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(Original Research)

A Study on Neoplastic and Non-neoplastic Masses in Companion Animal Patients in Malang Raya: Histological Classification and Case Proportion

Andreas Bandang Hardian[1], Syavira Ekdhiasmara[2], Saputra Jaka Prayoga[3], Nur Fitri Destriani[3], Tessa Saputri Marmanto[4], Eka Nur Prasetyawati[5], Pradhana Kurniawan Cadiwirya[6], Gamma Prajnia[7], Roosy Margaretha Riupassa[7], Winda Syafitri[8], Aneke Putri Yulie Dhayanti[9], Eko Budiarto[1], Galuh Herin Faranisa[1], and Chiara Palmieri[10]*

[1]Laboratory of Veterinary Anatomic Pathology, Faculty of Veterinary Medicine, Universitas Brawijaya, Jl. Puncak Dieng, Kunci, Kalisongo, Dau, Malang, East Java, Indonesia 65151
[2]Brawijaya Academic Animal Hospital, Universitas Brawijaya, Jl. Puncak Dieng, Kunci, Kalisongo, Dau, Malang, East Java, Indonesia 65151
[3]Bachelor Student of Faculty of Veterinary Medicine, Universitas Brawijaya ,Jl. Puncak Dieng, Kunci, Kalisongo, Dau, Malang, East Java, Indonesia 65151
[4]Malang Animal Care, Ruko Ditas, Jl. MT. Haryono No. Kav. 16, Dinoyo, Lowokwaru, Malang City, East Java, Indonesia 65145
[5]Luna Pethouse, Jl. Sunan Kalijaga No.1, Dinoyo, Lowokwaru, Malang City, East Java, Indonesia 65149
[6]Panthera Vet, Jl. Candi Sari II No.3, Mojolangu, Kec. Lowokwaru, Malang City, East Java, Indonesia 65142
[7]Okami Animal Clinic, Jl. Sudimoro No.10 A, Mojolangu, Lowokwaru, Malang City, East Java, Indonesia 65142
[8]Winadivet Petcare, l. Laksda Adi Sucipto No.221, Blimbing, Blimbing, Malang City, East Java, Indonesia 65126
[9]Pet House Drh, Jl. Agus Salim No.87, Sisir, Batu, Batu City, East Java, Indonesia 65311
[10]School of Veterinary Science, Faculty of Science, The University of Queensland, Australia

*Corresponding Author: andreasbandangh@ub.ac.id (Andreas Bandang Hardian)

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Abstract

Background: Understanding the case proportion of neoplastic and non-neoplastic disease in companion animals could reveal the trends; novel subtypes architectures, differential diagnoses and disease prognoses based on histopathological features for clinical purposes and future epidemiological study. This study aimed to explore trends, patterns, histopathological features and potential paraneoplastic syndromes associated with tumors in companion animals. **Methods:** Archived neoplastic tissue samples were collected over five years from an animal hospital, clinics, and veterinary practitioners in Malang Raya, Indonesia and processed through routine histopathologic examination. **Results:** A minimum of 30 feline and 40 canine patients with confirmed neoplastic lesions were identified, with glandular tumors being the most frequently reported in both species. Twenty-four non-neoplastic superficial masses were also reported including subcutaneous abscess, granulomatous dermatitis, pseudomycetoma/deep fungal mycosis, and panniculitis. Different regions worldwide may show varying prevalence rates and distinct patterns in the most frequently encountered abnormal superficial masses. **Conclusions:** Understanding these trends and considering the potential differential diagnoses can assist diagnosticians in systematically ruling out less likely conditions. The significant/notable proportion of neoplastic cases in companion animals underscores the importance of further investigations into possible environmental carcinogens, which may also pose risks to human health.

Keywords: Tumor, Animal, Non-neoplastic, Prevalence, Malang Raya

1. Introduction

Tumors – including abnormal nodules, lumps, and masses – are etymologically defined as abnormal

