Histopathological Investigation of Selective Neoplasm Found in Dog and Cat

Anamika Dhar

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Pathology.

Department of Pathology and Parasitology

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh.

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Authorization

I hereby declare that I am the sole author of the thesis submitted in fulfillment of the requirements for the Degree of Master of Science (MS) in the Department of Pathology and Parasitology (DPP), Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University (CVASU). I authorize CVASU to lend this thesis or to reproduce the thesis by Xeroxing or any other means, at the request of any other institutions or individuals for the purpose of scholarly research.

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The Author

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

.....	.....
Prof. Dr. Sharmin Chowdhury	**Associate Prof. Dr. Md. Shafiqul Islam**
Supervisor	Co-Supervisor
Department of Pathology and Parasitology	Department of Pathology and Parasitology

.....

Professor Dr. Md. Abdul Alim

Chairman of the Examination Committee & Head

Department of Pathology and Parasitology

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh.

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Abstract

Histopathological investigation of tumor cells plays a pivotal role in the diagnosis, prognosis, and treatment of various neoplastic conditions in companion animals. This study aims to explore the diverse cellular characteristics of suspected tumor cases includes lipoma in a cat, and a case of lymphoma and mammary gland carcinoma in two individual dogs using histopathological examinations. The tissue samples were collected after the surgical removal of the tumors from the respective animals that were documented at the Shahedul Alam Quadary Teaching Veterinary Hospital (SAQTVH) in Chattogram and the Teaching and Training Pet Hospital and Research Center (TTPHRC) in Dhaka. Grossly a large nodular mass were palpated in intra-abdominal region in a 5 years old local spayed cat and laparotomy was performed at TTPHRC, revealed a yellow, soft, lobulated mass with an oily surface. Besides, a 1.2 years old male Rottweiler dog with an around 5 cm nodular mass in left submandibular region and a local 8 years old unspayed dog with enlarged masses in mammary gland region were admitted in SAQTVH and TTPHRC respectively for surgery. Histopathological examination revealed mature adipocytes with eccentrically placed nuclei and less mitotic figures that indicate benign form of neoplasm in the cat called lipoma. In the Rottweiler dog the tumor tissue showed large number of pleomorphic cells, anisokaryosis, uneven distribution of chromatin materials, increased amount of binucleated cells indicating increased mitotic figure and fibroblast which is characteristics figure of lymphoma. The neoplastic cells also present within the blood vessels suggestive of metastasis and poor prognosis. In mammary gland tumor the ductal epithelial cells were proliferated, resulting in the creation of finger-like projections that extend into the inner space (lumen) and presence of pleomorphic cells with metaplasia within the milk duct indicative of invasive ductal carcinoma in dog. In Bangladesh, very few studies have been conducted on tumor in companion animals. Therefore, it’s essential to enhance our understanding of tumor biology and providing clinicians with valuable information to make perfect decisions in tumor management. Detailed investigation on the histopathology of abnormal cellular growth in this study will be helpful for diagnosis, prognosis and treatment of such cases in dogs and cats.

Keyword: histopathology, lipoma, lymphoma, mammary gland carcinoma, pleomorphic, metaplasia, metastasis, cat, dog.

Chapter 1: Introduction

Pets play a significant role in our society by fulfilling deep emotional and psychological needs. They often act as surrogate families, providing companionship in an increasingly impersonal and sometimes disheartening world. Particularly for children, pets offer a unique and appropriate form of companionship, which fosters attitudes of humanity, responsibility, and kindness (Rohitash et al., 2018). Additionally, having pets can teach individuals about biological realities and the circle of life. However, pets may suffer from many infectious and non-infectious diseases including various tumors. Abnormal cellular growth (in general known as tumor or cancer) in the form of benign and malignant might cause significant number of deaths in companions (Moulton, 1978). Cancer can be considered as a series of diseases triggered by the accumulation of genetic mutations combined with the disruption of regulatory epigenetic mechanisms that drive the progression of a normal cell into a highly malignant cancer cell (You et al., 2012).

Cancer is the leading cause of death in dogs (Gardner et al., 2016). It is estimated that 1 in 3 domestic dogs might develop cancer, which is the same incidence of cancer among humans (Pang et al., 2016). Dogs and cats develop spontaneous tumors with histopathologic and biological behavior similar to tumors that occur in humans. 50% of older dogs might develop the disease and eventually about 1 in 4 of them will die from the disease (Simkus et al., 2015). Multiple cancers are common to find in some breeds, on the other side, there is specific cancer for a specific breed. While it is widely acknowledged that there are breed-related disparities in the susceptibility to certain types of cancer in dogs, lymphoma, osteosarcoma, mast cell tumors, hemangiosarcoma, melanoma, and transitional cell carcinoma are among the most prevalent cancers in dogs Additionally, mammary tumors, soft tissue sarcomas, and lung cancers are frequently diagnosed. Commonly affected breeds encompass the Boxer, Scottish Terrier, Basset Hound, Airedale Terrier, Chow Chow, German Shepherd, Poodle, St. Bernard, Bulldog, Beagle, Rottweiler, and Golden Retriever (Morrison, 1998). The presence of additional hereditary diseases or issues specific to certain breeds poses a significant complicating factor. For instance, the seemingly lower cancer risk observed in bulldogs might be attributed to their shorter life

expectancy resulting from other health issues that are prevalent within the breed (Kennel Club, Purebred Dog Health Survey 2004).

Cancer is common in domestic cats, though likely somewhat less common than in dogs (Butler et al., 2012; MacVean et al., 1978). Most cat breeds developed within the past century and there are fewer genetically distinct breeds than in dogs (Lipinski et al., 2008; Kurushima et al., 2013). Cats can be affected by various types of cancer, such as lymphoma, squamous cell carcinoma, fibrosarcoma, and mammary tumors. Additionally, nasal tumors, mast cell tumors, and injection-site sarcomas are among the other cancer types that can commonly afflict cats (Thomas, 2015.) Tumors in cats most frequently develop in the skin and subcutaneous tissues. The skin, being the largest and most exposed organ of the body, is susceptible to various external insults. It is easily observed and felt, making it a readily accessible area for examination (Doddy et al., 1996).

Certain cat breeds are more prone to specific types of cancer. Siamese cats, for instance, have a higher reported occurrence of mammary tumors. Persian cats are known to be more susceptible to cancers such as squamous cell carcinoma and mammary tumors. Ragdolls may exhibit increased vulnerability to hypertrophic cardiomyopathy, a heart condition associated with a higher risk of heart tumors. Additionally, Burmese cats may have a heightened incidence of particular oral cancers (Hayes et al., 1981). The fact that there are very few specific breed predispositions reported for cancer in cats may reflect this, although Siamese cats are over-represented in cats with cancer in general, and specifically with mammary gland and intestinal neoplasia (Egenvall et al., 2010; Egenvall et al., 2009; Rissetto et al., 2011). Recently available feline genome may allow identification of genetic signatures of cancer risk in Siamese compared to other cats (Bertone et al., 2003).

Benign neoplasms typically grow slowly and have a compact structure, but they do not invade nearby tissues or spread throughout the body. On the other hand, malignant neoplasms exhibit unpredictability in growth, sometimes progressing rapidly. They invade and spread into surrounding tissues and have the potential to metastasize to other parts of the body. Defining a tumor is challenging but they can be broadly categorized into three types: (1) Benign tumors, which emerge in various body tissues, grow locally, and are clinically significant due to their potential to exert local

pressure or cause obstruction (Argyle et al., 2020) (2) In situ tumors, where the lesion appears to contain cancer cells but remains confined within the epithelial layer without invading the basement membrane or supporting mesenchyme; (3) Cancer, typically denoting a malignant tumor marked by uncontrolled cell division, capable of local invasion, and distant metastasis (Argyle et al., 2020).

Among various tumors in dog lymphoma is prevalent hematopoietic malignancy in dogs originating from lymphocytes, constitutes approximately 24% of all canine neoplasms, (Vail et al., 2001). The primary site frequently affected is the lymph nodes. However, mammary tumors are infrequent in cows, mares, goats, ewes, and sows. Distinct variations exist in the biological characteristics and histology of mammary tumors between dogs and cats. In dogs, about 45% of mammary tumors display malignancy. Additionally, dogs exhibit a greater prevalence of complex and mixed tumors compared to cats (Morrison, 2002; Haziroglu et al., 2010). The incidence of canine mammary tumours does vary by breed but breeds reported to be at risk vary between different studies and different geographical locations. Poodles (toy and miniature), spaniels (English springer, cocker, and Brittany), Puli, English setter, pointers, German shepherd, Maltese terrier, Yorkshire terrier, and dachshund have all been reported to be predisposed (Von et al., 2011).

Cancer diagnosis involves examining tissue and cytology specimens obtained through various procedures, such as surgical biopsy, needle biopsy, venipuncture, and tapping of pleural or ascitic fluids (Schrohl et al., 2003). Additionally, scraping tissue surfaces and collecting exfoliative cells from urine and sputum are common methods. While conventional histopathology, which assesses morphology, has been the standard for years, advanced technologies like mass spectrometry, microarray, and automated DNA sequencing have introduced new possibilities in cancer diagnosis and treatment (Reddy et al., 2009).The application of enzyme histochemistry and electron microscopy has expanded the initial micro-anatomic assessment to include biochemical and sub-cellular ultra-structural features (Zhao et al., 2010). Conventional radiography offers a straightforward method for diagnosing tumors in the gastrointestinal tract, lungs, brain, liver, urinary bladder, breast, bones, and joints of both domestic and pet animals (Pawaiya, 2005).

Histopathology continues to be the conventional and standard method for diagnosing cancer (Pawan et al., 2010). Nevertheless, a thorough histopathological examination conducted by a skilled oncologist can provide an accurate diagnosis regarding the tumor type and potential malignancy status. In cancer cells, observable cellular changes include hyperchromatic nuclei, an increased ratio of nucleus to cytoplasm, cell disorientation, and the presence of mitotic figures, all of which indicate neoplastic alterations in tissues (Pawan et al., 2010).

- **Objectives:**
    i. To examine the gross lesions and histopathological lesions of tumor.
    ii. To examine the alternations of cell size and shape.

Chapter 2: Review of Literature

2.1. Neoplasm:

A neoplasm is a type of abnormal and excessive growth of tissue. The process that occurs to form or produce a neoplasm is called neoplasia. The growth of a neoplasm is uncoordinated with that of the normal surrounding tissue, and persists in growing abnormally, even if the original trigger is removed. This abnormal growth usually forms a mass, which it may be called a tumor or tumor (Cooper, 1992).

Benign neoplasm: Benign tumors are non-cancerous growths that do not invade nearby tissues or spread to other parts of the body. They usually have a well-defined boundary and grow slowly. Although benign neoplasms are not cancerous, they can still cause problems depending on their size and location (Gerald et al., 2015).

Malignant neoplasm: Malignant tumors, on the other hand, are cancerous growths that have the ability to invade nearby tissues and spread to other parts of the body through a process called metastasis. Malignant neoplasms can be life-threatening if not detected and treated early (Gerald et al., 2015).

2.2. Challenges in Estimating Cancer Incidence Rates in Companion Animals:

A major condition that affects pets is cancer. However, it has been challenging to arrive at reasonably accurate estimates of cancer morbidity and mortality rates in companion animals. Veterinary cancer registries have existed in smaller numbers and have frequently been sporadic and short-lived, whereas cancer registries in human medicine have existed and developed since the 1940s. Additionally, there is little current knowledge on the incidence of the various types of cancer in companion animals. Cancer registries provide data for epidemiological studies as well as information for the evaluation of incidence and relative risk estimations. This data consists of identifying risk factors, assessing treatments, locating patients for clinical trials, and providing data for case-control studies (Dobson et al., 2002).

Few studies have attempted to estimate population-based cancer incidence rates, and the difficulties encountered in the computation of accurate companion animal populations were handled differently by each one (Merlo et al., 2008; Dobson et al., 2002). One of the largest, best-known, and most often cited veterinary cancer registries is the California Animal Neoplasm Registry (CANR). For 3 years after

1963, data were collected from a well-defined study area, recording more than 30,000 malignant neoplastic cases on which histopathology was performed free of charge (Dorn et al., 1968)

Publications derived from the CANR include estimates of cancer incidence from the reported cases in dogs and cats according to age, sex, and breed, showing that skin is the most frequently affected tissue in both cats and dogs and that purebred dogs seem to be more prone to develop neoplastic diseases in all sites (Dorn et al., 1968).In 2005, a new veterinary cancer registry was established at the Royal Veterinary and Agricultural University in Denmark. Data on the resident dog population was obtained from the Danish dog registry (Broenden et al., 2007).

2.3. Occurrence of Neoplasms in Companion Animals in Bangladesh:

The incidence of neoplasms in companion animals is relatively infrequent compared to other regions. However, there is a lack of comprehensive studies or extensive data on these neoplasm cases in Bangladesh. Only a few studies have attempted to estimate population-based cancer incidence rates in companion animals in the country. These studies faced challenges in accurately computing the population of companion animals. Additionally, veterinary cancer registries, which could provide valuable data for epidemiological studies and risk assessments, have been limited in number, often sporadic, and short-lived.

Several reported cases of neoplasms in pet animals in Bangladesh have been documented, highlighting the occurrence of abnormal cell growth in these animals. Here are a few reported cases:

A 5-year-old female dog was brought to the Teaching Veterinary Hospital of Chittagong Veterinary and Animal Sciences University in Bangladesh (Rakib et al., 2016). The dog was diagnosed with invasive ductal carcinoma, which is a type of breast cancer characterized by the abnormal growth of cells in the milk ducts. Another dog exhibited an inverted viral papilloma, initially presenting as a growth on the vulva. Over time, this papilloma progressed to full-thickness dysplasia and Bowenoid in situ carcinoma. Inverted viral papillomas are benign growths caused by a viral infection, but in rare cases, they can progress to dysplasia or carcinoma.

It is worth emphasizing that these cases are isolated reports and may not be representative of the overall prevalence or incidence of neoplasms in pet animals in Bangladesh. To obtain a comprehensive understanding of the occurrence of neoplasms in pets, it is necessary to conduct systematic studies and gather data from a larger sample size.

