

CASE REPORT

ISSN 1679-9216

Hemangiosarcoma in the Vastus Lateralis Musculature of a Bitch - Sonographic Findings

Rafael Kretzer Carneiro 1, Bruna Lima 2, Igor Cezar Kniphoff da Cruz 2, Brenda Santos Pompeu de Miranda 4, Bruno Watanabe Minto 2 & Marcus Antônio Rossi Feliciano 3

ABSTRACT

Background: A 10-year-old spayed bitch with hip dysplasia was referred for periarticular ultrasound evaluation. A poorly marginated structure of mixed echogenicity, predominantly hypoechoic, with heterogeneous echotexture, was identified in the vastus lateralis muscle of the right pelvic limb with increased stiffness, assessed using acoustic radiation force impulse (ARFI) elastography, compared to that of the adjacent muscle tissues, and intense vascularization by pulsed Doppler and contrast-enhanced ultrasound (CEUS). Guided biopsy was performed to confirm the diagnosis of hemangiosarcoma. The objective of this study was to describe B-mode ultrasound, CEUS, and ARFI findings of hemangiosarcoma in the vastus lateralis musculature of a bitch.

Case: A 10-year-old spayed bitch Golden Retriever weighing 36 kg was evaluated for pelvic limb lameness. The patient presented with bilateral pain during hip extension. The primary diagnostic suspicion was degenerative joint disease and secondary hip dysplasia. The patient was referred for imaging evaluation of the hip joints (radiography and ultrasound) under anesthesia. Right lateral and ventrodorsal projections with the pelvic limbs extended were obtained to confirm the diagnosis. In the right pelvic limb, an amorphous hypoechoic structure with irregular contours and heterogeneous echotexture was observed in the vastus lateralis muscle. In ARFI elastography, it was possible to identify differences in tissue stiffness between healthy and compromised portions. Pulsed-wave Doppler evaluation demonstrated an arterial waveform pattern with a peak systolic velocity of 38.8 cm/s, end-diastolic velocity of 6.9 cm/s, pulsatility index of 1.76, and resistive index of 0.82. CEUS study identified a mean peak of 27.26 %, mean time to peak of 39.95 s, and mean transmission time of 49.96 s. The populateal lymph node was hyperechoic and heterogeneous in B-mode. In ARFI elastography, the average stiffness was 2.52 m/s, and the CEUS obtained an average peak of 19.98%, average time to peak of 17.52 s, and mean transit time of 22.83 s. Doppler assessment revealed no clear vascularization in the lymph node. Thoracic radiography in 3 projections and abdominal ultrasonography were performed. Radiographic evidence of pulmonary nodules was not observed. On abdominal ultrasound evaluation, it was possible to identify changes in the spleen, which presented with splenomegaly, mixed echogenicity, and heterogeneous echotexture. The animal underwent ultrasound-guided muscle biopsy, which confirmed the presence of hemangiosarcoma in the muscle. The bitch was referred for splenectomy and lymphadenectomy of the right popliteal, which confirmed neoplastic involvement of the same neoplasm.

Discussion: On B-mode ultrasound, the observed changes were similar to those in a report of hemangiosarcoma in the muscles of the chest wall in dogs, which contributed to the diagnosis. Although it was only one patient, ARFI elastography results suggest that muscle hemangiosarcoma tends to follow the same elastographic characteristics as malignant lesions in other tissues. Additionally, the popliteal lymph node had a stiffness suggestive of malignancy (mean 2.52 m/s) because it presented a similar result and greater elasticity of metastatic axillary lymph nodes in bitches (>2.5 m/s) and women (> 1.44 m/s) with breast tumor. With CEUS, it was possible to identify vascularization in the sentinel lymph node that was not visible by pulsed Doppler, and in muscle mass. The findings of this report provided relevant results on muscle hemangiosarcoma in a bitch and demonstrated that the information obtained with the association of imaging methods supported the malignancy criteria described in other studies.

Keywords: canine, imaging, diagnosis, neoplasm.

