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Mammary neoplasms in female dogs: Clinical, diagnostic and therapeutic aspects

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Abstract: With the increase in the life expectancy of domestic animals and their increasingly affectionate relationship with their owners, it is possible to observe an increase in cases of neoplasms in these animals. Mammary neoplasia mainly affects older females who have not been castrated, due to hormonal dependence for the development of the tumour. The main form of treatment is surgery. This study aims to carry out an updated review on mammary neoplasms in female dogs covering the anatomy, physiology, prevalence, causes, diagnoses, treatments, prevention and prognosis, based on scientific articles by renowned researchers.

Keywords: dogs; mammary tumour; mammary lump; manual; mammary carcinoma

INTRODUCTION

The occurrence of neoplasms in domestic animals, especially in dogs, has increased due to the greater longevity of these animals. This may be due to the use of balanced diets, vaccination schedules, advanced diagnostic techniques, and increasingly specific and effective therapeutic protocols (Reis et al. 2010; Ribas et al. 2012; Biondi et al. 2014). This fact has allowed major advances in veterinary oncology, which stands out in the search for improvements in the prevention, diagnosis and treatment of neoplasms (Beauvais et al. 2012; Nagata et al. 2014).

Research involving mammary neoplasms in canine and feline females has gained importance, on the one hand, due to the similarities, in some aspects, to women and, on the other hand, due to the frequency with which they appear in companion animal clinics (Silva et al. 2004). Complete knowledge about the care and diagnosis of breast cancer in women is extremely important for public health, motivating studies on prevention and early diagnoses that seek to reduce morbidity and mortality related to this neoplasm (Humphrey et al. 2002). Also in veterinary medicine, there is great interest on the part of researchers, mainly due to the high casuistry of oncological care, often with late diagnoses, which compromises the success of treatment and reduces the survival rate of patients (Silva et al. 2004; Cavalcante and Cassali 2006; Andrade et al. 2010; Estrela-Lima 2010).

Mammary tumours are the most common neoplasms in veterinary oncology services in Brazil, representing more than 50% of the tumours diagnosed in dogs (Ribas et al. 2012; Biondi et al. 2014; Collivignarelli et al. 2021), the majority of which are malignant (Biondi et al. 2014; Valdivia et al. 2021). Metastases and recurrences develop in about 35–70% of bitches after excision (Collivignarelli et al. 2021).

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LITERATURE REVIEW

Surgical anatomy

The female dog has two mammary chains, with five glands each, arranged in parallel from the thoracic region to the inguinal region, named cranial thoracic (M1) and caudal (M2), cranial abdominal (M3) and caudal (M4) and inguinal (M5). Macroscopically, the mammary gland has bilaterally symmetrical and parallel glandular units in the ventral midline (Dyce et al. 2010), and, microscopically, it is a modified exocrine, tubuloalveolar sweat gland, responsible for the production and secretion of milk, specific to mammals (Getty 2008; Reese et al. 2011).

Regarding the blood supply to the mammary glands in bitches, the cranial and caudal thoracic mammary glands receive arterial blood from the internal thoracic, intercostal and lateral thoracic arteries; the cranial abdominals are irrigated by the cranial superficial epigastric artery and the caudal and inguinal abdominals are mainly irrigated by the caudal superficial epigastric artery (Dyce et al. 2010; Reese et al. 2011). Venous drainage of glands acts like arterial irrigation, however, small veins may cross the midline between the right and left glands (Reese et al. 2011).

The lymphatic drainage of the mammary chains in bitches is complex. In general, the cranial and caudal thoracic mammary glands drain into the ipsilateral superficial axillary and cervical lymph nodes (Lana et al. 2007; Dyce et al. 2010). The cranial abdominal breast drains mainly into the axillary lymph node, but simultaneously drains into the inguinal lymph node, both ipsilateral. The caudal and inguinal abdominal breasts drain into the ipsilateral superficial inguinal lymph node (Cassali et al. 2014). Drainage to the contralateral lymph node has already been described and may occur through lymphangiogenesis induced by the neoplasia (Sorenmo et al. 2011).

According to Sorenmo et al. (2011) some differences can be observed in the drainage of mammary glands without neoplasms when compared to neoplastic mammary glands (Table 1).

The lymphatic system is the main route for the generation of metastases from malignant mammary tumours in dogs (Queiroga and Lopes 2002), therefore, the clinician and surgeon require knowledge regarding mammary lymphatic drainage for the adequate surgical excision, as well as for prognostic determination (Cassali et al. 2014).

Inspection of the regional lymph node should be part of the routine evaluation of mammary neoplasms in bitches, as the presence of metastases has an impact on the neoplastic staging, survival and treatment to be recommended (Cassali et al. 2014).

Aetiology and predisposing factors

The aetiology of breast tumours may be related to dietary, genetic, environmental and hormonal factors (Henderson and Feigelson 2000; Silva et al. 2004; Uva et al. 2009; Andrade et al. 2010; Ribas et al. 2012; Toribio et al. 2012), with especial emphasis on this last factor, hormonal.

