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Research article

SEVEN-YEAR FOLLOW-UP OF TUMORS IN YOUNG DOGS IN THE REPUBLIC OF SERBIA

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Although the incidence of neoplasms increases with age, some tumors occur more commonly in younger dogs. Still, little information is available on the occurrence of neoplasms in dogs up to the age of 24 months. This study is a retrospective review of histopathological diagnoses of neoplasia at our laboratory during the last seven years (2017-2023) in two groups of dogs: group up to the age of 12 months and group from 13 to 24 months. In a total of 3704 neoplasms found in different dog tissues, 74 (2.00%) were identified in dogs up to 12 months and 135 (3.64%) in dogs aged 13 to 24 months. Canine cutaneous histiocytoma was the most frequent in both groups:41 cases (54.41%) in dogs up to 12 months, followed by 39 cases (28.89%) in those up to 24 months. In the present analysis of the group up to 12 months, 82.43% of tumors (n = 61) were benign, and 17.57% (n=13) were malignant, followed by 68.15% (n=92) benign neoplasms, and 31.85% (n=43) malignant in dogs aged from 13 to 24 months. French bulldogs and mixed-breed dogs were the most frequently affected. These data provide valuable epidemiological information on neoplasms in young dogs in the Republic of Serbia.

Keywords: young dogs, tumors, WHO classification, breed distribution

INTRODUCTION

Cancer is recognized in many studies as the leading cause of death in adult dogs [1]. Canine tumors have been increasing in our biopsy material in the past decade. Although neoplasms are mainly diseases of older dogs and peak approximately at the age of nine years [2], tumors can be diagnosed in all age categories. Besides, some authors have reported malignant tumors in only a few weeks-old puppies [3,4].

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However, little epidemiological information is available on different types of tumors in dogs aged up to 24 months, and data suggest that they occur in a low percentage. Thus, some authors [5] diagnosed tumors in dogs younger than 24 months in only 0.4%, even though it is well known that some neoplasms, such as histiocytoma and papillomas, occur more often in younger dogs [6]. According to different studies, approximately one-half of canine cutaneous histiocytomas (CCHs) occur under the age of 24 months [7,8] followed by another common tumor type in younger dogs, virally induced papillomas, which mostly appear as exophytic proliferation of squamous epithelium associated with CPV-2, CPV-6, and CPV-7 (Family *Papillomaviridae*). Although both leading tumors resolve spontaneously, the presence of papillomaviruses may later play an important role in canine squamous cell carcinoma development [9]. Over a nine-year period, Rigas et al. [10] found 16 cases of mastocytomas in dogs less than 12 months old, out of which 13 were cutaneous and three were subcutaneous mastocytomas. Similar to these results, cutaneous mastocytosis can also be diagnosed in dogs younger than 17 months [11].

Some authors revealed tumors of the hematopoietic system, brain and skin to be most common in post-mortem examinations of dogs up to six months old [12]. However, another study on biopsy samples derived from dogs up to 12 months of age in Germany revealed that mammary tumors and soft tissue sarcomas were the most common tumor types when CCHs and papillomas were excluded [13].

Considering the limited available data on canine pediatric oncology, the current study aims to investigate the presence and distribution of tumors in young dogs up to 24 months, submitted for evaluation in the past seven years. We would determine the absolute and relative frequency of each type of tumor, as well as the mean age, gender and breed distribution.

MATERIAL AND METHODS

The examined tissue samples were obtained from January 2017 to December 2023 by surgical biopsy in different veterinary clinics in Serbia. Tissue biopsies of tumor lesions from 209 dogs up to 24 months old were submitted to the Histopathology Laboratory of the Department of Pathology at the Faculty of Veterinary Medicine, University of Belgrade.

Collected tissue samples were immediately placed in 10% buffered formalin. After being delivered to the laboratory, they were processed in an automatic tissue processor, paraffin-embedded, and cut into 3-5 μm thick sections. The sections were stained with routine hematoxylin and eosin method, and in the case of suspicion of mast cell tumors, they were aditionally stained with the toluidine blue method. In some cases, immunohistochemistry was also used. Tumor diagnosis and classification were performed according to the World Health Organization [14,15] and Goldschmidt et al. (2018) [16] criteria.

