



# Epidemiology, Histopathological Classification, Hematological Alterations, and Treatment Outcomes of Canine Mammary Tumors in Ho Chi Minh City, Vietnam

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## ABSTRACT

Canine mammary tumors (CMTs) are among the most common neoplasms in female dogs. A clinic-based study was conducted to describe the occurrence, clinicopathological features, hematologic findings, and treatment outcomes of CMTs in dogs presented to the K9 Veterinary Clinic system in Ho Chi Minh City, Vietnam. From March 2023 to March 2024, 2,564 female dogs were examined. Mammary tumors were identified in 67 dogs through clinical examination, fine-needle aspiration cytology, and subsequent histopathological evaluation after surgical excision, of which 56 were classified as malignant. Canine mammary ductal carcinoma was the predominant histopathological type, characterized by proliferation of ductal epithelial cells with high cellular density, a high nuclear-to-cytoplasmic ratio, and poor differentiation. Of 56 dogs with malignant mammary tumors, 54 underwent treatment and were included in outcome evaluation. Stage II was the most common malignant stage (48.21%), followed by stage I (25.00%), stage III (14.28%), stage IV (8.93%), and stage V (3.58%). Higher tumor occurrence was recorded in dogs older than 10 years, intact females, and obese dogs. Hematologic abnormalities were frequently observed, including neutrophilia and thrombocytopenia in 27 cases each (40.30%), leukocytosis and reduced hematocrit in 20 cases each (29.85%), reduced red blood cell count in 16 cases (23.8%), and decreased hemoglobin concentration in 14 cases (20.89%). During the 12-month follow-up period, recovery was recorded in all treated dogs in stage I, in 81.48% of dogs in stage II, and in 25.00% of dogs in stage III, whereas no recovery was observed in stage IV. Findings highlight the importance of early detection and timely clinical management in dogs with mammary tumors.

**Keywords:** Cancer; Canine mammary tumor, Chemotherapy, Histopathology, Risk factor

## INTRODUCTION

Canine mammary tumors represent one of the most frequently diagnosed neoplastic conditions in intact female dogs, and approximately 50% of cases are reported to be malignant (Vazquez et al., 2023). Their marked heterogeneity—from benign lesions to highly aggressive carcinomas—requires accurate histopathological classification and grading to guide prognosis and therapy (Goldschmidt et al., 2011; Nosalova et al., 2024). Updated diagnostic frameworks highlight mitotic index, tumor architecture, and vascular invasion as key prognostic indicators (Rasotto et al., 2017). Canine mammary tumors share significant biological and molecular similarities with human breast cancer, supporting their value as a spontaneous comparative model (Vazquez et al., 2023).

Well-established risk factors include hormonal exposure, advanced age, and obesity; spayed dogs exhibit significantly lower tumor risk (Vazquez et al., 2023). Tumor-associated systemic inflammation is common, and hematologic abnormalities such as neutrophilia, anemia, and thrombocytopenia have been widely reported (Vazquez et al., 2023). Although modern imaging techniques such as Doppler ultrasound, elastography, and contrast-enhanced ultrasound have improved presurgical evaluation, fine-needle aspiration cytology (FNA) remains an essential first-line diagnostic tool due to its rapidity, minimal invasiveness, and ability to provide early suspicion of malignancy (Sontas et al., 2012; Feliciano et al., 2023). However, cytological assessment may show variable diagnostic accuracy and can be influenced by interpretative variability (Sontas et al., 2012). Therefore, definitive diagnosis and precise tumor classification still rely on histopathological examination, which remains the gold standard for determining tumor type, grade, invasion status, and guiding treatment planning (Cassali et al., 2020).

Although canine mammary tumors have been extensively investigated in many countries, published clinic-based data from Vietnam remain limited. Greater availability of local information on tumor frequency in veterinary practice, histopathological patterns, hematologic findings, and treatment outcomes may support more structured clinical

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assessment and management in Vietnamese canine patients. Thus, the present study aimed to investigate canine mammary tumors in female dogs presented to the K9 Veterinary Clinic system in Ho Chi Minh City, Vietnam.

