A Retrospective Study of the Prevalence, Associated Risk Factors and Histopathological Pattern of Squamous Cell Carcinoma (SCC) Cases Encountered in Hospital Pengajar Perubatan Veterinar, Universiti Malaysia Kelantan (HPVUMK) from the Year 2020 to 2024

By

Siti Nur Amany Ayuni Binti Shahrul Nizan

A Thesis Submitted in Fulfillment of the Requirement for the Degree of Doctor of Veterinary Medicine

Faculty of Veterinary Medicine
UNIVERSITI MALAYSIA KELANTAN

2024

ORIGINAL LITERARY WORK DECLARATION

I hereby certify that the work embodied in this thesis is the result of the original research and has not been submitted for a higher degree to any other University or Institution.

/	**OPEN ACCESS**	I agree that my thesis is to be made immediately available as hardcopy or online open access (full text).
/	**RESTRICTED**	(Contains restricted information as specified by the organisation where research was done)*
/	**EMBARGOES**	I agree that my thesis is to be made available as hardcopy or online (full text) for a period approved by the Post Graduate Committee. Dated from_until___.
/	**CONFIDENTIAL**	(Contains confidential information under the Official Secret Act 1972)*

I acknowledge that Universiti Malaysia Kelantan reserves the right as follows.

1. The thesis is the property of Universiti Malaysia Kelantan
2. The library of Universiti Malaysia Kelantan has the right to make copies for the purpose of research only.
3. The library has the right to make copies of the thesis for academic exchange.

SIGNATURE OF CANDIDATE	SIGNATURE OF SUPERVISOR
NRIC/PASSPORT NO. 011222031018	DR FATHIN FAAHIMAAH BINTI ABDUL HAMID
DATE: 12/02/2025	DATE: 12/02/2025

A Retrospective Study of the Prevalence, Associated Risk Factors and Histopathological Pattern of Squamous Cell Carcinoma (SCC) Cases Encountered in Hospital Pengajar Perubatan Veterinar, Universiti Malaysia Kelantan (HPVUMK) from the Year 2020 to 2024

ABSTRACT

Squamous cell carcinoma (SCC) is a type of cancer originating from squamous epithelial cells, often associated with chronic exposure to sunlight. This tumour is a significant health concern in veterinary medicine, as it ranks among the most commonly diagnosed skin cancers. This study aimed to determine the prevalence of SCC at Hospital Pengajar Perubatan Veterinar Universiti Malaysia Kelantan (HPVUMK) to assess whether the occurrence is a potentially public concern or remains within an acceptable range. Additionally, it was also conducted to investigate the risk factors contributing to the development of SCC and to explore the histopathological subtypes (grading) of SCC. A retrospective analysis of SCC cases diagnosed at HPVUMK from June 2020 to June 2024 focusing on felines and canines. Nine confirmed cases were identified, with the highest prevalence of 0.22% in felines in 2024 and 2.78% in canines in 2021. The lesions were mostly external, located on the head and limbs. The risk factors such as age, breed, neutering status, and management status was taken to test the association of the risk factor with SCC development. Fisher's exact test used to indicate significant correlations, suggesting that these variables could influence SCC development. However, this study found no significant association ($p>0.05$). This could be due to the limited geographical area, which restricted the number of SCC cases observed. Histopathological examination revealed that SCC cases were primarily well-differentiated and moderately differentiated, with no poorly differentiated subtype cases. This study provides valuable information on the prevalence, lesion locations, and histopathological subtypes (grading) of SCC, aiding in the understanding and management of this condition in veterinary practice.

Keywords: Squamous cell carcinoma, histopathological subtypes (grading), risk factors, prevalence, retrospective

Kajian Retrospektif Tentang Kadar Prevalens, Faktor Risiko Berkaitan dan Corak Histopatologi Kes Karsinoma Sel Skuamosa (SCC) yang Ditemui di Hospital Pengajar Perubatan Veterinar, Universiti Malaysia Kelantan (HPVUMK) dari Tahun 2020 hingga 2024

ABSTRAK

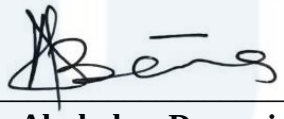
Karsinoma sel skuamosa (SCC) adalah sejenis kanser yang berasal daripada sel epitelium skuamosa, sering dikaitkan dengan pendedahan kronik kepada cahaya matahari. Tumor ini adalah mencetus kebimbangan kesihatan yang ketara dalam perubatan veterinar, kerana ia berada di antara kanser kulit yang paling kerap didiagnosis. Kajian ini bertujuan untuk menentukan kadar prevalens SCC di Hospital Pengajar Perubatan Veterinar Universiti Malaysia Kelantan (HPVUMK) untuk menilai sama ada SCC itu berpotensi menjadi kebimbangan orang ramai atau kekal dalam julat yang boleh diterima. Selain itu, ia juga dijalankan untuk menyiasat faktor risiko yang menyumbang kepada perkembangan SCC dan untuk meneroka jenis histopatologi bagi SCC. Analisis retrospektif kes SCC yang didiagnosis di HPVUMK dari Jun 2020 hingga Jun 2024 memfokuskan pada kucing dan anjing. Sembilan kes yang disahkan telah dikenal pasti, dengan kelaziman 0.22% pada kucing pada tahun 2024 dan 2.78% pada anjing pada tahun 2021. Lesi kebanyakannya adalah luaran, terletak pada kepala dan anggota badan. Faktor risiko seperti umur, baka, status pemandulan, dan status pengurusan telah diambil untuk menguji perkaitan faktor risiko dengan pembangunan SCC. Ujian tepat Fisher digunakan untuk menunjukkan korelasi yang signifikan, menunjukkan bahawa pembolehubah ini boleh mempengaruhi pembangunan SCC. Walau bagaimanapun, kajian ini mendapati tiada perkaitan yang signifikan ($p>0.05$). Ini mungkin disebabkan oleh kawasan geografi yang terhad, yang mengehadkan bilangan kes SCC yang dapat diperhatikan. Pemeriksaan histopatologi mendedahkan bahawa kes SCC terutamanya karsinoma sel skuamus perbezaan baik, sederhana dan tanpa kes SCC perbezaan lemah. Kajian ini memberikan maklumat yang berguna tentang kadar prevalens, lokasi lesi, dan jenis histopatologi SCC, yang membantu dalam pemahaman dan pengurusan keadaan ini dalam amalan veterinar.

Kata kunci: Karsinoma sel skuamosa, jenis histopatologi, faktor risiko, kadar prevalens, retrospektif

CERTIFICATION

This is to certify that we have read this research paper entitled **'A Retrospective Study of the Prevalence, Associated Risk Factors and Histopathological Pattern of Squamous Cell Carcinoma (SCC) Cases Encountered in Hospital Pengajar Perubatan Veterinar, Universiti Malaysia Kelantan (HPVUMK) from the Year 2020 to 2024'** by **Siti Nur Amany Ayuni Binti Shahrul Nizan**, and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirements for the course DVT 55204 – Research Project.

Dr Fathin Faahimaah Binti Abdul Hamid
DVM (UMK), MSC in Pathology (UMK)
Lecturer
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Supervisor)



Dr Abubakar Danmaigoro
DVM (UDUSOK), MSC in Veterinary Anatomy (UDUSOK),
PhD in Veterinary Anatomy & Histology (UPM)
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Co-supervisor)

ACKNOWLEDGMENTS

Alhamdulillah, I am grateful to have completed this thesis after facing and overcoming numerous challenges along the way. First and foremost, I am deeply grateful to my loving parents, Siti Rosnita Binti Sha'ari, Junaidi Bin Abdullah, and Shahrul Nizan Bin Doragi, for their endless love, sacrifices, and support. A special thanks also to my sister, Anis Anati, for always being there for me and believing in me.

I would like to thank my supervisor, Dr. Fathin Faahimaah Binti Abdul Hamid, and my co-supervisor, Dr. Abubakar Danmaigoro, for all their support and helpful feedback. A special thanks to Dr. Fathin for your guidance, patience, and continuous support throughout this journey, especially despite your busy schedule. Your help has been invaluable from start to finish.

In addition, I am thankful to Dr. Mimi Armiladiana Binti Mohamad, the head of HPV UMK and Dr. Nadiah Syuhada Binti Roslan, for their assistance in providing essential patient data and their ongoing support throughout the research process. A special note of thanks to the Histopathology and Rnomic laboratory assistants, Encik Mohd Faiz Bin Juha, Encik Ahmad Fadli Bin Ahmad Sanusi, and Encik Suhaimi Bin Omar, for their technical help and consistent support throughout this project.

I also would like to express my sincere gratitude to my friends, Ainurul Iman Binti Jasni and Nurulnajihah Binti Ahmad Jame, for your help and companionship. Without your support, this thesis would not have been possible. Finally, to everyone who has contributed, directly or indirectly, thank you for being part of this journey and for all your help along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SCC	Squamous Cell Carcinoma
OSCC	Oral Squamous Cell Carcinoma
dSCC	Digital Squamous Cell Carcinoma
PVs	Papilloma Viruses
FcaPVs	felis catus Papillomaviruses
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
SPSS	Statistical Package for the Social Sciences
UMK	Universiti Malaysia Kelantan
H&E	Hematoxylin & Eosin
FV	Feline visits
CV	Canine visits
FC	Feline cases
FP	Feline prevalence
CP	Canine prevalence
WD	Well-differentiated
MD	Moderately differentiated
PD	Poorly differentiated
IHC	Immunohistochemistry
LELCS	Lymphoepithelioma-like carcinoma of the skin
VC	Verrucous carcinoma
FIV	Feline immunodeficiency virus
CPVs	*Canis familiaris* papillomaviruses
HNSCC	Head and neck squamous cell carcinoma
DAB	3,3-diaminobenzidine
AEC	3-amino-9-ethylcarbazole
FeLV	Feline leukemia virus

LIST OF SYMBOLS

%	Percentage
>	Greater than
<	Lesser than
×	Times
⨯	Magnification
℃	Degree celcius

CHAPTER 1

1.0 INTRODUCTION

1.1 INTRODUCTION

Approximately one out of every four cats and dogs is prone to develop cancer or cancer-related illnesses (Lascelles & British Small Animal Veterinary Association, 2011). In veterinary medicine, squamous cell carcinoma (SCC) is a malignant tumour which is commonly diagnosed in both felines and canines. Besides SCC, the common skin tumours in felines include basal cell carcinomas, mast cell tumours and fibrosarcomas whereas in canines they include mast cell tumours, fibrosarcomas, melanomas, basal cell carcinomas and cutaneous lymphomas. Among cutaneous tumours, the rate of occurrence for cutaneous SCC in dogs constitutes 5.4% of cases, while in cats, it accounts for 17.5% of all cutaneous tumours (Kim *et al.*, 2022). Generally, the common cause of SCC is exposure to ultraviolet (UV) rays, particularly UVB radiation that directly to the skin and older animals (Murphy, 2013).

