



**UNIVERSITI PUTRA MALAYSIA**

**RETROSPECTIVE STUDY ON CANINE NEOPLASIA CASES  
SUBMITTED TO THE  
VETERINARY LABORATORY SERVICES UNIT,  
UNIVERSITI PUTRA MALAYSIA  
FROM YEAR 2015 TO 2019**

**SAFWA FARHANAH BINTI ZAINAL ABIDIN**

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**VETERINARY LABORATORY SERVICES UNIT,**

**UNIVERSITI PUTRA MALAYSIA**

**FROM YEAR 2015 TO 2019**

**SAFWA FARHANAH BINTI ZAINAL ABIDIN**

A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

in partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

43400 UPM Serdang, Selangor Darul Ehsan.

2020/2021

**CERTIFICATION**

It is hereby certified that we have read this project paper entitled “Retrospective Study on Canine Neoplasia Cases Submitted to The Veterinary Laboratory Services Unit, Universiti Putra Malaysia from Year 2015 To 2019”, by Safwa Farhanah Binti Zainal Abidin and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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## DEDICATIONS

**This project paper is dedicated to**

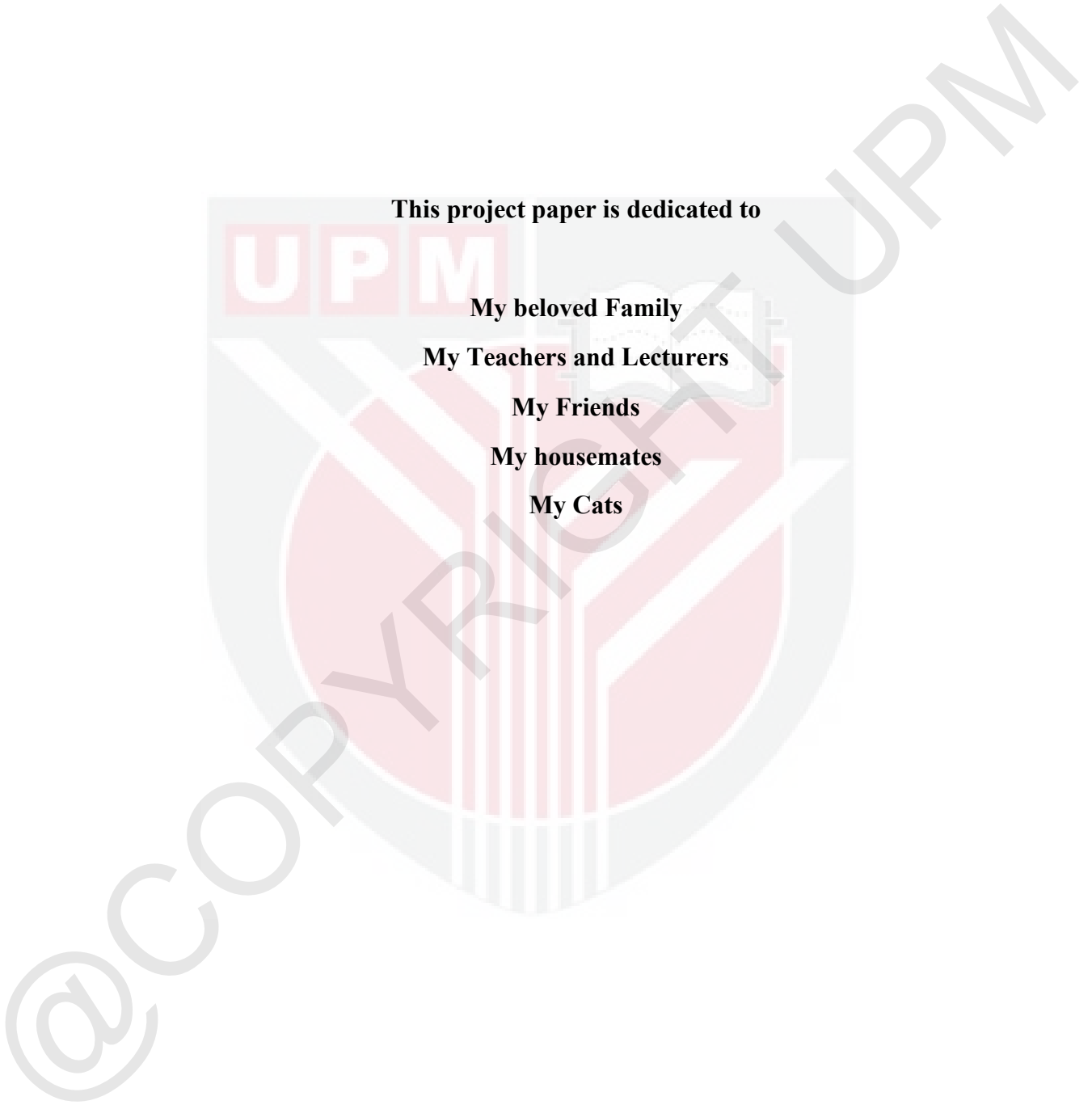
**My beloved Family**

**My Teachers and Lecturers**

**My Friends**

**My housemates**

**My Cats**



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Praise be to Allah for this opportunity to conduct this project. I would like to express my greatest gratitude to my supervisor Prof. Dr. Rasedee Abdullah for his continuous support, patience, great advice, immense knowledge, and assistance towards the success of this study.

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## TABLE OF CONTENTS

	<b>Page</b>
<b>TITLE</b>	<b>i</b>
<b>CERTIFICATION</b>	<b>ii</b>
<b>DEDICATIONS</b>	<b>iv</b>
<b>ACKNOWLEDGEMENTS</b>	<b>v</b>
<b>TABLE OF CONTENTS</b>	<b>vi</b>
<b>LIST OF TABLES</b>	<b>ix</b>
<b>LIST OF FIGURES</b>	<b>x</b>
<b>LIST OF ABBREVIATIONS</b>	<b>xi</b>
<b>ABSTRAK</b>	<b>xii</b>
<b>ABSTRACT</b>	<b>xiv</b>
<b>1.0 INTRODUCTION</b>	<b>1</b>
<b>2.0 LITERATURE REVIEW</b>	
2.1 Neoplasia in canine	
2.1.1 Age	<b>3</b>
2.1.2 Breed	<b>4</b>
2.1.3 Sex	<b>5</b>
2.1.4 Common sites of tumour	<b>5</b>
2.1.5 Common types of tumour	<b>7</b>
2.1.6 Differential diagnosis	<b>8</b>

2.2 Diagnostic cytology	
2.2.1 Cytology samples in tumour sampling	9
2.2.2 Method of sampling	9
2.2.3 Staining method	10
2.2.4 Benefits and limitations	10
2.2.5 Discrepancies between cytology and histopathology	11
<b>3.0 METHODOLOGY</b>	
3.1 Data retrieval	12
3.2 Statistical analysis	12
<b>4.0 RESULTS</b>	
4.1 Cases	13
4.2 Breed	14
4.3 Age	15
4.4 Sex	16
4.5 Common sites of tumour	16
4.6 Common types of tumour	18
<b>5.0 DISCUSSION</b>	19
<b>6.0 CONCLUSION</b>	20
<b>7.0 LIMITATION OF STUDY</b>	21
<b>8.0 RECOMMENDATIONS</b>	21

**9.0 REFERENCES**

**23**

**10.0 APPENDICES**

**26**



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**LIST OF TABLES**

<b>TABLE</b>		<b>Page</b>
Table 1	Common stains used in cytological diagnosis of tumours	<b>10</b>



**LIST OF FIGURES**

<b>Figure</b>		<b>Page</b>
<b>Figure 1</b>	Occurrence of neoplasia in dogs subjected to cytological diagnosis at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.	<b>13</b>
<b>Figure 2</b>	Frequency of breed of dogs presented and diagnosed with neoplasia at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.	<b>14</b>
<b>Figure 3</b>	Age of dogs presented and diagnosed with neoplasia at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.	<b>15</b>
<b>Figure 4</b>	Sex distribution of canine cases presented and diagnosed with or without neoplasia at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.	<b>16</b>
<b>Figure 5</b>	Site distribution of tumours in canine cases presented and diagnosed at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.	<b>17</b>
<b>Figure 6</b>	Distribution of tumour types in dogs presented for diagnosis at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.	<b>18</b>
<b>Figure 7</b>	Round cell tumour showing large lymphocytes and mitotic figures.	<b>26</b>
<b>Figure 8</b>	Epithelial Neoplasm showing nuclear molding and macronucleolus	<b>26</b>
<b>Figure 9</b>	Mesenchymal tumour showing pleomorphic cells and pink extracellular matrix.	<b>27</b>
<b>Figure 10</b>	Lipoma.	<b>27</b>
<b>Figure 11</b>	Lymphocytic leukemia.	<b>28</b>

