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UPDATES IN CLINICAL AND SONOGRAPHIC ASSESSMENT OF LOCOREGIONAL LYMPH NODES IN BITCHES WITH MAMMARY TUMORS.

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ABSTRACT

The incidence of mammary tumors in bitches has increased along with animal survival. Therefore, a thorough evaluation is essential for the diagnosis. One characteristic of mammary tumors is the great capacity of developing metastasis. Being responsible for drainage of the mammary glands, they play an important role in spreading tumor cells, being a negative prognostic factor. The development of new techniques to evaluate sentinel lymph nodes and early identification of alterations contribute positively to patient survival. This review discusses clinical evaluation and sonographic methods for assessment of locoregional lymph nodes in bitches with mammary neoplasms and diagnostic methods available.

KEYWORDS: neoplasia, lymphatic system, metastasis, ultrasonography, dogs.

INTRODUCTION

In the last decades, the increase of canine longevity, the close relationship with humans and changes in animals' lifestyle contributed negatively to the increase of neoplasms. However, it must be considered that advances in diagnostic techniques also contributed with the increase in the prevalence described (WITHROW and VAIL, 2007).

In bitches, mammary neoplasms represent, on average, 25 to 52% of diagnosed tumors (DALECK et al., 1998; DE NARDI et al., 2002; FELICIANO et al., 2012). The prevalence of malignant neoplasms is approximately 50% (DE NARDI et al., 2002). In Brazil, the rate can reach up to 70% (OLIVEIRA FILHO et al., 2010).

The most important characteristic of malignant neoplasms is the ability to develop metastasis. Regarding mammary tumors in bitches, its occurrence can reach up to 50% (PHILIBERT, 2003; DE NARDI, 2004; OLIVEIRA FILHO et al., 2010), contributing as the main mortality factor. Metastases are frequently identified in locoregional lymph

nodes, lungs, liver, spleen and other organs (DE NARDI et al., 2008; KLOPFLEISCH and GRUBER, 2009). The first affected lymph node from a lymphatic chain that drains the region affected by the tumor is called sentinel lymph node (WEAVER, 2010; CASSALI, 2014). The presence of tumor cells in locoregional lymph nodes is a negative prognostic factor in animals affected by mammary tumors (PHILIBERT, 2003; HELLER et al., 2005; CHANG et al., 2005). The identification of lymph nodes metastasis is based on histopathological examination after surgical excision (CASSALI et al., 2014), even though physical examination, ultrasonography, radiography and fine needle aspiration cytology (FNAC) can help presuming metastasis. So far, these methods are considered less accurate in cases of micro metastasis or clusters of tumor cells. (WEAVER, 2010).

There are few studies regarding the benefits of advanced imaging modalities such as computed tomography (CT), magnetic resonance imaging (MRI) and scintigraphy for the evaluation of sentinel lymph nodes in dogs (BALOGH et al., 2002; MAYER, et al., 2012; ROSSI et al., 2017; SOULTANI et al., 2013; SUGA et al., 2003.) However, the availability of these techniques is still restricted in some places, besides being rather expensive, making their application in clinical practice a bit restricted.

The postoperative complications reported in women after lymphadenectomy are unlikely in bitches and there are few descriptions in veterinary literature. According to a consensus in Veterinary Medicine, the treatment of mammary neoplasms consists of removing the primary tumor, the mammary glands and locoregional lymph nodes. (CASSALI et al., 2011). Therefore, the knowledge of lymphatic drainage is necessary for performing the surgical procedure and thus determine the prognosis. However, even though excision of axillary and inguinal lymph nodes is recommended, surgical invasiveness and limitations of the technique do not allow the procedure to be performed in all cases, such as the close relation of the axillary lymph node with the brachial plexus and large blood vessels, limiting in some cases its removal (CASSALI et al., 2014; INIC et al., 2014; TUOHY et al., 2009). For these reasons, early detection and identification of lymph node metastasis is considered important in order to improve the prognosis, treatment and patient survival (PATSIKAS, 2006).

Given the aforementioned information and the importance of lymphoid structures in breast tumors, this review aims to describe the morphophysiological characteristics and diagnostic aspects for clinical and sonographic assessment of locoregional lymph nodes in bitches with mammary tumors.

Morphological aspects of mammary gland locoregional lymph nodes

The lymphatic system is an important component of the blood drainage system, being composed of lymphatic vessels, lymph nodes and lymph. Lymph nodes are located throughout the body, surrounded by adipose tissue, and are associated with large blood vessels. They are reniform structures, covered by a thin capsule of smooth musculature and elastic fibers (HARDIE and PETRUS, 2007).

Bitches have five pairs of mammary glands identified in the cranial to caudal direction, being: cranial thoracic (M1), caudal thoracic (M2), cranial abdominal (M3), caudal abdominal (M4) and inguinal (M5), although they may only present four pairs (ZUCCARI et al., 2002; MURPHY, 2008). The mammary glands are arranged in two separate rows, extending from the thoracic to the inguinal regions (SWAIM, 2007). In healthy dogs, the lymphatic circulation promotes drainage of homolateral glands, and there is no direct contact between the right and left chains. All glands have independent drainage to the locoregional lymph node. However, the cranial abdominals are the only ones that drain simultaneously to the accessory axillary lymph node and to the superficial inguinal lymph nodes (ZUCCARI et al., 2002; MURPHY, 2008).

In bitches with mammary tumors, drainage to regional lymph nodes depends on the affected mammary gland. In relation to M1, M2 and M3, drainage occurs in the axillary lymph node and eventually to the superficial cervical lymph nodes. M4 and M5 have drainage for superficial inguinal lymph nodes. In some cases, the gland has double drainage, going to the axillary and superficial inguinal lymph nodes, which shows heterogeneity and plasticity of the lymphatic system (STAN et al., 2010). Drainage to the contralateral lymph node has already been described, which happens due to lymphangiogenesis induced by the neoplasm. (SORENMO., 2003). The Vascular Endothelial Growth Factor (VEGF) C plays an important part in promoting lymphangiogenesis and in promoting metastasis (SKOBE et al., 2001).

