Multiple Distinct Mammary Neoplasms with Different Histopathological Features in an 11-Year-Old Female Bulldog

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Abstract

Canine mammary tumors (CMTs) comprise a morphologically diverse group of neoplasms with variable epithelial, myoepithelial, and mesenchymal differentiation. Although multiple mammary masses are frequently identified in individual dogs, concurrent lesions within the same patient may represent distinct neoplastic entities with different histopathological characteristics and biological behavior. An 11-year-old female Bulldog weighing 27 kg was referred with three mammary masses located in the right inguinal, left caudal abdominal, and right cranial thoracic mammary glands. Following surgical excision, all specimens were submitted for histopathological evaluation. Routine hematoxylin and eosin (H&E) staining revealed three distinct mammary neoplasms: a well-differentiated squamous cell carcinoma (SCC), a grade I tubular carcinoma, and a benign mixed tumor (BMT). The simultaneous occurrence of three histologically distinct mammary neoplasms in a single senior dog represents a rare and noteworthy pathological finding. This case highlights the importance of submitting and evaluating each mammary mass individually, as multiple lesions within the same patient may represent distinct neoplastic entities requiring different diagnostic, prognostic, and therapeutic considerations.

Keywords: Benign mixed tumor; Bulldog; Canine mammary neoplasms; Squamous cell carcinoma; Tubular carcinoma

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Introduction

CMTs are among the most encountered neoplasms in female dogs and represent an important clinical concern in veterinary medicine because of their high frequency and variable biological behavior (Cassali *et al.,* 2014). Recent epidemiological data from the United Kingdom estimated the incidence of CMTs at approximately 1,340.7 cases per 100,000 female dogs in 2016, underscoring their substantial prevalence in the canine population (Varney *et al*., 2023). These tumors predominantly occur in middle-aged to older bitches, with the highest occurrence generally reported between 8 and 10 years of age (Sorenmo *et al.,* 2011). Age also appears to influence tumor phenotype, as benign mammary lesions are more frequently diagnosed in dogs approximately 7–9 years old, whereas malignant tumors tend to occur in comparatively older animals (Sorenmo *et al.,* 2009; Vascellari *et al.,* 2009, 2016). Breed is also considered a potential determinant of mammary tumor susceptibility in dogs, with some studies suggesting a greater occurrence among purebred animals than mixed-breed dogs. However, evidence regarding breed-specific predisposition remains inconsistent, likely reflecting differences in geographic populations, study designs, and underlying population characteristics (Vascellari *et al.,* 2016). The canine mammary gland is a specialized tubuloalveolar exocrine gland of apocrine origin, typically comprising five paired mammary glands, including two thoracic (M1 and M2), two abdominal (M3 and M4), and one inguinal pair (M5) (Patel *et al.,* 2019). CMTs typically present as discrete, firm nodules that may range considerably in size. Multifocal involvement is common, and lesions arising within the same mammary chain or even within a single gland may differ substantially in histological subtype and grade. The caudal mammary glands are more commonly affected, accounting for approximately 60% of CMTs (Cassali *et al.,* 2017). Metastatic potential is an important feature of malignant CMTs, with neoplastic cells capable of reaching regional lymph nodes and, subsequently, distant sites. The lungs are the predominant distant target, whereas involvement of abdominal organs, including the liver, spleen, and kidneys, is reported less frequently (Vazquez *et al.,* 2023). Histologically, CMTs encompass a broad spectrum, ranging from tumors with predominantly epithelial or mesenchymal differentiation to lesions containing combined epithelial and myoepithelial components. Notably, mesenchymal differentiation and prominent myoepithelial proliferation are relatively common features in CMTs but are uncommon in human breast neoplasms (Peña *et al.,* 2026; Gray *et al.,* 2020; Kim *et al.,* 2020).

SCC is a malignant epithelial neoplasm that can arise at various anatomical sites in dogs and accounts for approximately 3.9–10.4% of canine cutaneous tumors. Although most encountered in the oral cavity, SCC may also develop in the nasal cavity,

mammary glands, lungs, urinary bladder, and vagina (Jiménez-Alonso *et al.,* 2025). Cutaneous SCC primarily affects older dogs, with a mean age of 8.3-9.1 years, and appears more frequent in Dalmatians, Boxers, Bull Terriers, Beagles, Norwegian Elkhounds, Basset Hounds, and Pointers, without a clear sex predisposition. These tumors are typically locally invasive, with metastatic behavior and prognosis influenced by tumor location and progression (Webb *et al.,* 2009).