enlargement of tissues, which refer to either neoplastic or non-neoplastic growth [1]. Various tumor types have been reported worldwide presenting diverse consequences to the hosts due to their complex architectural features. Tumor statistics in companion animals could help clinicians to see the prevalence, forecast the pattern of occurrence and prognoses, as well as discriminate the neoplastic and non-neoplastic masses in terms of diagnostic pathology. Moreover, establishing curated animal cancer registry, such as the one developed by Australian Animal Cancer Registry (https://www.acarcinom.org.au/), could provide valuable epidemiological data to help correlating the occurrence of specific neoplastic cases with potential internal and external risk factors in the future [2]. Laboratory of veterinary anatomic pathology in the Faculty of Veterinary Medicine Universitas Brawijaya has been operating and serving for histopathology examination since 2021 for the veterinary hospital and clinics around Malang Raya area, East Java Province, Indonesia. Curation and analysis of our database and archival tissues are intended to serve and provide updated information of various neoplastic and non-neoplastic cases in submitted animal specimens. This study aimed to report the various neoplastic and non-neoplastic cases diagnosed in companion animals (pets and other domesticated animals e.g. ornamental birds and reptiles, domesticated rodents) in Malang Raya, Indonesia by analyzing medical records and archival tissue samples from various animal health centers. Curation of these data might also be a seed to the regional cancer registry establishment by compiling associating risk factors for epidemiological study. By analyzing the architectural characteristics of these tumors, we aim to gain insights into their malignancy, metastatic potential and other histological features. Processing tissues for histopathology examination spends more time, which requires clinical doctors to take initial treatment to support patients' life. Exploring the trend of neoplastic and non-neoplastic masses occurrence will assist clinicians to strategize the initial therapy based on common tumor cases.

2. Materials and Methods

2.1 Ethical Approval

This study was performed under approval of the research ethics committee (document number No: 041-KEP-UB-2023).

2.2 Study Period and Location

This study was conducted from April 2023 to November 2024. All examinations and analyses were performed at the laboratory of veterinary anatomic pathology Universitas Brawijaya, Indonesia.

2.3 Medical Records and Archival Tissue Collection

Medical records and archived tissues were collected from animal health facilities in Malang Raya including an animal hospital (67), animal clinics-veterinary practitioners (25), and direct submission to the laboratory (87). Most of the collected tissues were from externally visible masses in superficial organs (e.g. skin, mammary glands). Medical records, either digital or printed, were analyzed based on the species and final diagnosis. All medical records with a final diagnosis containing the keywords 'mass', 'tumor', 'growth', 'lump', 'nodule', and 'swelling' were selected. For all records with aforementioned keywords but no final diagnosis available or submitted tissues with no decisive diagnosis would be included in the 'unknown' category. We grouped the data based on the most common species recorded: canine, feline, avian. Other species such as exotic reptiles and rodents were included in the 'others' group. Samples with archived tissues and/or histopathology report of the masses were included in the final dataset. New submissions of masses without any conclusive clinical diagnosis underwent routine histopathological examination (paraffin embedding, haematoxylin-eosin staining-reviewed by ABH).

2.4 Tumor Classification and Histopathological Description

Each tumor was classified using standard classification systems as provided by Meuten *et al.*, in 2020 [3], World Health Organization (WHO) classification of tumors, SCC classification as described by Muller *et al.* in 2018 [4] and surgical pathology of tumors in domestic animals with reference to CL Davis-Thompson foundation [5,6,7,8]. All information gathered were summarized in a database, were visualized, and analyzed descriptively based on histological features.

3. Results

3.1 Case Proportion

A total of 179 medical records and specimens were initially collected, which were then narrowed down to 117 cases containing tumors. Of these, almost a quarter (24.17%) were identified as non-neoplastic masses. Among the remaining neoplastic cases, approximately one-third were classified as tumors with no specific characteristics and/or no further histological information available, leading to their categorization as unknown tumors (Fig. 1). The distribution of identified tumors in our database is presented in Table 1.