**LIST OF ABBREVIATIONS**

FNA	Fine Needle Aspiration
UVH	University Veterinary Hospital
VLSU	Veterinary Laboratory Services Unit
UPM	Universiti Putra Malaysia



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**ABSTRAK**

**KAJIAN RETROSPEKTIF TENTANG NEOPLASIA PADA ANJING  
BERDASARKAN KES YANG DIHANTAR KEPADA  
UNIT PERKHIDMATAN MAKMAL VETERINAR,  
UNIVERSITI PUTRA MALAYSIA,  
PADA TAHUN 2015 SEHINGGA 2019**

**Oleh**

**Safwa Farhanah Binti Zainal Abidin**

**2020**

**Penyelia: Professor Dr. Rasedee Abdullah**

**Penyelia bersama: Associate Professor Dr. Gayathri Thevi Selvarajah,**

**Dr. Azalea Hani Othman**

Insidens kes neoplasia pada anjing semakin meningkat. Walau bagaimanapun, tidak ada rekod mengenai prevalens neoplasia pada anjing di Malaysia. Justeru, objektif kajian ini ialah penentuan prevalens dan faktor risiko berkaitan neoplasia pada anjing yang di rujuk kepada Hospital Veterinar dan Unit Perkhidmatan Makmal Veterinar (VLSU), Universiti Putra Malaysia pada tahun 2015 hingga 2019. Data pesakit seperti

umur, baka, jantina, dan lokasi serta jenis tumor diperoleh daripada rekod Makmal Hematologi dan Biokimia Klinikal, VLSU. Daripada 745 kes, 432 telah didiagnosis mengidap neoplasia. Antara baka yang paling kerap didiagnosis mengidap neoplasia, mengikut kekerapan, ialah baka campuran tempatan (n = 124), Shih Tzu (n = 52), Golden Retriever (n = 42), German Shepherd (n = 24), Rottweiler (n = 23), Beagle (n = 16), Standard Schnauzer (n = 14), Labrador (n = 14), Terrier (n = 13), Poodle (n = 13), Doberman (n = 11), Husky (n = 10), Spitz (n = 8), dan Pomeranian (n = 5). Purata umur anjing yang menghidap neoplasia adalah  $8.38 \pm 3.0$  tahun dan dalam lingkungan umur 1 hingga 18 tahun. Tumor yang paling kerap didiagnosis ialah tumor sel bulat (46.76%), diikuti dengan tumor sel epitelium (32.18%), tumor sel mesenkima (11.57%), lipoma (0.23%), dan lain-lain (7.85%). Beberapa kes leukemia (1.39%) juga telah dicatat. Lokasi tumor yang dilaporkan dalam kajian ini adalah kelenjar (20.60%), kawasan oral (4.86%), penis (3.94%), trunkus (3.70%), kawasan nasal (3.01%), kawasan intra-abdomen (2.78%), digit (2.55%), prostat (2.31%), vulva (2.31%), and pundi kencing (2.31%). Tumor pelbagai lokasi dan diagnosis lain, masing-masing terdiri daripada 23.70% dan 27.75% daripada keseluruhan kes. Kesimpulannya, kajian ini menunjukkan bahawa tumor yang paling kerap didiagnosis pada anjing ialah tumor sel bulat dan sel epitelium, manakala lokasi paling kerap berlakunya tumor pada anjing ialah nodus limfa. Kajian tidak menunjukkan sebarang perkaitan antara prevalens neoplasia dengan umur atau jantina anjing. Anjing baka campuran tempatan paling kerap didiagnosis mengidap neoplasia.

**Kata kunci:** anjing, neoplasia, baka, umur, jantina.

**ABSTRACT**

**RETROSPECTIVE STUDY ON CANINE NEOPLASIA CASES SUBMITTED  
TO THE VETERINARY LABORATORY SERVICES UNIT,  
UNIVERSITI PUTRA MALAYSIA FROM YEAR 2015 TO 2019**

**By**

**Safwa Farhanah Binti Zainal Abidin**

**2020**

**Supervisor: Professor Dr. Rasedee Abdullah**

**Co-supervisors: Associate Professor Dr. Gayathri Thevi Selvarajah,**

**Dr. Azalea Hani Othman**

The incidence of canine neoplasia is on the increase. However, there is no record on the prevalence of neoplasia in dogs in Malaysia. Therefore, the objectives of the study were to determine the prevalence and associated risk factors of neoplasia in dogs presented to the University Veterinary Hospital (UVH) and Veterinary Laboratory Services Unit (VLSU), Universiti Putra Malaysia (UPM) for years 2015 to 2019. Patient data including age, breed, sex, and site and type of tumours were obtained from the record of cases submitted to the Haematology and Clinical Biochemistry Laboratory, VLSU. Out of 745 cases, 432 were diagnosed with neoplasia. The most commonly presented breeds

with neoplasia, in order of frequency, were the Local Mixed Breed (n= 124), Shih Tzu (n= 52), Golden Retriever (n= 42), German Shepherd (n= 24), Rottweiler (n=23), Beagle (n=16), Standard Schnauzer (n=14), Labrador (n=14), Terrier (n=13), Poodle (n=13), Doberman (n=11), Husky (n=10), Spitz (n=8), and Pomeranian (n=5). The mean age of dogs with neoplasia was  $8.38 \pm 3.0$  years with age range of 1 to 18 years. The most frequent tumours diagnosed were round cell tumours (46.76%), followed by epithelial cell tumour (32.18%), mesenchymal cell tumour (11.57%), lipoma (0.23%), and others (7.85%). A few cases of leukemia (1.39%) were also recorded. The tumour sites reported in this study, in order of frequency were the lymph nodes (20.60%), oral region (4.86%), penis (3.94%), trunk (3.70%), nasal region (3.01%), intra-abdominal region (2.78%), digits (2.55%), prostate (2.31%), vulva (2.31%), bladder (2.31%). Multiple sites and other diagnosis made 23.70 and 27.75% of all cases, respectively. In conclusion, the study showed that the most frequent tumours diagnosed in dogs were round and epithelial cell tumours while the most frequent site was the lymph nodes. There was no association between the prevalence of neoplasia with age or sex of dogs. Local mixed breed dogs were most frequently diagnosed with neoplasia.

**Keywords:** canine, neoplasia, breed, age, sex.

## 1.0 INTRODUCTION

Neoplasia is abnormal growth of cells, which can be either be benign or malignant. Benign neoplasia grows slowly and do not spread to other parts of body. Malignant neoplasia or cancer can invade surrounding tissues and spread to distant sites through the bloodstream and lymphatics. Neoplastic cells may be premalignant or malignant. Premalignant neoplasms have the potential of becoming aggressive and malignant, which is typically associated with apparent and often rapid damage of tissues and organs (Valent et al., 2017).

Neoplasms can also occur at various sites on the body, including the body, head, neck, bones, and internal organs. Neoplasms are characterised by high rate of cell division and loss of adhesive properties of the cell membrane. Malignant neoplasms occur when normal cells began to become less adhesive and separate from surrounding cells to invade other tissues and organs (Markert, 1968).

Classification of the tumours is based on the cell morphological characteristics and tissue-origin. Tumours, according to cytological identification, can be the mesenchymal, round cell, or epithelial tumour. In blood, the cancers are leukemias, which are of hematopoietic origin.

Neoplasias are more commonly seen in old than young dogs. Spontaneous neoplasms are also more common in dogs than in other domesticated animals, particularly

in the geriatrics (Prier and Brodey, 1963). Certain breeds are also predisposed to the development of neoplasms. The risk factors for neoplasia include age, sex, and breed.

Neoplasia in dogs are on the increase and it is not uncommon to find an animal with multiple primary tumours (Prier and Brodey, 1963). The increased incidence of neoplasia now observed in veterinary hospitals and clinics may also be due to the improved testing methods that had significantly increased the effectiveness of tumour detection.

The objectives of the study are to determine the:

- 1) rate of occurrence of neoplasia in dogs based on cytological diagnosis.
- 2) distribution in neoplasia in dogs based on age, breed, sex, and type and site of tumors in dogs.
- 3) type of canine tumours diagnosed at the Veterinary Laboratory Services Unit, Universiti Putra Malaysia (VLSU-UPM).