Tubular adenocarcinoma is characterized by malignant epithelial cells predominantly organized into tubular structures and is generally encountered in older bitches, with reported cases occurring mainly in dogs around 11 years of age (Misdorp *et al.,* 1972; kim et al., 2024). Histologically, these tumors may exhibit simple or complex architecture; the simple form consists mainly of tubular structures and may contain papillary or solid areas, whereas the complex form combines epithelial tubules with proliferating spindle or polygonal myoepithelial cells. Simple tubular adenocarcinomas may show marked local invasiveness, lymphatic invasion, and necrosis, while the complex form can exhibit a more lobular growth pattern and variable lymphatic involvement (Misdorp *et al.,* 1972).

Mixed mammary tumors are among the common mammary neoplasms in female dogs and encompass lesions with varying combinations of epithelial, myoepithelial, and mesenchymal components (Nunes *et al.,* 2019; Mitenko *et al*., 2023) . Based on the biological behavior of these components, this group includes BMTs, carcinomas arising in mixed tumors, and carcinosarcomas, which differ in the malignant potential of their epithelial and mesenchymal elements. BMTs contain benign epithelial, myoepithelial, and mesenchymal components, whereas carcinomas in mixed tumors are characterized by malignant epithelial proliferation within an otherwise benign mesenchymal component. In contrast, carcinosarcomas exhibit malignant transformation of both epithelial and mesenchymal components, representing the most aggressive form within this spectrum (Nunes *et al.,* 2019).

This case report describes the concurrent occurrence of three histologically distinct mammary neoplasms in a senior Bulldog, an unusual presentation that underscores the diagnostic complexity of CMTs. Accurate histopathological evaluation is essential for distinguishing tumor types, determining histological grade and prognosis, and guiding appropriate therapeutic management. Therefore, submission of excised mammary lesions and regional lymph nodes for anatomopathological examination is an essential component of the diagnostic and clinical management of CMTs.

Case presentation

Clinical presentation

An 11-year- and 2-month-old female Bulldog weighing 27 kg was referred with three mammary masses arising from the right inguinal, left caudal

abdominal, and right cranial thoracic mammary glands. The lesions were surgically excised at the Veterinary Hospital. Following excision, the tumor specimens were submitted to the Department of Pathology, where they were archived and subsequently evaluated by histopathological examination.

Histopathological procedures and tumor classification

Following surgical excision, the mammary tumor specimens were fixed in 10% neutral buffered formalin (Dr. Mojallali Chemical Industries Complex, Tehran, Iran) and routinely processed for histopathological examination. The tissues were dehydrated through ascending concentrations of alcohol, cleared in xylene, and subsequently embedded in paraffin wax (Tkaczyk-wlizło *et al*., 2023; Daneshnia *et al*., 2026). Serial sections approximately 5µm thick were prepared from the paraffin blocks using a Leica microtome (Leica RM2235, Wetzlar, Germany), mounted on glass slides, and stained with H&E. The stained sections were examined by light microscopy to evaluate the overall tumor architecture, cellular morphology, mitotic count, necrosis, and other relevant microscopic features. Histopathological classification and tumor grading were performed according to established diagnostic criteria (Goldschmidt *et al.,* 2011; Peña *et al.,* 2026).

Histopathological findings

In histopathological investigations, the tumor of right inguinal mammary gland was composed of neoplastic epithelial cells arranged in foci and sheets. The dermal layer was invaded multifocally by small clusters and individual neoplastic epithelial cells. These cells had a moderate to abundant slightly eosinophilic cytoplasm, round to oval vesicular nuclei and prominent nucleoli. The nests were consisted of immature polyhedral cells at the periphery and eosinophilic lamellated keratin pearls at the centre. Focal to multifocal areas of necrosis were seen. Neoplastic epithelial cells variably showed anisocytosis, anisokaryosis and increased numbers of mitotic figures from 1 to 3 per high power field. Diffuse mild mixed inflammatory cells (lymphocytes and macrophages) were observed within the stroma. The mass was diagnosed as a well differentiated SCC (Fig. 1).