The research findings help gain insight into the causes, development, and advancement of squamous cell carcinoma (SCC) in cats and dogs and may aid in devising more efficient diagnostic and therapeutic approaches. This, in turn, has the potential to enhance the well-being of affected animals by enabling earlier detection, offering improved treatment choices, and refining prognostic accuracy. Squamous cell carcinoma (SCC) represents a notable health challenge for cats and dogs, frequently resulting in discomfort, pain, and diminished quality of life. Research discoveries offer valuable perspectives on risk factors, preventative actions, and management approaches aimed at reducing the occurrence and consequences of SCC in these animal populations.

1.2 PROBLEM STATEMENT

Neoplasia is one of the leading causes of morbidity and mortality in companion animals which poses significant health challenges for both feline and canine populations. Despite advancements in veterinary medicine, the prevalence, risk factors, and patterns of neoplastic diseases in these animals remain inadequately understood to be correlated with the occurrence of squamous cell carcinoma (SCC). This gap in knowledge hinders the development of effective prevention, early detection, and treatment strategies. This research is conducted to address the notable gap in the literature regarding the understanding of how risk factors correlate with the various histopathological subtypes (grading) of SCC. Exploring this correlation is crucial for advancing our knowledge of SCC and could potentially contribute to more targeted prevention and treatment strategies.

1.3 RESEARCH QUESTIONS

I. What is the prevalence and trend of occurrence of squamous cell carcinoma (SCC) in each years in felines and canines presented in HPVUMK?

II. What are the risk factors that contribute to the development of squamous cell carcinoma (SCC) in felines and canines?

III. What are the histopathological features and subtypes (grading) of squamous cell carcinoma (SCC) observed at HPVUMK?

1.4 RESEARCH OBJECTIVES

I. To identify and analyse the prevalence of squamous cell carcinoma (SCC) in felines and canines over time at Hospital Pengajar Perubatan Veterinar, Universiti Malaysia Kelantan (HPVUMK).

II. To determine the risk factors involved with squamous cell carcinoma (SCC) in felines and canines.

III. To study the histopathological features and subtypes (grading) of squamous cell carcinoma (SCC) in felines and canines observed at HPVUMK.

1.5 RESEARCH HYPOTHESIS

I. The prevalence of squamous cell carcinoma (SCC) in felines and canines is increasing each passing year at HPVUMK.

II. There is evidence of risk factors such as age, breed, animal management and neutering status involved with the development of squamous cell carcinoma (SCC) in felines and canines.

III. The histopathological features and subtypes (grading) of squamous cell carcinoma (SCC) will vary significantly such as tumour size, location, and degree of differentiation.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 NEOPLASIA

Neoplasia is the process where abnormal cells are formed and continue to divide which leads to an increase in their number (Jasni & Al-Sultan, 2020). Patients diagnosed with neoplasia often present with a swollen mass. However, not all patients presenting with these conditions can be definitively diagnosed with a neoplasm (Sivaseelan, 2020). Neoplastic cells can be distinguished from normal cells through several characteristics, such as uncontrollable growth, impaired cellular differentiation, and alterations in cell communication and adhesion (Morris & Dobson, 2001).

Histopathology assessments are used to observe the tissue structure and architecture, thus it has a benefit to evaluate the tumour compared to the cytology evaluation (Meuten *et al.*, 2021). Several features are observed in histopathology including the changes in cellular characteristics, tumour architecture, presence of the cells invading the other tissues and indication of metastatic activity. The cellular characteristics of malignant tissue include poorly differentiated cells with a high nuclear to cytoplasmic ratio (Morris & Dobson, 2001), nuclear membrane irregularities, hyperchromasia, abnormal chromatin pattern (Fischer, 2020) abnormal mitotic changes, increased basophilia and vacuolation (Thrall, Rebar, & Cowell, 2003).

Malignant tumours typically exhibit rapid growth and invade surrounding tissues. They have poorly-defined borders, which allow them to spread into adjacent tissue. This growth often results in the destruction of normal tissue. Other than that, malignant tumours are capable of metastasizing through the lymphatic system, bloodstream, or transcoelomic spread (Morris & Dobson, 2001).

2.2 TYPES OF NEOPLASIA

Neoplasia has been divided into 3 groups which consist of the epithelial tumour, mesenchymal tumour and round cell tumour (Baba, 2007). There are two types of neoplasia including benign and malignant. The suffix "oma" is used for a benign neoplasm that originates from the epithelial tumour. In the mesenchymal tumour, the benign neoplasia suffix is "oma" whereas the suffix name for malignant neoplasia is "sarcoma" (Powers & Dernell, 1998).

Benign neoplasia does not invade the tissues, does not metastasize, and can be removed through surgery (Villalobos, 2018). Benign tumours have an existence in an encircling layer of connective tissue identified as a capsule that prevents them from penetrating the basement membrane (Perumpanani *et al.*, 1997). Various molecular processes allow malignant tumours to spread into nearby tissues and travel to other parts of the body, which can contribute to the spread of cancer throughout the body (Wittekind & Neid, 2005).

Tumours demonstrating a small size of less than 2 cm and minimal mitotic activity generally have a good prognosis, while malignant tumour with metastasis activity typically indicates a poor prognosis (Miettinen *et al.*, 2002). Malignant tumours can be spread through the blood and lymphatic vessels (Patel, 2020), iatrogenic (Parikh & Jagtap, 2006) and transcoelomic (Sugarbaker, 2011).

Benign neoplasia usually grows slowly and the growth of it may stop in some cases. However, malignant neoplasia often grows rapidly and rarely stops (Morris & Dobson, 2001). Benign neoplasia will cause harm to the patient as malignant neoplasia when the location of the growth impedes or disturbs the vital organ to function properly (Sivaseelan, 2020). Other than that, cytological features of the benign neoplasia show less cellular pleomorphism whereas the malignant neoplasia has more prominent cellular pleomorphism (Jasni & Al-Sultan, 2020).

2.3 SQUAMOUS CELL CARCINOMA (SCC)

Squamous cell carcinoma (SCC) is a type of neoplasm that arises from the squamous epithelial cells (Łojszczyk *et al*., 2021) which are known as epithelial flat cells located in the outer layer of the skin and in various mucous membranes throughout the body**.** There is about 20 to 30% oral squamous cell carcinoma (OSCC) occurring in dogs and 70% in cats which commonly affects both older species (Morris & Dobson, 2001). Oral tumours in cats are predominantly squamous cell carcinomas (69%), followed by fibrosarcomas (18%). Other types include osteosarcoma, acanthomatous ameloblastoma, and peripheral odontogenic fibromas (Cray *et al.*, 2020).

Oral cancer, predominantly squamous cell carcinoma (SCC), is the most common type of oral malignancy observed in cats, often affecting the tongue, gingiva, lips, and oropharynx, similar to its occurrence in humans (Bertone *et al.*, 2003). However, it commonly occurs in dogs in the oral cavity (44.9%) and skin (44.9%), while it's less common in the mammary gland, nasal cavity, lung, and bladder (Willcox *et al.*, 2019). Thus, SCC in cats might be easily mistaken for sporotrichosis due to they share the same predilection site of lesion (Han & Kano, 2020). Furthermore, SCC can be categorized into a few subtypes (grading) which mainly include well-differentiated, moderately differentiated and poorly differentiated (Marconato *et al.*, 2021).

2.4 CLINICAL SIGNS

Clinical signs of the animals that are diagnosed with squamous cell carcinoma (SCC) differ depending on the location of the tumour (Dickinson & Boldan, 2022). The common locations of SCC include the skin, eye, nasal cavity, urinary bladder, lung and reproductive organ. Furthermore, the squamous cell carcinoma (SCC) that occurs in the skin also known as cutaneous SCC. The animals were normally presented with scabs on the skin, ulcerated and become invasive toward the healthy tissue (Dickinson & Boldan, 2022). Other clinical presentation includes alopecia and itching. Moreover, squamous cell carcinoma (SCC) in the nasal cavity leads the animal to come with a nasal discharge, dyspnoea, protrude of one or both eyes and loss of vision (Sivaseelan, 2020). Cats diagnosed with oral SCC may show different signs such as mouth discomfort, dysphagia, drooling, loss of appetite, and even diagnosed with teeth problems like loosening or falling out (Bertone *et al.*, 2003).

2.5 GROSS AND HISTOPATHOLOGICAL FEATURES OF SQUAMOUS CELL CARCINOMA (SCC)

Squamous cell carcinoma (SCC) exhibits a loosely cohesive arrangement of highly atypical, immature basal, or para-basal-like squamous cells (Kokubun *et al.*, 2023). The gross appearance of the SCC commonly showed varying sizes, round, ovoid, irregular or cauliflower-like masses with necrosis and ulcerations. The tumour masses exhibited a rough surface and ranged in colour from pink to light brown (Chandrashekaraiah *et al.*, 2011).

SCC has various histologic subtypes (grading) which include well-differentiated SCC, moderately differentiated SCC, poorly differentiated SCC, clear-cell SCC, spindle cell SCC, and SCC with single cell infiltrates, de novo SCC, lymphoepithelioma-like carcinoma of the skin (LELCS), and verrucous carcinoma (VC) (Yanofsky *et al.*, 2010). The main subtypes (grading) discussed in this study are well-differentiated SCC, moderately differentiated SCC, and poorly differentiated SCC. Each type will exhibit different cytological and histological features. The term subtypes and grading of SCC resembles the same meaning in which it would express the differentiation of histological features (Yanofsky *et al.*, 2010; Ferreira, *et al.*, 2021). Both terms rely on the same histopathological features including the degree of invasion, keratinization, nuclear atypia, pleomorphic cell and observation of the intercellular bridges.

A well-differentiated tumour is observed and finds cells organized in a whorl pattern and cells with more prominent of intercellular bridges together with the prominent nucleoli (Garma-Aviña, 1994). Furthermore, the cells show a wide variety of maturity levels, with many having fully matured and undergoing keratinization (Begum *et al.*, 2023). Moderately differentiated squamous cell carcinoma (SCC) displayed large numbers of round cells, alongside angular cells, less prominent nuclei and sporadic keratinized cells retaining nuclei, observed both singularly and in clusters. The presence of occasional keratinized cells and an increased ratio of immature cells suggested the predominance of less mature cellular forms (Chandrashekaraiah *et al.*, 2011).

However, the poorly differentiated squamous cell carcinoma (SCC) does not show much of the solid cell nest as much as the well-differentiated SCC and moderately differentiated SCC. Instead, they were mostly organized in a pseudoacinar pattern and enclosed by cells resembling myoepithelial cells (María del Mar *et al.*, 2019). The areas with poor differentiation exhibited an anaplastic morphology, characterized by small, polygonal cells arranged in cords or individually, without keratin formation or dyskeratosis, and with a moderate infiltration of lymphocytes and plasma cells in the stroma (Belluco *et al.*, 2013).