All tumor samples originating from dogs younger than 24 months were divided into two groups: up to the age of 12 months and from 13 to 24 months. The obtained results were statistically analyzed using descriptive statistical parameters (arithmetic mean, minimum and maximum value). Samples without data for the selected parameter were not included in the calculation. The obtained results were statistically analyzed using Microsoft Excel 2016 software.

RESULTS

From 2017 to 2023, a total of 3704 tumor samples from dogs were examined, of which 209 (5.64%) were from dogs younger than two years. The trend in the number of tumors in young dogs over the years is shown in Table 1 and Figure 1.

Table 1. Distribution of tumors in dogs younger than 24 months over seven years

Year	2017.	2018.	2019.	2020.	2021.	2022.	2023.	Total
Total number of tumors in dogs	395	338	523	493	557	770	628	3704
Prevalence (%) of tumors in dogs up to 24 months	13 (3.29%)	35 (10.36%)	44 (8.41%)	24 (4.87%)	35 (6.28%)	29 (3.77%)	29 (4.62%)	209 (5.64%)

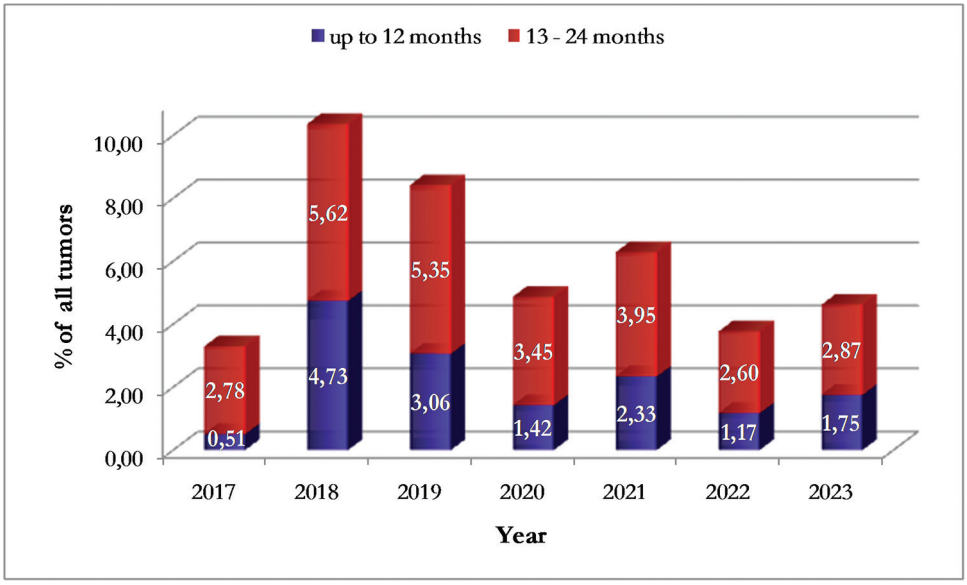

Figure 1. Percentage of tumors in dogs up to 12 months (blue) and from 13 to 24 months (red) of the total number of tumors

The average age of dogs with tumors in the group of dogs aged up to 2 years was 17.7 months. Tumors in young dogs were most often observed in males (116 samples, 55.50%), while in females, they were diagnosed in 88 (42.10%) cases. In 5 cases (2.39%), data on gender is missing. Tumors in dogs up to two years of age were diagnosed in 55 different breeds, most often in French bulldogs (33 cases, 15.79%), then mixed breeds (31 cases, 14.83%), American Stafford terriers (13 cases, 6.22%), Cane Corso (9 cases, 4.31%), Bichons (8 cases, 3.83%), Dobermans (8 cases, 3.83%) and Jack Russell Terriers (8 cases, 3.83%). The youngest dogs with tumors were only two months old – a female bichon with subcutaneous mastocytoma and a male French bulldog with papilloma. However, fibrosarcoma was diagnosed in a female Weimaraner who was only 4 months old. From the total number of submitted samples, the breed was unknown for four (1.91%) dogs.