DOI: 10.22456/1679-9216.126548

Received: 10 March 2023 Accepted: 10 July 2023 Published: 5 August 2023

¹Department of Veterinary Medicine, Universidade do Estado de Santa Catarina (UDESC), Lages, SC, Brazil. ²Department of Veterinary Surgery, Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil. ³Department of Veterinary Medicine, Universidade de São Paulo (USP), Pirassununga, SP. CORRESPONDENCE: M.A.R. Feliciano [marcusfeliciano@yahoo.com.br]. Department of Veterinary Medicine - USP. Rua Duque de Caxias n. 225. Jardim Elite. CEP 13635-000 Pirassununga, SP, Brazil.

INTRODUCTION

Hemangiosarcoma (HSA) is a malignant mesenchymal neoplasm with high metastatic potential that affects dogs [3,28]. HSA can affect any tissue, with the spleen being the most common primary site [28,29]. Although rare, muscle metastases have been reported in some patients [3,25], but without a literary description about the use of advanced ultrasound techniques such as elastography, contrasted ultrasound (CEUS) and Pulsed-wave Doppler, to evaluate this neoplasm and its metastases.

ARFI elastography makes it possible to differentiate morphostructurally affected tissues from normal tissues by changing tissue stiffness in a painless and non-invasive way [5]. This technique has been explored for various clinical purposes and routinely introduced for specific uses, such as evaluation of metastatic lymph nodes in humans [27] and characterization of malignancy of skin tumors in dogs [5].

CEUS assists in the assessment of tissue vascularization and perfusion by detecting neoplastic vascular changes not identified by B-mode ultrasound [17]. In medicine, CEUS contributed to the differentiation of benign and malignant nodules, demonstrating its contribution to diagnosis and therapy [19]. Furthermore, changes in systolic and diastolic values and pulsatility index by pulsed Doppler mode have been described as quantitative criteria for malignancy in neoplasms in dogs [6,10].

The aim of this work is to describe new findings of B-mode ultrasound, ARFI elastography, pulsed Doppler and CEUS in SAH in the vastus lateralis of a bitch, as well as the correlation with imaging findings with the sentinel lymph node.

CASE

A 10-year-old spayed bitch, Golden Retriever, weighing 36 kg, was referred to a Veterinary Medical Teaching Hospital - UNESP with lameness of the pelvic limbs. In the physical evaluation, the patient presented bilateral pain during hip extension. Other physiological parameters were within normal parameters. Based on the orthopedic evaluation, the main diagnostic suspicion was degenerative joint disease secondary hip dysplasia. A blood sample was taken for a complete blood count, as well as renal and hepatic profiles (alkaline phosphatase, alanine transaminase, creatinine, urea) and the patient was referred to an echocardiographic evaluation. No alterations were

found in any of these evaluations. The patient was referred to imaging evaluation of the hip joints (radiography and ultrasound) under anesthesia.

For the radiographic examination¹, the patient was positioned on the right lateral and ventrodorsal projections with the pelvic limbs extended. Radiographic findings were a slight remodeling of the head and thickening of the femoral neck, sclerosis of the subchondral bone in the cranial acetabulum confirming hip osteoarthrosis. For the evaluation of the periarticular structures, the patient was referred to musculoskeletal ultrasonography. During the B-mode² evaluation, a linear high-density transducer was used at a c and for the elastographic² and contrast-enhanced² study, a linear transducer of 9.0 M Hz was used.

The hips were shaved acoustic gel was applied prior to the sonographic evaluation. Scanning was performed in longitudinal and transversal views, with the patient positioned in lateral recumbency. The B-mode findings were irregularities in the bone surface of the cranial acetabulum and femoral head bilaterally. In the right pelvic limb, an amorphous hypoechoic structure with irregular contours and heterogenous echotexture was found in the vastus lateralis muscle, causing a loss in the muscle fibers and perimysium patterns (Figure 1A). This structure presented 3.97 cm (length) and 1.71 cm (height) [Figure 1B].