The table below shows the differentiation of squamous cell carcinoma (SCC) subtypes (grading) based on histopathological features using Brooder's grading system:

Table 2.1: Histopathological features of squamous cell carcinoma (SCC) for main subtypes (grading)

	WD SCC	**MD SCC**	**PD SCC**
Keratinization	Prominent keratinization	Less keratinization	Minimal or absent
Nuclear morphology	Abundant eosinophilic cytoplasm	Less eosinophilic cytoplasm	Amphophilic cytoplasm
Pleomorphism	Minimal pleomorphism	Moderate pleomorphism	Marked pleomorphism
Intercellular bridge	Prominent intercellular bridge	Less prominent intercellular bridge	No intercellular bridge
Degree of invasion	Invasion into dermis and subcutis by fibrous connective tissue proliferation	Prominent invasion with smaller neoplastic islands	Deep invasion with single or small clusters of cells
Mitotic figures	Mild	Moderate	Marked

Abbreviation: WD, well-differentiated; MD, moderately differentiated; PD, poorly differentiated

Sources: (Meuten, 2017)

Mitotic figures can help identify squamous cell carcinoma (SCC) subtypes (grading) depending on it rate of cell division. However, the factors such as tumour cellularity, cell size, section thickness, slicing techniques and the number of visual fields studied can affect the accuracy of mitotic rate estimation (Kapoor *et al*., 2013).

Sometimes, mitotic figures are difficult to identify due to the uncertainty in determining which phase they are in. Additionally, there are mitotic-like figures such as apoptotic bodies, hyperchromatic nuclei, deformed nuclei, karyorrhexis debris, inflammatory cells, and tissue artifacts that resemble mitotic figures. This resemblance makes it challenging for pathologists to accurately identify the truly mitotic figures. Given the various challenges in confirming mitotic figures, relying only on their evaluation may not be enough to accurately determine tumour subtypes (grading) (Donovan *et al*., 2021).

2.6 TUMOUR PREVALENCE AND ASSOCIATED FACTORS IN ASIA

In Asia, feline squamous cell carcinoma (SCC) is associated with the *Felis catus* Papillomaviruses (FcaPVs) with 21 biopsy samples collected in Japan (Yamashita-Kawanishi *et al.*, 2021). There are 5 types of FcaPVs including FcaPV-1, FcaPV-2, FcaPV-3, FcaPV-4 and FcaPV-5 which can be detected using a Polymerase Chain Reaction (PCR) (Vascellari *et al.*, 2019). In cats, papillomaviruses (PVs) occasionally prompt a transient surge in cell growth, resulting in self-resolving warts, but more commonly, they induce a less pronounced increase in cell growth, leading to raised patches, which can harbour increased DNA mutations predisposing to cancer, with uncertainty remaining regarding the direct role of PVs in causing DNA mutations in infected cat cells (Munday *et al.*, 2019).

A total of 21 of the cats diagnosed with squamous cell carcinoma (SCC) in Japan have collected the biopsy samples which resulted in seven of 21 cases being oral, nine of 21 cases are cutaneous and another five out of 21 were SCC at the other locations. However, there are no papillomaviruses observed in the histopathological examination but it is confirmed through the PCR (Yamashita-Kawanishi *et al.*, 2021). In Japan, there was a study among 1078 cats that were affected with tumours and the result from the study was 140 of the samples (13.1%) were benign and 938 out of 1078 (87.7%) were malignant tumours (Shida *et al.*, 2010). Mammary gland tumours, skin tumours, osteosarcomas, and hematopoietic tumours are among the most common malignancies found in dogs and cats (Todorova, 2006).

The researcher studied the association between the age (Salas *et al.*, 2015) and breed (Komazawa *et al.*, 2016) that contribute to tumour formation. Therefore, the age, breed and sex distribution may significantly contribute to the development of neoplasia cases in feline and canine species.

There has been a study examining the association between various factors contributing to the development of squamous cell carcinoma (SCC), including feline immunodeficiency virus (FIV) (Hutson, Rideout, & Pedersen, 1991) in feline and *Canis familiaris* papillomaviruses (CPVs) in canine (Christian, 2020).

2.7 TUMOUR PREVALENCE AND CHARACTERISTICS IN EUROPE AND NORTH AMERICA

A retrospective study was conducted in Switzerland from the year 1955 until 2008 on the rate of tumour cases that occur in canine species. Half of the patients diagnosed were diagnosed with a tumour. The finding shows that the most common tumour cases found in canines are adenoma (18.09%) followed by the mast cell tumour (6.5%), lymphoma (4.35%), melanocytic tumour (3.63%), fibroma (3.4%), haemangioma (2.8%), squamous cell carcinoma (1.95%) and osteoma (1.24%) (Gruntzig *et al.*, 2016). Large-breed dogs are most commonly associated with digital squamous cell carcinoma (dSCC) (Marconato *et al.*, 2021). However, the Schnauzer breed also can be affected by dSCC as there is a study conducted in Germany which shows Giant and Standard Schnauzer mostly affected with dSCC compared to the Miniature schnauzer (Aupperle-Lellbach *et al.*, 2023).

Consistent with other study, they discovered that the dark-coloured dog breeds such as Schnauzers, Briards, Rottweilers, Poodles, and Dachshunds are more predisposed to a skin cancer called digital squamous cell carcinoma (dSCC), while Jack Russell Terriers, often recognized for their white paws, showed a lower likelihood of developing this cancer compared to other mixed breeds (Cerezo-Echevarria *et al.*, 2023). The study conducted at the Veterinary Hospital of the University of Pennsylvania found that the maxilla emerged as the most frequent tumour location, contrasting with prior findings that identified the lingual or sublingual area and mandibular gingiva as the predominant sites (Soltero-Rivera *et al.*, 2014). However, the squamous cell carcinoma (SCC) of the digit commonly occurs in canines aged 6 to 13 years old with the presentation of an ulcer lesion, nodular masses at the nail bed, inflammatory thickening of the toe, nail deformation or loss, osteolysis, and lameness (Aupperle-Lellbach *et al.*, 2023).

CHAPTER 3

3.0 METHODOLOGY

3.1 STUDY AREA

This research was conducted at the UMK Veterinary Diagnostic Center (UVDC) Histopathology Laboratory at Faculty of Veterinary Medicine, UMK and Hospital Pengajar Perubatan Universiti Malaysia Kelantan (HPVUMK), Bachok, Kelantan. Analysis of the case and the histopathology slide observations are conducted at the histopathology laboratory.

3.2 STUDY DESIGN

This research study used the retrospective cohort study in which reliable records were taken from the histopathology laboratory and Hospital Pengajar Perubatan Veterinar (HPVUMK). The record was taken from the June year 2020 to June 2024. The data of patients diagnosed with squamous cell carcinoma (SCC) was recorded.

3.3 STUDY POPULATION

The study population involved in this research is felines and canines that were diagnosed with SCC at Hospital Pengajar Perubatan Veterinar (HPVUMK).

3.4 SELECTION CRITERIA

3.4.1 INCLUSION CRITERIA

- Availability of the historical data of the patient.
- Feline and canine patients that have been histopathology diagnosed with squamous cell carcinoma (SCC) in HPVUMK in between June 2020 to June 2024.

3.4.2 EXCLUSION CRITERIA

- Patients that are diagnosed with squamous cell carcinoma (SCC) outside the range of the year needed.
- Patients who diagnosed with squamous cell carcinoma outside the Hospital Pengajar Perubatan Veterinar, Universiti, Malaysia Kelantan (HPVUMK)
- Cases of SCC revisits were not counted multiple times which only the first diagnosis was included in the analysis.

3.5 SAMPLING METHOD AND PROCEDURE

3.5.1 DATA COLLECTION

The data for canines and felines diagnosed with squamous cell carcinoma (SCC) such as species, age, breed, clinical history and specimen types were collected from the histopathology laboratory at Universiti Malaysia Kelantan (UMK). The study focused on SCC cases recorded from the June 2020 to June 2024. Samples were sent to the histopathology laboratory of UMK for processing into histopathology slides, followed by staining process.

Data collection began at the histopathology laboratory to identify how many cases were suspected of squamous cell carcinoma (SCC). However, the information from the histopathology laboratory alone was insufficient, as patient signalment, clinical history, physical examination findings and tentative diagnoses were not fully recorded. Therefore, data collection was extended to the Hospital Pengajar Perubatan Veterinar Universiti Malaysia Kelantan (HPVUMK) to gather additional information on cases tentatively diagnosed with SCC and number of felines and canines visit to HPVUMK yearly. Both data of patients who definitive and tentatively diagnosed cases were recorded and compiled into an Excel sheet for analysis. The diagnosis of the SCC cases confirmed by pathologist at the UMK Veterinary Diagnostic Center (UVDC) to establish the definitive diagnosis.

The laboratory reference numbers have been collected, and the subsequent step involves locating the corresponding histopathology slides from the storage archives. Upon retrieval, only four slides of squamous cell carcinoma (SCC) cases were found. Three slides, along with their archive blocks, were missing, likely due to issues such as improper storage or accidental disposal. However, two slides could not be retrieved due to possible technical issues such as accidental disposal of the slides or broken slides. As a result, re-sectioning of two slides was attempted for the remaining tissue blocks to prepare new histopathology slides. The newly prepared slides from the tissue blocks were then stained with hematoxylin and eosin (H&E) for histopathological evaluations.

3.5.2 RE-SECTIONED THE TISSUE BLOCK FOR SLIDE INTERPRETATION

The tissue block is sectioned to obtain thin slices suitable for microscopic examination. This process is performed using a microtome, a precision instrument designed for cutting tissue sections. The block was placed on an ice plate to prevent tissue twitching or artifacts. Once chilled, the block was carefully trimmed with a blade to remove any frozen ice droplets adherence.

Next, the embedded tissue block was carefully placed onto the microtome stage, and trimmed using a standard Leica High Profile 818 microtome blade. The blade advanced across the tissue block, slicing thin sections with a thickness of 2.5 micrometers. These sections are then floated onto a warm water bath at around 40℃ to flatten and stretch them, ensuring uniform thickness and optimal adherence to glass slides. The sections were carefully transferred onto glass slides and left to dry.

3.5.3 HISTOPATHOLOGY SLIDE PREPARATION

The first step in histology slide preparation was tissue processing which involves running the glass slide through xylene 1 and xylene 2 for 2 minutes each. This is known as the deparaffinization steps to remove the paraffin. Then, the hydration step was done by placing the slide into 100% alcohol, and 75% alcohol for 2 minutes each to remove the xylene. Subsequently, the slide was rinsed under running tap water and then placed into distilled water for 3 seconds. Staining process was performed by staining the glass slide with hematoxylin for 3 minutes, followed by an 8-minute wash under running tap water and a brief immersion in an acid-alkaline solution. Afterward, the slide was rinsed again under running tap water and placed in distilled water for 3 seconds. The slide was then stained with 0.5% eosin, washed again in running tap water, and finally placed in distilled water for 3 seconds.

