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Original research

Pathomorphological diagnostic features of canine mast cell tumors

V. V. Logvinova, M. V. Kravtsova, M. O. Lieshchova
Dnipro State Agrarian and Economic University, Dnipro, Ukraine

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Dnipro State Agrarian and Economic University, Serhii Efremov Str., 25, 49600, Dnipro, Ukraine

Tel.: +38-050-273-84-74
E-mail: *lohvinova.v.v@dsau.dp.ua*

#**Corresponding author:**
V. V. Logvinova
lohvinova.v.v@dsau.dp.ua

Abstract. Mast cell tumour is one of the most common cutaneous neoplasms in dogs, ranging from well differentiated to aggressive tumors with metastases. Currently, prognosis and therapeutic definitions of canine cutaneous MCTs are based on the histological grade of malignancy. Clinical examination and treatment were performed in Dnipro veterinary clinics and pathological analysis in the Department of Animal Anatomy, Histology and Pathomorphology, Dnipro State Agrarian and Economic University (Dnipro, Ukraine). Thirty-six dogs with a history of skin neoplasia were examined. Skin MCTs were diagnosed in 23 males and 13 females, aged between 5 and 15 years. No breed predisposition was found out in tested cohort. MCTs were located on the trunk (44.4%), limbs (33.4%) and less frequently on the head and neck, axillary, inguinal and perineal areas (22.2%). MCT was detected in 8 animals where the malignancy was systemic and develops metastatic spread to internal organs. Pathological diagnosis included cytopathological examination of tumor material, pathological analysis of samples obtained by incisional biopsy using haematoxylin/eosin and toluidine blue staining. According to the diagnostic criteria (smear cellularity, presence of cells with basophilic cytoplasmic granularity, size and number of cytoplasmic granules, presence or absence of mitotic figures, nuclear pleomorphism, presence or absence of binucleations or multinucleations, anisokaryosis), benign mast cell tumour (high grade) was diagnosed in 23 animals and malignant mast cell tumour (low grade) in 13 animals. These results were confirmed by histopathological methods using haematoxylin and eosin staining. Additional staining of histological sections with toluidine blue allowed differentiation of benign mast cell tumours into high-grade (38.9%) and intermediate-grade (5%).

Keywords: cutaneous neoplasia; disseminated mastocytosis, metastases, classification of mast cell tumours.

Особливості патоморфологічної діагностики мастоцитом у собак

Анотація. Мастоцитома є одним із найчастіших новоутворень шкіри собак і може варіювати від високодиференційованих до агресивних пухлин з метастазами. В даний час прогностичні та терапевтичні визначення тучноклітинних пухлин шкіри у собак базуються на гістологічному ступені злоякісності. Клінічні дослідження і лікування проводили в ветеринарних клініках міста Дніпро, патоморфологічний аналіз – на кафедрі анатомії, гістології і патоморфології тварин Дніпровського державного аграрно-економічного університету (м. Дніпро, Україна). Досліджено 36 собак з попередньо виявленою неоплазією шкіри. Мастоцитому шкіри було діагностовано у 23 самців і 13 самок, вік тварин коливався від 5 до 15 років. Порідної схильності не виявлено. Мастоцитоми локалізувалися в області тулуба (44,4%), кінцівок (33,4%), а рідше на голові і шиї, пахвовій, пахвинній та промежинно-перианальній ділянках (22,2%). У 8 тварин мастоцитома носила системний характер, що супроводжувалося утворенням метастазів у внутрішніх органах. Патоморфологічна діагностика включала цитопатологічне дослідження матеріалу пухлини, патогістологічний аналіз зразків отриманих інцизійною біопсією з забарвленням гематоксиліном та еозином і толуїдиновим синім. За діагностичними критеріями (клітинність мазка, наявність клітин із базофільною зернистістю цитоплазми, розмір і кількість цитоплазматичних гранул, наявність чи відсутність фігур мітозу, ядерний поліморфізм, наявність чи відсутність бінуклеацій чи мультинуклеацій, анізокаріоз) встановлено доброякісну мастоцитому (високодиференційовану) у 23 тварин, і злоякісну низькодиференційовану у 13 тварин. Ці результати підтверджені гістопатологічно при забарвленні гематоксиліном і еозином. Додаткове забарвлення гістозрізів тулуїдиновим синім дозволило диференціювати доброякісну мастоцитому на високо- (38,9%) і середньодиференційовану (5%).

Ключові слова: неоплазії шкіри; системний мастоцитоз, метастази, класифікація мастоцитом.

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Introduction

Mast cell tumours (MCTs) are neoplasms that are composed of mast cells (mastocytes). They are the third most common type of tumor in dogs, after basal cell carcinoma and squamous cell carcinoma, and account for 11% of all skin cancers (Lieshchova et al., 2018; de Nardi et al., 2022).