## **2.0 LITERATURE REVIEW**

### **2.1 Neoplasia in dogs**

#### **2.1.1 Age**

The average age of dogs with primary liver tumour was reported to be 11.9 years, while the average age of dogs with metastatic liver cancers was 11.4 years (Vilkovyskiy et al., 2020). According to Schneider (1978), the age range of dogs for the acquisition of skin neoplasm, except histiocytoma, is between 6 to 14 years. The average age for the acquisition of lipoma is 8.0 years, for anal gland neoplasms, 10.9 years (Rothwell et al., 1987), and for lymphomas between 6 to 9 years (Yau et al., 2017). The occurrence of canine lymphoma increases with age and frequently affecting middle-aged dogs aged 6 to 8 years (Dorn et al, 1967; Teske, 1994). Some dogs with lymphomas of nodular pattern appeared to be younger than those with diffuse lymphomas, and the survival period for dogs with nodular lymphomas were shorter than those with the diffused type (Madewell, 1985).

The sarcomas, osteosarcoma or chondrosarcoma of the rib is not common in dogs, however, if they occur, young dogs aged 1.5 to 4 years are most affected. Limb bone sarcoma usually affects dogs aged 6 to 8 years (Cotchin, 1984). Another study suggested that lymphosarcomas and osteosarcomas in dog peaks at ages between 7 to 10 years (Cohen et al., 1974).

In a study on 130 dogs, it was shown that 15% of histiocytoma cases were in dogs less than 1 year of age while 19% were between 1 to 2 years old. Most histiocytomas (60-80%) occur in dogs less than 3 years old, although they can occur at any time during their lifespan (Rothwell et al., 1987; Moore and Affolter, 2005). Epithelial and melanocytic neoplasms were commonly diagnosed in dogs aged between 5 to 15 months (Aleksić-Kovačević et al., 2005).

### **2.1.2 Breed**

Certain breeds of dogs are more prone to the development of certain types of neoplasms (Prier and Brodey, 1963). Purebred dogs were substantially over-represented in cases of malignant skin melanoma and mammary gland tumour. These tumours have high age-specific incidence rates in purebred dogs (Dorn et al., 1968a and b). The probability of developing mammary gland tumours are also thrice as high in small than large breed dogs (Grüntzig et al., 2016).

The Australian Cattle dog and Doberman are highly predisposed to lymphomas (Yau et al., 2017) while the Boxer dog breed is more vulnerable to mast cell tumour (Ihrke et al., 2005). Boxers also have a high probability of developing osteosarcomas, lymphosarcomas, testicle, and gingival neoplasia (Cohen et al., 1974), cutaneous malignancies, and connective tissue cancers (Dorn et al., 1968a and b). Large breed dogs such as Great Danes and St. Bernards are susceptible to osteosarcomas. Highly pigmented breeds such as Cocker Spaniels, Scotch Terriers, and Airedale Terrier are predisposed to

melanomas (Cotchin, 1984) and Cocker Spaniels to adnexal skin tumours (Prier and Brodey, 1963). Aleksić-Kovačević et al., (2005) reported that squamous cell carcinoma was mostly diagnosed in Rottweilers, Giant Schnauzers, German Shepherds, and standard poodles.

### **2.1.3 Sex**

The overall prevalence of neoplasia is higher in female than male dogs, partly due to the high incidence of mammary gland tumours in bitches (Noury et al., 2020). Female dogs also have a higher tendency than males to acquire adenoma and adenocarcinoma while at lower risk to develop hemangioma and haemangiosarcoma, and squamous cell carcinoma (Grüntzig et al., 2016). On the other hand, males are at greater risk of getting mouth and pharynx cancers than female dogs (Dorn et al., 1968a and b). Skin tumours are also more prevalent in female than male dogs, irrespective of breed (Aleksić-Kovačević et al., 2005), while basal cell carcinoma and sebaceous adenoma were more prevalent in male than female dogs. Among female dogs, in order of frequency, mammary gland tumour, cutaneous melanoma, and connective tumours were the most prevalent (Dorn et al., 1968a and b).

### **2.1.4 Common sites of tumour**

While neoplasms are known to occur all over the body of the dog, some sites were reported to be more prone to the development tumours than others (Prier and Brodey, 1963). The most common sites for neoplasm in dogs are the skin and subcutaneous tissues

(Dorn et al., 1968a and b). Aleksić-Kovačević et al. (2005) stated, in the study on 211 canine cutaneous neoplasm cases, 49 (23.22%) were on skin of the head and neck, 53 (25.11%) at the limbs, 33 (15.64%) at the trunk, 33 (15.64%) at the anal and para-anal region, and 6 (2.84%) on the tail. Squamous cell carcinoma was also reportedly on the skin of the leg, trunk, head and neck, as well as on the poorly pigmented areas of the skin. Basal cell carcinoma was frequently found on the skin on head and neck regions, while the common sites of sebaceous adenoma were the head, neck, and limbs. Lipoma, histiocytoma, and mast tumour were commonly found on the skin and soft tissues. The mammary gland, and alimentary and respiratory tracts were reported to be prone to the development of adenocarcinomas. Rothwell et al. (1987) reported that the tail, perineum, prepuce and adjacent of anus were the general sites for perianal gland neoplasms. The trunk was usually the site for the occurrence of fibrosarcoma, squamous cell carcinomas, and lipomas, and the limbs for haemangiopericytomas.

Prostate cancers can aggressively metastasise to regional lymph nodes and lungs (Leav et al., 1968; Waters et al., 1998). Most tumours commonly metastasise haematogenously to the liver (30%). That study showed that more than 80% of all liver neoplasms were found in the left medial or left lateral lobes of the liver (Vilkovyskiy et al., 2020). Cotchin (1984) reported that glandular carcinomas are rare in dogs, if they occur, it will in the fundus and pylorus of the alimentary tract.

### 2.1.5 Common types of tumour

The most common tumours in dogs are mammary gland tumours followed by neoplastic and non-neoplastic skin tumours (Aleksić-Kovačević et al., 2005). Mammary tumours normally appear as clusters of cells with circular to the oval nucleus and clear cytoplasmic boundaries (Bhalla et al., 2011).

In clinical practice, haematopoietic and lymphatic system tumours are among the most often identified canine neoplasms and they pose great diagnostic and therapeutic challenges to clinicians (Madewell, 1985). The Royal Veterinary College reported that squamous cell carcinoma affecting the faucial tonsil as a common tumour in dogs (Cotchin, 1984).

In bone tumours, the incidence of osteosarcomas is nearly 10 times more than chondrosarcomas (Prier and Brodey, 1963). Bone tumours in dogs are mostly malignant; however, tumour metastasis were rarely reported (Cotchin, 1984).

Among malignant skin tumours in dogs, mast cell tumour was the most commonly reported (London and Seguin, 2003; Misdorp, 2004; Sledge et al., 2016). However, other studies suggested that haemangiosarcoma is most often diagnosed with skin neoplasm in dogs (Chikweto et al., 2011). The annual incidence rate of cutaneous histiocytoma accounted for 377 cases out of 100,000 dogs. Other less common cutaneous tumour include lipoma, adenoma, soft tissue sarcoma, mast cell tumour, and lymphosarcoma. The

incidence of epithelial tumours were higher than mesenchymal in dogs (Dobson et al., 2002).

In lymph nodes, lymphoma, metastatic neoplasms, example carcinoma and sarcoma were the major forms of tumours identified (Ku et al., 2017).

#### **2.1.6 Differential diagnosis**

In tumours involving lymphocytes, cancers that begin and predominate in the bone marrow are leukemias, while those originating in the lymph nodes are lymphomas and lymphosarcomas (Madewell, 1985). Lymphadenopathy may occur in chronic lymphocytic leukemia. Therefore, prominent lymphadenopathy and blood and bone marrow lymphocytosis may be associated with chronic lymphocytic leukemia or diffused, well-differentiated lymphocytic lymphoma (Mintzer and Hauptman, 1983).

In one study, among 225 skin masses from 207 dogs, the prevalence of skin neoplasms was 72%. Moreover, 15.6% were diagnosed as non-neoplastic tumours, and 12.4% were inflammatory conditions. The Inflammatory conditions comprised of pyogranulomatous dermatitis (50%), nodular and diffuse chronic dermatitis (25%), deep and superficial pyoderma (10.7%), sterile granuloma (10.7%), and allergic dermatitis (3.6%) (Chikweto et al., 2011).