In the ARFI elastography² (virtual touch tissue imaging quantification, 2D-Shear-wave technique), 8 regions of interest (ROIs) of healthy tissue and 6 ROIs of the lesion area were selected where it was possible to identify a difference in tissue stiffness between the healthy portion (mean 1.65 m/s) and the compromised portion (mean 2.45 m/s) [Figure 1C]. Pulsed-wave Doppler² evaluation was then performed and vascularization present demonstrated an arterial waveform pattern with peak systolic velocity (PSV) of 38.8 cm/s, end-diastolic velocity (EDV) of 6.9 cm/s, pulsatility index (PI) of 1.76 and resistive index (RI) of 0.82 (Figure 1D). For the contrast-enhanced ultrasound² (CEUS) study, catheterization of the cephalic was performed with a 20G catheter and then the contrast medium³ (0.01 mL/kg) was applied intravenously and the perfusion process was evaluated in real time with the software⁴ available in the ultrasound equipment for 180 s in an average area of 1 mm² (Figure 1E). It was possible to identify mean peak of 27.26%, mean time to peak 39.95 s, mean transmission time of 49.96 s (Figure 1F).

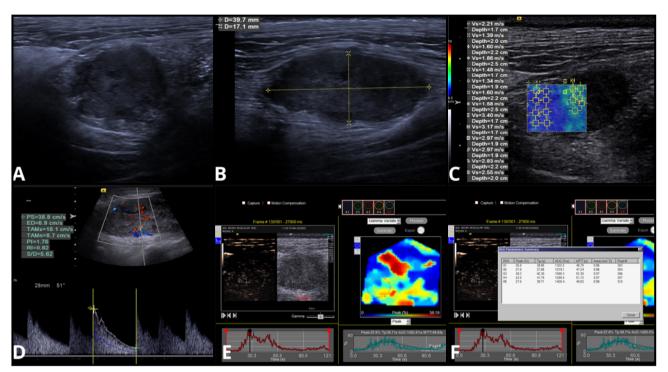


Figure 1. Amorphous hypoechoic structure with irregular contours and heterogenous echotexture in the vastus lateralis muscle causing a loss in the muscle fibers (A) measuring 3.97 cm [length] and 1.71 cm [height] (B). ARFI elastography demonstrating difference in tissue stiffness between the healthy portion [mean 1.65 m/s] and the compromised portion [mean 2.45 m/s] (C). Pulsed-wave Doppler evaluation demonstrating arterial waveform with peak systolic velocity (PSV) of 38.8 cm/s, end-diastolic velocity (EDV) of 6.9 cm/s, pulsatility index (PI) of 1.76 and resistance index (RI) of 0.82 (D). Evaluation of the perfusion process in real time for 180 s in an average area of 1mm² (E) with mean peak of 27.26%, mean time to peak 39.95 s, mean transmission time of 49.96 s (F).

After the evaluation of the muscle lesion, the sentinel lymph node (popliteal) was evaluated in B-mode, elastography ARFI, CEUS and pulsed doppler. In B-mode², the lymph node was hyperechoic and heterogeneous (Figure 2A) measuring approximately 0.95 cm (length) by 0.83 cm (height) [Figure 2B]. Using ARFI Elastography², in 6 selected ROIs, the average stiffness was 2.52 m/s (Figure 2C) and the CEUS² obtained an average peak of 19.98%, average time to peak 17.52 s, mean transit time of 22.83 s (Figure 2D). Color-coded Doppler² assessment was performed and no clear vascularization was present in the lymph-node, therefore, it was not possible to perform Pulsed-wave Doppler evaluation of the lymph node.

Due to the suspicion of muscle neoplasm, the patient was referred for tumor staging. Thoracic radiographic evaluation was performed in three projections and abdominal ultrasonography was performed. No radiographic evidence of pulmonary nodules was present. On the abdominal ultrasound evaluation, it was possible to identify changes in the spleen, which presented splenomegaly, mixed echogenicity and heterogenous echotexture.

Based on the imaging findings, the animal was submitted to ultrasound-guided muscle biopsy with a local anesthetic of lidocaine with no vasoconstrictor⁵ (7 mg/kg), which confirmed by histopathology the presence of hemangiosarcoma in the vastus lateralis muscle. After 7 days of the procedure, the bitch was referred for splenectomy and lymphadenectomy of the right popliteum, which confirmed neoplastic involvement by the same neoplasm. Instructions were given to the tutor to initiate a chemotherapic protocol, but to this date, the tutor has not initiated the indicated therapeutic protocol.

