UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2023. Volume 14, No. 1. P. 57-73

Received: 31.10.2022 Revised: 23.01.2023 Accepted: 22.02.2023

DOI: 10.31548/veterinary1.2023.57

UDC: 619:616-006.6:617-089.8:663.7

Clinical and morphological features of mastocyte diagnosis in dogs

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Abstract. Mastocytoma is one of the most common skin tumours in dogs. It is characterised by a specific course, unpredictable behaviour, and prognosis. This tumour is dangerous because it mimics the external signs of other neoplasms, from less-threatening lipoma to skin cancer. The purpose of the study is to conduct pathomorphological verification and classification of neoplasia in dogs diagnosed during a clinical examination and to establish the features of the morphological manifestation of the skin form of mastocytoma in them. The material for the studies was obtained by excisional biopsy during surgical interventions for skin neoplasms. The diagnosis of mastocytoma was established based on the results of a clinical examination of animals and the examination of biopsy material of neoplasms using cytological and histological research methods. It was established that the share of skin neoplasia in the structure of oncological diseases of dogs was 30.12%. Among the animals with skin neoplasia that were examined, mastocytoma was diagnosed in 17.55% of dogs aged 3 to 14 years. The highest incidence rates were established in dogs aged 7 to 12 years.

Suggested Citation:
Kolych, N., Hudz, N., & Tarasov, O. (2023). Clinical and morphological features of mastocyte diagnosis in dogs. *Ukrainian Journal of Veterinary Sciences*, 14(1), 57-73. doi: 10.31548/veterinary1.2023.57.

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Most often, skin mastocytoma was diagnosed in dogs of the following breeds: Shar Pei – 13.5%; Labrador – 9.5%; Boxer – 8.2%. Isolated cases of tumour formations were observed in animals of such breeds as: German shepherd, Spaniel, Central Asian Shepherd, and Pug. Depending on gender: males accounted for 52.2%, females – 47.8%. In 50% of the examined animals, mastocytomas developed with rapid dynamics, which indicates a substantial aggressiveness of tumour growth. Clinically, signs of dehydration and gastrointestinal disorders were detected in dogs, which indicates the development of paraneoplastic syndrome. According to the results of sonography, it was established that skin mastocytomas are visualised as heterogeneous hypoechoic structures with indistinct contours and uneven edges. Based on the results of the study, new information was obtained regarding the morphological features of the manifestation and development of mastocytes in dogs. Thus, the obtained results complement and expand knowledge about the pathogenesis of mastocytoma in dogs, the frequency of spread and features of the course of this oncological pathology in a separate geographical population of Ukraine

Keywords: cytological examination; histological examination; tumour; skin; metastasis

Introduction

In recent years, animal tumours have been used to test genetic engineering technologies as generally accepted models, which is important in the treatment of people with cancer (Akin, 2020). Over the past decade, there has been a growing interest in investigating neoplasia in dogs. This is due to large economic losses, which are caused by the need for examinations, diagnostics, and treatment of animals with neoplasms. Measures to prevent tumour growth also require scientific justification. The investigation of "natural models" of neoplasia in animals plays a leading role in expanding the understanding of the essence of tumour growth (Leguit *et al*., 2020).

In animals, over the past 30 years, the rate of growth of diseases for malignant tumours and mortality from them has increased (Leguit *et al*., 2020; Tamlin *et al*., 2020).

Dogs are located in the closest climatic and living conditions to humans and are also exposed to the same negative environmental factors in large and technogenically saturated cities. According to the World Health Organisation, animal tumours meet many criteria for "natural model candidates" for human tumours (Tamlin *et al*., 2020). Animal tumours have many features of the clinical course, risk factors, biochemical characteristics, and some pathogenetic and prognostic factors in common with human tumours (Willmann *et al*., 2019). Indications on the frequency of occurrence, morphology, pathogenesis, clinical course, diagnosis, and treatment of tumours in dogs are insufficient and sometimes scattered, which makes it difficult to solve a number of issues of comparative oncology (Akin, 2020; Balima & Vairamuthu, 2020).

Problems in solving most oncology issues are associated with a large histogenetic and histological specificity of malignant and benign neoplasms and are the result of versatile approaches to grouping tumours according to different classifications based on different evaluation criteria (Smiech *et al*., 2018). There are only a few banks in the world that store a substantial number of tumour samples from various tissues obtained from dogs that differed in breed, age, region, and habitat conditions. In Ukraine, almost no studies have been conducted on various

aspects of neoplasms in dogs. Therefore, there is a need to identify the regional features of the manifestation of neoplasia in dogs, their spread, and features of behaviour in the body, which is quite valuable clinical material. Conducting these studies will help to clarify some issues of carcinogenesis and contribute to obtaining new data on the role of individual environmental factors in these processes due to technogenic human activities. These data will also be of biological interest since the processes that determine the geographical accumulation of cases of neoplasms in humans and dogs are to some extent completely different and not always interdependent (Garden *et al*., 2018).