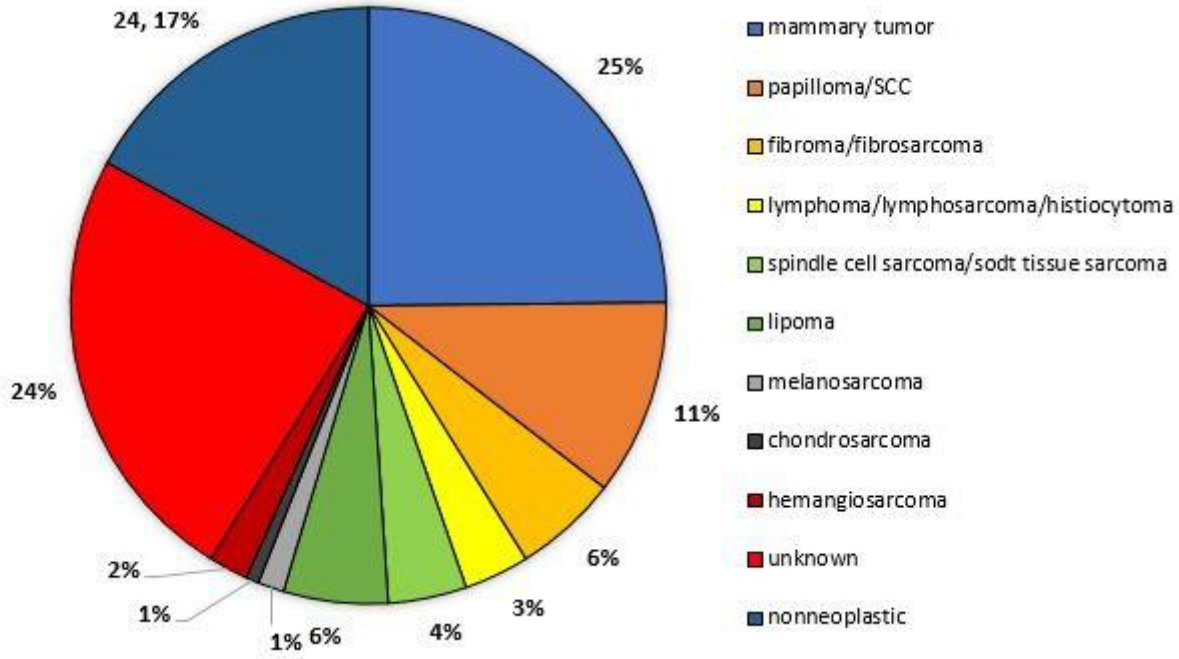

Figure 1. Proportion of neoplastic and non-neoplastic masses. Proportion of non-neoplastic masses included almost a quarter of total superficial masses cases collected.

Table 1. Summary of superficial masses collected in this study.

Diagnosis	Canine	Feline	Avian	Others	Total
Mammary tumor	21	12		2	35
Papilloma/SCC	1	8		6	15
Fibroma/fibrosarcoma	5	2		1	8
Lymphoma/lymphosarcoma/ histiocytoma	3	2			5
Spindle cell sarcoma/soft tissue sarcoma	2	1	1	2	6
Lipoma	2	3	2	1	8
Melanosarcoma (cutaneous)	1			1	2
Chondrosarcoma	1				1
Hemangiosarcoma	1		1	1	3
Unknown	22	12			34
Non-neoplastic					24

Sixty-two medical reports with a clinical diagnosis of suspected neoplasm were collected from the canine patient group; however, only 37 were histologically confirmed as neoplastic masses (Fig. 2). The most common tumor type in this group was mammary gland neoplasia (21 cases). Three of them were classified as atypical malignant tumors due to poor differentiation of cancer cells.

patients were diagnosed with mammary tumors at first examination. Information regarding potential surgeries or reproductive interventions was not available at the time of clinical examination. Three cases of gland-type tumors (adenoma and adenocarcinoma) were excluded from the category of mammary tumors due to lack of information about location of the mass and presence of atypical structural patterns.

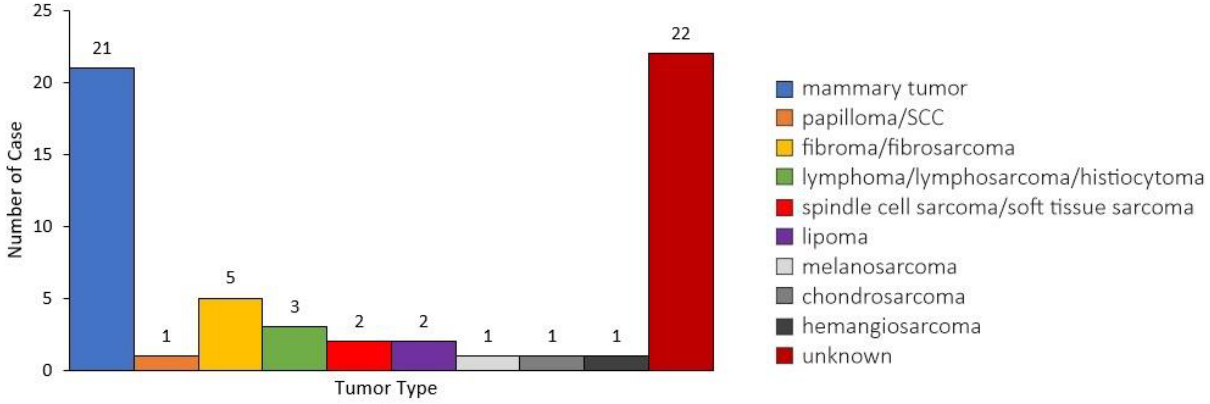

Figure 2. Case proportion of tumors in canine patients submitted in our facility from Malang Raya in the last five years. The most common specimen submitted for histopathologic examination was diagnosed as a mammary tumor with different subtypes. Other common tumors found in canine

In the feline cohort, 56 records and/or specimens of tumors were collected with 28 consisting of confirmed medical records and/or available archival tissue samples (Fig. 3). Twelve

The dataset also included 12 avian patients, although archival tissues were available for only four of them. Four reptilian patients were reported with masses on extremities and abdomen; however