Despite the epidemiological importance of breast tumours in bitches in Brazil, medical records of the care for these animals are often incomplete (Biondi et al. 2014), missing important information about the reproductive life such as castration, occurrence of illnesses reproductive disorders (pseudocyesis,

Table 1. Normal and neoplastic lymphatic drainage of mammary gland tumours in bitches

Mammary gland	Normal lymphatic drainage	Neoplastic lymphatic drainage
M1	axillary lymph node	axillary and sternal lymph node
M2	axillary lymph node	axillary and sternal lymph node
M3	axillary and superficial inguinal lymph node	axillary, superficial inguinal and medial iliac lymph nodes
M4	superficial inguinal lymph node	superficial inguinal and axillary lymph node
M5	superficial inguinal lymph node	superficial inguinal, popliteal and medial lymph nodes on the thigh

Source: Adapted from Sorenmo et al. (2011)

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abortion and others) and use of contraceptives which are often related to the aetiology of these tumours in bitches (Ribas et al. 2012; Toribio et al. 2012).

In both normal and neoplastic breasts, tissue growth is stimulated by hormones, oestrogen, progesterone and prolactin, and growth factors (Misdorp 2002; Sorenmo et al. 2013; Cassali et al. 2014; de Araujo et al. 2015).

Oestrogen plays an important role in oncogenesis as it stimulates the mitotic activity of the mammary epithelium, which increases the risk of neoplasms in this organ. The expression of the oestrogen receptor (ER) is undoubtedly an important biomarker in human breast cancer, as its presence determines the sensitivity index to hormonal treatment (Buitrago et al. 2011). In bitches, the proportion of benign mammary tumours that present an increase in ERs and progesterone receptors (PRs) is high, while carcinomas present a lower concentration of these receptors (Horta et al. 2012).

Progesterone acts to suppress the activity of the myometrium, stimulates the growth of endometrial glands and promotes the development of breast alveolar tissue (Martins and Lopes 2005). When associated with oestrogen, it plays an important role in the development of breast neoplasms, due to the proliferative factor (Misdorp 2002; Queiroga et al. 2010). When associated with prolactin, it increases the number of oestrogen receptors, facilitating the mitotic action of this hormone (Misdorp 2002).

Given the above, one of the main risk factors for tumour development are sex hormones (Beauvais et al. 2012). Furthermore, the occurrence of pseudocyesis and the use of progestins as contraception may contribute to the formation of these tumours. When administering exogenous progesterone to dogs or cats, there is stimulation of the growth hormone synthesis in the mammary gland, lobuloalveolar proliferation and consequent hyperplasia of the myoepithelial and secretory elements, inducing the formation of benign nodules in young animals (Rutteman et al. 2001). Breast tissues can undergo malignant metaplasia when constantly exposed to progesterone (Misdorp 2002).

In pseudocyesis, the non-pregnant female presents an increase in serum levels and/or sensitivity to prolactin, associated with a faster than normal decrease in progesterone. In bitches that present recurrent pseudocyesis, high concentrations of prolactin cause milk retention and the possible formation of mammary neoplasms (Martins and Lopes 2005).

Some studies show a possible relationship between obesity and the development of mammary neoplasms in bitches (Cleary et al. 2010; Lim et al. 2015; Chandler et al. 2017). Obesity in the development of these neoplasms may be related to the actions of leptin and adiponectin proteins (Cleary et al. 2010). Leptin inhibits apoptosis and stimulates cell proliferation while adiponectin reduces cell proliferation and promotes apoptosis. These substances produced by the adipose tissue undergo changes in concentrations according to the body weight, so when the weight and body mass index increase, the serum concentrations of leptin increase and adiponectin decrease (Cleary et al. 2010; Lim et al. 2015).

Obesity may also be associated with a decrease in the serum concentration of sex hormones bound to the globulin, resulting in an increase in the serum concentrations of free oestrogen. Furthermore, adipose tissue can be a source of increased oestrogen production via aromatase mediated androgen conversion (De Nardi et al. 2016).

In a study carried out by Sirivaidyapong (2003), 91.3% of bitches that received snacks or were fed a homemade diet mixed or not with food, had mammary tumours. Homemade food, rich in meat and animal fat, causes, in addition to obesity, an increase in the emergence of mammary neoplasms, with these factors having a greater role in promoting carcinogenesis (Cotran et al. 2000; Sirivaidyapong 2003).

There are few studies correlating the type of diet with carcinogenesis in animals, however, there are some reports in humans, and the diet can cause 20% to 30% of all cancers in humans in economically developed countries. Some research reveals that animals that received homemade food, mainly meat, especially pork¸ increased the development of dysplasia and mammary tumours. There is a higher prevalence of mammary neoplasia in bitches that are obese and that received a diet rich in red meat (De Nardi et al. 2016).