Ambiguous assessments and certain discrepancies in the diagnosis and prognosis of the biological behaviour of mastocytes indicate the relevance of conducting more in-depth and diverse studies of this neoplasia. Notably, this issue is quite important for veterinary science and practice. The study of pathomorphological and clinical-biological features of mastocytoma in different geographical populations of Ukraine is not only of scientific interest but also of great practical importance for veterinary medicine.

Considering the above, the purpose of the study was to examine the features of the clinical and morphological manifestation of the cutaneous form of mastocytoma, the frequency of spread, breed, and gender predisposition of dogs to the cutaneous form of mastocytoma in dogs under conditions of the city of Kyiv and the suburban area.

Literature review

Mastocytoma (mast cell tumour, histiocytic mastocytoma, mast cell sarcoma, *mast cell tumour*, MST, *Mastocytoma*) got its name from the type of cells from which it is formed – mastocytes (Macewen & Withrow, 2013). Mast cells are oval or round cells of mesenchymal nature, their diameter is 10-13 microns, with secretory granules that are located in the cytoplasm (Sledge *et al*., 2016; Balima & Vairamuthu, 2020). Mast cell precursors are formed in the bone marrow, then they migrate through the walls of blood vessels to the tissues, where they differentiate. Their final maturation occurs in the connective tissue. Mast cells are found in almost all body tissues, but their greatest concentration is around blood and lymphatic vessels in places of contact with the external environment (skin, respiratory tract, intestines). Mast cells are one of the main cells that perform a protective function in the animal body, and they also regulate the homeostasis of connective tissue. These cells produce biologically active substances and mediators: heparin, histamine, serotonin, tumour necrosis factor-α (TNF-α), proteases, interleukins, glycoproteins, chemotactic migration factor of eosinophils, etc. and are able to deposit and store them in cytoplasmic granules. The release of biologically active substances from mast cells occurs by exocytosis. Two types of mast cell degranulation are described: partial, which lasts the whole day, and rapid, which takes several minutes. During the activation and degranulation of mastocytes, these biologically active substances are released, which play a leading role in the course of physiological and pathological processes (Garden *et al.*, 2018).

Mast cell activation syndrome is a fairly common disorder in the pathogenesis of both rare and common diseases. Therewith, it is noted in the literature that the number of mast cells increases substantially with inflammation and immune responses (Valent *et al.*, 2012; Bautista-Quach *et al*., 2013; Akin, 2020).

An important function of these cells is their involvement in the manifestation of adaptive immunity. On their surface, mast cells carry antibodies (Class E immunoglobulins), which are released by plasma cells under the action of antigens, and in the process of cell life, they

actively bind to the surface of mast cells. There can be from 5,000 to 500,000 IgE molecules on the surface of mast cells simultaneously, which are directed against various antigens (Broudy, 1997).

The course of diseases involving activated mastocytes in animals and humans has certain differences (Willmann *et al.*, 2018; Tamlin *et al*., 2020).

Mastocytoma can also occur in other tissues of the reticuloendothelial system: in the bone marrow, kidneys, and liver, referred to by the term "systemic mastocytosis". The biological behaviour of systemic mastocytosis is very different from that of cutaneous mastocytes (Lisickaja & Sedov, 2011).

According to the medical literature (Pardanani, 2016; Frolova *et al*., 2018; Leguit *et al*., 2020), mastocytosis affects both adults and children. Adults experience systemic mastocytosis, which affects not only the skin, but also the spleen, liver, and bone marrow. A benign period is rare. The disease has an unfavourable prognosis and a very aggressive course. In children, the prognosis is more favourable and the disease occurs mainly in the skin form and in the form of pigmented urticaria.

Skin neoplasms in dogs occupy one of the first places in terms of frequency of occurrence and spread and account for 1/3 of all cancer cases (Sledge *et al.*, 2016). Most often, dogs are diagnosed with mastocytoma of the skin. This type of tumour is observed in 16-21% of cases of all skin neoplasms (Balima & Vairamuthu, 2020).

Factors that lead to the risk of skin mastocytoma in dogs remain unclear. Therewith, genetic factors play a substantial role, indicating a different frequency of neoplasia in different breeds of dogs. Another important endogenous risk factor for tumours is the age of animals. Tumours in dogs can be detected throughout their lives, but the largest proportion of animals with mastocytoma is diagnosed at the age of 7-12 years (Withrow & MacEwen's, 2013).

Other factors that may be important in the etiology of tumours in dogs are carcinogenic environmental hazards, parasitic and viral infection, and injuries (Leguit *et al.*, 2020). There is also an assumption about the viral aetiology of mastocytes (Lisickaja & Sedov, 2011). However, to date, there are no studies that would support this theory. Receptors for sex hormones (testosterone and estrogens) have been established in the cytoplasm of mast cells and, together with data on a more favourable prognosis of the disease in females, may indicate the role of hormones in tumour development (Balima & Vairamuthu, 2020).