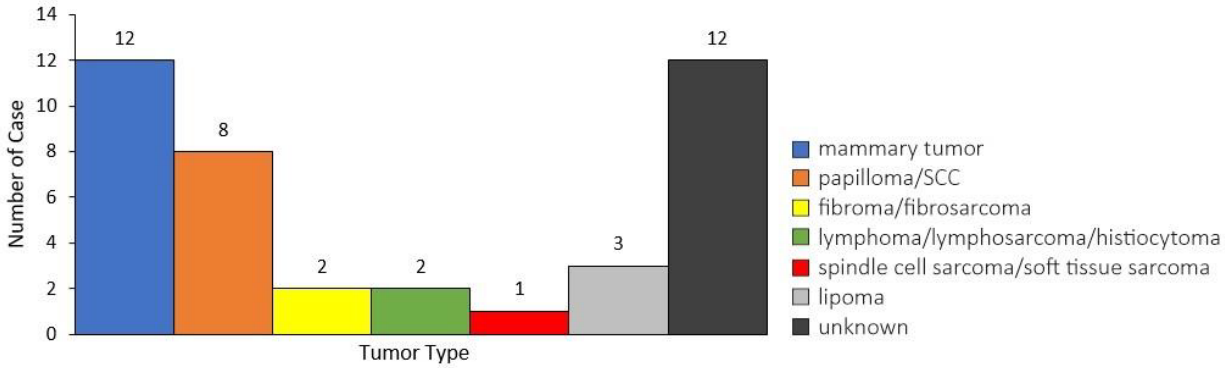

Figure 3. Case proportion of tumors in feline patients submitted in our facility from Malang Raya in the last five years. Similar to the trend observed in canine patients, mammary tumor was the most common reported tumor in feline patients.

no archival tissue was available. Two additional tumors were reported in a rabbit and a hamster and diagnosed as squamous cell carcinoma and adenocarcinoma, respectively.

3.2 Common Tumors and Subtypes

3.2.1 Squamous cell carcinoma (SCC)

Three cases of SCC were identified in canine, feline, and rodent patients, showing subtypes similar to human oral SCC. Histological variants were well-differentiated, moderately-differentiated, and papillary-type SCC (Fig. 4). Well-differentiated SCC consisted of islands and trabeculae of neoplastic epithelial cells invading the surrounding stromal tissue. Cells were round

