UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE VETERINÁRIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

ASPECTOS PATOLÓGICOS DE MASTOCITOMAS CUTÂNEOS COM METÁSTASES EM CÃES

PAULA REIS RIBEIRO

PORTO ALEGRE

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Orientador: Saulo Petinatti Pavarini

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PAULA REIS RIBEIRO

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RESUMO

O mastocitoma é uma das neoplasias cutâneas mais frequentemente diagnosticadas em cães e possui comportamento biológico amplo, variando de tumores bem diferenciados a tumores com comportamento maligno e doença metastática disseminada. As principais localizações anatômicas das metástases em cães são pouco estudadas, principalmente devido à falta de realização de necropsia nesses casos. O objetivo deste estudo foi avaliar os principais sítios metastáticos de mastocitomas cutâneos caninos e descrever os aspectos macroscópicos e histológicos das metástases. Foi conduzido um estudo retrospectivo nos arquivos de necropsia do Laboratório de Patologia Veterinária da Universidade Federal do Rio Grande do Sul de Janeiro de 2008 a Março de 2020 em busca de casos de mastocitomas cutâneos com metástases em cães. No período de 12 anos foram detectados 49 casos, todos classificados como mastocitomas cutâneos de alto grau. Os principais sítios metastáticos relatados foram os linfonodos (47/49; 95,9%), seguidos do baço (33/49;67,3%), fígado (29/49; 59,2%), medula óssea (20/49; 40,8%), rim (16/49; 32,7%) e coração (14/49; 28,6%). Outras localizações incomuns incluíram os pulmões (9/49; 18,4%), glândulas adrenais (4/49; 8,2%), músculo esquelético (4/49; 8,2%), osso (3/49; 6,1%), bexiga (2/49; 4.1%), próstata (2/49; 4.1%), esôfago (2/49; 4.1%), intestino delgado (2/49; 4.1%) e palato mole (1/49; 2%). Macroscopicamente, os linfonodos afetados apresentavam, em todos os casos, leve a acentuado aumento de volume, frequentemente com perda de distinção corticomedular. O padrão de metástase esplênica foi predominantemente caracterizado por esplenomegalia (28/33; 84,8%), por vezes associado a nódulos (13/33; 39,4%) e áreas puntiformes brancas (4/33; 12,1%). Hepatomegalia foi a principal apresentação macroscópica de metástase hepática (28/29; 96,5%), associada a áreas puntiformes brancas (9/29; 31%) e nódulos (2/29; 6,9%). Metástases renais eram caracterizadas por nódulos unilaterais ou bilaterais (9/16; 56,3%), áreas puntiformes brancas (3/16; 18,8%) ou palidez difusa do parênquima (1/16; 6,3%). O coração exibia nódulos na superfície epicárdica (6/14; 42,9%), palidez difusa (2/14; 14,3%) ou múltiplas áreas brancas no miocárdio (1/14; 7,1%). Nódulos metastáticos pulmonares foram observados em dois casos, enquanto nos demais sete cães as lesões eram apenas microscópicas e envolviam os vasos dos septos alveolares. Histologicamente, os linfonodos apresentavam obliteração do parênquima por mastócitos neoplásicos, os quais também estavam presentes nos seios medulares. No baço, as células neoplásicas estavam dispersas no parênquima (16/33; 48,5%), formando nódulos (10/33;

30,3%) ou difusamente distribuídas (9/33; 27,3%). No fígado, os mastócitos estavam principalmente nos sinusoides (24/29; 82,8%), formavam nódulos (10/29; 34,5%) e ocupavam espaços periportais (5/29; 17,2%). Nos rins e no coração, metástases intersticiais e nodulares foram observadas. Todas os mastocitomas apresentaram marcação imuno-histoquímica positiva para proteína KIT, com padrão de marcação KIT III em 29 casos (59,2%) e KIT II em 20 casos (40,8%).

Palavras-chave: metástases, mastócitos, células redondas, canino, diagnóstico, necropsia, oncologia veterinária.

ABSTRACT

Mast cell tumor (MCT) is one of the most common cutaneous neoplasms of dogs, and it has a variable biological behavior, ranging from well differentiated to more aggressive tumours with metastatic disease. Nonetheless, the main metastatic sites of MCT are poorly described because of the lack of necropsy in cases with MCT-related disease and death. This study aimed to evaluate the metastatic sites of canine MCT and describe the macroscopic and histologic aspects of the metastases. A retrospective study was performed using the necropsy database of the pathology laboratory of the Universidade Federal do Rio Grande do Sul between January 2008 and March 2020 in search for cases of metastatic cutaneous MCTs. In the 12-year period, 49 cases were selected, and all tumours were classified as high-grade MCTs. The main metastatic sites were the lymph nodes (47/49; 95.9%), followed by spleen (33/49; 67.3%), liver (29/49; 59.2%), bone marrow (20/49; 40.8%), kidneys (16/49; 32.7%) and heart (14/49; 28.6%). Other sites included the lungs (9/49; 18.4%), adrenal glands (4/49; 8.2%), skeletal muscle (4/49; 8.2%), bone (3/49; 6.1%), urinary bladder (2/49; 4.1%), prostate gland (2/49; 4.1%), esophagus (2/49; 4.1%;), small intestine (2/49; 4.1%) and soft palate (1/49; 2%). Grossly, there was mild to severe lymphadenomegaly in all cases, which was frequently accompanied by loss of the nodal corticomedullary differentiation. Splenic metastases were mainly characterized by splenomegaly (28/33; 84.8%), occasionally associated with nodules (13/33; 39.4%) and white pinpoint areas (4/33; 12.1%). Hepatomegaly was the most common gross feature of hepatic metastasis (28/29; 96.5%) and was associated with white pinpoint foci (9/29; 31%) and nodular areas (2/29; 6.9%). Renal metastases were characterized by unilateral or bilateral nodules (9/16; 56.3%), white pinpoint areas (3/16; 18.8%) or pale discoloration of the parenchyma (1/16; 6.3%). The heart had nodules in the epicardial surface (6/14; 42.9%), pale myocardium (2/14; 14.3%) or multifocal white areas in the myocardium (1/15; 7.1%). The lungs had metastatic nodules in 2 dogs, while in the remaining cases there were only histologic lesions with neoplastic mast cells in the blood vessels of alveolar septa. Histologically, the lymph nodes were obliterated by neoplastic mast cells, which were also in the medullary sinus. In the spleen, neoplastic mast cells were multifocally scattered (16/33; 48.5%), arranged in nodules (10/33; 30.3%) or diffusely distributed (9/33; 27.3%). In the liver, neoplastic cells were mainly in the sinusoids (24/29; 82.8%), but also formed nodules (10/29; 34.5%) and were arranged in a periportal pattern (5/29; 17.2%). In the kidneys and heart, interstitial and nodular metastases were observed. All neoplasms had positive immunolabeling for KIT

protein, with KIT III staining pattern in 29 cases (59.2%) and KIT II staining pattern in 20 cases (40.8%).

Keywords: metastases, mast cells, round cells, canine, diagnosis, necropsy, veterinary oncology.

SUMÁRIO

1. INTRODUÇÃO

No Brasil, estima-se que a população total de caninos seja em torno de 52,2 milhões de animais, com pelo menos um cão em cerca de 44,3% dos domicílios (BRASIL, 2013a, 2013b). Nos últimos anos, observou-se uma mudança na relação humano-cão, com maior investimento de tutores em cuidados sanitários (DOTSON & HYATT,2008), e no Brasil isso tem sido corroborado pelo aumento do faturamento anual no mercado pet, o qual inclui serviços veterinários, acessórios, equipamentos e produtos de higiene e beleza animal (ABINPET, 2018). Esses fatores somados ao aumento da expectativa de vida dos cães (INOUE *et al.*, 2015) culminaram com o aumento do diagnóstico de doenças crônicas, como doenças neoplásicas e degenerativas (ADAMS *et al*., 2010; O'NEILL *et al.*, 2013).

Mastocitomas estão entre as neoplasias comumente diagnosticadas em caninos e correspondem aos tumores de pele mais frequentes em cães, perfazendo cerca de 16 a 21% do total de neoplasmas cutâneos nessa espécie (BOSTOCK, 1986; LONDON & THAMM, 2013; DALECK; ROCHA; FERREIRA, 2016; KIUPEL, 2017). Os mastócitos são originários de células hematopoiéticas pluripontes da medula óssea e migram posteriormente para a pele e mucosas do trato alimentar e respiratório, nos quais exercem resposta imune predominantemente frente à alérgenos ambientais e parasitários. Na pele, os mastócitos são observados predominantemente na derme em torno de folículos e vasos sanguíneos, além do estrato basal da epiderme (LONDON & SEGUIN, 2003; WELLE *et al.*, 2008; KIUPEL, 2017). Mastócitos são células redondas, com grânulos intracitoplasmáticos contendo aminas biogênicas, enzimas e proteoglicanos, os quais exibem metacromasia quando corados por azul de toluidina e Giemsa (KUMAR & SHARMA, 2010; POHLMAN, 2010; KIUPEL, 2017).

A etiopatogênese do mastocitoma cutâneo canino é pouco compreendida (LONDON & SEGUIN, 2003; BLACKWOOD *et al.*, 2012; LONDON & THAMM, 2013; DALECK; ROCHA; FERREIRA, 2016), mas acredita-se que o desenvolvimento dos tumores possa ter relação com receptor do fator de células-tronco (KIT), devido a mutações em seu gene codificador (c-*kit*) (ZEMKE; YAMINI; YUZBASIYAN-GURKAN, 2002; LETARD *et al.*, 2008; WELLE *et al.*, 2008). O mastocitoma ocorre principalmente em cães adultos, com média de nove anos e não tem predisposição por sexo. Uma ocasional predisposição racial tem sido descrita, especialmente em Boxers,

Labradores, Golden Retrievers, Shar-peis, Buldogues, Pitbulls, Pugs, entre outros (KIUPEL, 2017).

A forma cutânea é a apresentação mais comum do mastocitoma, seguida pela forma subcutânea; todavia, também podem ser afetados raramente de forma primária o trato gastrointestinal, nasofaringe, laringe, conjuntiva, medula espinhal, uretra, ureter, baço, fígado e pulmões (LONDON & THAMM, 2013; KIUPEL, 2017). Na pele, o mastocitoma ocorre principalmente na região de tronco, nas extremidades/membros e menos frequentemente na cabeça e pescoço (HOTTENDORF & NIELSEN, 1967; O'KEEFE *et al.*, 1987; SHAW; KUDNIG; FIRESTONE, 2018). Há ampla variação clínica, mas geralmente os tumores são caracterizados por nódulos únicos, alopécicos a eritematosos (LONDON & SEGUIN, 2003; LONDON & THAMM, 2013; KIUPEL, 2017). Clinicamente, podem ser observados sinais paraneoplásicos locais a sistêmicos relacionados à degranulação de mastócitos e liberação de histamina, heparina, proteases e outras aminas vasoativas, incluindo edema, eritema, ulceração e formação de pápulas, além de úlceras gastroduodenais e distúrbios de coagulação e de cicatrização (BLACKWOOD *et al.*, 2012; LONDON & THAMM, 2013; DALECK; ROCHA; FERREIRA, 2016; KIUPEL, 2017).