Recent advances in diagnostic imaging, anatomical and histological dissection have enabled detailed information on the lymphatic system (HARDIE and PETRUS, 2007). The lymphatic pathway plays an important role, as it is the carrier of tumor cells and it is most commonly affected by metastasis (PEREIRA et al., 2003; PATSIKAS et al., 2006). Axillary lymph node involvement in mammary tumors is of great importance, as it is close to other lymphatic centers, such as superficial cervical and ventral thoracic (PEREIRA et al., 2003). Inspection of the locoregional lymph node should be part of the routine evaluation of mammary tumors in bitches, as the presence of metastasis has an impact on tumor staging, survival and treatment (CASSALI et al., 2014).

Sentinel lymph node and its identification

The sentinel lymph node is the first lymph node to drain a tumor, and for this reason it is expected to be the first site to develop metastasis after lymphatic spread (PAZ et al., 2001; TUOHY et al., 2009). In animals, it was first described in cats by SORENMO. (2003).

In women, the use of lymphatic mapping to identify sentinel lymph nodes has significantly improved the treatment of breast cancer, as it determines a more specialized therapeutic approach (SORENMO., 2003). In this sense, the techniques for staging lymph nodes in bitches need to be reconsidered, since the evaluation of locoregional lymph nodes becomes essential for staging in this species, with the identification of the sentinel lymph node being a prognostic factor that will guide therapeutic approaches. (CASSALI et al., 2014).

The mapping, as the technique for trans operative identification of these structures is called, consists of the objective and individualized detection of lymph nodes that drain a tumor (SORENMO,2003). For this purpose, different types of dyes applied directly to tumor or peri-tumor tissue are used. Among them, patent blue is the only one approved for use in humans and for this reason the most used in research and in the clinic (Figure 1). The benefits of assisting the identification of the sentinel lymph node or performing the mapping with dye are: facilitating the identification of lymph nodes, minimizing surgical incisions and increasing efficiency, especially when evaluating axillary lymph nodes (SORENMO, 2003).

Figure 1. Photograph showing the application of patent blue for identification of the axillary lymph node of a bitch with mammary tumor (Personal File).

Usually, 0.5 ml of 2.5% patent blue is administered in bitches up to 15 kg, and 1 ml in bitches weighing more than 15 kg, by intradermal injection in the peri-tumor region (BESERRA, et al. 2011). After the injection, the dye is drained through the lymphatic route, allowing the visualization of the regional lymphatic chain during the surgical procedure (EL KATIB, et al 2011).

Besides patent blue, methylene blue, iopamidol, indocyanine green (DE SOUZA et al., 2012), indirect lymphography using iodized oil as a marker (BRISSOT and EDERY, 2016) and scintigraphy (TUOHY and WORLEY, 2014) can also be used for mapping and identifying sentinel lymph nodes. After application, the dye is drained by the lymphatic system, allowing visualization of the regional lymphatic chain during the surgical procedure (EL KHATIB et al., 2011). The dye application technique is considered easy to perform, of low cost, does not require prior training and can be successfully applied in the surgical routine of small animals (EL KHATIB et al., 2011).

In small animal clinical practice, lymph nodes are submitted to puncture or biopsy if they are altered on physical examination, which leads to inappropriate staging. Studies have shown that lymph nodes that drain regions with neoplasms might not present changes in size and consistency but may already be metastasized (BESERRA et al. 2016). It was observed that collecting samples only from the lymph node closest to the nodule has not been sufficient for a correct tumor staging and the presence of locoregional disease can be neglected (SORENMO et al. 2013).

In dogs, surgical techniques for excision of tumors are chosen according to the ease of access and/or the presence of palpable lymph nodes. The inguinal lymph node is usually removed next to the inguinal mammary gland while the axillary is rarely removed because it is located next to important vascular and nervous structures (TUOHY et al., 2009).

In women with breast cancer it is considered that the condition of the axillary lymph node is the most important prognostic factor and has been repeatedly shown as an indicator of survival (GIULIANO et al., 1994). BUITRAGO et al. (2011) describe that

about 70% of patients with axillary lymph node involvement will develop relapse within 10 years, compared with 20% to 30% of patients without a negative nodule.

The identification of these nodules is a challenge for staging, due to the inability to visualize, which results in failures that can lead to an inadequate treatment, recurrence and distant metastasis. Identification of metastasis in locoregional lymph nodes prior to surgical removal of the mammary gland in women is extremely important, as axillary lymphadenectomy commonly develops lymphedema, arm paralysis and/or chronic pain (ROCKSON, 2008).

Currently, surgical excision and histopathological analysis of the axillary lymph node is recommended in women affected by breast tumors greater than five centimeters in diameter (QUADROS, 2007; BOUGHEY, 2013). This approach is considered to have prognostic and therapeutic value, since the identification of tumor cells in the lymph node is related to tumor aggressiveness and the potential for distant metastasis, respectively, enabling the reduction of regional and distance recurrence (PAULINELLI, 2017).

In general, changes or complications are observed, such as: seroma, lymphedema, restriction of shoulder movements, changes in scapular elevation, sensory changes in the upper limb and even induction of new malignant tumors such as angiosarcomas, in women and in bitches (PINHEIRO, 2003; PAULINELLI, 2017). However, data in the literature are insufficient to describe specific changes in bitches.

Evaluation of locoregional lymph nodes in mammary tumors

In Veterinary Medicine, complete evaluation of the patient is established for the proper conduct of the diagnosis and prognosis of neoplastic animals. In this context, anamnesis, physical examination and complementary cytology, radiography, ultrasound and histopathology techniques are necessary for tumor identification and treatment (MORRISON, 1998).