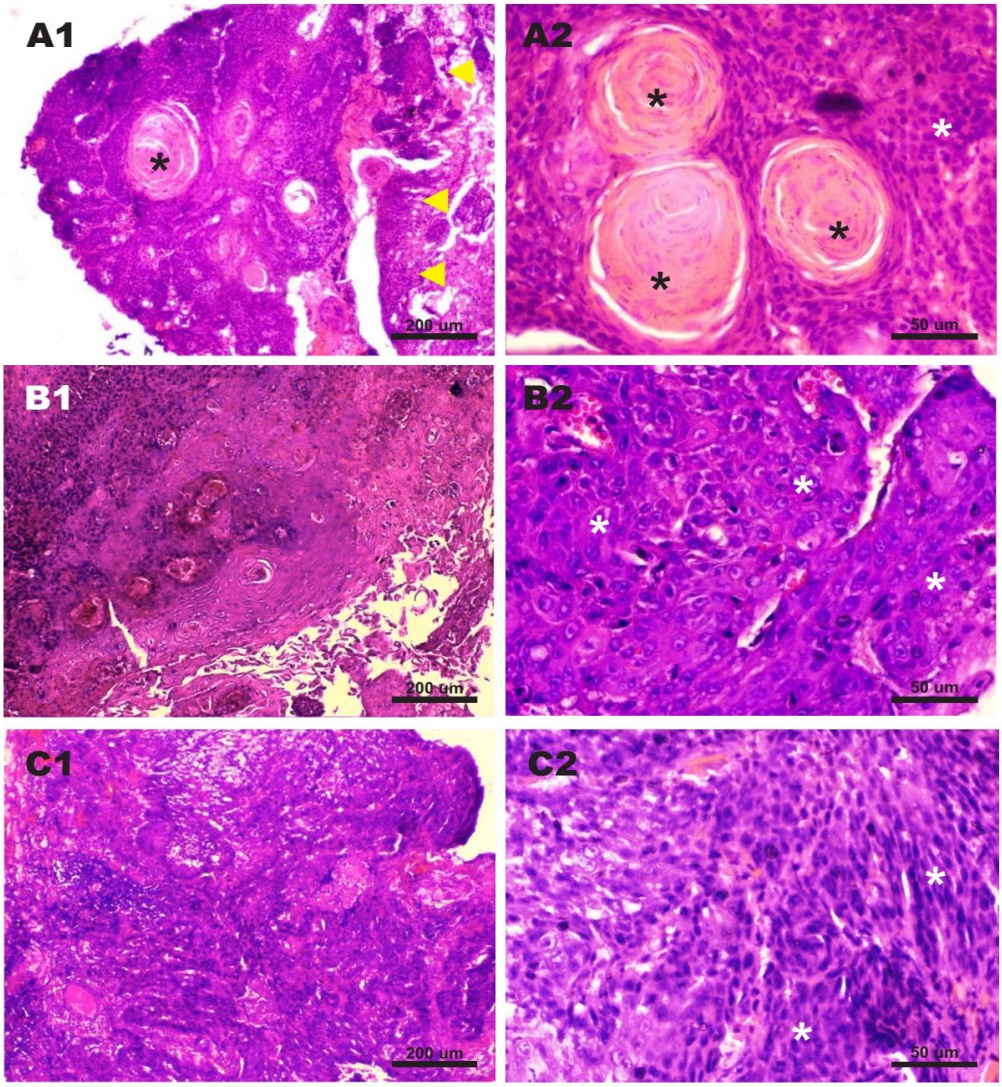

Figure 4. Histopathological architecture of putative subtype of squamous cell carcinoma in companion animal patients from Malang Raya. At least, three subtypes were reported as histopathological variation of SCC: well-differentiated (cat, A1-A2, 100x-400x magnification respectively), moderately-differentiated (hedgehog, B1-B2, 100x-400x magnification respectively), and papillary-type SCC (cat, C1-C2, 100x-400x magnification respectively). Black and white asterisks indicate keratin pearls and neoplastic epidermal cells consecutively. The structure is suspected of remaining epidermal layers (yellow arrowheads).

to polygonal with large vesicular nuclei, prominent nucleoli and moderate amount of eosinophilic cytoplasm. High anisocytosis and anisokaryosis were observed in the densely cellular areas, together with multifocal randomly scattered keratin pearls. Moderately-differentiated SCC presented similar characteristics to well-differentiated SCC, with minimal keratinization and lack of keratin pearls. Inverted papillary projections were present in papillary-type SCC. The papillary projections were formed by fibrovascular tissues with multiple layers of neoplastic epidermal cells.

3.2.2 Mammary adenocarcinoma

Adenocarcinoma was the most frequent tumor type in canine and feline species. Four subtypes were identified based on the classification system for canine and feline tumors from the Davis-Thompson Foundation: Solid type adenocarcinoma, mixed tumor with the proliferation of myoepithelial component/adenomyoepithelioma, and tubulopapillary carcinoma (Fig. 5). Solid-type adenocarcinoma (Fig. 5, A1-A2, C1-C2) consists of