Then, the next procedure was continued with the dehydration and clearing process by placing the glass slide through the alcohol 75%, alcohol 100%, and xylene 1 for 2 minutes. Afterward, running the glass slide through the xylene 2. After that, the glass slide was dipped into the xylene 1 once. Then, the mounting process was done by adding one drop of DPX mounting medium on the tissue sample. Finally, place the coverslip on top of the glass slide. The tissue was observed for microscopic changes using a Olympus CX21LED digital microscope and the slides were scanned using a Panoramic MIDI II scanner to retrieve high-quality images.

3.6 DATA ANALYSIS

The data sample taken was analysed using the Statistical Package for the Social Sciences (SPSS) software using the Fisher's exact test to examine the association between the two categorical variables. The significance level was set at ($p<0.05$). The two variables include the dependent and independent, in categorical data. The dependent variable is histopathological differentiation, while the independent variables consist of age, breed, management status, and neutering status of the patient. The age variable was converted into categorical data by categorizing the age range as follows:

Table 3.1: Classification of age range in felines	
Kitten	Birth to 1 years
Young Adult	1 years to 6 years
Mature Adult	7 years to 10 years
Senior	More than 10 years

(Quimby, 2021)

Table 3.2: Classification of age range in canines	
Puppy	Birth to 6 months
Juvenile	6 months to 1 years
Young Adult	1 years - 2 years
Mature Adult	2 years - 6 years
Senior	7 years - 11 years
Geriatric	More than 12 years

(Harvey, 2021)

Prevalence was calculated using the number of existing cases of a particular condition in a population divided by the total population at a specific time. The formula used was:

$$\text{Prevalence} = \frac{\text{Number of cases in the population at one time}}{\text{Total population at the same point in time}} \times 100 \quad (3.1)$$

(Ford, 2020)

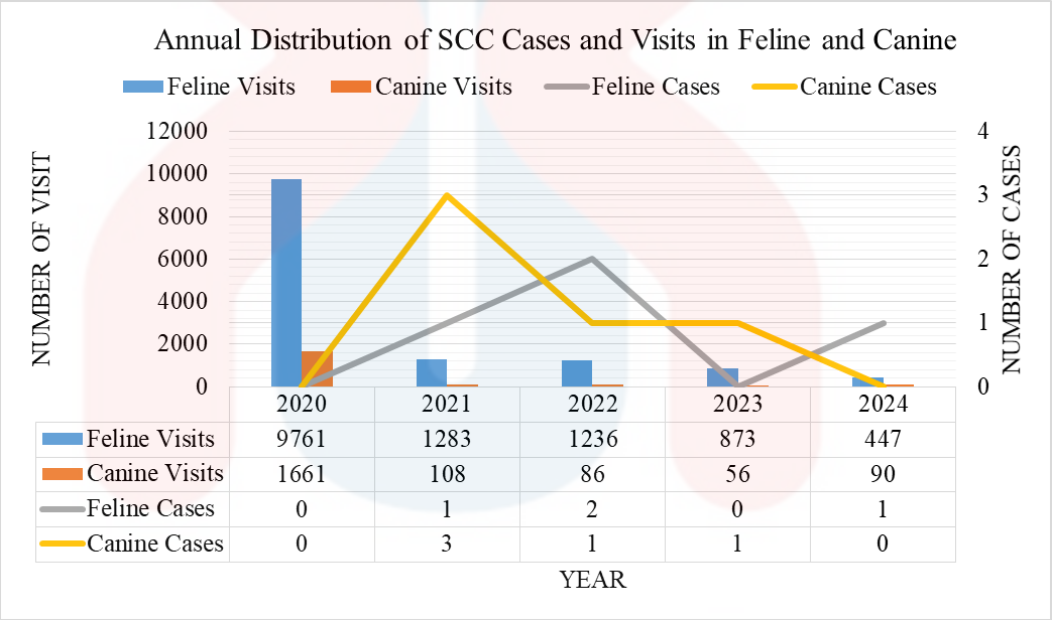
The data analysis included a pie chart illustrating the distribution of lesion locations in felines and canines squamous cell carcinoma (SCC) cases and a clustered column-line chart showing the annual distribution of SCC cases and visits in felines and canines. Both charts were created using Microsoft Excel to summarize and present the findings clearly.

CHAPTER 4

4.0 RESULT AND DISCUSSION

4.1 RESULTS

4.1.1 YEARLY DISTRIBUTION OF SQUAMOUS CELL CARCINOMA (SCC) CASES AND VISITS IN FELINES AND CANINES



	2020	2021	2022	2023	2024
Feline Visits	9761	1283	1236	873	447
Canine Visits	1661	108	86	56	90
Feline Cases	0	1	2	0	1
Canine Cases	0	3	1	1	0

Figure 4.1: Total number of feline and canine diagnosed with squamous cell carcinoma (SCC) from the year 2020 to 2024 in HPVUMK

Based on data obtained from the Hospital Pengajar Perubatan Veterinar, Universiti Malaysia Kelantan (HPVUMK), there were a total of nine cases of squamous cell carcinoma (SCC) in both felines and canines from the year 2020 to 2024. In 2020, no cases were reported. In 2021, one case in felines and three cases in canines were reported. For 2022, three cases of felines and one case of canines were reported. In 2023, only one case in canines and in 2024, one case in felines was reported.

4.1.2 PREVALENCE OF SQUAMOUS CELL CARCINOMA IN FELINE AND CANINE AT HPVUMK

Table 4.1: Prevalence of squamous cell carcinoma (SCC) in feline and canine at Hospital Pengajar Veterinar, Universiti Malaysia Kelantan from the year 2020 to 2024

	FV	CV	FC	CC	FP	CP
2020	9761	1661	0	0	0.0%	0.0%
2021	1283	108	1	3	0.08%	2.78%
2022	1236	86	2	1	0.16%	1.16%
2023	873	56	0	1	0.0%	1.79%
2024	447	90	1	0	0.22%	0.0%

Abbreviation: FV, feline visits; CV, canine visits; FC, feline cases; FP, feline prevalence; CP, canine prevalence

The highest prevalence in canines was observed in 2021 at 2.78%, while in felines, it was highest in 2024 at 0.22%. In felines, there is no consistent pattern of increasing or decreasing cases of squamous cell carcinoma (SCC), whereas in canines, a decreasing trend is observed, with the number of cases steadily declining in subsequent years. In 2020, both felines and canines show 0% of SCC due to certain limitations discussed in the discussion.

4.1.3 STATISTICAL ANALYSIS FINDINGS

Table 4.2: Statistical analysis test of the risk factors with squamous cell carcinoma (SCC) in felines (p-value)

Variable	Squamous Cell Carcinoma Cases (n = 4), n (%)	p-value
Age		**0.446**
Kitten	1 (25.0%)	
Young adult	2 (50%)	
Mature adult	1 (25.0%)	
Senior	0 (0.0%)	
Breed		**0.362**
Domestic short-hair	3 (75.0%)	
British short-hair	1 (25.0%)	
Persian	0 (0.0%)	
Siamese	0 (0.0%)	
Neutering status		**0.223**
Yes	1 (25.0%)	
No	2 (50.0%)	
Not in listed	1 (25.0%)	
Management status		**0.721**
Indoor	1 (25.0%)	
Outdoor	1 (25.0%)	
Semi indoor	1 (25.0%)	
Not in listed	1 (25.0%)	

The p-values obtained for the risk factors in felines associated with squamous cell carcinoma (SCC) development were greater than ($p>0.05$), suggesting that none of the factors were significantly correlated to SCC in felines. However, it was observed that young adult felines had a higher number of cases affected by SCC. In terms of breed, domestic short-haired cats were more commonly affected compared to other breeds. Regarding neutering status, unneutered felines showed a higher prevalence of SCC. For management, there was no variation as each variable had only one case, which had no significant patterns.

Table 4.3: Statistical analysis test of the risk factors with squamous cell carcinoma (SCC) in canines (p-value)

Variables	Squamous Cell Carcinoma Cases (n = 5), n (%)	p-value
Age		**0.741**
Puppy	1 (20.0%)	
Juvenile	0 (0.0%)	
Young adult	0 (0.0%)	
Mature adult	1 (20.0%)	
Senior	3 (60.0%)	
Geriatric	0 (0.0%)	
Not in listed	0 (0.0%)	
Breed		**0.306**
Golden retriever	1 (20.0%)	
Shih-tzu	1 (20.0%)	
English bull dog	1 (20.0%)	
Pitbull	1 (20.0%)	
Belgian malinois	0 (0.0%)	
Schnauzer	1 (20.0%)	
Not in listed	0 (0.0%)	
Neutering status		**0.333**
Yes	1 (20.0%)	
No	4 (80.0%)	
Not in listed	0 (0.0%)	

Table 4.3 (continued): Statistical analysis test of the risk factors with squamous cell carcinoma (SCC) in canines (p-value)

Variables	Squamous Cell Carcinoma Cases (n = 5), n (%)	p-value
Management status		**0.549**
Indoor	2 (40.0%)	
Outdoor	0 (0.0%)	
Semi indoor	2 (40.0%)	
Not in listed	1 (20.0%)	

The p-values for the risk factors in canines diagnosed with SCC revealed no significant associations ($p>0.05$) between the risk factors and SCC development in canines. However, senior dogs were more frequently affected by SCC. In terms of breed, all breeds had one case each, with no specific breed showing a higher predisposition. Unneutered dogs had a higher number of cases. For management, both indoor and semi-indoor dogs had an equal number of cases recorded.

4.1.4 DISTRIBUTION OF MASS LOCATIONS AND LESIONS APPEARANCE IN FELINES AND CANINES DIAGNOSED WITH SQUAMOUS CELL CARCINOMA (SCC)

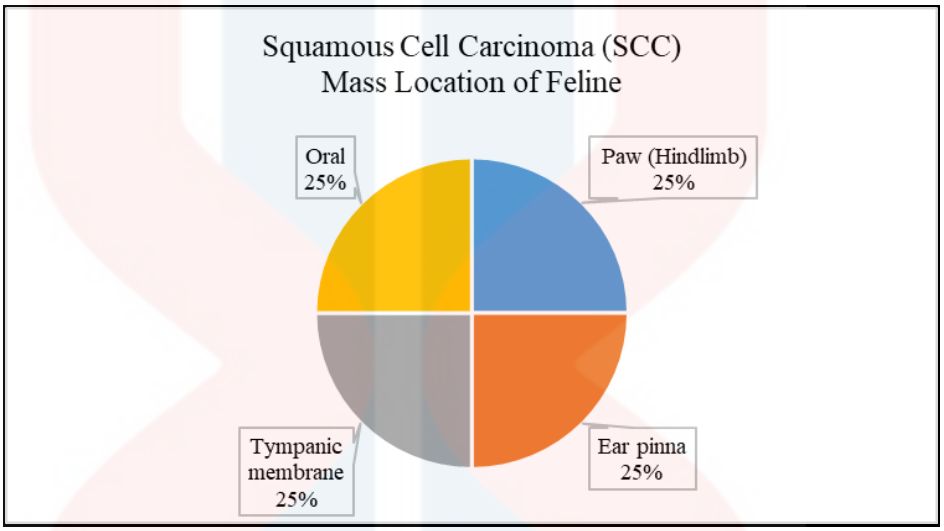


Figure 4.2: Distribution mass location in feline diagnosed with squamous cell carcinoma (SCC), (n = 4), n (%)

The mass locations in felines diagnosed with squamous cell carcinoma (SCC) were distributed across the oral cavity, paw (hindlimb), tympanic membrane, and ear pinna, with each location representing 25% of the cases. The lesions varied in presentation, with some appearing as pedunculated growths, while others were ulcerated and cauliflower-like in shape. These masses exhibited a moist appearance and a reddened surface, typical of the inflammatory response associated with SCC.