This type of tumour has a breed and age-related predisposition. For example, some breeds have a high risk of developing MCTs, including Boxer, Bull Terrier, Golden Retriever, Labrador Retriever, French Bulldog, Shar-Pei and Dachshund, whereas this tumor is rare in German Shepherd, Chihuahua, Poodle, Yorkshire Terrier and Cocker Spaniel (Mochizukiet et al., 2016; Pierini et al., 2019). The disease occurs in older dogs, with an average age of onset of 8-9 years, but it can also occur in younger dogs (Pierini et al., 2019). As MCTs are more common in certain breeds, genetic factors have been suggested to be involved in their development (Oliveira et al., 2020; Ciaputa et al., 2022; Talavera Guillén et al., 2023; Nishibori et al., 2023; Galietta et al., 2023).

MCTs are mainly located in the skin and subcutaneous tissue, most are solitary and 10-15% are multiple. The skin of the trunk, extremities and perineum are the most common sites, but MCTs can develop in any part of the body. Cases have been reported in the conjunctiva, salivary glands, nasal and oral cavities, spinal column, etc. There are also MCTs that originate in the abdominal cavity, such as gastrointestinal or hepatic-splenic MCTs (Ke et al., 2023; Cino et al., 2023).

There is considerable variability in the appearance of MCTs, and they can sometimes be confused with non-tumoral skin problems. High-grade MCTs tend to be single, small, slow-growing tumors, whereas more malignant MCTs can grow at a rapid rate. By releasing biologically active substances from the granules, mast cells can cause other local or systemic clinical symptoms in addition to visible lesions. When histamine, heparin and other related vascular regulatory factors are activated, the tumor itself and the surrounding tissue can become swollen, red and itchy, and the tumor can appear to be larger in size. This is something that is observed by many owners, so that a tumor on the body of a dog can change in size as it grows and shrinks (Ciaputa et al., 2022; Bellamy & Berlato, 2022; Cino et al., 2023).

Excessive histamine release can cause systemic symptoms leading to increased gastric hydrochloric acid secretion and gastrointestinal ulceration. Thus, several MCTs-affected dogs may suffer with vomiting and diarrhoea. Excessive heparin release can also cause coagulopathy, leading to gastrointestinal bleeding or bleeding problems elsewhere. The sudden release of large amounts of histamine and other vasoregulatory factors can cause hypervasodilatation and hypotensive effects leading to acute coma and shock in dogs (Kiupel et al., 2011; London & Thamm, 2013; Kiupel, 2016). Despite a considerable number of reports on the prevalence, clinical manifestations and different treatment methods of MCTs, some aspects of their histogenesis and pathological manifestations are not sufficiently covered. Therefore, the aim of this study was to determine the characteristics of the pathological diagnosis of MCTs in private veterinary practices.

Materials and methods

Totally 36 cases of cutaneous MCTs in dogs were analyzed in current study. Diagnostic and therapeutic measures were carried out in 2021-2023 in veterinary clinics of Dnipro city (Ukraine). The Department of Animal Anatomy, Histology and Pathomorphology of the Dnipro State Agrarian and Economic University performed the pathological examination of the material.

The diagnosis of MCTs was completed comprehensively taking into the account the anamnesis, previous clinic observations, cytological examination results and additional diagnostic methods with ultrasound analysis application. The tumors were pathomorphologically examined after surgical resection.

During the clinical examination, attention was given to the location, size, consistency of the tumor and the condition of the surrounding tissue. Ultrasound was used when systemic involvement was suspected. The device used was the ESAOTE MyLab 40 with linear and convex probes. The frequency range was 5.0-7.5 and 15 MHz at different depths. Ultrasound examination included not only tumor itself but also surrounding tissue, regional lymph nodes, abdominal and pelvic organs to detect distant metastases.

The preliminary diagnosis of MCTs was confirmed by cytopathological examination. Classic fine needle aspiration (FNA) was used to obtain tissue samples. The smears were stained with Romanowsky-Giemsa after drying and fixation (5 min).

Following the tumor ectomy by incisional biopsy, 3×3×3 cm pieces were obtained and fixed in 10% neutral aqueous formalin solution for 24-36 hours. They were dehydrated through isopropyl alcohol and embedded in paraffin. A sliding microtome was used to cut thin histological slices (5-7 μm). The conventional method was used to stain the histological slices with haematoxylin and eosin. The degree of mastocyte differentiation was determined by additional staining with toluidine blue. The slices were deparaffinized and embedded in xylene (3 min), ethyl alcohol and distilled water. An aqueous 0.1% toluidine blue solution was then applied to the slice for 10 minutes and rinsed with distilled water. Slices were cleared in xylene and embedded in polystyrene after dehydration in 96% ethyl alcohol.

The slices were examined using a Micromed XC-3330 light microscope (×40, ×100, ×400, ×1000 magnification). Microphotographs were obtained with using a Micromed MDS 500 digital camera and the software for picture design. The Kiupel histological classification (Kiupel et al., 2010) was used to determine the pathological confirmation of neoplasia, and neoplasms (MCTs) with low, medium and high differentiation were identified.