## **2.2 Diagnostic cytology**

### **2.2.1 Cytology samples in tumour diagnosis**

Cytology involves the microscopic evaluation of cells and tissues (Bhalla et al., 2011). The type of samples to be obtained for cytological diagnosis is dependent on the tissue and organ location of the tumour. In cases of suspected respiratory neoplasms, the cytology samples to be collected include nasal flushes, transtracheal aspirates, bronchial brushings, and lung aspirates. For reproductive system tumours, the samples to be collected include prostatic fluids, testicular aspirates, vaginal scrapings, and mammary discharges (Wellman, 1990).

### **2.2.2 Sampling method**

Fine needle biopsy that can be achieved with or without aspiration are commonly used to obtain samples from the lymph node, salivary gland, mammary gland and other subcutaneous glandular organs, and proliferative lesions (Cowell et al., 2008). Not all biopsies would yield an adequate number of cells for diagnosis. For example, aspirates or histiocytoma imprints usually yield very few cells, although it may be enough to provide clues to the diagnosis (Duncan and Prasse, 1979). Fine-needle aspiration of the lymph nodes can provide good cytological evidence of non-Hodgkin's lymphoma in dogs (Teske and Van Heerde, 1996).

### 2.2.3 Staining method

The general type of stains used in cytology are the Romanowski type-stains, especially Wright's, Giemsa, Diff-Quik, and Papanicolaou, Sano's trichrome stains, new methylene blue (Cowell et al., 2008; Wellman, 1990) (Table 1). Wright's stain is good for the diagnosis of mast cell tumours, because it stains the granules metachromatically making them easily recognisable (Duncan and Prasse, 1979).

Table 1. Common stains used in the cytological diagnosis of tumours. (Wellman, 1990)

Stain	Description
Wright's	Provides strong colour contrast and is a permanent stain with outstanding cytoplasmic detail and appropriate nuclear detail
Papanicolaou	Provide brilliant details of nucleus for the classification of malignancies
New methylene blue	Better nuclear details, low colour contrast, and not a permanent stain

### 2.2.4 Benefits and limitation

Cytological diagnosis can be performed rapidly, cheaply, and repeatedly (Bhalla et al., 2011). Although fine needle aspiration is rapid, precise, and cost-effective for cytology diagnosis in humans, in veterinary medicine it can be weakly diagnostic. However, smears from fine needle aspirations were more cellular with better preservation of tissue structures than scrappings, discharge fluids, or impression smears (Allen et

al.,1986). Fine needle aspiration provides a rapid look into the tissues and cells without the involvement of surgical procedures (Duncan and Prasse, 1979).

### **2.2.5 Discrepancies between cytology and histopathology**

Cytology is a valuable complement to histopathology, and for some neoplasms the cytology can be easier than histopathology in the diagnosis of tumours. This is true for round cell tumours, because they do not have specific features that require histological identification. The cell morphology of round cell tumours is also clear in smears and imprints, that is in tissue sections. Tissue imprints and needle aspirates do not show morphological variations. Poorly granulated cells of anaplastic tumours are more readily identifiable showing higher resolution of cytoplasmic granules in smears and imprints than in tissue sections (Duncan and Prasse, 1979).

Neoplasia cytology shows a sensitivity of 66.6%, specificity of 91.5%, and accuracy of 77.2%, with 93.0% probability accuracy in the diagnosis of malignancy (Ku et al., 2017). In one study, cytology of fine needle biopsy managed to correctly diagnose 108 of 147 cases presented (Griffiths et al., 1984). In 36 stromal tumours only 18 were correctly diagnosed.

### **3.0 METHODOLOGY**

#### **3.1 Data retrieval**

Canines cases with suspected tumours submitted to the Haematology and Clinical Biochemistry Laboratory, Veterinary Laboratory Services Unit, Universiti Putra Malaysia from 2015 to 2019, for cytology for diagnosis were collected and compiled. Data of the canine patient including age, sex, breed, neuter status, and site of tumour were recorded. The neoplasia cases were classified according to the type of tumours based cytological diagnoses. Cases with repeat visits and the same cytological diagnosis or tumours with different cytological diagnosis or at different anatomical sites were recorded as one case. Other cases recorded included suspected neoplasia cases with cytological results that showed non-tumour diagnosis or no significant findings. Cases without data on the sex and age of the dog were excluded from the study. However, cases of dogs of unknown breed were included.

#### **3.2 Statistical analysis**

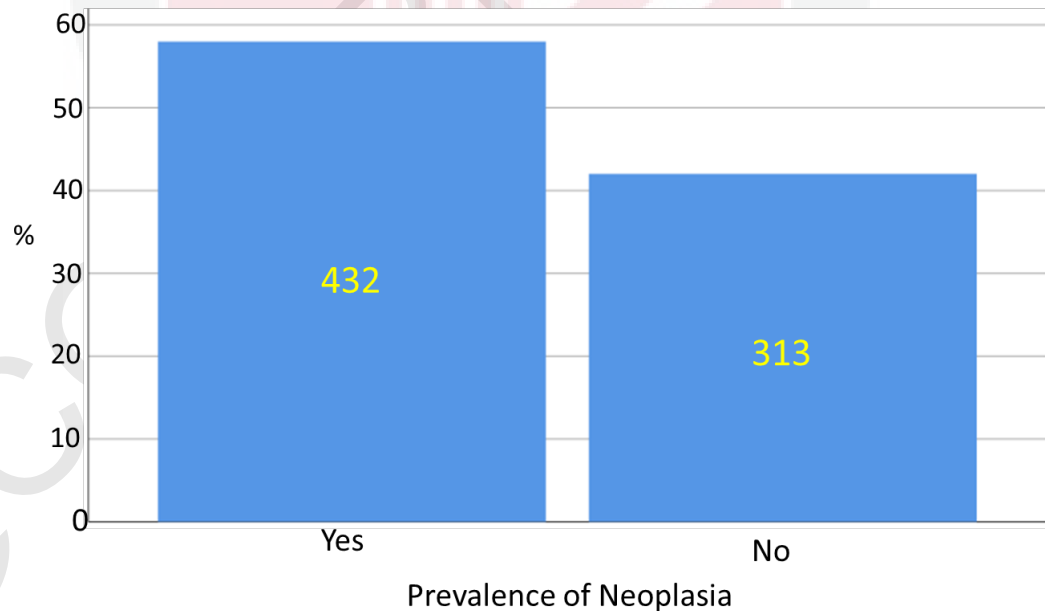
The frequency and associated risk factors were presented as bar charts and tables. The association between the occurrence of neoplasia and age was determined using the normality and Mann-Whitney U tests. Other information such as location and type of tumours were described and tabulated as additional information to the complement laboratory data. The association between the prevalence of tumours and breed and sex

were determined using the Pearson Chi-Square test. The statistical analyses were done using SPSS software version 19.0 (SPSS, IBM Inc, USA) at  $\alpha=0.05$ .