2.4. The factor that can play an important role to form a tumor:

Cell damage can arise from a diverse range of events, often resulting from a combination of factors. The following are some potential contributing variables:

- **Genetics:** Certain breeds, including Golden Retrievers, Boxers, Bernese Mountain Dogs, and Rottweilers, show a higher frequency of specific types of tumors. This observation implies a potential hereditary or genetic influence, but further research is required to accurately identify the breeds that are more susceptible to developing particular cancers (Morrison, 1998).
- **Age:** Cancer is more prevalent in older pets, although the precise relationship between cancer and age is not entirely clear. It is suspected to be associated with the weakening of the immune system as pet age. As the body's defense mechanisms become less robust with age, the likelihood of mutated cells evading detection increases, leading to the development of cancer (Von, 2011).
- **Environment:** Exposure to environmental hazards or chemicals can elevate the cancer risk in both humans and their companion pets. Some instances include various herbicides, pesticides, and other chemical agents. Certain substances like nickel, uranium, radiation, and asbestos have demonstrated carcinogenic properties. Additionally, prolonged exposure to the sun and UV light can also contribute to an increased risk of cancer.
- **Tumor microenvironment**: The tumor microenvironment is the ecosystem that surrounds a tumor inside the body. It includes immune cells, the extracellular matrix, blood vessels, and other cells, like fibroblasts. A tumor and its microenvironment constantly interact and influence each other, either positively or negatively (Buck et al., 2017). The growth, progression, and metastasis of tumors can be significantly influenced by several variables that are present in the tumor microenvironment (TME). The extracellular matrix (ECM), different cell types, growth factors, cytokines, chemokines, cancer-

associated fibroblasts (CAFs), and immune cells like macrophages and T cells are among these variables. Similar to this, by encouraging angiogenesis and invasion, the ECM and inflammatory cells can also aid in the development of tumors. T cells and other immune cells. There are many different T cell populations within the TME that infiltrate the tumor areas, at the invasive tumor margin and in draining lymphoid organs. Among these, cytotoxic CD8+ memory T cells (CD8+, CD45RO+), which are normally antigen 'experienced' and capable of killing tumor cells, are strongly associated with a good prognosis (Fridman et al., 2012).The disruption of tissue homeostasis creates dynamic changes in the cellular metabolism and function of both stromal and immune cells (Buck et al., 2017). This highly-trafficked network constitutes the tumor microenvironment (TME) (Figure: 1).

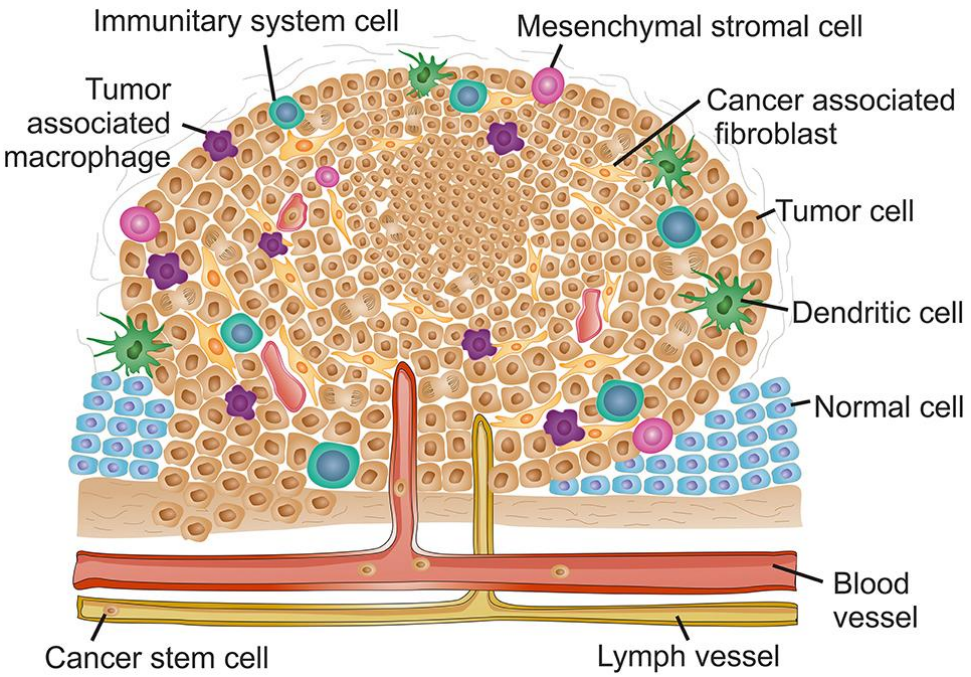


Figure 3: Tumor microenvironment

2.5. Types of Genes that Cause Cancer:

Proto-oncogene, tumor suppressor genes, and DNA repair genes are the three primary gene groups that are usually affected by the genetic alterations that cause cancer. These modifications are referred to be cancer's "drivers" at times.

Proto-oncogene: Proto-oncogene play a role in regular cell division and proliferation. However, these genes may develop into cancer-causing genes (or oncogene), allowing cells to grow and survive when they shouldn't by being changed in specific ways or being more active than usual (Adamson, 1987).

Tumor-suppressing gene: Genes that decrease tumors are also involved in regulating cell division and proliferation. Certain tumor suppressor gene mutations can cause cells to divide uncontrollably (Fearon, 2020).

DNA repair gene: DNA damage must be repaired using DNA repair genes. It is common for cells with mutations in these genes to also have mutations in other genes and chromosomal abnormalities including duplications and deletions of chromosomal segments. These alterations might work together to turn the cells malignant (Doroshow et al., 2020).

2.6. Types of cancer in pet animals:

In dog:

Lymphoma, bone tumors (including osteosarcoma), mast cell tumors, soft tissue sarcomas, and tumors of the oral cavity are among the most common of the numerous cancers that can be detected in dogs and cats. More, less prevalent cancers can still affect dogs.

Here are the top seven canine cancers, tumors, and neoplasia, along with descriptions of how they appear:

- **Melonema:** Melanoma tumors are spherical masses that can develop anywhere on a dog's body and range in size from a quarter inch to two inches. Cutaneous melanoma occurs more commonly in dogs with heavily pigmented skin, with Schnauzers (both miniature and standard) and Scottish terriers at increased risk (Goldschmidt et al., 1992)
- **Osteosarcoma:** The most prevalent kind of bone cancer in dogs is osteosarcoma. This type of cancer has the potential to spread rapidly throughout the body and be quite aggressive. Although there are many adverse effects, acute lameness is the main danger indicator (Joseph, 2018).
- **Canine transmissible venereal tumor (CTVT):** CTVT is species-specific and highly contagious. The cancer is prevalent in populations of stray dogs or

environments of uncontrolled copulation. The tumors occur around the area of the external genitalia and can grow up to 15 cm in area (Ganguly, 2013).

- **Mast cell tumor**: Basal tumors, also known as mast cells, are found in the connective tissues just beneath the skin's surface. Mast cell tumors are common, accounting for 20% of all canine skin tumors (Vail et al., 2019).
- **Lymphoma:** Similar to non-Hodgkin's lymphoma in humans, canine lymphoma is a blood cell and lymphoid tissue malignancy (Simon et al., 1996).

  There are four primary forms of canine lymphoma:

  - Alimentary lymphoma, which is rather common, affects the gastrointestinal tract.
  - Mediastinal lymphoma, which is extremely uncommon, affects the chest.
  - Multicentric, The most prevalent type of lymphoma and one that affects the entire body.
  - Extranodal, A rare form that affects organs outside the lymphatic system (such as the kidney, lung, or eyes).
- **Mammary gland cancer**: A common kind of cancer in dogs is mammary gland cancer, particularly in unspayed animals. A mass or lump in the mammary glands, pain, warmth, or pain where the tumor is located, lethargy, decreased appetite, and weight loss are all signs of mammary gland tumors in dogs (Michael, 2022). In dogs, Mammary Gland Tumors (MGTs) are among the most prevalent diagnosed neoplasms in elderly unsprayed bitches (MacEwen, 2000). In these animals, the mammary gland appears to be more predisposed to develop tumors compared with other animal species (Kumar et al., 2010). The tumor itself and its covering skin may be traumatized and ulcerated (MacEwen et al., 1996; Simon et al., 1996).
- **Hemangiosarcoma:** Hemangiosarcoma is an extremely dangerous and fast-moving cancer of the blood vessel walls that can cause cancerous tumors in dogs. The heart, spleen, and skin are the most affected areas, but hemangiosarcoma can be found anywhere in and on the dog's body. In 1980, Priester and McKay reported breed-associated risks for the development of hematopoietic neoplasms, including HSA, based on data from the Veterinary Medical Databases (VMDB). Breeds that represented the highest risk included

Boxers, Basset hounds, and St. Bernards. Scottish terriers, Bulldogs, Airedales, Weimaraners, Golden retrievers, Doberman pinschers, Labrador retrievers, English setters, and Great Danes were among other breeds at risk, whereas mixed breeds, miniature and Toy poodles, Pomeranians, Chihuahuas, Boston terriers, Cocker spaniels, and Dachshunds were considered less likely to develop these tumors (relative risk < 1) (Priester et al.,1980).

- **Type of cancer in cats:**

Numerous cancers, including lymphoma, squamous cell carcinoma, fibrosarcoma, and mammary tumors, are prevalent in cats. Nasal tumors, mast cell tumors, and injection-site sarcomas are other cancer types that can affect cats. It's necessary to remember that cats are susceptible to skin cancers like fibromas and collagenous nevi (Haziroglu et al., 2010).

- **Lymphoma:** One of the most typical cancers found in cats is lymphoma. It is a malignancy of the lymphoid tissues and lymphocytes, a particular type of blood cell. Feline immunodeficiency virus (FIV)-positive cats are more likely to develop lymphoma. Alimentary lymphoma (AL) targets the gastrointestinal tract with variable involvement of extra-intestinal sites including lymph nodes, liver, and spleen (Ettinger, 2003; Guillermo, 2001).
- **Squamous cell carcinoma**: The most commonly seen oral tumor in cats is squamous cell carcinoma (SCC), which develops from the cells lining the mouth canal. It often develops from the tissues lining the mouth, such as the tonsils, gums, and tongue. Depending on the location and severity of the malignancy, different cats may exhibit different signs of squamous cell carcinoma (Mikiewicz et al., 2019).
- **Fibrosarcoma:** In cats, fibrosarcoma is a soft tissue cancer that typically affects the skin, subcutaneous tissue (the area just beneath the skin), and occasionally internal organs. Fibrosarcoma is typically identified as a localized tumor or lump on the cat's body, and it can be low- or high-grade, which affects treatment planning and prognosis (Roccabianca et al., 2021).

The total number of canine cancer cases is widely acknowledged, but specific figures for individual cancer types remain uncertain. Due to instances where dogs and cats may pass away suddenly without a definitive diagnosis, it

becomes challenging to estimate precise numbers. Future insights and treatment options are expected to emerge from continuous research endeavors.

2.7 Classification of cancer:

The International Classification of Diseases for Oncology, Third Edition (ICD-O-3) is the globally recognized standard for categorizing and naming histologies. This classification system is rooted in the principles outlined in the ICD-O-3.

Cancers may be classified according to:

- Their primary site of origin,
- Their histological
- Tissue types
- Grade
- Stages

❖ **Classification by site of origin:**

Cancers can be classified into different kinds depending on where they first developed, including mammary gland, lung, prostate, liver, renal cell, oral, and brain cancers.

❖ **Classification by tissue type:**

Cancers can be categorized into six primary classifications based on the forms of tissues they affect.

- **Carcinoma:** This particular type of cancer develops from cells in the epithelial layer that line internal organs and the external surfaces of bodily parts. Since epithelial tissues are most frequently found in the body, from being present in the skin to be present in the covering and lining of organs and internal passageways, such as the gastrointestinal system, carcinomas, and malignancies of epithelial tissue, make up 80 to 90% of all cancer cases. Adenocarcinoma and squamous cell carcinoma are the two forms of carcinoma. Squamous cell carcinoma begins in the squamous epithelium, whereas adenocarcinoma develops in an organ or gland.
- **Sarcoma:** These malignancies originate in the fat, muscles, bones, cartilage, and connective and supporting tissues. One of the sarcomas known as osteosarcoma is bone cancer. Sarcomas resemble the tissue in which they develop in appearance.

Examples of sarcomas are:

- Osteosarcoma or osteogenic sarcoma (bone)
- Chondrosarcoma (cartilage)
- Leiomyosarcoma (smooth muscle)
- Rhabdomyosarcoma (skeletal muscle)
- Mesothelial sarcoma or mesothelioma (membranous lining of body cavities)
- Fibrosarcoma (fibrous tissue)
- Angiosarcoma or hemangioendothelioma (blood vessels)
- Liposarcoma (adipose tissue)

- **Myeloma:** These originate in the bone marrow's plasma cells. Different antibodies can be produced by plasma cells in response to infections. A kind of blood cancer is myeloma.
- **Leukemia:** This is a group of cancers that are included within the blood cancer category. The bone marrow, which is the location of blood cell formation, is affected by several malignancies. When malignant, the bone marrow starts to overproduce immature white blood cells, which are unable to carry out their typical functions and frequently leave the patient vulnerable to infection.
- **Lymphoma:** Lymphoma is described as a clonal growth of malignant lymphoid cells that primarily affects the liver, spleen, and solid visceral organs such as the lymph nodes and bone marrow. In about 80% of cases, peripheral lymph nodes are involved.
- **Mixed types**: These contain two or more cancer-related factors. Examples include teratocarcinoma, mixed mesodermal tumor, carcinosarcoma, and adenosquamous carcinoma. Another type of tumor that uses tissues from the embryo is a blastoma.

❖ **Classification by grade**:

Additionally, cancers can be categorized by grade. The grade of the malignancy is based on how aberrant the cells are in comparison to the surrounding normal tissues. The grade ranges from 1-4 and rises with increasing irregularity.

Well-differentiated cells are found in low-grade tumors and closely resemble normal, specialized cells. Undifferentiated cells are significantly aberrant compared to the tissues around them. These tumors are of a high grade.

Grade 1 – well-differentiated cells with a slight abnormality

Grade 2 – cells are moderately differentiated and slightly more abnormal

Grade 3 – cells are poorly differentiated and very abnormal

Grade 4 – cells are immature and primitive and undifferentiated.

- **Classification by stages**:

According to their stage, cancers are also divided into several categories. There are various staging techniques. The most popular classification system employs

- ✓ Tumor size (T),
- ✓ Node involvement (N), and
- ✓ Distant metastasis (M) to determine the extent of regional spread. The staging of the TNM is this.

- **Tumor size:**

  T0, for instance, denotes the absence of any tumor evidence.T1 to 4, increasing tumor size and involvement, and T is, carcinoma in situ or restricted to surface cells.

- **Node involvement**:

  Similarly, N0 denotes no lymph node involvement, while N1 through N4 denotes varying degrees of lymph node involvement. Nx denotes that it is difficult to evaluate node involvement.

- **Metastasis:**

  Metastasis is further divided into two categories. M0 denotes the absence of distant spread. M1 denotes the presence of distant spread.

  According to the TNM staging categorization, stages can be subdivided. Stage 0 cancer is in situ, or limited to surface cells. Stage I cancer is limited to the tissue of origin. Stage II denotes localized restricted dissemination. Stage III denotes substantial local and regional spread. Stage IV denotes metastatic, advanced cancer with distant distribution.

2.8. Insights into Lipoma, Lymphoma, and Mammary Gland Carcinoma:

The objective of this study was to examine malignant tumors in dogs and cats. Specifically, we will delve into the histopathological discoveries concerning lymphoma, lipoma, and mammary gland carcinoma in these animals.