Recently, considerable attention from researchers has been focused on finding genes that may be associated with the development of mastocytoma (Mohammad & Joanne, 2016). Mutations in the c-kit gene have recently been identified in some dog, human, rat, and mouse mastocytoma cell lines and tissue samples (Webster *et al.*, 2006). The gene encodes a receptor on the mast cell progenitor membrane in the bone marrow that binds the mast stem cell growth factor (SCF). This factor stimulates the proliferation and differentiation of mast cell progenitor cells into mature cells. Mutations in the gene cause changes in the structure of the receptors, which leads to the launch of a cascade of biochemical reactions without binding SCF itself (semke *et al.*, 2001; Jones *et al.*, 2004). Based on the results of a study in the field of humane genetics, it was established that point mutations of the gene in children are often associated with mastocytosis in adults (Jones *et al.*, 2004).

Currently, there are several types of classification of skin tumours, including cutaneous mastocytes. Classification by stages of the process is conducted according to the international clinical TNM classification (Owen, 1980), which is used to describe the anatomical spread of the tumour process and is based on three components: T – the size and spread of the primary

tumour; N – the absence or presence of metastases in regional lymph nodes; M – the absence or presence of metastases.

The figures added to these three main components indicate the spread of the process: T_0 (there is no tumour); T_1 (tumour up to 2 cm in diameter); T_2 (tumour with a diameter of 2-5 cm); T_3 (the diameter of the tumour is from 5 cm, they note germination into the subcutaneous tissue, regardless of the size); T_4 (the tumour spreads to muscles or other structures); N_0 (there are no metastases in regional lymph nodes); N_1 (there are small metastases in the nearest regional lymph nodes, but their mobility is preserved); N_2 (there are metastases in several regional lymph nodes); N_3 (damage to all regional lymph nodes, Immobile lymph nodes); M_0 (there are no metastases in other organs); M_1 (metastases were established in other organs, including the bone marrow).

In addition to the TNM classification, the WHO clinical classification is widely used, which provides for mastocyte division according to 5 stages (Table 1).

Table 1. WHO classification of skin mastocytes in dogs

0	The tumour is absent or completely removed, confirmed histologically
I	Well-limited single node without lymph node metastases
II	Well-limited single node, but with lymph node metastases
III	Single large node or multiple nodes with signs of infiltrative growth without / with lymph node metastases
IVa	A tumour with distant metastases, but no common clinical symptoms
IVb	A tumour with distant metastases with common clinical symptoms present

Source: *compiled by the author based on data from (Owen, 1980)*

According to the WHO International histological classification of canine skin tumours, mastocytomas belong to cutaneous lymphoproliferative tumours and are divided according to the degree of differentiation. According to the degree of differentiation, there is no single gradation of mastocytes. Today, three systems are used simultaneously: according to Patnaik (1984), according to Bostock (1986), and a more modern classification according to Kiupel (2011). They are all based on tumour size, cell morphology, and mitotic index, with the difference only in assigning a degree of gradation from 1 to 3 (Table 2).

Table 2. Histological classification of mastocytes according to Patnaik and Bostock

Mastocytoma	Gradation according to		Cell morphology
	Patnaik	Bostock	
Anaplastic / low-grade	3	1	A substantial number of mitotic figures; cytoplasm is poorly restricted; asymmetrical nuclei, few secretory granules.
Intermediate / medium-differentiated	2	2	A small number of mitotic figures; the ratio of the nucleus to the cytoplasm is less than the anaplastic type; the average number of secretory granules is noted
Highly differentiated	1	3	A small number of mitotic figures; large secretory granules are pronounced, the nuclei are rounded, the cytoplasm is limited

Source: *compiled by the author based on the studies by Patnaik (1984), Bostock (1986)*

According to the classification of Kiupel *et al*. (2011), mastocytomas are divided into two groups: high or low degree of differentiation and completely ignore moderately differentiated tumours. Notably, animal tumours meet similar neoplasms in humans by many criteria. The similarity in the aetiology, pathogenesis, symptoms, and morphology of tumours in dogs and humans gives researchers the opportunity to use animal models to examine the course of the tumour process and develop modern diagnostic and therapeutic measures for the treatment of humans (Garden *et al*., 2018; Gargiulo, 2018).

Materials and Methods

The study was conducted based on a veterinary clinic "Animal health" (Kyiv region, u.t.s. Hlevakha) and the laboratory of the Volodymyr Kasianenko Department of Animal Anatomy, Histology, and Pathomorphology throughout 2021-2022. During this study period, 1,205 dogs with surgical pathology were registered.