Results

Cutaneous mast cell tumours were diagnosed in 23 males and 13 females out of 36 dogs. The age of the dogs ranged from 5 to 15 years. There was no breed predisposition. MCTs were localised on the trunk (44.4%), limbs (33.4%) and less frequently on the head and neck, axillary, inguinal and perineal areas (22.2%) (Fig. 1).

The clinical appearance of MCT is a mass of varying sizes and shapes. They can be delineated as nodules ranging in size from 0.6 to 12 cm in size and are elevated above the skin (Fig. 2). The consistency can be firm as well as soft. The hyperemia, swelling and pain on palpation are often associated with tumour growth.

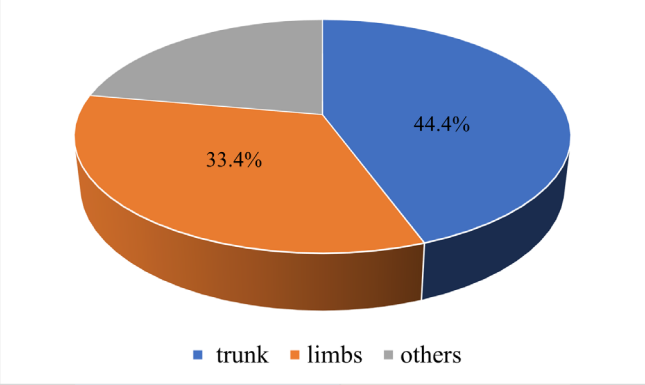

Fig. 1. The ratio of MCTs locations.

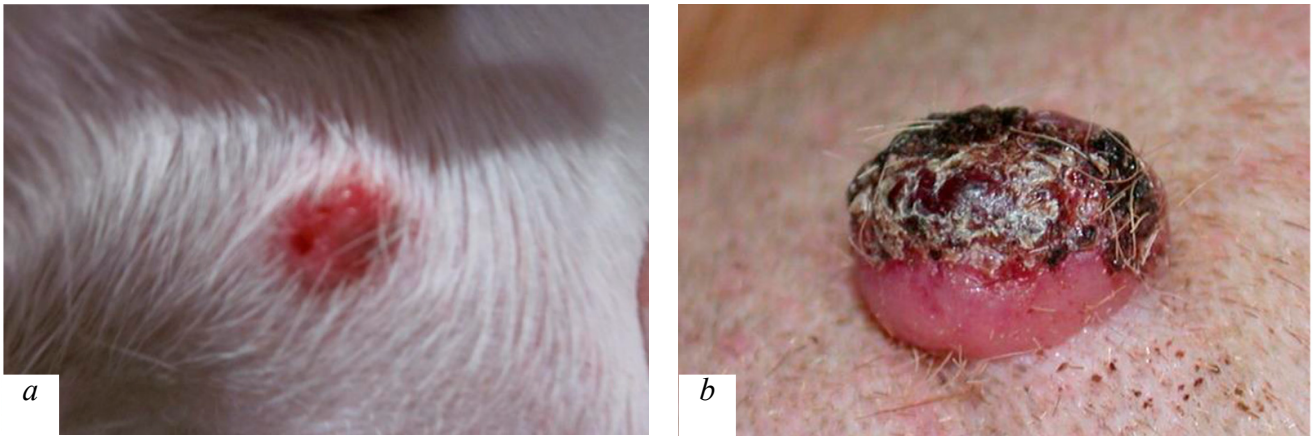

Fig. 2. Location and macroscopic appearance of MCTs in dogs:
a – the initial stage of MCT formation, unformed nodule; *b* – necrotic MCT on the trunk.

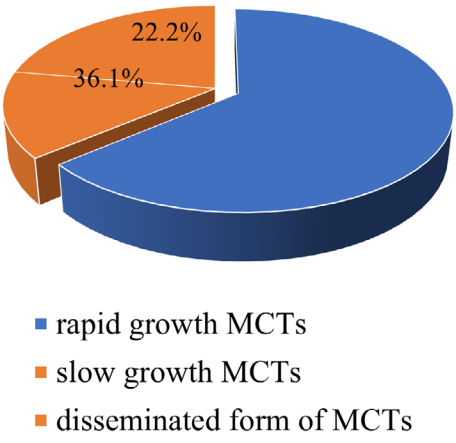

Fig. 3. The ratio of various forms of MCTs.

The results of present study showed that rapid tumour growth, local inflammation and oedema of the surrounding tissue was observed in 13 animals (36.1%) of all tested. Slow tumour growth, growing separately from the surrounding tissue, was observed in 23 (63.9%) animals. The disseminated form of MCTs was identified in 8 (22.2%) animals (Fig. 2).