Diagnosis

CLINICAL APPROACH AND STAGING

The majority of bitches with mammary neoplasms are clinically healthy at the time of diagnosis and the nodules can be identified by owners or veteri-

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narians during routine clinical examination (Cassali et al. 2011). However, according to Oliveira et al. (2021), approximately 25% to 50% of dogs with malignant mammary tumours already have metastases at the time of diagnosis.

The early diagnosis of mammary gland neoplasms is essential, through a complete physical evaluation, laboratory tests, imaging diagnoses and the microscopic evaluation of the nodule, which will be fundamental for establishing adequate treatment, essential in preventing recurrence or metastasis (Novosad 2003).

Information regarding the time of onset and progression of the lesion, previous treatment and reproductive history is important (Cassali et al. 2014). After the anamnesis and complete physical examination to determine the general condition (Cassali et al. 2014), the female must undergo a specific physical examination of the mammary gland, which consists of palpation of the mammary chains and regional lymph nodes to identify nodules or changes in the size, shape or consistency, macroscopically defining the lesions (Giuliano et al. 2011; Cassali et al. 2014; Brissot and Edery 2017).

After palpation of the mammary gland and lymph nodes, a fine needle cytopathological examination (FCAA) of the nodules and lymph nodes should be used as a complementary part of the diagnosis (De Nardi et al. 2016). According to Lana et al. (2007), there is no predisposition regarding the mammary chains affected in the neoplastic process, however, some authors reported that approximately 60% of neoplasms occur in the last two pairs of glands, that is, the caudal and inguinal abdominals, as they have a greater volume of glandular tissue (Misdorp 2002; Goldschmidt et al. 2017).

Regarding the morphology, they can appear as small or large nodules, plaques, adherent or mobile, single or multiple, ulcerated, or circumscribed, depending on the biological behaviour of the tumour (Misdorp 2002; Sorenmo et al. 2011; Goldschmidt et al. 2017).

Nodules larger than five centimetres, fast-growing, adherent, with large areas of ulceration and with metastases to the lymph nodes are associated with the worst prognosis (Ferreira et al. 2009; Estrela-Lima 2010; Beserra et al. 2016). Breasts with multiple nodules can present different histological types, both benign and malignant, these aspects must be taken into consideration, as they influence the prognosis (Cassali et al. 2014).

Regarding the fact that there is no breed predisposition (Dore et al. 2003), however, some studies demonstrate a higher incidence in Cocker Spaniel, Dachshund, English Setter, Pointer, Fox Terrier, Boxer and Beagle bitches (Dore et al. 2003). However, with the breed, it is important to consider that breed predisposition is not a determining factor, as there is a large percentage of dogs of no defined breed being affected by mammary neoplasia, concluding that it is complex to establish a standard of predisposed breeds (Misdorp 2002; Zatloukal et al. 2005).

Clinical staging of bitches with mammary neoplasms must be carried out so that planning can be carried out regarding treatment options and the prognosis can be determined (Batschinski and Tedardi 2016). The World Health Organization (WHO) proposed the TNM system whose variables include the assessment of the primary tumour (T), involvement of the regional lymph nodes (N) (axillary and superficial inguinal) and identification of distant metastases (M) (Rutteman et al. 2001; Batschinski and Tedardi 2016; De Nardi et al. 2016; Cassali et al. 2020). This classification system defines five clinical stages according to the tumour progression (Table 2).

Table 2. Clinical staging for bitches with mammary neoplasms according to the TNM system

T – Primary tumour	
T1:	< 3 cm diameter
T2:	3–5 cm diameter
T3:	> 5 cm diameter
N – Regional lymph nodes	
N0:	absence of metastasis (cytology or histology)
N1:	presence of metastasis (cytology or histology)
M – Distant metastasis	
M0:	absence of metastasis
M1:	distant metastasis
Stages	
I:	T1N0M0
II:	T2N0M0
II:	T2N0M0
III:	T3N0M0
IV:	Any TN1M0
V:	Any T any N M1

Source: Adapted from Cassali et al. (2011)

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Patients with stage IV or V, as well as those with nodules larger than five centimetres (T3), have a negative impact on the prognosis, with shorter survival times than others (Cassali et al. 2020). According to Nunes et al. (2018), dogs in clinical stage IV had a median survival time of 331 days compared to 1 149 days for dogs in stage I.

Palpation of the superficial axillary and inguinal lymph nodes should be performed in search of changes. It is important to highlight that the absence of inflammation and lymph node enlargement does not exclude neoplastic involvement of the lymph nodes, and cytology should be performed on the regional lymph nodes and a histopathological examination should be performed when a mastectomy is performed, being considered important factors in the prognosis having a great impact on the survival of dogs (Nunes et al. 2018; Cassali et al. 2020).