The study material included 98 dogs with skin tumours, including 9 animals with mastocytoma. The dogs were of different breeds and genders, ranging in age from 3 to 14 years. Cancer-stricken animals were examined in accordance with the criteria of clinical TNM classification of tumours using clinical and laboratory research methods (Owen, 1980).

Ultrasound examination was performed using the ESAOTE MyLab 40 device (Italy). Sonographic examination was performed on the tumour itself, surrounding tissues, and regional lymph nodes. The organs of the abdominal and pelvic cavities were examined to detect distant metastases. Sonographic images were used to analyse the echostructure of the examined objects.

Radiography of the tumour site, abdominal cavity, and chest was performed using a digital X-ray machine "ZooMax LC" (Hungary) in two standard (straight and lateral) perpendicular projections. Material for cytological examination was obtained by fine-needle puncture biopsy of neoplasia. This method is based on taking a cytological sample, by puncture, during which cells are sucked in from the neoplasm. A 10 mL plastic syringe and needle No. 22 were used to do this.

Before performing the puncture, the tumour was palpated, its interaction with surrounding tissues and blood vessels was determined, its mobility and convenient position for performing the puncture were examined. Next, the tumour was fixed with the fingers of the left hand. A needle with or without a syringe (the plunger is lowered) was passed perpendicularly through the skin directly into the tumour. Fingers checked the correctness of the puncture. If the syringe was not attached, it was attached with the plunger lowered. 2-3 sudden suction movements were performed, removing the syringe from the needle each time after lifting the plunger.

The syringe was removed along with the needle. Therewith, ensuring that the plunger of the syringe occupies a lowered position before removing the needle. After removing the needle, a syringe with a raised plunger was attached to it. The contents of the syringe were carefully squeezed out onto a slide. The distribution of cellular material on the glass surface was conducted in one direction using a needle.

The resulting smear should be oblong in shape and of medium thickness so that the cells are placed in a single layer and can be differentiated. The smear was air-dried and stained according to Poppenheim. The main criterion for mastocytoma verification is the detection of round tumour cells in cytological preparations that contain azurophilic granules in the cytoplasm.

Experiments using live animals were conducted in accordance with the recommendations of Arrive. For digital processing of the results, the statistical analysis method using Microsoft Excel 2019 and Statistica 6.0 computer programmes was used.

Results and Discussion

Among the examined animals, the average cancer rate was 12.5%. From the total number of sick animals with cancer problems (n = 295), 108 dogs were diagnosed with tumours and tumour-like skin lesions. The results of the study (Hosseini *et al.,* 2014) confirm that among animals with oncological pathologies, dogs are at risk of developing cutaneous mastocytoma, which accounts for up to 21% of all skin tumours. The aetiology of mastocytes in dogs is unknown, but assumably, is multifactorial.

According to the results of a clinical examination of animals, and based on cytological examination of biopsy punctates of neoplasia in 13 dogs, the diagnosis of mastocytoma of the skin was confirmed. In dogs, most of these tumours occur as primary skin tumours.

According to the registration data, the sick animals were of different breeds and genders, aged from 3 to 14 years. Most often, skin mastocytoma was recorded in dogs of the following breeds: Shar Pei – 3 cases, Staffordshire terrier – 2, Boxer – 2, Labrador – 3, Doberman – 1, and Rottweiler – 2 cases. German shepherd, Spaniel, Pug, Alabai, and French bulldog dogs had isolated cases (3.9%). Among animals with mastocytoma, males accounted for 52.2% and females – for 47.8%. The vast majority of animals admitted to the clinic underwent a full course of vaccinations, but the animals were also vaccinated only once in their lifetime. Not all animals were also regularly treated with antiparasitic agents. The sample includes animals of both residential and street maintenance, mainly with a mixed type of feeding.

In the age aspect, the number of cases of the disease was distributed as follows: animals aged 3 years – 1 case; 5-6 years – 2; 7-8 years – 2; 9-10 years – 4; 11-12 years – 3; 13-14 years – 1 case. The highest incidence rates were established in animals aged 7 to 13 years. It can be noted that the results of this study coincide with the data of foreign researchers (Sledge *et al.*, 2016; Smiech *et al.*, 2018). According to the literature data (Smiech *et al.*, 2018), dogs of such breeds as Boxer, American Staffordshire Terrier, Labrador Retriever, Beagle, French and English Bulldogs, Pug, and Shar Pei are susceptible to the development of the skin form of mastocytoma. It is also known that along with the above-mentioned dog breeds, high morbidity rates are noted among other breeds-these are Rottweilers, Dobermans, German Shepherds, and Cocker Spaniels (Shoop-Worrall *et al.*, 2015). Perhaps such discrepancies in the literature data can be explained by the distribution of certain breeds in different geographical populations.

In clinical practice, mastocytoma is considered a skin neoplasia with a specific clinical course, unpredictable biological behaviour and prognosis. The priority area of research in this study was to identify the prognostic factors of this disease.