Ultrasound analysis showed that metastases had formed in the liver, lymph nodes, spleen and several other organs. The analysis with ultrasound the liver showed focal lesions up to 3 cm or most large that accompanied by islands of calcification, necrosis and haemorrhage (Fig. 3).

Cytopathological studies have shown that MCT smears contain a significant number of mastocytes. These are loose connective tissue cells. Mastocytes are cells of mesenchymal origin with a diameter of 10 to 13 microns and a large number of basophilic granules in the cytoplasm. The following diagnostic criteria were used to analyse the microscopic specimens: cellularity of the smear,

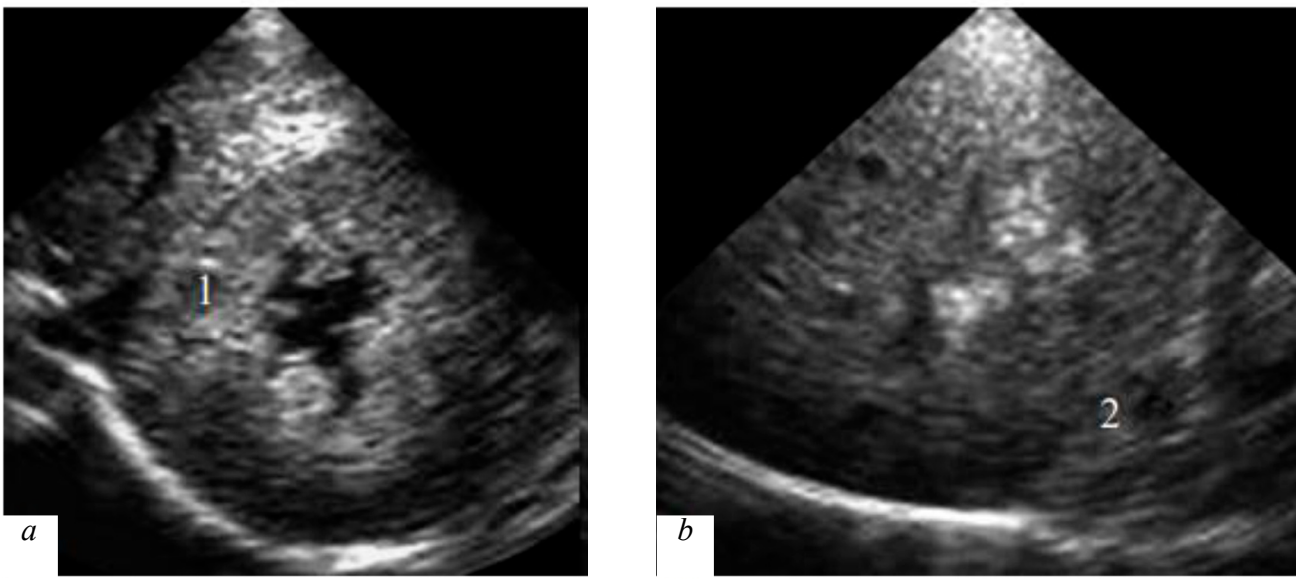

Fig. 4. Ultrasound sonograms of focal lesions in liver with visceral form mastocytosis: *a* – a large isoechoic lesion (1) with a cavity in the centre, *b* – grouped hyperechoic lesions (2) with hypoechoic margins – metastases.