2.8.1. Lipoma:

Lipomas are benign fatty tumors of mesenchymal origin, comprised of mature fat cells (adipocytes), mostly seen in adult female or elderly obese dogs (Goldschmid et al., 2002). Approximately 16% of dogs suffer from lipomas (Randall et al., 1998). Simkus et al., (2015) reported high incidence of lipoma in female dogs (66.7%) than in male dogs (33.3%) and the incidence is higher in old aged females (Julie et al., 2013), while O'Neill et al., (2018) also reported that the incidence of lipoma more in aged dogs. While lipomas occur often in dogs, they do so much less common in cats. Patients with them are often middle-aged or older when they occur in cats. Though the exact etiology is unknown (Ludwig et al., 2017), the proposed factors are hereditary, hormonal, congenital, and trauma (Rapidis, 1982). These are the most common soft tissue tumors, usually surrounded by a thin capsule (Hoseini et al., 2010), and may be localized in any region of the body, superficial or deep (Hakim et al., 1994). These are the most common soft tissue tumors, usually surrounded by a thin capsule (Hoseini et al., 2010), and may be localized in any region of the body, superficial or deep (Hakim et al., 1994). The growth of lipoma is gradual and painless (Malik et al., 2020).

Location: They may occur over the thorax, abdomen, thighs, and proximal limbs and several lipomas can be single or multiple (Cowell et al., 2008). The lipomas located between skin and muscle layers are generally soft on palpation and freely movable while intermuscular lipomas are not mobile as such (Veena et al., 2013).

Appearance: Most lipomas are small, well-circumscribed, and movable, but not painful. However, large lipoma masses may become a cause of worrying concern, as they can restrict feed intake and their presence in affected areas may cause pain to animals (Subapriya et al., 2020). It has been reported that inter-regional injections of 10% calcium chloride solution cause lipoma regression, but this treatment is not recommended because irritation and skin necrosis may occur (Albers et al., 1985). So,

surgical excision is the right choice of treatment (Veena et al., 2013). Lipomas usually do not recur following complete excision.

Symptoms:

- Although lipomas are typically oval or spherical, soft, and movable, they can sometimes be harder and connected to adjacent tissues or structures.
- The appearance of a slow-growing lump beneath the skin of the cat.
- Lipomas typically appear on the chest, neck, upper legs, and abdomen (but they can sometimes develop on internal organs;
- If the lipoma grows very large, it may compromise the cat's blood flow and, in severe circumstances, cause tissue death in the affected area.

2.8.2. Lymphoma:

Lymphoma is one of the most common malignant tumors to occur in dogs. The most frequently affected breeds include the Boxer, Scottish Terrier, Basset Hound, Airedale Terrier, Chow Chow, German Shepherd, Poodle, St. Bernard, Bulldog, Beagle, Rottweiler, and Golden Retriever (Morrison, 1998). Dogs can develop lymphoma in several various locations on their bodies. Multicentric lymphoma, which affects lymph nodes all over the body and may spread to those below the chin, in front of the shoulder blades, and behind the knees, is the most common type of lymphoma in dogs. However, lymphoma may additionally occur in other forms and impact many body organs, including the spleen, skin, and gastrointestinal system. The type and subtype of lymphoma determine the exact location of the disease in dogs.

Location: The four location types are multicentric, mediastinal, gastrointestinal, and extranodal (involving the kidney, central nervous system, skin, heart, or eye).

Classification:

- **Multicentric Lymphoma**: It is the most common type found in the lymph nodes, with or without involvement in the liver, spleen, or bone marrow. Approximately 80 to 85 percent of lymphomas in dogs are multicentric. This type of cancer affects the lymph nodes, and in the majority of cases, the most obvious clinical manifestation is the rapid enlargement of the lymph nodes.
- **Mediastinal Lymphoma**: It occurs in the lymph nodes in the thorax and possibly the thymus. Mediastinal lymphoma is rare. In this disease, either or

both the thymus and the mediastinal lymph nodes in the area of the chest become enlarged, caused by high-grade malignant T lymphocytes.

- **Alimentary Lymphoma**: It occurs as either a solitary tumor or diffuse invasion of the stomach or intestines, with or without involvement in the surrounding lymph nodes, liver, or spleen (Lowe, 2004). The second most common form of lymphoma is alimentary lymphoma, which accounts for less than 10 percent of canine lymphomas.
- **Extranodal Lymphoma**: In dogs, it refers to lymphoma that targets a specific organ, such as the skin, eyes, kidneys, lungs, or central nervous system. The most common extranodal lymphoma affects the skin and is called cutaneous lymphoma.

Symptoms:

As there are so many different kinds of lymphoma, symptoms of lymphoma in dogs might be problematic to diagnose at first.

- **Multicentric Lymphoma:**

  Swollen lymph nodes tend to be the initial sign of multicentric lymphoma in dogs. Dogs with lymphoma frequently have lymph nodes that are three to ten times larger than normal. These enlargements feel like a solid, rubbery lump that can move freely beneath the skin but are not uncomfortable. As multicentric lymphoma advances, dogs may also experience lethargy, fever, anorexia, weakness, and dehydration.
- **Alimentary Lymphoma:**

  Anorexia, diarrhea, weight loss, vomiting, gastrointestinal pain, and alimentary lymphoma, which affect the intestines, can be symptoms in canines.
- **Mediastinal Lymphoma:**

  Mediastinal lymphoma frequently causes breathing problems in canines. This could be a result of a significant mass inside the chest or an accumulation of fluid inside the chest (pleural effusion). Along with increased thirst and urine, affected dogs can also show swelling of the face or front legs.

- **Extranodal Lymphoma**:

  According to the organs involved, extranodal lymphoma symptoms vary. The most common type of cutaneous lymphoma affects the skin. Individually detectable elevated nodules or, more commonly, distributed scaly lesions are telltale indications of cutaneous lymphoma. The gums, lips, and roof of the mouth are frequently affected by cutaneous lymphoma in the mouth.

2.8.3. Mammary gland cancer:

A common variety of neoplasms, the breast tumor, develops from the glandular epithelium of the mammary gland. In elderly, intact female dogs and cats who have not been spayed, as well as in other animals, they are frequently discovered. Consequently, these malignancies provide a significant challenge for veterinary medicine. Two histologic classification systems for canine mammary tumors and dysplasias have been published: the first in 1974 and the modification in 1999 (Tran et al., 2016). Approximately 45% of mammary tumors are malignant in dogs, whereas 90% are malignant in cats, and dogs have a much higher number of complex and mixed tumors than do cats (Morrison, 2002; Haziroglu et al., 2010). Canine mammary gland tumors are widespread in different parts of the world. Besides, different evidence showed that the incidence is higher in intact female dogs than in spayed or neutered dogs (Kamble et al., 2016). Estrogen and progesterone play a major role in normal mammary gland development, but these hormones have also been implicated in tumor development (Ali et al., 2016). Estrogens are promoters of initiated cells in addition to regulating the transcription of several nuclear protooncogenes (Garden et al., 2018).

Etiology:

The exact causes of canine mammary cancers remain largely unknown, but it seems that hormones related to the estrous cycle play a role. Mammary tumors are more commonly observed in female dogs that have not been spayed or were spayed after their first heat cycle. The reported incidence of mammary tumors in dogs is approximately 3.4 percent. Dogs spayed before their first heat have 0.5 percent of this risk, and dogs spayed after just one heat cycle have 8 percent of this risk (Klopfleisch et al., 2010). The tumors are often multiple. The average age of dogs with mammary tumors is seven to eleven years old (Morrison, 2002). Obesity at one year of age and

eating red meat has also been associated with an increased risk for these tumors (Joshi et al., 2012).

Symptoms:

Clinically, mammary tumors appear as single or multiple nodules in the parenchyma, with or without the involvement of nipples. Grossly, tumors appear as single or multiple nodules (1-25 cm) in one or more glands. The cut surface is usually lobulated, gray-tan, and firm, often with fluid-filled cysts. Mixed mammary tumors may contain grossly recognizable bone or cartilage on the cut surface. More than 50% of canine mammary tumors are benign mixed tumors; a smaller percentage of malignant mixed tumors are seen. In the latter, epithelial or mesenchymal components, either singly or in combination, may produce metastases (Ali et al., 2016; Cassali et al., 2015). It is impossible to distinguish between benign and malignant tumors because they might be either small, firm or have well-defined nodules. Tumors are either benign or malignant. Malignancy is characterized by rapid development, local tissue invasion, and ulceration.

2.9. Difference between normal cells and cancer cells:

The primary distinctions between healthy and cancerous cells lie in their development, communication, ability to repair and undergo cell death, adhesion and spreading characteristics, appearance, maturation process, evasion from the immune system, functions, and angiogenesis.

- **Cell Growth Rate:**

  When there are enough cells, normal cells stop reproducing and start growing again. Cancer cells keep growing even after there are enough cells. A cell cluster created by this excessive proliferation results in the creation of a tumor.

- **Duration of Cell Life:**

  Cancer cells can proliferate uncontrolled, have an irregular lifespan, and frequently invade other body regions. Normal cells develop, live a consistent amount of time, and eventually die from aging or damage.

- **Communication between Cells**:

Cancer cells do not respond to signals sent by other cells indicating the need to stop growing, unlike normal cells which typically halt their growth in response to these signals.

- **Cell appearance:**

Normal cells typically exhibit a regular and consistent shape, maintaining a well-defined morphology that is appropriate for their specific function within the body. These cells typically have a uniform size, shape, and organization. However, cancer cells, on the other hand, often display irregularities in their shape and structure.

- **Cell shape:**

Nucleus: The nucleus is a smooth structure that consistently maintains a spheroid shape in normal cells. The control of nuclear morphology involves many structural elements. The nuclear lamina is one of these structural elements. Nuclear membrane bulges known as "blebs" are frequently seen in cancer cell nuclei, which are typically deformed (Chloe et al., 2012). According to research, this "blebbing" is brought on by an imbalance in the proteins that make up the nuclear lamina, which causes the lamina fibers to separate.

Chromatin: In cancer cells, the fine, uniformly distributed chromatin that is present in normal cells aggregates into irregular clumps that vary in size and shape.

Nucleolus: Cancer cells have a nucleolus that is increasingly larger and irregular; some cells have several nucleoli.

- **Restoration of Cells:**

When normal cells are injured or get old, they either repair the damage or die. Either the cancer cells are not fixed or they do not die.

- **Ability to Metastasize (Spread) :**

Normal cells remain in their proper locations within the body. Some cancer cells can separate and transfer to different parts of the body through the lymphatic and circulatory systems.

- **The Immune System's Evasion:**

The immune system has the ability to identify and remove healthy cells that are damaged. However, cancer cells can employ tactics to evade detection by the immune system, allowing them to grow and form tumors over time.

- **Stickiness:**

Normal cells release chemicals that make them adhere to one another. Cancer cells can float away to neighboring places or through the circulation or lymphatic system to distant regions of the body because they are unable to produce these chemicals.

- **Angiogenesis:**

When new tissue is required to heal injured tissue, normal cells recruit blood vessels to form and feed that tissue as a natural element of growth and development. Even when growth is not required, cancer cells continue to do this.

- **Function:**

Normal cells complete the specified tasks**.** Cancer cells fail to perform their assigned activities.

2.10. Tissue changes that are confused with cancer cells:

In certain instances, specific tissue changes may occur in the body are not initially cancerous. However, if left untreated, these alterations have the potential to advance and develop into cancer. Two examples of such tissue changes are hyperplasia and dysplasia.

Hyperplasia:

Hyperplasia refers to a condition where cells within a tissue multiply at a faster rate than usual, resulting in an accumulation of extra cells. When examined under a microscope, the cells and structure of the tissue still appear normal. Hyperplasia can

be caused by various factors or diseases, with chronic irritation being one of them. (Zachary et al., 2013).

Dysplasia:

Dysplasia, on the other hand, involves the presence of abnormal cells within a tissue or organ. It is important to note that dysplasia itself is not cancer, but it has the potential to develop into cancer. Dysplasia represents a more advanced disorder compared to hyperplasia, where there is an accumulation of extra cells. However, in dysplasia, there are abnormalities in the organization of the tissue and the cells appear aberrant or atypical. The likelihood of developing cancer increases as the cells and tissue exhibit greater levels of abnormality (Zachary et al., 2013).

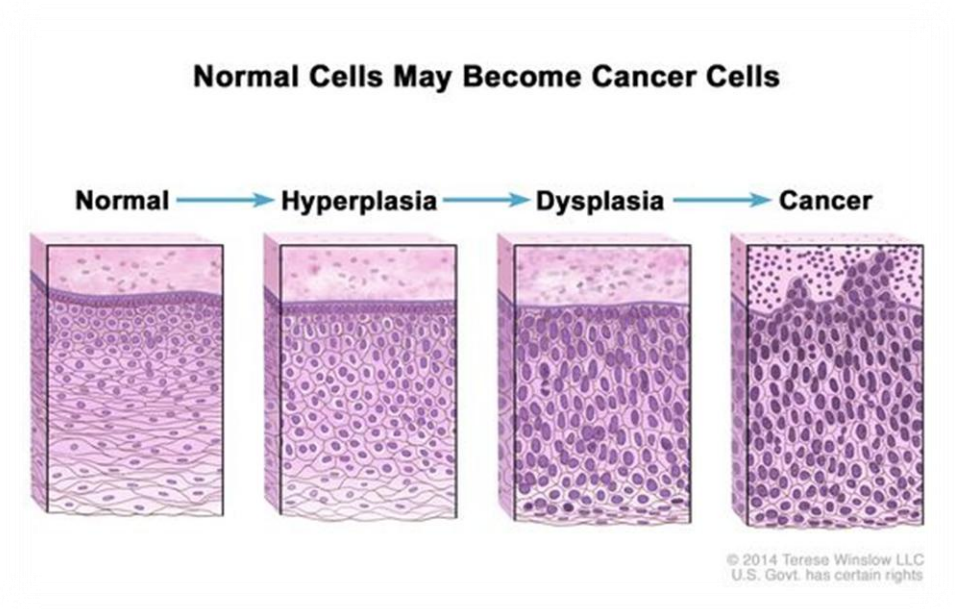


Figure 4: Tissue change.

2.11. Background and Significance of histopathology in oncology:

Histopathology refers to the examination of a biopsy or surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides (Carson et al., 2009). This technique is widely used in veterinary medicine to aid in the diagnosis, staging, prognosis, and treatment of various types of cancer in animals.

Background:

During the early 19th century, microscopy was already being used in veterinary medicine. However, it was not until the late 1800s that histopathology gained widespread adoption in the field (Rissetto, 2011). Renowned German pathologists like Rudolf Virchow and Julius Cohnheim played significant roles in popularizing histopathology, making it a standard practice in veterinary medicine (Pawan et al., 2010).Throughout the 20th century, histopathology continued to progress and became an indispensable tool in veterinary practice. Advancements in staining techniques allowed for more precise differentiation of various diseases based on specific tissues and cells. The introduction of immunohistochemistry (IHC) techniques in the 1980s was particularly groundbreaking, as it enabled veterinarians to diagnose even more complex diseases by differentiating specific antigens (Reddy et al., 2009)

Through histopathology, they can determine the cell type, grade, and stage of the tumor, which are crucial factors in designing an appropriate treatment plan and predicting the prognosis for the affected animal (Turek et al., 2005).