According to the results of clinical examinations of dogs during the diagnostic and treatment period, a substantial polymorphism in the appearance and clinical manifestation of mastocytes was established.

The examined dogs showed neoplasia, which had the appearance of small dense nodules (Fig. 1), and nodes of 3-5 cm in size, usually similar to lipomas and giant tumours of 9-10 cm or more in size.

Figure 1. Mastocytoma of the body area in the form of a dense nodule

In some animals, eczema-like skin lesions were noted, which were very similar in appearance to nodular panniculitis (Fig. 2).

Figure 2. Eczema-like skin lesions. Boxer dog, age – 9 years

It was established that in 92.7% of dogs, mastocytomas had the appearance of single nodes and only in 7.3% – multiple (Fig. 3).

Figure 3. Mastocytoma on the pelvic limb. Pug dog, age – 7 years

Most often, mastocytomas were localised on the trunk – 44.7% and limbs – 38.5%, less often in the head and neck (Fig. 4), and inguinal and perineal-perianal areas (16.8%).

Figure 4. Mastocytoma of the neck area. Russian spaniel dog, age –10 years

Mastocytomas are located in the groin areas, especially those involving the foreskin or scrotum, and preputial mastocytomas can usually have more malignant biological behaviour.

During the studies, it was established that small neoplasms (up to 2.5-3.0 cm) were mainly located in the thickness of the skin. These neoplasms had the appearance of a node that was mobile, well delimited from the surrounding tissues, and mostly rounded or ellipsoid in shape. In some animals, at the initial stages of mastocytoma development, it manifested itself as a nodular seal in the thickness of the skin, the contours of which were almost not palpated and already based on this seal, the formation of a tumour node took place.

Based on anamnestic data and the results of the study, it was established that in seven examined animals, the rate of mastocytoma development was rapid – in two months, the tumours doubled their size. Within 8-9 weeks, small seals or nodules formed tumours with a diameter of 4-5 cm or more, indicating a substantial tumour growth aggressiveness. The growth of neoplasia was also rapid in four dogs. The time of doubling the size of the primary tumour reached an average of four months. Therewith, according to the medical history, it was established that in two dogs, the neoplasm development

period lasted about a year and a half. It is believed that one of the clinical prognostic factors in the assessment of mastocytoma is the rate of doubling of the tumour mass of neoplasia.

During the study of the local process and the results of monitoring the course of the disease, a variety in the clinical manifestation of mastocytes was noted. On palpation at the initial stage of development, the consistency of the tumours was soft, and over time they acquired a dense elastic consistency. The surface of the tumour went from smooth to bumpy. In 88% of cases, the characteristic signs of mastocytoma were swelling and inflammation. Inflammation was observed both in the tumour itself and in the surrounding tissues. Alopecia, erythema, the presence of skin ulcers, and pain on palpation, were visualised on the tumour. Notably, in addition to pain, mastocytes are characterised by itching of the skin in the tumour area, which is caused by the release of biologically active substances, in particular, histamine. Therefore, the animals constantly licked the tumour, tried to scratch and even gnaw, thereby provoking its growth. Constant irritation and trauma of the neoplasia led to the periodic exacerbation of the inflammatory process: increased oedema, ulceration, and, as a result, accelerated growth of tumours. It was established that the progressive course of the disease occurred due to the localisation of tumours in the groin, pelvic extremities, perineum, and neck.

The main indicators that indicate mastocyte malignancy are the size of neoplasms, their growth rate, the degree of germination in surrounding tissues, damage to regional lymph nodes and metastasis to internal organs (Beer *et al*., 2018). During the clinical examination of animals, the condition of the mandibular, axillary, superficial inguinal, popliteal, and other (regional) lymph nodes was considered.

According to the results of the initial examination of 13 dogs, six of them did not show any changes in the lymph nodes. They were almost not palpated, which indicates that there is no reaction to the pathological process. In five dogs, the regional lymph nodes were slightly enlarged, but did not ache, were mobile and not compacted. In two dogs with large tumours, the lymph nodes were enlarged, sedentary, and had dense consistency, which indicates the generalisation of the pathological process.

In accordance with the international clinical TNM classification of tumours, the scheme of examination of animals for oncological diseases provides for conducting a clinical study in combination with radiation diagnostics. In the available Ukrainian literature, it is not always possible to find information on the use of X-ray and ultrasonographic research methods, and their effectiveness in differential diagnosis of individual skin pathologies in dogs. Each test animal was subjected to X-ray and ultrasound examinations at the time of admission to the veterinary clinic and at certain intervals. This allowed tracing the dynamics of the pathological process and external changes in tumours. According to the results of X-ray imaging, three types of mastocyte growth were identified-limited nodular, infiltrative nodular, and diffuse infiltrative types.