O diagnóstico de mastocitoma pode ser realizado inicialmente pelo exame citológico, o qual é considerado um método de diagnóstico rápido, fácil e eficiente para essa neoplasia (BAKER-GABB; HUNT; FRANCE, 2003; LONDON & THAMM, 2013; KIUPEL, 2017). Entretanto, para que o comportamento neoplásico e o prognóstico neoplásico possam ser estimados, é necessário realizar a biópsia incisional ou excisional para graduação histológica (LONDON & SEGUIN, 2003; BLACKWOOD *et al.*, 2012; LONDON & THAMM, 2013). Em relação à graduação histológica, Patnaik, Ehler & MacEwen (1984) propuseram um sistema de classificação em três graus (I, II e III), os quais eram determinados de acordo com as características histomorfológicas de cada neoplasma. Os principais aspectos analisados por essa classificação são arranjo, densidade e diferenciação celular, infiltração em subcutâneo e tecidos subjacentes, quantidade e aspecto dos grânulos intracitoplasmáticos, além de contagem mitótica (PATNAIK; EHLER; MACEWEN, 1984). Devido à considerável variação diagnóstica entre os patologistas ao utilizar o sistema de classificação de três graus (NORTHRUP *et al.*, 2005a, 2005b; KIUPEL *et al.*, 2011) e à grande quantidade de diagnósticos de mastocitoma grau II, o qual informa pouco sobre o prognóstico (KIUPEL *et al.*, 2011; SABATTINI *et al.*, 2015), um novo sistema de graduação foi proposto, com uma

classificação de mastocitomas em baixo grau e alto grau (KIUPEL *et al.*, 2011). Nesse sistema, considera-se alto grau quando há pelo menos sete mitoses, três células multinucleadas (mais de três núcleos), três núcleos bizarros e/ou cariomegalia acima de 10% das células em dez campos de maior aumento (2,37 mm²) (KIUPEL *et al.*, 2011).

Existem diversos fatores para prever o comportamento biológico e determinar o prognóstico dos mastocitomas caninos, como graduação histológica, estadiamento clínico, taxa de proliferação celular e de crescimento, recidiva, sinais sistêmicos, raça, tamanho do tumor e mutação no *c-kit* (LONDON & THAMM, 2013). Estudos prévios demonstraram que métodos complementares, como análise de índices de proliferação, imuno-histoquímica para Ki-67, estadiamento clínico e análise molecular de mutação no gene c-kit, podem ser associados à graduação histológica para determinar o prognóstico (VASCELLARI *et al.*, 2012; WARLAND *et al.*, 2014; SABATTINI *et al.*, 2015). Em ambos os sistemas de classificação, a expressão de Ki67 está associada ao prognóstico (VASCELLARI *et al.*, 2012) e a proliferação celular possui importante papel na progressão de mastocitomas (WEBSTER *et al*., 2007). Além disso, o prognóstico pode ser avaliado pelo padrão de marcação membranar ou intracitoplasmática de KIT, em que a marcação intracitoplasmática está associada com maior ocorrência de metástases e morte relacionado ao mastocitoma (KIUPEL *et al.*, 2004; PREZIOSI; MORINI; SARLI, 2004).

Apesar de apresentarem comportamento amplamente variável, os mastocitomas na maioria das vezes não causam doença metastática disseminada (BAKER-GABB; HUNT; FRANCE, 2003; BLACKWOOD *et al.*, 2012; KIUPEL *et al.*, 2017). Quando há acometimento sistêmico, a pele normalmente é o sítio primário da neoplasia (O'KEEFE *et al.*, 1987). Além disso, o grau histológico possui relação direta com a ocorrência de metástases, já que mastocitomas de alto grau possuem maior chance de desenvolver metástases (KIUPEL *et al.*, 2011; STEFANELLO *et al.*, 2015), e o mesmo é observado em mastocitomas grau III, nos quais metástases podem ser observadas em mais de 80% dos casos (BLACKWOOD *et al.*, 2012). Os principais sítios de metástase de mastocitomas cutâneos em cães são pouco estudados, principalmente devido à falta de realização de necropsia em cães que morreram por doença relacionada ao mastocitoma (KIUPEL, 2017). No entanto, estudos prévios descrevem linfonodos, baço, fígado e medula óssea como os órgãos mais frequentemente acometidos (HOTTENDORF & NIELSEN, 1968; O'KEEFE *et al.*, 1987; LONDON & SEGUIN, 2003; BLACKWOOD *et al.*, 2012; WARLAND *et al.*, 2014; DALECK; ROCHA; FERREIRA, 2016).

Metástases em linfonodos regionais são comuns, enquanto metástases distantes são infrequentes, e na maioria das vezes precedidas por acometimento dos linfonodos regionais (HOTTENDORF & NIELSEN, 1968; WARLAND *et al.*, 2014; STEFANELLO *et al.*, 2015; PIZZONI *et al.*, 2017). De forma distinta a outras neoplasias, metástases pulmonares não são comuns (MARCONATO *et al.*, 2008; LONDON & THAMM, 2013; WARLAND *et al.*, 2014). Dessa forma, a ocorrência de metástases tem relação direta com o prognóstico e redução do tempo de sobrevida (KRICK *et al.*, 2009; STEFANELLO *et al.*, 2009; PIZZONI *et al.*, 2017; MOORE *et al.*, 2020). Considerando esses aspectos, o objetivo do presente estudo foi estimar a frequência de casos de mastocitomas cutâneos com metástases em cães necropsiados no Setor de Patologia Veterinária no período de janeiro de 2008 a março de 2020, determinar os principais órgãos acometidos pelas metástases e avaliar as características macroscópicas e histológicas das metástases.

2. ARTIGO

Nesse item é apresentado o artigo intitulado "Pathological aspects of cutaneous mast cell tumours with metastases in 49 dogs" a ser submetido ao periódico "Veterinary and Comparative Oncology".

Pathological aspects of cutaneous mast cell tumours with metastases in 49 dogs

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Abstract

Cutaneous mast cell tumour (MCT) is one of the most frequently diagnosed cutaneous neoplasms of dogs, with clinical and pathological characteristics that vary from solitary, well differentiated to more aggressive tumours and metastatic disease. We retrospectively described the gross and histologic aspects of metastatic MCT in 49 dogs. Primary cutaneous MCT was mainly identified in the inguinal region, and at necropsy multiple cutaneous nodules were more frequently reported. All primary MCT were classified as high grade neoplasms, and metastases mainly involved the lymph nodes (47/49; 95.9%), followed by the spleen (33/49; 67.3%), liver (29/49; 59.2%), bone marrow (20/49; 40.8%), kidneys (16/49; 32.7%), and heart (14/49; 28.6%). Uncommon sites included the lungs (9/49; 18.4%), adrenal glands (4/49; 8.2%), and other organs (32.7%). The main gross findings included: lymphadenomegaly in all 47 cases of nodal metastases; splenomegaly in 28 cases, with splenic nodules in 13 of these dogs; hepatomegaly in 28 cases, with white pinpoint foci in 9 of the cases; nodules in the capsular surface of the kidneys in 9 dogs; and epicardial nodules in 6 cases. Histologically, the lymph nodes were mainly obliterated by neoplastic mast cells, while in the spleen these cells were multifocally scattered (48.5%), arranged in nodules (30.3%), or obliterating the parenchyma (27.3%). In the liver, the neoplastic cells mainly infiltrated the sinusoids (82.8%), but often formed random nodules (34.5%). Interstitial and nodular metastases were observed in the kidneys and the heart. In the lungs, neoplastic mast cells were mainly within the blood vessels of the alveolar septa, while gross changes were rare. All cutaneous MCTs had positive immunolabeling for KIT protein, with KIT III staining pattern in 29 cases and KIT II staining pattern in the remaining 20 cases.

Keywords: canine, metastatic disease, necropsy, round cells, tumour, veterinary oncology

Introduction

Mast cell tumour (MCT) is one of the most frequently diagnosed cutaneous neoplasm in dogs, accounting for 16% to 21% of all skin tumours diagnosed in this species.[1-3] MCT has a heterogeneous clinical and biological behaviour, ranging from solitary, well differentiated tumours to more aggressive tumours with metastatic disease. Given its broad clinical presentation, prognostic factors, such as histologic grade, are often used in the diagnosis and classification of MCT.[1-3] MCT grading is widely used and it includes a three-tier system, with grade I (well differentiated tumours), II (intermediately differentiated), and III (poorly differentiated tumours),[4] and a more recent two-tier system that classifies MCTs as low-grade and high-grade.[5] Other prognostic indicators include KIT protein immunohistochemistry (IHC), proliferation markers, such as mitotic count, Ki67, and AgNOR, and molecular detection of c-*kit* mutation via polymerase chain reaction.[1,6,7] Nonetheless, no factor is entirely and solely predictive of clinical outcome, and therefore, clinical features must also be considered, such as clinical history of tumor recurrence, clinical staging, breed, lymph node status, and anatomic site.[8]

Metastatic MCT is directly related to these prognostic indicators, in which high-grade MCTs are more prone to metastasize than low-grade tumours.[5,9] This behaviour is also observed in grade III MCT, in which more than 80% will evolve to metastatic disease, while grade I MCTs rarely metastasize.[1,9] Moreover, it is well known that the presence of metastasis carries a poor prognosis.[10-12] Regarding KIT protein IHC, staining patterns II (focal to stippled cytoplasmic) and III (diffuse cytoplasmic) were associated with increased recurrence and decreased survival time, while staining pattern I (membrane-associated) is associated with a better prognosis.[6]

Canine MCT metastases are reported to frequently involve the regional lymph nodes and less often the liver, spleen, and bone marrow,[1,9,11,13] but only a few investigations have described the pathological features of metastases detected during the necropsy.[3] Most of these studies focus on the clinical[11,13] or imaging aspects of metastatic disease,[14-16] or on nodal metastasis.[10,17] To our knowledge, there is one study that investigated metastatic disease associated with MCT using necropsy data,[18] but that study lacks further investigation on the gross and histologic features of the metastases. The current study aimed to describe the pathological features of cutaneous MCTs with metastases detected in dogs submitted to necropsy in a 12-year period, and further define the main metastatic sites, as well as the gross and histologic features of each metastasis.

Materials and methods

Case selection and analysis

A retrospective study of the necropsy database (book records and digital search) from the Veterinary Pathology Laboratory of the Universidade Federal do Rio Grande do Sul (UFRGS) was conducted to search for cases of cutaneous MCTs with metastases detected in dogs at the necropsy from January 2008 to March 2020. Information regarding age, breed, sex, and clinical history was retrieved from the necropsy reports and selected cases were grouped according to age range, sex and breed. Cases in which a cutaneous MCT was diagnosed via biopsy with complete surgical excision or limb amputation, but that had no cutaneous MCT detected during necropsy were also included in this study when histologic evaluation of necropsy samples revealed MCT metastasis from the cutaneous tumour. Additionally, when a diagnosis of MCT was established via biopsy or cytology, the report was retrieved and the survival time was estimated based on the time of diagnosis.

Gross evaluation

Gross lesions from each case were obtained from the necropsy reports and an analysis based on the available gross photographs taken at necropsy was made. When the primary cutaneous site of the tumour was informed, cases were grouped according to location – head and neck, limbs, thorax, abdomen and inguinal region (which included the prepuce and scrotum) (Figure 1). Moreover, the distribution of the cutaneous neoplasm was evaluated and characterised as multiple nodules, focal nodules or focally extensive nodules (with diffuse enlargement of the area around the neoplasm). MCT-related progressive disease was considered as recurrence at the primary site and/or development of MCT at a different cutaneous site. Recurrence was identified based on the presence of a previous MCT diagnosis at the same location in the biopsy records, clinical history, or during necropsy.

The gross features of the metastases were assessed in order to determine the main aspects in each site. Metastases were characterised by diffuse enlargement, pale discoloration, multifocal white pinpoint foci, and/or focal to multifocal nodules in the affected organs. In the lymph nodes, loss of corticomedullary differentiation was also evaluated. Muscular metastasis was only considered if the affected muscular tissue was distant from the cutaneous neoplasm.

The presence of gastrointestinal (GI) ulcers was assessed during the necropsy, and later confirmed by histology. The location and distribution of the ulcers were investigated and divided as affecting the stomach and small intestine.

Histologic evaluation and grading

All retrieved haematoxylin-eosin-stained glass were reviewed by 3 pathologists (PRR, MVB, SPP) in order to confirm the initial diagnosis of MCT, provide a detailed

histologic description of the tumour, and determine the presence of microscopic metastases. The original histologic grade was reviewed and tumours were reclassified according to the Patnaik *et al.* (1984) and Kiupel *et al.* (2011) grading systems. Tumours were classified as high-grade in Kiupel grading system if there were at least 7 mitoses in 10 high-power fields (HPF; 2.37 mm^2); at least 3 multinucleated cells in 10 HPF; at least 3 bizarre nuclei in 10 HPF; and/or karyomegaly >10%.[5] The histologic features of the metastases, such as the distribution pattern of the neoplastic mast cells, were also assessed in all metastatic sites. The organs were considered metastatic sites when the neoplastic mast cells were diffusely distributed throughout the parenchyma, multifocally scattered or arranged in nodules.