Significance:

1. **Diagnosis and Classification**: Histopathology allows veterinary professionals to accurately diagnose cancer and classify the type of tumor. Different types of cancer can have varying behavior and responses to treatment, so an accurate diagnosis is essential for determining the most effective therapeutic approach.
2. **Grading and Staging**: Histopathological examination helps grade the tumor, indicating its level of aggressiveness and differentiation. Additionally, it aids in staging the cancer, which assesses the extent of tumor spread and the presence of metastasis.
3. **Treatment Guidance**: Oncologists use histopathology results to tailor treatment plans for individual animals. This can include surgical resection, chemotherapy, radiation therapy, or a combination of these modalities. Knowing the tumor's characteristics through histopathology helps in selecting the most effective and least invasive treatment.
4. **Prognostic Information**: The histopathological analysis of cancer samples provides valuable prognostic information, helping owners and veterinarians understand the expected outcome and potential survival time for the animal.

5. **Research and Advancements**: Histopathological investigation contributes to ongoing research in veterinary oncology. The data obtained from studying tumor samples helps in understanding the biology of cancer in animals, leading to advancements in diagnostic techniques and treatment options.

2.12. Diagnostic procedure of neoplasm:

Diagnosis procedure of cancer:

1. **Physical examination**:

The first stage involves conducting a comprehensive physical examination, during which the animal's body is carefully examined by touch to detect any unusual growths or masses.

2. **Imaging Techniques:**
   - **X-rays**: These help visualize internal structures like bones and soft tissues, aiding in identifying tumors, bone density changes, and signs of metastasis.
   - **Ultrasound:** Using sound waves, it creates real-time images of organs and tissues, particularly useful for evaluating abdominal and thoracic areas and differentiating solid masses from fluid-filled structures.
   - **Computed Tomography (CT) Scan**: Produces detailed cross-sectional images, allowing precise assessment of tumor size, location, and involvement with surrounding structures.
   - **Magnetic Resonance Imaging (MRI**): Utilizing powerful magnets and radio waves, MRI provides detailed images of soft tissues, especially useful for brain and spinal cord tumor evaluation.

3. **Biopsy:**

Tissue samples are collected from the tumor for examination. Various biopsy techniques include:

- **Fine Needle Aspiration (FNA):** A thin needle collects cell samples, commonly used for superficial or challenging tumors.
- **Core Needle Biopsy**: A larger needle obtains a small tissue core for a more comprehensive evaluation.

- **Surgical Biopsy**: In some cases, the entire tumor or a substantial part is surgically removed for more accurate diagnosis.

4. **Cytology:**

This involves examining individual cells obtained from the tumor or its fluid content, often done in conjunction with fine needle aspiration, providing rapid preliminary results.

5. **Histopathology**:

The collected biopsy samples are collected for microscopic examination to determine the tumor type, grade, and other characteristics.

6. **Blood Tests:**

Blood work assesses the animal's overall health and may detect changes in blood cell counts or tumor markers indicating the presence of a tumor. Blood cell analysis is a valuable diagnostic tool in veterinary medicine, similar to its role in human medicine, to confirm the presence of cancer in animals. Veterinarians commonly employ various methods of blood cell analysis as part of their diagnostic workup for potential cancer cases in animals. These methods include:

- **Complete Blood Count (CBC):** Just like in humans, a CBC in animals provides essential information about the types and quantities of blood cells present. Abnormalities in blood cell counts can raise suspicion of cancer. For instance, certain cancers may lead to changes in white blood cell counts or the presence of abnormal cells.
- **Tumor Markers**: Similar to human medicine, some animals may produce specific tumor markers when they have cancer. These markers can be detected in the blood, and elevated levels may indicate the possibility of cancer. However, it's important to note that not all types of animal cancers produce easily detectable tumor markers.
- **Peripheral Blood Smear**: Examine a peripheral blood smear under a microscope to observe any irregularities in the size, shape, or number of blood cells. This examination can provide insights into certain types of cancer, such as leukemia.

- **Bone Marrow Examination**: If veterinarians suspect bone marrow involvement, they may perform a bone marrow aspiration and biopsy to assess the cells within the bone marrow. This procedure helps in detecting cancers like leukemia and lymphoma.
- **Genetic Testing**: Genetic testing can be used in animals to identify specific genetic mutations associated with certain types of cancers. Blood cells can carry these genetic mutations, and analyzing them can aid in diagnosis and guide treatment decisions.

7. **Endoscopy and Colonoscopy**:

These procedures visualize and biopsy tumors inside the gastrointestinal or respiratory tract or other body cavities.

8. **Staging and Imaging Modalities**:

If the tumor is malignant, additional imaging tests and staging procedures may assess the extent of tumor spread (metastasis) to other parts of the body.

2.13. Histopathology assessment in radiology and chemotherapy:

Radiotherapy:

Histopathological assessment plays a crucial role in the planning and monitoring of radiotherapy treatment for animals with cancer. Prior to initiating radiotherapy, veterinarians often perform a biopsy or surgical removal of the tumor to obtain tissue samples. These samples are then sent to a veterinary pathologist for histopathological examination (Zhao et al., 2010).

Role in planning treatment: In terms of treatment planning, the histopathology report provides vital information about the tumor type, grade, and stage. This information helps veterinary oncologists design a customized radiotherapy treatment plan that specifically targets the unique characteristics of the tumor. Tumors with higher grades may require higher radiation doses, and the stage of cancer influences the scope and extent of the radiotherapy (Knapp et al., 1997).

Role in treatment monitoring: Furthermore, histopathological examination also plays a significant role in treatment monitoring. Throughout and after the radiotherapy sessions, follow-up biopsies or fine-needle aspirates may be conducted to assess how the treatment is progressing. By examining these samples, veterinarians can evaluate

the degree of tumor shrinkage or changes in cellular characteristics, which provide valuable insights into the tumor's response to radiation treatment. Similarly, another study involving cats with fibrosarcoma revealed that the combination of histopathological and radiological evaluation played a crucial role in predicting the tumor's local control after radiation therapy (Turek et al., 2005).

Chemotherapy:

Chemotherapy is a common treatment modality in many veterinary cancer patients in addition to surgery and radiation therapy. Cytotoxic drugs can lead to complete remissions for some disseminated cancers (lymphoma, for example), be effective in decreasing tumor size, and may prolong life in many other types of metastatic cancers, such as osteosarcoma. Histopathological assessment is valuable in various stages of chemotherapy treatment in veterinary science (Reddy et al., 2009).

Role in Treatment Monitoring: Throughout the course of chemotherapy, periodic histopathological evaluation of tumor samples may be conducted to assess the treatment's effectiveness. The presence of viable cancer cells or changes in tumor characteristics informs veterinarians if adjustments to the chemotherapy protocol are necessary.

Side effects of chemotherapy in veterinary science: In veterinary medicine, chemotherapy can lead to a range of side effects that can impact the quality of life for companion animals. These common side effects include bone marrow suppression, which affects the production of blood cells and can lead to decreased immunity and anemia. Gastrointestinal problems, such as nausea, vomiting, and diarrhea, are also frequently observed (Chun et al., 2007).

Additionally, animals undergoing chemotherapy may experience a loss of appetite, fatigue, and allergic reactions. One common side effect is alopecia, which refers to hair loss. These side effects can vary in intensity and duration, and veterinarians often work to manage and mitigate them to improve the overall well-being of the animals during their cancer treatment (Chun et al., 2007).

Chapter 3: Materials and Methods

3.1. Study area and Duration:

The study was carried out for a duration of 6 months, spanning from November 2022 to April 2023. The initial 3 months were allocated to collecting the required samples, and the subsequent 3 months were dedicated to laboratory work. The samples used in the study were sourced from two distinct veterinary hospitals: the Teaching and Training Pet Hospital and Research Center (TTPHRC) situated in Purbachol, Dhaka, and the Shahedul Alam Quadary Teaching Veterinary Hospital (SAQTVH) located at Chattogram Veterinary and Animal Sciences University in Chattogram.

3.2. Sample size:

The study's sample population comprised of three tissue samples from dogs and cats tentatively diagnosed with neoplastic conditions. The samples were obtained from animals of different sexes, age groups, and breeds, all of which were reported to the Shahedul Alam Quadary Teaching Veterinary Hospital (SAQTVH) in Chattogram and the Teaching & Training Pet Hospital and Research Center (TTPHRC) in Dhaka.

3.3. Case History:

A female cat that had been spayed and was five years old presented at the TTPHRC in Dhaka. The cat exhibited a tumor-like growth on its skin situated within the abdominal area, and was suspected a lipoma.

At the SAQTVH in Chattogram, a one-year and two-month-old male Rottweiler was brought in by the owner. The dog exhibited a tumorous cutaneous growth situated in the submandibular region. The owner reported that the growth was observed one month ago and has been progressively increasing in size since then. The lesions had a diameter of 5 centimeters. The preliminary assessment suggests lymphoma, relying on the patient's medical history and the observation of symptoms.

A female dog, aged eight years, that had been unspayed, was presented at the TTPHRC in Dhaka. With a history marked by reduced intake of food and water, the identification of palpable masses in the mammary glands, and indications of pain in the affected area, the patient's background suggests a progressive growth in tumor size over the four months.

3.4. Sample collection and preservation:

Surgically extracted suspected neoplastic tumors from dogs and cats were collected. All samples were placed in Bouin's solution to preserve them for histological examination. The samples were stored in labeled plastic containers. Histological examination involved routine Hematoxylin and Eosin (H&E) staining following the method described by Luna in 1968.

3.5. Process of Histopathology:

3.5.1. Tissue fixation:

The first step in slide preparation is tissue fixation, which is a vital process aimed at preventing tissue autolysis and putrefaction. Tissue fixation is a critical step in preparing histopathological slides. It involves preserving the tissue specimen in its natural state by using chemical agents or other methods. Tissue samples should be transferred into fixative immediately after collection. Although there are many types of fixative, but here Bouin’s solution is used for fixation of tissue.

3.5.2. Washing:

Allowing the specimens to soak in distilled water for an extended period, typically overnight, to facilitate thorough washing. Then, assign numbers to the individual samples and organize them in a connected series to create a tissue "garland."

3.5.3. Dehydration:

The objective of dehydration is to remove the water from the tissue. For this reason, tissue is run through a specific concentration of alcohol for a specific time. The concentration and time allowance are given below.

i. Alcohol 80% for two hours
ii. Alcohol 95% for one hour (two changes)

3.5.4. Clearing:

Xylene is used as a clearing reagent. The purpose of clearing is to remove the alcohol from the tissue. Tissue is run through the xylene solution for a specific time.

i. Xylene one hour (two changes).
ii. Xylene for two hours

3.5.5. Impregnation:

It is done to remove clearing agents. It is done by bathing in paraffin having a temperature of 56-58 degrees Celsius. Here three changes are done where tissue is kept for two hours before every change. After completing impregnation, the tissue kept for rest overnight.

3.5.6. Embedding:

It was done by placing the tissue in melted paraffin to make the block, which after solidification provided a firm medium for keeping all parts of the tissue intact when sections were cut.

3.5.7. Preparation of tissue section:

For the preparation of tissue sections following processes are followed:

i. A tissue block embedded in paraffin was set in the microtome machine. Then sections were cut at 3-5 μm thickness until suitable tissue film was formed.
ii. Flim of tissue sections were placed in the warm water bath (55-58) and allowed to spread.
iii. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. Sections were picked up on grease-free clear slides.

3.5.8. Preparation of Harri's Hematoxylin Solution:

The hematoxylin and the alum were dissolved in alcohol and in water respectively by the aid of heat. Both the solutions were thoroughly mixed just after removal from heat and boiled as soon as possible. Then mercuric oxide was added slowly after removal from heat. The solution was again heated to simmer and stopped heating when it became dark purple in color. Then the vessel was plunged into a basin of cold water and made it cool. 2-4 ml of glacial acetic acid was added per 100 ml solution just before use to increase the precision of nuclear stain.

3.5.9. Preparation of Eosin Solution:

For working solution 1 part of Eosin stock solution was mixed with 3 parts of 80% alcohol. Just before use 0.5 ml of glacial acetic acid per 100ml of stain solution was added.

3.5.10. Hematoxylin Eosin Staining Procedure:

The tissue section that is taken into the slide is prepared for staining. The following procedure is followed for the staining of the slide.

A regressive staining procedure was followed to stain the tissue slides. In the regressive staining technique, the sections were first overstained with a relatively neutral solution of hematoxylin. Then the excess stain was removed by using an acid alcohol solution. After that sections were neutralized with an alkaline solution (weak ammonia water) for better differentiation. Then the sections were counterstained with eosin followed by the removal of excess eosin by alcohol. After staining and mounting cover slip the slides were air dried and then examined under microscope.

After that put DPX on the stain tissue and place a cover slip on it. The tissue is ready to study for histopathological changes. Then under the microscope in different magnifications, the study was done and found the abnormal changes in cellular levels.

Chapter 4: Results

Histopathological examination holds significant importance in the identification, prognosis, and therapeutic approach for different neoplastic conditions in companion animals. The objective of this study is to investigate the varied cellular features observed in suspected tumor cases, encompassing a feline lipoma, along with instances of lymphoma and mammary gland carcinoma in two distinct dogs, through histopathological analyses.

4.1. Lipoma:

Gross lesion:

The neoplasm displayed irregular shapes and sizes, growing to a substantial extent. They presented as round or oval masses, sometimes with a pedunculated attachment. The tumor displayed a yellow hue and was clearly demarcated by a capsule. Its surface exhibited irregularities and protrusions (Figure: 3). Upon cutting into the tumor, there were multiple soft, yellow-colored lobules, and the cut surface had an oily appearance.

Microscopic lesions:

The subcutaneous mass exhibited sheets of numerous large, pleomorphic cells (Figure: 4 and 5), surrounded by thin fibrous partitions. Each adipocyte within the mass contained a large fat globule, causing the nucleus to be pushed towards the outer edge and flattened due to compression (Figure: 6). The adipocytes displayed clear cell boundaries and contained a single large vacuole in their cytoplasm with nuclei positioned eccentrically (Figure: 7). Presence of reactive cells within the fat tissue that is indicating inflammation (Figure: 8).