The physical examination of the mammary glands consists of palpation of the mammary chains and regional lymph nodes in search of nodules or changes in size, shape and consistency. In addition, information on the time of appearance and progression of the lesion, previous treatment and reproductive history are important (CASSALI et al., 2014). After palpation of the mammary glands and lymph nodes, fine needle aspiration cytology (FNAC) can be used as a complementary part of the diagnosis. However, this test has a high rate of false negative results, especially when there is the presence of micro metastases or tumor cells clusters (WEAVER, 2010).

In the veterinary practice, most of the time the regional lymph nodes of the mammary chain are submitted to puncture or biopsy only when they are altered on palpation, leading to an inappropriate staging, as the palpation does not allow the identification of metastasis (TUOHY et al., 2009; WILLIAMS and POCKER, 2003).

The gold standard evaluation for the diagnosis of metastasis in regional lymph nodes is histopathological analysis after surgical excision (QUEIROGA and LOPES, 2002). The criteria used to consider malignancy are: high cell density in the smear, with low degree of cohesion and the presence of "naked" cells in abundance; variability in the dimensions of the nuclei (anisocariosis); irregular patterns of chromatin distribution (granular or reticular), normal and abnormal mitosis figures; presence of several nucleoli in the same nucleus or macro nucleus (ALLEN, 1986).

However, the technique is invasive (TUOHY et al., 2009) and the method is not used in many cases. On the other hand, some non-invasive diagnostic methods can be introduced in the evaluation procedure, such as the radiographic exam that is used to detect distant metastasis, mainly in the thoracic cavity. (FERGUSON et al., 1995).

In human medicine, the sonographic characteristics of the axillary lymph node and mammary glands are well defined, such as shape, contour, size and architecture (JALES et al., 2005). However, in Veterinary Medicine, ultrasonographic examination has been performed mainly to investigate the presence of abdominal metastasis (NYMAN et al., 2006), addressing little information about the evaluation of these locoregional structures in the affected bitches.

Ultrasonography provides the clinician with important information about lymph node architecture, but definitive diagnosis is only obtained by histopathology (CHAMMAS et al., 2004; NYMAN et al., 2005). However, over the years, ultrasonography has gained space and disseminated new techniques that can also help in the diagnosis of tissues with neoplastic growth. NYMAN et al. (2005) sonographically evaluated 318 superficial lymph nodes from 142 dogs using transducers ranging from 7 to 14 MHz and obtained important information regarding lymph node characterization, although reporting difficulty in identifying axillary and inguinal lymph nodes. These structures have an oval or flat shape, predominantly hypoechoic, with homogeneous echotexture, well-defined hyperechoic contours and, occasionally, a central hyperechogenic line identified in the hilum region. (Figure 2) (FELICIANO et al., 2015).

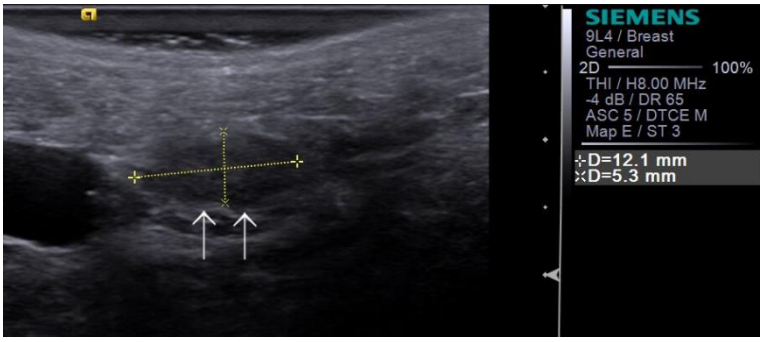

Figure 2. Sonographic image of a normal axillary lymph node of a bitch with mammary tumor. Note the hypoechoic structure (arrows) with homogenous echotexture and S / L ratio of 0,43 (Personal File).

When evaluating abnormal tissues, the sonographic aspect of the enlarged lymph node may be similar in a neoplastic, inflammatory or infectious process, thus being an unused parameter in the differentiation of benign or malignant processes. (DE SWARTE et al., 2011). Conversely, the shape of the lymph node can be considered a valuable parameter in the differentiation between malignant and benign characteristics (NYMAN and O´BRIEN, 2007). In general, in inflammatory processes, lymph nodes tend to increase proportionally in all planes, maintaining an oval shape. In neoplastic processes, they tend to disproportionately increase the short axis, becoming more rounded (CHAMMAS et al., 2004). The shape of the lymph node is calculated by dividing the short axis (S) by the long axis (L), being considered oval when the S / L ratio is less than 0.5 (Figure 2) and rounded when the S / L ratio is greater than 0.5, which tends to malignancy (Figure 3) (FELICIANO et al., 2015).

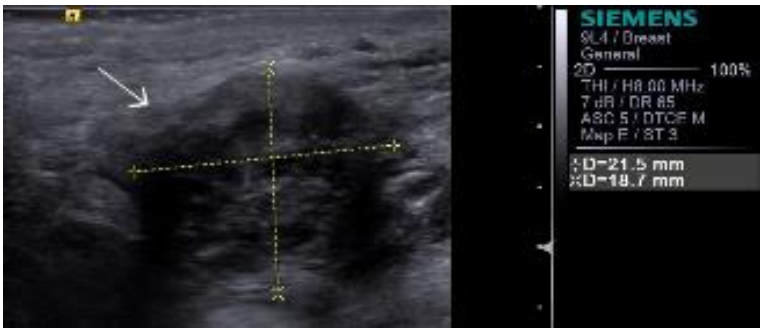

Figure 3. Sonographic image of a metastatic axillary lymph node of a bitch with mammary tumor. Note the presence of a rounded structure (arrow), presenting a heterogenous echotexture and S / L ratio of 0,87, which indicates malignancy (Personal File).

Changes in lymph node architecture may also suggest metastasis, especially when there is loss of visualization of the central hyperechogenic hilum, with replacement by a diffusely hypoechogenic image (FELICIANO et al., 2015). NYMAN et al. (2005) reported that the infiltration of neoplastic cells can cause irregularities in the outline of the lymph node and that for benign characteristics, the structures usually have regular contours. Additionally, when there is inflammatory reaction, there is diffuse cortical thickening, with a probable increase in its size, but maintaining the oval shape. Metastatic lymph nodes will normally present heterogeneous echotexture, due to the presence of cystic areas, resulting from hemorrhages and tissue necrosis (Figure 3) (CARVALHO, 2004; CHAMMAS et al., 2004; NYMAN et al., 2005).