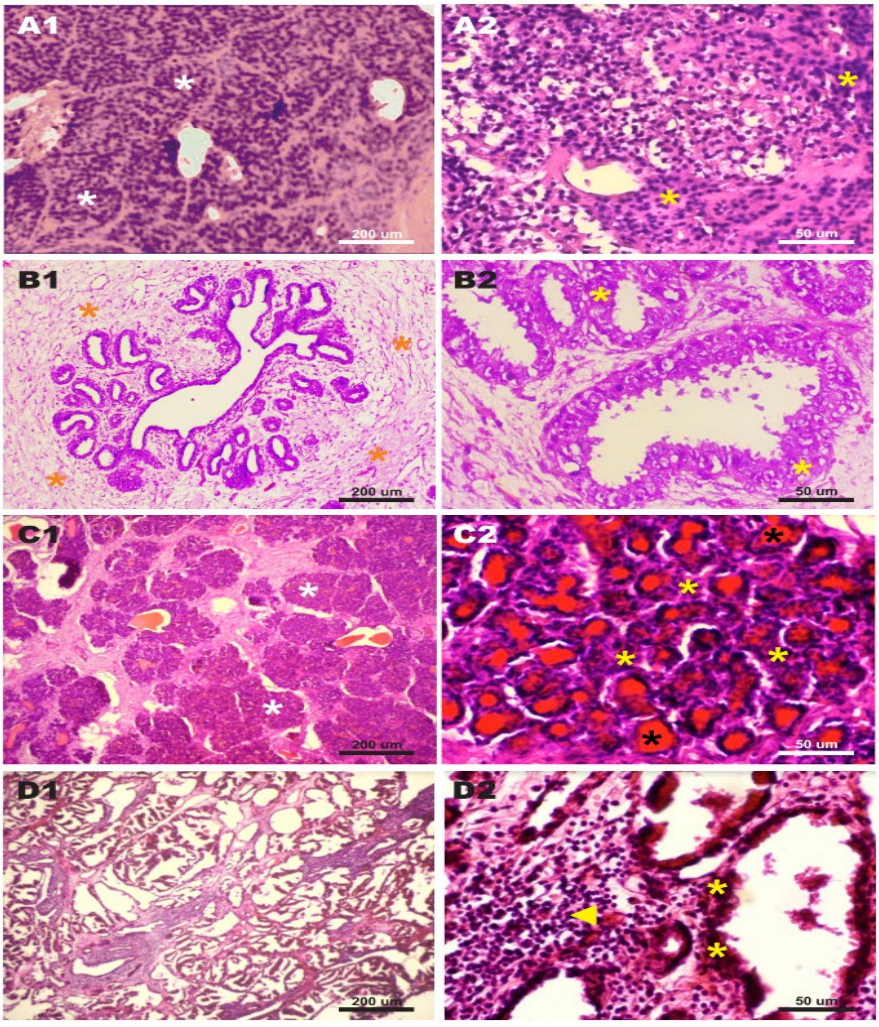

Figure 5. Histopathological architecture of putative subtype of mammary gland tumors in companion animal patients. Four subtypes were reported: solid type adenocarcinoma (dogs, A1-A2, C1-C2, 100x-400x magnification respectively) with some suspected secretory glands (black asterisks), mixed tumor (cat, B1-B2, 100x-400x magnification respectively) with proliferation of the myoepithelial component (orange asterisk), tubulopapillary carcinoma (dog, D1-D2, 100x-400x magnification respectively). Lower magnification shows nest formation (white asterisks) in solid type adenocarcinoma. Various epithelial cell proliferation patterns are present (yellow asterisks).

a dense population of epithelial cells supported by scant stroma, with a nest of invading cells that form solid masses with rare tubular formations. This form of tumor has polygonal to oval shaped cells with high number, exhibiting high anisokaryosis and anisocytosis. Nuclei are oval and often hyperchromatic with prominent nucleoli. Thin septa-like layers separated the hypercellular clusters and the outer margins showed irregular contours with subtle connective tissue capsulation (suggesting an *in-situ* malignancy). In some cases, a group of malignant cells were infiltrating adjacent tissues including fat tissues.

Mixed tumor with myoepithelial proliferation (Fig. 5, B1-B2) showed a mixture of more than two cell populations and variable amount of fibrous stroma. The first population consisted of tubules lined by cuboidal to columnar cells with moderate anisocytosis and anisokaryosis. The second population included spindle cells with poorly demarcated cell borders. Higher magnification revealed disorganized tubule formations, with pleomorphic arrangements of epithelial cells and projections extending into the luminal part of the tubules. Thin loose connective tissue was observed at the margins of the masses.

The last subtype of mammary carcinoma found in this study was tubulopapillary adenocarcinoma (Fig. 5, D1-D2), characterized by tubular and papillary structures. Higher magnification revealed adenomer-like structures with broad luminal space and simple cuboidal-columnar epithelial cells. The interstitial areas contained fibrovascular tissue and hypercellular clusters with moderate numbers of lymphocytes. Various thicknesses of connective tissues were present at the margin of the mass.

3.3 Non-neoplastic Superficial Masses

Records and specimens containing non-neoplastic superficial masses were assessed, which constituted 24.17% of the masses identified. Various types of non-neoplastic lesions were observed in including: nodular-granulomatous dermatitis with adnexal inflammation (n=14), pseudomycetoma - deep fungal mycosis (n=5), panniculitis (n=3), glandular hyperplasia (n=1), and cysts (n=1). Histopathological features of these non-neoplastic superficial masses are illustrated in Figure 6.

4. Discussion

Most submitted neoplastic masses in our histopathology service originated from superficial organ systems including skin and mammary glands. In all patient species, mammary gland tumor was the most common reported neoplasm followed by papilloma-SCC. However, this finding apparently is not reflecting the natural prevalence of neoplastic cases. There is a tendency that clients and or clinician will be more aware of visible superficial masses rather than profound or visceral masses contained in patients. According to our experience, visceral masses were more frequently encountered during necropsy in some deceased patients. Therefore, the natural-occurred (no experimental) animal tumor prevalence in Malang Raya is possibly greater than we expected.

Tumors can exhibit a wide range of structural and cellular variations depending on their location and pathogenesis. While many tumor subtypes are still under investigation, their clinical significance is increasingly in concern, with different subtypes leading to different outcomes and prognoses [9]. The cellular composition of a neoplastic mass plays a critical role in determining its aggressiveness, physical characteristics, functional behavior and prognosis, which are further influenced by cell communications through specific signaling proteins. As a result, molecular based identification methods for tumor subtypes are being proposed for integration into clinical practice. Katz *et al.* in 2018 [10] reported that sarcomas exhibit a wide range of responses to therapeutic agents. Most sarcomas are sensitive to chemotherapy, while others show better response to a combination of chemotherapy and anti-angiogenic agents, and others are resistant to chemotherapy but still responsive to anti-angiogenic agents or targeted therapies. Immunotherapy has also been developed to inhibit tumor development of poorly differentiated sarcoma types by targeting immune checkpoints.