## MATERIALS AND METHODS

### Ethical approval

The present study was conducted in accordance with animal welfare guidelines and approved by the Scientific Committee of the Faculty of Veterinary Medicine, Ho Chi Minh City University of Technology (HUTECH), Vietnam. All procedures involving animals were carried out under the supervision of licensed veterinarians, and informed consent was obtained from all dog owners prior to sample collection.

### Study design and population

An observational study was performed from March 2023 to March 2024 at the K9 Veterinary Clinic system in Ho Chi Minh City, Vietnam. Female dogs presented during the study period were examined for health conditions by a veterinarian. Physical examination was carried out in all cases, and abnormal mammary nodules were further evaluated by fine-needle aspiration cytology. Mammary masses with cytologic findings suggestive of neoplasia underwent complete surgical excision and histopathological examination for definitive diagnosis. Tumor-related analyses were limited to dogs with histopathologically confirmed mammary tumors.

### Inclusion and exclusion criteria

Dogs were eligible for inclusion when an abnormal mammary nodule was detected on clinical examination, and cytologic findings were suggestive of mammary neoplasia. Additional requirements included complete surgical excision of the lesion, histopathological confirmation of mammary tumor, clearly documented medical history, and absence of concurrent disease with possible effects on hematologic findings. Only dogs in which a mammary tumor was diagnosed for the first time during the study period were included. Previous mammary tumor history and prior tumor-related treatment constituted exclusion criteria. Additional exclusion criteria included incomplete records, unclear clinical history, lack of histopathological confirmation, and systemic disorders capable of altering hematologic parameters. Hematologic analysis was limited to pre-treatment blood data and was interpreted descriptively owing to the absence of a healthy age-matched control group.

### Epidemiological data collection

Epidemiological information, including age, body condition, and reproductive status, was obtained through case history and physical examination. Age was stratified into two epidemiologically relevant categories, including  $\leq 10$  years and  $> 10$  years, based on the distribution of cases in the study population and in alignment with the age-grouping criteria applied for risk factor assessment. Body condition was evaluated according to the World Small Animal Veterinary Association (WSAVA) body condition score (BCS), in which dogs scoring 7/9 or higher were classified as obese, acknowledging the role of excess adiposity in mammary tumor risk. Reproductive status was recorded as intact or spayed, given the well-established influence of hormonal exposure on mammary tumor development.

### Cytological examination

Fine-needle aspiration cytology was carried out in all dogs presenting with abnormal mammary nodules detected during clinical examination. Aspirated cellular material was obtained with a 23-gauge needle after aseptic site preparation, evenly smeared on glass slides, air dried, and processed with May-Grünwald-Giemsa stain following standard laboratory methods for veterinary cytology (Sontas *et al.*, 2012). Slide interpretation formed part of the routine diagnostic workflow and was performed by clinicians experienced in veterinary cytology. Cytologic assessment served as a preliminary approach for recognizing mammary lesions with morphologic features consistent with neoplasia and for guiding the selection of cases for surgical removal. Final diagnosis, histopathological classification, and tumor grading were determined exclusively after excision and histopathological evaluation of the lesion (Cassali *et al.*, 2020).

### Hematological analysis

Blood samples were collected from dogs with confirmed mammary tumors during diagnostic evaluation, before surgery or administration of any tumor-directed treatment. Approximately one mL of blood was drawn from the cephalic vein without sedation and placed into Ethylene Diamine Tetra Acetic Acid (EDTA) containing tubes for complete blood count analysis. Hematological parameters, including total leukocyte count, neutrophil count, erythrocyte count, hemoglobin concentration, hematocrit, and platelet count, were measured using an automated hematology analyzer (BC-

5000Vet, Mindray, China). Results were interpreted according to the laboratory reference intervals. Owing to the absence of a healthy control group, hematologic findings were described descriptively rather than used for causal inference.

### Tumor staging

Tumors were staged according to the Tumor - Nodes - Metastasis system proposed by Owen (1981) and are presented in Table 1, widely applied in canine mammary oncology and recommended in updated classification guidelines (Cassali et al., 2020).

Clinical stages (I-V) were assigned according to tumor size (T), regional lymph node involvement (N), and distant metastasis (M), with T1 defined as  $\leq 3$  cm, T2 as 3-5 cm, and T3 as  $\geq 5$  cm; N0 indicating absence of regional lymph node metastasis and N1 indicating its presence; and M0 indicating absence of distant metastasis and M1 indicating distant metastasis.