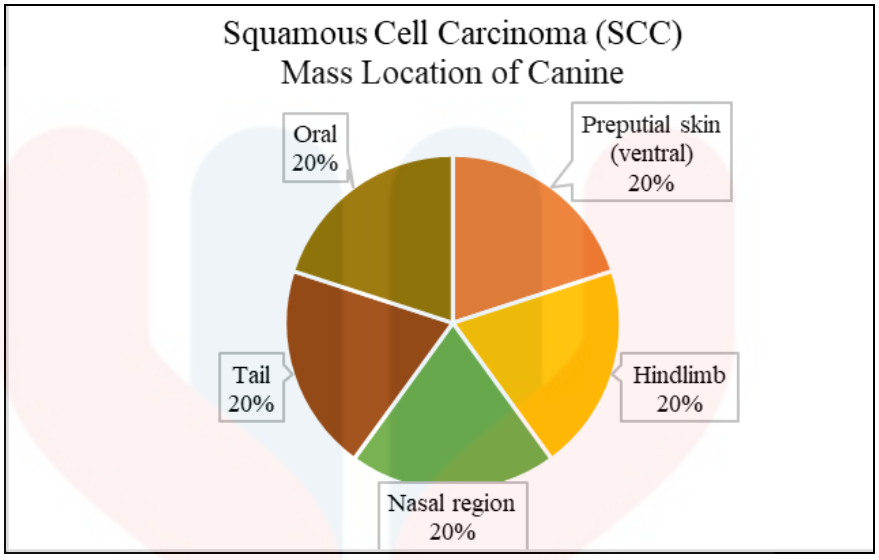


Figure 4.3: Distribution mass location in feline diagnosed with Squamous Cell Carcinoma (SCC), (n = 5), n (%)

In canines, the masses were found in the oral cavity, preputial skin, tail, hindlimb, and nasal region, with one case from each location. The lesions were heterogeneous, with some showing ulcerated surfaces and others presented as non-ulcerated, dark red, moist masses. Oral lesions, in particular, were associated with hypersalivation and a foul odour, common in advanced SCC cases in canines.

4.1.5 HISTOPATHOLOGICAL FEATURES OF DIFFERENT SUBTYPES (GRADING) OF SQUAMOUS CELL CARCINOMA (SCC)

A. Well-differentiated squamous cell carcinoma (SCC) in felines and canines

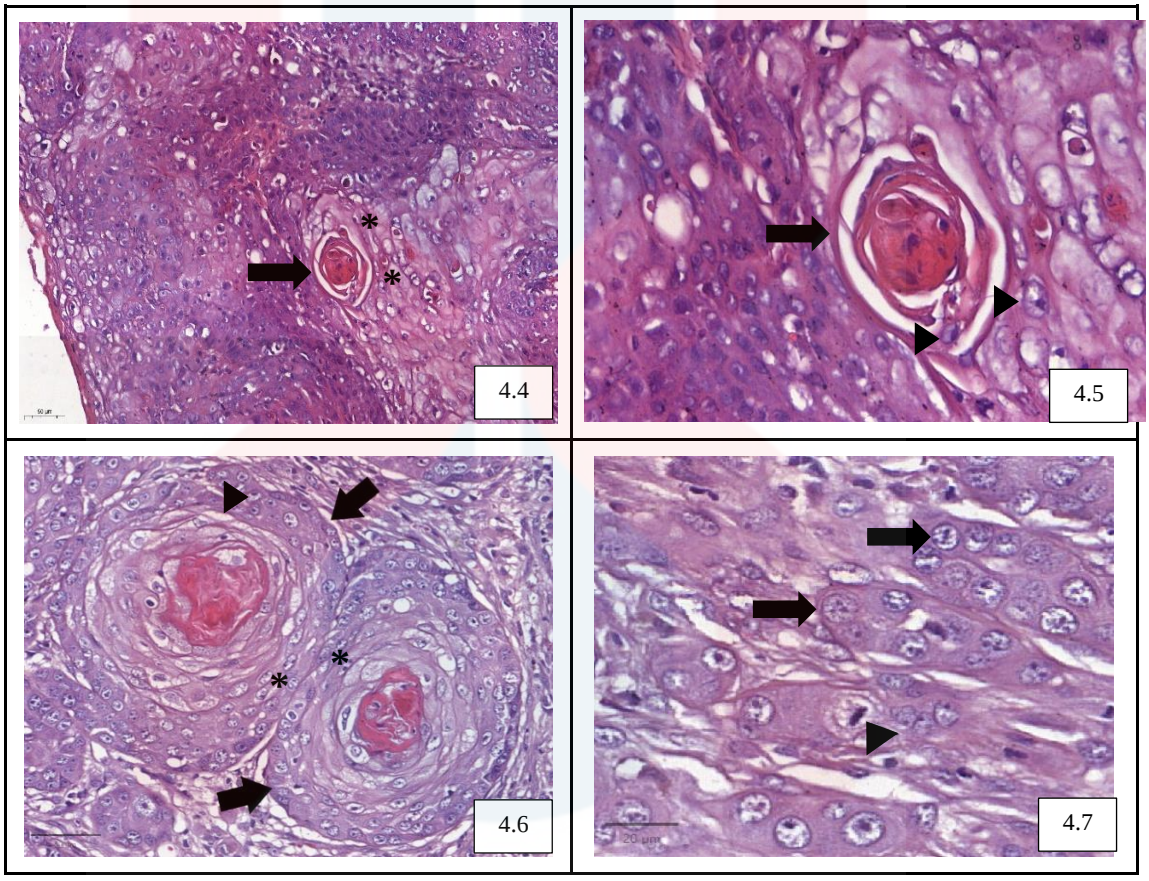


Figure 4.4: Presence of newly formed keratin pearl (arrow) with irregularly arranged in an islet, along with the proliferation of round to spindle-shape squamous epithelial cells (asterisk) (H&E, 40×) (feline). **Figure 4.5:** Presence of keratin pearls (arrow) surrounded by pleomorphic squamous cells, characterized by round to oval nucleoli (arrowhead) (H&E, 100×) (feline). **Figure 4.6:** Presence of multiple keratin pearls (arrow) with epithelial nest arranged in concentric rings and epithelial proliferation arranged in an islet with pleomorphic cell (asterisk) showing a round to oval in shape with vesicular and prominent nucleoli (arrowhead) (H&E, 100×) (canine).**Figure 4.7:** Presence of a mitotic figure (arrowhead) in metaphase, where the nucleus is visible with condensed chromatin and other adjacent cell show features such as round, vesicular and prominent nucleoli (arrow) (H&E, 100×) (canine).

In general, histopathological features of well differentiated squamous cell carcinoma in both felines and canines were presented with the keratinization, pleomorphic squamous cells, prominent nucleoli and mitotic figures activity. Formation of the keratin pearl was observed by the arrangement of irregular cell shape in a nest like appearance due to the proliferative growth (Figures 4.4 to 4.6). Besides, the squamous cells which exhibit a notable nuclear pleomorphism arranged in round to spindle shape cell together with marked atypical forms were observed (Figures 4.4 to 4.6). Additionally, the mitotic figures that were identified in metaphase are one of the well differentiated characteristics of histopathological features (Figure 4.7).

B. Moderately differentiated squamous cell carcinoma in felines

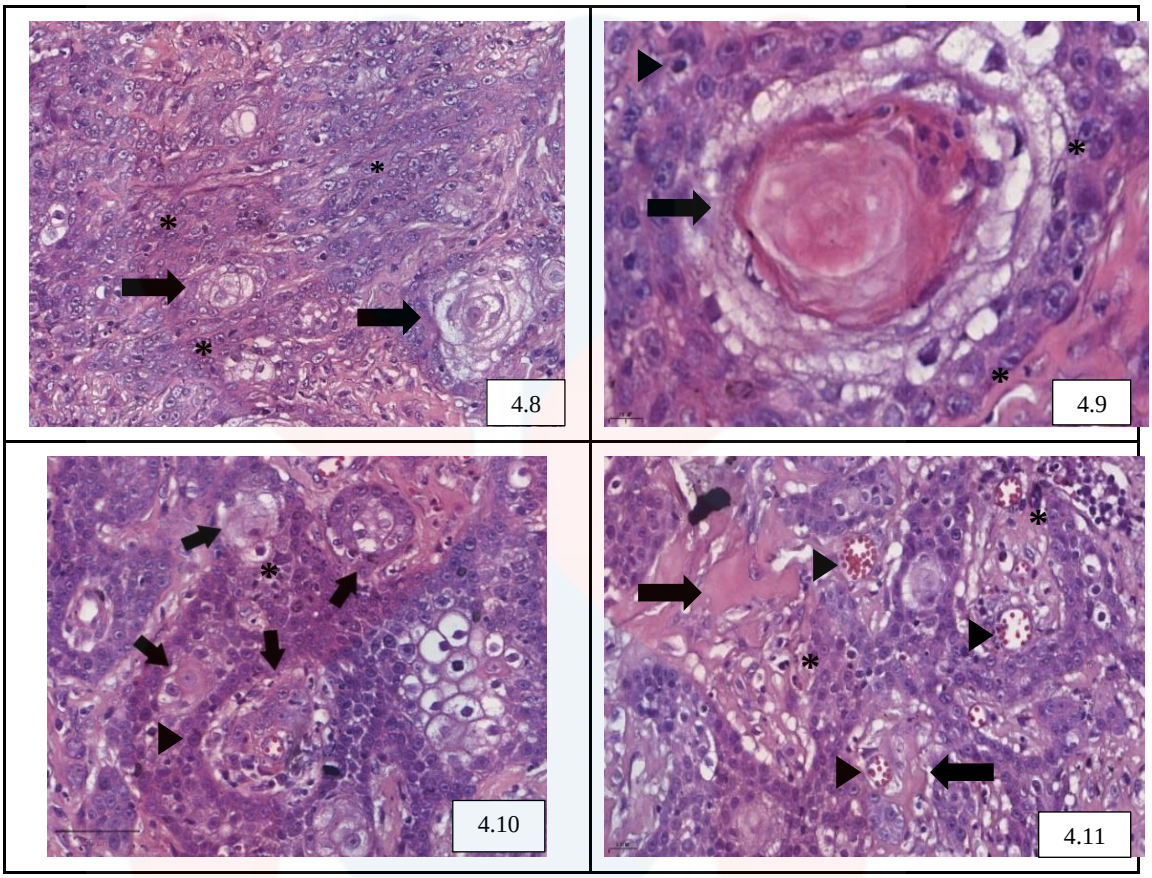


Figure 4.8: Presence of irregularly newly arranged squamous epithelial nest (arrow) with cellular infiltration (asterisk) pattern of squamous epithelial cells (H&E, 40✕) (feline). **Figure 4.9:** Presence of keratin pearl (arrow) surrounded by pleomorphic cells (asterisk) with prominent nucleoli (arrowhead) arranged in concentric pattern with less prominent intercellular bridges observed (H&E, 100✕) (feline). **Figure 4.10:** Initial stage of squamous epithelial cell differentiation (arrow) with prominent nucleoli (asterisk) and round to oval nucleoli (arrowhead) (H&E, 100✕) (feline). **Figure 4.11:** Presence of cellular infiltration (asterisk) of epithelial cells within the collagenous stroma (arrow) along with multiple blood capillary (arrowhead) indicating angiogenesis within the mass (H&E, 100✕) (feline).