Tumor subtype determination has become the foundation of protein-based treatments such

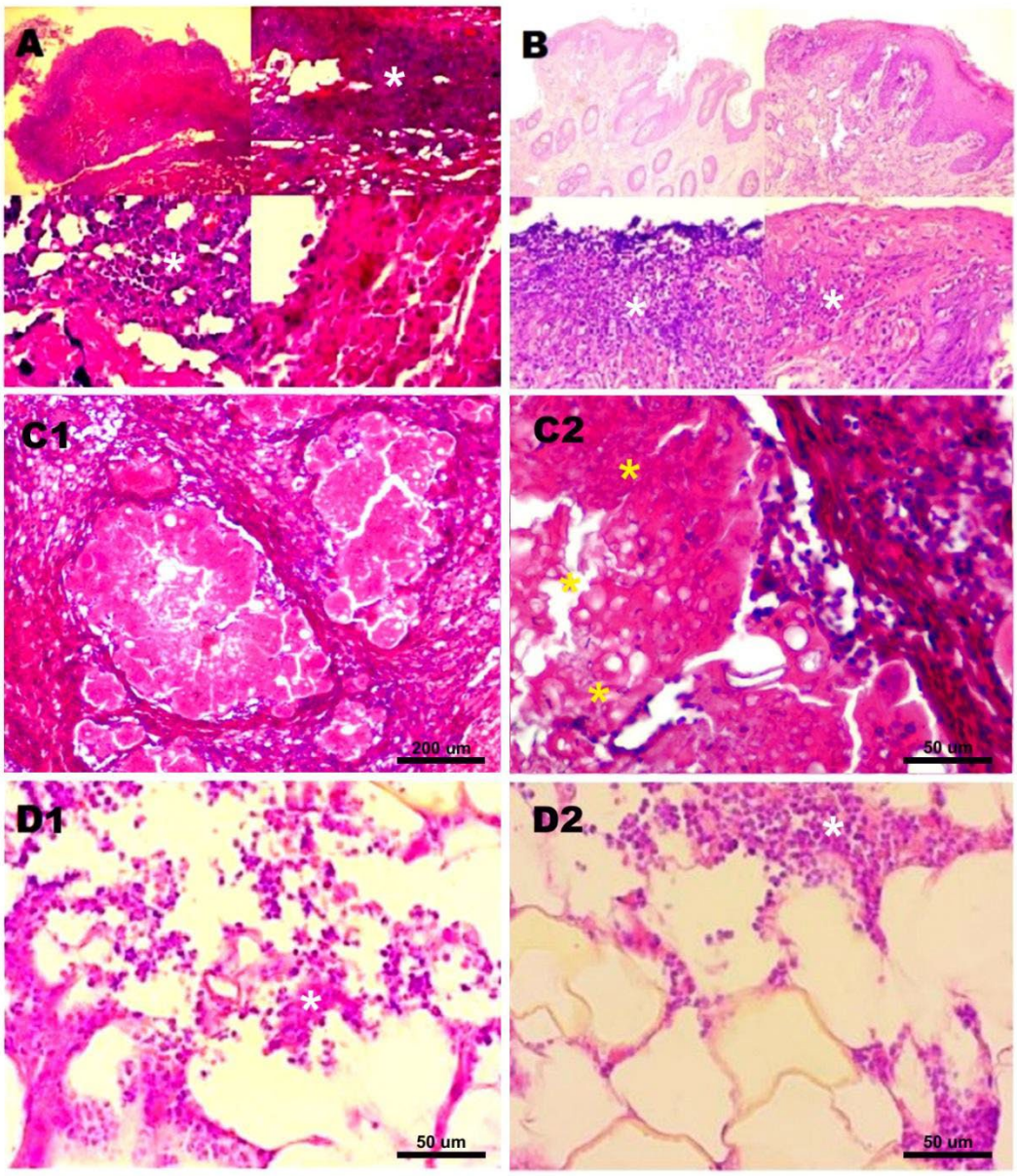

Figure 6. Most common non-neoplastic masses submitted: subcutaneous abscess, with granulomatous dermatitis (cats and dogs, A,B, various magnifications), pseudomycetoma (cat, fungal hyphae in yellow asterisks)/deep fungal mycosis (cat, C1-C2, 100x-400x magnification respectively), panniculitis (dog, D1-D2, 100x-400x magnification respectively). Hypercellularity containing clusters of inflammatory cells were present (white asterisks).

as hormonal therapy and immunotherapy. Ambs in 2010 [11] highlighted that biologically diverse cancers, such as those affecting the mammary gland, can lead to different outcomes. This has opened new avenues for developing cancer therapies tailored to specific tumor subtypes, as different subtypes exhibit distinct behaviors and structural characteristics [12, 13].