According to Oliveira Filho et al. (2010), more than 25% of cases of mammary cancer present with lymph node metastasis. The distribution of these metastases is determined according to the blood and lymphatic drainage of the primary tumour, with the preferred sites for metastasis being regional lymph nodes and mainly the lungs (De Nardi et al. 2013; Nunes et al. 2018), justified due to the intense flow of blood that passes through this organ, in which a network of capillaries slows circulation and acts as a filter for aggregates of tumour cells, which lodge in the vascular tree, inserting pseudopods between the endothelial cells and migrating to the lung parenchyma (Cotram et al. 2000).

Imaging exams play a fundamental role in helping both the initial diagnosis and the therapeutic response in oncological cases (Oliveira et al. 2021); The radiographic examination is extremely important for the staging of animals, as one of the sites most affected by distant metastasis is the lung. Therefore, all bitches with mammary tumours need to undergo radiography in three views (right and left lateral and ventrodorsal). However, pulmonary micrometastases (nodules between 0.2 mm and 2 mm) are not visualised using chest radiography. Therefore, if metastases are not identified on the chest X-ray examination, the presence of metastases cannot be ruled out and tomography examinations may be indicated (Dobson and Lascelles 2011).

An abdominal ultrasound is also indicated for metastasis research, as it allows for the evaluation of the echotexture of the tumour process and its invasiveness, which will help in planning the surgical procedure (Nunes et al. 2018). Computed tomography allows the identification of metastatic nodules smaller than 6 mm, being considered the gold standard for researching metastasis in both the thoracic and abdominal cavities (Cassali et al. 2014).

The investigation of distant metastasis is essential to determine the clinical staging and therapeutic plan. Dogs with distant organs infiltrated by tumour cells may not benefit from surgery (Nunes et al. 2018).

MICROSCOPIC EVALUATION

The microscopic characteristics of the tumour associated with clinical staging are important tools for determining the prognosis and defining the best therapeutic strategy (Cassali et al. 2020).

HISTOLOGIC EVALUATION

A histopathological evaluation, commonly performed after surgery, is recommended for all cases and, in addition to the primary tumour, all mammary glands and regional lymph nodes must be evaluated (Cassali et al. 2020). In case of multiple nodules, the histopathological diagnosis should be performed on all of them, which will be useful in determining the prognosis and additional treatment options (Litterine-Kaufman et al. 2019).

Despite the existence of different tumour classification methods and the absence of uniform criteria to differentiate types of neoplasms, in Brazil, Cassali et al. (2020) published an updated histological classification (Table 3).

The histopathological classification of mammary neoplasms in bitches is an important tool to indicate the biological behaviour of the neoplasm, however, it is controversial among pathologists due to the histogenetic complexity. The most used classifications are those described by Moulton (1990), from the World Health Organization (WHO) and later Goldschmidt et al. (2011), with some divergences between them. Histological types such as solid carcinoma, micropapillary carcinoma, carcinosarcomas and sarcomas are associated with an unfavourable prognosis with shorter survival time and greater risk of developing metastasis (Horta et al. 2012; Cassali et al. 2014; Nunes et al. 2014; de Oliveira Gamba et al. 2017; Nunes et al. 2019).

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Table 3. Histological classification of canine mammary tumours

Histological classification of canine mammary tumours	
Non-neoplastic epithelial lesions	
Ductal hyperplasia	Nodular hyperplasia
Adenosis	Ductal ectasia
Columnar cell injuries:	
- Columnar cell changes	- Columnar cell hyperplasia
- Atypical columnar cell lesion	
Benign tumours	
Adenoma	Adenomyoepithelioma
Myoepithelioma	Basaloid adenoma
Fibroadenoma	Benign mixed tumour
Ductal papilloma	Phyllodes tumour
Malignant tumours	
- Carcinomas	
Carcinoma *in situ*: ductal carcinoma *in situ*, lobular carcinoma in situ	
Carcinoma in mixed tumour	
Papillary carcinoma (inva sive and non-invasive)	
Tubular carcinoma	Solid carcinoma
Basaloid carcinoma	Cibriform carcinoma
- Special types of carcinomas	
Micropapillary carcinoma	Pleomorphic lobular carcinoma
Secretory carcinoma	Mucinous carcinoma
Lipid-rich carcinoma	Glycogen-rich carcinoma
Squamous cell carcinoma	Fusiform cell carcinoma
Carcinoma with sebaceous differentiation	
- Myoepithelial neoplasms	
Malignant adenomyoepi thelioma	Malignant myoepithelioma
- Sarcomas	
Fibrosarcoma	Osteosarcoma
Carcinosarcoma	Sarcoma in mixed tumour
- Other sarcomas	
Condrosarcoma, Liposar coma, Hemangiosarcoma, Phyllodes sarcoma	

Source: Cassali et al. (2020)

IMMUNOHISTOCHEMISTRY

The immunohistochemistry technique is used in veterinary medicine to identify various markers related to the biological behaviour of tumours to determine prognostic and predictive factors for neoplasms (Ramos-Vara et al. 2008). According to Cassali et al. (2020), the immunohistochemical panel for canine and feline mammary carcinomas should be composed of the expressions of the oestrogen receptor (ER), progesterone receptor (PR), Ki-67 and COX-2.