## 4.0 RESULTS

### 4.1 Cases

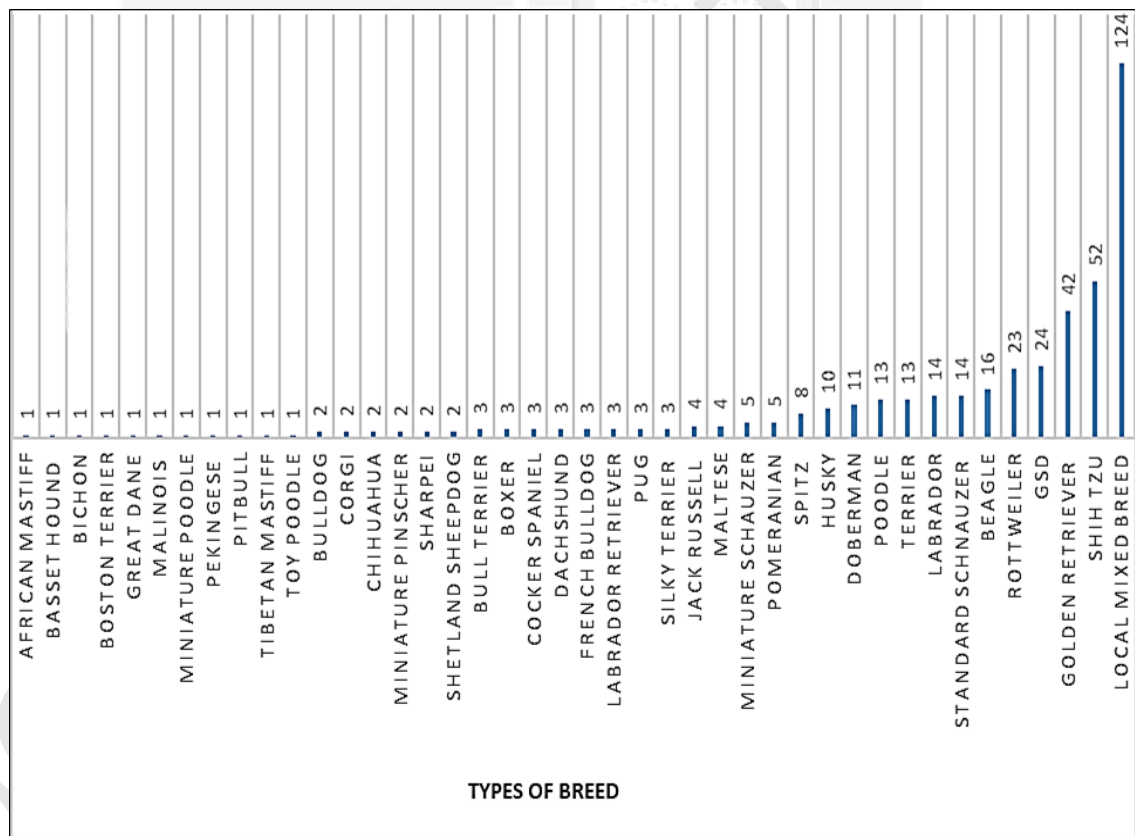
The results showed that among 745 canine cases of suspected neoplasia from the year 2015 to 2019 and based on cytological diagnosis, only 58% were diagnosed with neoplasia and 42% were non-tumour cases, which include infections, inflammations, haemorrhages, and non-significant findings (Figure 1).



**Figure 1.** Occurrence of neoplasia in dogs subjected to cytological diagnosis at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.

## 4.2 Breed

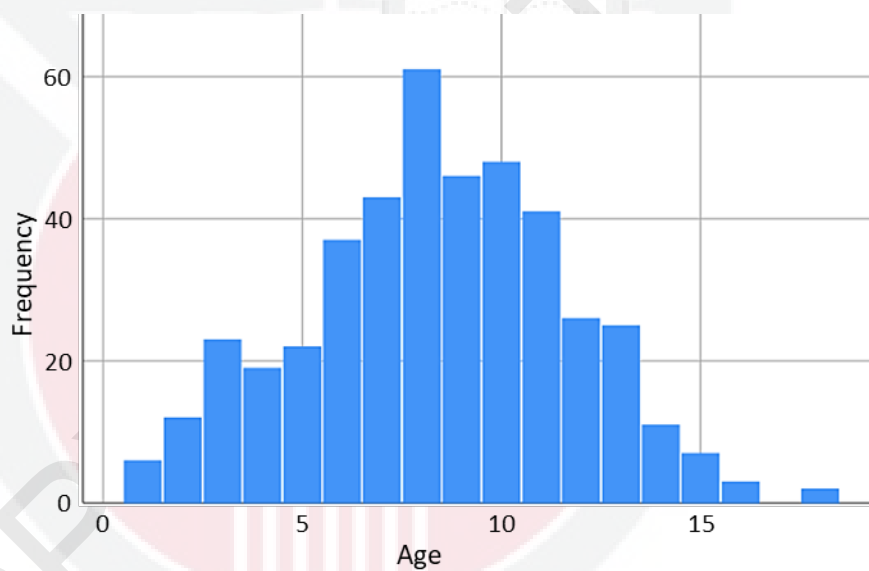
All cases were represented by 42 breeds of dogs. The most frequent breeds in the study were Local Mixed Breed, Shih Tzu, Golden Retriever, German Shepherd, Rottweiler, Beagle, Standard Schnauzer, Labrador, Terrier, Poodle, Doberman, Husky, and others. The highest number of dogs significantly ( $p < 0.05$ ) presented in this study were Golden Retriever, German Shepherd, Rottweiler (Figure 2).



**Figure 2.** Frequency of breed of dogs presented and diagnosed with neoplasia at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.

### 4.3 Age

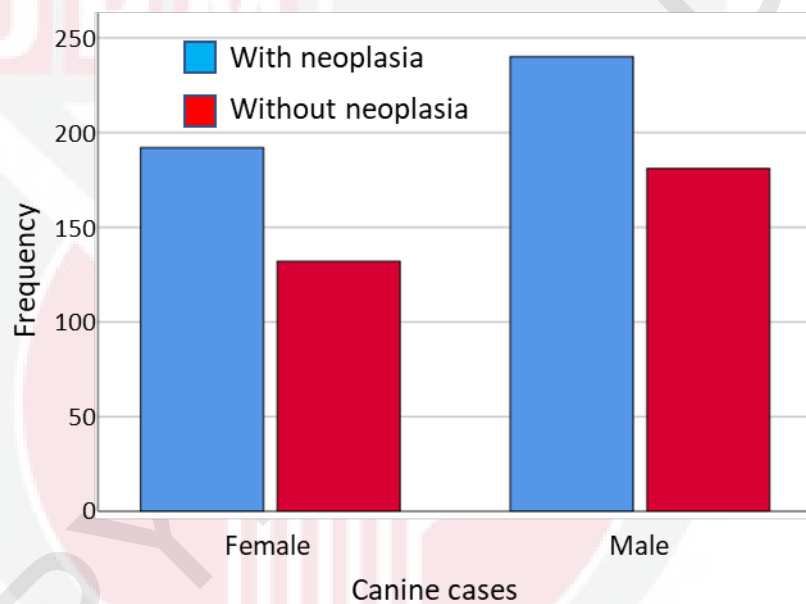
There was no significant ( $p>0.05$ ) difference in the prevalence of tumours among dogs of various ages. Dogs of age 8 were overrepresented ( $n= 61$ ) in the study. Meanwhile, out of 432 cases with the final diagnosis of neoplasia, age 16 ( $n= 3$ ) and 18 ( $n=2$ ) are underrepresented. The mean age of dogs with neoplasia was  $8.38 \pm 3.31$  years, with a minimum age of 1 year and maximum age of 18 years. (Figure 3).



**Figure 3.** Age of dogs presented and diagnosed with neoplasia at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.

#### 4.4 Sex

There was no significant association ( $p > 0.05$ ) between the prevalence of neoplasia and the sex of dogs. Approximately 32% of male dogs and about 26% of bitches were diagnosed with neoplasia in the 745 cases reported. (Figure 4).

