas that are too small to be caught in the capillary bed, but sufficiently large to enhance the reflection of flowing blood. High molecular weight gases, such as perfluorocarbons, are used for the microbubbles. Microbubbles measure between 1 and 6 μm in diameter, a size that enables free passage through all capillary beds. The advantage of ultrasound contrast agents vs contrast agents used in magnetic resonance or computed tomography is that the bubbles behave in the same way as red blood cells and do not diffuse out of the vascular space (Kalantarinia and Okusa, 2007).

Ultrasound waves directed at the vasculature are scattered upon interaction with intravenously-infused contrast agents, resulting in a bright image of the vasculature. Therefore, using contrast agents with ultrasound enables detecting microcirculation changes, improves Doppler imaging in tissues with slow blood flow rates, and provides more detailed information on vasculature structure during imaging in B-mode. Ultrasound contrast agents have proven to be safe and free of hemodynamic effects, making them ideal for the study of tissue blood flow, micro and macrovascular flow patterns, velocity and perfusion (Lindner, 2002). Ultrasonography evaluation with contrast agent takes about 20 min (Haers and Saunders, 2009).

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

Microbubble contrasted ultrasound has been considered in some cases to be superior to magnetic resonance imaging and computerized tomography (contrasted) in the assessment of focal nodules in parenchymal organs in humans. Furthermore, its use does not require anaesthesia, there is no radiation emission, and it provides real-time images (Haers and Saunders, 2009). In humans, this technique has been used in testicular diseases to obtain higher diagnostic accuracy when ultrasound findings are inconclusive (Souza *et al.,* 2014).

Microbubble contrasted ultrasound is a new technique in veterinary medicine and has been used to evaluate liver (O'Brien, 2007), spleen (Ohlerth *et al*., 2007), abnormal spleen (Ivancic-Arndt *et al.,* 2007; Maronezi *et al.,* 2015a), kidneys (Waller *et al*., 2007), pancreas (Rademacher *et al.,* 2015), lymph nodes (Schärz *et al.,* 2005), and portosystemic shunts in dogs (Salwei *et al.,* 2003).

The information obtained by contrast examination enables to establish parameters related to homogeneous or heterogeneous tissue filling (Volta *et al.,* 2014). Furthermore, in conjunction with microbubble contrast-enhanced ultrasonography, B-mode is used to determine wash-in, wash-out and peak enhancement time of the contrast in the tissue, which was correlated with tissular perfusion (Takeda *et al.,* 2012).

The microvascular fill pattern of contrast-enhanced ultrasonography enables detecting small masses at early stages of evolution and hypervascularization in aggressive tumours, aiding in non-invasive differentiation of malignant and benign tumours (Lock *et al.,* 2009).

Contrast-enhanced ultrasonography of the spleen revealed mean wash-in time in healthy dogs to be 13.2 s and peak enhancement time 29.8 ± 11.6 s (Ohlerth *et al.,* 2007; Figure 4). In humans, wash-in time is 12.0 s and approximately 50 s after contrast injection the splenic parenchyma becomes homogeneous (peak enhancement) and a dense enhancement persists for 5 to 7 min (wash out; Catalano *et al.*, 2004).

Contrast-enhanced ultrasonography evaluation of the spleen in dogs with subclinical ehrlichiosis revealed wash-in time of 5.31 ± 0.7 s, peak enhancement time of 18.56 ± 2.90 s, and wash-out time of 94.56 ± 35.21 s; which were lower than those reported in the literature (Maronezi *et al.,* 2015a; Figure 5).

Non-invasiveness, no anaesthesia, and real-time evaluation are the main advantages of contrast-enhanced ultrasonography. The disadvantages are the equipment requirements and high cost of ultrasound contrast agents. Additionally, pulmonary contrast elimination may limit its use in patients with pulmonary diseases. Some

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

adverse effects of contrast were observed in humans (headaches, diarrhea, neutropenia, nausea, skin reactions, dyspnoea, rhinorrhagia), but not in small animals (Wdowiak *et al*., 2010). In experimental studies with sheep, dexamethasone was used to prevent pseudo-anaphylaxis and pulmonary hypertension due to acute and transient reactions (right ventricular dilatation and pulmonary pressure increase) reported in pigs after contrast administration (Antoine *et al.,* 2014).