DISCUSSION

To the author's knowledge, no reports were found describing findings of elastography ARFI, contrast-enhanced ultrasound (CEUS) and Pulsed-wave Doppler of muscle hemangiosarcoma in dogs. Musculoskeletal ultrasonography has been commonly used in equine [8,30] and human medicine [1,20,23] and has become increasingly present in small animals clinical practice [2,22]. In this report, the ultrasound examination and the association of ultrasound techniques brought unprecedented and important information

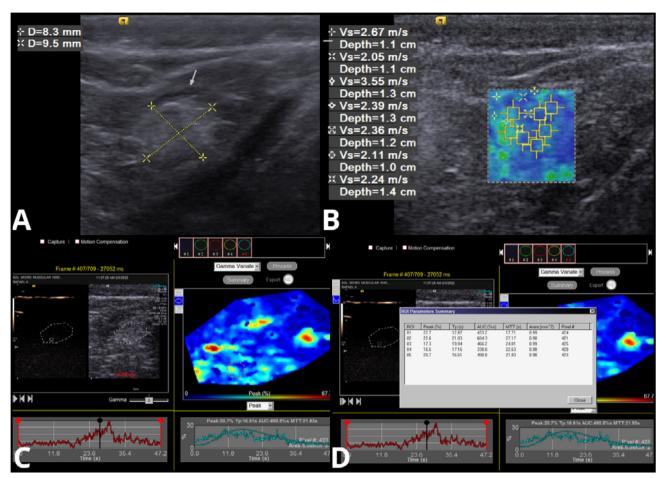


Figure 2. Popliteum lymph node hyperechoic and heterogeneous [white arrow] (A) measuring 0.95 cm [length] by 0.83 cm [height] (B). ARFI elastography, in 6 selected ROIs, average stiffness of 2.52 m/s (C) and the CEUS with average peak of 19.98%, average time to peak 17.52 s, mean transit time of 22.83 s (D).

for decision making and correct diagnosis of muscle neoplasia.

In dogs, muscle metastases are mainly diagnosed by biopsy [25] and computed tomography [3], but information on the sonographic characteristics of hemangiosarcoma is scarce. On B-mode ultrasound, the observed changes were similar to a report of hemangiosarcoma described in muscles of the chest wall in dogs [9], which contributed to the diagnosis. Increased echogenicity of the popliteal lymph node is relevant information, as healthy and metastatic lymph nodes in dogs are usually hypoechogenic [4,13,26]. The authors believe that the diagnostic moment and pathological processes involved, such as inflammation, tissue necrosis and the type of tumor [11] are factors that affected the echographic aspect of the evaluated lymph node.

ARFI Elastography is a noninvasive and non-operator-dependent technique that has shown an increase in stiffness in malignant lesions in dogs [5,18] and humans [7,21]. Although it is only 1 patient, ARFI

elastography results suggested that muscle hemangiosarcoma tends to follow the same elastographic character of malignant lesions of other tissues, as there was an increase in the shear-wave velocity of the tumor area compared to healthy tissue. In addition, the popliteal lymph node had a stiffness suggestive of malignancy (mean 2.52 m/s) because it presented a similar result and greater elasticity of metastatic axillary lymph nodes in bitches (> 2.5 m/s) [26] and women (> 1.44 m/s) [27] with breast tumor, respectively.

On pulsed Doppler, it was possible to verify vascularization in the muscle structure with PI, PS and ED suggestive of malignancy. The increase in PI (> 0.93) has been associated with malignancy in cutaneous and subcutaneous neoplasms in dogs [5] whereas the increase in systolic (> 21.2 m/s) and diastolic (> 4.8 m/s) values are quantitative criteria for malignancy in female dogs with mammary tumors [10]. The authors corroborate that the alterations found on Doppler may be correlated with the biology of hemangiosarcoma because it

is a neoplasm of vascular origin [12]. In addition, it is possible that this fact may interfere and accelerate the process of angiogenesis and formation of arteriovenous shunts, generating turbulent flows as already observed in human thyroid tumors [14].