Regarding lymph node vascularization, NYMAN et al. (2005) found a predominantly hilar flow in benign process and peripheral flow in malignant ones. The metastatic lymph node usually presents unorganized vascularization, with subcapsular peripheral distribution, with vessels of tortuous and irregular architecture (Figure 4) (NYMAN and O´BRIEN, 2007).

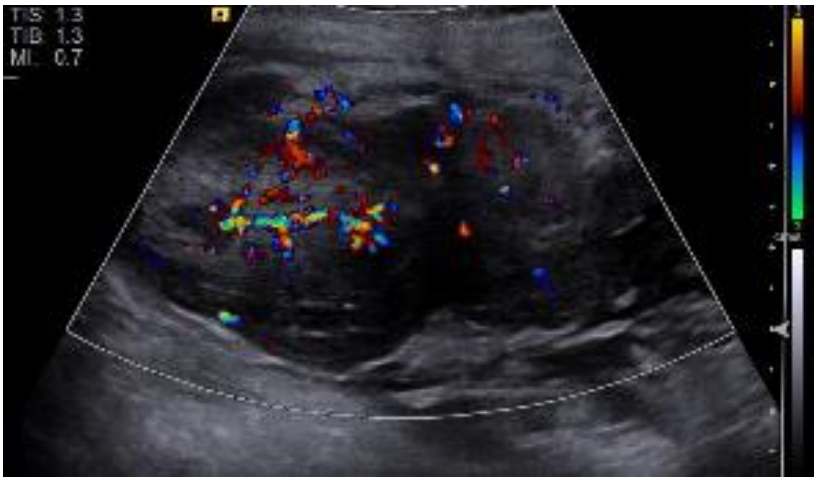

Figure 4. Color-doppler image of a metastatic axillary lymph node of a bitch with mammary tumor. Note the presence of a structure with irregular surface, heterogenous echotexture and presence of an unorganized neovascularization (Personal File).

The elastography technique emerged as a method that allows non-invasive assessment of tissue elasticity and has become an efficient tool in the identification of malignant breast lesions in humans and animals (FELICIANO et al. 2017; TOZAKI et al.

2011) Recently, clinical studies have evaluated the accuracy of this technique to identify metastasis in axillary lymph nodes of women with breast cancer and have shown extremely promising results (WOJCINSKI et al. 2012; YOUK et al. 2017). The sonoelastography technique was recently used in attempt to detect malignancy in superficial lymph nodes of dogs with neoplasia. In this type of compressive elastography, lymph nodes were classified into four scores according to a qualitative chromatic scale. A higher frequency of benign lymph nodes with score one (soft elasticity) and a higher frequency of malignant lymph nodes with score four (rigid elasticity) was found. The study pointed out that, although it is a subjective method, sonoelastography is another complementary imaging test for the presumptive diagnosis of malignancy (BELOTTA, 2019).

Silva et al. (2018) described the use of the ARFI elastography technique (Acoustic Radiation Force Impulse) in evaluating axillary and inguinal lymph nodes in bitches affected by mammary tumors and obtained excellent sensitivity and specificity in the results. Cut-off values were determined to verify the malignancy of these lymph nodes, which can differentiate them from benign and reactive ones: SWV (Shear Wave Velocity) values greater than 2.5 m / s could identify malignancy in axillary lymph nodes with 95% sensitivity and 87% specificity. Thus, the ARFI elastography technique can be used as an important tool for the evaluation of changes in lymph nodes in bitches with mammary tumors.

CONCLUSION

The assessment of locoregional lymphoid tissues in bitches with mammary tumors is of great importance for determining a more reliable prognosis performed by the responsible veterinarian. This review brought relevant information that can contribute to a better understanding of the lymph nodes in question.

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REFERENCES

ALLEN, S. W.; PRASSE, K. W.; MAHAFFEY, E. A. Cytologie differentiation of benign from malignant canine mammary tumors. **Veterinary pathology**, v. 23, n. 6, p. 649-655, 1986. <DOI: 10.1177/030098588602300601>.

BALOGH, L.; THURÓCZY, J.; ANDÓCS, G.; MÁTHÉ, D.; CHAUDHARI, P.; PERGE, E.; BIKSI, I.; POLYÁK, A.; KIRÁLY, R.; JÁNOKI, G. A. Sentinel lymph node detection in canine oncological patients. Nuclear Medicine Review, v. 5, n. 2, p. 139-144, 2002.

BELOTTA, A. F.; GOMES, M. C.; ROCHA, N. S.; MELCHERT, A.; GIUFFRIDA, R.; SILVA, J. P.; MAMPRIM, M. J. Sonography and sonoelastography in the detection of malignancy in superficial lymph nodes of dogs. **Journal of veterinary internal medicine**, v. 33, n. 3, p. 1403-1413, 2019. <DOI: 10.1111/jvim.15469>.

BESERRA, H. E. O.; CAVALCANTE, R. V.; PESSOA, A. W. P.; PINHEIRO, L. G. P. Técnica de detecção do linfonodo sentinela da glândula mamária de cadelas utilizando azul patente e Tecnécio 99m Tc. **Vet Zootec**, v.18, n.2, p.57-59, 2011.