Limited nodular growth of mastocytes was characterised by a homogeneous, heterogeneous radiological shadow. The tumour node was mostly oval, less often rounded in shape and was located in the thickness of the skin. Infiltrative nodular type of tumours was characterised by visualisation of the tumour shadow without clear contours, and structural changes were well expressed in the skin thickness and subcutaneous tissue. According to the diffuse infiltrative type of tumour growth, the X-ray shadow of neoplasms was compacted. Due to the swelling and thickening of the skin, it was almost impossible to establish clear boundaries of the neoplasm. In X-ray imaging, infiltrative nodular

and diffuse infiltrative types of neoplasia characterise the process of mastocyte malignancy.

During ultrasound examination, it was established that the sonographic structure of mastocytes depends on the location of the tumour, the degree of spread of the pathological process and the stage of the neoplastic process. Mostly mastocytomas localised in the thickness of the dermis were visualised as heterogeneous hypoechoic structures. These structures had uneven edges and indistinct contours. They showed signs of infiltration into the surrounding tissues, which may indicate a low ability of such tumours to form capsules. Increased peripheral vascularisation of mastocytoma and diffuse hypoechoic infiltration of surrounding tissues in such cases was a sign of inflammatory oedema. Due to sonographic studies of some mastocytes, which were characterised by prevailing inflammation, deposits of calcium salts in the thickness of the tumour were noted.

During the generalised process and the spread of mastocytoma to internal organs, the liver and spleen were primarily affected, which may indicate a preference for the hematogenic pathway of metastasis. Sonographically, such foci had similar structures and were visualised as heterogeneous dense formations with clear contours and uneven edges, with increased chaotic vascularisation.

The high variability of clinical symptoms in animals with the development of mastocytoma substantially complicates the determination of the diagnosis for a veterinarian. Therefore, determining the features of clinical changes and blood parameters in dogs with this pathology will contribute to the development of a complex of diagnostic markers.

One of the main steps in the diagnosis of mastocytes is cytological examination. Mastocytoma is characterised by heterogeneous behaviour. Highly differentiated tumours have a low metastasis potential, and the metastatic potential of low-differentiated tumours is high and amounts to up to 95% (Leguit *et al.,* 2020).

In most cases, diagnosing cutaneous mastocytoma by cytological or histological studies is not difficult, but forming an accurate prognosis is more complicated. Prognostic substantiality factors included classification (cytology and histopathology), stage (regional and distant metastases), breed, tumour location, and treatment (surgery, radiation, and chemotherapy). Cytological examination after fine needle aspiration is useful for making a diagnosis, but histopathology is necessary for evaluation. Cytological examination often helps in the diagnosis of mast cells due to the characteristic appearance of mast cells with regular staining. As with other species, Wright-Giemsa staining results in more intense granule staining in tumour mast cells. The mechanism of differences in staining is unclear. Several mast cell subtypes were identified in humans and dogs based on granule content and biological function. (Hosseini *et al.,* 2014).

In the case of the examined neoplasms, a typical cytological picture was observed. Round cells were noted against the background of a substantial number of red blood cells and eosinophils, which contained a large number of small metachromatic granules and a homogeneous nucleus in their cytoplasm, without pronounced atypia. In some cells, metachromatic granules completely covered the nucleus and made it invisible. Granules were established both in the cytoplasm and outside the cells (Fig. 5, 6).

The cells themselves were more often arranged in groups and had pronounced anisocariosis and anisocytosis. 2-3 figures of mitosis were identified on the glass. Basically, the degree of mastocytoma differentiation is determined during a histological examination, but given the individual mitosis patterns and a large number of granules, it can be assumed that all samples were highly differentiated.

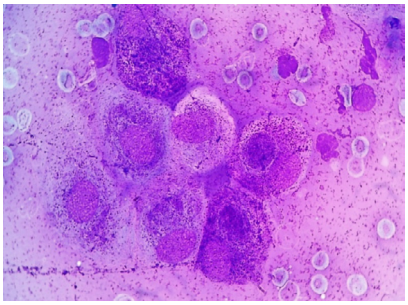

Figure 5. Highly differentiated mastocytoma. Pappenheim staining, ×100

Notes: *cells with small granules in the cytoplasm and a typical nucleus (indicated by arrows)*

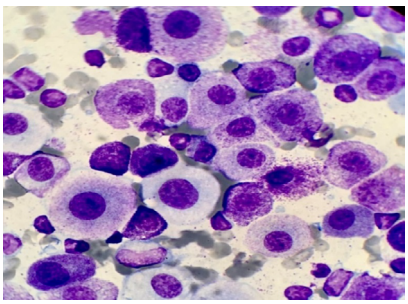

Figure 6. Highly differentiated mastocytoma. Pappenheim staining, ×100

Notes: *cells with small granules in the cytoplasm and a typical nucleus (indicated by arrows)*

According to the results of the cytological study, mastocytoma can be considered potentially aggressive, the cells of which contain few metachromatic granules and there are two or more of the following signs: the presence of multinucleated and mirror cells in the preparation; nuclear polymorphism in more than 50% of the population cells; a large number of mitoses.

