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PATHOHISTOLOGICAL CHARACTERISTICS OF SPONTANEOUS MAMMARY TUMORS IN THE BITCH IN BULGARIA

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ABSTRACT

The pathohistological characteristics of 264 spontaneous mammary tumors in the bitch were examined and analyzed at the Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University – Stara Zagora. The neoplastic formations were fixed in a 10% solution of neutral buffered formalin and processed according to the classical histological technique. We classified the tumors according to the histological classification for canine mammary tumors published by Goldschmidt *et al.* (2011). 5 types of benign and 11 types of malignant tumors were diagnosed. Mixed mammary gland tumors (14.39%) and fibroadenomas (8.71%) predominated among the benign ones, and tubulopapillary carcinoma (22.73%) and solid carcinoma (11.74%) prevailed among the malignant ones. Based on the conducted research, we can conclude that mammary gland tumors are an important problem in the pathology of dogs. We believe that tracking their pathohistological characteristics will contribute to modern diagnostics and therapy.

Key words: mammary gland tumors in the bitch, pathohistological studies.

Introduction

Canine mammary tumors represent a heterogeneous group of neoplastic diseases (Soler *et al.*, 2016; Nakagaki *et al.*, 2019; Varallo *et al.*, 2019; Devarathnam *et al.*, 2021; Valdivia *et al.*, 2021; Tancoš *et al.*, 2023; Vazquez *et al.*, 2023) and are among the most common oncological diseases in pets (Nemceva, 2021; Varfolameeva and Khankhasykov, 2017). They are the most common neoplasia in unneutered female dogs (Salas *et al.*, 2015; Fish, 2019).

The main risk factors leading to the development of this type of pathology are advanced age, breed, hormonal imbalance, obesity, environmental influences (Pérez Alenza *et al.*, 1998; Torres *et al.*, 2021; Nemceva, 2021). Mammary tumors are thought to occur most frequently in bitches aged 5 years and older (Raskin and Meyer, 2001), with the average age of affected animals being between 8 and 11 years (Sorenmo *et al.*, 2011). An increased incidence of mammary tumors has been found in purebred dogs compared to mixed breeds (Dorn *et al.*, 1968; Zatloukal *et al.*, 2005; Salas *et al.*, 2015; Burrai *et al.*, 2020). Epithelial neoplasms predominate (about 95%) (Brodey *et al.*, 1983) and an increase in the number of malignant tumors has been registered in recent years (Nunes *et al.*, 2018; Srisawat *et al.*, 2024). Approximately 50% of carcinomas metastasize to regional lymph nodes, and distant metastases are most often to the lungs (Gray *et al.*, 2020).

The most common form of manifestation of mammary gland tumors in bitches is nodular. It is estimated that 70% of neoplasms are multinodular and only 30% of them are single nodules. The nodular form is characterized by the presence of one or several tumor nodes (Yakunina, 2011), and the abdominal and inguinal glands are most often affected (Nguyen *et al.*, 2018; Nunes *et al.*, 2019; Dinev *et al.*, 2002).

The aim of the present study was to investigate the pathohistological characteristics of spontaneous mammary tumors in bitches. After 2014 in Bulgaria, there is not enough information about the histogenesis of this type of neoplasia (Simeonov, 2014).

Materials and Methods

We conducted the research on surgically removed tumors of the mammary gland in dogs provided by the University Veterinary Hospital, Trakia University – Stara Zagora, as well as by private veterinary clinics from all over Bulgaria. The studies were conducted in the period 2022–2025, examining a total of two hundred and sixty-four spontaneous mammary gland tumors. The pathohistological studies were carried out at the Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University – Stara Zagora. Neoplastic formations were fixed in a 10% solution of neutral buffered formalin and processed according to the classical histological technique. Routine histological staining with hematoxylin-eosin was performed (Dzhurov *et al.*, 1989; Dyakov *et al.*, 1989). Tumors were classified according to the histological classification for canine mammary tumors published by Goldschmidt *et al.* (2011) (Table 1).