Of all diagnosed tumors in dogs younger than two years, 153 (73.21%) were benign, and 56 (26.79%) were malignant. The most commonly diagnosed benign tumors were cutaneous histiocytomas (79 cases, 37.80%), papillomas (40 cases, 19.14%) and lipomas (7 cases, 3.35%), while the most frequently observed malignant tumors were lymphomas (7 cases, 3.35%), adenocarcinomas of the sebaceous gland (5 cases, 2.39%) and fibrosarcomas (5 cases, 2.39%).

Cutaneous histiocytomas were most often diagnosed in French Bulldogs (19 cases or 23.75% of all diagnosed cutaneous histiocytomas in dogs younger than two years), followed by mixed breeds (12 cases, 15.00%), Cane Corso (6 cases, 7.50%), American Stafford Terriers (4 cases, 5.00%), Labrador retrievers, and Pit Bull terriers (3 cases each, 3.75%).

Papillomas were most often diagnosed in French bulldogs (7 cases or 17.07% of all diagnosed papillomas in dogs under two years), followed by mixed breeds (6 cases or 14.63%), American Stafford Terriers, Bichons, and Jack Russell Terriers with 3 cases each (7.32%).

Mast cell tumors were diagnosed in mixed breeds (2 cases, 25% of all diagnosed mast cell tumors in dogs under two years), followed by single cases (12.5%) in American Stafford Terrier, Bichon, Cane Corso, Minsterlander, Pug and Golden Retriever.

Tumors in dogs up to the age of 12 months

Out of a total of 209 diagnosed tumors in dogs aged up to 2 years, 74 (2.00% of all diagnosed tumors in dogs) were in dogs up to one year old. Of these, 61 (82.43%) were benign, and only one-fifth (13 cases, 17.57%) were malignant. The prevalence of specific tumors is shown in Table 2.

Considering the breed of dogs with tumors in the group up to the age of year, tumors were most often diagnosed in French bulldogs (14 cases, 18.29%), mixed breeds (9 cases, 12.16%), American Staford terriers (6 cases, 8.11%), Dobermans (4 cases, 5.41%), Bichons (3 cases, 4.05%), and Jack Russell Terriers (3 cases, 4.05%). Of the

total number of tumors observed in dogs under one year of age, two-thirds were diagnosed in males (47 cases, 63.51%), while 26 cases (35.14%) were diagnosed in females. In one case (1.35%), there was no data on gender.

The average age of dogs with cutaneous histiocytomas as the most common type of diagnosed tumor in this group was 9.78 months.

Table 2. Tumor prevalence in dogs up to the age of 12 months

Tumor type	Number	%
Cutaneous histiocytoma	41	54.41
Papilloma	13	17.57
Sebaceous adenocarcinoma	2	2.70
Basosquamous carcinoma	2	2.70
Rectal adenocarcinoma	1	1.35
Renal adenocarcinoma	1	1.35
Mammary mix adenoma	1	1.35
Rectal adenoma	1	1.35
Anaplastic carcinoma	1	1.35
Fibroma	1	1.35
Fibrosarcoma	1	1.35
Haemangioma cavernosum	1	1.35
Haemangiosarcoma	1	1.35
Lipoma	1	1.35
Liposarcoma	1	1.35
Subcutaneous mastocytoma	1	1.35
Melanoacanthoma	1	1.35
Gigantocellular osteosarcoma	1	1.35
Synovial sarcoma	1	1.35
Trichoepithelioma	1	1.35

Tumors in dogs from 13 to 24 months of age

Out of 209 tumors in young dogs analyzed in this study, 135 samples were from dogs aged one to two years (3.64% of all diagnosed tumors in dogs). In this age category, an increase in the number of malignant tumors was observed compared to the group with younger dogs. They were diagnosed in one-third of cases (43 cases, 31.85%). Benign tumors were dominantly present in this age group as well (92 cases, 68.15%) (Table 3).