The Ki-67 proliferation index is a cell cycle-related marker widely used in canine neoplasms (Kaszak et al. 2018). This marker estimates the cellular proliferation potential of breast neoplasms and is related to the prognosis of these tumours. In general, tumours with higher proliferative rates exhibit more aggressive behaviour with an increased risk of metastasis (Nowak et al. 2016; Kaszak et al. 2018).

Ki-67 is a nuclear protein, non-histone, detected in cells during all the active phases of the cell cycle (G1, S, G2 and M) that disappears rapidly after mitosis. Ki-67 is considered the main prognostic marker of the cell proliferation index in canine mammary neoplasms (Cassali et al. 2014).

According to the latest Brazilian consensus, the proliferation index should be determined by Ki-67 nuclear staining evaluated in at least 1 000 neoplastic cells in high power fields (400×), also considering the size of the microscopic field and the suggested cut off point is ≥ 20% (Cassali et al. 2020).

COX-2 plays an important role in several neoplasms contributing to tumour development and angiogenesis (Lavalle et al. 2009; Carvalho et al. 2017). It participates in the metabolism of arachidonic acid, generating prostaglandins that are responsible for cell proliferation, apoptosis, modulation of the immune system and angiogenesis. Increased expression of COX-2 in canine mammary neoplasms has been associated with aggressive characteristics, such as histological grades, the mitotic index and lymph node metastasis and, consequently, a worse prognosis (Queiroga et al. 2010; Guimaraes et al. 2014; Milanta et al. 2016).

Lavalle et al. (2009) observed that the high expression of COX-2 in canine mammary neoplasms correlates with a shorter survival time. Similarly, Queiroga et al. (2010) observed that the higher the expression of COX-2 in canine mammary neoplasms, the greater the chance of lymph node and

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distant metastasis formation, and consequently, a shorter survival time.

Oestrogen and progesterone, in addition to being essential for the normal development of breast tissue, also influence tumour growth, since the majority of mammary neoplasms express ER and/or PR (Kim et al. 2014). Animals with mammary tumours that express ER and PR, or only ER, have a better prognosis when compared to those that express only PR, as positive ERs correlate with well-differentiated tumours (Kim et al. 2014; Mohr et al. 2016). A decreased ER/PR ratio in mammary carcinomas has been associated with decreased cellular differentiation and disease progression (Kim et al. 2014).

Sentinel lymph node

The importance of evaluating regional lymph nodes to quantify the metastatic spread of neoplasia is well recognised in veterinary medicine, however, harvesting the correct lymph node may not be possible without mapping the sentinel lymph node (SLN) (Beer et al. 2023).

The SLN is defined as the first lymph node to drain a neoplasm, and, for this reason, it should be the first site to receive tumour cells if lymphatic dissemination occurs (Paz et al. 2001; Tuohy et al. 2009; Beer et al. 2023).

Studies show that lymph nodes corresponding to regions that have neoplasia, even without changes in size and consistency, may already have metastasis (Beserra et al. 2016).

The difficulty of lymph node resection, especially in the axillary chain, can lead to serious errors in the prognosis and the identification of metastatic focus (Matos et al. 2012). Therefore, lymph node staging techniques in bitches need to be considered, since the assessment of regional lymph nodes becomes essential for the prognosis and therapeutic approaches (Cassali et al. 2014).

The use of dyes (patent blue and methylene blue) applied to peri-neoplastic tissue has been used to identify the SLN, as they facilitate the identification of lymph nodes, in addition to minimising surgical incisions, especially when evaluating the axillary lymph node (Sorenmo et al. 2013).

The administration of patent blue dye (2 mg/kg), intradermally peritumoral, helps identify the lymphatic flow and indicates the correct SLN (Pinheiro et al. 2003; Cassali et al. 2020). For such administration, the nodule must be divided into four quadrants, and the dye distributed equally in each of them, in the intradermal region. Five to ten minutes after applying the dye, it is now possible to identify the local lymphatic drainage and the corresponding regional lymph node, through the skin or below it, after the incision (Sorenmo et al. 2013; Worley 2014; Cassali et al. 2020).

The dose of methylene blue dye used by the team of Maues et al. (2016), in bitches with mammary neoplasms, to identify the axillary SLN, was 0.5 ml for a bitch weighing up to 15 kg and 1.0 ml for those weighing more than 15 kg, in two or more applications, in the cranial thoracic breast of the mammary chain affected by the neoplasm.

According to Beserra et al. (2016), the peritumoral intradermal application of patent blue resulted in 100% specificity and 89.5% sensitivity in identifying the ipsilateral axillary lymph node in bitches with mammary neoplasms.