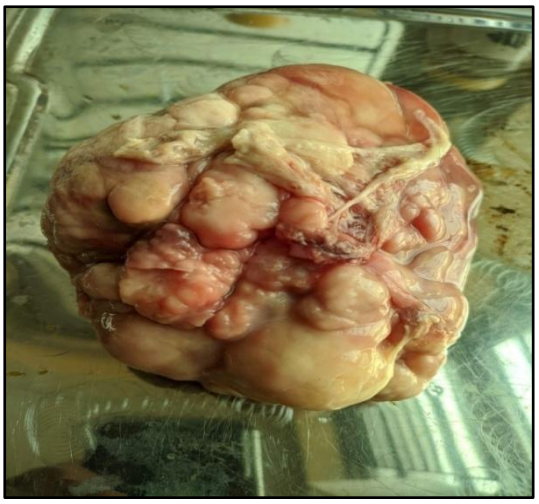


Figure 3: A soft and yellow-colored intraabdominal multilobulated mass

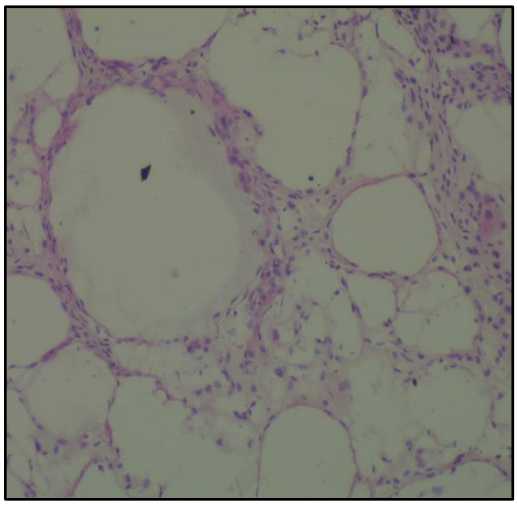


Figure 4: Presence of fat cells with distinct nucleus

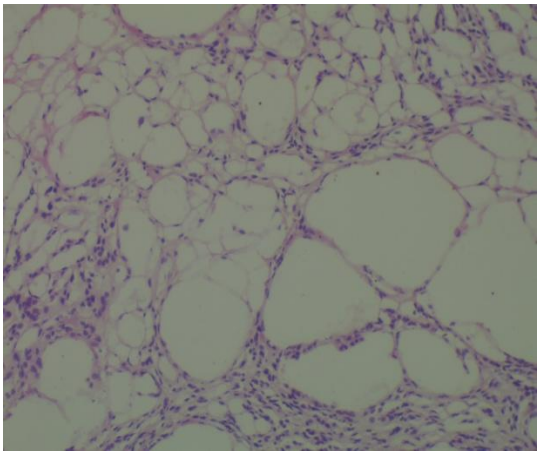


Figure 5: Adipocyte displaying well defined cell boundaries

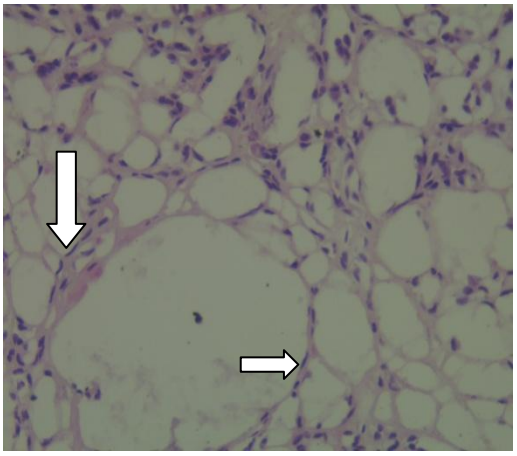


Figure 6: A singular lipid vacuole with compressed nuclei at the periphery

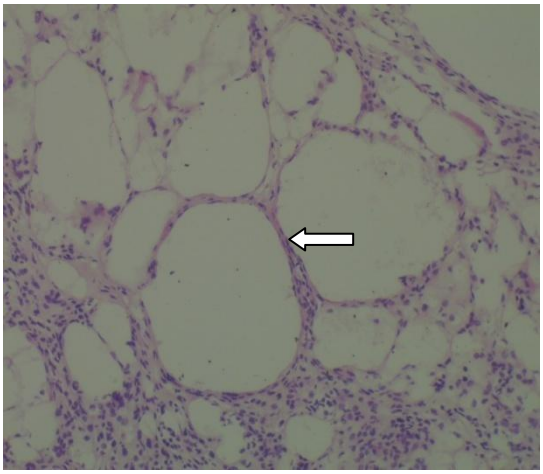


Figure 7: Adipocyte with compressed nuclei

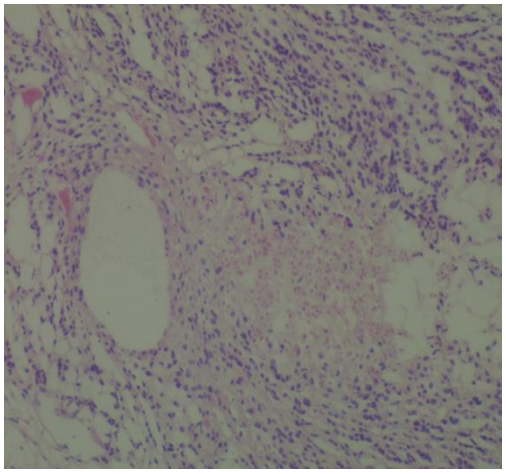


Figure 8: Infiltration of reactive cells in fat tissue indicating inflammation

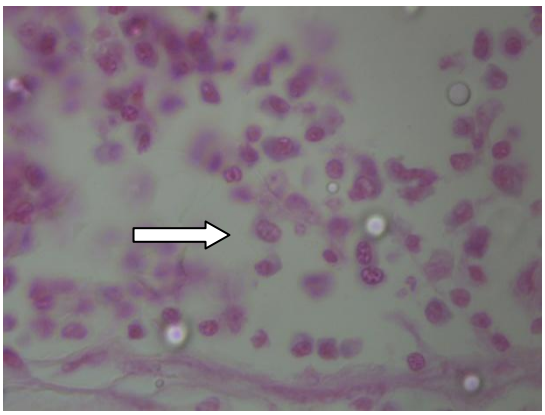
4.2. Lymphoma:

Gross lesion:

The dog had a tumor-like growth on its skin located in the area beneath the jaw, specifically in the submandibular region. On examination, the lymph nodes were visibly swollen and firm to the touch, located in the submandibular region, and did not appear to cause the dog any pain.

Microscopic lesion:

In this microscopic image, there is evident clustering of pleomorphic anaplastic cells (Figure: 9 and 10). The neoplastic cell nuclei are of medium size with moderate variation in size (anisokaryosis), and the chromatin material within the nuclei is finely spread out. Binucleated cells are also observed in this microscopic sample with a relatively largeer size and slightly bluish-tinted cytoplasm (Figure: 11). Fragmented nuclei, referred to as karyorrhexis, are evident in the observation, suggesting the presence of necrotic cells (Figure: 12). Furthermore, neoplastic cells are detected within the blood vessel in the examined specimen (Figure: 13). The capsule has undergone thickening (Figure: 14 and 17), and the presence of mitotic activity in the cells suggests ongoing cellular division (Figure: 15). Fibroblasts cell are present (Figure: 16). Accumulation of reactive cells and tumor cells (Figure: 18).



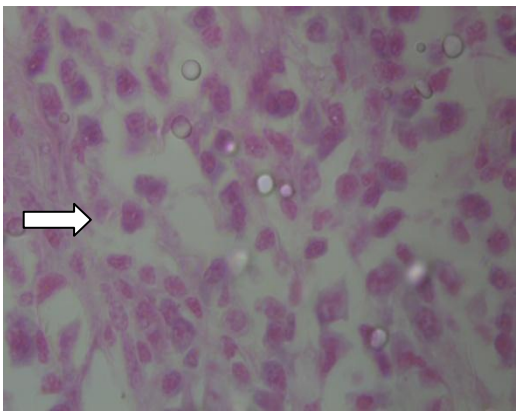


Figure 9 and 10: Presence of pleumorphic cells

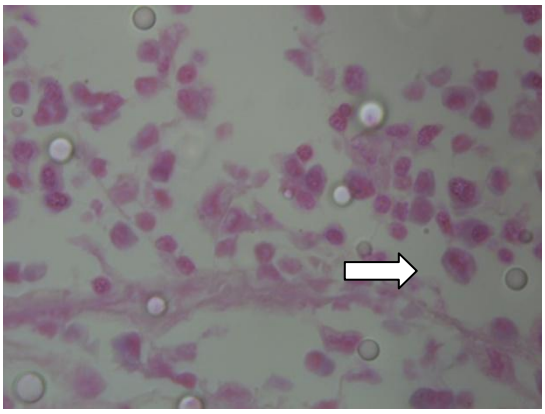


Figure 11: Presence of binucleated cell

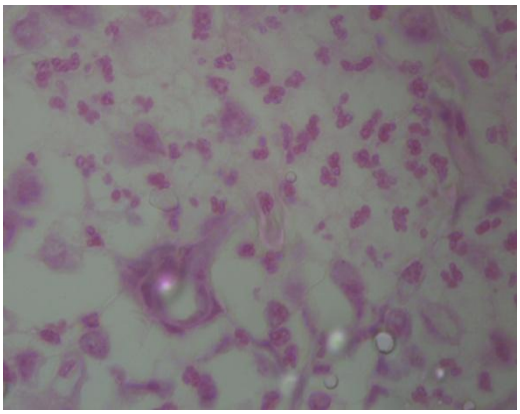


Figure 12: Nuclear fragmentation of (karyorrhexis) lymphoid cells

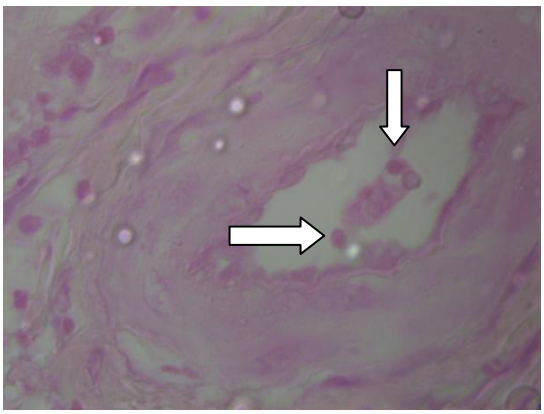


Figure 13: Neoplastic cells are observed within blood vessel

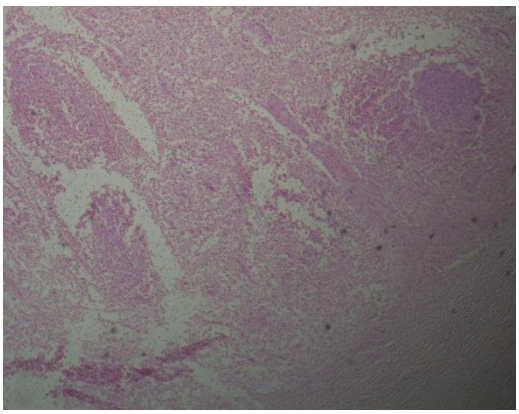


Figure 14: Thicken capsule

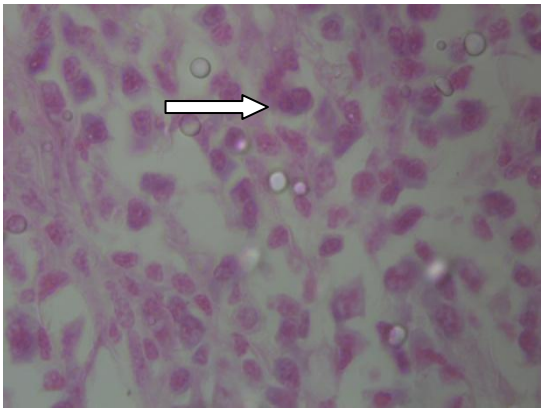


Figure 15: Presence of mitotic activity and large nuclei with coarse chromatin

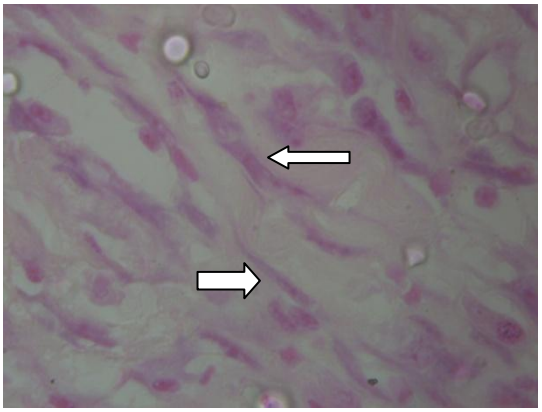


Figure 16: Fibroblasts are present

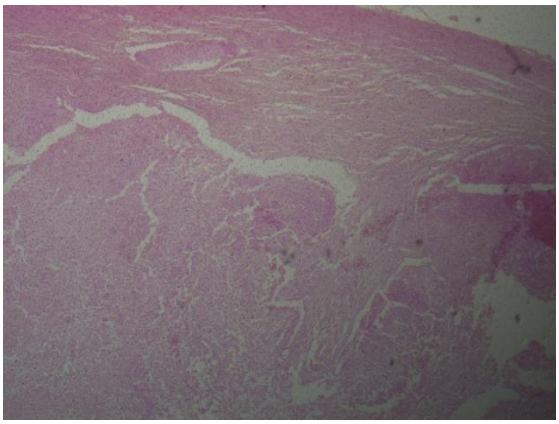


Figure 17: Thickened capsule with inward moving and destruction of medullary cord

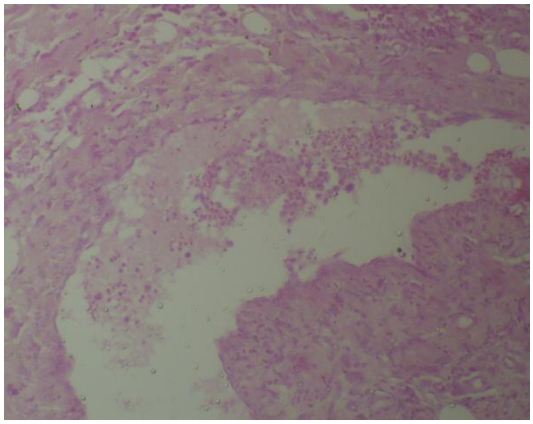


Figure 18: Accumulation of reactive cells and tumor cells in blood vessel

4.3. Mammary gland carcinoma:

Gross lesions:

On a macroscopic level, the lesions exhibit continuous, solid, and reddened patches without distinct boundaries. Additional symptoms encompass itchiness, an increase in local temperature, varying degrees of pain (ranging from intense to moderate), swelling, and redness of the skin above the mammary gland. The tumor was clearly defined, possessing a firm texture, and its cut surface displayed a grayish-white coloration (Figure: 19).

Microscopic lesions:

The tumors exhibited the development of tubular structures accompanied by papillary extensions, as illustrated in Figure: 20 & 21. Additionally, there was growth observed in the surrounding stromal tissue. These tubular structures were covered by epithelial cells ranging from cuboidal to columnar in shape, along with certain polygonal cancerous cells. These cells displayed eosinophilic cytoplasm and contained prominently hyperchromatic nuclei. The infected region is infiltrated by tumor infiltrating lymphocytes (Figure: 22). Hemorrhage and fibrosis are observed in the examined sample (Figure: 23). Moreover, congestion is also observed (Figure: 24). Fibroblast cells are also identified in this microscopic specimen (Figure: 25). There is an observable yet moderate variability in the size of nuclei. Pleomorphic cells are observed with milk duct and acini (Figure: 26, 27 & 28). Figure 29 & 30 illustrate a transformation in which the lining cells of a duct are transitioning into an increased quantity of squamous cells. Neoplastic ductal epithelial cells exhibit proliferation, resulting in the development of papillary projections extending into the lumen, as depicted in Figure: 31. There is also also observed proliferation of lining cells in milk duct (Figure: 32).