**Table 1.** Staging of canine mammary tumors

Stage	Tumor size	Regional lymph node metastasis	Distant metastasis
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
Stage IV	Any T	N1	M0
Stage V	Any T	N0 or N1	M1

The staging is prepared according to Owen et al. (1981)

### Histopathology

Excised mammary tissue samples measuring at least  $1 \times 2$  cm were fixed in 10% neutral-buffered formalin and submitted to the Department of Pathology, Veterinary Hospital of Ho Chi Minh City University of Agriculture and Forestry, Vietnam. Following routine histopathological processing, tissue sections were stained with hematoxylin and eosin using Leica BioSystems reagents (USA) and examined under a light microscope (Optika B-193PL, Italy). Histopathological classification was performed according to internationally accepted criteria for canine mammary tumors, including assessment of tumor type, degree of differentiation, mitotic activity, necrosis, and lympho-vascular invasion (Goldschmidt et al., 2011; Peña et al., 2013). Histopathological evaluation was based on representative sections of the excised tumor. Slide interpretation was performed under blinded conditions, with pathologists masked to clinical findings, cytology results, and hematologic data.

### Treatment and follow-up

All dogs with histopathologically confirmed malignant mammary tumors received postoperative adjuvant chemotherapy following surgical excision. Surgical treatment ranged from lumpectomy to regional mastectomy according to tumor location and multiplicity. Concurrent ovariectomy was performed in intact females during the same procedure (Kristiansen et al., 2013). Because advanced tools for metastatic risk stratification, such as computed tomography, magnetic resonance imaging, and tumor-associated biomarkers, were not available in routine clinical practice, all malignant tumors were considered eligible for adjuvant treatment. Carboplatin 150 mg/15 mL (Bidiphar, Vietnam) was administered intravenously at 300 mg/m<sup>2</sup> every 21 days for three cycles, and firocoxib 227 mg (Boehringer Ingelheim, France) was given orally at 5 mg/kg once daily for six months; the dosage schedule was based on Lavallo et al. (2012). Follow-up examinations were performed every three months for one year and included physical examination, thoracic radiography, and abdominal ultrasonography. Recovery was defined as the absence of local recurrence or distant metastasis on thoracic radiography and abdominal ultrasound at the 12-month follow-up examination.

### Statistical analysis

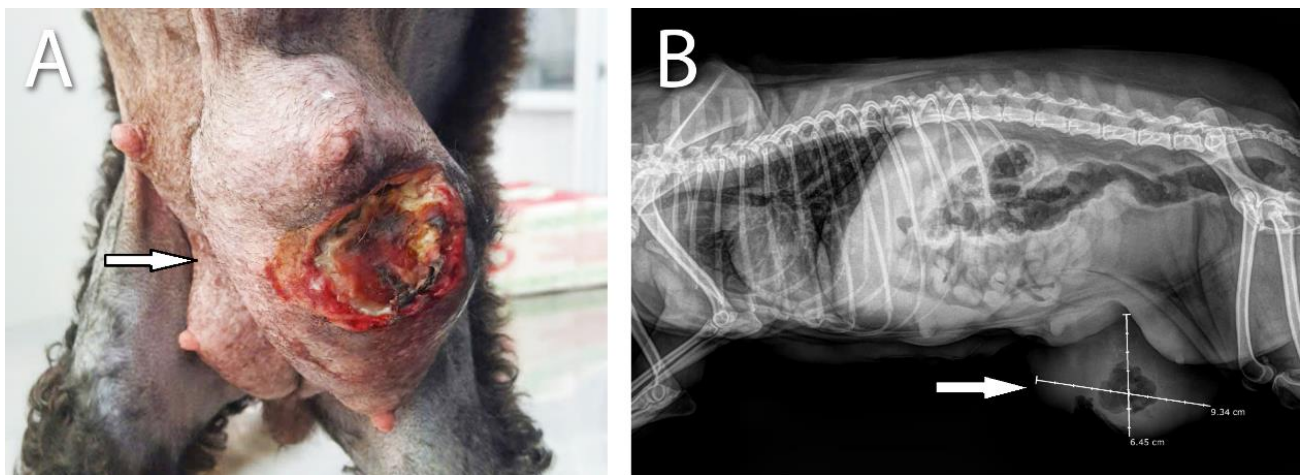
Data were compiled and analyzed using Microsoft Excel 2016 and Minitab 17. Quantitative variables were summarized as mean  $\pm$  standard deviation. Relationships between epidemiological factors and mammary tumor occurrence were evaluated using univariable binary logistic regression with the Fit Binary Logistic Regression procedure