In a study conducted by Maues et al. (2016), 2% methylene blue administered intradermally to mammary tumours located in the cranial thoracic gland was 76.27% effective for identifying the axillary lymph node. These authors cited the advantages of this dye as being low cost, easy to access and simple to apply.

Other non-colorimetric methods for marking the SLN include indirect lymphography with iodinated oil followed by radiography (Brissot and Edery 2017), indirect computed tomographic lymphography with iopamidol (Majeski et al. 2017) or with iohexol (Rossi et al. 2018), indirect assessment using M-mode ultrasound and elastography (Silva et al. 2004; Belota et al. 2019), in addition to lymphoscintigraphy with technetium 99 (Tc-99) (Beer et al. 2023).

This last method, lymphoscintigraphy with Tc-99, is considered the standard technique for detecting SLN in human oncology (Pereira et al. 2008), but has the disadvantage of the investment costs in facilities for handling radioactive materials, in addition to the operating costs for each procedure. Such factors make its use difficult in routine veterinary oncology, and it is therefore replaced by colorimetric markers.

Buitrago et al. (2011) described that around 70% of patients with axillary lymph node involvement will develop recurrence within 10 years, compared to 20% to 30% of patients with a negative node.

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Identification of these lymph nodes is a challenge for staging due to the inability to visualise, which results in failures that can lead to inadequate treatment, recurrence and distant metastasis.

Paulinelli et al. (2017) considers that this approach regarding SLN research has prognostic and therapeutic value, as the identification of neoplastic cells in the lymph node is related to neoplastic aggressiveness and potential for distant metastasis, respectively, enabling the reduction of regional and distant recurrence. For Maues et al. (2016) and Collivignarelli et al. (2021), the detection of SLN is of great importance for the surgeon, not only for the most appropriate surgical excision, but also for determining an accurate surgical prognosis, and should be incorporated into the surgical routine.

Therapy

Surgical excision is the primary treatment indicated in the control of breast neoplasms in which the objective is to remove the tumour(s) with clean margins and, depending on the case, prevent the development of new nodules in the remaining glands (Monteiro et al. 2011; Horta et al. 2014; Horta et al. 2015; Sorenmo et al. 2020). This technique can be curative in dogs without lymphatic involvement and distant metastasis or with less aggressive histological types (Horta et al. 2014; Sorenmo et al. 2020).

However, the surgery for dogs with distant metastases detected before surgery will not prolong the dog's survival time, but may increase the quality of life of patients with ulcerated and/or painful lesions (Cassali et al. 2020).

Surgical techniques include mastectomy, which can be simple, regional, complete unilateral and complete bilateral, depending on the involvement and severity of the neoplasm. Simple mastectomy aims to remove only the affected mammary, while regional mastectomy removes mammary glands with the same lymphatic drainage. Complete mastectomy aims to remove a unilateral or bilateral chain, whether only some glands are affected or all, this technique also has a prophylactic nature, since neoplastic cells are not seen macroscopically, and can be implanted in breasts that are apparently not affected. by neoplasia (Monteiro et al. 2011).

Experts in the field have not yet reached a consensus on which technique offers better local control and reduces the risk of metastases and tumour recurrence, requiring more research (Cassali et al. 2020).

Some oncologists advocate radical procedures, considering the possibility of developing new tumours in the remaining mammary glands. Additionally, small nodules may be associated with aggressive biological behaviour. Unilateral or bilateral mastectomy, even providing greater local control of the tumour, is associated with a greater risk of surgical complications due to the aggressiveness of the technique and is not always related to a longer survival time (Horta et al. 2014; Horta et al. 2015).

Regional mastectomy assumes that some mammary glands share the same lymphatic and venous drainage and must be removed simultaneously in the bloc along with their respective superficial regional lymph nodes (Monteiro et al. 2011; Cassali et al. 2020). This technique is indicated in cases of neoplasms smaller than 3 cm (T1), slow growing, not adhered to adjacent tissues, and without signs of inflammation (Cassali et al. 2020).

Unilateral mastectomy involves the removal of all glands from a mammary chain, associated with the ipsilateral superficial regional lymph nodes (axillary and inguinal), and is indicated for multiple nodules, lesions located in M3 and tumours with poor clinical prognosis factors factors of poor clinical prognosis, such as growth rapid and/or presence of lesions larger than 3 cm (T2 and T3) (Sorenmo et al. 2020). If the patient presents nodules in both breast chains, their removal is recommended in two surgical procedures, with an interval of four to six weeks between them (Cassali et al. 2020).

For patients in clinical stage II to V, a unilateral or bilateral mastectomy may be indicated; and stage I bitches can benefit from a regional mastectomy, always considering the lymphatic drainage of the mammary chain (Cassali et al. 2020).

Table 4 shows some guidelines for determining the surgical technique for mastectomy according to Cassali et al. (2020).