Table 1: Histological classification of canine mammary gland tumors (Goldschmidt *et al.* 2011)

1. Hyperplasia/dysplasia
Duct ectasia
Lobular hyperplasia (adenosis)
Epitheliosis
Papillomatosis
Fibroadenomatous changes
Gynecomastia
2. Benign neoplasms
Adenoma – simple
Intraductal papillary adenoma
Ductal adenoma (Ductal adenoma with squamous differentiation)
Fibroadenoma
Myoepithelioma
Complex adenoma
Benign mixed tumor
3. Malignant epithelial neoplasms
Carcinoma – in situ
Carcinoma – simple
a. tubular; b. tubulopapillary; c. cystic-papillary; d. cribriform
Carcinoma – micropapillary
Carcinoma – solid
Comedocarcinoma
Carcinoma – anaplastic
Carcinoma arising in a complex adenoma/mixed tumor
Carcinoma – complex type
Carcinoma and malignant myoepithelioma
Carcinoma – mixed type
Ductal carcinoma
Intraductal papillary carcinoma
4. Malignant epithelial neoplasms
Squamous cell carcinoma
Adenosquamous carcinoma

Mucinous carcinoma
Lipid-rich carcinoma
Spindle cell carcinoma
Inflammatory carcinoma
5. Malignant mesenchymal neoplasms – sarcomas
Osteosarcoma
Chondrosarcoma
Fibrosarcoma
Haemangiosarcoma
Other sarcomas
6. Carcinosarcoma – malignant mixed mammary tumor
7. Neoplasms of the nipple
Adenoma
Carcinoma
Carcinoma with epidermal infiltration
8. Hyperplasia/dysplasia of the nipple

Results

The study covered 264 mammary neoplastic formations, 262 of which were found in female dogs, and 2 of the cases involved male dogs. The age of the affected animals ranged from 3 to 17 years. The largest number of neoplastic formations were diagnosed in dogs aged 7–10 years (125 animals). Twenty-three of the studied animals were castrated.

Pathohistologically, 5 types of benign and 11 types of malignant tumors were diagnosed, as well as precarcinomatous changes in a male dog. Benign neoplasms were represented by benign mixed tumor (14.39%), fibroadenoma (8.71%), adenoma – simple (5.68%), ductal adenoma (3.4%), complex adenoma (2.65%). Malignant included tubulopapillary carcinoma (22.73%), solid carcinoma (11.74%), tubular carcinoma (7.95%), complex carcinoma (6.82%), malignant mixed tumor/carcinosarcoma (6.44%), spindle cell carcinoma (3.03%), squamous cell carcinoma (1.89%), comedocarcinoma (1.14%), lipid-rich carcinoma (1.14%), fibrosarcoma (1.14%), anaplastic carcinoma (0.76%) (Fig. 1). Out of the total number of mammary tumors 78.03% were epithelial, 1.14% were mesenchymal and 20.83% were mixed.

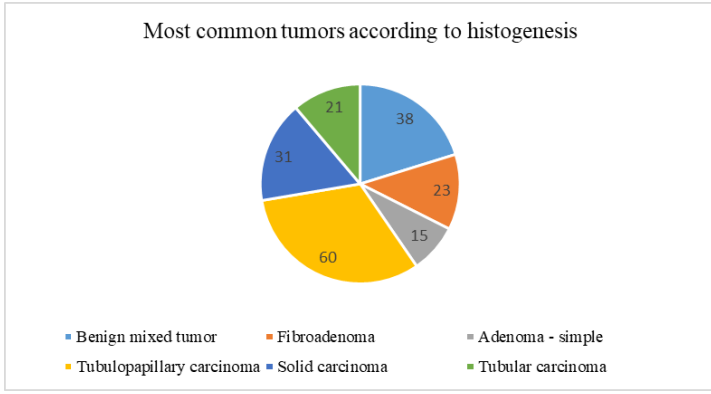

Figure 1: Most common tumors according to histogenesis.

Pathohistological characteristics of individual tumor types:

Benign tumors

Benign mixed tumor

We found a benign mixed tumor in 38 female dogs (14.39%). The most commonly affected breeds were Yorkshire Terrier, Crossbreeds and Pinscher. Macroscopically, the mixed benign tumors in most cases were single, well circumscribed, encapsulated, with a firm consistency and granular structure. Pathohistologically, their parenchyma consisted of several types of cell populations-well-differentiated epithelial cells admixed with myoepithelial cells and mesenchymal cells producing cartilage and/or bone tissue (Fig. 2). The ratio between the epithelial and mesenchymal components varied. The stroma was represented by well-differentiated connective tissue. Nuclei were round to oval in shape, anisocytosis and anisokaryosis were minimal.