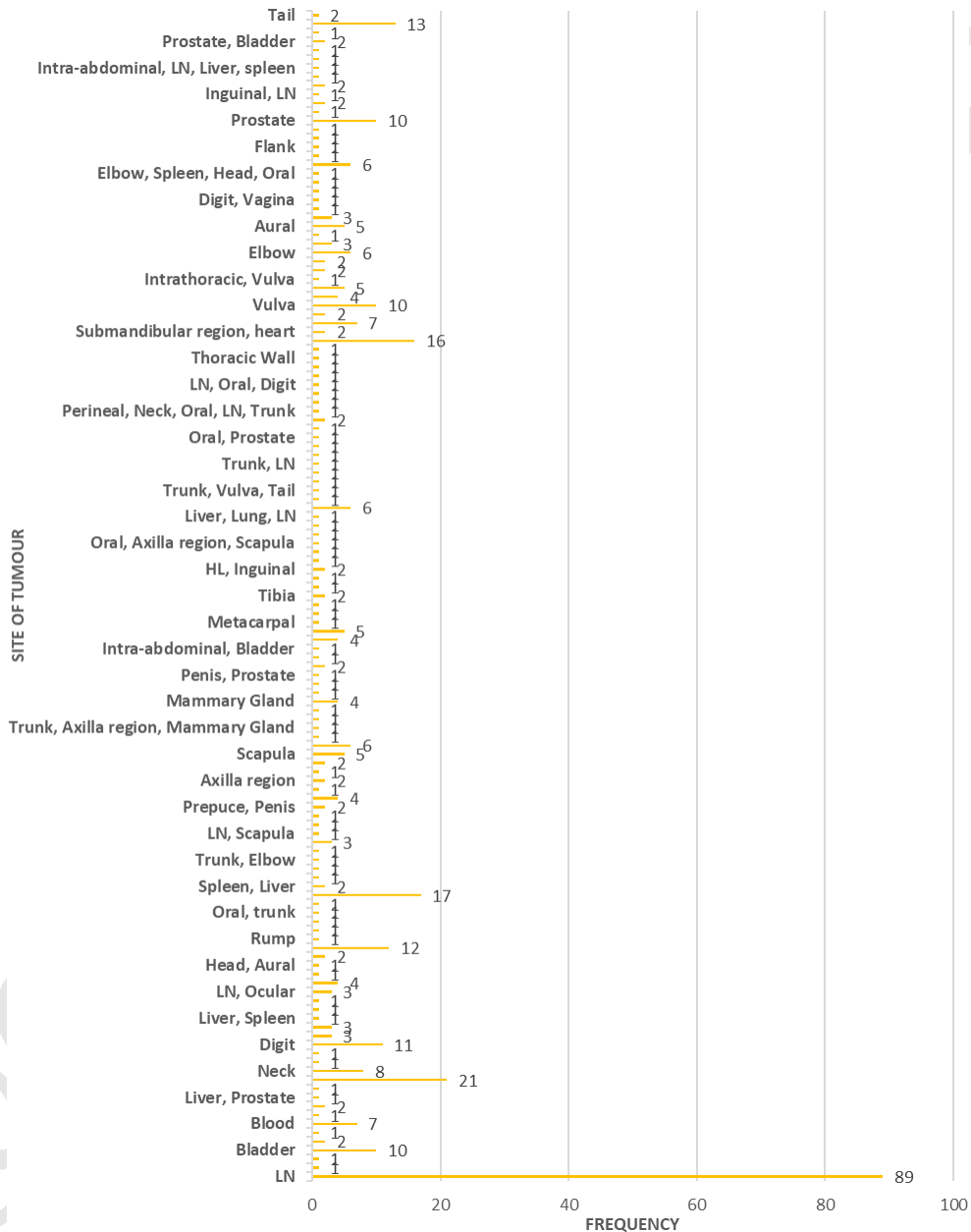


**Figure 4.** Sex distribution of canine cases presented and diagnosed with or without neoplasia at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.

#### 4.5 Common sites of tumours

Based on sampling and cytology diagnosis, among the 432 neoplasia cases recorded, lymph node ( $n=89$ ) was the most common of site of tumour, followed by the oral region ( $n=21$ ), penis ( $n=17$ ), trunk area ( $n=16$ ), nasal region ( $n=13$ ), intra-abdominal area ( $n=12$ ), digit ( $n=11$ ), bladder ( $n=10$ ), vulva ( $n=10$ ), neck area

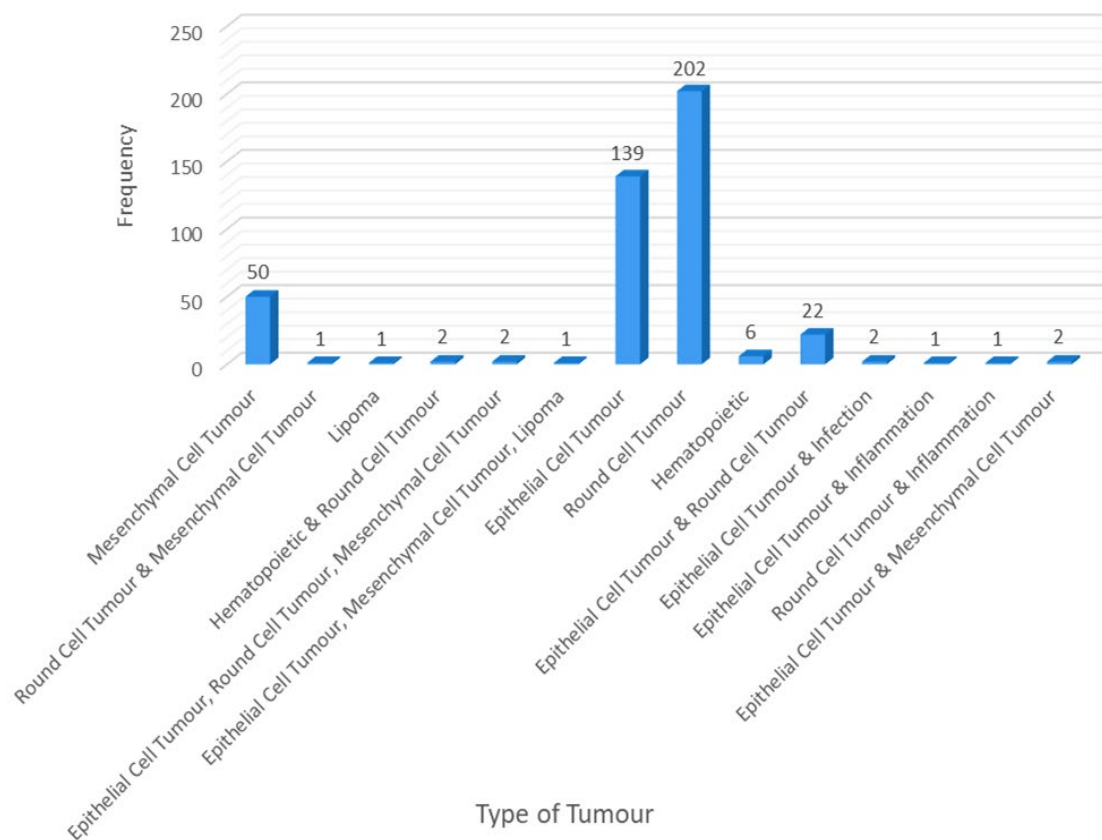
(n=8), blood (n=7), intrathoracic area (n=7), forelimb area (n=6), perineal area (n=6), spleen (n=6), and hindlimb area (n=5). (Figure 5).



**Figure 5.** Site distribution of tumours in canine cases presented and diagnosed at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.

#### 4.6 Common types of tumour

Cases suspected with neoplasia were classified according to the cytology reports and the results showed that among the most frequent types of canine neoplasia were round cell (46.76%), epithelial cell (32.18%), and mesenchymal cell tumour (11.57%). A few cases of leukemia (1.39%), lipoma (0.23%), and mixed tumours (7.85%) were recorded. Another 314 cases were classified as non-tumour or no significant findings cases. Non-tumour cases include infections and inflammations (Figure 6).



**Figure 6.** Distribution of tumour types in dogs presented for diagnosis at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.

## 5.0 DISCUSSION

Previous studies showed that 55.2% of tumours were in dogs of the 6 to 10 year age range (Grüntzig et al., 2016). In our study, the mean age of dogs with neoplasia was  $8.38 \pm 3.0$  years. The study shows that old dogs are more susceptible than young dogs to develop neoplasia.

Among cases presented to VLSU-UPM, the Local Mixed breed (28.7%) were most frequently diagnosed with tumours. This may not represent the true prevalence of tumours in dogs in Malaysia, instead, it may be due to the Local Mixed breed being the predominant breed of dogs with tumours presented to the University Veterinary Hospital and Haematology and Clinical Biochemistry Laboratory, VLSU-UPM.

Overall, there were more male than female dogs presented with tumours in this study. This could probably be due to the fact many cases of suspected mammary gland tumours, without final diagnosis, due to lack of cytological evidence were excluded from the study. A previous study reported that female dogs showed a higher occurrence of tumours than male dogs (Baioni et al., 2017). This observation was attributed to the high occurrences of mammary gland tumours in female dogs. In fact, Noury et al. (2020) did report that mammary gland tumours are the most common neoplasia in female dogs that had contributed to the overall higher prevalence of tumours in female than male dogs (Merlo et al., 2008; Shida et al., 2008). In our study, several cases that were also excluded due to lack of information including age and sex, which may contribute to the differences in result between our study and those of previous studies.

In this retrospective study, round (46.76%) and epithelial (32.18%) cell tumours were most commonly seen in tissue samples of dogs submitted to the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM. Round cell tumours were largely contributed by lymphomas, which is consistent with the findings of other investigators (Zandvliet, 2016; Mingus, 2019). Besides lymphoma, our study showed that other round cell tumours such as transmissible venereal, mast cell, and plasma cell tumours, and histiocytoma, were also frequent in dogs.

The most frequent locations of lymphomas in dogs in this study were the lymph nodes. This observation is similar to that reported by Ku et al. (2017).

## **6.0 CONCLUSION**

The study allowed us to determine the occurrence of neoplasia in dogs presented to UVH-UPM and VLSU-UPM, based on cytological diagnosis and frequency of tumours based on age, breed, and sex of dogs. The most frequent tumours diagnosed in these dogs were round and epithelial cell tumours while the most frequent site of tumours were the lymph nodes. However, there was no association between the prevalence of neoplasia and the age or sex of dogs. The local mixed breed of dogs was most frequently diagnosed with neoplasia.