Contrast-enhanced ultrasonography has shown important results in the diagnosis of breast neoplasms and renal alterations in dogs [6,10] allowing the evaluation of tissue perfusion, especially microcirculation [16,31]. With this technique, it was possible to identify vascularization in the sentinel lymph node, not visible by pulsed doppler, and in muscle mass. However, the qualitative data obtained did not help in the diagnosis of malignancy, since they presented characteristics of both aggressive and benign lesions when compared to healthy cervical lymph nodes affected by nasopharyngeal carcinoma in humans [15] and aggressive and benign ovarian lesions in women [24].

The findings of this report brought relevant results on muscle hemangiosarcoma in a bitch and

demonstrated that the information obtained with the association of imaging methods supported the malignancy criteria already described in other studies. Thus, although tumor diagnosis and characterization are based on histology, alternative imaging tests are increasingly necessary to assist in making surgical and therapeutic decisions.

MANUFACTURERS

¹Agfa-Gevaert Group. Mortsel, Belgium.

²Siemens. Munich, Germany.

³Bracco Imaging do Brasil Importação e Distribuição de Medicamentos Ltda. São Paulo, SP, Brazil.

⁴Cadence Design Systems, San Jose, CA, USA.

⁵Cristália Produtos Químicos Farmacêuticos. Fortaleza, CE, Brazil

Acknowledgements. The authors would like to thank the Higher Education Personnel Improvement Coordination (CAPES) and National Council for Scientific and Technological Development (305182/2020-0).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

REFERENCES

- 1 Barbuto L., Di Serafino M., Della V.N., Rea G., Esposito F., Vezzali N., Ferro F., Caprio M.G., Vola E.A., Romeo V. & Vallone G. 2019. Pediatric musculoskeletal ultrasound: a pictorial essay. *Journal of Ultrasound*. 22(4): 491-502. DOI: 10.1007/s40477-018-0337-v.
- **2 Barella G., Lodi M. & Faverzani S. 2018.** Ultrasonographic findings of shoulder tenomuscular structures in symptomatic and asymptomatic dogs. *Journal of Ultrasound*. 21(2): 145-152. DOI: 10.1007/s40477-017-0271-4.
- 3 Carloni A., Terragni R., Morselli-Labate A.M., Paninarova M., Graham J., Valenti P., Alberti M., Albarello G., Millanta F. & Vignoli M. 2019. Prevalence, distribution, and clinical characteristics of hemangiosarcoma-associated skeletal muscle metastases in 61 dogs: A whole body computed tomographic study. *Journal of Veterinary Internal Medicine*. 33(2): 812-819. DOI: 10.1111/jvim.15456.
- **4 Choi M., Yoon J. & Choi M. 2020.** Contrast-enhanced ultrasound sonography combined with strain elastography to evaluate mandibular lymph nodes in clinically healthy dogs and those with head and neck tumors. *The Veterinary Journal*. 257: 105447. DOI: 10.1016/j.tvjl.2020.105447.
- 5 Cruz I.C.K., Carneiro R.K., De Nardi A.B., Uscategui R.A.R., Bortoluzzi E.M. & Feliciano M.A.R. 2022. Malignancy prediction of cutaneous and subcutaneous neoplasms in canines using B-mode ultrasonography, Doppler, and ARFI elastography. *BMC Veterinary Research*. 18(10): 1-13. DOI: 10.1186/s12917-021-03118-y.
- 6 Cruz I.C.K., Gasser B., Maronezi M.C., Uscategui R.A.R., Feliciano M.A.R., Padilha-Nakaghi L.C., Aires L.P.N. & Silva P.D.A. 2021. Applicability of B-mode ultrasonography, ARFI elastography and contrast-enhanced ultrasound in the evaluation of chronic kidney disease in dogs. *Pesquisa Veterinária Brasileira*. 41: 1-7. DOI: 10.1590/1678-5150-PVB-6785.
- 7 D'Onofrio M., Crosara S., De Robertis R., Canestrini S., Demozzi E. & Pozzi R.M. 2014. Elastography of the pancreas. *European Journal of Radiology*. 83(3): 415-419. DOI: 10.1016/j.ejrad.2013.04.020.
- 8 Ehrle A., Lilge S., Clegg P.D. & Maddox T.W. 2021. Equine flexor tendon imaging part 1: Recent developments in ultrasonography, with focus on the superficial digital flexor tendon. *The Veterinary Journal*. 278: 105764. DOI: 10.1016/j.tvjl.2021.105764.
- 9 Fabbi M., Di Palma S., Manfredi S., Gnudi G., Miduri F., Daga E., Melis G.C., Bianchi E., Voccia S. & Volta A. 2017. Imaging diagnosis-ultrasonographic appearance of skeletal muscle metastases in a dog with hemangiosarcoma. *Veterinary Radiology & Ultrasound.* 58(6): E64-E67. DOI: 10.1111/vru.12432.