BESERRA, H. E. O.; GRANDI, F.; DUFLOTH, R. M.; PINHEIRO, L. G. P.; MIOT, H. A.; VEXENAT, S. C. O. R.;

ROCHA, N. S. Metastasis of mammary carcinoma in bitches: evaluation of the sentinel lymph node technique. **Adv. Breast Cancer Res**. v. 5, n. 2, p. 58-65, 2016. <DOI: 10.4236/abcr.2016.52006>

BOUGHEY, J. C.; SUMAN, V. J.; MITTENDORF, E. A.; AHRENDT, G. M.; WILKE, L. G.; TABACK, B.; LEITCH, A. M.; KUERER, H. M.; BOWLING, M.; FLIPPO-MORTON, T. S.; BYRD, D. R.; OLLILA, D. W.; JULIAN, T. B.; MCLAUGHLIN, S. A.; MCCALL, L.; SYMMANS, W. F.; LE-PETROSS, H. T.; HAFFTY, B. G.; BUCHHOL, T. A.; NELSON, H.; HUNT, K. K.; Sentinel lymph node surgery after neoadjuvant chemotherapy in patients with node-positive breast cancer: the ACOSOG Z1071 (Alliance) clinical trial. **Journal of the American Medical Association**, v. 310, n. 14, p. 1455-1461, 2013. <DOI: 10.1001/jama.2013.278932>

BRISSOT, H. N.; EDERY, E. G. Use of indirect lymphography to identify sentinel lymph node in dogs: a pilot study in 30 tumours. **Veterinary and comparative oncology**, v. 15, n. 3, p. 740-753, 2017. <DOI: 10.1111/vco.12214>

BUITRAGO, F.; UEMURA, G.; SENA, M. C. F. Fatores prognósticos em câncer de mama. **Comunicação em ciência e saúde**, p. 69-81, 2011.

CARVALHO, C. F. Ultrassonografia em linfonodos. In: CARVALHO, C. F. **Ultrassonografia em Pequenos Animais**.1 ed. São Paulo: Roca, 2004, cap. 13, p. 175-179.

CASSALI G. D; LAVALLE, G. E. FERREIRA, E. ESTRELA-LIMA, A. NARDI, A. B. FERNANDES, C.G. SOBRAL, R. A. AMORIM, R. L. OLIVEIRA, L.O. SUEIRO, F. A. R. BESERRA, H. E. O. BERTAGNOLLI, A. C. GAMBA, C.O. DAMASCENO, K.A. CAMPOS, C.B. ARAUJO, M.R. CAMPOS, L.C. MONTEIRO, L. N. NUNES, F. C. HORTA, R.S. REIS, D. C. LUVIZZOTO, M. C. R. MAGALHÃES, G.M. RAPOSO, J.B. FERREIRA, A. M. R. , TANAKA, N.M. GRANDI, F. UBUKATA, R. BATSCHINSKI, K. TERRA, E.M. SALVADOR, R. C. L. JARK, P. C. DELECRODI, J. E. R. NASCIMENTO, N. A. SILVA, D. N. SILVA, L. P. FERREIRA, K. C. R. S. FREHSE, M. S. SANTIS, G. W. SILVA, E. O. GUIM, T. N. KERR, B. CINTRA, P. P. SILVA, F. B. F. LEITE, J. S. MELLO, M. F. V. FERREIRA, M. L. G. FUKUMASU, H. SALGADO, B. S. TORRES, R. ; Consensus for the Diagnosis, Prognosis and Treatment of Canine Mammary Tumors - 2013. **Brazilian Journal of Veterinary Pathology**, v. 7, p. 38-69, 2014.

CASSALI, G. D.; CASSALI, G. D. LAVALLE, G. E. DE NARDI, A. B. FERREIRA, E. BERTAGNOLLI, A. C. LIMA, A. E. S. ALESSI, A.C. DALECK, C.R. SALGADO, B.S. FERNANDES, C.G. SOBRAL, R.A. AMORIM, R.L. GAMBA, C.O. DAMASCENO, K.A. AULER, P.A. MAGALHÃES, G.M. SILVA, J.O. RAPOSO, J.B. FERREIRA, A. M. R. OLIVEIRA, L.O. MALM, C. ZUCCARI, D.A.P.C. TANAKA, N.M. RIBEIRO, L.G.R. CAMPOS, L.C. , SOUZA, C. M. DE LEITE, J.S. SOARES, L.M.C. CAVALCANTI, M. F. FONTELES, Z.G. SCHUCH, I.D. PANIAGO, J.D.G. OLIVEIRA, T.S. TERRA, E.M. CASTANHEIRA, T.L.L. FELIX, A.O.C. CARVALHO, G.D. GUIM, T.N. GUIM, T.N. GARRIDO, E. FERNANDES, S.C. MAIA, F. C. L. DAGLI, M.L.Z. ROCHA, N.S. FUKUMASU, H. GRANDI, F. MACHADO, J.P. SILVA, S. M. M. S. BEZERRIL, J.E. FREHSE, M.S. PAES DE ALMEIDA, E.C. CAMPOS, C.B. ; Consensus for the Diagnosis, Prognosis and Treatment of Canine Mammary Tumors. **Brazilian Journal of Veterinary Pathology**, v. 4, p. 153-180, 2011.

CHAMMAS, M. C.; LUNDBERG, J. S.; JULIANO, A. G.; SAITO, O. C.; MARCELINO, A. S. Z.; CERRI, G. G. Linfonodos cervicais: um dilema para o ultra-sonografista. **Radiologia Brasileira**, v. 37, n. 5, p. 357-364, 2004. <DOI: 10.1590/S0100-39842004000500010>

CHANG, S. C.; CHANG, C. C.; CHANG, T. J.; WONG, M. L. Prognostic factors associated with survival two years after surgery in dogs with malignant mammary tumors: 79 cases (1998–2002). **Journal of the American Veterinary Medical Association**, v. 227, n. 10, p. 1625-1629, 2005. <DOI: 10.2460/javma.2005.227.1625>

DALECK, C. R.; FRANCESCHINI, P. H.; ALESSI, A. C.; SANTANA, A. E.; MARTINS, M. I. M. Aspectos clínico e cirúrgicos do tumor mamário canino. **Ciência Rural**, v. 28, n. 1, p. 95-100, 1998. <DOI: 10.1590/S0103-84781998000100016>

DE NARDI, A. B.; RAPOSO, T. M. M.; ASSUNCAO, K. A. Neoplasias Mamárias. In: DALECK, C. R.; DE NARDI, A. B. **Oncologia em Cães e Gatos**. 2ed.Rio de Janeiro: Roca, 2016, v. , p. 499-516.