Table 3. Tumor prevalence in dogs from 13 to 24 months old

Tumor type	Number	%
Cutaneous histiocytoma	39	28.89
Papilloma	28	20.74
Lymphoma	7	5.19
Lipoma	6	4.44
Mammary adenoma	5	3.70
Planocellular carcinoma	4	2.96
Fibrosarcoma	4	2.96
Mastocytoma gr. I	4	2.96
Mammary adenocarcinoma	3	2.22
Sebaceous adenocarcinoma	3	2.22
Ameloblastoma	2	1.48
Fibromatous epulis	2	1.48
Fibromyxoma	2	1.48
Subcutaneous mastocytoma	2	1.48
Malignant melanoma	2	1.48
Rectal adenocarcinoma	2	1.48
Perianal adenoma	1	0.74
Papillary ovarian adenoma	1	0.74
Anaplastic carcinoma	1	0.74
Basosquamous carcinoma	1	0.74
Hepatocellular carcinoma	1	0.74
Sebaceous epithelioma	1	0.74
Ossificant epulis	1	0.74
Fibroma	1	0.74
Haemangioendothelioma	1	0.74
Haemangiosarcoma	1	0.74
Histiocytoma malignum	1	0.74
Leiomyoma	1	0.74
Malignant fibrous histiocytoma	1	0.74
Mastocytoma gr. II	1	0.74
Chondroblastic osteosarcoma	1	0.74
PNST/Schwannoma	1	0.74
Rhabdomyosarcoma	1	0.74
Trichoblastoma	1	0.74
Trichoepithelioma malignum	1	0.74
TVT	1	0.74

Tumors in dogs aged from 1 to 2 years were most often diagnosed in mixed breeds (22 cases, 16.30%), French bulldogs (18 cases, 13.33%), American Stafford terriers (7 cases, 5.19%), Cane Corso (6 cases, 4.44%) and German shepherds (6 cases, 4.44%). Tumors in this group of dogs were represented in approximately equal numbers in males (69 cases, 51.11%) and females (62 cases, 45.93%). In 4 cases (2.96%), there was no data on gender.

The average age of dogs with cutaneous histiocytomas in this group was 21.13 months.

DISCUSSION

Tumors are the most common finding in biopsy samples of canine tissue that have been histopathologically examined in the past decade at the Department of Pathology, Faculty of Veterinary Medicine, University of Belgrade. According to our previous study, skin tumors were the most frequently present, making up 81.58% of all evaluated cutaneous lesions at our laboratory [17]. The average age of dogs with cutaneous tumors in that study was 7.32 years, but in the current study, we have found skin tumors in puppies aged only 8 weeks. The well-known fact is that approximately 50% of canine cutaneous histiocytomas occur in dogs under the age of 2 years [7]. Similar to this data, CCHs, were the most frequently diagnosed tumors in our previously examined biopsy specimens which frequently occurred at an average age of 2.32 years (from 6 months to 13 years), with the French Bulldog being the most commonly affected breed [17]. However, in the current study, the mean age of dogs with CCHs in the first group was 9.78 months, and in the second group was 21.13 months. This tumor was followed by papillomas in both of the investigated groups of young dogs, and it was also found in French bulldog puppie aged only 8 weeks.

In contrast, the average age of dogs with other epithelial tumors observed in our previous study, such as apocrine adenoma, was 12 years, and the average age of dogs with malignant trichoepithelioma was 11 years [17], suggesting the potential role of aging in skin tumor development. In addition, the high incidence of skin tumors could be related to prolonged exposure to various environmental insults.

The two most frequently found tumors in the current study, CCHs and papillomas, usually resolve spontaneously as a result of a cell-mediated immune response. In regressing papillomas, we often obtained a lymphocytic infiltrate within the epidermis, which was also described by other authors [18]. However, some authors [9] have found canine papillomavirus DNA in canine oral squamous cell carcinomas in dogs with a known history of oral papillomatosis. An increase in lymphocyte infiltration could be correlated with spontaneous regression of transmissible venereal sarcoma [19], as well.

Regarding cutaneous mast cell tumors (CMCT,s), the mean age of the dogs with CMCT in our previous study was 7.56±2.45 years, with Golden Retrievers being most frequently affected [20]. In that five-year study (before 2013), no mast cell tumors were observed in dogs up to the age of two years. Regardless of the age of the dogs diagnosed with mast cell tumors, no c-kit mutations were detected in any of the

examined samples using molecular methods. Although mast cell tumors (MCT) have been reported in dogs of any age, some authors found that predilection for young Shar Pei may exist [21]. In our current study, MCTs were primarily present in mixed breeds of young dogs, followed by individual cases in American Stafford Terrier, Bichon, Cane Corso, Minsterlander, Pug (mops) and Golden Retriever. In our study, most of the diagnosed MCTs in dogs up to 24 months of age in our study were graded (using propositions of both Patnaik [22] and Kiupel grading systems) as CMCT grade I, indicating a good prognosis and behavior.