In conclusion, this review provides information on the ultrasonographic methods used for splenic exam in dogs. Conventional ultrasonography is useful for providing information on splenic parenchyma while Doppler is emerging as an important and suitable non-invasive tool for blood flow assessment. The ARFI elastography of the spleen in dogs may provide valuable criteria for the evaluation and diagnosis of splenic abnormalities, and the specificity of this imaging technique could aid differentiating malignant splenic neoplasias. Contrast-enhanced ultrasonography can also contribute to evaluate changes in the splenic tissue, perfusion and malignant differentiation.

References

1. Bhatia KS, Tong CS, Cho CC, Yuen EH, Lee YY, Ahuja AT. Shear wave elastography of thyroid nodules in routine clinical practice: Preliminary observations and utility for detecting malignancy. Eur Radiol 2012; 22:2397-2406.
2. Brito MBS, Feliciano MAR, Coutinho LN, Uscategui RR, Simoes APR, Maronezi MC, Almeida VT, Crivelaro RM, Gasser B, Pavan L, Vicente WRR. Doppler and contrast-enhanced ultrasonography of testicles in adults domestic felines. Reprod Domest Anim 2015; 50:730-734.
3. Buergelt CD. Nodular splenic disease in dogs. Vet Med 2001; 96:766-773.
4. Couto CG, Hammer AS. Diseases of the lymph nodes and the spleen. In: Ettinger SJ, Feldman EC, editors. Textbook of veterinary internal medicine. 4th ed. Philadelphia: W.B. Saunders Company; 2010. p. 1930-1946.
5. Carrillo J, Soler M, Lucas X, Agut A. Colour and pulsed Doppler ultrasonographic study of the canine testis. Reprod Domest Anim 2012; 47:655-659.
6. Carvalho CF, Chammas MC, Cerri G. Princípios físicos do Doppler em ultrassonografia. Ciênc Rural 2008; 38:872-879.
7. Carvalho CF, Chammas MC. Elastography – a new technology associated with ultrasonography. Clín Veterinária 2013; 17:62-70.
8. Comstock C. Ultrasound elastography of breast lesions. Ultrasound Clin 2011; 6:407-415.
9. Dudea SM, Giurgiu CR, Dumitriu D. Value of ultrasound elastography in the diagnosis and management of prostate carcinoma. Med Ultrason 2011; 13:45-53.