DE NARDI, A. B.; RODASKI, S.; SOUSA, R. S.; COSTA, T. A.; MACEDO, T. R.; RODIGHERI, S. M.; RIOS, A.; PIEKARZ, C. H. Prevalência de neoplasias e modalidades de tratamento em cães atendidos no Hospital Veterinário da Universidade Federal do Paraná. **Archives of Veterinary** Science, v. 7, n. 2, p. 15-26, 2002. <DOI: 10.5380/avs.v7i2.3977>

DE NARDI, Andrigo Barbosa. **Expressão da ciclooxigenase-2 nas neoplasias de mama em cadelas (*Canis familiaris,* LINNAEUS, 1758)**. 2004. 54p. Dissertação (Mestrado em Cirurgia Veterinária), Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, 2004.

DE SWARTE, M.; ALEXANDER, K.; RANNOU, B.; D'ANJOU M. A.; BLOND, L.; BEAUCHAMP, G. Comparison of sonographic features of benign and neoplastic deep lymph nodes in dogs. **Veterinary Radiology & Ultrasound**, v. 52, n. 4, p. 451-456, 2011. <DOI: 10.1111/j.1740-8261.2011.01808.x>

EL KHATIB, E. M.; PIRES, P. F. T. A.; DE LIMA, A. F. K. T.; REPETTI, C. S. F.; FRANCO, R. P.; HATAKA, A. Uso do azul de metileno na identificação do linfonodo sentinela em cadelas com neoplasias mamarias. **Veterinaria e Zootecnia**, v. 18, n. 2, p. 60-66, 2011.

FELICIANO, M. A. R.; CANOLA, J. C.; VICENTE, W. R. R. **Diagnóstico por imagem em cães e gatos**. 1.ed. São Paulo: MedVet, 2015.

FELICIANO, M. A. R.; JOÃO, C. F.; CARDILLI, D. J.; CRIVELARO, R. M.; VICENTE, W. R. R. Neoplasia mamária em cadelas: revisão de literatura. **Rev Cient Eletr Med Vet**, n.1, 2012.

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. Ultrasonography methods for predicting malignancy in canine mammary tumors. **PloS one**, v. 12, n. 5, 2017. <DOI: 10.1371/journal.pone.0178143>

FERGUSON, H. R. Canine mammary gland tumors. **Veterinary Clinics of North America: Small Animal Practice**, v. 15, n. 3, p. 501-511, 1985. <DOI: 10.1016/S0195-5616(85)50053-4>

GIULIANO, A. E.; KIRGAN, D. M.; GUENTHER, J. M., MORTON, D. L. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. **Annals of surgery**, v. 220, n. 3, p. 391, 1994. <DOI: 10.1097/00000658-199409000-00015>

HARDIE, R.J.; PETRUS, D.J. **Manual de Cirurgia de Pequenos Animais.** 3.ed. São Paulo: Manole, 2007.

HELLER, D. A.; CLIFFORD, C. A.; GOLDSCHMIDT, M. H.; HOLT, D. E.; SHOFER, F. S.; SMITH, A. SORENMO, K. U. Cyclooxygenase-2 expression is associated with histologic tumor type in canine mammary carcinoma. **Veterinary pathology**, v. 42, n. 6, p. 776-780, 2005. <DOI: https://doi.org/10.1354/vp.42-6-776>

INIC, Z.; ZEGARAC, M.; INIC, M.; MARKOVIC, I.; KOZOMARA, Z.; DJURISIC, I.; INIC, I.; PUPIC, G.; JANCIC, S. Difference between luminal A and luminal B subtypes according to Ki-67, tumor size, and progesterone receptor negativity providing prognostic information. **Clinical Medicine Insights: Oncology**, v. 8, 2014. <DOI: 10.4137/CMO.S18006>

JALES, R. M.; MARUSSI, E. F.; DE ANDRADE, K. C.; DERCHAIN, S. F. M. Desempenho da ultra-sonografia na detecção das metástases em linfonodos axilares em mulheres com câncer de mama. **Revista Brasileira de Ginecologia e Obstetrícia**, v. 27, n. 9, p. 534-540, 2005. <DOI: 10.1590/S0100-72032005000900006>

KLOPFLEISCH, R.; GRUBER, A. D. Differential expression of cell cycle regulators p21, p27 and p53 in metastasizing canine mammary adenocarcinomas versus normal mammary glands. **Research in veterinary science**, v. 87, n. 1, p. 91-96, 2009. <DOI: 10.1016/j.rvsc.2008.12.010>

MAYER, M. N.; KRAFT, S. L.; BUCY, D. S.; WALDNER, C. L.; ELLIOT, K. M.; WIEBE, S. Indirect magnetic resonance lymphography of the head and neck of dogs using Gadofluorine M and a conventional gadolinium contrast agent: A pilot study. The Canadian Veterinary Journal, v. 53, n. 10, p. 1085, 2012.

MORRISON, W. B. Canine and feline mammary tumors. In: MORRISON, W. B. **Cancer in dogs and cats: Medical and Surgical Management** 2.ed. Baltimore :Willians & Wilkins, 1998. Cap.39. p.591-598.