in Minitab. Odds ratios, 95% confidence intervals, and corresponding P values were obtained directly from the output of the Fit Binary Logistic Regression procedure. Statistical significance was defined at  $p < 0.05$ .

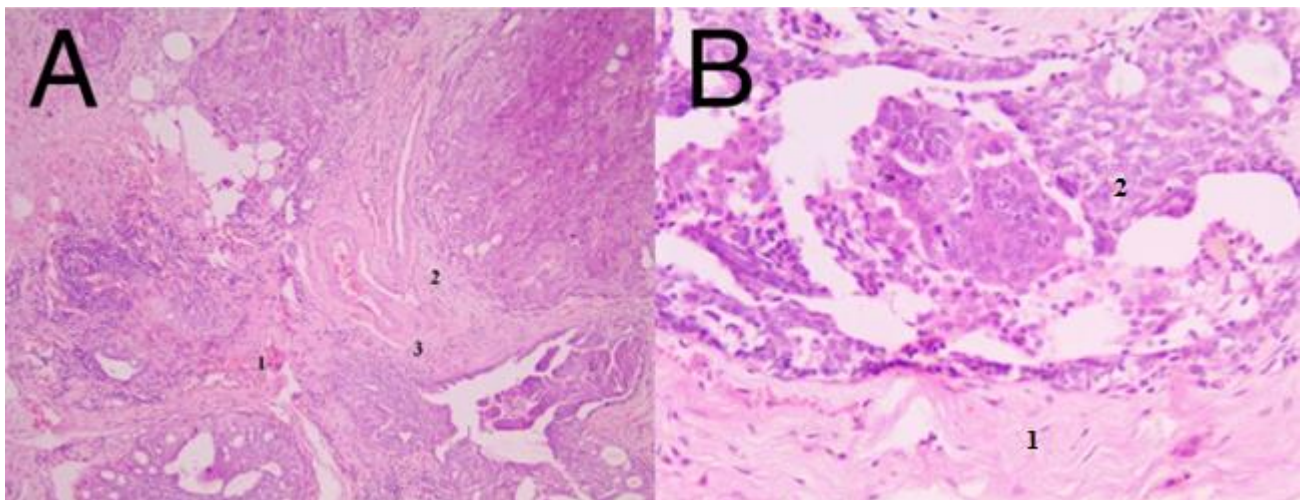
## RESULTS

### Clinical staging of malignant mammary tumors

Clinical examination identified abnormal mammary nodules in 84 dogs (Figure 1). Fine-needle aspiration cytology supported a diagnosis of mammary tumor in 67 cases, corresponding to 2.61% of the female dogs examined at the clinic system during the study period. Histopathological examination after surgical excision confirmed malignancy in 56 cases (2.18%), and those cases were subsequently staged according to the TNM system (Figure 2). Stage II tumors accounted for the highest proportion, representing 48.21% of malignant cases, followed by stage I at 25.00%. Stage III tumors comprised 14.28%, while advanced stages were less common, with stage IV representing 8.93% and stage V only 3.58%. The overall distribution showed a clear decline in case frequency with increasing stage severity, suggesting that most tumors were detected before the onset of distant metastasis.



**Figure 1.** Clinical presentation (A) and radiographic assessment (B), indicating the lesion sites, of a stage III mammary tumor in an 8-year-old female Poodle dog at a veterinary clinic in Ho Chi Minh City, Vietnam (May 2023).