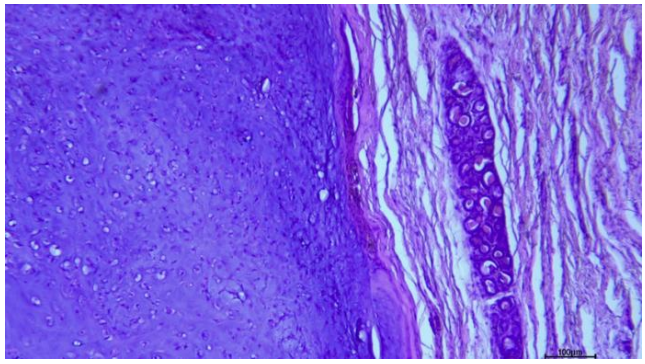

Figure 2: Pathohistological finding in benign mixed tumor. The tumor parenchyma consisted of well-differentiated epithelial cells admixed with myoepithelial cells and mesenchymal cells producing cartilage tissue. H/E staining, bar=100µm.

Fibroadenoma

Fibroadenoma was observed in 23 cases (8.71%). The most commonly affected breeds were Yorkshire Terrier, Crossbreeds and Siberian Husky. Macroscopically, the fibroadenomas included in the study were single, solid, well-demarcated masses. Pathohistologically, the parenchyma of the neoplastic formations was composed of glandular tubules surrounded by extensive connective tissue stroma (Fig. 3).

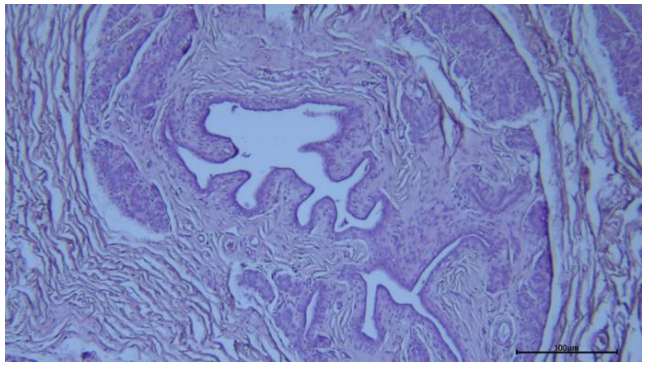

Figure 3: Pathohistological finding in fibroadenoma. The tumor parenchyma consisted of glandular tubules surrounded by extensive connective tissue stroma. H/E staining, bar=100µm.

Adenoma – simple

We identified simple adenoma in 15 dogs (5.68%), the majority of which were of the Yorkshire Terrier, Pomeranian, Samoyed and Golden Retriever breeds. Macroscopically, this type of tumors were solid, single, circumscribed masses. Pathohistologically, the parenchyma consisted of fully differentiated epithelial cells forming tubular structures. Cell nuclei were round to oval in shape, centrally located. Anisocytosis and anisokaryosis were minimal. The stroma was made up of sparse connective tissue and blood vessels.

Ductal adenoma

Ductal adenoma was observed in 9 animals (3.4%). The breeds suffering from this type of neoplasia were Cocker Spaniel, Crossbreeds, German Spitz, Border Collie. Macroscopically, the ductal adenomas were single, solid masses. Pathohistologically, they were composed of neoplastic epithelial cells located in the lumen of the tubules and a supporting fibrovascular stroma. The cells were usually arranged in two rows, basal and luminal (Fig. 4). Nuclei were round to oval in shape, centrally located.

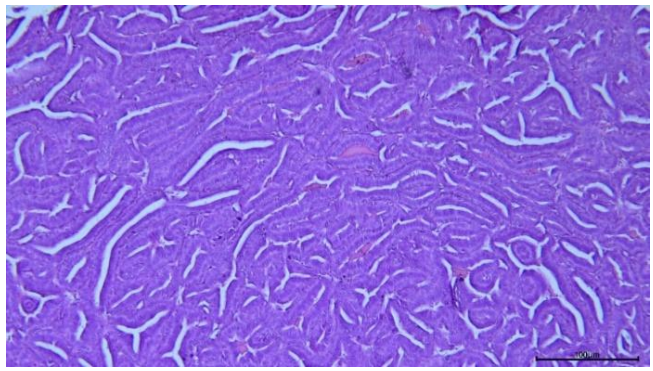

Figure 4: Pathohistological finding in ductal adenoma. The cells were usually arranged in two rows, basal and luminal. H/E staining, bar=100µm.