In this study, two cases of moderately differentiated squamous cell carcinoma (SCC) were diagnosed, one in a canine and one in a feline which shows the histopathological features of a newly formed squamous epithelial nest, keratinization, prominent nucleoli, cellular infiltration pattern, pleomorphic cell with less prominent intercellular bridge. The newly arranged squamous epithelial nest was observed with significant nuclear pleomorphic characteristic (Figure 4.8 to 4.10). Besides, the moderately formation of keratin pearl was observed with less prominent intercellular bridge compared to the well-differentiated SCC surrounded with pleomorphic cell characterised by round to spindle cell shape (Figure 4.9). Lastly, the histopathological feature observed from this study was the moderate cellular infiltration of the epithelial cell indicating moderate invasion of the cell within the mass (Figures 4.8, 4.11) together with the angiogenesis for the tumour growth (Figure 4.11).

4.2 DISCUSSION

Squamous cell carcinoma (SCC) is one of the common malignant epithelial tumours that occur in both feline and canine. Squamous cell carcinoma (SCC) originates from the squamous cell epithelium and indicates that this tumour can arise in any location with the presence of squamous cells (Jie *et al.,* 2021). However, the exact causes of SCC development are still not fully understood and only a few have specifically explained the underlying reason why certain risk factors of the animals are susceptible to developing the tumours.

Squamous cell carcinoma (SCC) was diagnosed in felines and canines over five years, with four cases reported in felines and five cases in canines. In 2020, no cases were reported. The lower prevalence observed in certain years could potentially reflect limitations in diagnostic procedures or a reduced number of cases being diagnosed which may lead to inaccurate comparisons when looking at data across different years. The diagnostic equipment needed for diagnosing SCC includes a biopsy kit for sample collection and a digital microscope for examining the histopathological patterns. At HPVUMK, the services provided are limited to histopathology, where tissue samples are processed and analysed to confirm the presence and subtypes (grading) of SCC. Besides, it is also may attribute to owner-related constraint such as financial problems or limited awareness of the SCC. Furthermore, the increased number of biopsies performed at HPVUMK in recent years, likely due to improved referral practices, could have contributed to more precise diagnoses. Consequently, the chances of detecting SCC cases were lower during 2020 due to the movement control order (MCO) in response to COVID-19.

Based on the figure 4.4 to 4.11, squamous cell carcinoma (SCC) subtypes (grading) are categorised based on the degree of keratinization, mitotic figure observation, histological cell architecture and degree of invasion of neoplastic cells. There are two main subtypes (grading) of the SCC including well-differentiated and moderately differentiated were the main features observed in both felines and canines in this study. Squamous cell carcinoma (SCC) subtypes (grading) cannot be differentiated solely based on their macroscopic appearance (Lin, 2014). Further diagnostic tests, such as cytology and histopathology, are necessary to accurately classify the subtypes (grading) (Kokubun *et al.*, 2023).

From figure 4.4 to 4.7, well-differentiated SCC is characterized by cords or nests of neoplastic cells with a prominent presence of keratin pearls at the centre, prominent intercellular bridges, and abundant stromal tissue, whereas from figure 4.8 to 4.11 are characterized into moderately differentiated SCC which displays thinner fibrous stroma, irregular cell orientation, and prominent individual keratinization with presence of mitotic activity.

In contrast, poorly differentiated SCC lacks keratin pearls or cord formation, presenting highly pleomorphic spindle and polygonal cells, minimal fibroplasia, and marked cellular anaplasia and mitotic activity, reflecting increasing malignancy and loss of differentiation. Thus, specialized markers like immunohistochemistry (IHC) are often used to help in the case of tentative diagnosis made in order to provide confirmatory diagnosis into tumour differentiation (Chandrashekaraiah *et al.*, 2011).

For non-tonsillar head and neck squamous cell carcinoma (HNSCC), the prognosis is generally good with early intervention, such as surgery, if the tumour is detected at an early stage. However, in cases of late-stage HNSCC, where the disease has progressed, the prognosis becomes grave, with treatment options offering limited success (Supsavhad, *et al.*, 2016). As comparison, the oral squamous cell carcinoma (OSCC) has a good prognosis in the early stage. In contrast, later diagnosis of OSCC resulting the OSCC being difficult to treat, and has a poor prognosis due to spreading and increased invasiveness (Bramati, *et al.*, 2021). One study stated that well-differentiated squamous cell carcinoma (SCC) has a better prognosis compared to the poorly differentiated SCC (Ferreira, *et al.*, 2021). However, the publications on prognosis for well-differentiated, moderately differentiated and poorly differentiated SCC are still limited at the moment.

In this study, a total of nine squamous cell carcinoma (SCC) cases were identified with four characterized as well-differentiated SCC, with one case from feline and three from canines. Two moderately differentiated SCC cases were detected, one from each species. Additionally, two cases from each species were tentatively diagnosed with SCC, but their subtypes (grading) could not be definitively classified. This classification could be further confirmed using immunohistochemistry (IHC), which is essential for a more precise determination of the histopathological subtypes (grading) of SCC. Immunohistochemistry (IHC) is an advanced method used for further clarification when the histopathological diagnosis is difficult to interpret.

Immunohistochemistry (IHC) uses two different chromogens in order to visualize the colour such as DAB (3,3-diaminobenzidine)**,** which stains tissues in brown colour, and AEC (3-amino-9-ethylcarbazole), which results in a red colour (Chengmin, Jiayu, Jun, Yang, & Zhou, 2024). Thus, it is used for detecting cytokeratins and keratins (epithelial cells), vimentin (mesenchymal cells), and desmin (muscle cells) to help confirm the diagnosis of neoplasms in both human and veterinary medicine (Chandrashekaraiah *et al.*, 2011). Additionally, IHC is useful for all subtypes (grading) of SCC by helping to accurately classify each subtype. However, it is very important in cases of poorly differentiated SCC where there is a loss of cell architecture making the visualization characteristics of SCC difficult to be observed.

Age is one of the factors that play a role in making felines and canines more susceptible to squamous cell carcinoma (SCC). Squamous cell carcinoma (SCC) can be developed at any age for both feline and canine. However, there is a study that observed SCC was commonly diagnosed in middle-aged and older felines (Ryan Veterinary Hospital of the University of Pennsylvania, 2017). As for canines, SCC primarily occurs in the older canines with an approximate age about 8 years old (Seok J, 2024). In this study, age was not a significant factor for either species. However, young adult cats were most commonly diagnosed with SCC, whereas senior dogs were more frequently affected. The results observed from table 4.2 and table 4.3 were relevant to the other study as both species affected with SCC mostly range from 1 to 11 years old.

Although squamous cell carcinoma (SCC) typically occurs in older animals, certain predisposing factors may increase its occurrence in younger animals. For example, feline immunodeficiency virus (FIV) infection, commonly associated with high-stress environments and close confinement, such as restricted living space, can weaken immune defenses even in younger animals (Hosie, 2017). This immune suppression impairs the body's ability to counteract carcinogenic factors, such as ultraviolet (UV) exposure, or viral infections like papillomavirus (PV), which are known contributors to SCC development. Thus, PV may play a role in initiating SCC by disrupting key tumour-suppressor pathways (Inês Sequeira, 2022).

A study conducted in Germany proposed a possible association between feline leukemia virus (FeLV) and squamous cell carcinoma (SCC). However, the evidence remains inconclusive due to the limited number of FeLV positive cats diagnosed with SCC, making it difficult to establish a strong association (Reinacher, 1989). Thus, further research on the association between retroviral infections and SCC could provide valuable insights into the potential link between these infections and SCC, particularly in younger animals. Such studies would help clarify the role of retroviruses in the development of SCC in this demographic.

Unlike felines, which have no specific breed predisposition for squamous cell carcinoma (SCC), certain canine breeds are more frequently affected (Pavlin *et al.*, 2018). The breeds most commonly associated with SCC include Pit Bulls, Dalmatians, Terriers, Beagles, Schnauzers, Basset Hounds, and Collies have been reported to have a higher likelihood of being diagnosed with SCC (Alves *et al.*, 2022). These breeds are more prone to having areas of unpigmented skin, potentially increasing their direct exposure to sunlight. Data from Hospital Pengajar Veterinar, Universiti Malaysia Kelantan (HPVUMK) indicates that three domestic shorthair cats and one British shorthair cat were diagnosed with SCC. In dogs, one case of SCC was diagnosed in each of these breeds which included Golden Retriever, Shih Tzu, English Bulldog, Pitbull, and Schnauzer highlighting the diversity of breeds affected by this condition.

There is no significant association between neutering status and the likelihood of developing squamous cell carcinoma (SCC), as both neutered and unneutered animals have an equal risk of being affected (Belanger *et.al*, 2017; Smith, 2014; Sones, 2019). However, a retrospective study conducted in France has shown that neutered cats are more likely to develop SCC compared to intact cats (Lino & Lanore, 2019). Although this study shows unneutered felines and canines are more frequently diagnosed with SCC, there is no evidence to prove an association between neutering status and SCC development. Few studies stated no association of neutering status with development of the SCC which strongly supported the reason behind this insignificant p-value from this study.