Often, mastocytoma granules are not stained with Diff-Quik paint and require staining with Wright dye. During Diff-Quik cytology, if eosinophils are observed together with large round cells without granules, mastocytoma should be suspected and a different staining method should be used.

The main disadvantage of cytological examination is that it does not allow for determining the degree of cell differentiation, which is an important prognostic feature. Therefore, it is believed that in the diagnosis of mastocytes, the most accurate method is the use of histological examination.

A study (Hosseini *et al.*, 2014) clearly showed cellular infiltration of mast cells, mononuclears, and eosinophils in the thickened, hyperplastic, and hyperkeratinised epidermis. In contrast to delayed-hypersensitivity mononuclear cells, mast cells and eosinophils prevailed, indicating an immediate-type hypersensitivity reaction. Once activated, mast cells at the wound edge are known to release inflammatory mediators in the damaged tissue by degranulation. Thus, immediate hypersensitivity reactions may be responsible for the development of skin damage due to a tumour in dogs. However, the results show that intensive proliferation of mast cells after the tumour occurs mainly in the skin.

For single mastocytes, surgical excision of the tumour with a wide capture of healthy tissues is always recommended to radically remove it (Lisickaja & Sedov, 2011; Pardanani, 2016). Therewith, it is necessary to remove at least 3 cm of healthy tissue bordering the tumour. After its removal, a 0.5 × 1 cm piece of tissue is cut off from the edge of this place from 3-5 sides, placed in a 10% aqueous solution of formalin and sent for research. Histological examination of excisional mastocytoma should necessarily examine the edges of resection where it will be determined whether the tumour cells remain in the border tissue. If the result of histological examination is negative, then "clean edges" are indicated. During fine-needle aspiration of surgical excision and over-manipulation of the mastocytoma site, local bleeding often occurs, assumably due to coagulation defects caused by the release of heparin. Indeed,

there was an extended clotting time of blood taken from or around the tumour. Since bleeding can be a serious complication of surgery, doctors should anticipate local bleeding and try to avoid over-manipulating the tumour during a biopsy or during its removal.

Mastocytomas are very radiosensitive, and numerous studies show that fractionated radiation (daily or every other day for 15-18 procedures with a total dose of 46 to 54 Gray) is an effective adjunct therapy for incomplete edges after surgery) in almost 75-96% of dogs with topical treatment (no signs of re-growth 3-5 years after exposure) (Laura D Garrett, 2014). However, disagreements about what "incomplete" edges mean and how likely such dirty edges are to lead to tumour re-growth cast doubt on the need for radiation therapy in some cases. If the tumour is located in a place where only minimal resection can be obtained, then in practice the author suggests radiation therapy. Radiation therapy can also be very useful when used in weekly doses of large fractions for four to six procedures for treating bulky non-surgical mastocytes.

According to the conducted histological studies, a diverse picture was established in the samples. Cell morphology depends on the degree of differentiation, but most neoplastic cells have a clear cell membrane, a centrally located round nucleus, and a different number of cytoplasmic granules, which are coloured light grey when stained with hematoxylin and eosin, or purple with metachromatic spots when stained with toluidine blue. The connective tissue stroma varies from small to large and may look swollen or hyalinised.

However, most often indistinct boundaries of the neoplasm were determined. The surrounding area was diffusely infiltrated by tumour tissues under the skin, a small number of mitoses (no more than one in a large field of view) and massive destruction of the dermis. In some cases, the shape of the tumour cells was only round, while in others it was polygonal. The cytoplasm of cells is clearly defined and granular, in such cells, there is a separate nucleus located in the centre, with two nucleoli. Therewith, cells that had an inconspicuous cytoplasm with a dark small nucleus were observed. These cells were very similar to small lymphocytes. In the vast majority of cases, two cell types were established simultaneously in the same sample. The cells were arranged in groups, often forming nests, chains, and "paths", and were separated by bundles of collagen fibres.

On histological sections stained with hematoxylin and eosin, the nuclei of mast cells are very similar and resemble the nuclei of fibroblasts or pericytes, so special types of staining are required to establish the correct diagnosis.

The histological picture of all forms of mastocytosis is manifested by infiltrates consisting mainly of mast cells. A different number of eosinophils are either dispersed throughout the tumour mass or form aggregates. Metachromasia of mast cell granules was detected using toluidine, methylene blue, and naphthol-as-D-chloroacetate esterase, which turns the cytoplasmic granules of mast cells red.

According to the gradation of mastocytes according to Patnaik (1984), according to the degree of differentiation, all neoplasms can be attributed to the second degree. According to the Kiupel classification (2011), all neoplasms corresponded to mastocytomas with a high degree of differentiation.