Complex adenoma

Complex adenoma was found in 7 of the cases (2.65%). The breed distribution was as follows: Yorkshire Terrier, Pinscher, Bulgarian Shepherd Dog, Pomeranian and Jack Russell Terrier. Macroscopically, the complex adenomas were single, solid masses. Pathohistologically, they consisted of neoplastic epithelial cells, myoepithelial cells and myxoid substance. Nuclear and cellular pleomorphism was minimal.

Malignant tumors

Tubulopapillary carcinoma

Tubulopapillary carcinoma was found in 60 dogs (22.73%). The most commonly affected breeds were Yorkshire Terrier, Crossbreeds, Pinscher, Cocker Spaniel, Jack Russell Terrier, Pomeranian, Golden Retriever. Macroscopically, the tubulopapillary carcinomas in most cases were multinodular formations with a dense consistency, mainly affecting the abdominal and inguinal gland complexes. The largest formation reached 15 cm. Pathohistologically, the parenchyma consisted of neoplastic epithelial cells that formed tubulopapillary structures surrounded by a fine connective tissue stroma (Fig. 5). The tumor cells showed clear histological signs of malignancy.

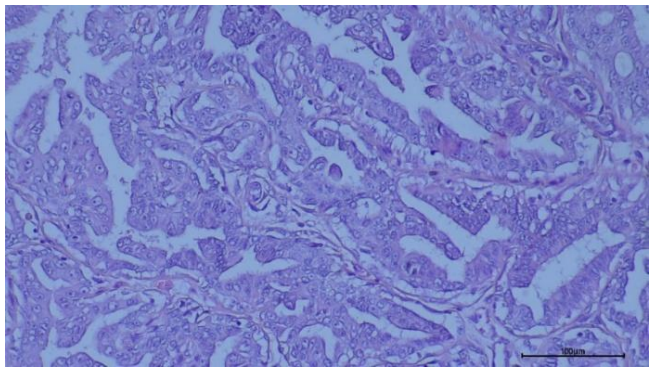

Figure 5: Pathohistological finding in tubulopapillary carcinoma. The tumor parenchyma consisted of neoplastic epithelial cells that formed tubulopapillary structures surrounded by a fine connective tissue stroma. H/E staining, bar=100µm.

Solid carcinoma

Solid carcinoma was found in 31 dogs (11.74%). The most commonly affected breeds were Yorkshire Terrier, Crossbreeds, Pinscher, Cocker Spaniel, German Shepherd, Cane Corso. Macroscopically, solid carcinomas were single or multiple, solid tumor formations, mainly affecting the abdominal and inguinal mammary glands. The largest formation reached 13 cm. Pathohistologically, their parenchyma was composed of dense nests of tumor cells forming small lobules surrounded by fine connective tissue (Fig. 6). Neoplastic cells were polygonal to oval with poorly demarcated cell borders and sparse cytoplasm, pleomorphic with numerous mitotic figures.

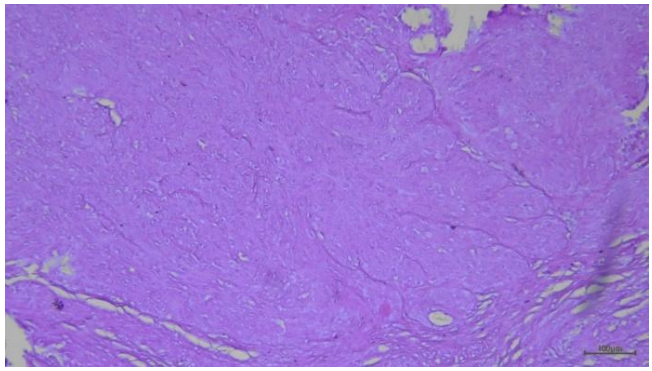

Figure 6: Pathohistological finding in solid carcinoma. The tumor parenchyma consisted of dense nests of neoplastic cells forming lobules surrounded by fine connective tissue. H/E staining, bar=100µm.

Tubular carcinoma

Tubular carcinoma was observed in 21 animals (7.95%). The most commonly affected dog breeds were Yorkshire Terrier, Crossbreeds, Pinscher, German Shepherd, Cane Corso, Chihuahua. Macroscopically, the tubular carcinomas were single, solid masses. The neoplastic epithelial cells were arranged in tubular structures surrounded by fibrovascular stroma. The tumor cells showed clear histological signs of malignancy.