Sunlight exposure is one of the causes of squamous cell carcinoma (SCC) development. Ultraviolet (UV) radiation acts as a carcinogen by triggering photochemical reactions that damage deoxyribonucleic acid (DNA), disrupt immune responses, and lead to mutations. This damage can result in actinic keratosis (AK), a preneoplastic condition caused by prolonged sun exposure, which may progress to SCC (Alves *et al.,* 2022). Animals with outdoor management have greater exposure to sunlight, increasing their risk of developing SCC compared to indoor or semi-indoor animals. From this study, one out of four felines diagnosed with SCC was managed outdoors. In contrast, none of the five canines diagnosed with SCC were kept outdoors. Thus, this study shows no significant association of the management status with the developing SCC.

One limitation of this study is the small sample size of patients diagnosed with squamous cell carcinoma (SCC), coupled with incomplete data retrieved from the Hospital Pengajar Veterinar, Universiti Malaysia Kelantan (HPVUMK). A small sample size renders the data statistically insignificant, making it difficult to establish a reliable association between risk factors and the development of SCC. Another limitation of this study is the increasing number of veterinary clinics in Kelantan each year, leading to fewer cases being diagnosed at HPVUMK as pet owners prefer clinics closer to their homes. Furthermore, data from the year 2024 may be underrepresented due to the study period only covering a partial year. Additionally, pet owners may hesitate to seek treatment due to concerns over high costs or financial constraints, potentially leading to underdiagnosed SCC cases in this region.

This study provides the important association of age, breed, neutering status and management status which could have contributed to the animal becoming more susceptible in developing SCC.

CHAPTER 5

5.0 CONCLUSIONS

In conclusion, squamous cell carcinoma (SCC) can occur across various ages and breeds, though certain age groups and breeds may be more predisposed to developing the condition. This study found no significant correlation between neutering status or management practices and the development of SCC. However, it highlights findings from other studies that may help explain the potential links between these risk factors and SCC.

Thus, the prevalence of squamous cell carcinoma (SCC) in felines has increased over the years at HPVUMK, and the prevalence in canines has shown a decreasing trend. The highest prevalence of SCC in felines was recorded in 2024 at 0.22%, while in canines, the highest prevalence occurred in 2021 at 2.78%. It also can be concluded that there is no significant association between risk factors such as age, breed, animal management, and neutering status with the development of SCC in felines and canines. Histopathological analysis confirmed that SCC subtypes (grading) do indeed vary based on factors such as tumour size, location, and degree of differentiation, supporting the hypothesis that these characteristics influence tumour appearance and behaviour.

To address the limitations, it is recommended to expand the geographical area of the study to include more cases, improving the statistical significance of the data. Besides, a broader study on the histopathological subtypes (grading) of squamous cell carcinoma (SCC) would be beneficial, particularly with the incorporation of immunohistochemistry (IHC) to enhance diagnostic accuracy. Furthermore, it is suggested to retain medical records for at least 10 years before disposal. This would allow for easier calculation of prevalence rates and ensure data accuracy, which could be utilized for publications and shared widely for educational purposes.

This research contributes to the field of small animal medicine by understanding the subtypes (grading) of SCC that plays a crucial role in predicting the prognosis of the patient and guiding treatment decisions. Furthermore, it offers valuable insights for pet owners, helping them reduce their pets' risk of SCC by minimising exposure to factors that may increase susceptibility, ultimately promoting preventive care and better overall health management.

REFERENCES

Alves, D. S., Cavalca, A. M. B., & Alves, C. E. F. (2022, March 7). A Critical Review of the risk factors associated with Canine Squamous Cell Carcinoma development. *Brazilian Journal of Veterinary Pathology*, *15*(1), 1-10. doi: 10.24070/bjvp.1983-0246.v15i1p1-10

Aupperle-Lellbach, H., Heidrich, D., Conrad, D., Beitzinger, C., Masala, N., Klopfleisch, R., & Muller, T. (2023). Comparative Study of Digital Squamous Cell Carcinoma in Giant, Standard, and Miniature Schnauzers. *Animal 2023, 13*(12). doi: 10.3390/ani13121990

Baba, A. I. (2007). Comparative Oncology. Publishing House of the Romanian Academy.

Bramati, C., Abati, S., Bondi, S., Lissoni, A., Arrigoni, G., Filipello, F., & Trimarchi, M. (2021). Early diagnosis of oral squamous cell carcinoma may ensure better prognosis: A case series. *Clinical Case Reports*, *9*(10). doi:10.1002/ccr3.5004

Begum, S., Deka, A., Tamuli, S. M., Dutta, B., & Buragohain, M. (2023). Histopathological And Cytopathological Findings Of Canine Squamous Cell Carcinoma. *Haryana Vet*, *62*(SI-2), 162-164.

Belanger, J. M., Bellumori, T. P., Bannasch, D. L., Famula, T. R., & Oberbauer, A. M. (2017). Correlation of neuter status and expression of heritable disorders. *Canine Genetics and Epidemiolog, 4*. doi:10.1186/s40575-017-0044-6

Belluco, S., Brisebard, E., Watrelot, D., Pillet, E., Marchal, T., & Ponce, F. (2013). Digital Squamous Cell Carcinoma in Dogs: Epidemiological, Histological, and Immunohistochemical Study. *Veterinary Pathology*, *50*(6), 1078-1082. doi: 10.1177/0300985813490757

Bertone, E. R., Snyder, L. A., & Moore, A. S. (2003). Environmental and Lifestyle Risk Factors for Oral Squamous Cell Carcinoma in Domestic Cats. *Journal of Veterinary Internal Medicine*, *17*(4), 557-562. doi: 10.1111/j.1939-1676.2003.tb02478.x

Bilgic, O., Duda, L., Sánchez, M. D., & Lewis, J. R. (2015). Feline Oral Squamous Cell Carcinoma: Clinical Manifestations and Literature Review. *Journal of Veterinary Dentistry*, *32*(1), 30-40. doi: 10.1177/089875641503200104

Cerezo-Echevarria, A., Kehl, A., Beitzinger, C., Müller, T., Klopfleisch, R., & Aupperle-Lellbach, H. (2023). Evaluating the Histologic Grade of Digital Squamous Cell Carcinomas in Dogs and Copy Number Variation of KIT Ligand—A Correlation Study. *Veterinary Sciences*, *10*(2), 88. doi: 10.3390/vetsci10020088

Chandrashekaraiah, G. B., Rao, S., Munivenkatappa, B. S., & Mathur, K. Y. (2011). Canine Squamous Cell Carcinoma: a Review of 17 Cases. *Brazilian Journal of Veterinary Pathology*, *4*(2), 78-86.

Chengmin, Z., Jiayu, L., Jun, H., Yang, L., & Zhou, Y. (2024, September 24). Application of Different Staining Methods in the Diagnosis of Pigment-Rich Melanoma. *Journal of Clinical Laboratory Analysis, 38*(19-20). doi:10.1002/jcla.25106

Cray, M., Selmic, L. E., & Ruple, A. (2020, August 3). Demographics of dogs and cats with oral tumors presenting to teaching hospitals: 1996–2017. *Journal of Veterinary Science, 21*(5). doi: 10.4142/jvs.2020.21.e70

Christian D.B.T Alves, M. N. (2020, Jun 4). Canine papillomavirus type 16 associated to squamous cell carcinoma in a dog: virological and pathological findings. *Brazilian Journal of Microbiology, 51*(4), 2087-2094. doi:10.1007/s42770-020-00310-4

Dickinson, K., & Boldan, M. (2022, October 26). *Squamous Cell Carcinomas in Cats*. PetMD. https://www.petmd.com/cat/conditions/cancer/squamous-cell-carcinomas-cats

Ferreira, I. G., Boff, A. L., Luzzato, L., Souza, P. R., & Bevilaqua, M. (2021, April 12). Well-Differentiated Squamous Cell Carcinoma: Is Histological Differentiation a Relevant Prognostic Parameter? *2*. doi:10.5826/dpc.1102a34

Fischer, E. G. (2020). Nuclear Morphology and the Biology of Cancer Cells. *Acta Cytologica, 64*(6), 511-519. doi:10.1159/000508780

Ford, G. (2020, November 6). *Prevalence vs. Incidence: what is the difference? - Students 4 Best Evidence*. Students 4 Best Evidence. https://s4be.cochrane.org/blog/2020/11/06/prevalence-vs-incidence-what-is-the-difference/

Ganta, C. (2018, March). *Cutaneous Squamous Cell Carcinoma in Cats: Associated Factors*. Kansas State Veterinary Diagnostic Laboratory. https://ksvdl.org/resources/news/diagnostic_insights/march2018/squamous.html

Garma-Aviña, A. (1994). The cytology of squamous cell carcinomas in domestic animals. *Journal of Veterinary Diagnostic Investigation*, *6*(2), 238-246. doi: 10.1177/104063879400600216

Gruntzig, K., Graf, R., Boo, G., Guscetti, F., Hassig, M., Axhausen, K. W., Fabrikant, S., Welle, M., Meier, D., Folkers, G., & Pospischil, A. (2016). *Swiss Canine Cancer Registry 1955e2008: 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. doi: 10.1016/j.jcpa.2016.05.011

Han, H. S., & Kano, R. (2020). Feline sporotrichosis in Asia. *Brazilian Journal of Microbiology*. doi: 10.1007/s42770-020-00274-5

Harvey, N. D. (2021, April 27). How Old Is My Dog? Identification of Rational Age Groupings in Pet Dogs Based Upon Normative Age-Linked Processes. *Front Veterinary Science, 8*. doi: 10.3389/fvets.2021.643085

Hosie., M. J. (2017). GUIDELINE for Feline immunodeficiency virus. *ABCD*. https://www.abcdcatsvets.org/guideline-for-feline-immunodeficiency-virus/

Hutson, C. A., Rideout, B. A., & Pedersen, N. C. (1991, November 15). Neoplasia associated with feline immunodeficiency virus infection in cats of Southern California. *J Am Vet Med Assoc, 199*(10), 1357-1362.

Inês Sequeira, M. d. (2022, October 11). Feline Oral Squamous Cell Carcinoma: A Critical Review of Etiologic Factors. *Veterinary Sciences, 9*(10). doi:10.3390/vetsci9100558

Jasni, S., & Al-Sultan, I. I. A. (2020). *Veterinary Pathology Is Exciting: The Journey Is Begins = Menariknya Patologi Veterinar: Bermulanya Satu Kembara*. UMK Press.

Jie, T. J., Reduan, M. F. H., Jasni, S., Shaari, R., Rajiendren, S. K., Shaharulnizim, N., Roslan, N. S., Kamaruzaman, I. N. A., & Aziz, A. R. (2021, February 20). Squamous Cell Carcinoma in a Left Periorbital Region in a Domestic Short Hair Cat: A Case Report. *Journal of Animal Health and Production, 9*(2), 136-139. doi: 10.17582/journal.jahp/2021/9.2.136.139

Kapoor, D. K., Puri, D. A., Prakash, D. A., Jazib, D., & Sharma, D. G. (2013). Mitotic Counting and its Significance in Histopathological. *Heal Talk, 5*(6).