Immunohistochemistry

IHC for KIT protein was performed in all cases using sections of formalin-fixed paraffin-embedded cutaneous MCT. Antigen retrieval was performed by boiling the sections in citrate buffer (pH 6.0) for 40 minutes at 96°C, and later the sections were incubated with polyclonal anti-KIT antibody (code A04502, Dako) at a 1:200 dilution. The amplification signal was obtained with the peroxidase-labelled antibody method (MACH4, Universal HRP polymer, Biocare Medical, Pacheco, CA, USA), and the reactions were revealed with 3-amino-9-ethylcarbazole chromogen kit (AEC, Biocare Medical). For positive control, a previously confirmed case of MCT was used. Negative control consisted of incubating the tissue sections with irrelevant polyclonal antibody, in addition to a case of cutaneous lymphoma.

Cases were classified according to the KIT-immunolabeling pattern as pattern I (predominantly membrane-associated staining), pattern II (focal to stippled cytoplasmic staining) and pattern III (diffuse cytoplasmic staining), as previously described.[6]

Results

Cases evaluation

Forty-nine cases of cutaneous MCT with metastasis to other organs were identified in dogs from January 2008 to March 2020. Affected dogs had ages ranging from 2 to 16 years (median age = 8 years; mean age = 10.3 years) and the frequency was slightly higher in males, with 27 cases (55.1%), compared with 22 females (44.9%). A wide range of breeds were reported, with mixed-breed dogs represented in 28 cases, followed by Boxers (3/49), Labrador Retrievers (3/49), German Shepherds (3/49), Pitbull Terriers (3/49), and Cocker Spaniels (2/49), Bulldogs, Dachshunds, Belgian Malinois, Pekineses, Shar-Peis and Tosas (1 case each).

Assessment of MCT progressive disease showed that 20 dogs (40.8%) developed new MCT at a different cutaneous site after excision and/or had local recurrence of excised tumour. Recurrence at primary site was noted in 10 of these cases, and new MCT at different cutaneous sites were present in 15 dogs, of which 5 had concomitant local recurrence and MCT at different cutaneous site. In 20 other dogs (40.8%), the MCT observed during necropsy was non-recurring, and in 9 cases (18.4%) this information was not available.

A previous diagnosis of MCT was available in 25 cases (51%), either via incisional or excisional biopsy (11/25; 44%) or cytology (14/25; 56%). In these cases, the survival time ranged from 5 to 1324 days (mean survival time = 150.6 days; median survival time = 69 days). The clinical history reported that chemotherapy was employed in 12 cases (24.5%), in which the chemotherapeutic drugs were informed in 4 cases and included vinblastine, lomustine and prednisone.

We were able to determine the primary cutaneous location of the MCT in 35 cases (71.4%). The main location was the inguinal region (14/35; 40%; Figures 2A-B), which

concomitantly affected the scrotum and prepuce in 9 dogs, followed by the limbs (11/35; 31.4%), in which the pelvic limbs were affected in 6 cases and the thoracic limbs in 5 cases (Figure 2C). Other locations included the thorax (7/35; 20%), and head and neck (3/35; 8.6%). The tumour location could not be determined in the remaining 14 cases (28.6%), either because of the presence of multiple nodules, averting the definition of the primary tumour, or because of the absence of proper clinical history or previous diagnosis.

The distribution of the cutaneous MCT was assessed during necropsy, and the main presentation was of multiple cutaneous nodules throughout the body (23/49; 47%; Figure 2D), followed by focally extensive nodules, with diffuse enlargement of the region surrounding the neoplasm (13/49; 26.5%) (Figure 2E-F), and single nodules in the remaining 13 cases (26.5%).

Paraneoplastic syndrome included GI ulcers in 16 dogs (32.7%), with perforation in 3 cases (18.8%) (Figure 3A) and focal (1/3) or diffuse peritonitis (2/3). The stomach was affected in all 16 cases (Figure 3B), followed by the duodenum in 10 dogs (Figure 3C). The distribution of the ulcers was available in 15 cases and the gastroduodenal region was affected in 10 dogs (66.7%), which was associated with ulcers in the cardia region in 2 cases and in the oesophagus in 1 case. Moreover, the pylorus or antrum-pylorus region was affected in 5 dogs (33.3%) (Figure 3D).

Histologic analysis of the cutaneous MCT revealed that according to Patnaik *et al*. (1984) grading system 46 tumours were considered grade 3 (93.9%), while a minority was classified as grade 2 MCTs (3/49; 6.1%). In line with Kiupel *et al.* (2011) grading system, all evaluated tumours consisted of high-grade MCTs. A mitotic count (MC) higher than 7 in 10 HPF was observed in all cases and it was the only malignant criteria of high-grade MCTs in 23 cases (46.9%). The MC in 10 HPF varied from 7 to 150 (mean MC = 39.8; median MC = 30). The second most common high-grade feature was

karyomegaly of at least 10% (24/49; 49%), followed by the presence of at least 3 multinucleated cells (3 or more nuclei) in 10 HPF (12/49; 24.5%), and by the presence of at least 3 bizarre nuclei in 10 HPF (11/49; 22.4%) (Figure 4A).

Metastases (gross and histologic analysis)

Metastasis to the lymph nodes (LNs) was observed in 47 cases (95.9%) (Table 1), in which there was lymphadenomegaly in all of these dogs (Figure 4B) and the nodal parenchyma had loss of corticomedullary differentiation in 32 cases (68.1%) (Figure 4C). Histologically, nodal metastasis was mainly characterised by total replacement of the parenchyma by neoplastic mast cells in 43 cases (91.5%) (Figure 4D). Moreover, partial replacement and/or neoplastic mast cells effacing the medullary sinus were detected in 4 dogs (8.5%).

MCT metastasis to the spleen was detected in 33 cases (67.3%), in which diffuse enlargement of the splenic parenchyma (splenomegaly) was noted in most of the cases (28/33; 84.8%), but no gross abnormalities were observed in the 5 remaining cases. Additionally, white to red, capsular, single (12/13) or multiple (1/13) nodules that extended into the parenchyma were seen in 13 dogs (39.4%; Figure 5A). Uncommon lesions included 4 cases (12.1%) with white pinpoint randomly distributed foci in the capsule and splenic infarcts in 3 cases (9.1%; Figure 5B). Histologic evaluation revealed multifocal scattered neoplastic cells in the red pulp and along the edges of the trabeculae (16/33; 48.5%) (Figure 5C), nodular formation (10/33; 30.3%) (Figure 5D), and diffuse distribution of neoplastic cells with total replacement of the parenchyma (9/33; 27.3%).

Hepatic metastasis was reported in 29 dogs (59.2%), of which diffuse enlargement of the hepatic parenchyma (hepatomegaly) was noted in most cases (28/29; 96.5%), but no gross abnormalities were observed in the remaining case. White pinpoint multifocal

foci up to 0.5 cm in diameter were observed in 9 dogs (31%; Figure 6A), which were associated with few nodules up to 1.0 cm in 2 of these dogs. Less common lesions included multifocal white to yellow nodules up to 6 cm in 2 cases (6.9%; Figure 6B). Histologically, hepatic metastasis was mainly characterised by neoplastic cells within the sinusoids (24/29; 82.8%) and arranged in nodular areas (10/29; 34.5%), wherein both patterns occurred concomitantly in 8 cases (Figure 6C). Additionally, these cells were observed expanding the portal tracts in 5 dogs (17.2%) (Figure 6D).

The bone marrow was affected in 20 cases (40.8%), with no gross abnormalities reported during necropsy. Histologically, the metastases were characterised by increased number of neoplastic mast cells, mainly scattered within the marrow cells (17/20; 85%), but occasionally arranged in cohesive groups (3/20; 15%) (Figure 7A).

MCT metastasis to the kidneys was detected in 16 dogs (32.7%), but in 3 of these cases no gross lesions were observed (18.8%). Grossly, randomly distributed, single or multiple, capsular and parenchymal white nodules were noted in 9 cases (56.2%), and involved one (6/9) or both kidneys (3/9) (Figure 7B). Less common changes included white pinpoint areas in the capsular surface in 3 dogs (18.8%), of which pale discoloration was associated in 1 case, and only pale discoloration in another case (6.2%). Renal metastasis was histologically composed of nodular areas within the parenchyma (10/16; 62.5%), which involved both cortical and medullary regions, and of neoplastic cells occupying the renal interstitium (6/16; 37.5%) (Figure 7C).

Cardiac MCT metastasis was observed in 14 cases (28.6%), of which 5 had only histologic changes (35.7%). The metastases were mainly characterised by single or multiple white nodules in the epicardium in 6 dogs (42.9%) (Figure 7D), of which diffuse pale discoloration occurred in 1 case. In 2 cases (14.3%), the myocardium was diffusely pale and in the remaining case (7.1%), the heart had multifocal to coalescent white areas

in the myocardium (Figure 7E). Histologically, neoplastic mast cells were arranged as nodules in the myocardium which frequently extended into the epicardium (7/14; 50%) and at the interstitium between the cardiac myocytes (7/14; 50%) (Figure 7F).

MCT metastasis to the lungs was detected in 9 cases (18.4%), but grossly visible changes were only observed in 3 cases, in which 2 dogs had focal to multifocal white nodules extending into the pulmonary parenchyma and another dog had a focally extensive unilateral white area. Histologically, colonization of the parenchyma was observed only in 3 dogs, with nodular formation in 2 cases and invasion of alveoli by neoplastic cells in the other dog. In the remaining cases (6/9; 66.7%), the neoplastic cells were within the blood vessels and mildly thickened the alveolar septa.

Other organs with metastasis included the adrenal glands in 4 cases (8.2%), with a unilateral white nodule grossly observed in 1 dog, and the remaining cases were confirmed by histologic identification of multifocal areas of neoplastic mast cells within the parenchyma. Muscular metastasis was observed in 4 dogs (8.2%), of which 3 affected the skeletal muscles surrounding the vertebrae, and 2 involved the intercostal muscles. In 3 of these cases, the neoplasm also affected the axial bone, involving the thoracic vertebrae in 2 cases and the lumbar vertebrae in the other dog. The gastrointestinal tract had metastases in 5 cases (10.2%), of which the oesophagus and the small intestine (ileum) were affected in 2 cases each, and the soft palate in 1 case. Nodular formation was grossly noted in the 2 cases of oesophageal metastasis, which primarily affected the submucosa in 1 case and the serosa in the other. Intestinal metastases presented as a nodule in the serosa in 1 dog and the other was confirmed by histologic identification of neoplastic mast cells in the lamina propria of the intestinal mucosae. The soft palate was affected in 1 dog with nodular formation expanding the submucosa layer. The prostate gland had metastasis in 2 cases (4.1%), which were only histologically detected with

neoplastic mast cells interspersed among the prostatic glands. The urinary bladder was affected in 2 dogs (4.1%), wherein it was grossly haemorrhagic in 1 case and, histologically, both showed neoplastic mast cells interspersed in the muscular layer.

Immunohistochemistry

All cutaneous MCTs had positive immunolabeling for KIT protein at the IHC. KIT-staining pattern III was observed in 29 cases (59.2%), and KIT-staining pattern II was observed in the remaining 20 cases (40.8%).