Microscopically, in the left caudal abdominal mass, sheets of tubular or gland-like structures were observed. The lining of the tubules was 1-2 cells thick. Neoplastic cells had an eosinophilic cytoplasm, and cell margins were distinct. The fibrovascular intertubular stroma consisted of vessels, fibroblasts and collagen fibres. The tubules were lined by cuboidal epithelium with large, oval, vesicular nuclei, a prominent nucleolus, little cellular pleomorphism and few mitotic figures. The mass was diagnosed as a tubular carcinoma (grade I) (Fig. 2).

In microscopical findings of the right cranial thoracic mammary gland mass, there were both epithelial and myoepithelial proliferation with foci of mesenchymal cells producing cartilage and bone, and variable amounts of fibrovascular stroma.

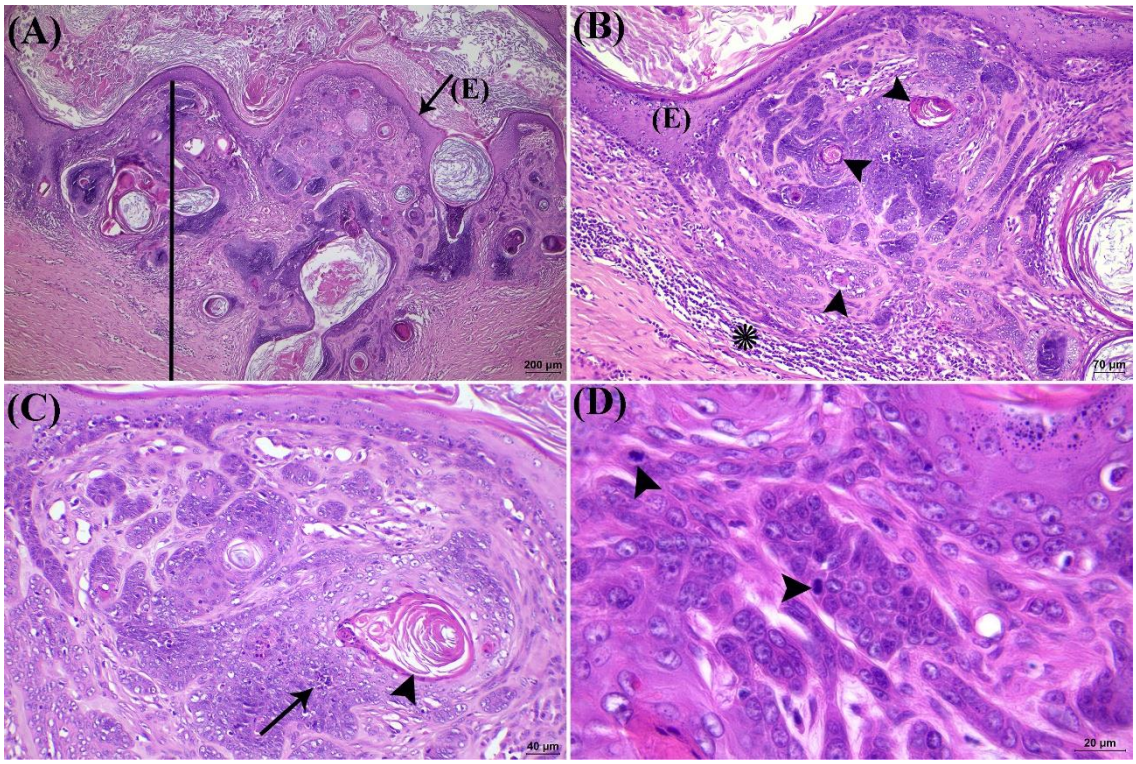

Figure 1: (A-D): Histopathological findings of squamous cell carcinoma in the right inguinal mammary gland. (A-B): Note the dermal layer (black line) invaded by tumor, keratin pearl (arrowheads), mixed inflammatory cells infiltration (*), epidermal layer (E). (C): Higher magnification of keratin pearl (arrowhead) and necrotic cells (arrow). (D): Mitotic figures (arrowheads), H&E.