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

10. Feliciano MAR, Garcia PHS, Vicente WRR. Introdução à ultrassonografia. In: Feliciano MA, Canola JC, Vicente WRR, editors. Diagnóstico por imagem em cães e gatos. 1st ed. São Paulo: MedVet; 2015a. p. 53-57.
11. Feliciano MAR, Maronezi MC, Simões APR, Uscategui RR, Maciel GS, Carvalho CF, Canola JC, Vicente WRR. Acoustic radiation force impulse elastography of prostate and testes of healthy dogs: Preliminary results. J Small Anim Pract 2015b; 56:320-324.
12. Feliciano MAR, Maronezi MC, Brito MS, Simões APR, Maciel GS, Castanheira TLL, Garrido E, Uscategui RR, Miceli NG, Vicente, WRR. Doppler and elastography as complementary diagnostic methods for mammary neoplasms in female cat. Arq Bras Med Vet Zootec 2015c; 67:935-939.
13. Feliciano MAR, Maronezi MC, Pavan L, Castanheira TL, Simões APR, Carvalho CF, Canola JC, Vicente WRR. ARFI elastography as complementary diagnostic method of mammary neoplasm in female dogs – preliminary results. J Small Anim Pract 2014a; 55:504-508.
14. Feliciano MAR, Maronezi MC, Crivellenti LZ, Crivellenti SB, Simões APR, Brito MBS, Garcia PHS, Vicente WRR. Acoustic radiation force impulse (ARFI) elastography of the spleen in healthy adult cats – a preliminary study. J Small Anim Pract 2014b; 56:180-183.
15. Feliciano MAR, Nepomuceno AC, Crivelaro RM. Foetal echoencephalography and Doppler ultrasonography of the middle cerebral artery in canine foetuses. J Small Anim Pract 2013; 54:149-152.
16. Feliciano MAR, Muzzi LAL, Leite CAL. Two-dimensional conventional, high resolution two-dimensional and three-dimensional ultrasonography in the evaluation of pregnant bitch. Arq Bras Med Vet Zootec 2007; 59:1333-1337.
17. Fry MM, Mcgavin MD. Bone marrow, blood cells, and lymphatic system. In: Mcgavin MD, Zachary JF. Pathologic basis of veterinary disease. 4th ed. St Louis: Mosby Elsevier; 2007:743-832.
18. Garcia PHS, Feliciano MAR, Carvalho CF, Crivellenti LZ, Maronezi MC, Almeida VT, Uscategui RR, Vicente WRR. Acoustic radiation force impulse (ARFI) elastography of kidneys in healthy adult cats: Preliminary results. J Small Anim Pract 2015; 56:505-509.
19. Gil MEU, Froes TR, Feliciano MAR Baço. In: Feliciano MAR, Canola JC, Vicente WRR. Diagnóstico por imagem em cães e gatos. 1st ed. São Paulo: Med Vet; 2015:579-601.
20. Goddi A, Bonardi M, Alessi S. Breast elastography: A literature review. J Ultrasound 2012; 15:192-198.
21. Haers H, Saunders JH. Review of clinical characteristics and applications of contrast-enhanced ultrasonography in dogs. J Am Vet Med Assoc 2009; 234:460-470.
22. Hecht S, Mai W. Spleen. In: Penninck D, D' Anjou MA. Atlas of small animal ultrasonograph. 2nd ed. Oxford: Wiley-Blackwell: 2015. p. 239-258.

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

23.Holdsworth A, Bradley K, Birch S. Elastography of the normal canine liver, spleen, and kidneys. Vet Radiol Ultrasound 2014; 55:620-627.
24.Hörmann M. Preface. In: Albrecht T, Thorelius L, Solbiati L, Cova L, Frauscher F. 1nd ed. Contrast-enhanced ultrasound in clinical practice: Liver, prostate, pancreas, kidney, and lymph nodes. Berlin, Germany: Springer; 2006.
25.Ivancic-Arndt M, Seiler G. Contrast harmonic ultrasound of splenic hemangiosarcoma and associated liver nodules in dogs. American College of Veterinary Radiology Annual Meeting 2007:19.
26.Jeon S, Lee G, Lee SK, Kim H, Yu D, Choi J. Ultrasonographic elastography of the liver, spleen, kidneys, and prostate in clinically normal beagle dogs. Vet Radiol Ultrasound 2015; 56:425-431.
27.Kalantarinia K, Okusa MD. Ultrasound contrast agents in the study of kidney function in health and disease. Drug Discov Today Dis Mech 2007; 4:153-158.
28.Lindner JR, Song J, Jayaweera AR, Sklenar J, Kaul S. Microvascular rheology of definity microbubbles after intra-arterial and intravenous administration. J Am Soc Echocardiogr 2002; 15:396-403.
29.Lock G, Schmidt C, Helmich F, Stolle E, Dieckmann KP. Early experience with contrastenhanced ultrasound in the diagnosis of testicular masses: A feasibility study. Urology 2009:1049-1053.
30.Maronezi MC, Feliciano MAR, Crivellenti LZ, Borin-Crivellenti S, Silva PES, Zampolo C, Pavan L, Gasser B, Simões APR, Maciel GS, Canola JC, Vicente WRR. Spleen evaluation using contrast enhanced. Arq Bras Med Vet Zootec 2015a; 67:1528-1532.
31.Maronezi MC, Feliciano MAR, Crivellenti LZ, Simões APR, Bartlewski PM, Gill I, Canola JC, Vicente WRR. Acoustic radiation force impulse elastography of the spleen in healthy dogs of different ages. J Small Anim Pract 2015b; 56:180-183.
32.Mattoon JS, Nyland TG. Spleen. Small animal diagnostic ultrasound. 3rd ed. St Louis: Elsevier; 2015. p. 400-327.
33.Moris J, Dobson J. Tumores variados. Oncologia em pequenos animais. São Paulo: Roca, 2007. p. 272-278.
34.Nogueira AC, Morcerf F, Moraes AV, Carrinho M, Dohmann H. Ultrassonografia com agentes de contrastes por microbolhas na avaliação da perfusão renal em indivíduos normais. Rev Bras Ecocard 2002; 15:74-78.
35.O'Brien RT. Improved detection of metastatic hepatic hemangiosarcoma nodules with contrast ultrasound in three dogs. Vet Radiol Ultrasound 2007; 48:146-148.
36.Ohlerth S, Rüefli E, Poirier V. Contrast harmonic imaging of the normal canine spleen. Vet Radiol Ultrasound 2007; 48:451-456.
37.Ophir J, Alam KS, Garra BS. Elastography: imaging the elastic properties of soft tissues with ultrasound. J Med Ultrason 2002; 29:155-171.
38.Rademacher N, Schur D, Gaschen F, Kearney M. Contrast-enhanced ultrasonography of the pancreas in healthy dogs and in dogs with acute pancreatitis. Vet Radiol Ultrasound 2015; 57:58-64.