Discussion

MCTs have a wide biological behaviour, and may occur as malignant neoplasms with potential to metastasize.[2,3] The histologic grade is one of the most important and commonly used prognostic factors for MCTs[1,19] of which grade III (Patnaik system) and high-grade (Kiupel system) MCTs are associated with a higher metastatic potential and MCT-related mortality.[4,5,8-10,13] Similarly, in the present study, all cases had metastases and were classified as high-grade tumours. Although the histologic grade is an important prognostic tool for MCT, in dogs with advanced and metastatic MCT, as the ones of the present investigation, it should not be used solely for prognostication, as dogs with low-grade/grade 1 or 2 MCT may present metastatic disease.[9,11,21] Therefore, the histologic grade should be correlated with other prognostic factors, such as proliferation markers[1,3] and mitotic count (MC), which have been associated with MCT metastasis and higher lethality rates.[8] In the current study, MC was the only indicator of proliferation and a cut-point of 7 mitoses in 10 HPF was considered.[5] A MC higher than 7 was the only criterion indicative of high-grade MCT that was present in all cases in this investigation, which reinforces its relevance in detecting MCTs with more aggressive potential. The use of MC as the sole indicator of proliferation may result in both false-positive and false-

negative results because it may also represent karyologic abnormalities instead of only proliferating cells.[19] However, MC may be as helpful as histologic grading for prognostic assessment, acting as an independent negative prognostic factor,[12,22] and MC higher than 5 in 10 HPF has been associated with a poor clinical outcome,[23] and increased metastatic disease.[22]

Tumour recurrence has been associated with poor prognosis in MCT[8] and should be considered if there is tumour regrowth at the primary site of an excised MCT.[3] The rate of local recurrence in apparently completely resected MCTs ranges from 5% to 23%[1] and may be up to 36% in high grade MCT.[24] In the current investigation, local recurrence was reported in 20.4% of dogs, similarly to previous studies[8,20] but lower than what has been reported in dogs with distant metastases diagnosed during necropsy.[18] Development of new MCTs at distant cutaneous sites was more frequent than local recurrence in the present study, similarly to that reported.[25] It is unclear if these neoplasms represent distant recurrence, metastases or *de novo* tumours,[3,25] however, they should not be considered metastases until proven by molecular markers.[3]

The mean age of 10.3 years in this investigation is similar to that observed by other studies conducted on necropsied dogs with MCT[18] and on dogs clinically diagnosed with MCT metastatic disease.[11] Although males were slightly overrepresented, no sex predilection was identified in the present study, as previously described.[3] Although some breeds, such as Boxers, Bulldogs, Retrievers, Pugs and Shar-Peis, have been described as predisposed to develop MCTs,[1,3] no such predisposition was observed in this investigation.

The main anatomic locations of the primary cutaneous MCTs in the current investigation were the inguinal region and the limbs, comprising half of the cases. Although previous studies have not established an association between tumour location

and clinical outcome,[8,11,12] MCTs in the inguinal area have been regarded as more aggressive tumours.[1,3] However, this presumption has been mainly based on clinical experience rather than statistical analysis[25]. Considering that the inguinal region was frequently affected in our study, MCTs in this location should prompt clinical veterinarians and oncologists for a higher risk of metastatic disease; nonetheless, the anatomic location should not be the only factor to be considered for prognosis, as neoplasms located at other cutaneous sites also had metastases in our investigation. Moreover, MCTs on the inguinal region frequently showed diffuse enlargement of the affected area with local invasion, which is similar to that observed in other studies.[18] A worse prognosis may be explained in these cases because of the difficulty of adequate surgery in this region,[1] as locoregional control has improved the outcome in grade 3 MCT.[23] The main gross features of the cutaneous MCTs at necropsy in this study included multiple nodules throughout the body, comprising almost half of the cases. Even though solitary nodules are the most common clinical presentation of MCTs,[3,19] the higher frequency of multiple nodules in our study, when compared to rates of 10-21% in other investigations,[1,26] may be related to the higher proportion of dogs presenting MCT at distant cutaneous site, as the prognostic value of multiple nodules is uncertain with conflicting results.[13,25,26]

The LN was the most common metastatic site of cutaneous MCTs in this investigation, as previously described.[9,11,13,18] This finding emphasises the relevance of LN evaluation during clinical staging of MCTs, as this is most likely the first organ to be affected by metastatic disease. MCTs involving the inguinal region (9/14; 64.3%) and pelvic limbs (4/6; 66.7%) often metastasized to the medial iliac and hypogastric LNs, which is a pattern that has also been described in dogs with MCTs of the caudal portion of the body.[18,27] This feature reinforces the relevance of assessing this chain of LNs during

clinical staging of MCTs, especially those in the caudal portions of the body. According to the classification system of node metastasis,[17] 91.5% of the cases were classified as HN3 (overt metastasis), while the remaining cases were defined as HN2 (early metastasis), which indicates that all cases represented true metastases, mostly represented by overt and advanced metastatic disease.

Distant metastases of MCT are less common than nodal metastases, and are more difficult to be clinically detect,[3] with frequencies as low as 4.1%,[9] 6.8%,[13] 11.6,[23] or it may reach higher levels such as 19.2%.[14] The most common metastatic sites for canine cutaneous MCTs are the spleen, liver, bone marrow, and non-regional LNs,[1,9,11,13,18,28] similar to what we observed in the current study. Further, renal, cardiac metastases were also detected in our study, which have been rarely reported for MCTs.[18,27] Distant metastases are usually preceded by local LN metastasis,[13,18] which is in accordance with our findings, since in 95.9% of the cases with distant metastases there was nodal involvement.

Splenic metastasis was frequently observed (67.3%), as previously found by other authors in necropsied dogs (75% to 79%),[18,27] and it may be as high as 86.7% in dogs with stage IV MCT.[11] Splenic nodules, which was a common gross finding in the current investigation, may represent nodular hyperplasia, abscesses, granulomas, or neoplasms.[30] As old dogs frequently have hyperplastic nodules,[18,30] and this age category is overrepresented in this study, differential diagnosis and histologic confirmation is essential. The liver was the third most common metastatic site, as previously observed in necropsied dogs with a similar rate (62%).[18] However, a higher frequency has been described by fine-needle aspiration (84.4%).[11] Hepatomegaly was the most common gross finding in the current investigation, even though it was not a constant lesion in another study;[18] nevertheless, subjective increase in liver size was described during

ultrasound investigation of MCT metastases.[14,31] Nodular formation was uncommon in the affected livers of the current investigation, and this might explain why nodules were not frequently noted in ultrasound of dogs with hepatic metastases of MCT.[14,16,31] Histologic evaluation of the affected livers showed that in some cases neoplastic cells were only seen as small foci or scattered within the sinusoids, which may explain why liver cytology may be non-diagnostic.[15]

Bone marrow involvement in MCT is considered rare, carries a poor prognosis,[11,28] and the rate of metastatic mast cells through cytologic evaluation ranges from 4.5% to 20%.[11,33] However, this percentage increases when the diagnosis is performed via necropsy, in which rates of 48% and 58% were observed by other authors,[18,27] similarly to the current investigation. Metastasis to the bone marrow is usually accompanied by multi-organ involvement,[11,28] as in the present study, in which all dogs with bone marrow involvement had metastases to other organs. Our results reinforce the hypothesis that bone marrow involvement may indicate multi-organ involvement and, therefore, advanced disease with poor prognosis.

Although detection of MCT metastases to the kidney via ultrasound has been reported in lower levels, ranging from 2.2% to 8.3%,[11,31] the kidneys were the fifth most common site affected by metastatic disease in this investigation, in a similar manner to that previously described in necropsied dogs.[18,27] Even though in our study pinpoint white areas were scarcely reported, this was the only gross renal lesion described during necropsy for dogs with metastatic MCT in other investigation.[18] Additionally, mild lesions, such as pale discoloration, or absence of kidney lesions does not rule out metastases, and, therefore, histologic evaluation is essential to establish a definitive diagnosis. Cardiac metastasis of MCT are not frequently described and the reports comprise only cases diagnosed during necropsy, with rates of 21% and 8.3%.[18,27]

Although epicardial nodules were more frequently observed, some cases may have an absence of gross lesions,[18] as seen in 35.7% of our cases, indicating that micrometastasis may occur. To the authors' opinion, the lack of clinical studies with cardiac metastasis of MCT may be explained by the frequent absence of cardiac gross lesions or only subtle changes in the heart colour.

Differing from other neoplasms, such as hemangiosarcoma, osteosarcoma, histiocytic sarcoma and carcinoma,[29] the lungs are not common metastatic sites for MCT, [2,11,13,27,28,29] as in our study. This is similar to that observed in other investigations of MCT metastases in dogs, in which the rate of pulmonary metastasis ranged from 16.6% to 21.4%.[27,28] Nevertheless, previous reports have indicated even lower frequencies of pulmonary involvement (2.2%)[11] or absence of detection,[13] but these were only diagnosed through thoracic radiograph. This corroborates that pulmonary metastasis of MCT is not common and it is mostly present in advanced MCT disease with multi-organ involvement, which was the main pathological presentation in the current investigation. Moreover, in the present work, the lungs often did not have any gross lesions, which reinforces that nodular formation in the lungs is not frequently observed,[13] in addition to emphasize the relevance of histologic confirmation, as demonstrated by other authors.[28]

Even though involvement of the GI tract has been described, the rate in the current investigation was low (5 cases) and other reports of necropsied dogs with MCT did not find evidence of metastases in the GI tract;[18,27] consequently, metastatic spread to these sites should be considered particularly rare. We considered these cases as metastases of cutaneous MCT rather than visceral MCT, as there was a diagnosis of cutaneous MCT and metastases to other organs in all cases, which may assert the GI tract as part of a multi-organ involvement.[3]

Paraneoplastic syndrome, such as GI ulcers, may occur with MCTs and results from histamine release by neoplastic cells, which stimulates gastric histamine receptors and leads to hydrochloric acid oversecretion and increased gastric motility.[1,3] GI ulcers may be expected especially in recurrent and metastatic MCTs,[33,34], as the ones of the present research, which reinforces that these should be properly investigated during necropsy.[34] In the current investigation, the frequency of GI ulcers (32.7%) was similar to that observed in necropsied dogs with MCT of another study (33.3%),[27] but it can be up to 83.3%[33]. The prevalence of GI ulcers should be interpreted with caution, as these studies investigated populations of dogs affected by MCT at necropsy, suggesting that advanced disease and long-term treatment could also be associated with these ulcers.

The aetiology of MCT is unknown, but stem cell factor (SCF), KIT protein, and c-*kit* have shown to play a major role in the oncogenesis of these tumours. The SCF binds to the KIT protein, which is encoded by c-*kit*, and this binding results in a signalling cascade that is essential for mast cell proliferation and survival.[3] In the current study, all MCTs exhibited KIT II and KIT III IHC staining pattern, which was an expected finding because all of our cases were metastatic MCTs. Similarly, other studies have revealed that increased KIT cytoplasmic staining is associated with poor prognosis[6] and increased risk of metastatic disease and MCT-related death.[8]

MCT is one of the most common cutaneous tumours in dogs, and, therefore, it represents a frequent diagnosis for oncologists and pathologists. The wide clinical and biological behaviour represents a challenge to evaluate the prognosis, as aggressive metastatic disease may occur, as observed in this study. The most common metastatic sites for MCT included the lymph nodes, spleen, liver and bone marrow, although the kidneys and the heart might also be affected. Pulmonary metastasis was uncommon and it mainly showed histologic lesions. The most common gross changes included

lymphadenomegaly, splenomegaly with white to red capsular nodules, hepatomegaly with white pinpoint multifocal foci, renal focal to multifocal white nodules, and white nodules in the epicardial surface of the heart. The confirmation of MCT metastases through histologic evaluation is essential, especially for some organs that might only present histologic lesions, such as the lungs, the kidneys and the heart. Additionally, KIT IHC helped to endorse the final diagnosis and to determine the staining patterns, with increased cytoplasmic staining in all cases (KIT II and III).

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References

1. Blackwood L, Murphy S, Buracco P, et al. European consensus document on mast cell tumours in dogs and cats. *Vet Comp Oncol*. 2012; 10(3):e1-e29.

2. London CA, Thamm DH. Mast Cell Tumors. In: Withrow SJ, Vail DM, Page RL, eds. *Withrow and Macewen's Small Animal Clinical Oncology*, 5th ed. St. Louis, Elsevier; 2013:335-355.

3. Kiupel M. Mast cell tumors. In: Meuten DJ, eds. *Tumors in Domestic Animals*, 5th ed. Ames, Willey Blackwell; 2017:176-202.

4. Patnaik AK, Ehler WJ, Macewen EG. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Vet Pathol*. 1984; 21:469-474.

5. Kiupel M, Webster JD, Bailey KL, et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet Pathol*. 2011; 48(1):147-155.