## **7.0 LIMITATION OF STUDY**

All data collected were entirely from the forms and cytology diagnostic reports archived at the Haematology and Clinical Biochemistry Laboratory, VLSU-UPM. Among limitations in this study is the lack of information and incomplete data. There were several cases with missing information including sex and age of the dogs and these cases were excluded in the study.

Therefore, to overcome the limitations, complete information of the patients including age, sex, breed, and type and site of samples must be obtained from the records at UVH, UPM and private clinics for cases referred to the Haematology and Clinical Biochemistry laboratory, VLSU-UPM.

## **8.0 RECOMMENDATIONS**

It is recommended that future studies should be conducted to also include cats with neoplasia and use the information to compare the prevalence between canine and feline tumours. The tumour cases should be classified as benign or malignant. Currently, the accuracy of cytological diagnosis between fine needle aspirate samples and impression smears of cases presented to VLSU-UPM is not known. This aspect of the study should be included in future investigations.

Future studies should also compare the accuracy of tumour diagnosis between cytological and histopathological diagnoses. This would provide information on the

specificity and sensitivity of the cytology and histopathology methods in providing the accurate diagnosis of tumours.



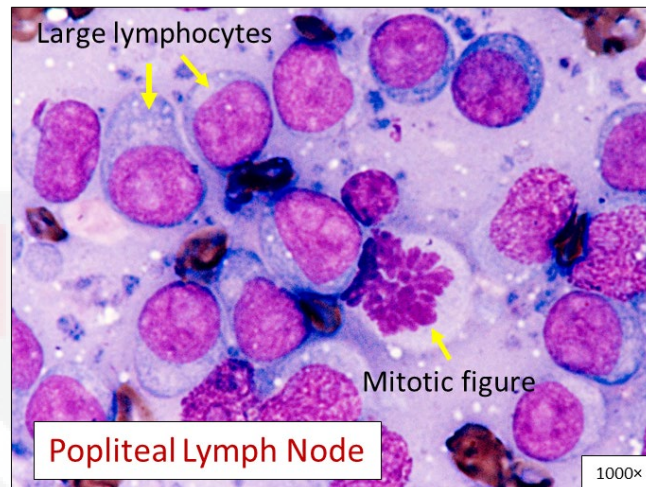
## 9.0 REFERENCES

- Aleksić-Kovačević, S., Kukulj, V., Marinković, D., and Knežević, M. (2005). Retrospective study of canine epithelial and melanocytic tumours. *Acta Veterinaria-Beograd*, 55(4), 319-326.
- Allen, S. W., Prasse, K. W., and Mahaffey, E. A. (1986). Cytologic differentiation of benign from malignant canine mammary tumours. *Veterinary Pathology*, 23(6), 649-655.
- Baioni, E., Scanziani, E., Vincenti, M. C., Leschiera, M., Bozzetta, E., Pezzolato, M., Desiato, R., Bertolini, S., and Ru, G. (2017). Estimating canine cancer incidence: findings from a population-based tumour registry in northwestern Italy. *BMC Veterinary Research*, 13(1), 1-9.
- Bhalla, S., Singh, A., Sood, N. K., and Gupta, K. (2011). Specificity and sensitivity of cytological techniques for rapid diagnosis of neoplastic and non-neoplastic lesions of canine mammary gland. *Brazilian Journal of Veterinary Pathology*, 4(1), 13-22.
- Chikweto, A., Mcneil, P., Bhaiyat, M. I., Stone, D., and Sharma, R. N. (2011). Neoplastic and nonneoplastic cutaneous tumours of dogs in Grenada, West Indies. *ISRN Veterinary Science*, 2011, 416435.
- Cohen, D., Reif, J. S., Brodey, R. S., and Keiser, H. (1974). Epidemiological analysis of the most prevalent sites and types of canine neoplasia observed in a veterinary hospital. *Cancer Research*, 34(11), 2859-2868.
- Cotchin, E. (1984). Veterinary oncology: A survey. *Journal of Pathology*, 142(2), 101-127.
- Cowell, R. L., Tyler, R. D., Meinkoth, J. H., and DeNicola, D. B. (2008). Diagnostic cytology and hematology of the dog and cat. 3rd Edition, Elsevier, Canada.
- Dobson, J. M., Samuel, S., Milstein, H., Rogers, K., and Wood, J. L. N. (2002). Canine neoplasia in the UK: Estimates of incidence rates from a population of insured dogs. *Journal of Small Animal Practice*, 43(6), 240-246.
- Dorn C.R., Taylor D.O.N., and Hibbard H.H. (1967). Epizootiologic characteristics of canine feline leukaemia and lymphoma. *American Journal of Veterinary Research*, 28: 91001.
- Dorn, C. R., Taylor, D. O. N., Frye, F. L., and Hibbard, H. H. (1968a). Survey of animal in Alameda and Contra Costa countries, California. I. Methodology and description of cases. *Journal of the National Cancer Institute*, 40(2), 295-305.
- Dorn, C. R. Taylor, D. O. N. Schneider, R. Hibbard, H.H., and Klanker, M. R. (1968b). Survey of neoplasms in Alameda and Contra Costa Counties, California. II. Cancer morbidity in dogs and cats from Alameda County. *Journal of the National Cancer Institute* 40, 307-318.
- Duncan, J. R. and Prasse, K. W. (1979). Cytology of canine cutaneous round cell tumours. *Veterinary Pathology*, 16(6), 673-679.
- Griffiths, G. L., Lumsden, J. H., and Valli, V. E. (1984). Fine needle aspiration cytology and histologic correlation in canine tumors. *Veterinary Clinical Pathology*, 13(1), 13-7.

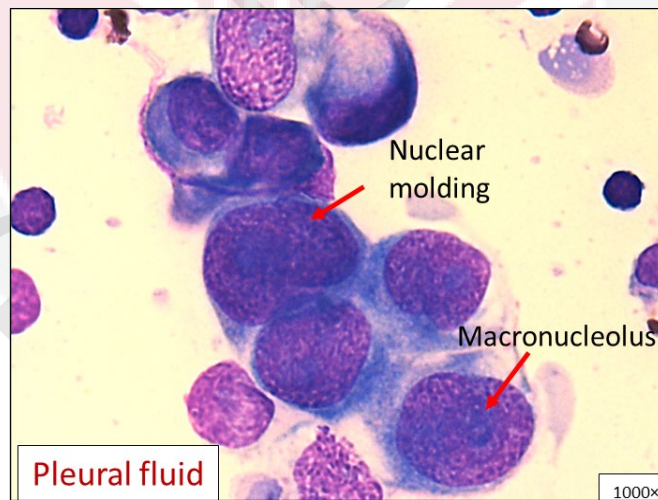
- Grüntzig, K., Graf, R., Boo, G., Guscetti, F., Hässig, M., Axhausen, K. W., Fabrikant, S., Welle, M., Meier, D., Folkers, G., and Pospischil, A. (2016). Swiss Canine Cancer Registry 1955–2008: Occurrence of the most common tumour diagnoses and influence of age, breed, body size, sex and neutering status on tumour development. *Journal of Comparative Pathology*, 155(2-3), 156-170.
- Ihrke, P. J., Walder, E. J., Affolter, V. K., and Gross, T. L. (2005). *Skin diseases of the dog and cat: Clinical and histopathologic diagnosis*. 2<sup>nd</sup> Edition, Wiley-Blackwell.
- Ku, C. K., Kass, P. H., and Christopher, M. M. (2017). Cytologic–histologic concordance in the diagnosis of neoplasia in canine and feline lymph nodes: a retrospective study of 367 cases. *Veterinary and Comparative Oncology*, 15(4), 1206–1217.
- Leav, I. and Ling, G.V. (1968). Adenocarcinoma of the canine prostate. *Cancer*, 22(6), 1329-1345
- London, C. A. and Seguin, B. (2003). Mast cell tumours in the dog. *Veterinary Clinics of North America - Small Animal Practice*, 33(3), 473-489.
- Madewell, B. R. (1985). Canine lymphoma. *Veterinary Clinics of North America - Small Animal Practice*, 15(4), 709-722.
- Markert, C. L. (1968). Neoplasia: a disease of cell differentiation, *Cancer Research*, 28(9), 1908-1914.
- Merlo, D. F., Rossi, L., Pellegrino, C., Ceppi, M., Cardellino, U., Capurro, C., Ratto, A., Sambucco, P. L., Sestito, V., Tanara, G., and Bocchini, V. (2008). Cancer incidence in pet dogs: findings of the Animal Tumor Registry of Genoa, Italy. *Journal of Veterinary Internal Medicine*, 22(4), 976-984.
- Mingus, L. (2019). Common cancers in dogs. Flint Animal Cancer Centre, Colorado State University, USA. <https://www.csuanimalcancercenter.org/2019/11/14/common-cancers-in-dogs/> (Retrieved on 23 December, 2020).
- Mintzer, D. M. and Hauptman, S. P. (1983). Lymphosarcoma cell leukemia and other non-Hodgkin's lymphomas in leukemic phase. *American Journal of Medicine*, 75(1), 110-120.
- Misdorp W. (2004). Mast cells and canine mast cell tumours. A review. *Veterinary Quarterly*, 26(4), 156-69.
- Moore, P. F. and Affolter, V.K (2005). Canine and feline histiocytic diseases. In: Ettinger SJ, Feldman EC, (Editors). *Textbook of veterinary internal medicine*. St Louis: Elsevier Saunders, 2005, 779-782.
- Noury, S., Bouayad, H., Tligui, N., and Azrib, R. (2020). A retrospective study on frequency of canine tumors at Veterinary Teaching Hospital in Rabat (Morocco). *Moroccan Journal of Agricultural Sciences*, 1(1), 34-38.
- Prier, J. E. and Brodey, R. S. (1963). Canine Neoplasia. A prototype for human cancer study. *Bulletin of the World Health Organization*, 29(3), 331–344.
- Rothwell, T. L., Howlett, C. R., Middleton, D. J., Griffiths, D. A., and Duff, B. C. (1987). Skin neoplasms of dogs in Sydney. *Australian Veterinary Journal*, 64(6), 161-164.
- Schneider R (1978). *Tumors in domestic animals*, 2<sup>nd</sup> Edition, Moulton, J. E. (Editor), University of California Press, Berkeley.