Complex carcinoma

Complex carcinoma was found in 18 dogs (6.82%). The most commonly affected dog breeds were Yorkshire Terrier, Crossbreeds, Pinscher, Cocker Spaniel, German Shepherd, French Bulldog.

Macroscopically, complex carcinomas were most often single masses. Pathohistologically, they consisted of neoplastic epithelial cells, myoepithelial cells, and myxoid substance, differing from complex adenoma by the significant pleomorphism of the neoplastic epithelium and the greater number of mitotic figures.

Malignant mixed tumor/Carcinosarcoma

We found a malignant mixed tumor in 17 dogs (6.44%). Affected dogs were mainly Yorkshire Terrier, Crossbreeds, Pinscher, Cocker Spaniel, German Shepherd. Macroscopically, carcinosarcomas were single or multiple tumor formations with a solid consistency. Pathohistologically, they consisted of malignant neoplastic epithelial cells, most often forming tubular structures and a malignant mesenchymal component.

Spindle cell carcinoma

We found spindle cell carcinoma in 8 dogs (3.03%). The breed distribution was as follows: French Bulldog, Pinscher, Yorkshire Terrier, Crossbreeds, Belgian Shepherd, Beagle. Macroscopically, the spindle cell carcinomas were mostly solitary formations, with one of them measuring up to 10 cm. Pathohistologically, their parenchyma consisted of neoplastic cells with elongated nuclei and eosinophilic cytoplasm, moderate anisocytosis and anisokaryosis and a variable number of mitoses. The stroma is made up of moderately developed connective tissue.

Squamous cell carcinoma

We found squamous cell carcinoma in 5 dogs (1.89%) from the German Shepherd, Siberian Husky, Labrador, Crossbreed and Cocker Spaniel breeds. Four of the tumors were in female dogs and one in a male dog. Macroscopically, the squamous cell carcinomas were ulcerated multiple masses. Pathohistologically, the parenchyma consisted of squamous epithelium. Epithelial cells formed islands of horny substance (keratin pearls) (Fig. 7). In the periphery of the visual field, we observed an inflammatory cell infiltrate.

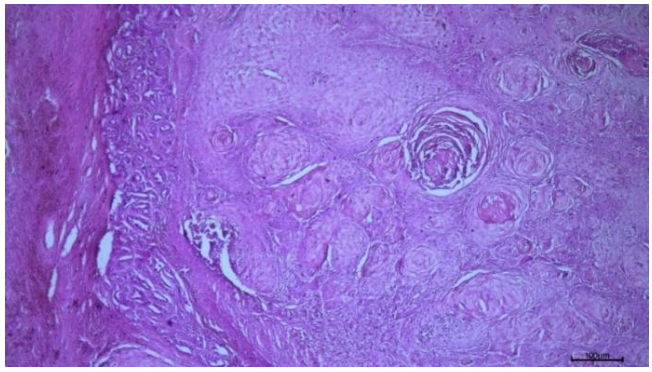

Figure 7: Pathohistological finding in squamous cell carcinoma. Epithelial cells formed islands of horny substance (keratin pearls). H/E staining, bar=100µm.

Comedocarcinoma

We found comedocarcinoma in 3 dogs (1.14%) from the Pinscher, Crossbreed and Pitbull breeds. Macroscopically, comedocarcinomas were single, solid tumor masses. Pathohistologically, the parenchyma was characterized by central necrosis, and in the periphery densely arranged neoplastic cells were observed that formed nests (Fig. 8). The signs of malignancy were pronounced.

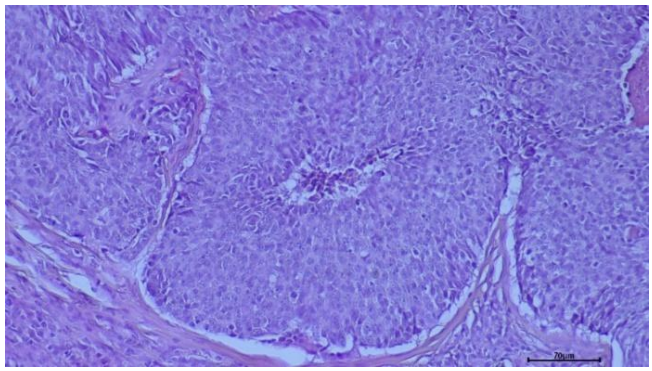

Figure 8: Pathohistological finding in comedocarcinoma. The tumor parenchyma was characterized by central necrosis and in the periphery densely arranged neoplastic cells forming nests. H/E staining, bar=70µm.