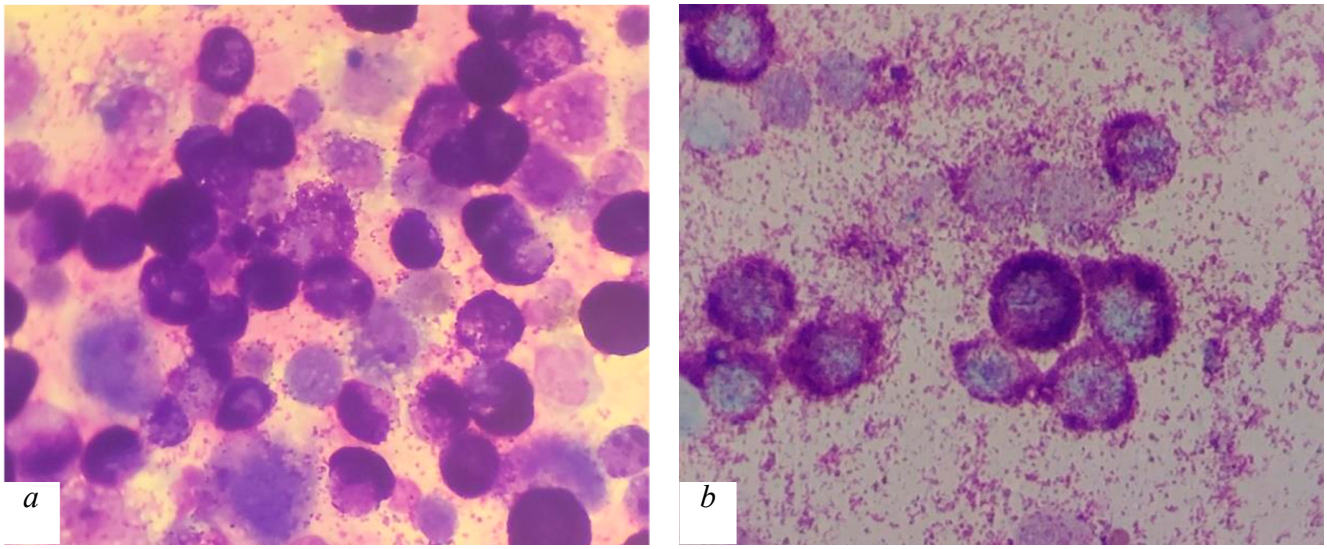

Fig. 5. Cytological examination of a differentiated MCT of the canine skin. Stained according to Romanovski-Gimza. Clusters of rounded cells with basophilic cytoplasmic granularity (granules on the background of the slice). *a* – × 100; *b* – × 400

presence of cells with basophilic cytoplasmic granules, size and number of cytoplasmic granules, presence or absence of mitotic figures, nuclear polymorphism, presence or absence of binucleation or multinucleation, anisokaryosis (Fig. 5).

Cytopathological examination according to these criteria resulted in the diagnosis of benign (high-grade) MCTs in 23 (63.9%) animals and malignant (low-grade) MCTs in 13 (36.1%) animals (Table).

Hematoxylin and eosin-stained slices showed low-grade and high-grade MCTs. High-grade MCTs are represented by rows or clusters of neoplastic mastocytes that are well differentiated, uniform in size and shape, with rounded monomorphic nuclei and small intracytoplasmic granules. These MCTs do not show mitotic figures. Binucleate and multinucleate cells are not characteristic, and in most observed samples there is minimal stromal reaction or necrosis.

Heterogeneous clusters of polymorphic cells partially presented with granular cytoplasm (metachrome-stained cytoplasmic granules) are detected as low-grade (anaplastic) MCTs. The nuclei of the cells are large and vacuolated. They are heterogeneous in

Table – The ratio of benign and malignant forms of canine MCTs

MCTs clinical and morphological features		Diagnostic methods					
		Cytology		Pathohistology (staining methods)			
				Hematoxylin and eosin		Toluidine blue	
		Animals	%	Animals	%	Animals	%
Benign	High-grade MCTs	23	63.9%	23	63.9%	14	38.9%
	Intermediate-grade MCTs	–	–	–	–	9	25%
Malignant	Low-grade MCTs	13	36.1%	13	36.1%	13	36.1%

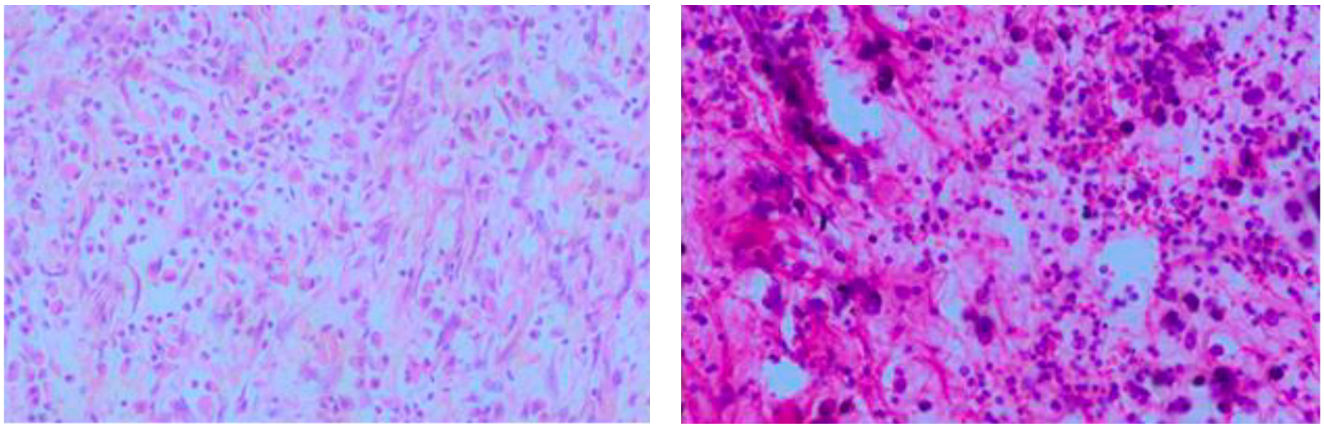

Fig. 6. Histological slice of canine skin low-grade MCT. Clusters of polymorphic round cells with metachromatic cytoplasmic granularity. Hematoxylin and eosin stain, ×100.