MURPHY, S. Mammary tumours in dogs and cats. **In practice**, v. 30, n. 6, p. 334-339, 2008. <DOI: 10.1136/inpract.30.6.334>

NYMAN, H. T.; KRISTENSEN, A. T.; LEE, M. H.; MARTINUSSEN, T.; MCEVOY, F. J. Characterization of canine superficial tumors using gray-scale B mode, color flow mapping, and spectral doppler ultrasonography—a multivariate study. **Veterinary Radiology & Ultrasound**, v. 47, n. 2, p. 192-198, 2006. <DOI: 10.1111/j.1740-8261.2006.00127.x>

NYMAN, H. T.; KRISTENSEN, A. T.; SKOVGAARD, I. M.; MCEVOY, F. J. Characterization of normal and abnormal canine superficial lymph nodes using gray-scale B-mode, color flow mapping, power, and spectral Doppler ultrasonography: a multivariate study. **Veterinary Radiology & Ultrasound**, v. 46, n. 5, p. 404-410, 2005. <DOI: 10.1111/j.1740-8261.2005.00074.x>

NYMAN, H. T.; O'BRIEN, R. T. The sonographic evaluation of lymph nodes. **Clinical techniques in small animal practice**, v. 22, n. 3, p. 128-137, 2007. <DOI: 10.1053/j.ctsap.2007.05.007>

OLIVEIRA FILHO, J. C.; KOMMERS, G. D.; MASUDA, E. K.; MARQUES, B. M. F. P. P.; FIGHERA, R. A.; IRIGOYEN, L. F.; BARROS, C. S. L. Estudo retrospectivo de 1.647 tumores mamários em cães. **Pesquisa Veterinária Brasileira**, v. 30, n. 2, p. 177-185, 2010. <DOI: 10.1590/S0100-736X2010000200014>.

PATSIKAS, M. N.; KARAYANNOPOULOU, M.; KALDRYMIDOY, E.; PAPAZOGLOU, L. G.; PAPADOPOULOU, P. L.; TZEGAS, S. I.; TZIRIS, N. E.; KAITZIS, D. G.; DIMITRIADIS, A. S.; DESSIRIS, A. K. The lymph drainage of the neoplastic mammary glands in the bitch: a lymphographic study.

Anatomia, histologia, embryologia, v. 35, n. 4, p. 228-234, 2006. <DOI: 10.1111/j.1439-0264.1996.tb00072.x/abstract>

PAULINELLI, R. R.; FREITAS-JUNIOR, R.; RAHAL, R. M. S..; OLIVEIRA, L. F. P.; VILELA, M. H. T.; MOREIRA, M. A. R.; ALVES, K. L.; PELEJA, M. B. DE RESENDE, T. C. C. A prospective randomized trial comparing patent blue and methylene blue for the detection of the sentinel lymph node in breast cancer patients. **Revista da Associação Médica Brasileira**, v. 63, n. 2, p. 118-123, 2017. <DOI: 10.1590/1806-9282.63.02.118>

PAZ, W. A.; PAIM, S. P.; DE MELLO, G., L.; RANGEL, K. K.; CHRISTO, R. R.; GONÇALVES, R.; SILVA, M. E.; BARROSO, A. A.; DE SOUZA, A. F. et al. Biópsia de linfonodo sentinela; experiência clínica. **Revista Brasileira de Cancerologia**, v. 47, p. 309-15, 2001.

PEREIRA, C. T.; RAHAL, S. C.; DE CARVALHO BALIEIRO, J. C.; RIBEIRO, A. A. C. M. Lymphatic drainage on healthy and neoplasic mammary glands in female dogs: can it really be altered?. **Anatomia, histologia, embryologia**, v. 32, n. 5, p. 282-290, 2003. <DOI: 10.1046/j.1439-0264.2003.00485.x>

PHILIBERT, J. C.; SNYDER, P. W.; GLICKMAN, N.; GLICKMAN, L. T.; KNAPP, D. W.; WATERS, D. J. Influence of host factors on survival in dogs with malignant mammary gland tumors. **Journal of Veterinary Internal Medicine**, v. 17, n. 1, p. 102-106, 2003. <DOI: 10.1111/j.1939-1676.2003.tb01330.x>

PINHEIRO, L. G. P.; DE MORAES, M. O.; SOARES, A. H.; LOPES, A. J. T.; NAGUÉRE, M. A. S. P.; GONDIM, F. A. L.; BRANDÃO, C. B.; NASCIMENTO, D. C. H.; SOARES, J. P. H.; SILVA, J. M. M. Estudo experimental de linfonodo sentinela na mama da cadela com azul patente e Tecnécio Tc99m. **Acta cirúrgica brasileira**, v. 18, n. 6, p. 545-552, 2003. <DOI: 10.1590/S0102-86502003000600006>

QUADROS, L. G. A.; GEBRIM, L. H. A pesquisa do linfonodo sentinela para o câncer de mama na prática clínica do ginecologista brasileiro. **Revista Brasileira de Ginecologia e Obstetrícia**, v. 29, n.3, p.158-164, 2007. <DOI: 10.1590/S0100-72032007000300008>

QUEIROGA, F.; LOPES, C. Tumores mamários caninos – novas perspectivas. In: Congresso de Ciências Veterinárias, 1., 2002, Oeiras, PI. **Anais...** Oeiras: Revista Científica eletrônica de medicina veterinária, 2002. p.183-190.

ROCKSON, S. G. Diagnosis and management of lymphatic vascular disease. **Journal of the American College of Cardiology**, v. 52, n. 10, p. 799-806, 2008. <DOI: 10.1016/j.jacc.2008.06.005>

ROSSI, F.; KÖRNER, M.; SUÁREZ, J.; CAROZZI, G.; MEIER, V. S.; ROOS, M.; BLEY, C. R. Computed tomographic-lymphography as a complementary technique for lymph node staging in dogs with malignant tumors of various sites. Veterinary Radiology & Ultrasound, v. 59, n. 2, p. 155-162, 2018. <DOI: 10.1111/vru.12569>.

SILVA, P.; USCATEGUI, R. A. R.; MARONEZI, M. C.; GASSER, B.; PAVAN, L.; GATTO, I. R. H.; DE ALMEIDA, V. T.; VICENTE, W. R. R.; FELICIANO, M. A. R. Ultrasonography for lymph nodes metastasis identification in bitches with mammary neoplasms. **Scientific reports**, v. 8, n. 1, p. 1-8, 2018. <DOI: 10.1038/s41598-018-34806-9>

SKOBE, M.; HAWIGHORST, T.; JACKSON, D. G.; PREVO, R.; JANES, L.; VELASCO, P.; RICCARDI, L.; ALITALO, K.; CLAFFEY, K.; DETMAR, M. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis.