6. Kiupel M, Webster JD, Kaneene JB, Miller R, Yuzbasiyan-Gurkan V. The use of KIT and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. *Vet Pathol*. 2004; 41(4):371-377.

7. Webster JD, Yuzbasiyan-Gurkan V, Miller R, Kaneene JB, Kiupel M. Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. *Vet Pathol*. 2007; 44(3):298-308.

8. Horta RS, Lavalle GE, Monteiro LN, et al. Assessment of canine mast cell tumor mortality risk based on clinical, histologic, immunohistochemical, and molecular features. *Vet Pathol.* 2018; 55(2):212-223.

9. Stefanello D, Buracco P, Sabattini S, et al. Comparison of 2- and 3-category histologic grading systems for predicting the presence of metastasis at the time of initial evaluation in dogs with cutaneous mast cell tumors: 386 cases (2009–2014). *J Am Vet Med Assoc*. 2015; 246(7):765-769.

10. Krick EL, Billings AP, Shofer FS, Watanabe S, Sorenmo KU. Cytological lymph node evaluation in dogs with mast cell tumours: association with grade and survival. *Vet Comp Oncol.* 2009; 7(2):130-138.

11. Pizzoni S, Sabattini S, Stefanello D, et al. Features and prognosis impact of distant metastases in 45 metastases in 45 dogs with de novo IV cutaneous mast cell tumours: A prospective study. *Vet Comp Oncol*. 2017; 16:28-36.

12. Moore AS, Frimberger AE, Taylor D, Sullivan N. Retrospective outcome evaluation for dogs with surgically excised, solitary Kiupel high-grade, cutaneous mast cell tumours. *Vet Comp Oncol*. 2020; 1-7.

13. Warland J, Amores-Fuster I, Newbury W, Brearley M, Dobson J. The utility of staging in canine mast cell tumours. *Vet Comp Oncol*. 2014; 12(4):287-298.

14. Stefanello D, Valenti P, Faverzani S, et al. Ultrasound-Guided cytology of spleen and liver: a prognostic tool in canine cutaneous mast cell tumor. *J. Vet Intern Med*. 2009; 23:1051-1057.

15. Book AP, Fidel J, Wills T, Bryan J, Sellon R, Mattoon J. Correlation of ultrasound findings, liver and spleen cytology, and prognosis in the clinical staging of high metastatic risk canine mast cell tumors. *Vet Radiol Ultrasound*. 2011; 52(5):548-554.

16. Pecceu E, Varela JCS, Handel I, Piccinelli C, Milne E, Lawrence J. Ultrasound is a poor predictor of early or overt liver or spleen metastasis in dogs with high-risk mast cell tumours. *Vet Comp Oncol.* 2020; 18(3):389-401.

17. Weishaar KM, Thamm DH, Worley DR, Kamstock DA. Correlation of nodal mast cells with clinical outcome in dogs with mast cell tumour and a proposed classification system for the evaluation of node metastasis. *J Comp Pathol*. 2014; 151:329-338.

18. Hottendorf GH, Nielsen SW. Pathologic report of 29 necropsies on dogs with mastocytoma. *Vet Pathol*. 1968; 5:102-121.

19. Kiupel M, Camus M. Diagnosis and prognosis of canine cutaneous mast cell tumors. *Vet Clin North Am Small Anim Pract*. 2019; 49:819-836.

20. Bostock DE. The prognosis following surgical removal of mastocytomas in dogs. *J Small Anim Pract*. 1973; 14:27-40.

21. Bae S, Milovancev M, Bartels C, et al. Histologically low-grade, yet biologically high-grade, canine cutaneous mast cell tumours: A systematic review and meta-analysis of individual participant data. *Vet Comp Oncol*. 2020; 18(4):580-589.

22. Romansik EM, Reilly CM, Kass PH, Moore PF, London CA. Mitotic index is predictive for survival for canine cutaneous mast cell tumours. *Vet Pathol*. 2007; 44:335-341.

23. Hume CT, Kiupel M, Rigatti L, Shofer FS, Skorupski KA, Sorenmo KU. Outcomes of dogs with grade 3 mast cell tumours: 43 cases (1997-2007). *J Am Anim Hosp Assoc.* 2011; 47(1):37-44.

24. Donnely L, Mullin C, Balko J, et al. Evaluation of histological grade and histologically tumour-free margins as predictors of local recurrence in completely excised canine mast cell tumours. *Vet Comp Oncol.* 2013; 13(1):70-76.

25. Kiupel M, Webster JD, Miller RA, Kaneene JB. Impact of Tumour depth, tumour location and multiple synchronous masses on the prognosis of canine cutaneous mast cell tumours. *J Vet Med A Physiol Pathol Clin Med*. 2005; 52:280-286.

26. Murphy S, Sparkes AH, Blunden AS, Brearley MJ, Smith KC. Effects of stage and number of tumours on prognosis of dogs with cutaneous mast cell tumours. *Vet Rec.* 2006; 158:287-291.

27. O'Keefe DA, Couto CG, Burke-Schwartz C, Jacobs RM. Systemic mastocytosis in 16 dogs. *J Vet Intern Med*. 1987; 1:75-80.

28. Marconato L, Bettini G, Giacoboni C, Romanelli G, Cesari A, Zini E. Clinicopathological features and outcome for dogs with mast cell tumors and bone marrow involvement. *J Vet Intern Med*. 2008; 22:1001-1007.

29. Lamb CR, Whitlock J, Foster-Yeow ATL. Prevalence of pulmonary nodules in dogs with malignant neoplasia as determined by CT. *Vet Radiol Ultrasound*. 2019; 60:300-305.

30. Valli VEO, Kiupel M, Bienzle D. In: MG Maxie, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals,* 6th ed. St. Louis, Elsevier; 2016:102-268.

31. Sato AF, Solano M. Ultrasonographic findings in abdominal mast cell disease: A retrospective study of 19 patients. *Vet Radiol Ultrasound*. 2003; 45:51-57.

32. Endicott MM, Charney SC, McKnight JA, Loar AS, Barger AM, Bergman PJ. Clinicopathological findings and results of bone marrow aspiration in dogs with cutaneous mast cell tumours: 157 cases (1999 – 2002). *Vet Comp Oncol*. 2007; 5:31-37.

33. Howard EB, Sawa TR, Nielsen SW, Kenyon AJ. Mastocytoma and gastroduodenal ulceration. gastric and duodenal ulcers in dogs with mastocytoma. *Pathol Vet.* 1969; 6:146-158.

34. Uzal FA, Plattner BL, Hostetter JM In: MG Maxie, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals,* 6th ed, St. Louis, Elsevier; 2016:1-257.

Table 1. Metastatic sites of cutaneous mast cell tumor and their gross aspects in 49 necropsied dogs

Dog nº	Metastatic sites								
	Nodal metastasis	Distant metastasis	Spleen	Liver	Bone marrow	Kidneys	Heart	Lungs	Others
1	+ (D)	+	+ (D; SI)	+ (D)	-	-	+ (PD)	-	Urinary bladder
2	+ (D; LCD)	+	-	-	-	+ (PA)	-	-	-
3	+ (D)	-	-	-	-	-	-	-	-
4	+ (D; LCD)	+	+ (D)	+ (D)	-	+	-	-	-
5	-	+	+ (D; N)	+ (D)	-	-	-	-	-
6	+ (D)	+	-	+ (D)	-	-	-	+	Urinary bladder
7	+ (D; LCD)	+	-	+ (D; N)	-	+ (PD; PA)	-	-	-
8	-	+	-	-	+	-	-	-	Bone, skeletal muscle
9	+ (D)	+	-	+ (D)	+	-	+ (PD)	-	-
10	+ (D; LCD)	+	+ (D)	-	+	+ (N)	+ (N; PD)	-	Bone, skeletal muscle
11	+ (D; LCD)	+	+	+ (D; PA)	+	+	-	+ (WA)	-
12	+ (D; LCD)	-	-	-	-	-	-	-	-
13	+ (D)	+	+ (D; N)	-	-	+ (N)	-	-	-
14	+ (D; LCD)	+	+ (D; N)	+ (D; PA)	+	-	-	-	-
15	+ (D; LCD)	+	+ (D; N)	+ (D)	+	-	+ (N)	-	-
16	+ (D; LCD)	+	+ (D; PA)	+ (D)	+	-	-	+	-
17	+ (D)	+	+	+ (D; PA)	+	-	-	-	-
18	+ (D; LCD)	+	+ (D; N)	+ (D)	+	-	+	-	-
19	+ (D)	+	+ (D; PA; SI)	+ (D; PA)	+	+ (PA)	-	+	Adrenal glands
20	+ (D; LCD)	+	-	+ (D)	-	-	-	-	-
21	+ (D; LCD)	+	+ (D; N)	-	-	-	-	+ (N)	-
22	+ (D; LCD)	+	-	-	-	-	+ (PD; WA)	-	-
23	+ (D; LCD)	+	+ (D; N)	+ (D; PA)	+	+ (N)	-	-	Bone, skeletal muscle, prostate, oesophagus
24	+ (D; LCD)	+	+ (D)	+ (D; N)	-	+ (N)	+ (N)	-	Small intestine
25	+ (D; LCD)	+	+ (D; N)	+ (D; PA)	+	-	-	-	
26	+ (D; LCD)	+	+ (D; N)	+ (D)	+	+ (N)	+ (N)	+ (N)	Adrenal glands, oesophagus, soft palate
27	+ (D)	+	-	-	+	-	+ (N)	-	-
28	+ (D)	+	-	+ (D)	-	-	-	-	-

29	+ (D; LCD)	+	+ (D)	-	-	-	-	-	-
30	+ (D; LCD)	+	+	+	-	-	+	+	-
31	+ (D)	+	+ (D; N)	-	-	-	-	-	-
32	+ (D; LCD)	+	+ (D; SI)	+ (D)	-	+ (N)	-	-	-
33	+ (D; LCD)	+	+ (D)	+ (D)	-	-	-	-	-
34	+ (D; LCD)	+	+ (D)	+ (D)	-	-	-	-	-
35	+ (D)	+	+ (D; PA)	+ (D; PA)	+	+	+	+	-
36	+ (D; LCD)	+	+ (D)	-	-	+ (N)	+ (PD)	-	-
37	+ (D)	+	+ (D)	-	-	-	-	-	-
38	+ (D; LCD)	+	-	+ (D; PA)	-	+ (N)	-	+	Adrenal glands
39	+ (D)	-	-	-	-	-	-	-	-
40	+ (D; LCD)	+	+	+ (D)	+	+ (PD)	-	-	Bone
41	+ (D; LCD)	+	+	-	-	-	-	-	-
42	+ (D)	+	+ (D; PA)	+ (D)	+	-	-	-	Prostate
43	+ (D; LCD)	+	+ (D; N)	-	+	-	-	-	-
44	+ (D; LCD)	+	+ (D)	+ (D)	+	-	-	-	Small intestine
45	+ (D; LCD)	+	-	-	-	-	+ (N)	-	
46	+ (D)	+	-	-	+	+ (N)	-	-	Adrenal glands (N), skeletal muscle
47	+ (D; LCD)	+	+ (D; N)	-	-	-	+	-	
48	+ (D; LCD)	-	-	-	-	-	-	-	-
49	+ (D; LCD)	+	+ (D; N)	+ (D; PA)	-	-	+	-	-
Total	47	45	33	29	20	16	15	9	

D: diffuse enlargement of the parenchyma; LCD: loss of corticomedullary distinction; N: nodules; PA: pinpoint areas; SI: splenic infarcts; PD: pale discoloration; WA: white areas

Figure legends

Figure 1. Primary sites of cutaneous mast cell tumour (MCT): 1, head and neck (dark grey); 2, thorax (white with black dashed lines); 3, abdomen (white under view); 4, limbs (light grey); 5, inguinal region (black under view)

Figure 2. Gross findings of MCT with metastases in dogs. (A-B) Primary cutaneous MCT located in the inguinal region. (A) The neoplasm involves the penis, prepuce, and scrotum, with diffuse enlargement of the affected area. (B) On the cut surface, a white to reddish multinodular mass involves and expands the prepuce. (C) Primary cutaneous MCT involving the thoracic limb with diffuse enlargement of the axillary region. (D) Multiple cutaneous MCT distributed throughout the body, mainly affecting the inguinal and abdominal regions. (E) Focally extensive inguinal mass with diffuse enlargement of the region surrounding the neoplasm. (F) Focally extensive mass initially involving the thorax (arrowhead) with multifocal to coalescent nodules (arrow) extending into the axillary region.