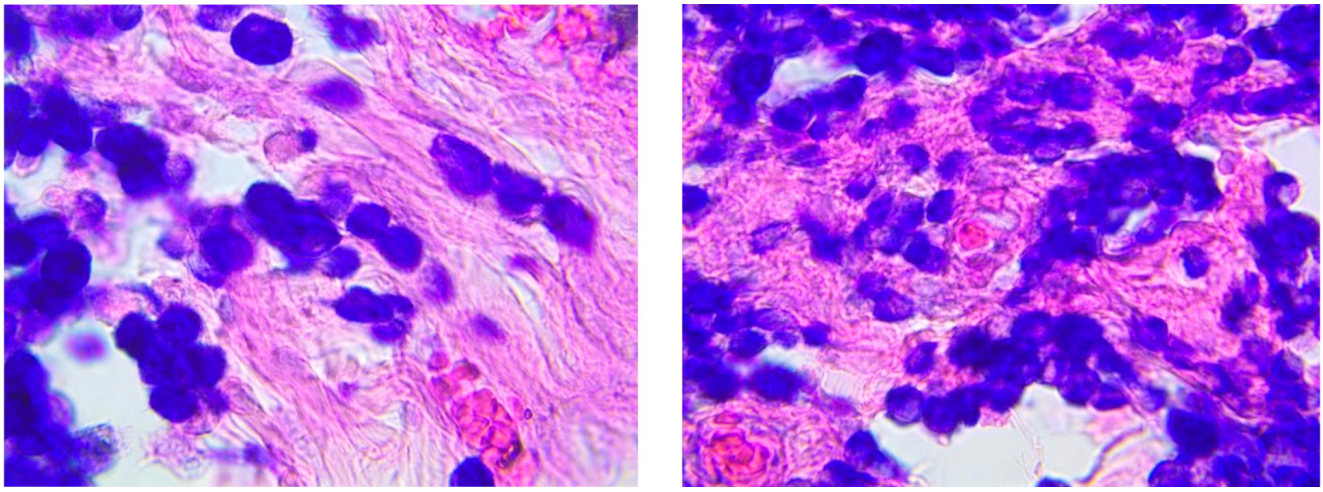

Fig. 7. Histological slice of a dog skin mast cell tumour showing a high-grade cell differentiation. Toluidine blue stain, × 400.

shape. The granularity of the cytoplasm varied considerably in size – both large, few granules and small, multiple, dusty granules were found (Fig. 6). Mitotic figures were often present in the intercellular spaces. Changes were observed not only in the composition of the cells, but also in the connective tissue. Signs of acute serous inflammation, oedema, haemorrhage and cellular infiltrates were often associated with MCTs.

Benign (high-grade) mast cell tumours were confirmed in 23 (63.9%) dogs and malignant (low-grade) mast cell tumours in 13 (36.1%) dogs by pathological analysis of haematoxylin and eosin-stained histological slices.

Proposed manner to detect tumours is potent to differentiate the intermediate level of differentiation by analysing toluidine blue-stained slices of MCTs in addition to low-grade and high-grade tumours identification. The presence of atypical polymorphic mast cells (mastocytes) is characteristic of high-grade differentiation. These cells are located in the dermis, between the hair follicles. They are arranged in strands, small groups separated by dermal collagen fibres (Fig 7). The cells are round, monomorphic. They contain medium-sized granules with rounded nuclei and condensed chromatin in the cytoplasm. The oedema of the tissue was minimal as well as the signs of both necrosis and mitosis was not observed.

The MCTs of intermediate differentiation were characterised by an infiltrative growth with a deep involvement of the dermis and of the subcutaneous fat. The cells were rounded, oval (sometimes spindle-shaped), moderately polymorphic. They were arranged in clusters with areas of hyalinisation with intensely coloured granules (Fig. 8). The nuclei were oval, sometimes irregular in shape, and hyperchromatic. The tissue was in a state of oedema and necrosis with 0-2 mitoses in the field of view. A variety of granularity in the cytoplasm (from the smallest to the largest) is characteristic of a low-grade mast cell tumour. Some cells are undergoing mitosis. There are characteristic changes in the connective tissue: inflammation, oedema, haemorrhage, cellular infiltrates, and necrotic changes.

Using an additional method of staining the histological samples (toluidine blue), we found that of 36 animals with MCT, 23 (63.9%) had a benign tumour. Of these, 14 (38.9%) had high-grade MCT and 9 (25%) had intermediate-grade tumours. Low-grade mast cell differentiation was confirmed in 13 (36.1%) dogs that evidence the high malignant potential in the tumour growth.