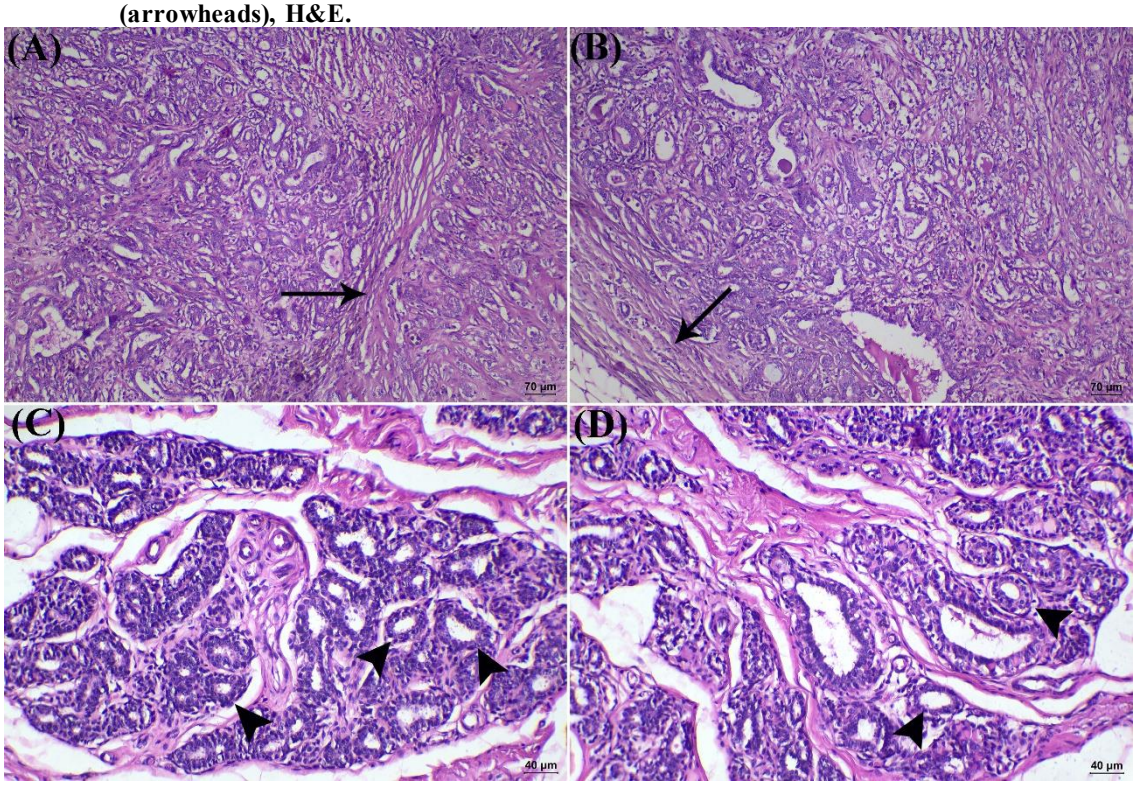

Figure 2: (A-D): Microscopical findings of tubular carcinoma in the left caudal abdominal mammary gland. (A-B): Note the sheets of tumor with fibrovascular stroma (arrows). (C-D): Higher magnification of tubular or gland-like structures lined by cuboidal epithelium (arrowheads), H&E.

The epithelium was composed of tubules and cords lined by cuboidal to columnar cells with eosinophilic cytoplasm. Nuclei were round to oval with a single central basophilic nucleolus. Anisokaryosis and anisocytosis were minimal. The myoepithelium was composed of spindle to round cells. Basophilic myxoid matrix, large multifocal cartilages and bone tissue were also seen. Based on histopathological findings, the mass was diagnosed as a BMT (Fig. 3).