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.

39.Rodaski S, Piekarz CH. Diagnóstico e estadiamento clínico. In: Daleck CR, De Nardi A B, Rodaski S. Oncologia em cães e gatos. 11th ed. São Paulo: Roca; 2009 p.52-73.
40.Salwei RM, O'Brien RT, Matheson JS. Use of contrast harmonic ultrasound for the diagnosis of congenital portosystemic shunts in three dogs. Vet Radiol Ultrasound 2003; 44:301-305.
41.Schärz M, Ohlerth S, Achermann R. Evaluation of quantified contrast-enhanced color and power Doppler ultrasonography for the assessment of vascularity and perfusion of naturally occurring tumours in dogs. AJVR 2005; 66:21-29.
42.Schneider AG, Calzavacca P, Schelleman A, Huynh T, Bailey M, May C, Bellomo R. Contrast-enhanced ultrasound evaluation of renal microcirculation in sheep. Intensive Care Med Exp 2014; 2:1-14.
43.Sharpley JL, Marolf AJ, Reichle JK, Bachand AM, Randall EK. Color and power Doppler ultrasonography for characterization of splenic masses in dogs. Vet Radiol Ultrasound 2012; 53:586-590.
44.Souza MB, Mota Filho AC, Sousa CVS, Monteiro CLB, Carvalho GG, Pinto JN, Linhares JCS, Silva LDM. Triplex Doppler evaluation of the testes in dogs of different sizes. Pesq Vet Bras 2014; 34:1135-1140.
45.Takeda CSI, Carvalho CF, Chammas MC. Ultrassonografia contrastada na medicina veterinária – revisão. Clín Veterinária 2012; 17:08-114.
46.Volta A, Manfredi S, Vignoli M, Russo M, England G, Rossi F, Bigliardi E, Di Ianni F, Parmigiani E, Bresciani C, Gnudi G. Use of contrast-enhanced ultrasonography in chronic pathologic canine testes. Reprod Domest Anim 2014; 49:202-209.
47.Waller K R, O'Brien RT, Zagzebski JA. Quantitative contrast ultrasound analysis of renal perfusion in normal dogs. Vet Radiol Ultrasound 2007; 48:373-377.
48.Warren-Smith CMR, Andrew S, Mantis P, Lamb CR. Lack of associations between ultrasonographic appearance of parenchymal lesions of the canine liver and histological diagnosis. J Small Anim Pract 2012; 53:168-173.
49.Wdowiak M, Rychlik A, Nieradka R, Nowicki M. Contrast-enhanced ultrasonography (CEUS) in canine liver examination. Pol J Vet Sci 2010; 13:767-773.

ISBN: 978-608-66191-0-7
IMSC-2018, May 26, 2018. Tetovo. Macedonia.