The tumors can be found in all age categories, but the incidence has been reported the most often in adult dogs. Authors in a recent comprehensive study have reported 8.5 years as the mean age of cancer diagnosis based on 3452 cases of dogs with cancer, ranging from <1–20 years [2]. The current seven-year study (2017-2023) reveals the presence of 2.00% of all diagnosed tumors in the I group of dogs up to the age of 12 months. In this group, the majority of the tumors were benign (82.43%) and close to one-fifth (17.57%) were malignant. In tumor samples originating from the II group of dogs aged between 13 and 24 months we found an increasing number of malignant tumors (31.85%) compared to benign tumors, which were still dominant (68.15%). It can be assumed that the immaturity of the immune system could play a role in the development of tumors in puppies as well.

Regarding the breeds in both investigated age categories of young dogs, tumors were most often diagnosed in French bulldogs (18.29% of dogs up to 12 months and 13,33% of dogs aged 13 to 24 months) and in mixed breeds (12.16% of dogs up to 12 months and 16.30% of dogs aged 13 to 24 months). However, larger sample size studies in young dogs are necessary to obtain knowledge regarding the breed disposition of individual tumor types.

In both of the analysed groups, the gender distribution of tumors was higher in young male dogs (55.50%) than in females (42.10%). Although we have recorded eight mammary tumors in female dogs aged 13 to 24 months and only one mix adenoma was in the group up to 12 months, more data on hormonal status need to be considered in future studies for a better understanding of that issue.

Although genetic damage is a universal component of the pathogenesis of neoplasia, neoplastic disease progression results from the relation between genetic alterations of transformed cells and cancer immunoediting by the host's immune defense mechanisms [23].

Multiple human and canine studies have proven to date that the dysfunction of the immune system enables tumor growth and metastasis [24]. which also could be related to tumor development in both age groups that contributed data to the current study.

Our results provide updated information on the type, frequency, sex and breed distributions of the tumors in two investigated groups of young dogs: I group up to the age of 12 months and II group from 13 to 24 months, both evaluated in the past seven years at Department for Pathology, Faculty of Veterinary Medicine, University of Belgrade.

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Authors' contributions

VK and SAK conceived and designed the study and made substantial contributions to writing and revising the manuscript. VK, SG, and IV were responsible for data collection, performed statistical analysis, and made significant contributions to data interpretation. All authors read the manuscript, made appropriate suggestions, and approved the final version.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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PRAĆENJE TUMORA KOD MLADIH PASA U REPUBLICI SRBIJI U PERIODU OD SEDAM GODINA

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Iako se učestalost neoplazmi povećava sa godinama, neki tumori se češće javljaju kod mlađih pasa. Ipak, malo je informacija o pojavi neoplazmi kod pasa do 24 meseca starosti. Ova studija predstavlja retrospektivni pregled histopatoloških dijagnoza tumora u našoj laboratoriji tokom poslednjih sedam godina (2017-2023) kod dve grupe pasa: grupe do 12 meseci starosti i grupe od 13 do 24 meseca starosti. Od ukupno 3704 neoplazme dijagnostikovane u različitim tkivima pasa, 74 (2,00%) su identifikovane kod pasa do 12 meseci i 135 (3,64%) kod pasa starosti od 13 do 24 meseca. Histiocitom kože kod pasa bio je najčešći tumor u obe grupe: 41 slučaj (54,41%) kod pasa do 12 meseci, odnosno 39 slučajeva (28,89%) kod pasa do 24 meseca. U prikazanoj analizi u grupi do 12 meseci starosti benigni tumori bili su zastupljeni u 82,43% (n=61) slučajeva, a kod 17,57% (n=13) pasa ustanovljeni su maligni tumori. U grupi pasa starosti od 13 do 24 meseca 68,15% (n=92) su bile benigni tumori i 31,85% (n=43) maligni tumori. Od svih rasa, tumori su najčešće dijagnostikovani kod francuskog buldoga i mešanaca. Ovi podaci daju dragocene epidemiološke podatke o neoplazmi kod mladih pasa u Republici Srbiji.