Figure 19: Gross appearance of resected mammary tumor

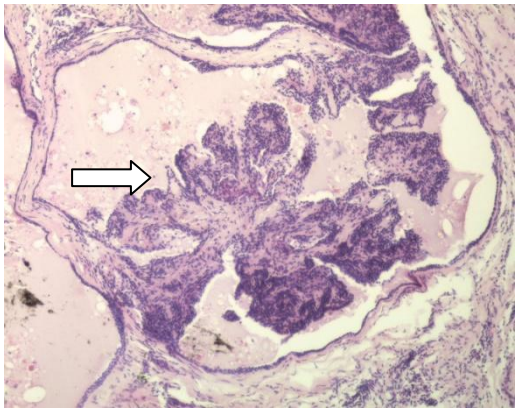


Figure 20: Proliferation is observed forming papillae projecting towards the center

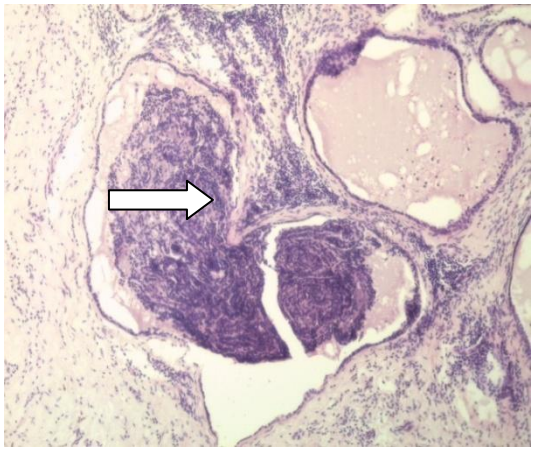


Figure 23: Proliferation is observed forming papillae projecting towards the center

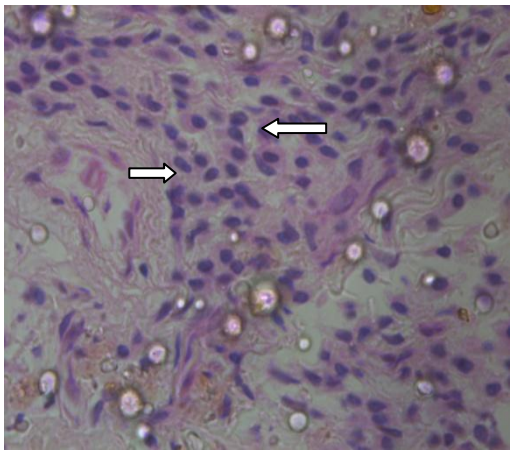


Figure 22: Presence of tumor infiltrating cells in mammary gland

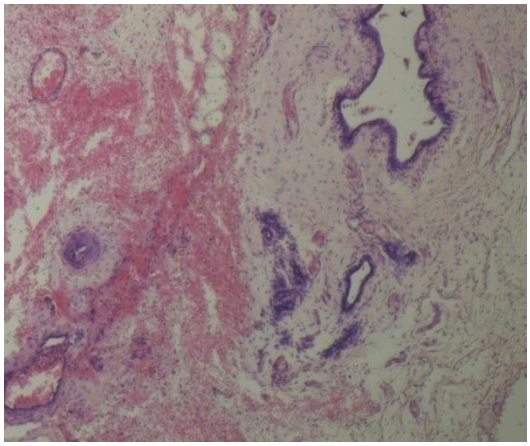


Figure 23: Hemorrhage and fibrosis observed in mammary gland

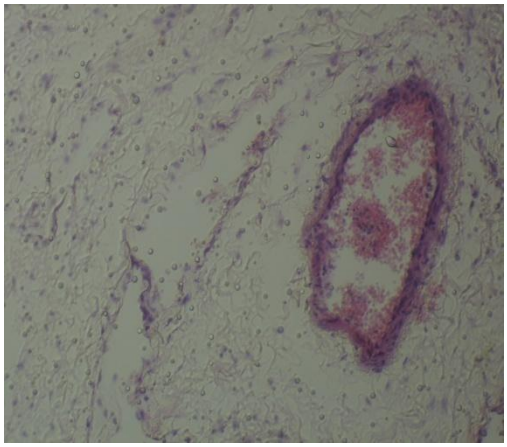


Figure 24: Congestive blood vessel in tumor area

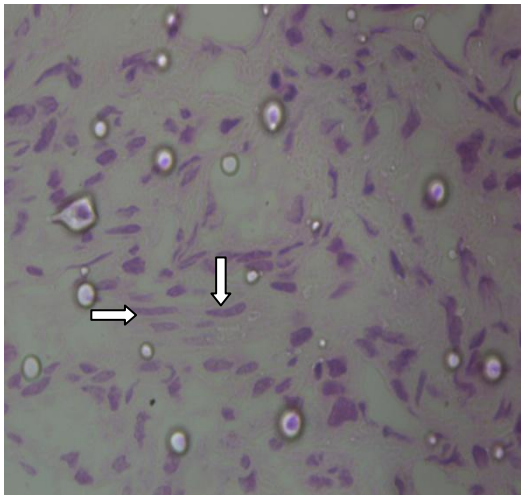


Figure 25: Presence of fibroblasts

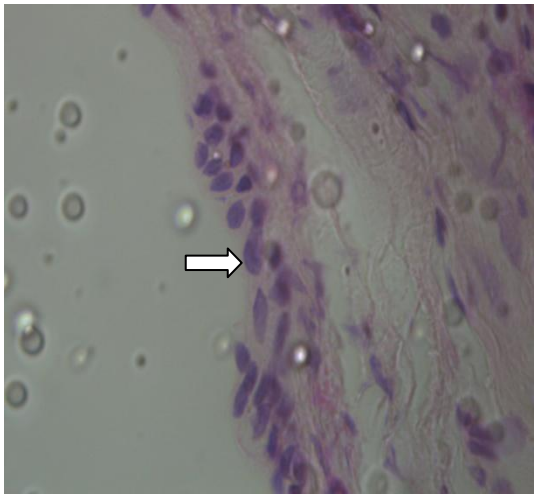


Figure 26: Presence of pleomorphic cell in the duct

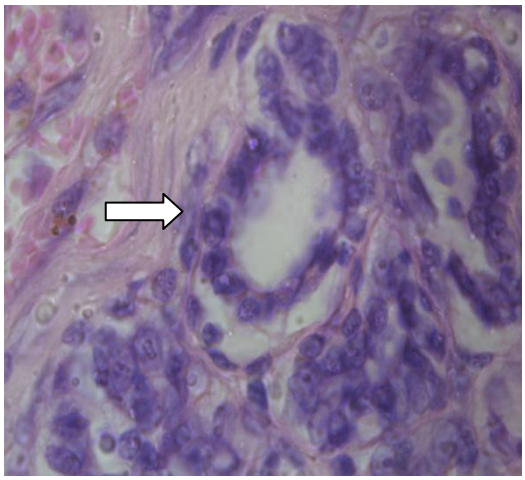


Figure 27: Presence of pleomorphic cell in milk duct

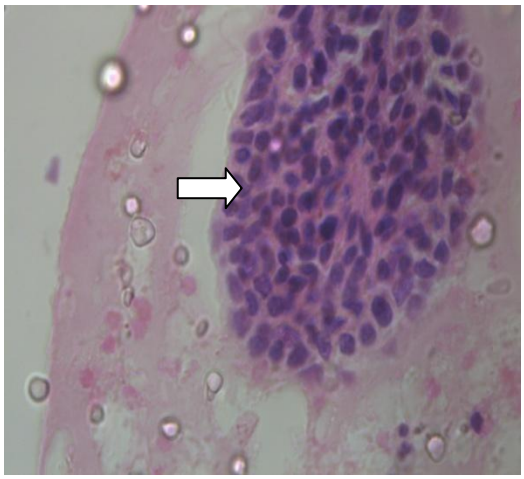
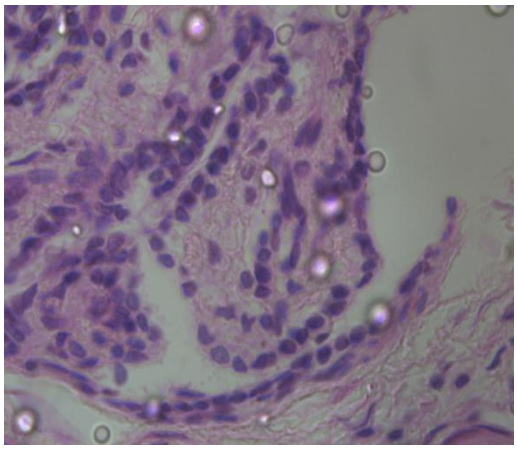


Figure 28: Presence of pleomorphic cell within milk acini



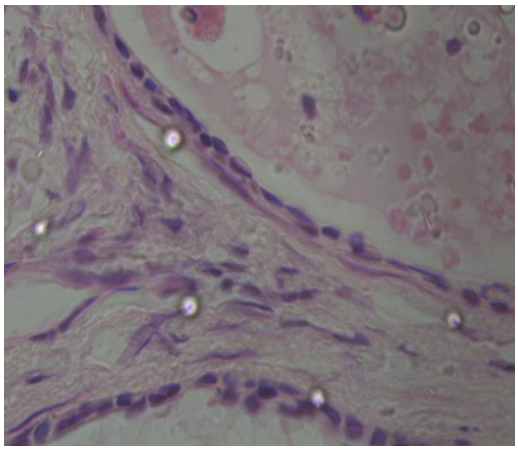


Figure 29 and 30: Micrograph showing squamous cell metaplasia

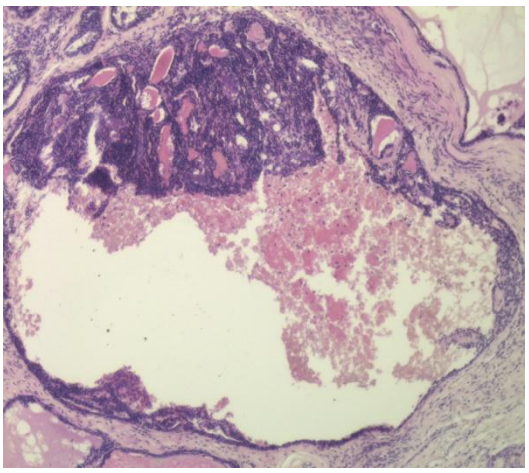


Figure 31: Proliferation of ductal epithelial cells forming papillary growth

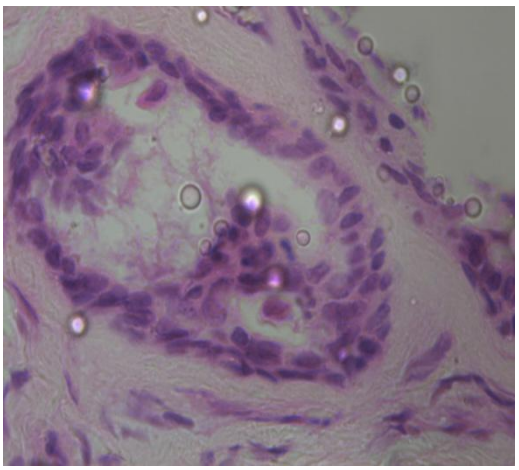


Figure 32: Proliferation of lining cell in duct

Chapter 5: Discussion

5.1. Lipoma:

Lipoma is a common non-cancerous growth that originates from mature fat cells of mesenchymal origin. These tumors tend to occur most frequently in subcutaneous (beneath the skin) regions of the trunk, gluteal area, and proximal limbs. Lipomas are typically identified in older dogs, usually around 9 to 11 years of age (Gupta et al., 2009). Out of 175 cases examined in a survey, only four lipomas were found in locations other than the subcutaneous tissue. Lipomas within the thoracic cavity have also been documented in dogs by several researchers, including Teunissen in 1977, Woolfson and others in 1984, Wilson and Hawe in 1986, and Anderson and Lippincott in 1989. Lipoma is most common in overweighed dogs especially in adult female obese dogs. It was also reported in a non-descript, non-obese male dog and is contrary with the early reports in which the tumours were recorded in female, pure breeds and obese dogs (Lahrach et al., 2013; Lamagna et al., 2012). But, in this current case, the lipoma was observed in a spayed cat of a local breed, which is in contrast to the earlier reports that documented such tumors in female, purebred, and overweight dogs (Lahrach et al., 2013).

The macroscopic examination revealed a distinct, encapsulated mass with several lobes, composed of yellow and greasy adipose tissue. These gross characteristics align with the findings reported by Goldsmith and Hendrick (2002), as well as Sasikala and Arulmozhi (2020) in their respective studies. The lipomas in the study were typically found to have a round, ovoid, or pedunculated shape. They were frequently observed to have multiple lobes, and their appearance was well-defined, with a distinct boundary and encapsulated structure. The texture of these lipomas was described as soft or flabby (as shown in Figure 3). Similar observations were reported by Hupes et al., 2016.

Microscopically, the lipoma mass showed delicate fibrous partitions that formed irregular lobules (as shown in figure 5). The supporting structure contained blood vessels responsible for providing nutrients. Each adipocyte within the lipoma was filled with a large fat droplet, which displaced the nucleus towards the outer edge, causing it to flatten due to the pressure from the fat droplet (as shown in figure 7). The

current study's observations are consistent with the results reported in the studies conducted by Ashley et al., 1990 and Dadhich et al., 2018. Both Moulton (1978) and Degloorkar et al., (1992) conducted studies that revealed comparable results, confirming the existence of fibrous partitions, irregular lobules, and compression-induced flattening of the nucleus within the adipocytes present in the lipoma mass.

5.2. Lymphoma:

Research carried out in the North of Paraná revealed that dogs between two and five years old were the most frequently affected age group by the condition (Moreno et al., 2007). The same age category experienced the highest frequency of occurrences in a study conducted in the United States (Teske, 1994). In this research, the affected canine participant was a mere one year and two months of age. The neoplasms tend to spread early to the submandibular lymph nodes, which serve as a primary site for metastasis. Lymphoma is a common hematopoietic neoplasm of dogs that arises from lymphocytes and accounts for up to 24% of all canine neoplasms (Vail et al., 2001). The most common site of involvement is lymph nodes. The notable high malignancy level observed in most lymphomas (65%) in this research aligns with observations made by other researchers (Teske et al., 1994; Fournel-Fleury et al., 2002; Suzano et al., 2010; Ponce et al., 2010).

Microscopically, the appearance of lymphoma cells displays considerable diversity, forming groupings that range from small to large in size, and may even encompass immunoblastic cell varieties. Significant variation in nuclear size is evident (anisokaryosis). The frequency of cell division varies greatly, aligning with the specific cell type or tumor grade (Gross et al., 2006). In this particular instance, the cellular heterogeneity was notably extensive. In this study, there is an observable yet moderate degree of nucleus size variation (anisokaryosis), with genetic material inside the nuclei appearing finely dispersed. Neoplastic cells have been identified inside the blood vessel in the analyzed sample. The capsule has experienced thickening, and the existence of mitotic activity in the cells indicates ongoing cellular division.