**Figure 2.** Biopsy findings from a stage IV malignant mammary tumor in a 10-year-old female Chihuahua dog. **A:** Scattered foci of hemorrhagic necrosis (1); Proliferation of fibrous connective tissue (2); Proliferation of ductal epithelial cells with high cellular density, high nuclear-to-cytoplasmic ratio, and poor differentiation (3). **B:** Proliferation of fibrous connective tissue (1); Proliferation of ductal epithelial cells with high cellular density, high nuclear-to-cytoplasmic ratio, and poor differentiation (2).

### Epidemiological risk factors

Epidemiological analysis demonstrated strong associations between tumor occurrence and the evaluated risk factors (Table 2). Age was identified as an important factor associated with mammary tumor occurrence, with dogs older than 10 years showing a higher frequency of tumors than dogs aged 10 years or younger (3.41% versus 1.44%). The results

indicated that dogs older than 10 years had a significantly greater risk of mammary tumor occurrence than dogs in the younger age group ( $p < 0.05$ ).

Mammary tumor occurrence was also significantly influenced by reproductive status. A higher proportion of tumors was recorded in intact females than in spayed females (3.39% versus 1.34%), and the difference between both groups reached statistical significance ( $p < 0.05$ ). Body condition showed the strongest relationship with mammary tumor occurrence among all evaluated factors. Obese dogs had a significantly higher tumor frequency than non-obese dogs (4.25% versus 1.32%), with a clear difference between the two groups ( $p < 0.05$ ). Overall, age, reproductive status, and body condition were associated with mammary tumor occurrence.

**Table 2.** Risk factors associated with canine mammary tumors observed in a veterinary clinic located in Ho Chi Minh City, Vietnam

Risk factor	Number of cases	Number examined	Prevalence (%)	OR (95% CI)	P value
Age $\leq$ 10 years	15	1040	1.44	Reference	—
Age $>$ 10 years	52	1524	3.41	2.41 (1.35-4.31)	$< 0.05$
Spayed	13	972	1.34	Reference	—
Intact (not spayed)	54	1592	3.39	2.59 (1.41-4.77)	$< 0.05$
Non-obese	19	1434	1.32	Reference	—
Obese	48	1130	4.25	3.30 (1.93-5.65)	$< 0.05$

### Hematological alterations

Among 67 dogs with mammary tumors, hematological abnormalities were frequently observed (Table 3). Neutrophilia was the most common leukocyte abnormality, recorded in 27 dogs (40.30%), with neutrophil counts ranging from 12.63 to 21.94  $\times 10^9/L$ . Leukocytosis was detected in 20 dogs (29.85%), with a mean leukocyte count of  $21.22 \pm 1.78 \times 10^9/L$ . Erythrocyte-related abnormalities were also identified. Reduced red blood cell counts were found in 16 dogs (23.88%), with values ranging from 2.03 to 5.04  $\times 10^{12}/L$ . Decreased hemoglobin concentration was observed in 14 dogs (20.89%), and reduced hematocrit was recorded in 20 dogs (29.85%). Platelet abnormalities were also common. Thrombocytopenia was recorded in 27 dogs (40.30%), with platelet counts ranging from 24.00 to 115.00  $\times 10^9/L$ . Overall, neutrophilia and thrombocytopenia were the most frequently observed hematological abnormalities among dogs with mammary tumors.

### Treatment outcomes according to clinical stage

Recovery during the 12-month follow-up period was observed in all treated dogs in stage I (14/14; 100.00%), in 22 of 27 treated dogs in stage II (81.48%), and in 2 of 8 treated dogs in stage III (25.00%). No recovery was recorded in treated dogs with stage IV disease during the 12-month follow-up period (Figure 3). Both untreated cases were classified as stage V and were excluded from treatment outcome evaluation. A progressive decline in recovery was observed with advancing clinical stage, as presented in Table 4.