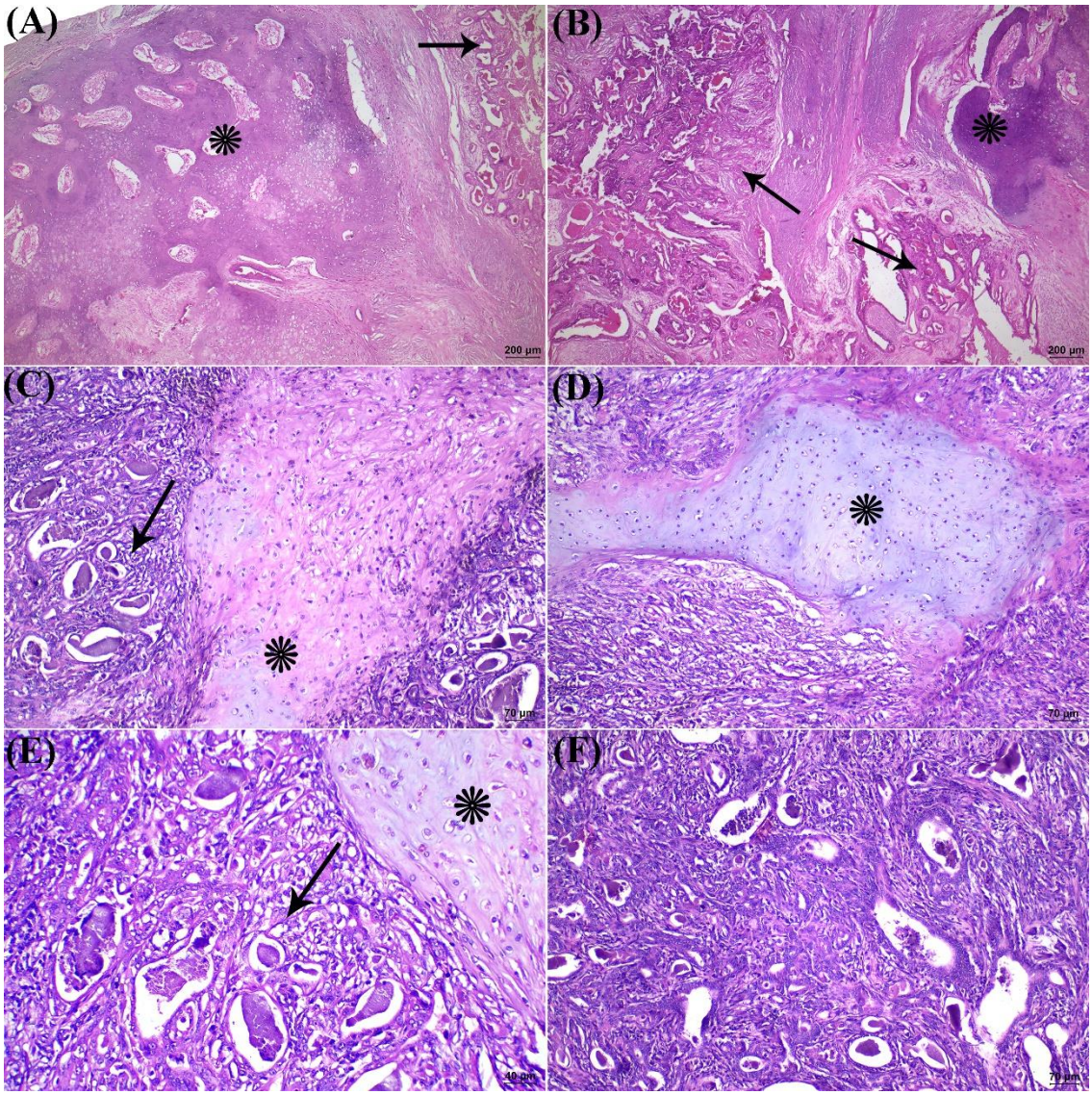

Figure 3: (A-F): Histopathological findings of benign mixed tumor in the right cranial thoracic mammary gland mass. (A-D): Both epithelial and myoepithelial proliferation (arrows) with cartilage (*). (E): Higher magnification of figure C. (F): The epithelial and myoepithelial structures of tumor, H&E.

Discussion

Neoplastic disorders are increasingly recognized in companion animals, with dogs being among the most affected species (Ciaputa *et al.,* 2022). Among female dogs, mammary gland tumors represent the most frequently encountered neoplasms, comprising approximately 25-50% of all tumors diagnosed in this species (Collivignarelli *et al.,* 2021). The present case highlights the remarkable histological diversity of canine mammary neoplasms, with three morphologically distinct tumors identified within a single dog.