Currently, histopathology remains the most accessible diagnostic standard for identifying cancer subtypes, however clinical decision-making based solely on histopathology findings can sometimes be inconclusive. Histopathological assessment of tumor subtypes involved parameters such as cell shapes, pleomorphism, cellular composition, differentiation rate, and overall architecture. For example, SCC has numerous subtypes based on differentiation rates and histological features. Pereira *et al.* in 2007 [14] classified SCC into well, moderately, and poorly differentiated subtypes, while Cassarino *et al.* in

2006 [15] grouped SCC by risk potential into low, intermediate, high, and indeterminate malignant levels. Hemangiosarcomas (HSA) is classified into several subtypes based on histopathology (capillary, cavernous, and solid subtype) [16, 17]. In some cases, conventional haematoxylin-eosin staining is sufficient for clinical practice, especially for evaluating excision margins. Our contributors reported two recurrent cases of tumor, which were consistent with incomplete margin excision of the masses marked by absence of tissue transition between neoplastic mass and normal adjacent tissues or capsules.

Molecular profiling of tumor subtypes holds the potential to improve diagnostic accuracy and therapy recommendations. Studies exploring molecular alterations and signaling pathways within cancer cells and their microenvironment provide deeper insights into cancer types and their potential targets for treatment [11]. Retrospective studies on cancer prognosis, such as those on colorectal and gastric cancers, have already explored the implications of tumor subtypes and consensus molecular profiles [18, 19, 20].

Tumor molecular profiling involves studying signaling pathways, which play a key role in the pathogenesis of neoplastic events and influence prognosis. Certain proteins drive cell proliferation, affecting tumor size, vascularization, and metastatic potential. Perou *et al.* in 2000 [21] and Sorlie *et al.* in 2001 [22] identified five subtypes of mammary gland cancer according to gene expression profiles, including two estrogen receptor-positive (ER+) subtypes (luminal A and B), and three ER-negative subtypes (HER2-positive, and ER-negative subtypes). These subtypes are associated with different clinical outcomes and therapeutic options. Ambs in 2010 [11] emphasized that protein expression analysis by IHC can guide therapy selection, particularly for systemic adjuvant and gene repair strategies.

Genetic aberrations are strongly linked to certain tumors such as liposarcomas. Dei Tos, in 2014 [23] reported that specific liposarcoma subtypes, including well differentiated, de-differentiated, myxoid, and pleomorphic, are associated with distinct molecular alterations. Similarly, the WHO identified lymphoma as a type of tumor with the largest number of subtypes, each characterized by unique protein expressions. Valli *et al.* in 2011 [24] reported that several protein markers such as CD79a, CD20, CD3, anaplastic lymphoma kinase (ALK), and CD30 could effectively differentiate lymphoma subtypes in dogs.

Our finding also revealed a relatively high prevalence of non-neoplastic masses (24.17%) within our abnormal superficial masses cohort. These cases led to misdiagnosis, with practitioners initially suspecting neoplastic masses before submitting them for histopathology investigation. For example, deep fungal mycosis might mimic the metastatic feature of neoplastic mass due to recurrent mass growth after first excision. Thick connective tissue capsules in granulomas may have been falsely interpreted as tumors due to the hard consistency of mass margins. These findings highlight the potential for misdiagnosis when relying solely on external examination of masses. Histopathology remains the definitive method for determining the nature of these masses, guiding therapeutic actions and improving patient outcomes. However, diagnostic accuracy is not solely reliant on sophisticated facilities and testing.

5. Conclusion

A five-year analysis of medical records from companion animals diagnosed with tumors revealed mammary tumors as the most common neoplasia in the study population. Considering the various differential diagnoses of a merely superficial mass, it is crucial to adopt a comprehensive strategy and clinical reasoning approach for diagnosis determination. Developing a structured thinking algorithm can help ensure diagnostic accuracy. By consciously avoiding cognitive biases and maintaining a systematic approach to clinical decision-making, we can minimize the risk of misdiagnosis, ensuring more reliable outcomes and preventing malpractice in the process.

Ethics Approval and Consent to Participate

This study was performed under approval of the research ethics committee of Universitas Brawijaya Bioscience Institute (document number No: 041-KEP-UB-2023).

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Conflict of Interest

The authors declare no conflict of interest.

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