Lipid-rich carcinoma

Lipid-rich carcinoma was observed in 3 dogs (1.14%) of the following breeds: Yorkshire Terrier, Pinscher and Beagle. This type of tumor macroscopically represented multiple formations involving the inguinal mammary glands. Pathohistologically, the parenchyma was composed of densely arranged neoplastic cells containing multiple vacuoles or a single large vacuole in their cytoplasm (Fig. 9). Their nuclei are often vesiculated. Cellular pleomorphism was moderately to highly pronounced. The stroma was poorly developed.

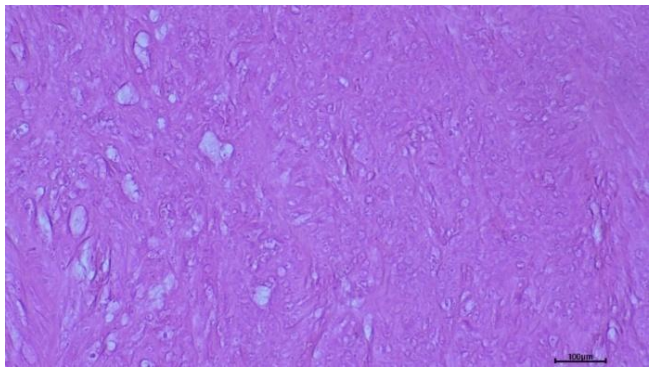

Figure 9: Pathohistological finding in lipid-rich carcinoma. The tumor parenchyma was composed of densely arranged neoplastic cells containing multiple vacuoles or a single large vacuole in their cytoplasm. H/E staining, bar=100µm.

Fibrosarcoma

We found fibrosarcomas in 3 dogs (1.14%). The breed distribution was as follows: Cocker Spaniel, Bichon Frisé and Rottweiler. Macroscopically, in two of the dogs, the tumor masses were single, solid masses, and in one of them, they were multiple growths. Pathohistologically, the parenchyma was represented by interwoven bundles of spindle-shaped or round connective tissue cells, elongated nuclei, and indistinct cell borders, showing clear signs of malignancy.

Anaplastic carcinoma

We found anaplastic carcinoma in 2 dogs (0.76%) from the Yorkshire terrier and Pitbull breeds. Macroscopically, the neoplastic formation was multiple growths with ulceration. Pathohistologically, the parenchyma consisted of poorly differentiated epithelial cells with pronounced pleomorphism (Fig. 10), often with eccentrically located nuclei, abundant cytoplasm, and the presence of numerous mitotic figures. The stroma was poorly developed.

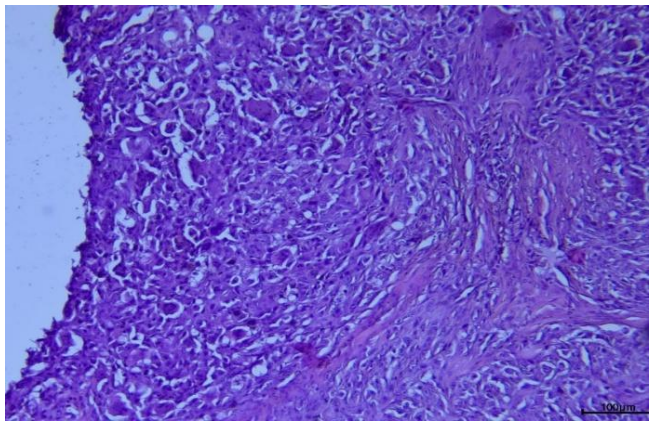

Figure 10: Pathohistological finding in anaplastic carcinoma. The tumor parenchyma consisted of poorly differentiated epithelial cells with pronounced pleomorphism with eccentrically located nuclei and abundant cytoplasm. H/E staining, bar=100µm.