Figure 3. Gross findings of gastrointestinal ulcers associated with MCT in dogs. (A) Multifocal perforated gastroduodenal ulcers mainly affecting the cardiac and gastroduodenal regions, which measure up to 2.0 cm in diameter. (B) Multifocal gastric ulcers characterised by round cavities in the mucosa, with elevated borders, mild fibrin deposition and diffuse gastric hyperaemia. (C) Intestinal ulcers affecting the duodenum, characterised by round to linear cavities in the mucosa, associated with fibrin deposition and mucosal hyperaemia. (D) Gastric ulcers affecting the antrum-pylorus region, associated with mucosal hyperaemia and haemorrhage, with dark-red content over the ulcerated mucosa (compatible with digested blood).

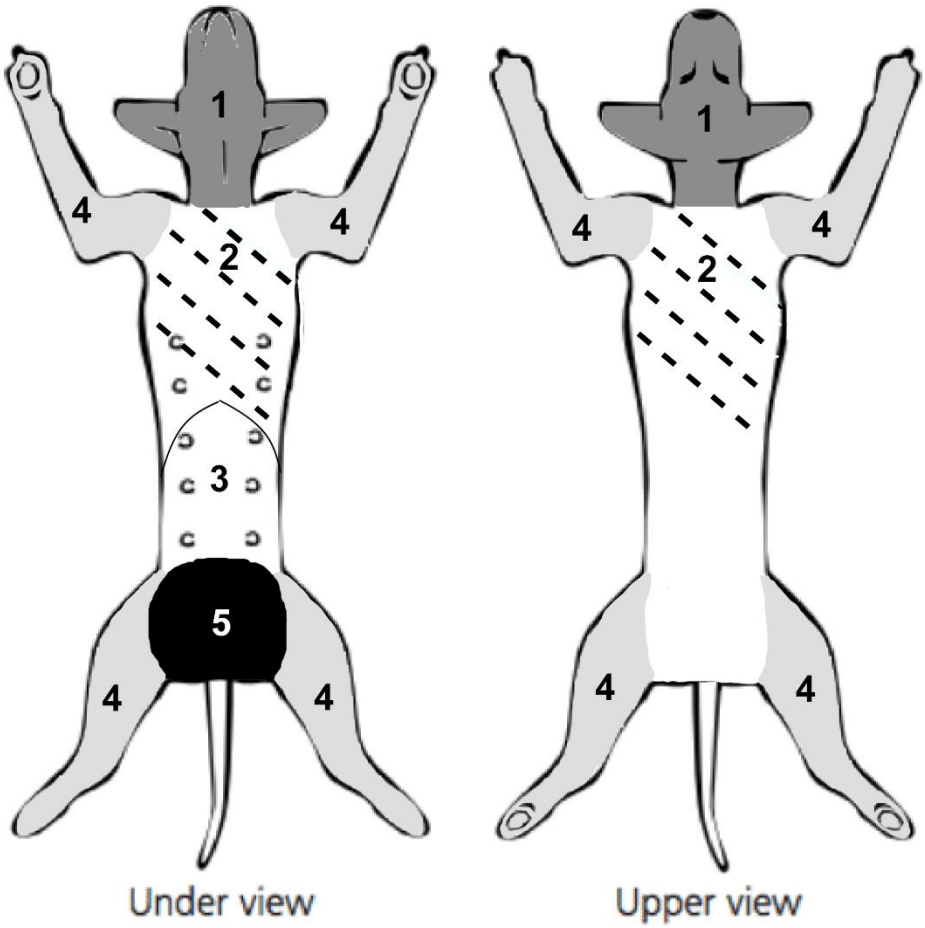
Figure 4. Gross and histologic findings of the metastatic sites of cutaneous MCT in dogs. (A) Malignant criteria of high-grade tumours, which includes multinucleated cells (more than 3 nuclei), bizarre nuclei, mitotic figures and karyomegaly. Haematoxylin and eosin (HE), 400×. (B) Severe enlargement of medial iliac and hypogastric lymph nodes, which are diffusely white. (C) Severe lymphadenomegaly affecting the superficial cervical and axillary lymph nodes, which are diffusely white and reveals loss of corticomedullary differentiation. (D) Total obliteration of nodal parenchyma by neoplastic mast cells, with a remaining lymphoid follicle (asterisk). HE, 200×. Inset: marked immunolabeling of neoplastic mast cells in the cortical region of the lymph node, preserving the lymphoid follicle. Immunohistochemistry (IHC) for KIT protein, 100×.

Figure 5. Gross and histologic findings of the metastatic sites of cutaneous MCT in dogs. (A) The spleen is diffusely and severely enlarged, with multiple white to red nodules up to 0.2 cm in diameter. (B) Severe splenomegaly with a focal splenic infarct associated with fibrin deposition, in addition to multifocal white pinpoint foci. (C) Multifocal scattered neoplastic mast cells in the splenic red pulp and along the edges of the trabeculae. IHC for KIT protein, 100×. (D) Nodular formation (asterisk) by neoplastic mast cells expanding the splenic parenchyma. HE, 100×.

Figure 6. Gross and histologic findings of the metastatic sites of cutaneous MCT in dogs. (A) The liver is moderately enlarged with multiple white pinpoint foci up to 0.5 cm in diameter. (B) Moderate hepatomegaly associated with multiple white to yellowish nodules ranging from 1 to 5 cm in diameter. (C) Neoplastic mast cells arranged in nodular areas and expanding the sinusoids. HE, 100×. (D) Neoplastic mast cells expanding the portal tracts and occasionally within the sinusoids. HE, 200×. Inset: marked immunolabeling of neoplastic mast cells in the portal tract. IHC for KIT protein, 200×.

Figure 7. Gross and histologic findings of the metastatic sites of cutaneous MCT in dogs. (A) Bone marrow involvement in cutaneous MCT with neoplastic mast cells arranged in small and cohesive groups within the marrow cells (asterisk). HE, 200×. (B) Multifocal white nodules in the capsular surface with up to 1 cm in diameter affecting both kidneys. (C) Neoplastic mast cells within the cortical renal interstitium. HE, 200×. (D) Multifocal white nodules in the epicardial surface of the right ventricle and auricle, measuring up 1.5 cm in diameter. (E) Multifocal to coalescent white areas in the myocardium associated with mild pale discoloration. (F) Cardiac metastasis of cutaneous MCT metastasis shows neoplastic mast cells interspersed with cardiac myocytes. HE, 200×.

Figure 1.



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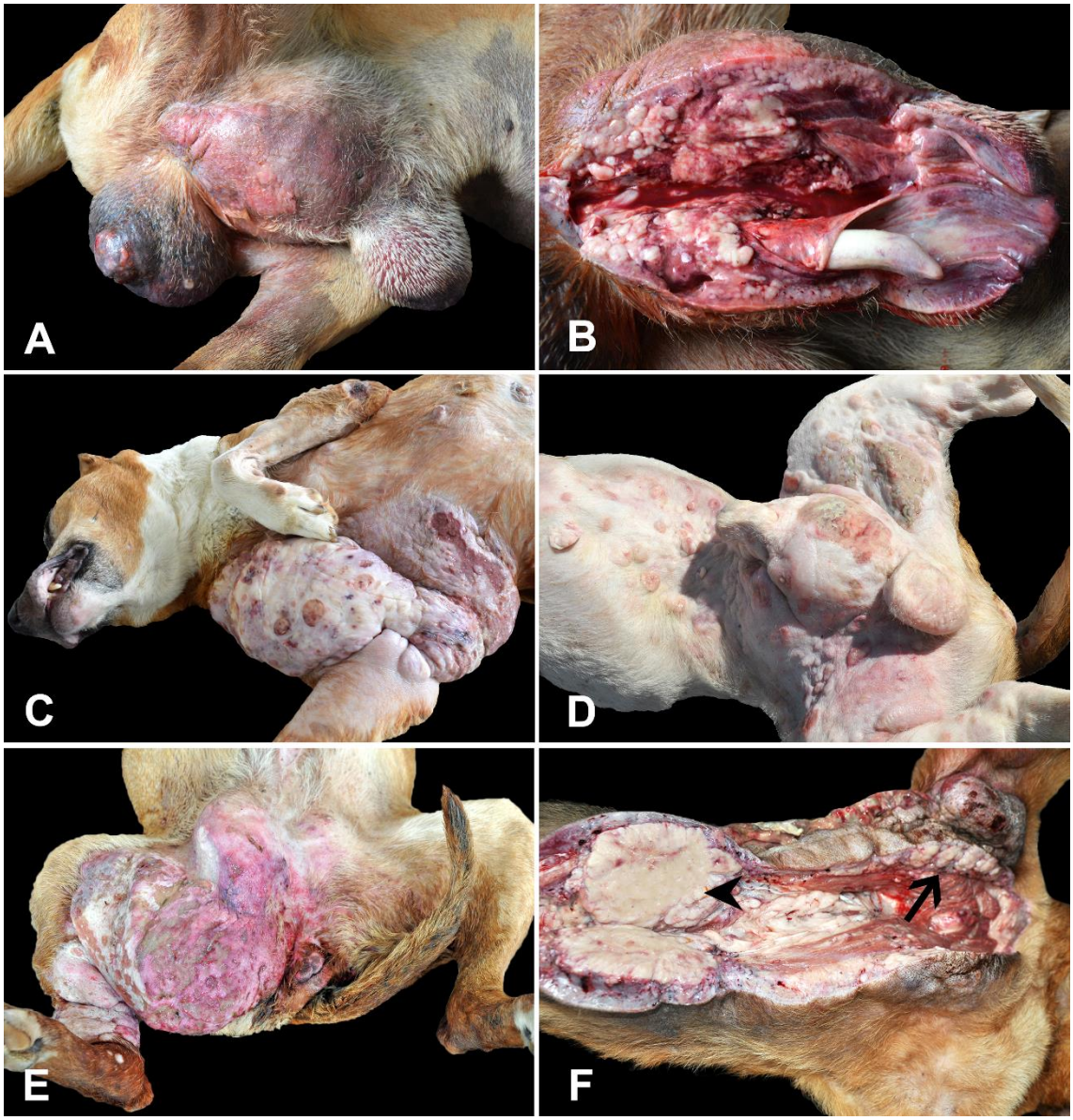

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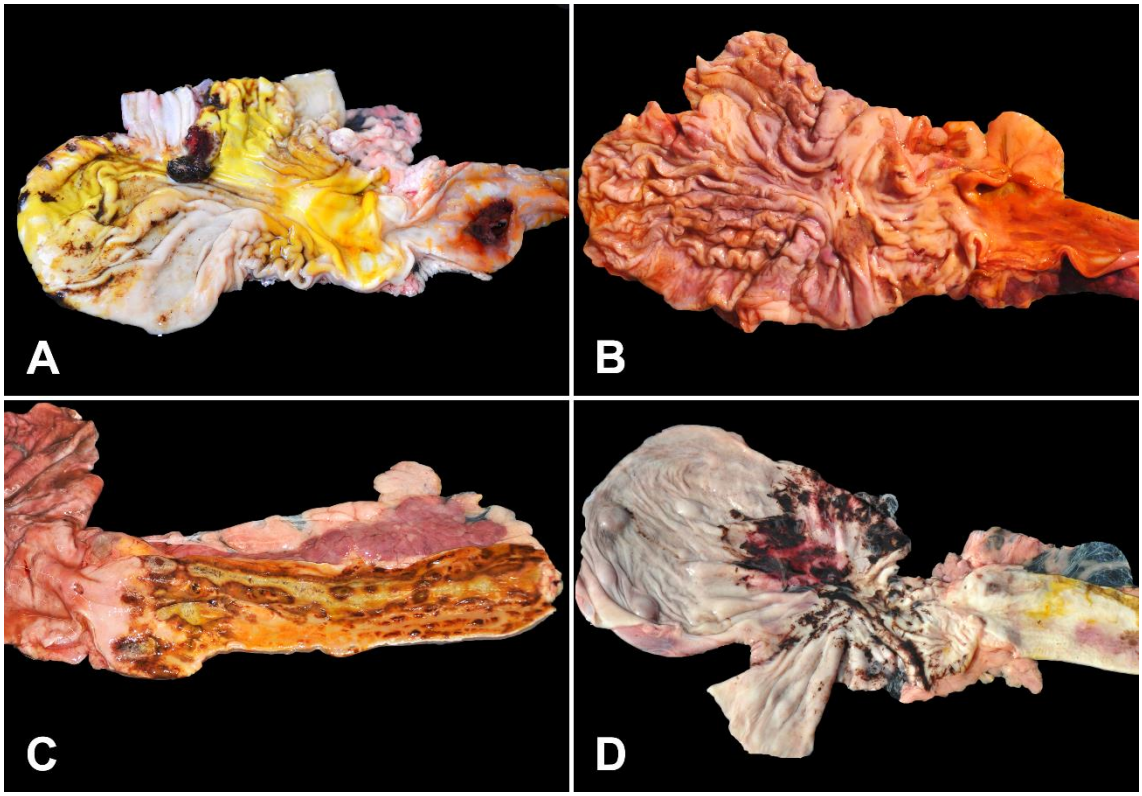