**Table 3.** Alterations in selected blood physiological parameters in dogs with mammary tumors presented to a veterinary clinic located in Ho Chi Minh City, Vietnam

Parameter	Unit	Alteration	Number of dogs	$\bar{X} \pm SD$ (Min - Max)	Percentage (%)
Total leukocytes	$10^9/L$	Increased	20	$21.22 \pm 1.78$ (18.88 – 24.80)	29.85
Neutrophils	$10^9/L$	Increased	27	$16.13 \pm 2.51$ (12.63 – 21.94)	40.30
Erythrocytes	$10^{12}/L$	Decreased	16	$3.86 \pm 0.80$ (2.03 – 5.04)	23.88
Hemoglobin	g/L	Decreased	14	$83.00 \pm 16.26$ (51.00 – 107.00)	20.89
Hematocrit	%	Decreased	20	$26.29 \pm 25.54$ (14.00 – 32.90)	29.85
Platelets	$10^9/L$	Decreased	27	$68.56 \pm 25.54$ (24.00 – 115.00)	40.30

$\bar{X} \pm SD$ : Mean  $\pm$  Standard deviation; Min-Max: Minimum to maximum values.



**Figure 3.** Pulmonary metastasis (white arrows) detected by radiography in a 12-year-old female mixed-breed dog at a Veterinary Clinic in Ho Chi Minh City, Vietnam (January 2024) with stage IV mammary carcinoma despite previous surgical and chemotherapeutic treatment.

**Table 4.** Therapeutic outcomes of malignant canine mammary tumors in dogs presented to a veterinary clinic located in Ho Chi Minh City, Vietnam

Stage	Number of dogs	Number recovered during follow-up	Recovery rate (%)
I	14	14	100.00
II	27	22	81.48
III	8	2	25.00
IV	5	0	0.00

## DISCUSSION

The present study provides one of the first clinic-based descriptions of canine mammary tumors in female dogs presented to the K9 Veterinary Clinic system in Ho Chi Minh City, Vietnam. The frequency of mammary tumor diagnoses in the examined clinic population was 2.61%, which falls within the range of hospital-based frequencies reported in previous international studies (Vazquez et al., 2023). Such findings support the clinical relevance of mammary neoplasia in female dogs presented for veterinary care. Malignant tumors accounted for the majority of histopathologically confirmed cases, and the stage distribution showed a greater proportion of cases detected at earlier clinical stages than at advanced metastatic stages, in agreement with patterns reported in previous studies (Nosalova et al., 2024). Interpretation of the reported 2.61% frequency should remain confined to the examined clinic population, because the study population consisted of female dogs brought to a single private clinic system for various health conditions and therefore represented a convenience sample rather than a random or population-representative canine sample. Under such conditions, the reported percentage reflects the frequency of mammary tumor diagnoses within the K9 Veterinary Clinic system during the study period and cannot be generalized as a population estimate for dogs in Ho Chi Minh City, Vietnam. Risk factor analysis in the evaluated cohort also mirrors global findings. Older age significantly increased tumor occurrence, supporting previous reports that cumulative hormonal exposure, genomic instability, and chronic inflammation promote carcinogenesis (Salas et al., 2015; Rodríguez et al., 2022). The strong association between tumor occurrence and intact reproductive status corresponds closely with classical reproductive oncology studies showing a dramatic reduction in tumor development in dogs spayed early in life (Zheng et al., 2022; Guirguis and Beggs, 2025). Obesity, identified as the strongest risk factor in the present study, has likewise been associated with mammary tumor initiation, hormone

dysregulation, and pro-inflammatory metabolic pathways in previous reports (Marchi et al., 2022; Tamarindo et al., 2023). These converging patterns reinforce obesity as a modifiable risk factor and highlight the need for targeted preventive strategies.

Hematological abnormalities recorded in dogs with mammary tumors, including neutrophilia, leukocytosis, thrombocytopenia, and reductions in erythrocyte-related parameters, are comparable to patterns previously described in canine mammary neoplasia (Uçmak et al., 2021; Vazquez et al., 2023). Such findings may be consistent with inflammatory or paraneoplastic processes reported in malignant disease. Nevertheless, the absence of a healthy age-matched control group precluded definitive determination of whether those abnormalities were directly attributable to the tumor. Interpretation should therefore remain limited to the presence of hematologic changes within the affected cohort. An additional limitation was the lack of stratified analysis according to clinical stage. Neutrophilia was identified in the cohort, but no stage-based comparison was performed to assess whether neutrophil elevation was more common or more pronounced in advanced-stage tumors than in early-stage tumors.