- 10 Feliciano M.A.R., Uscategui R.A.R., Maronezi M.C., Simões A.P.R., Silva P., Gasser B., Pavan L., Carvalho C.F., Canola J.C. & Vicente W.R.R. 2017. Ultrasonography methods for predicting malignancy in canine mammary tumors. *PLoS ONE*. 12(5): e0178143. DOI: 10.1371/journal.pone.0178143.
- **11 Figueiredo C.R.L.V. 2019.** The unusual paradox of cancer-associated infammation: an update. *Jornal Brasileiro de Patologia e Medicina Laboratorial*. 55(3): 321-332. DOI: 10.5935/1676-2444.20190029.
- **12 Hammond T.N. & Pesillo-Crosby S.A. 2008.** Prevalence of hemangiosarcoma in anemic dogs with a splenic mass and hemoperitoneum requiring a transfusion: 71 cases (2003–2005). *Journal of the American Veterinary Medical Association*. 232(4): 553-558. DOI: 10.2460/javma.232.4.553.
- **13 Hristov T. 2020.** Ultrasound findings in dogs with splenic hemangiosarcoma. *Tradition and Modernity in Veterinary Medicine*. 2(9): 15-20. DOI: 10.5281/zenodo.4317322.
- **14 Kalantari S. 2018.** The diagnostic value of color Doppler ultrasonography in predicting thyroid nodules malignancy. *International Tinnitus Journal*. 22(1): 35-39. DOI: 10.5935/0946-5448.20180006.
- 15 Ling W., Nie J., Zhang D., Yang Q., Jin H., Ou X., Ma X. & Luo Y. 2020. Role of Contrast-Enhanced Ultrasound (CEUS) in the Diagnosis of Cervical Lymph Node Metastasis in Nasopharyngeal Carcinoma (NPC) Patients. *Frontiers in Oncology*. 10: 972. DOI: 10.3389/fonc.2020.00972.
- 16 Linta N., Pey P., Baron T.M., Pietra M., Felici M., Bettini G., Cipone M. & Diana A. 2021. Contrast-enhanced ultrasonography in dogs with inflammatory bowel disease. *Journal of Veterinary Internal Medicine*. 35(5): 2167-2176. DOI: 10.1111/jvim.16202.
- 17 Madruga G.M., Da Cruz I.C.K., Carneiro R.K., Feliciano M.A.R., Maronez M.C., Uscategui R.R., Abreu T.G.M. & Perlmann E. 2022. Intraocular Lymphoma in Dogs Findings of Contrast Enhanced Ultrasound and ARFI Elastography. *Acta Scientiae Veterinariae*. 50(1): 782. DOI: 10.22456/1679-9216.121902.
- 18 Maronezi M.C., Carneiro R.K., Cruz I.C.K., Oliveira A.P.L., De Nardi A.B., Pavan L., Silva P.D.A., Uscategui R.A.R. & Feliciano M.A.R. 2022. Accuracy of B-mode ultrasound and ARFI elastography in predicting malignancy of canine splenic lesions. *Scientific Reports*. 12(1): 4252. DOI: 10.1038/s41598-022-08317-7.
- 19 Pang T., Huang L., Deng Y., Wang T., Chen S., Gong X. & Liu W. 2017. Logistic regression analysis of conventional ultrasonography, strain elastosonography, and contrast-enhanced ultrasound characteristics for the differentiation of benign and malignant thyroid nodules. *PLoS One*. 12(12): e0188987. DOI: 10.1371/journal.pone.0188987.
- **20 Perone M.V. & Yablon C.M. 2021.** Musculoskeletal Ultrasound in the Emergency Department: Is There a Role? *Seminars Roentgenology*. 56(1): 115-123. DOI: 10.1053/j.ro.2020.09.004.
- 21 Pu H., Zhao L., Yao M., Xu G., Liu H., Xu H.X. & Wu R. 2017. Conventional US combined with acoustic radiation force impulse imaging (ARFI) for prediction of triple-negative breast cancer and the risk of lymphatic metastasis. *Clinical Hemorheology and Microcirculation*. 65(4): 1-13. DOI: 10.3233/CH-16196.
- 22 Rossignoli P.P., Feliciano M.A.R., Minto B.W., Maronezi M.C., Uscategui R.A., Ido C.K., Rolemberg D.S., Faria, L.G., Cruz I.C.K. & Aires L.P.N. 2020. B mode ultrasonography and elastography in the evaluation of the pectineus muscle in dogs with hip dysplasia. *Turkish Journal of Veterinary and Animal Science*. 44(5): 1142-1149. DOI:10.3906/vet-2004-109.
- 23 Sahlani L., Thompson L., Vira A. & Panchal A.R. 2016. Bedside ultrasound procedures: musculoskeletal and non-musculoskeletal. *European Journal of Trauma and Emergency Surgery*. 42(2): 127-138. DOI: 10.1007/s00068-015-0539-3.
- 24 Sconfienza L.M., Perrone N., Delnevo A., Lacelli F., Murolo C., Gandolfo N. & Serafini G. 2010. Diagnostic value of contrast-enhanced ultrasonography in the characterization of ovarian tumors. *Journal of Ultrasound*. 13(1): 9-15. DOI: 10.1016/j.jus.2009.09.007.
- 25 Shiu K.B., Flory A.B., Anderson C.L., Wypij J., Saba C., Wilson H., Kurzman I. & Chun R. 2011. Predictors of outcome in dogs with subcutaneous or intramuscular hemangiosarcoma. *Journal of the American Veterinary Medical Association*. 238(4): 472-479. DOI: 10.2460/javma.238.4.472.
- 26 Silva P., Uscategui R.A.R., Maronezi M.C., Gasser B., Pavan L., Gatto I.R.H. Almeida V.T.A., Vicente W.R.R. & Feliciano M.A.R. 2018. Ultrasonography for lymph nodes metastasis identification in bitches with mammary neoplasms. *Scientific Reports*. 8(1): 17708. DOI: 10.1038/s41598-018-34806-9.