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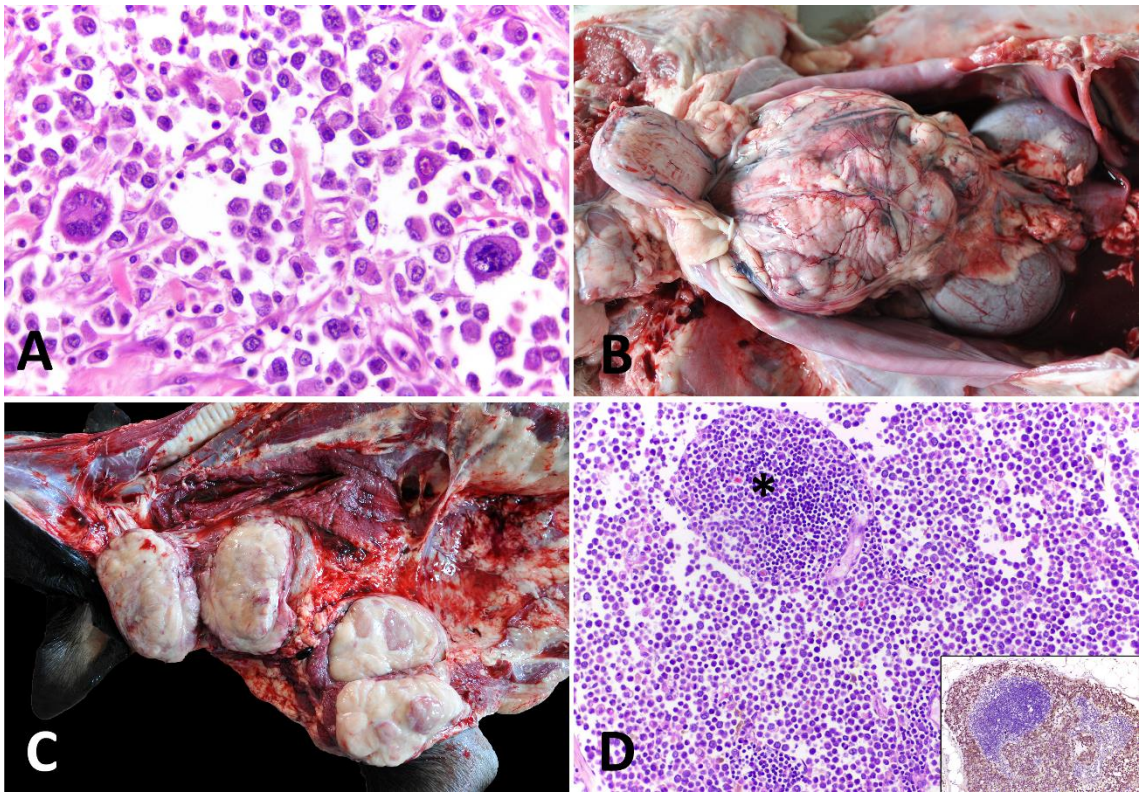

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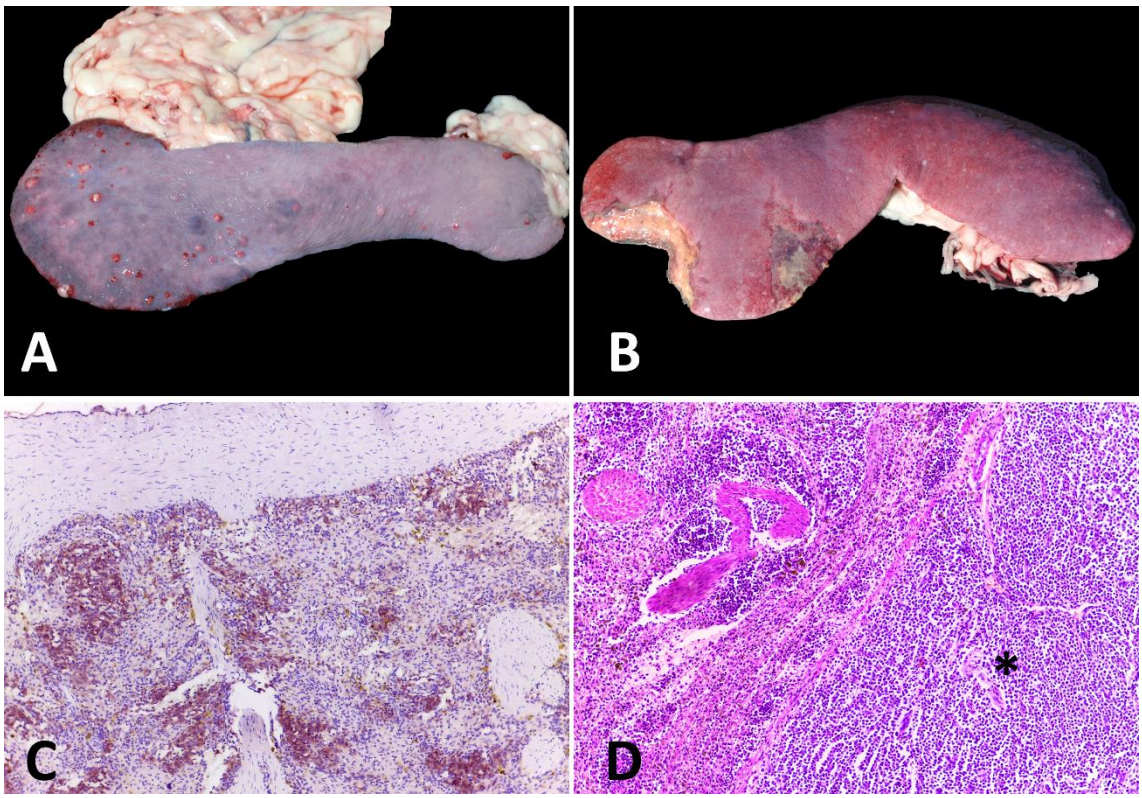


Figure 6.

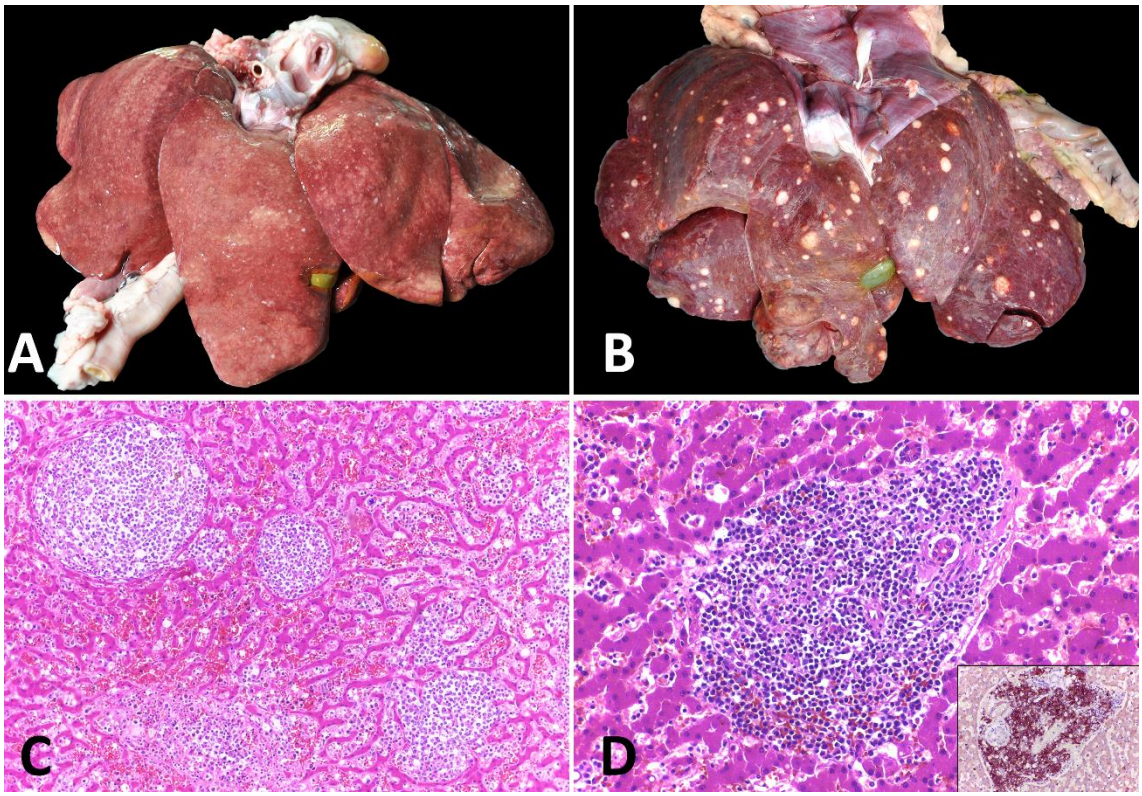
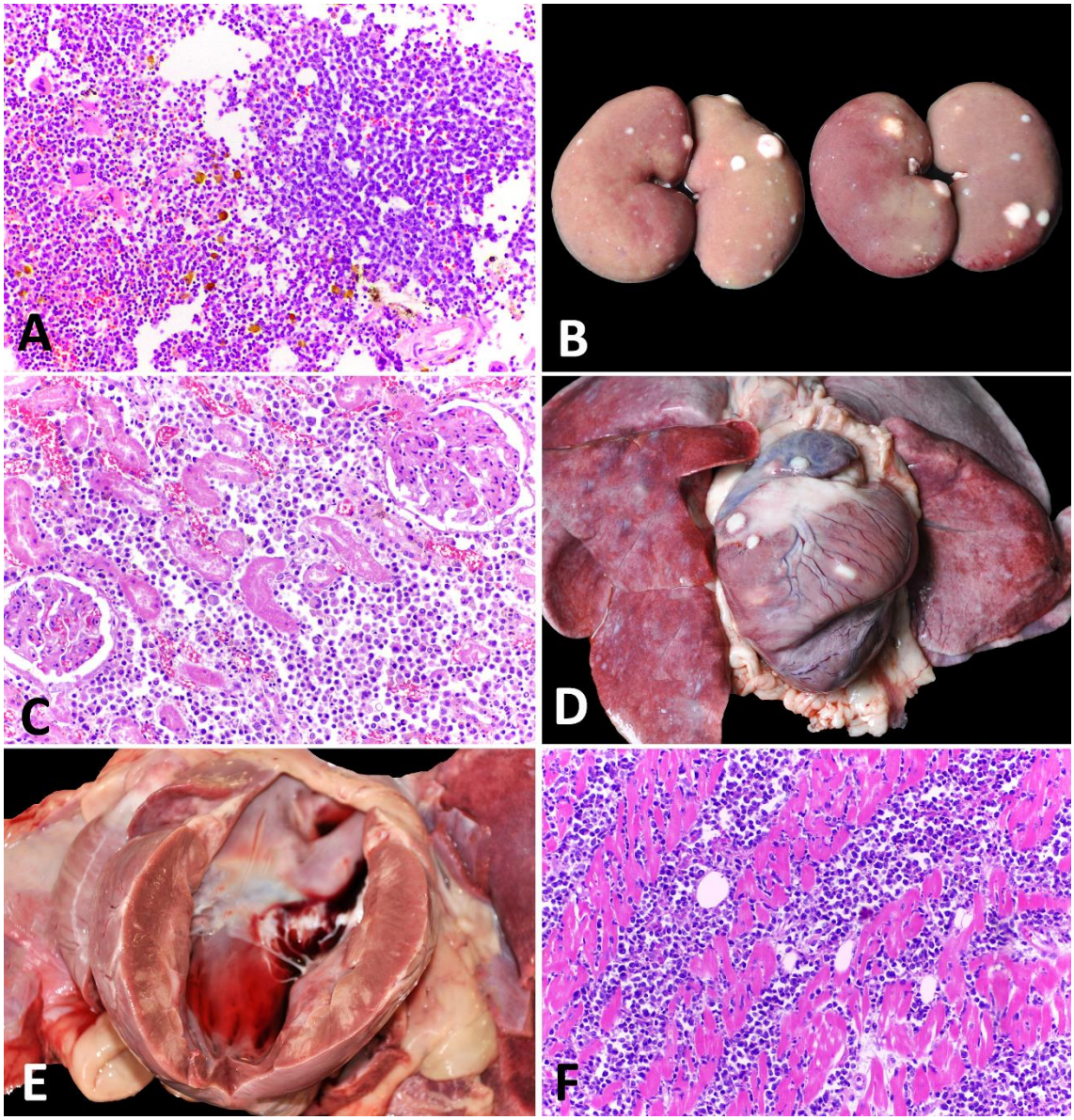