The therapeutic outcomes observed in the present cohort underscore the clinical importance of early detection. More favorable recovery rates were recorded in stages I and II than in advanced-stage disease, in agreement with previous reports indicating improved outcomes when mammary tumors are identified and managed before nodal or distant spread (Nunes et al., 2019; Nosalova et al., 2024). In contrast, the poor outcomes in stages III and IV correspond to evidence that lympho-vascular invasion, nodal metastasis, and tumor aggressiveness sharply reduce survival probability (Dias et al., 2016; Cassali et al., 2020). The two dogs in stage V whose owners declined treatment were consistent with the poor prognosis commonly associated with advanced canine mammary tumors, especially in the presence of metastatic disease or aggressive histological subtypes (Soares et al., 2023).

Beyond conventional staging and histopathology, recent advances offer additional context to interpret these findings. Molecular and immunohistochemical biomarkers, including *Ki-67*, *PCNA*, *HER2*, *p53*, *Cyclin D1/E*, *E-cadherin*, and *β-catenin*, have been shown to correlate with tumor grade, proliferative activity, metastatic potential, and survival outcomes (Valdivia et al., 2021). Studies on miRNA expression, transcriptomic signatures, and metabolomic alterations further demonstrate that CMTs encompass complex molecular pathways influencing progression and therapeutic response (Tamarindo et al., 2023). Although advanced biomarkers were beyond the scope of the present study, the incorporation of molecular characterization in future studies in Vietnam may strengthen diagnostic and prognostic accuracy.

The diagnostic workflow in this study, relying on cytology as an initial screening tool followed by definitive histopathology, also reflects widely accepted best practices. While cytology is rapid and minimally invasive, diagnostic accuracy may be limited by sampling heterogeneity, inadequate cellular yield, and variability in cytologic interpretation, as documented in previous studies (Dolka et al., 2018; Sontas et al., 2012). Histopathology remains the gold standard for precise tumor typing and grading (Rasotto et al., 2017). Advances in imaging, including Doppler ultrasound, elastography, and contrast-enhanced ultrasound, have also been shown to improve preoperative characterization of mammary masses (Feliciano et al., 2023). Such tools may contribute substantially to earlier tumor detection and more refined treatment stratification in future Vietnamese clinical settings, in view of the increasing need for accurate diagnosis and therapeutic decision-making for canine mammary tumors as the disease becomes more frequently recognized in the dog population in Vietnam.

## CONCLUSION

The present study provides a clinic-based description of canine mammary tumors in female dogs presented to the K9 Veterinary Clinic system in Ho Chi Minh City, Vietnam. Older age, intact reproductive status, and obesity were associated with greater mammary tumor occurrence. Hematologic abnormalities were frequently identified in affected dogs. Recovery during the 12-month follow-up period was more commonly observed in early-stage disease, whereas advanced stages showed less favorable outcomes. Results underscore the value of early detection, reproductive management, and timely clinical intervention. Extrapolation of the findings should be made with caution because the study population represented a clinic-based convenience sample, and follow-up was limited to 12 months. Further studies with longer follow-up and analysis by clinical stage, histological grade, and tumor type are needed to clarify the clinical significance of hematologic abnormalities in canine mammary tumors in Vietnam.

## DECLARATIONS

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## Author's contributions

Vu Thanh Du and Loan Vu Thuy Hong Nguyen conceptualized the study and developed the methodological framework. Tran Thi Ngoc Tran, Tam Huu Nguyen, Vy Le Thuy Nguyen, Nghia Hieu Hong, Phat Cong Nguyen, Thong Minh Le, and Khoi Hoang Dang Vo conducted clinical investigations, collected samples, and performed diagnostic procedures. Loan Vu Thuy Hong Nguyen, Tam Huu Nguyen, Trieu Van Pham, and Vu Thanh Du curated, analyzed, and interpreted the data. Loan Vu Thuy Hong Nguyen and Vu Thanh Du drafted the manuscript. All authors contributed to manuscript revision, approved the final version, and agreed to be accountable for the revision processing.

## Competing interests

The authors declare no conflict of interest.

## Availability of data and materials

All data supporting the findings of this study are available upon reasonable request from the corresponding author.

## Ethical considerations

The manuscript was written originally without any copy from data from published articles and books, and no artificial intelligence tools were used in the writing or preparation of the manuscript.

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