SCC is an uncommon malignant mammary neoplasm in dogs, characterized by proliferation of neoplastic squamous epithelial cells showing variable degrees of keratinization. Previous studies have described these tumors as being composed of infiltrative nests, islands, or cords of epithelial cells, often with peripheral basaloid cells and central areas of concentric keratin accumulation forming characteristic keratin pearls (Goldschmidt *et al.,* 2011; Cassali *et al.,* 2014). In the present case, the right inguinal mammary mass exhibited multifocal dermal invasion by neoplastic epithelial cells arranged in nests and sheets, with prominent keratin pearl formation, supporting the diagnosis of a well-differentiated SCC. Although distinguishing mammary-derived SCC from cutaneous SCC extending into the mammary tissue can be challenging, careful evaluation of the lesion origin and surrounding tissues is essential for accurate classification (Goldschmidt *et al.,* 2011). The morphological findings observed in this case, including epithelial nests containing peripheral immature cells and centrally located eosinophilic keratin material, were consistent with previously reported features of well-differentiated canine mammary SCC (Handrashekaraiah *et al.,* 2011).

Tubular carcinoma is characterized by a predominant tubular growth pattern and is a well-recognized epithelial mammary carcinoma in dogs (Goldschmidt *et al.,* 2011). Histologically, the neoplastic tubules are commonly lined by one to two layers of epithelial cells, while cellular morphology, degree of tubular differentiation, and mitotic activity contribute to histological grading (Goldschmidt *et al.,* 2011). In the present case, the left caudal abdominal mammary mass showed a predominantly tubular architecture, with tubules lined by a single to double layer of cuboidal neoplastic cells and supported by fibrovascular stroma, consistent with the characteristic morphology of tubular carcinoma (Goldschmidt *et al.,* 2011). The relatively uniform cellular population, minimal pleomorphism, and low mitotic activity observed in this lesion were compatible with its classification as a grade I carcinoma (Goldschmidt *et al.,* 2011). Although tubular carcinomas may exhibit variable stromal composition, inflammatory infiltration, and invasive behavior, none of these features were prominent in the lesion described here, further supporting

its low histological grade (Goldschmidt *et al.,* 2011).

Mixed tumors are among the common mammary neoplasms in female dogs (Cassali *et al.,* 2017; Esteves-Monteiro *et al*., 2025). BMTs typically contain relatively well-differentiated epithelial and myoepithelial components together with variable mesenchymal differentiation, including cartilage, bone, and myxoid tissue, while the epithelial component generally exhibits minimal atypia and low mitotic activity (Cassali *et al.,* 2017). In the present case, the right cranial thoracic mammary mass demonstrated epithelial and myoepithelial proliferation accompanied by abundant myxoid matrix and multifocal cartilage and bone formation, with minimal anisocytosis and anisokaryosis, supporting its classification as a BMT. The spindle to round shaped myoepithelial proliferation observed in this lesion is consistent with the reported association between myoepithelial differentiation, myxoid matrix formation, and subsequent mesenchymal differentiation in canine BMTs (Bertagnolli *et al.,* 2011; Damasceno *et al.,* 2014; Cassali *et al.,* 2017). The development of cartilage and bone in these tumors has been attributed to mesenchymal differentiation involving myoepithelial or stromal cells, with ossification occurring through either endochondral or intramembranous mechanisms (Goldschmidt *et al.,* 2011). From a clinical perspective, complete surgical excision is generally considered curative for BMTs, whereas delayed removal may permit malignant transformation of the epithelial component and progression to carcinoma arising in a mixed tumor (Cassali *et al.,* 2017).

In the present case, the three lesions exhibited markedly different morphological and biological characteristics despite occurring in the same dog. Their concurrent development in separate mammary glands further illustrates the heterogeneity of canine mammary neoplasia and the potential for multiple lesions to represent distinct tumor types. Histopathological classification is an important predictor of tumor behavior; therefore, all mammary nodules should undergo histopathological evaluation, regardless of size, to provide reliable prognostic information and inform appropriate therapeutic planning.

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