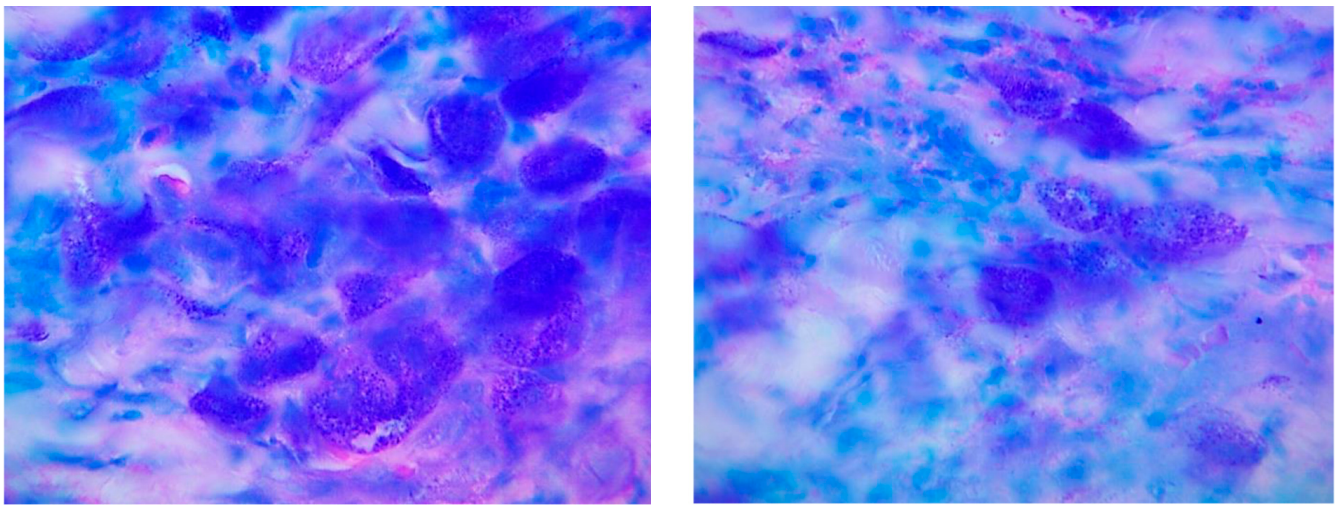

Fig. 8. Histological slice of a dog skin mast cell tumour showing an intermediate-grade level of cell differentiation. Toluidine blue staining, × 1000.

Discussion

Mast cells are derived from haematopoietic stem cell precursors and have granules in the cytoplasm containing histamine, heparin, tumour necrosis factor-alpha (TNF-alpha) and other vasoactive substances, the release of which causes local (erythema, oedema) and systemic (gastric and duodenal ulcers) reactions (Jackson et al., 2021; Ciaputa et al., 2022; Cino et al., 2023).

Accounting for approximately 16-25% of skin and adjacent tissue tumours, MCTs are the most common neoplasm of the skin and subcutaneous tissues in dogs. Genetic factors are thought to be responsible for the high incidence of MCTs. Although Bulldogs are at a high risk of developing MCTs, the majority of MCTs are benign. Recently, it has been confirmed that several types of MCTs in Pugs are benign. On the contrary, MCTs in Shar-Pei are more aggressive (Oliveira et al., 2020; Galietta et al., 2023).

Symptoms depend on the location, degree of malignancy and cellular differentiation of the tumour. Cutaneous MCTs are very variable in appearance and are sometimes confused with non-neoplastic lesions. This makes diagnosis based on external examination unreliable (Bellamy & Berlato, 2022). The majority of neoplasms are single lesions, but 11–14% are multiple lesions. A lump that remains for several days or months appears on the skin or subcutaneous tissue. After a period of rest for several months, the mass varies in size and may grow rapidly. Erythema and oedema are most common in the early stages. The changes may mimic other skin or subcutaneous tumours (benign or malignant), insect bites or allergic reactions. The malignant progress starts with a single lesion on the skin or subcutaneous tissue, but it can look like several. In our study, single nodules ranging in size from 0.6 to 12 cm were seen. They were accompanied by hyperemia, swelling and pain evoked by local palpation. In 36.1% of cases, tumours were characterised by rapid growth, inflammation and swelling of the surrounding tissues, and in 63.9% by slow tumour growth with clear separation from the surrounding tissues. Approximately 50% of cases occur in the trunk and perineum, 40% in the extremities and 10% in the head and neck. MCTs can also be found in other sites: conjunctiva, salivary glands, nasopharynx, larynx, oral cavity, ureters, spine (de Nardi et al., 2022). In this study, we found that in 44.4% of tested animals while MCT was located in the trunk, 33.4% in the extremities, and 22.2% in the head and neck, axillary, groin, and perineal regions.

Regional lymph node involvement may occur in low-grade tumours. Hepatosplenomegaly is a characteristic feature of dissemination. The term disseminated or systemic is often used for visceral MCTs. Infiltration of the intra-abdominal lymph nodes, spleen, liver and bone marrow is observed. Vomiting, diarrhoea and melena have been reported in dogs with gastrointestinal tumours (de Nardi et al., 2022; Ke et al., 2023).