5.3. Mammary gland carcinoma:

Dogs with mammary gland tumors are typically older, approximately 9 to 11 years old, sexually intact, or spayed later in life (Dantas et al., 2012). It is well established that intact dogs are at more risk of developing mammary neoplasia than spayed

bitches (Sorenmo, 2003). In the current research, an occurrence of a mammary tumor was noted in an unsprayed 8-year-old dog. This observation aligns with the discoveries made by Broady et al., 1983. However, Karayannopoulou et al., 1990 stated that the highest frequency of mammary gland tumors was seen in dogs aged between 8 and 13 years. It has been reported that the posterior pair of mammary glands are more prone to trauma when the animal moves or when it lies down and this may be the reason for more propensity of tumor development in these glands (Runnells et al., 1965).

The Gross lesions exhibit continuous, solid, and reddened patches without distinct boundaries. Microscopically the cells vary from polygonal to round to cuboidal, and they may have a scant amount of eosinophilic cytoplasm and a high nuclear-cytoplasmic ratio. Nuclei may be hyperchromatic, central, round to oval with clumped chromatin and a single central basophilic nucleolus, or they may be vesicular with a small nucleolus. Anisokaryosis and anisocytosis are moderate, and mitoses are found but vary in number. As is commonly found with carcinoma–in situ in other organs, there is loss of cellular and nuclear polarity and loss of the normal architecture, with layering of cells within the ducts. There are often areas of lobular hyperplasia within the mammary tissue adjacent to the areas of neoplasia (Mouser et al., 2010). The current study's observations are consistent with the results.

There is a growth of neoplastic ductal epithelial cells, leading to the formation of papillary projections that extend into the lumen. The cells exhibit nuclei that are notably hyperchromatic. Tumor infiltrating lymphocytes have invaded the affected area. There is also a transformation occurring, wherein the cells lining a duct are undergoing a shift towards an augmented presence of squamous cells. The characteristics of both the macroscopic and microscopic aspects of the current lesions correspond closely to those documented by previous researchers (Misdrop, 2002; Krithiga et al., 2005; Reddy et al., 2009; Chavan et al., 2016; Raval et al., 2018; and Patel et al., 2019). Tumor infiltrating lymphocytes are believed to represent the local immune response, a key mechanism in controlling tumor growth and metastasis, and an independent prognostic indicator for many tumors (Canna et al., 2005). In this study, tumor infiltrating lymphocytes have penetrated the affected area. The findings of the present study align with the outcomes.

Chapter 6: Conclusion

This thesis has delved into the vital significance of histopathological examination within the field of veterinary science, contributing significantly to our understanding of tumor cells. Through meticulous analysis of tissue samples, histopathology has provided invaluable insights into tumor diagnosis, classification, and treatment for animals. The research findings highlighted in this thesis underscore the essential nature of precise tumor diagnosis, serving as the basis for developing personalized treatment strategies for affected animals. By evaluating the tumor type, grade, and extent of invasion, histopathological assessment plays a critical role in determining the tumor's behavior and its potential response to therapeutic interventions. In this study, a histological analysis revealed the presence of a significant accumulation of pleomorphic cells, anisokaryosis, uneven distribution of chromatin materials, and an increased number of binucleated cells. These findings suggest the occurrence of neoplastic changes. Moreover, the identification of neoplastic cells within blood vessels indicates potential metastasis. The observation of mitotic figures further supports the presence of neoplastic alterations in the tissues.

Chapter 7: Limitations

Obtaining sufficient and representative tissue samples is essential for precise cancer diagnosis and staging. The accuracy of histopathological assessments depends on obtaining adequately sized tissue samples that truly reflect the tumor's nature. However, in the restricted timeframe of the study, acquiring enough tissue proved to be a significant challenge.

The clinical history data provided by the pet's owner was insufficient. Without a comprehensive clinical history, the tumor's characteristics might not be fully understood, resulting in uncertainty in the diagnosis and potentially inappropriate treatment choices

References

Adams VJ, Evans KM, Sampson J, Wood J. 2010. Methods and mortality results of a health survey of purebred dogs in the UK. Journal of Small Animal Practice.

Adamson ED. 1987. Oncogenes in development. Development 99, 449–471.

Ali MR, Ibrahim MI, Hala RA, Salah AS, Mostafa ES. 2016. Treatment of natural mammary gland tumors in canines and felines using gold nanorods-assisted plasmonic photothermal therapy to induce tumor apoptosis. International journal of nanomedicine. 11: 4849-4863.

Anderson Lippinco CL. 1989. lntrathoracic lipoma in a dog. California Veterinarian. 436.

Argyle DJ, Khanna C, Giancristofaro N. 2020. Tumor Biology and Metastasis. In: Vail D.M., Thamm D.H., Liptak J.M., editors. Withrow and MacEwen's Small Animal Clinical Oncology. 6th ed. W.B. Saunders; St. Louis, MO, USA. pp. 36–60.

Ashley DJB. 1990. Evan's histopathological appearance of tumors, 4th Edn. Churchil Livingstone. Edinburgh London Melbourne and Newyork.

Barrs V, Beatty J. 2012. Feline alimentary lymphoma: Classification, risk factors, clinical signs and non-invasive diagnostics. Journal of Feline Medicine and Surgery. 14:182–90.

Birbrair A, Zhang T, Wang ZM, Messi ML, Olson JD, Mintz A, Delbono O. 2014. "Type-2 pericytes participate in normal and tumoral angiogenesis". American Journal of Physiology-Cell Physiology. 307 (1).

Boldizsar HO, Szenci TM, and Csenki J. 1992. "Studies on canine mammary tumors. in: age, seasonal and breed distribution," The Veterinary Bulletin, vol. 63, p. 4028.

Brodey RS, Goldschmidt MA, and Roszel JR. 1983. "Canine mammary gland neoplasm,". Journal of the American Animal Hospital Association, vol. 19, pp. 61–90.

Bronden LBFA, Kristensen AT. 2007. Veterinary cancer registries in companion animal cancer: A review Veterinary Comparetive Oncology. 5: 133–144.

Buck MD, Sowell RT, Kaech SM, Pearce EL. 2017. Metabolic Instruction of Immunity. 169:570–8.

Butler, LM, Bonnett BN, Withrow RL and MacEwen. 2012. Small Animal Clinical Oncology, 5th ed.; Withrow, S.J., Vail, D.M., Page, R.L., Eds.; Elsevier Saunders: St Louis, MO, USA. pp. 68–82.

Canine Malignant Lymphoma: Introduction". The Merck Veterinary Manual. Retrieved 2007-01-28.

Canna K, McArdle PA, McMillan DC. 2005.The relationship between tumour T-lymphocyte infiltration, the systemic inflammatory response and survival in patients undergoing curative resection for colorectal cancer. Brazilian Journal Cancer. 92:651–654.

Carson, Freida L, Hladik, Christa. 2009. Histotechnology: A Self-Instructional Text (3rd ed.). Hong Kong: American Society for Clinical Pathology Press.

Cassali GD, Lidianne N, Conrado OG, Karine D, Cecília B. 2015. Cytologic analysis of the mammary papillar discharge in a canine micropapillary carcinoma. Veterinary clinical pathology. 44(3): 448-451.

Chavan CA, Kaore MP, Bhandarkar AG, Dhakate MS, & Kurkure NV. 2016. Cytological, histological and immunohistochemical evaluation of canine mammary tumors. Indian Journal of Veterinary Pathology. 40(2), 139-143.

Chun R, Garret l, Vail D. 2007. Cancer chemotherapy. In: Withrow SJ, Vail DM, editors. Withrow & MacEwen's Small Animal Clinical Oncology. 4th ed. Philadelphia: WB Saunders. pp. 163–192.

Connolly, James L, Schnitt, Stuart J, Wang, Helen H, Longtine, Janina A, Dvorak, Ann, Dvorak, Harold F. 2003. "Role of the Surgical Pathologist in the Diagnosis and Management of the Cancer Patient". In Holland, James F.; Frei, Emil; Kufe, Donald W. (eds.). Holland-Frei Cancer Medicine (6 ed.)

Cooper GM. 1992. Elements of human cancer. Boston: Jones and Bartlett Publishers. p. 16.

Cowell RL, Tyler RD, Meinkoth JH and DeNicola DB. 2008. Diagnostic cytology and hematology of the dog and cat. 2nd ed. Elsevier Health Sciences. pp 20-51.

Coyle KA, Steinberg H. 2004. Characterization of lymphocytes in canine gastrointestinal lymphoma. Veterinary Pathology. 41(2):141–146.

Dadhich R, Dadhic H, Sharma GD, Singh G, Rathore A, Dholpuria S. 2018. Pathological and HaematoBiochemical studies of Lipoma in Dogs. Veterinary Practitioner. 19(1):35-36

Dantas CG, Angélica CB, Enio F, Karine D, Conrado OG. 2012. Canine mammary mixed tumours: A review. Veterinary medicine international.

Dobson JM, Samuel S, Milstein H, Rogers K, Wood JLN. 2002. Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. Journal of Small Animal Practice. 43:240–246.

Doddy FD, Glickman LT, Glickman NW, Janovitz EB. 1996. Feline fibrosarcomas at vaccination sites and non-vaccination sites. Journal of Comparative Pathology. 114, 165–174.

Dorn CR, Taylor DO, Frye FL, Hibbard HH. 1968. Survey of animal neoplasm in Alameda and Contra Costa counties, California. I. Methodology and desciption of cases. Journal of the National Cancer Institution. 40:295–305.

Dorn CR, Taylor DO, Schneider R, Hibbard HH, Klauber MR.1968. Survey of animal neoplasm in Alameda and Contra Costa countries, California. II. Cancer morbidity in dogs and cats from Alameda country. Journal of the National Cancer Institution 40:307–318.

Egenvall A, Bonnett BN, Haggstrom J, Ström Holst B, Möller L, Nødtvedt A. 2010. Morbidity of insured Swedish cats during 1999–2006 by age, breed, sex, and diagnosis. Journal of Feline Medicine and Surgery. 12, 948–959.

Egenvall A, Nødtvedt A, Haggstrom J, Ström HB, Möller L, Bonnett BN. 2009. Mortality of life-insured Swedish cats during 1999–2006: Age, breed, sex, and diagnosis. Journal of Veterinary Internal Medicine. 23, 1175–1183.

Ettinger SN. 2003. Principles of treatment for feline lymphoma. Clinical Techniques Small Animal Practice. 18:98–102.

Fidler IJ and Brodey RS. 1967. “The biological behavior of canine mammary neoplasms,” Journal of the American Veterinary Medical Association. vol. 151, no. 6, pp. 1311–1318.

Fighera RA, Souza TM, Rodrigues A and Barros CSL. 2006. Aspectos clinicopatológicos de 43 casos de linfoma em cães. MedveP, Revta Cient. Medicina Veterinaria de Pequenos Animals Estimation.

Filippo G, Jesus VA, Belinda P, Svetomir NM, Paola N. and Alberto S. 2020. Relevance of immune cell and tumor microenvironment imaging in the new era of immunotherapy. Journal of experimental and clinical cancer research. 39,89.

Funkhousera M , Rastko S, Takeshi S, Anne EG, Robert DG and Monica OC. 2013. Mechanical model of blebbing in nuclear lamin meshworks. Proceedings of the National Academy of Sciences of the United States of America. 110(9): 3248–3253.

Gabor LJ, Malik R, Canfield PJ. 1968. Clinical and anatomical features of lymphosarcoma in 118 cats. Australia Veterinary Journal. 76:725–32.

Galway K, Black A, Cantwell M, Cardwell CR, Mills M, Donnelly M. 2012. "Psychosocial interventions to improve quality of life and emotional wellbeing for recently diagnosed cancer patients". The Cochrane Database of Systematic Reviews. 11(11).

Garden O, Volk SW, Mason NJ, Perry JA. 2018. Companion animals in comparative oncology: One Medicine in action. The Veterinary Journal. 240: 6-13.

Gardner, Heather L, Fenger, Joelle M, Cheryl A. 2016. "Dogs as a Model for Cancer". Annual Review of Animal Biosciences. 4 (1): 199–222.

Gerald, Abrams., "Neoplasia I". Archived from the original on 31 October 2015.

Goldschmidt MH and Hendrick MJ. 2002. Tumors of the skin and soft tissues. In: Tumors of Domestic Animals. 4th edn., DJ Meuten, edn., Iowa State Press, Iowa, pp45-117.

Goldsmith MH, Hendrick MJ. 2003. Tumors of the skin and soft tissues. In: Meuten DJ editor: Tumors in domestic animals, Lowa State Press: Blackwell. 45-117.

Greenlee PG, Filippa DA, Quimby FW, Patnaik AK, Calvano SE, Matus RE, Kimmel M. 1990. Lymphomas in dogs. A morphologic, immunologic and clinical study. American cancer society journal. 66, 480 – 490.

Guillermo CC. 2001. What is new on feline lymphoma? Journal of Feline Medicine and Surgery. 3:171–6.

Gupta N, Tiwari SK. 2009. Studies on Incidence, Histopathological features and Surgical management of Neoplasms in Canine. Veterinary World. 2(10):392-395.

Hakim E, Kolander Y, Meller Y, Moses M and Sagi A. 1994. Gigantic lipomas. Plastic and Reconstructive Surgery. 94(2): 369-371.

Hayes HJr, Milne KL, Mandell CP. 1981. Epidemiological features of feline mammary carcinoma. Veterinary Record. 108, 476–479.

Haziroglu R, Yardimci B, Aslan S, Yildirim MZ. 2010. Cytological Evaluation of canine mammary tumours with fine needle aspiration biopsy. Revue de medecine veterinaire. 161(5): 212-218.

Hoseini AT, Razavi SM and Khabazian A. 2010. Lipoma in oral mucosa: two case reports. Dental Research Journal. 7(1): 41-43.

Hupes RR, Pietro ND, Wittmaack MC, Sembenelli G, Bueno CM, Pazzini JM, Jark PC, Nardi AB and Castro JL. 2016. Intermuscular Lipoma in dogs. Acta Scientiae Veterinariae. 44(Suppl. 1): 127.

Joshi S, Bhadauria RS, Gunjan Jadon, Diwaker AK. 2012. Introduction to neoplasm: 'tumor classification' a review article. International Journal of Advanced Research in Pharmaceutical & Bio Sciences. 1(3): 227-264.

Kamble M, Dhakate MS, Upadhye SV, Akhare SB. 2016. Surgical management of mammary tumor in a dog. Intas Polivet. 17(1): 196-197.

Karayannopoulou ME, Kaldrimida A, Dessiris S. 1990. “Some epidemiological aspect of canine mammary tumour, treatment and prognosis,” European Journal of Companion Animal Practice. vol. 1, no. 1, pp. 41–47.

Klopfleisch R, Euler HV, G Sarli G, Pinho S, Gärtner F. 2010. Molecular Carcinogenesis of Canine Mammary Tumors: News From an Old Disease. Veterinary Pathology. 48(1) 98-116.

Knapp DW. 1997. Assessment of histological grade, proliferative activity, and cell-cycle distribution in canine osteosarcoma. Journal of Veterinary Diagnostic Investigation. 9(3):258-64.