A study (Hosseini *et al.,* 2014) showed cytohistopathological features of cutaneous mastocytes in dogs in the skin area. Histological features of these tumours indicate that most of them should be classified as mastocytomas with a high degree of differentiation. The use of a classification system by tumour type can be useful for differentiating this type of neoplasm from other malignancies with similar morphology. In addition, the study demonstrated that

skin mastocytomas in dogs can be used as a sensitive prognostic indicator of tumour behaviour after surgery, which is associated with other histopathological variables that are widely recognised as having prognostic value.

Conclusions

Among the dogs admitted to the veterinary clinic "Animal health" (Kyiv region, u.t.s. Hlevakha) with surgical diseases, the proportion of neoplasia was 12.5%. According to the structure of oncological diseases, skin tumour lesions in dogs accounted for 32.16%. As a result, mastocytoma was diagnosed in 13 dogs aged 3 to 14 years. The highest incidence rates were established in dogs aged 7 to 12 years. Notably, in 92.7% of dogs, mastocytomas were isolated and only in 7.3% of cases they were multiple.

The most common places of localisation of neoplasia are the trunk, limbs, less often the head and neck, and groin area. The behaviour of mastocytes is very diverse and depends on the degree of cell differentiation. Low-differentiated tumours have a high potential for metastasis and often recur. A high frequency of mastocyte recurrence after excision is noted with insufficient capture of healthy tissues, and with tumours of a low degree of differentiation. Based on the results of a comparative analysis of the clinical stages and histology of cutaneous mastocytosis, a direct correlation was established between the size of the tumour and the degree of its malignancy. A more malignant course of the disease was characterised by mastocytomas in dogs that were localised in areas of the body accessible for permanent injury (limbs, groin area, neck). According to the pathomorphological assessment of mastocytoma, it is necessary to consider the clinical stage of neoplasia to determine diagnostic accuracy.

During X-ray imaging, the infiltrative nodular and diffuse infiltrative types of mastocytes characterise the process of their malignancy. According to the gradation of mastocytes by the degree of differentiation, all the examined neoplasms can be attributed to the second degree. However, according to another classification, all neoplasms corresponded to mastocytomas of a high degree of differentiation. Prospects for further research are to examine the prognostic factors for the biological behaviour of cutaneous mastocytoma in dogs during surgery.

Acknowledgements

None.

Conflict of Interest

None.

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Клініко-морфологічні особливості діагностики мастоцитом у собак

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Анотація. Мастоцитома є однією з найпоширеніших пухлин шкіри в собак. Вона характеризується специфічним перебігом, непередбачуваною поведінкою та прогнозом. Ця пухлина небезпечна тим, що імітує зовнішні ознаки інших новоутворень, від менш загрозливої ліпоми до раку шкіри. Мета досліджень – провести патоморфологічну верифікацію та класифікацію неоплазій у собак, які діагностовано під час клінічного обстеження, а також встановити особливості морфологічного прояву в них шкірної форми мастоцитоми. Матеріал для досліджень отримували методом ексцизійної біопсії під час хірургічних втручань з приводу новоутворення шкіри. Діагноз на мастоцитому встановлювали за результатами клінічного обстеження тварин і дослідження біопсійного матеріалу новоутворень за допомогою цитологічних і гістологічних методів дослідження. Встановлено, що частка неоплазій шкіри у структурі онкологічних захворювань собак становила – 30,12%. Серед тварин із неоплазіями шкіри, які проходили обстеження, мастоцитома була діагностована у 17,55% собак, віком від 3 до 14 років. Найвищі показники захворюваності встановлено у собак віком від 7 до 12 років. Найчастіше шкірну мастоцитому діагностували у собак породи: шарпей – 13,5%; лабрадор – 9,5%; боксер – 8,2%. Поодинокі випадки пухлинних утворень спостерігали у тварин таких порід, як: німецька вівчарка, спаніель, середньоазіатська вівчарка та мопс. Залежно від статі: кобелі становили – 52,2%, суки – 47,8%. У 50% досліджуваних тварин мастоцитоми розвивалися з стрімкою динамікою, що свідчить про значну агресивність пухлинного росту. Клінічно у собак визначали ознаки дегідратації та розлади зі сторони шлунково-кишкового тракту, що вказує на розвиток паранеопластичного синдрому. За результатами сонографії встановлено, що мастоцитоми шкіри візуалізуються як неоднорідні гіпоехогенні структури

з нечіткими контурами і нерівними краями. За результатами дослідження отримані нові відомості щодо морфологічних особливостей прояву та розвитку мастоцитом у собак. Таким чином, одержані результати доповнюють та розширюють знання щодо патогенезу мастоцитоми в собак, частоти поширення та особливості перебігу цієї онкологічної патології в окремій географічній популяції України

Ключові слова: цитологічне дослідження; гістологічне дослідження; пухлина; шкіра; метастаз