Discussion

Based on the results of our study, it is confirmed that mammary tumors in dogs represent a heterogeneous group of neoplastic diseases, which is in accordance with the studies of a number of authors (Soler *et al.*, 2016; Nakagaki *et al.*, 2019; Varallo *et al.*, 2019; Devarathnam *et al.*, 2021; Valdivia *et al.*, 2021; Tancoš *et al.*, 2023; Vazquez *et al.*, 2023). According to researchers such as (Nunes *et al.*, 2018; Srisawat *et al.*, 2024), an increase in the number of malignant tumors has been registered in recent years. Our study is in line with their observations. Malignant tumors found by us represent 64.77%, and benign tumors – 35.23%, in a ratio of 1:1.8 in favor of malignant ones.

The literature shows that the largest proportion of canine mammary gland tumors are represented by benign mixed tumors, followed by adenoma, and by malignant tumors – various subtypes of simple carcinomas and solid carcinomas (Srisawat *et al.*, 2024). Pathohistologically, we diagnosed 5 types of benign and 11 types of malignant tumors. Of the benign ones, mixed mammary gland tumors predominated (14.39%) and fibroadenomas (8.71%) and of the malignant tumors, tubulopapillary carcinoma (22.73%) and solid carcinoma (11.74%).

Female dogs have been found to be at a significantly higher risk of developing mammary tumors than male dogs (Srisawat *et al.*, 2024). In our study, 99.24% of all dogs with neoplastic formations were female and only 0.76% were male. The majority of the studied animals were not castrated (241 dogs), which is a prerequisite and an important factor for the development of mammary gland neoplasia. The differences in prognosis between intact and spayed female dogs with mammary cancer may be due to the lower content of estrogen receptors in mammary tumors found in spayed females (Peña *et al.*, 2012). In our study, castrated animals were dominated by mixed

mammary gland tumor and solid carcinoma. No correlations were found between castration status and histological subtype of the neoplasms.

The age range of the affected animals varied from 3 to 17 years. These results were similar to those observed by Srisawat *et al.* (2024), in which the affected dogs ranged in age from one to seventeen years. Our research showed that largest number of animals were at 7 years old – 28 dogs, at 8 years old – 36 dogs, at 9 years old – 31 dogs and at 10 years old – 30 dogs. In the this age group (7–11 years), malignant tumor formations prevailed. In this way, our study is in agreement with the studies of other researchers working in this direction (Raskin and Meyer, 2001; Sorenmo *et al.*, 2011). The high number of middle-aged and older dogs diagnosed with mammary neoplasia can be explained by the fact that the risk of developing this type of tumor increases with age.The youngest (3 years old) and the oldest dog (17 years old) were Yorkshire Terriers. The diagnoses given to them were fibroadenoma and anaplastic carcinoma, respectively, in the three-year-old and seventeen-year-old dog. From the presented data it is clear that in older animals the diagnoses are more malignant.

An increased incidence of mammary tumors has been found in purebred dogs compared to mixed breeds (Dorn *et al.*, 1968; Zatloukal *et al.*, 2005; Salas *et al.*, 2015; Burrai *et al.*, 2020). In the present study, the largest number of affected dogs were Yorkshire Terriers – 41, followed by Crossbreeds – 39, Pinschers – 26, Cocker Spaniels – 13 and others. Our results can be explained by the fact that the Yorkshire Terrier is among the most common companion dog breeds in Bulgaria, which suggests a higher incidence of various diseases, in this case mammary gland tumors. However, the exact reason for the overrepresentation of the Yorkshire Terrier breed in our and many other studies is still unknown.

Epithelial tumors were most often formed. Out of the total number of mammary neoplasms, 206 (78.03%) were epithelial, 3 (1.14%) were mesenchymal and 55 (20.83%) were mixed, which is consistent with authors such as Brodey *et al.* (1983); Simeonov (2014), who consider that the majority of mammary neoplasms are epithelial. The abdominal and inguinal mammary glands are most commonly affected (Dinev *et al.*, 2002; Nguyen *et al.*, 2018; Nunes *et al.*, 2019). A large number of the registered neoplasms in our study were found precisely with such anatomical localization.

Based on the study we conducted, we can conclude that mammary gland tumors are an important problem in the pathology of dogs, due to their increasingly frequent occurrence and widespread distribution. The study of mammary tumors offers a unique opportunity to enrich knowledge and the possibilities for predicting the outcome of this type of neoplasia. According to the pathohistological characteristics, malignant neoplasms predominate, of which the majority are epithelial tumors. From the presented results, we can also conclude that the age of onset of the tumor disease and castration status are of great importance. We believe that tracking their pathohistological characteristics will contribute to modern diagnostics and therapy.

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