High-grade tumours tend to be solitary, small, slow-growing, and have a low malignant potential. Low-grade tumours tend to grow rapidly, form ulcers and cause considerable irritation. The surrounding tissue may be inflamed and swollen, and small nodules may develop around the lesion. Intermediate-grade mast cell tumours are soft to the touch and have a similar texture to flesh. Clinical confusion with lipoma is common (Ciaputa et al., 2022; Cino et al., 2023).

Histological examination is the single most important prognostic factor in dogs with MCTs. Biopsy is considered to be the "gold standard" method of sampling, although a high degree of agreement between cytology and histopathology has been observed. MCTs are heterogeneous, ranging from single, well-differentiated tumours to aggressive ones with metastatic lesions. Due to the wide range of clinical manifestations, prognostic factors are often used in the diagnosis and classification of MCTs, in addition to histological evaluation. There is a 3-stage classification system with grades I, II and III (from high to low-grade tumours), as well as a newer 2-stage system that only classifies tumours as low or high-grade (London & Thamm, 2013; Kiupel et al., 2011; Kiupel, 2016). Other group of prognostic indicators include immunohistochemical detection of the type III receptor protein-tyrosine kinase (Kit), proliferative markers such as mitotic count, Ki67 and AgNOR, and molecular detection of the c-kit mutation by polymerase chain reaction (Webster et al., 2007; Blackwood et al., 2012). However, none of these factors is 100% predictive of clinical outcome. Therefore, clinical features such as history of tumour recurrence, stage, race, lymph node status and anatomical location should also be considered (Horta et al., 2018). High-grade MCTs were found in 63.9% and low-grade MCTs in 36.1% of animals on cytopathological examination. The sensitivity of the method is based on the characteristics of metachromasia of mast cell granules. However, mastocytes have been detected in allergic reactions and in inflammatory reactions (Ribeiro et al., 2022). This may lead to a false conclusion, as mastocytes degranulate on palpation or mechanical stimulation. On the other hand, it is not only mastocytes but also other cells, such as melanocytes, that may have granularity in the cytoplasm. The cytological findings were confirmed by our subsequent histopathological studies using the haematoxylin and eosin staining technique. However, the use of toluidine blue staining provided more informative data: a high degree of MCT differentiation was found in 38.9% of cases, intermediate in 25%, and low in 36.1%, with systemic mastocytosis confirmed in 22% of animals. The use of toluidine blue staining made it possible to diagnose the disease according to a 3-stage classification.

In recent years several therapeutic approaches have been proposed for the treatment of MCT in dogs and cats. These tools supplementation is dependent on the degree of differentiation including low, intermediate and high grade. The results of them application have been variable. In common, the results reported in respect to animal treatment were vary depending on the grade of malignancy (Oliveira et al., 2020; Nishibori et al., 2023). After surgical resection, high-grade MCTs have a favourable prognosis. Low-grade MCTs require further tumor management in addition to surgical resection and may result in death (Ciaputa et al., 2022).

Metastatic MCTs, especially if they are highly malignant, have a poor prognosis compared to low-grade tumours. More than 80% of grade III MCTs will develop metastases, whereas metastases are rare in grade I MCTs (Pizzoni et al., 2018; Moore et al., 2020). It has been reported that metastases often involve regional lymph nodes. Less commonly, they involve the liver, spleen and bone marrow (Webster et al., 2007). In our study, metastases were found in the liver, spleen and other organs in 22% of the dogs. In the liver, tumors of limited growth up to 3 cm and larger were observed. There were islands of calcification, necrosis and haemorrhage. Several studies have described the pathological features of metastases found at autopsy. Most studies have focused on clinical or imaging aspects of metastatic disease or exclusively on nodal metastases (Warland et al., 2014; Pizzoni et al., 2018). To date, only one study has investigated metastatic disease associated with MCTs using autopsy data. However, this study lacks further definition of the histological characterisation of metastases (Pecceu et al., 2020).

Although the majority of cases can be treated with adequate local therapy alone, some neoplasms show a biologically aggressive behaviour that is associated with local recurrence or metastasis (Bellamy & Berlato, 2022; Ribeiro et al., 2022). In this context, the most effective treatment regimen can be selected using the 3-stage diagnosis.

Conclusion

The obtained results of the study showed that among of 36 cases of MCTs in dogs 23 (63.9%) were benign and 13 (36.1%) were malignant. The localisation of MCTs in the tested animals was as follows: trunk – 44.4%, limbs – 33.4%, head, neck, axillary, inguinal and perineal – 22.2%. An additional histopathological staining technique (toluidine blue) allowed a more detailed differentiation of MCTs – of 23 (63.9%) dogs with benign MCTs, 9 (25%) were diagnosed with intermediate-grade differentiation and 14 (38.9%)

with high-grade differentiation. Proposed in our study 3-stage diagnosis has implications for the prescription of effective treatment.

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