Krithiga K, Murali MB, and Balachandran C. 2005. Cytological and histological diagnosis of canine mammary tumours. Indian Journal Veterinary Patholology. 29(2).

Lahrach KEL, Kadi KI, Mezzani A, Marzouki A, Boutayeb F. 2013. An unusual case of an intramuscular lipoma of the biceps brachii. The Pan African Medical Journal. 15(4):1-4.

Lamagna B, Grec A, Guardascione A, Navas L, Ragozzino M, Paciello O. 2012. Canine lipomas treated with steroid injections: Clinical findings. Plos One. 7(11):1-5.

Lowe AD. 2004. "Alimentary lymphosarcoma in a 4-year-old Labrador retriever". The Canadian Veterinary Journal, la Revue Veterinaire Canadienne. 45 (7): 610–612.

Ludwig EK, Byron CR, Lahmers KK and Santos MP. 2017. Frontal and caudal maxillary sinus lipoma in a horse. Canadian Veterinary Journal. 58(5): 503-507.

MacDonald V. 2009.Chemotherapy: managing side effects and safe handling. Canadian Veterinary Journal. 50(6):665-8.

MacVean, DW, Monlux AW, Anderson PS, Silberg SL, Roszel JF. 1978. Frequency of canine and feline tumors in a defined population. Veterinay Pathology. 15, 700–715.

Mathieu V, Lucile C, Jérémy B, Sophie L, Maxence D, Marie-Od, Julie B, Nadine C, Georgia M, Christophe C, Ghita B, Laurent T, Alexander JG, Olivier H. and Valérie F. 2011. Feline low-grade alimentary lymphoma: an emerging entity and a potential animal model for human disease. BMC Veterinary Research. 11;14(1):306

Matsumoto I, Uchida K, Nakashima K. 2018. Pathological features of intestinal T-cell lymphoma in Shiba dogs in Japan. Veterinary Comparative Oncology. 16(4):417–423.

Merlo DF, Rossi L, Pellegrino C, Ceppi M, Cardellino U, Capurro C, Ratto A, Sambucco PL, Sestito V, Tanara G, Bocchini V. 2008. Cancer Incidence in pet dogs: findings of the animal tumour registry of Genoa, Italy. Journal of Veterinary Internal Medicine. 22:976–984.

Meuten DJ. 2002. Tumours in Domestic Animals. 4th edn. Iowa State Press, Ames, IA, USA.

Mikiewicz M, Paździor-Czapula K, Gesek M, Lemishevskyi V, Otrocka-Domagała I. 2019. Canine and Feline Oral Cavity Tumours and Tumour-like Lesions: A Retrospective Study of 486 Cases (2015–2017). Journal Comparattive Pathology. 172:80–87.

Misdrop. 2002. Tumours of mammary glands In: Tumour in Domestic Animals 4th edition. Iowa State Press. pp: 575-606.

Moore PF, Affolter VK, Olivry T, Schrenzel MD. 1998. The use of immunological reagents in defining the pathogenesis of canine skin disease involving proliferation of leukocytes. In: Kwochka K. W., Willemse T. envon Tscharner C. (eds.). Advances in Veterinary Dermatology.

Moore PF, Rodriguez-Bertos A, Kass PH. 2011. Feline gastrointestinal lymphoma: mucosal architecture, immunophenotype, and molecular clonality. Veterinary Pathology. 49:658–68.

Moreno K and Bracarense AP. 2007. Linfoma canino de células T: aspectos epidemiológicos, clínicos e morfológicos de 38 casos. Brazilian. Journal Veterinary Research and Animal Science. 44(Suppl.):103-110.

Morrison WB. 2002. Cancer in dogs and cats: medical and surgical management. Teton New Media.

Morrison WB. 1998. Cancer in Dogs and Cats (1st ed.).

Moulton JE. 1978. Tumors in Domestic animal. 2nd ed. University of California Press. Berkeley and Los Angeles.

Moulton JE. 1990. Tumours in domestic animals, 3rd Edition. University of California press, Berkely. 31-33.

Mouser P, Miller MA, Antuofermo E, Badve SS, Mohammed SI. 2010. Prevalence and classification of spontaneous intraepithelial lesions in dogs without clinical mammary disease. Veterinary Pathology. 47:475–284.

Mouser P, Miller MA, Antuofermo E, Badve SS, Mohammed SI. 2010. Prevalence and classification of spontaneous intraepithelial lesions in dogs without clinical mammary disease. Veterinary Pathology. 47:475–284.

Nandini, DB. 2017. Cancer Cell Nucleus: An Insight. Journal Molecular Biomarkars Diagnosis. S2:026.

Pang, Lisa Y, Argyle, David J. 2016. "Veterinary oncology: Biology, big data and precision. Veterinary journal. 213.

Papetti M, and Herman I. 2002. Mechanisms of normal and tumor-derived angiogenesis. American Journal Physiology - Cell Physiology. 282(5),C947-C970.

Patel MP, Ghodasara DJ, Raval SH and Joshi BP. 2019. Incidence, Gross Morphology, Histopathology and Immunohistochemistry of Canine Mammary Tumors. Indian Journal of Veterinary Sci and Biotechnology. 14(4), 40-44.

Pawaiya RVS. 2005. Pathology of chemically induced neoplasms and evaluation of molecular markers in diagnosis of animal tumours. Indian Journal of Veterinary Pathology. 29(1), 63.

Pawan K. and Pawaiya RVS. 2010. Advances in Cancer Diagnostics. Brazilian Journal Veterinary Pathology. 3(2), 142-153.

Pawan K, Kumar R, Pawaiya RVS, Madhu BP. 2010. Diagnostic significance of mitotic index and AgNOR count in canine mammary tumours. Brazalian Journal Veterinary Pathology. 3(1), 41-45.

Pickup MW, Mouw JK, Weaver VM. 2014. The extracellular matrix modulates the hallmarks of cancer. European Molecular Biology Orgainaization Reports. 15:1243–53.

Priester WA, McKay FW. 1980. The occurrence of tumors in domestic animals. National Cancer Institution Monographes. 54:1–210.

Purebred Dog Health Survey. 2004. Kennel Club, British Small Animal Veterinary Association Scientific Committee.

Rakib TM, Islam MS, Rahman MM, Siddiki AZ, Sutradhar BC, Hossain MA, Masuduzzaman M. 2016. A case report on invasive ductal carcinoma in dog. Advance Animal Veterinary Science. 4(1): 1-4.

Rapidis AD, 1982. Lipoma of the oral cavity. International Journal Oral Surgery. 11(1):30-35.

Raskin R and Meyer D. 2010. Canine and Feline Cytology—A Color Atlas and Interpretation Guide, 2nd edition. St. Louis, MO: Saunders Elsevier, p. 77-104.

Raval SH, Joshi DV, Parmar RS, Patel BJ., Patel JG, Patel VB, Ghodasara DJ, Chaudhary, PS, Kalaria, VA and Charavada AH. 2018. Histopathological classification and immunohisto chemical characterization of canine mammary tumours. Indian Journal Veterinary Pathology. 42(1), 19-27.

Reddy, GM, Pawan K, Kumar R, Pawaiya RVS, Ravindran R. 2009. Histopathological classification and incidence of canine mammary tumours. Indian Journal Veterinary Pathology. 33(2), 152-155.

Reddy GM, Kumar P, Kumar R, Pawaiya RVS and Ravindran R. 2009. Histopathological classification and incidence of canine mammary tumours. Indian Journal Veterinary Pathology. 33(2), 152-155.

Rissetto K, Villamil JA, Selting KA, Tyler J, Henry CJ. 2011. Recent trends in feline intestinal neoplasia: An epidemiologic study of 1,129 cases in the veterinary medical database from 1964 to 2004. Journal of American Animal Hospital Association. 47, 28–36.

Ritt MG. 2009. Epidemiology of hematopoietic neoplasia. In: Weiss D.J., Wardrop K.J., editors. Schalm's Veterinary Hematology. 6th ed. Blackwell Publishing; Ames, IA, USA.

Roccabianca P, Schulman FY, Avallone G, Foster RA, Scruggs JL, Dittmer K, Kiupel M. 2021. In: Surgical Pathology of Tumors of Domestic Animals:

Volume 3: Tumors of Soft Tissue. 3rd ed. Kiupel M., editor. Davis-Thompson DVM Foundation; Gurnee, IL, USA.

Rohitash D, Dadhich H, Sharma GD, Singh G, Adholpuria S, Abhilasha D and Manish A. 2018. Pathological and haematobiochemical studies of lipoma in dogs. Veterinary Practitioner. Vol. 19 No. 1.

Runnells RA, Monlux WS, Monlux AW. 1965. Principles of Veterinary Pathology, 7 th edition. Scientific Book Agency, Calcutta.

Sasikala M, Arulmozhi A, Balasubramaniam GA, Kathirvel S and Vigneshwaran S. 2020. Pathology of giant lipoma in a non-descript dog: A case report. Journal of entomology and zoology studies.

Schrohl AS, Anderson MH, Sweep F, Schmitt M, Harbeck N, Foekens J. 2003. Tumor markers- From laboratory to clinical utility. Molecular and Cell Proteomics. 378-87.

Seelig D, Webb T, Avery P, Avery A. 2014. Canine T-zone lymphoma: unique immunophenotypic features, outcome and population characteristics. Journal of Veterinary Internal Medicine. 28(3).

Simon D 2006. "Malignant lymphoma in the dog: Short and long term chemotherapy" . Proceedings of the North American Veterinary Conference.

Sorenmo K. 2003. Canine mammary gland tumors. Veterinary Clinics of North American Small Animal Practice. 33:573-596

Stedman's medical dictionary (28th ed.). Philadelphia: Lippincott Williams & Wilkins. 2006. p. Neoplasm.

Subapriya S, Vairamuthu S, Pazhanivel N, George RS and Gokulakrishnan M. 2020. Cutaneous lipoma in dogs. Journal of Entomology and Zoology Studies. 8(3): 17-19.

Suzano SMC, Sequeira JL, Rocha NS and Pessoa AWP. 2010. Classificação citológica dos linfomas caninos. Brazilian Journal Veterinary Research and Animal Science. 47(1):47-54.

Taylor, Elizabeth J. 2000. Dorland's Illustrated medical dictionary (29th ed.) Philadelphia: Saunders. p. 1184.

Teunisse GHB. 1977. lntrathoracic liporna in a dog. Tijdschrift voor Diegeneeskunde 102,113116.

The BT, Fearon ER, Niederhuber JE, Armitage JO, Doroshow JH, Kastan MB, Tepper JE. 2020. Chapter 14: Genetic and Epigenetic Alterations in Cancer, eds. Abeloff's Clinical Oncology. 6th ed. Philadelphia, Pa: Elsevier.

Thomas R. 2015. Cytogenomics of Feline Cancers: Advances and Opportunities. Veterinary Science. 246-258.

Tran C, Moore AS, Frimberger AE. 2016. Surgical treatment of mammary carcinomas in dogs with or without postoperative chemotherapy. Veterinary and comparative oncology. 14(3): 252-262.

Turek MM. 2005. Correlation between radiographic and histologic findings in cats with vaccine-associated sarcoma. Veterinary Radiology Ultrasound. 46(1):64-8.

Vail DM, Thamm DH, Liptak JM, Withrow and MacEwen. 2019. Small Animal Clinical Oncology. 6th ed. Elsevier; St. Louis, MO, USA.

Vail DM, MacEwan EG, Young KM. 2001. Canine lymphoma and lymphoid leukemias. In: Withrow SJ, MacEwan EG, editors. Small Animal Clinical Oncology. Philadelphia: Saunders. pp. 558–579.

Vail DM, Moore AS, Ogilvie GK, Volk LM. 1998. Feline lymphoma (145 cases): proliferation indices, cluster of differentiation immunoreactivity, and their association with prognosis in 90 cats. Journal Veterinary Internal Medicine. 12:349–54.

Valenciano A, Cowell R, Cowell and Tyler. 2020. Diagnostic Cytology and Hematology of the Dog and Cat, 5th edition. St. Louis, MO: Elsevier. p. 171-181.

Veena P, Bharathi S and Devarathnam J. 2013. Surgical management of lipoma in a dog. Intas Polivet. 14(2): 475-476.

Von Euler H. 2011. Tumours of the mammary glands. In: Dobson J, Lascelles, editors. BSAVA Manual of Canine and Feline Oncology. 3rd edition. chapter 16. British Small Animal Veterinary Association. pp. 237 –247.

Wlfson WJM, Dulisch JM, ML and TAMS, TR. 1984. lntrathoracic lipoma in a dog. Journal of the American Veterinary Medicine. 185(9).

Warner MR. 1976. Age, incidence and site distribution of mammary dysplasia's in young beagle bitches. Journal of the National Cancer Institute. 57:57-61.

Wilson DS, and HAWE RS. 1986. lntrathoracic lipoma in a dog. Journal of the American Animal Hospital Association. 22, 9597.

You JS, Jones PA. 2021. Cancer genetics and epigenetics: Two sides of the same coin? Cancer Cell. 22:9–20.

Zachary JF, McGavin MD. 2013. Pathologic Basis of Veterinary Disease. 5th edition. Canadian Veterinary Journal. 55(4):372.

Appendix

❖ **Compositions of fixative used to preserve tissue sample**

➢ **Bouin's solution (fixative)**

- Picric acid, saturated aqueous solution – 750 ml.
- 37-40% formalin – 250 ml
- Glacial acetic acid – 50 ml

❖ **Reagents and solutions used in staining of tissue sections**

➢ **Harris Hematoxylin**

- Hematoxylin Crystals: 5.00g
- 100% Alcohol: 50.0ml
- Ammonium alum: 100g
- Distilled water: 1000ml
- Mercuric oxide (red): 2.5g.

❖ **Preparation of Eosin Solution:**

➢ 1% Stock Alcoholic Eosin:

Yellow eosin, water-soluble: 1g

Distilled water: 20ml

Dissolved and add 95% Alcohol: 80 ml.

❖ **Acid alcohol**

- Alcohol 70% - 1000 ml.
- Hydrochloric acid, concentrated - 10 ml.

❖ **Ammonia water**

Distilled water - 1000 ml.

Ammonium hydroxide, 28% - 2-3 ml.

❖ **Staining procedure**

- Xylene ..... two changes each for 5 mins
- 100% alcohol..... two changes each for 5 mins
- 95% alcohol..... 2mins
- Tap water.....5mins
- Harris hematoxylin.....10mins
- Running tap water.....10mins
- 1% acid alcohol.....2dips
- Running tap water.....5mins
- Ammonia water.....3dips
- Running tap water.....10mins
- Eosin stain.....2mins
- 95% alcohol.....3mins
- 100% alcohol.....3mins
- 100% alcohol.....3mins
- Xylene.....two changes each for two mins.

Biography

Anamika Dhar passed the Secondary School Certificate examination in 2011 followed by Higher School Certificate examination in 2013. She obtained her Doctor of Veterinary Medicine Degree in 2019 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now she is a candidate for the degree of MS in Veterinary Pathology, under the Department of Pathology and Parasitology, Faculty of Veterinary Medicine, CVASU. She holds a deep interest in the fields of Oncology.