Figure 7.



3. CONSIDERAÇÕES FINAIS

Mastocitoma é uma das neoplasias cutâneas mais comuns em cães e devido ao seu amplo comportamento clínico e biológico, ocorrem casos de tumores agressivos e com potencial metastático, como os observados no presente estudo.

Os principais sítios metastáticos de mastocitomas cutâneos em cães foram os linfonodos regionais, baço, fígado e medula óssea; entretanto, uma quantidade razoável de casos com metástases renais e cardíacas também foram observadas. Locais incomuns de metástases incluem os pulmões, glândulas adrenais, próstata, bexiga, ossos axiais, musculatura esquelética distante do sítio cutâneo primário e trato gastrointestinal (esôfago, palato mole e intestino delgado).

Macroscopicamente, os principais achados na necropsia referentes às metástases foram de linfadenomegalia, esplenomegalia com nódulos brancos a vermelhos na cápsula, hepatomegalia com áreas multifocais puntiformes brancas, nódulos brancos focais a multifocais nos rins e nódulos brancos na superfície epicárdica.

O exame microscópico é essencial para confirmação diagnóstica da neoplasia e dos sítios metastáticos, especialmente em órgãos em que apenas lesões histológicas podem ser observadas, como nos pulmões, rins e coração.

A técnica de imuno-histoquímica para proteína KIT auxiliou na validação diagnóstica de mastocitoma, permitindo descartar diagnósticos diferenciais de outras neoplasias cutâneas. Ademais, a detecção dos padrões de marcação está associada com o prognóstico, e todos os casos do presente estudo apresentaram marcação KIT II e KIT III, com marcação intracitoplasmática aumentada.

REFERÊNCIAS

ABINPET. Associação Brasileira da Indústria de Produtos para Animais de Estimação. 2018. Disponível em: http://abinpet.org.br/download/abinpet_folder_2018_d9.pdf. Acesso em: 29 de junho de 2019.

ADAMS, V. J. *et al.* Methods and mortality results of a health survey of purebred dogs in the UK. **Journal of Small Animal Practice**, v. 51, n. 10, p. 512-524, 2010.

BAKER-GABB, M.; HUNT, G. B.; FRANCE, M. P. Soft tissue sarcomas and mast cell tumours in dogs; clinical behaviour and response to surgery. **Australian Veterinary Journal**, v. 81, n. 12, p. 732-738, 2003.

BRASIL. Instituto Brasileiro de Geografia e Estatística. 2013. Disponível em: http://www.agricultura.gov.br/assuntos/camaras-setoriais-tematicas/documentos/camaras-tematicas/insumos-agropecuarios/anos-anteriores/ibge-populacao-de-animais-de-estimacao-no-brasil-2013-abinpet-79.pdf. Acesso em: 29 de junho de 2019.A

BRASIL. Instituto Brasileiro de Geografia e Estatística. 2013. Disponível em: https://biblioteca.ibge.gov.br/visualizacao/livros/liv94074.pdf. Acesso em: 29 de junho de 2019.B

BLACKWOOD, L. *et al.* European consensus document on mast cell tumours in dogs and cats. **Veterinary and Comparative Oncology**, v. 10, n. 3, p. e1-e29, 2012.

BOSTOCK, D. E. Neoplasms of the skin and subcutaneous tissues in dogs and cats. **British Veterinary Journal**, v. 142, n. 1, p. 1-19, 1986.

DALECK, C. R.; ROCHA, N. S.; FERREIRA, M. G. P. A. Mastocitona. In: DALECK, C. R.; DE NARDI, A. B. ***Oncologia em cães e gatos***. 2ª ed. Roca, Rio de Janeiro, 2016. p. 647-659.

DOTSON, M. J.; HYATT, E. M. Understanding dog-human companionship. **Journal of Business Research**, v. 61, n. 5, p. 457-466, 2008.

HOTTENDORF, G. H.; NIELSEN, S. W. Pathologic survey of 300 extirpated canine mastocytomas*. **Zentralblatt für Veterinärmedizin. Reihe A**, v. 14, n. 3, p. 272-281, 1967.

HOTTENDORF, G. H.; NIELSEN, S. W. Pathologic report of 29 necropsies on dogs with mastocytoma. **Pathologia Veterinaria**, v. 5, n. 2, p. 102-121, 1968.

INOUE, M. *et al.* A current life table and causes of death for insured dogs in Japan. **Preventive Veterinary Medicine**, v. 120, n. 2, p. 210-218, 2015.

KIUPEL, M. *et al.* The use of KIT and tryptase Expression patterns as prognostic tools for canine cutaneous mast cell tumors. **Veterinary Pathology**, v. 41, n. 4, p. 371-377, 2004.

KIUPEL, M. *et al.* Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. **Veterinary Pathology**, v. 48, n. 1, p. 147-155, 2011.

KIUPEL, M. Mast cell tumors. In: MEUTEN, D. J. ***Tumors in Domestic Animals.*** 5ª ed. Willey Blackwell, Ames, 2017. p. 176-202.

KUMAR, V.; SHARMA, A. Mast cells: emerging sentinel innate immune cells with diverse role in immunity. **Molecular Immunology**, v. 48, n. 1-3, p. 14-25, 2010.

KRICK, E. L. *et al*. Cytological lymph node evaluation in dogs with mast cell tumours: association with grade and survival. **Veterinary and Comparative Oncology**, v. 7, n. 2, p. 130-138, 2009.

LETARD, S. *et al.* Gain-of-function mutations in the extracellular domain of KIT are common in canine mast cell tumors. **Molecular Cancer Research**, v. 6, n. 7, p. 1137-1145, 2008.

LONDON, C. A.; SEGUIN, B. Mast cell tumors in the dog. **Veterinary Clinics of North America: Small animal practice**, v. 33, n. 3, p. 473-489, 2003.

LONDON, C. A.; THAMM, D. H. Mast Cell Tumors. In: WITHROW, S. J.; VAIL, D. M.; PAGE, R. L. ***Withrow and Macewen's Small Animal Clinical Oncology***. 5ª ed. Elsevier, St. Louis, 2013. p. 335-355.

MARCONATO, L. *et al.* Clinicopathological features and outcome for dogs with mast cell tumors and bone marrow involvement. **Journal of Veterinary Diagnostic Investigation**, v. 22, n. 4, p. 1001-1007, 2008.

MOORE, A. S. *et al.* Retrospective outcome evaluation for dogs with surgically excised, solitary Kiupel high-grade, cutaneous mast cell tumours. **Veterinary and Comparative Oncology**, v. 18, n. 3, p. 402-408, 2020.

NORTHRUP, N. C. *et al.* Variation among pathologists in histologic grading of canine cutaneous mast cell tumors. **Journal of Veterinary Diagnostic Investigation**, v. 17, n. 3, p. 245-248, 2005.A

NORTHRUP, N. C. *et al.* Varation among pathologists in the histologic grading of canine cutaneous mast cell tumors with uniform use of a single grading reference. **Journal of Veterinary Diagnostic Investigation**, v. 17, n. 6, p. 561-564, 2005.B

O'KEEFE, D. A. *et al.* Systemic mastocytosis in 16 dogs. **Journal of Veterinary Internal Medicine**, v. 1, n.2, p. 75-80, 1987.

O'NEILL, D. G. *et al.* Longevity and mortality of owned dogs in England. **The Veterinary Journal**, v. 198, n. 3, p. 638-643, 2013.

PATNAIK, A. K.; EHLER, W. J.; MACEWEN, E. G. Canine Cutaneous Mast Cell Tumor: morphologic Grading and Survival Time in 83 dogs. **Veterinary Pathology**, v. 21, n.5, p. 469-474, 1984.

PIZZONI S. *et al.* Features and prognostic impact of distant metastases in 45 dogs with de novo stage IV cutaneous mast cell tumours: A prospective study. **Veterinary and Comparative Oncology**, v. 16, n. 1, 28-36, 2018.

POHLMAN, L. M. Basophils, mast cells, and their disorders. In: WEISS, D. J.; WARDROP K. J. ***Schalm's Veterinary Hematology***. 6ª ed. Wiley-Blackwell, Ames, 2010. p. 290-297.

PREZIOSI, R.; MORINI, M.; SARLI, G. Expression of the KIT protein (CD117) in primary cutaneous mast cell tumors of the dog. **Journal of Veterinary Diagnostic Investigation**, v. 16, n. 6, p. 554-561, 2004.

SABATTINI S. *et al.* Histologic grading of canine mast cell tumor: Is 2 better than 3?. **Veterinary Pathology**, v. 52, n. 1, p. 70-73, 2015.

SHAW, T.; KUDNIG, S. T.; FIRESTONE, S. M. Diagnostic accuracy of pre-treatment biopsy for grading cutaneous mast cell tumours in dogs. **Veterinary and Comparative Oncology**, v. 16, n.2, p. 214-219, 2018.

STEFANELLO, D. *et al.* Ultrasound-guided cytology of spleen and liver: A prognostic tool in canine cutaneous mast cell tumor. **Journal of Veterinary Internal Medicine**, v. 23, n. 5, p. 1051-1057, 2009.

STEFANELLO, D. *et al.* Comparison of 2- and 3-category histologic grading systems for predicting the presence of metastasis at the time of initial evaluation in dogs with cutaneous mast cell tumors: 386 cases (2009–2014). **Journal of the American Veterinary Medical Association**, v. 246, n. 7, p. 765-769, 2015.

VASCELLARI, M. *et al.* Expression of Ki67, BCL-2, and COX-2 in canine cutaneous mast cell tumors: association with grading and prognosis. **Veterinary Pathology**, v. 50, n. 1, p. 110-121, 2012.

WARLAND, J. *et al.* The utility of staging in canine mast cell tumours. **Veterinary and Comparative Oncology**, v. 12, n. 4, p. 287-298, 2014.

WEBSTER, J. D. *et al*. Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. **Veterinary Pathology**, v. 44, n. 3, p. 298-308, 2007.

WELLE, M. M. *et al.* Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment. **Veterinary Dermatology**, v. 19, n. 6, p. 321-339, 2008.

ZEMKE, D.; YAMINI, B.; YUZBASIYAN-GURKAN, V. Mutations in the juxtamembrane domain of c-*KIT* are associated with higher grade mast cell tumors in dogs. **Veterinary Pathology**, v. 39, n. 5, p. 529-535.