- Shida, T., Maruo, T., Kawamura, H., Takeda, H., Madarame, H., Kayanuma, H., and Sukanuma, T. (2008). An epidemiological study on tumors in 5,819 dogs. *Journal of the Japan Veterinary Medical Association*, 61(11), 876-872.
- Sledge, D. G., Webster, J., and Kiupel, M. (2016). Canine cutaneous mast cell tumours: A combined clinical and pathologic approach to diagnosis, prognosis, and treatment selection. *Veterinary Journal*, 215, 43–54.
- Teske, E. (1994) Canine malignant lymphoma: A review and comparison with human non-Hodgkin's lymphoma, *Veterinary Quarterly*, 16(4), 209-219,
- Teske, E. and van Heerde, P. (1996). Diagnostic value and reproducibility of fine-needle aspiration cytology in canine malignant lymphoma. *Veterinary Quarterly*, 18(3), 112–115.
- Valent, P., Akin, C., Arock, M., Bock, C., George, T. I., Galli, S. J., Gotlib, J., Haferlach, T., Hoermann, G., Hermine, O., Jäger, U., Kenner, L., Kreipe, H., Majeti, R., Metcalfe, D. D., Orfao, A., Reiter, A., Sperr, W. R., Staber, P. B., Sotlar, K., Schiffer C., Superti-Furga, G., and Horny, H. (2017). Proposed terminology and classification of pre-malignant neoplastic conditions: A consensus proposal. *EBioMedicine*, 26, 17-24 .
- Vilkovyskiy, I. F., Vatnikov, Y. A., Kulikov, E. V., Sotnikova, E. D., Yagnikov, S. A., Seleznev, S.B., Krotova, E. A., Byakhova, V. M., Grishin, V. N., and Avdotin, V. P. (2020). Influence of hepatic neoplasia on life expectancy in dogs. *Veterinary World*, 13(3), 413-418.
- Waters, D.J., Sakr, W. A., Hayden, D. W., Lang, C. M., McKinney, L., Murphy, G. P., Radinsky, R., Ramoner, R., Richardson, R.C., and Tindall, D. (1998). Workgroup 4: Spontaneous prostate carcinoma in dogs and nonhuman primates. *Prostate* 1998; 36(1), 64-67.
- Wellman, M. L. (1990). The cytologic diagnosis of neoplasia. *The Veterinary Clinics of North America - Small Animal Practice*, 20(4), 919-938.
- Yau, P., Dhand, N. K., Thomson, P. C., and Taylor, R. M. (2017). Retrospective study on the occurrence of canine lymphoma and associated breed risks in a population of dogs in NSW (2001–2009). *Australian Veterinary Journal*, 95(5), 149-155.
- Zandvliet, M. (2016). Canine lymphoma: A review. *Veterinary Quarterly*, 36(2), 76-104.

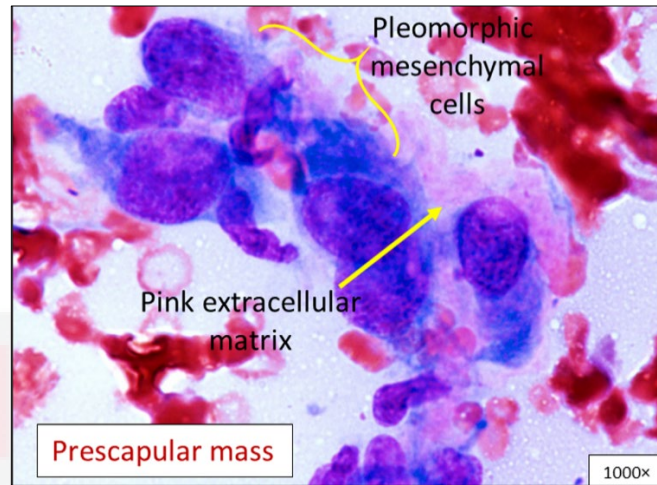
## 10.0 APPENDICES



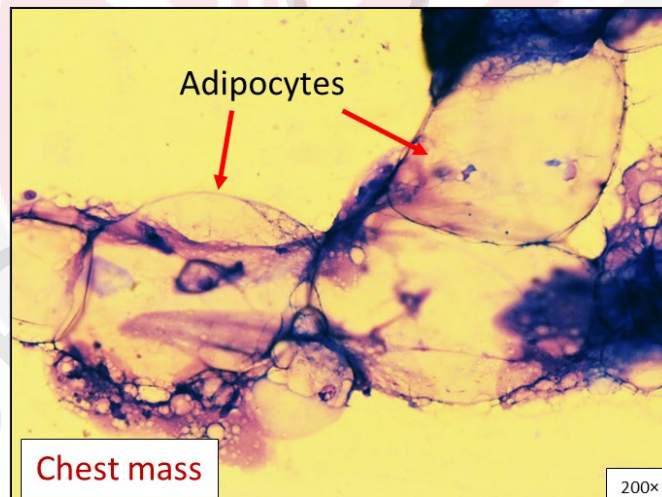
**Figure 7.** Round cell tumour showing large lymphocytes and mitotic figures.



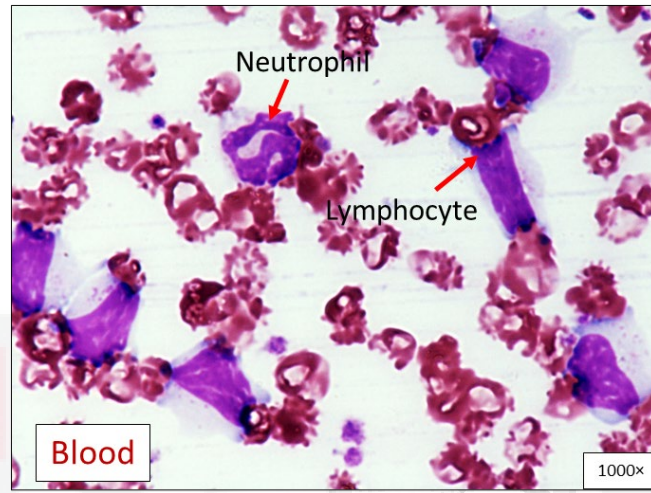
**Figure 8.** Epithelial Neoplasm showing nuclear molding and macronucleolus



**Figure 9.** Mesenchymal tumour showing pleomorphic cells and pink extracellular matrix.



**Figure 10.** Lipoma.



**Figure 11.** Lymphocytic leukemia.