Kim, S., Hong, S. S., Kim, J.-H., & Na, K.-J. (2022, April 30). Well-Differentiated Squamous Cell Carcinoma Found on the Tail of a Captive Leopard (Panthera Pardus): The Importance of Diagnostic Tests. *Journal of Veterinary Clinics, 39*(2), 70-74. doi: 10.17555/jvc.2022.39.2.70

Kokubun, K., Nakajima, K., Yamamoto, K., Akashi, Y., & Matsuzaka, K. (2023, March 11). Evaluation of oral brush liquid-based cytology for oral squamous cell carcinoma: a comparative study of cytological and histological diagnoses at a single center. *BMC Oral Health, 23*(1), 145. doi: 10.1186/s12903-023-02839-w

Komazawa, S., Sakai, H., Itoh, Y., Kawabe, M., Murakami, M., Mori, T., & Maruo, K. (2016). Canine tumor development and crude incidence of tumors by breed based on domestic dogs in Gifu Prefecture. *The Journal of Veterinary Medical Science, 7*(8), 1269-1275. doi: 10.1292/jvms.15-0584

Lascelles, B. D. X., & British Small Animal Veterinary Association. (2011). *BSAVA Manual of Canine and Feline Oncology* (J. Dobson & B. D. X. Lascelles, Eds.; Third edition ed., pp. 1). Wiley.

Lin, M. J., Pan, Y., Jalilian, C., & Kelly, J. W. (2014). Dermoscopic characteristics of nodular squamous cell carcinoma and keratoacanthoma. *Dermatology practical & conceptual*, *4*(2), 9–15. doi: 10.5826/dpc.0402a02

Lino, M., & Lanore, D. (2019). Prognostic factors for cats with squamous cell carcinoma of the nasal planum following high-dose rate brachytherapy. *Journal of Feline Medicine and Surgery*, *21*(12). doi: 10.1177/1098612X18822409

Łojszczyk, A., Łopuszyński, W., Szadkowski, M., Orzelski, M., & Twardowski, P. (2021). Aggressive squamous cell carcinoma of the cranium of a dog. *BMC Veterinary Research*. doi: 10.1186/s12917-021-02843-8

Marconato, L., Murgia, D., Finotello, R., Meier, V., Morello, E. M., Pisoni, L., Foglia, A., Guerra, D., Chalfon, C., Aralla, M., Mesto, P., Annoni, M., Albanese, F., Bettini, G., & Sabattini, S. (2021, April 30). *Clinical Features and Outcome of 79 Dogs With Digital Squamous Cell Carcinoma Undergoing Treatment: A SIONCOV Observational Study*. Front Vet Sci. doi: 10.3389/fvets.2021.645982

María del Mar, L.-M., Aloma, M.-F., David, V., Elena, M., & Joaquín, O. (2019). Highly invasive and poorly differentiated corneal squamous cell carcinoma in a dog. *BMC Veterinary Research*, *15*. doi: 10.1186/s12917-019-1790-3

Meuten, D. J., Moore, F. M., Donovan, T. A., Bertram, C. A., Klopfleisch, R., Foster, R. A., Smedley, R. C., Dark, M. J., Milovancev, M., Stromberg, P., Williams, B. H., Aubreville, M., Avallone, G., Bolfa, P., Cullen, J., Dennis, M. M., Goldschmidt, M., Luong, R., Miller, A. D., Whitley, D. (2021). International Guidelines for Veterinary: A Call to Action. Tumor Pathology: A Call to Action. *Veterinary Pathology*, *58*(5), 766-794. doi: 10.1177/0300985821101371

Miettinen, M., El-Rifai, W., Sobin, L. H., & Lasota, J. (2002). Evaluation of Malignancy and Prognosis of Gastrointestinal Stromal Tumors: A Review. *Human Pathology*, *33*(5), 478-483. doi: 10.1053/hupa.2002.124123

Morris, J., & Dobson, J. (2001). *Small Animal Oncology* (pp.4-110). Wiley.

Munday, J. S., Sharp, C. R., & Beatty, J. A. (2019). Novel viruses: Update on the significance of papillomavirus infections in cats. *Journal of Feline Medicine and Surgery*, *21*(5), 409-418. doi: 10.1177/1098612X18808105

Murphy, S. (2013). Cutaneous Squamous Cell Carcinoma in the Cat: Current understanding and treatment approaches. *Journal of Feline Medicine*, *15*(5), 401-407. doi/10.1177/1098612X13483238

Parikh, R. M., & Jagtap, M. W. (2006). Fine Needle Aspiration Causes Iatrogenic Spread of Malignancy. *Hypothesis*, *4*(2), 8.

Patel, A. (2020, July 30). Benign vs Malignant Tumors. *JAMA Oncology Patient Page*. doi: 10.1001/jamaoncol.2020.2592

Pavlin, D., Dolenšek, T., Švara, T., & Nemec, A. (2018, January 22). Solid type primary intraosseous squamous cell carcinoma in a cat. *BMC Veterinary Research*. doi: 10.1186/s12917-018-1344-0

Perumpanani, A. J., Sherratt, J. A., & Norbury, J. (1997). Mathematical modelling of capsule formation and multinodularity in benign tumour growth. *Nonlinearity*, *10*(6), 1599-1614. doi: 10.1088/0951-7715/10/6/009

Powers, B. E., & Dernell, W. S. (1998). Tumor Biology and Pathology. *Clinical Techniques in Small Animal Practice*, *13*(1), 4-9. doi: 10.1016/S1096-2867(98)80020-0

Quimby, J., Gowland, S., Carney, H. C., DePorter, T., Plummer, P., & Westropp, J. (2021). 2021 AAHA/AAFP Feline Life Stage Guidelines. *Journal of Feline Medicine and Surgery*, *23*(3), 211-233. doi: 10.1177/1098612X21993657

Reinacher, M. (1989). Diseases Associated with Spontaneous Feline. *Veterinary Immunology and Immunopathology, 21*, 85-95. doi:10.1016/0165-2427(89)90132-3

Ryan Veterinary Hospital of the University of Pennsylvania. (2017, November). *Squamous Cell Carcinoma in Cats*. Penn Vet Ryan Hospital. https://www.vet.upenn.edu/docs/default-source/ryan/comprehensive-cancer-care/squamous-cell-carcinoma-in-cats.pdf?sfvrsn=dbb2e0ba_2

Salas, Y., Márquez, A., Diaz, D., & Lomero, L. (2015). Epidemiological Study of Mammary Tumors in Female Dogs Diagnosed during the Period 2002-2012: A Growing Animal Health Problem. *PLoS ONE*, *10*(5). doi:10.1371/journal.pone.0127381

Seok, J., & Lee, S. (2024, July 26). Case report: Evaluation of cutaneous squamous cell carcinoma metastasized to lymph nodes using 18F-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography in a dog. *Front. Vet. Sci*, *11*. doi: 10.3389/fvets.2024.1429094

Shida, T., Yamada, T., Maruo, T., Ishida, T., Takeda, H., Kawamura, H., Ishikawa, T., Sugiyama, H., Madarame, H., Ito, T., Kayanuma, H., & Suganuma, T. (2010). A Retrospective Study in 1,070 Feline Tumor Cases of Japan. *Japan Veterinary Cancer Society*, *1*(1), 1-7.

Sivaseelan, S. (2020). *Animal Oncology* (pp. 3-168). Astral International Pvt. Limited.

Smith, A. N. (2014). The Role of Neutering in Cancer Development. *Vet Clin Small Anim*. doi: 10.1016/j.cvsm.2014.06.003

Soltero-Rivera, M. M., Krick, E. L., Reiter, A. M., Brown, D. C., & Lewis, J. R. (2014). Prevalence of regional and distant metastasis in cats with advanced oral squamous cell

carcinoma: 49 cases (2005–2011). *Journal of Feline Medicine and Surgery*, *16*(2), 164-169. doi: 10.1177/1098612X1350297

Sones, E. (2019, September). Neoplastic considerations for spaying and neutering dogs. *Clinical Theriogenology*, *11*. https://clinicaltheriogenology.net/index.php/CT/article/download/9494/15386

Sugarbaker, P. H. (Ed.). (2011). *Peritoneal Carcinomatosis: Principles of Management* (Vol. 82). Springer US. doi: 10.1007/978-1-4613-1247-5_6

Supsavhad, W., Dirksen, W. P., Martin, C. K., & Rosol, T. J. (2016). Animal models of head and neck squamous cell carcinoma. *The Veterinary Journal*, 7-16. doi:10.1016/j.tvjl.2015.11.006

Thrall, M. A., Rebar, A. H., & Cowell, R. L. (2003). Cytologic criteria of malignancy. *The North American Veterinary Conference*. doi:10.5555/20063240060

Todorova, I. (2006). Prevalence And Etiology Of The Most Common Malignant Tumours In Dogs And Cats. *Bulgarian Journal of Veterinary Medicine*, *9*(2), 85-98. doi: 10.30539/iraqijvm.v43i2.533

Vascellari, M., Mazzei, M., Zanardello, C., Melchiotti, E., Albanese, F., Forzan, M., Croce, M. F., Alberti, A., & Abramo, F. (2019, November). Felis catus Papillomavirus Types 1, 2, 3, 4, and 5 in Feline Bowenoid in Situ Carcinoma: An In Situ Hybridization Study. *Veterinary Pathology*, *56*(6), 818. doi: 10.1177/0300985819859874

Villalobos, A. E. (2018). *Tumors of the Skin in Dogs - Dog Owners*. MSD Veterinary Manual. https://www.msdvetmanual.com/dog-owners/skin-disorders-of-dogs/tumors-of-the-skin-in-dogs

Willcox, J. L., Marks, S. L., Ueda, Y., & Skorupski, K. A. (2019). Clinical features and outcome of dermal squamous cell carcinoma in 193 dogs (1987-2017). *Veterinary and Comparative Oncology*, *17*(2), 130-138. doi:10.1111/vco.12461

Wittekind, C., & Neid, M. (2005). Cancer Invasion and Metastasis. *Oncology*, *69*(1), 14-16. doi: 10.1159/000086626

Yamashita-Kawanishi, N., Sawanobori, R., Matsumiya, K., Uema, A., Chambers, J. K., Uchida, K., Shimakura, H., Tsuzuki, M., Chang, C.-Y., Chang, H.-W., & Haga, T. (2021). Detection of felis catus papillomavirus type 3 and 4 DNA from squamous cell 4 carcinoma cases of cats in Japan. *The Journal of Veterinary Medical Science*, *80*(8), 1229–1233. doi: 10.1292/jvms.21-0153

Yanofsky, V. R., Mercer, S. E., & Phelps, R. G. (2010, November 4). Histopathological Variants of Cutaneous Squamous Cell Carcinoma: A Review. *Journal of Skin Cancer*, 1-13. doi:10.1155/2011/210813