A total bilateral mastectomy consists of the simultaneous removal of the two mammary chains together with their bilateral superficial lymph nodes (axillary and inguinal), being indicated for dogs with a flat chest and elastic skin in which there is little compromise in surgical synthesis, but those animals with a deep chest will require reconstructive surgical techniques (Bartels et al.

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Table 4. Guidelines for determining the surgical technique and extent for single canine mammary tumours, depending on the location

Tumour location	Tumour size	Surgery
M1*	< 3 cm (T1)	regional mastectomy (M1 – M2 and axillary lymph node)
	> 3 cm (T2 or T3)	unilateral mastectomy
M2*	< 3 cm (T1)	regional mastectomy (M1 – M2 – M3 and axillary lymph node)
	> 3 cm (T2 or T3)	unilateral mastectomy
M3*	any size (T1, T2 or T3)	unilateral mastectomy
M4*	< 3 cm (T1)	regional mastectomy (M3 – M4 – M5 and inguinal lymph node)
	> 3 cm (T2 or T3)	unilateral mastectomy
M5*	< 3 cm (T1)	regional mastectomy (M4 – M5 and inguinal lymph node)
	> 3 cm (T2 or T3)	unilateral mastectomy

*Tumours associated with other prognostic factors should undergo unilateral mastectomy (Cassali et al. 2020)

1978). According to Cassali et al. (2020), this technique causes extensive tissue damage and should be avoided, except in cases of neoplastic invasion in the contralateral mammary chain.

The recommended surgical margin for breast tumours is 1–2 cm of healthy tissue, which may involve the adjacent muscular tissue in the deep plane (pectoral muscles, abdominal obliques or rectus abdominis), in case of tumour adherence. To plan adjuvant therapies, it is recommended to analyse the surgical margins, which can be done with nankin ink. If there are neoplastic cells in the stained area, the sample is considered to have "compromised margins" (Papazoglou et al. 2014).

When undergoing the surgical mastectomy procedure, the inguinal lymph node, due to its anatomical position, is removed next to the corresponding mammary gland. However, the axillary lymph node is only removed when there is a change in its palpation. This occurs, in part, due to the difficulty of locating it (Matos et al. 2012; Cassali et al. 2014). Studies carried out by Bianchi et al. (2018) and Collivignarelli et al. (2021) demonstrated that axillary lymph node removal should be included as a routine technique to allow better staging of mammary neoplasms in bitches.

An ovariohysterectomy can be performed at the time of mastectomy, but studies indicate that not all bitches will benefit from an increased survival time or recurrence of the disease. Those bitches who have ER-positive tumours, increased the serum oestrogen or reproductive changes unrelated to the mammary tumour may benefit from this procedure (Kristiansen et al. 2016). It should be considered that an ovariohysterectomy can lead to increased surgical trauma when performed together with a mastectomy, especially in patients who have comorbidities or an advanced stage of the disease (Cassali et al. 2020).

A palliative mastectomy aims to promote quality of life and pain control and may be indicated in patients with distant metastases and ulcerated tumours. In these cases, surgery will not influence the survival rate, promoting only the local control of neoplastic dissemination (Boston and Henderson 2014).

Although surgery is the treatment of choice, chemotherapy can help prevent recurrence and metastasis formation (Novosad 2003), and is also indicated in cases of inoperable tumours and when the patient already has distant metastases (Karayannopoulou and Lafioniatis 2016).

Bitches diagnosed with micropapillary carcinoma, solid carcinoma, carcinosarcoma and pleomorphic lobular carcinoma are at an increased risk of metastases and, in these cases, adjuvant chemotherapy is recommended, regardless of the clinical stage.

Patients with clinical stages IV and V, who have metastasis in the lymph nodes or lungs, will always benefit from chemotherapy (Cassali et al. 2011; Cassali et al. 2020).

Chemotherapy protocols using carboplatin, doxorubicin and gemcitabine are indicated for bitches with mammary neoplasms and can be used alone or in combination with cyclophosphamide and COX-2 inhibitors (Lavalle et al. 2012; Cassali et al. 2020). Suryawanshi (2021) concluded, in their

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study, that surgical excision combined with chemotherapy with cyclophosphamide is an effective protocol for the treatment of malignant mammary tumours in dogs, with minimal toxicity and it may be possible to increase the quality and survival of patients.

In both human and veterinary medicine, selective COX-2 inhibitors are indicated in the treatment of mammary carcinomas, associated with other antitumour drugs. To obtain good treatment results with COX-2 inhibitors, COX-2 expression in tumours must be previously determined (Kaszak et al. 2018).

Patients with breast cancer should be monitored every two months. In the first six months with clinical evaluations, chest X-rays in three positions and an abdominal ultrasound; subsequently, such exams must be carried out every three months, for two years (Cassali et al. 2020).

Inflammatory carcinoma

Inflammatory mammary carcinoma (IMC) is a highly aggressive subtype of mammary gland tumour that occurs spontaneously in women and bitches (Pena et al. 2003; Cassali et al. 2014), characterised as a progressive disease with a high mortality rate (Marconato et al. 2009).