- 27 Tamaki K., Tamaki N., Kamada Y., Uehara K., Miyashita M., Sm Chan M., Ishida T., Ohuchi N. & Sasano H. 2013. Non-invasive evaluation of axillary lymph node status in breast cancer patients using shear wave elastography. *The Tohoku Journal of Experimental Medicine*. 231(3): 211-216. DOI: 10.1620/tjem.231.211.
- **28 Thamm D.H. 2013.** Hemangiosarcoma. In: Withrow S.J., Vail D.M. & Page R.L. (Eds). *Withrow and MacEwen's Small Animal Clinical Oncology*. St. Louis: Saunders, pp.679-688.
- 29 Wendelburg K.M., Price L.L., Burgess K.E., Lyons J.A., Lew F.H. & Berg J. 2015. Survival time of dogs with splenic hemangiosarcoma treated by splenectomy with or without adjuvant chemotherapy: 208 cases (2001-2012). *Journal of the American Veterinary Medical Association*. 247(4): 393-403. DOI: 10.2460/javma.247.4.393.
- 30 Yamada A.L.M., Pinheiro M., Marsiglia M.F., Hagen S.C.F, Baccarin R.Y.A. & Silva L.C.L.C. 2020. Ultrasound and clinical findings in the metacarpophalangeal joint assessment of show jumping horses in training. *Journal of Veterinary Science*. 21(3): e21. DOI: 10.4142/jvs.2020.21.e21.
- 31 Zhang W., Wang L. & Xin Z. 2018. Combination of serum CA19-9 and CA125 levels and contrast-enhanced ultrasound parametric data facilitates to differentiate ovarian serous carcinoma from ovarian malignant epithelial cancer. *Medicine*. 97(16): e0358. DOI: 10.1097/MD.0000000000010358.