Nature medicine, v. 7, n. 2, p. 192-198, 2001.

SORENMO, K. U. Canine mammary gland tumours. **Veterinary Clinics of Small Animal Practice**, v. 33, p. 573-596, 2003. Available from: < http://dx.doi.org/10.1016/S0195-5616(03)00020-2>. Accessed: Aug. 12, 2017. doi: 10.1016/S0195-5616(03)00020-2.

SORENMO, K. U.; WORLEY, D. R.; GOLDSCHMIDT, M. H. Tumors of the mammary gland. In: WITHROW, S.J. et al. **Withrow & MacEwen`s small animal clinical oncology**. 5.ed. St. Louis: Elsevier. p.538-556. 2013

SOULTANI, C.; PATSIKAS, M. N.; KARAYANNOPOULOU, M.; JAKOVLJEVIC, S.; CHRYSSOGONIDIS, I.; PAPAZOGLOU, L.; PAPAIOANNOU, N.; PAPADOPOULOU, P.; PAVLIDOU, K.; ILIA, G. A.; KAITZIS, D.; ILIA, T. M. Assessment of sentinel lymph node metastasis in canine mammary gland tumors using computed tomographic indirect lymphography. **Veterinary Radiology & Ultrasound**, v. 58, n. 2, p. 186-196, 2017. <DOI: 10.1111/vru.12460>

SOUZA, F. W.; BRUN, M. V.; DE NARDI, A. B.; HUPPES, R. R.; QUARTERONE, C.; RAPOSO, T. M. M.; KASPER, P. N.; DE OLIVEIRA, M. T.; GUEDES, R. L. Linfadenectomia laparoscópica em cadela com neoplasia mamária. **Ciência Rural**, v. 43, n. 4, p. 750-753, 2013. <DOI: 10.1590/S0103-84782013005000028>

STAN, F.; GUDEA, A.; IOANA, C.; DAMIAN, A.; PAPUC, I.; DEZDROBITU, C.; BOCHIS, I. et al. Identifying The Limphatic Vascular Model Of The Mammary Gland In Bitches. **Lucaristiinlifice Medicina Veterinara**, v. 43, n. 2, p. 356-363, 2010.

SUGA, K.; YUAN, Y.; OGASAWARA, N.; OKADA, M.; MATSUNAGA, N. Localization of breast sentinel lymph nodes by MR lymphography with a conventional gadolinium contrast agent: Preliminary observations in dongs and humans. **Acta Radiologica**, v. 44, n. 1, p. 35-42, 2003. <DOI: 10.1258/rsmacta.44.1.35>.

SWAIM, S. F. Enxertos cutâneos. In: SLATTER, D. **Manual de cirurgia de pequenos animais**. 3.ed: São Paulo: Manole; 2007. p. 405-15.

TOZAKI, M.; ISOBE, S.; YAMAGUCHI, M.; OGAWA, Y.; HOMMA, K.; SAITO, M.; JOO, C.; FUKUMA, E. Ultrasonographic elastography of the breast using acoustic radiation force impulse technology: preliminary study. Japanese **Journal of Radiology**, v. 29, n. 6, p. 452-456, 2011. <DOI: 10.1007/s11604-011-0561-2>

TUOHY, J. L.; MILGRAM, J.; WORLEY, D. R.; DERNELL, W. S. et al. A review of sentinel lymph node evaluation and the need for its incorporation into veterinary oncology. **Veterinary and comparative oncology**, v. 7, n. 2, p. 81-91, 2009. <DOI: 10.1111/j.1476-5829.2009.00183.x>

TUOHY, J. L.; WORLEY, D. R. Pulmonary lymph node charting in normal dogs with blue dye and scintigraphic lymphatic mapping. **Research in veterinary science**, v. 97, n. 1, p. 148-155, 2014. <DOI: 10.1016/j.rvsc.2014.07.002>

WEAVER, D. L.; KRAG, D. N.; ASHIKAGA, T.; HARLOW, S. P.; O'CONNELL, M. Pathologic analysis of sentinel and nonsentinel lymph nodes in breast carcinoma: a multicenter study. **Cancer: Interdisciplinary International Journal of the American Cancer Society**, v. 88, n. 5, p. 1099-1107, 2000. <DOI: 10.1002/(SICI)1097-0142(20000301)88:5<1099::AID-CNCR22>3.0.CO;2-7>

WILLIAMS, L. E.; PACKER, R. A. Association between lymph node size and metastasis in dogs with oral malignant melanoma: 100 cases (1987–2001). **Journal of the American**

Veterinary Medical Association. v.222, n.9, p.1234–1236, 2003. <DOI: /10.2460/javma.2003.222.1234>.
WITHROW, S.J.; VAIL, D.M. **Small Animal Clinical Oncology**. St. Louis: Elsevier, 2007.
WOJCINSKI, S.; DUPONT, J.; SCHMIDT, W.; CASSEL, M.; HILLEMANNS, P. Real-time ultrasound elastography in 180 axillary lymph nodes: elasticity distribution in healthy lymph nodes and prediction of breast cancer metastases. **BMC medical imaging**, v. 12, n. 1, p. 35, 2012. <DOI: 10.1186/1471-2342-12-35>
YOUK, J. H.; SON, E. J.; KIM, J. A.; GWEON, H. M. Pre-operative evaluation of axillary lymph node status in patients with suspected breast cancer using shear wave elastography. **Ultrasound in medicine & biology**, v. 43, n. 8, p. 1581-1586, 2017. <DOI: 10.1016/j.ultrasmedbio.2017.03.016>
ZUCCARI, D. A. P. C.; SANTANA, A. E.; ROCHA, N. S. Expressão dos filamentos intermediários no diagnóstico dos tumores mamários de cadelas. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 54, n. 6, p. 586-591, 2002. <DOI: 10.1590/S0102-09352002000600005>