Its clinical presentation is like mastitis or dermatitis, a hardened plaque with an appearance that resembles an orange peel, presenting extensive inflammation of the skin overlying the mammary glands, oedema and pain involving the axillary, mammary and inguinal regions. Patients with IMC may present with systemic signs of generalised weakness, anorexia and metastasis (Perez Alenza et al. 2001).

It is characterised by the presence of any subtype of carcinoma associated or not with an intense inflammatory reaction, infiltration in the dermis and its superficial lymphatic vessels, with lymphatic tumour emboli, observed on histopathology (Marconato et al. 2009; Cassali et al. 2014). It is noteworthy that for a correct diagnosis of IMC, the histopathological evaluation must be associated with the observation of inflammatory clinical signs (pain, heat, oedema, redness) (Goldschmidt et al. 2011).

Although the recommended treatment for breast carcinomas is surgical excision, in the case of IMC, it is difficult to define the surgical margins due to the extensive inflammation. In this case, chemotherapy and/or palliative treatments are preferable (Raposo et al. 2017).

There is no consensus on the best chemotherapy protocol to be used in dogs with IMC (Raposo et al. 2017), but clinical studies have shown a satisfactory response in relation to the prognosis of dogs treated with COX-2 inhibitors (piroxicam, firocoxib), alone or in combination with other chemotherapy drugs such as doxorubicin, carboplatin, cyclophosphamide and mitoxantrone (Clemente et al. 2009; Marconato et al. 2009; Gregorio et al. 2013). Carprofen at a dose of 4.4 mg/kg, orally, every 24 h and firocoxib at a dose of 5 mg/kg, orally, every 24 h, also provide clinical improvement in these patients (De Nardi et al. 2016).

This carcinoma has a poor prognosis and the average survival time varies from weeks to a few months (Marconato et al. 2009). According to Raposo et al. (2017), prognostic studies in dogs with IMC are challenging, because most of these animals will be euthanised, given the severity of the clinical signs presented, rather than dying from advanced metastatic disease.

Prevention

The role of sex hormones in the development of mammary tumours in bitches is supported by their high incidence in unspayed bitches or when ovariohysterectomy (OH) is performed after the second oestrous cycle (Lana et al. 2007; Kamiguchi et al. 2016).

Due to this, early castration (before the first oestrus) is recognised as the main measure for preventing mammary neoplasms in bitches (Lana et al. 2007; Kamiguchi et al. 2016), reinforcing that hormonal exposure during life increases the predisposition to developing mammary tumours.

According to Misdorp (2002), young bitches castrated before the first oestrus have a 0.5% risk of developing mammary cancer, while those that underwent the castration procedure after the first oestrous cycle have an 8% risk, and those that underwent castration after the second oestrus have a 26% greater risk of developing the neoplasm. OH performed after two and a half years of age, is not prophylactic, as it is no longer possible to inhibit hormonal action (Withrow et al. 2014).

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Some research has reported adverse effects of OH before the first oestrus, such as urinary incontinence and increased risk of other types of neoplasms, such as lymphoma, mast cell tumour and osteosarcoma (Riva et al. 2013; Hart et al. 2020a; Cooley et al. 2002).

Cooley et al. (2002) reported that Rottweiler bitches, castrated before one year of age, had a three to four times higher risk of developing osteosarcoma when compared to non-castrated bitches.

However, Riva et al. (2013) observed that when castration in bitches is carried out after 12 months of age, the risk of developing hemangiosarcoma is four times greater when subjected to early castration or those not castrated.

In a recent study carried out by Hart et al. (2020b), with mixed-breed dogs, the authors assessed the risk of developing orthopaedic problems and neoplasms such as hemangiosarcoma, mast cell tumour, lymphoma and osteosarcoma according to the animal's reproductive status and weight. These researchers did not observe an increase in the incidence of cancer related to early castration, however, dogs over 20 kilograms castrated before one year of age showed a high incidence of orthopaedic conditions, suggesting the need for a personalised surgical castration approach, according to the size of the animals.

However, in another study, this same group of researchers, observed that Shih-Tzu bitches castrated between six and 11 months increased the risk of developing neoplasms (Hart et al. 2020a).

For Cassali et al. (2020), more studies are still needed to determine the best time to perform castration. These researchers suggested that, when the main objective of castration is the prevention of mammary neoplasia, it should be carried out between the first and second oestrus. However, specific characteristics of each breed must be considered when making a decision.

FINAL CONSIDERATIONS

Mammary neoplasms in bitches are frequently diagnosed in clinical surgical care for small animals. In this way, knowledge about the main aspects of this tumour, its diagnosis, as well as therapeutic and prophylaxis options become fundamental for the success of treatment and maintenance of the patient's quality of life.

Conflict of interest

The authors declare no conflict of interest.

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